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Effects of dietary camelina, flaxseed, and canola oil supplementation on plasma fatty acid concentrations and health parameters in horses

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ABSTRACT

Camelina (*Camelina sativa*) is a hardy, low-input oilseed crop that provides a rich source of the n-3 fatty acid, α-linolenic acid (**ALA**). The primary purpose of the present study was to assess the effects of dietary camelina oil (**CAM**) consumption on various health parameters, as compared to horses fed canola oil (**OLA**) or flax oil (**FLX**). Secondly, to determine how dietary CAM, FLX, and OLA alter circulating plasma total lipids across time. Thirty horses, from three separate herds, were used for this study [14.9 years ± 5.3 years; 544 ± 66 kg calculated BW (mean ± SD)]. After a 4-week gradual acclimation period using sunflower oil mixed with soaked hay cubes, horses were balanced by location, age, sex, weight, and breed and randomly allocated to one of three treatment oils (CAM, OLA, or FLX) at an inclusion of 370 mg of oil/kg BW/day. Horses had *ad libitum* access to hay and/or pasture for the duration of the study. Body condition score (**BCS**), BW, oil intake, complete blood counts, plasma biochemical profiles, and plasma total lipids were measured on weeks 0, 2, 4, 8, and 16 throughout the 16-week treatment period. BW, BCS, and oil intake were analyzed using an ANOVA using PROC GLIMMIX in SAS Studio. Complete blood counts and biochemical profiles were analyzed using an ANCOVA, and fatty acids were analyzed using an ANOVA in PROC MIXED in SAS Studio. No differences were observed among treatment groups for BW, BCS, oil intake, complete blood counts, and biochemical parameters. Individual fatty acids that differed among treatments and/or across time were largely reflective of the different FA profiles of the oils provided. Most notably, plasma ALA was greater for FLX than OLA, but neither differed from CAM ($P = 0.01$). Linoleic acid did not differ among treatments or over time ($P > 0.05$). The n-6:n-3 ratio decreased over time for both CAM and FLX, and ratios were lower for FLX than OLA at week 16, but not different from CAM ($P = 0.02$). These results suggest that dietary CAM had no adverse effects on health parameters and that daily supplementation of CAM and FLX at 370 mg of oil/kg BW/day induces positive changes (a decrease) in the n-6:n-3 status of the horse. Consequently, CAM may be considered as an alternative oil to FLX in equine diets.

Implications

Camelina (*Camelina sativa*) is a hardy, low-input crop that grows well in Northern countries (ie. Canada) and has the potential to be utilized as an alternative ingredient to flaxseed or canola oil in equine nutrition. Feeding camelina oil to horses for 16 weeks did not negatively impact their health, and in fact, camelina and flaxseed oil positively impacted the fatty acid status of the horses. Overall, this suggests that camelina oil can be used as an environmentally, economically, and socially sustainable alternative oil supplement for horses.

Introduction

The recent search for new sources of n-3 fatty acids that are environmentally, economically, and socially sustainable has sparked a renewed interest in camelina oilseeds (*Camelina sativa*). Camelina, also referred to as 'false flax' or 'gold of pleasure', is an ancient crop from the *Brassicaceae* family that has gained attention

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for its high concentration of α-linolenic acid (**ALA**, 18:3n-3) and low corresponding n-6 to n-3 ratio (∼1:2) (Zubr and Matthäus, 2002; Berti et al., 2016; Burron et al., 2021). Camelina oil additionally possesses high concentrations of polyphenols and tocopherols, which support the oxidative stability of the oil and improve the shelf-life (Matthäus, 2002; Zubr and Matthäus, 2002; Abramovič et al., 2007). While previous reports are varied, in general, the oxidative stability of camelina oil is higher than other n-3 rich oils (fish and flaxseed oil), but less stable than some n-6 or n-9 rich oils (olive, sunflower, sesame, and corn oil) (Eidhin et al., 2003b). There are additionally many agronomic benefits to *Camelina sativa* that are supportive of its use as a sustainable alternative oilseed crop. Camelina is a hardy crop that has a short growing season and has a tolerance to drought and low temperature and, as such, can be grown in various climates, seasons, and soil types (Berti et al., 2016; Gugel and Falk, 2006; Moser, 2010; Saucke and Ackermann, 2006; Vollmann and Eynck, 2015). Camelina is resistant to many diseases and pests that commonly impact other oilseed crops and also demonstrates an ability to suppress key annual weeds. Camelina seeds, like many oilseeds, contain some anti-nutritional factors, such as glucosinolates, sinapine, phytates, and trypsin inhibitors. However, these anti-nutritional factors are not present in significant concentrations in the oil extracted from the seed (Pozzo et al., 2022). The dietary consumption of oil or meal from this novel crop has been previously investigated in many species, including dogs (Burron et al., 2021), rabbits (Peiretti et al., 2007), pigs (Habeanu et al., 2010), and fish (Morais et al., 2012; Hixson et al., 2013), though to the authors knowledge, the use of camelina oil in equine diets has not been previously investigated.

Lipids serve many functional roles in equine diets such as increasing dietary energy density, serving as a caloric substitute for hydrolysable and rapidly fermentable carbohydrates, providing a source of fatty acids and antioxidants, and facilitating the absorption of fat-soluble vitamins (Mélo et al., 2016; National Research Council, 2007). Most commonly, these are delivered as supplemental oils, though less frequently, they can be included as part of a complete feed. Compared to a hay and grain diet with a digestible energy to net energy conversion efficiency of less than 60% (NRC, 2007), absorbed lipids have greater metabolic utilization. Specifically, a conversion efficiency of over 85% has been reported in ponies fed supplemental corn oil (Kane et al., 1979).

The benefits of dietary lipid supplementation include improving skin and coat health (O'Neill et al., 2002; Goh et al., 2004), supporting athletic performance (Kronfeld, 1997; Godoi et al., 2010; Bazzano et al., 2015; Manso Filho et al., 2019; Mowry et al., 2022), as well as providing beneficial anti-inflammatory and immune-modulating effects (Hall et al., 2004; Piccione et al., 2019). It is worth noting that the unique fatty acid profile of each supplemental oil can affect the type and magnitude of these benefits. Horses are natural foragers; therefore, their diets contain a substantial quantity of n-3 fatty acids from pasture grasses (Kitessa et al., 2010; Dal Bosco et al., 2014; Piccione et al., 2019). However, the n-6:n-3 ratio of equine diets changes quickly when horses no longer have access to pasture and are fed a diet high in concentrates, often over-supplying n-6 fatty acids and minimizing the supply of n-3 fatty acids. This imbalance can be problematic as in general, the n-6 and n-3 fatty acid pathways work in opposition to each other to maintain homeostasis in the body, since most n-6 fatty acids support innate inflammation in the body, while most n-3 fatty acids have anti-inflammatory properties (Sinclair et al., 2002). As such, adding a dietary oil source that is rich in n-3 fatty acids to an equine diet can help balance out this ratio, maximizing the benefits of both the n-6 and n-3 fatty acids, in addition to meeting other functional benefits that a dietary oil source can provide. Many oils commonly used in equine diets have a high n-6:n-3 ratio, such as soybean oil (8:1), corn oil (57:1), sunflower oil (64:0), or rice-bran oil (26:1) (Eidhin et al., 2003a; Glencross, 2009; Mowry et al., 2022). Conversely, flaxseed oil has a low n-6:n-3 ratio (∼1:3), thus making it a suitable plant-based n-3 supplement (Mowry et al., 2022), though additional plant-based n-3 rich ingredients would help diversify ingredients available to horse owners.

The first objective of this study was to assess the effects of dietary camelina oil supplementation on health parameters by comparing it to flaxseed oil and canola oil, two commonly used sources of ALA and linoleic acid, respectively, in equine diets. Since camelina oil has been approved as safe for use in many species, and because flaxseed oil and canola oil are commonly used in equine diets, we hypothesize that camelina oil supplementation in healthy, adult horses has no negative effects on oil intake, calculated BW, body condition score (**BCS**), complete blood counts, and plasma biochemistry profiles. The second objective of this study was to understand how these oils affect circulating fatty acid concentrations that provide the basis of the metabolic effects of these different oils. We hypothesize that circulating plasma fatty acids are reflective of the fatty acid profile of each oil supplement, in that the n-6:n-3 ratios are the lowest in the group of horses fed flaxseed oil, moderate in the camelina oil group, and highest in the canola oil group.

Material and methods

Animals and housing

The present study was approved by the University of Guelph's Animal Care Committee (Animal Utilization Protocol #4481) and was conducted in accordance with national (Canadian Council on Animal Care) and institutional guidelines for the care and use of animals. Thirty (30) adult horses [23 mares, 7 geldings; 14.9 years ± 5.3 years; 544 ± 66 kg calculated BW (mean ± SD)] of various breeds (17 Standardbreds; 2 Thoroughbreds; 4 Draft Crosses; 3 Quarter Horses; 2 Arabians; 1 Paint) were used. All horses met the inclusion criteria of being clinically healthy on assessment, showing no abnormalities on routine blood biochemistry and complete blood counts, and were not receiving anti-inflammatory pharmaceuticals. Horses used for this study were from three herds housed at separate locations: Arkell Research Station (Arkell, Ontario, Canada; 17 mares, 4 geldings), Equine Sports Medicine and Reproduction Centre (University of Guelph, Guelph, Ontario, Canada; 3 mares), and CJ Equestrian Centre (Rockwood, Ontario, Canada; 3 mares, 3 geldings). Horses at all locations were group housed outdoors in mixed herds of up to nine horses per paddock or pasture, and with sufficient access to shelters. Horses at Arkell Research Station and Equine Sports Medicine and Reproduction Centre were not on an exercise program but could exercise voluntarily within their paddock or pasture. Horses at CJ Equestrian Centre were exercised at a moderate weekly workload (3–5 hours/week; ∼90 beats/min), which remained consistent throughout the course of the study (NRC, 2007). The study period (wash-in and treatment periods) of the Arkell Research Herd was from June to December, with six horses starting the wash-in diet at the beginning of June and 15 horses starting the wash-in diet in mid-August. The study period of the CJ Equestrian Herd was from the end of July to early December, and the study period for the Equine Sports Medicine and Reproduction Centre horses was from the end of August to the beginning of January. Treatment diets were evenly distributed among these locations and for different start dates.

Diets, experimental design, and oil intake

In order to acclimate the horses to a high dietary oil inclusion, supplementation of sunflower oil (Table 1) was slowly introduced

over a 4-week period. During this wash-in period, the oil inclusion gradually increased from 37 mg oil/kg calculated BW/day to the final inclusion of 370 mg oil/kg calculated BW/day over the 4-week period. In determining the dosage of the oil supplement (370 mg/kg BW) for the present study, we considered the existing body of research indicating that horses have a capacity to tolerate high-fat diets, as well as the recommended upper limit of 700 mg/kg BW/day by the NRC (Harris et al., 1999; NRC, 2007). This dosage was selected to accommodate the specific qualities of the horses in our study population and to avoid potential adverse effects associated with high-fat intake in horses with low activity levels and high BCS. Additionally, this level of supplemental oil is greater than current recommendations on commercial flaxseed oil marketed to the equine sector. After the wash-in period, horses were grouped by location, age, sex, weight, and breed and randomly assigned to one of three treatment groups (Table 1) [flaxseed oil (**FLX**), camelina oil (**CAM**), canola oil (**OLA**)]. Oil (370 mg oil/kg calculated BW/day) was mixed with water-soaked hay cubes (Premium Timothy™ Hay Cubes, Ontario Dehy, Goderich, ON, Canada) to create a mash before feeding, and daily portions were provided to horses once or twice daily depending on location and start date. Feeding of treatment diets continued for 16 weeks.

All horses in the study received *ad libitum* access to hay or pasture throughout the study period; however, small differences in management practices between locations should be noted. From the beginning of June, horses at Arkell Research Station were housed on pasture with no access to hay, then in early October, *ad libitum* access to hay was additionally provided to supplement pastures. In mid-November, horses were moved from the pastures to winter housing and had *ad libitum* access to hay, but no pasture, for the remainder of the study (Supplementary Tables S1 and S2). The horses at Equine Sports Medicine and Reproduction Centre were fed hay *ad libitum,* with intermittent access to pasture throughout the study period. Horses at CJ Equestrian had *ad libitum* access to hay with 2.54 cm slow feed netting, as well as intermittent access to pasture and/or electrolytes.

Assuming horses at maintenance will consume 1.5–2% of their BW in DM per day (NRC, 2007), then the horses in the present study with a mean BW of 544 kg would be consuming an average of 11 kg of DM (pasture or hay) per day at the upper end of the range. Given the relatively low fat content of hay (Average mid-quality hay (Ontario, Canada): 2.8% crude fat (DM basis); MadBarn FeedBank) and pasture (Average pasture (Ontario, Canada): 3.8% crude fat (DM basis); MadBarn FeedBank), we can then assume that the horses in the present study were consuming at most between 30 g and 40 g crude fat per day on a DM basis in their basal diet of hay, pasture, or hay and pasture. Daily monitoring of the forage intake was not feasible for this project due to the horses being group housed with free access to forage, as is common and best practice in equine nutrition and welfare. However, given the low crude fat content in the basal diet in comparison to the >200 g of oil supplementation provided daily and the standardized basal diet among treatment groups, the daily intake of forage and the fatty acid profile of the forage is unlikely to affect the primary outcomes of the present study, which is to compare three dietary oil supplements in terms of health parameters and total lipid profiles.

Oil intake was recorded daily, and then, intake values were averaged for each horse over 4-week intervals to be analyzed. On occasions where horses refused treatment diets for more than two consecutive days, oil was syringe fed to horses using a 60 mL syringe to ensure delivery of treatment oils. Partial refusals of feed were rare during this study and typically would be freely consumed by the horse at a later time when offered again by researchers on the same day.

BW and body condition scores

A flexible measuring tape was used to measure heart girth and body length in centimeters (cm) and the formula $[BW\ (kg) = ((Heart\ Girth\ (cm))^2 \times Body\ Length\ (cm))/11\ 877]$ by Carroll and Huntington (1988) was used to calculate BW (Carroll and Huntington, 1988). Calculated BW is considered an accurate way to measure BW in horses when weight scales are not available (Carroll and Huntington, 1988). However, it must be noted that BW in the present study was calculated and could vary slightly from BW measurements using a weight scale. These BW calculations were used to determine the amount of oil provided to the horse each day. However, this paper will only report calculated BW values from the start of treatment oil supplementation (week 0) to the end of the study (week 16), as the goal is to assess the impacts of the treatment oils, not the wash-in oil, on BW. Body condition score was assessed on weeks 0, 2, 4, 8, and 16 using a 9-point scale over six anatomical locations via physical and visual assessment. A score of 1 was considered extremely thin and 9 was considered extremely obese (Henneke et al., 1983).

Blood collection and handling

Blood was collected on weeks 0, 2, 4, 8, and 16 via jugular venipuncture using a 20- or 21-gauge × 3.8 cm VACUETTE™ Multi-Drawing Blood Collection Needle (Greiner Bio-One, Kremsmünster, Upper Austria, Austria) and a Multi-Sample Needle Holder (Globe Scientific, Mahwah, NJ, USA). Blood samples were drawn at the same time for each collection day and at each location (between 0800 and 0830) before daily feeding of study diets. Hay and water were not restricted before blood collection. First, approximately 6 mL of blood was collected into a lithium heparin vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) to be subsequently analyzed for plasma biochemical indices and fatty acid concentrations. Next, approximately 2 mL of blood was collected into a K_2 EDTA 10.8 mg Vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for complete blood counts. All blood tubes were stored on ice until the end of blood collection; then, the lithium heparin blood was centrifuged at 3 500 *g* for 15 minutes using an accuSpin Micro 17 centrifuge (Thermo Fisher Scientific, Waltham, MA, USA). After centrifugation, plasma aliquots were collected and stored for subsequent analysis. Plasma for fatty acid concentrations was stored at −80 °C before analysis. Plasma for biochemical indices was analyzed the same day at the Animal Health Laboratory (University of Guelph, Guelph, Canada) or frozen at −80 °C until submission to the laboratory was available. Additionally, EDTA whole blood was submitted to the Animal Health Laboratory on collection day unless the laboratory was closed, in which case blood smears were done and stored at room temperature until submission, as is recommended by the Animal Health Laboratory.

Complete blood count and biochemistry analysis

Complete blood cell count (hematology) values were determined at the Animal Health Laboratory using an automated hematology system (Advia 2120; Siemens Global, Munich, Germany). Parameters analyzed include blood leukocyte counts, erythrocyte counts, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelets, mean platelet volume, total solids protein, and segmented neutrophil, lymphocyte, monocyte, and eosinophil counts. Plasma biochemistry concentrations were determined using an automated biochemical analyzer (Cobas 6000 c501 Analyzer; Roche Diagnostics International AG, Rotkreuz,

Table 1
Fatty acid profiles of oil types used in an equine feeding trial (camelina, canola, flax, and sunflower oil).

Fatty Acid (% composition)	Sunflower Oil[1]	Canola Oil[1]	Flax Oil[1]	Camelina Oil[2]
16:0	5.00	4.39	5.91	5.50
16:1n-7	–	0.23	0.20	0.00
18:0	3.25	1.47	3.12	2.55
18:1 *cis*-9	53.3	57.7	17.2	14.0
18:1n-7	–	–	0.89	1.02
18:2n-6	38.0	22.7	16.0	17.2
18:3n-3	0.28	12.0	56.0	34.9
20:0	0.10	0.51	0.33	1.53
20:1n-9	0.04	1.01	0.33	15.8
20:1n-7	–	–	–	0.71
20:2n-6	–	–	–	2.14
20:3n-3	–	–	–	1.53
22:1n-9	–	0.10	–	3.16
Unsaturated fatty acids	91.6	93.6	90.6	90.4
SFAs	8.38	6.37	9.36	9.57
MUFAs	53.4	59.0	18.6	34.6
PUFAs	38.2	34.6	72.0	55.8
∑ n-6	38.0	22.7	16.0	19.3
∑ n-3	0.28	12.0	56.0	36.5
∑ n-9	53.4	58.8	17.5	32.9
∑ n-7	0.00	0.23	1.10	1.73
n-6/n-3 ratio	135	1.89	0.29	0.53

Abbreviations: MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; SFAs = saturated fatty acids.
[1] Numerical values represent average analytical values that are adapted from Olomu and Baracos, 1991; Teh and Birch, 2013; Ghazani et al., 2014; Mungure and Birch, 2014; and Rueda et al., 2014.
[2] Gas chromatography analysis by Smart Earth Camelina Corp. (Saskatoon, Saskatchewan, Canada).

Switzerland). Biomarkers assessed include calcium, phosphorus, magnesium, sodium, potassium, chloride, carbon dioxide, anion gap, sodium:potassium ratio, total protein, albumin, globulin, albumin:globulin ratio, urea, creatinine, glucose, cholesterol, total bilirubin, conjugated bilirubin, free bilirubin, alkaline phosphatase, gamma-glutamyl transferase, aspartate aminotransferase, creatine kinase, glutamate dehydrogenase, and calculated osmolarity. Reference intervals used by the Animal Health Laboratory for both complete blood count and biochemistry analytes were previously determined based on 102 mixed-breed horses whose samples had been submitted for routine health checks. Biochemical reference intervals were determined using serum, whereas lithium heparin plasma was used in the current study. Heparinized plasma and serum have been shown to be similar in most biochemical analyte concentrations (Mohri et al., 2007).

Plasma fatty acid analysis

Stored plasma aliquots were removed from the freezer; then, 1 mL of plasma was freeze dried and sent to Smart Earth Camelina (Saskatoon, Saskatchewan, Canada) for routine analysis using gas chromatography. Gas chromatography fatty acid methyl ester analysis was performed based on King et al. (2008). Briefly, the freeze-dried plasma was reconstituted in 0.5 mL of hexane containing 0.295 mg 19:0 (MJS BioLynx) as an internal standard. Samples were methylated by adding 2.0 mL 0.75 N Methanolic Acid and incubated for 2 hours at 80 °C. Tubes were allowed to cool and settle; then, 100 µL of the hexane phase was transferred to gas chromatography vials and run using a gas chromatography Agilent 7890 N equipped with flame ionization detector and DB23 column (0.25 mm × 30 m, 0.25 um thickness; J&W Folsom, California, USA).

Statistical analysis

Statistical analyses for BCS, oil intake, and calculated BW were performed using an ANOVA with PROC GLIMMIX of SAS® (v.9.4., SAS Institute Inc., Cary, NC, USA), where horse was treated as the experimental unit, treatment oil was a fixed effect, week was a repeated measure, and location was a random effect. Statistical analyses for complete blood count, biochemistry, and plasma fatty acids were performed using PROC MIXED of SAS® (v.9.4., SAS Institute Inc., Cary, NC, USA), where horse, week, and treatment oil were treated as fixed effects and location was treated as a random effect. Week was also treated as a repeated measure. For biochemistry and complete blood count data, an analysis of covariance was used, with baseline as the covariate, to account for individual variation between horses. For all data, least-square means were used to assess differences among means of treatment, week, and treatment-by-week interactions. When fixed effects were significant, means were separated using Tukey-Kramer Adjustments. Assumptions of residuals for all parameters were assessed with Q-Q and scatter plots via visual assessments and data were not transformed. Significance was declared at a $P < 0.05$. However, 95% confidence intervals are also provided to establish significance for complete blood counts and biochemistry analytes. Results are reported as least square means ± SEM. All statistical models and statistical codes are available in Supplementary Material S1.

Results

Health parameters

BW, body condition score, and oil intake

No differences were observed among treatments, across weeks, or for treatment-by-week interactions for BCS, oil intake, or calculated BW throughout the 16-week feeding period ($P > 0.05$) (Supplementary Tables S3–S5). Average daily oil intake throughout the study period was 207.67 g/day for CAM, 203.63 g/day for FLX, and 198.73 g/day for OLA (Supplementary Table S5). While palatability was outside the scope of the current investigation, it is worth noting that eight horses began refusing treatment oils and subsequently needed to be syringe fed the oil for the remainder of the study period. Distribution of horses among treatment groups that required syringe feeding for a period longer than 7 days was as follows: five horses in the FLX treatment group, three

horses in CAM treatment group, and zero horses in OLA treatment group. Moreover, those that were syringe fed were distributed across locations (Arkell Research Station, n = 4; Equine Sports Medicine and Reproduction Centre, n = 1; CJ Equestrian, n = 2).

Complete blood count and biochemistry

Cholesterol did not differ among treatments, though there was a treatment-by-week interaction. Specifically, concentrations of cholesterol for the FLX group were lower at week 16 compared to weeks 2, 4, and 8. Additionally, FLX was lower than OLA at week 16, though neither differed from CAM ($P = 0.01$). No other complete blood count or biochemical analytes differed among treatments or for treatment-by-week interactions ($P > 0.05$; Table 2). Mean concentrations of complete blood count and biochemical analytes stayed within the Animal Health Laboratory reference range at all time points, with the exception of potassium, total bilirubin, free bilirubin, and glutamate dehydrogenase.

Plasma fatty acids

Saturated fatty acids

Palmitic acid (16:0) % composition was greater for CAM than FLX, but not different from OLA ($P = 0.03$; Table 3). No other saturated fatty acids (**SFA**) differed among treatments ($P > 0.05$; Table 3). Arachidic acid (20:0) % composition was greater on weeks 2, 8, and 16 compared to week 0 and 4 ($P < 0.01$; Table 4). No other SFA differed across time ($P > 0.05$; Table 4). Margaric acid (17:0) % composition was greater at week 16 compared to week 0 for the OLA group, but not different from any other time points ($P = 0.01$; Table 5). No other treatment-by-week interactions were observed ($P > 0.05$; Table 5).

Monounsaturated fatty acids

Oleic acid (18:1n-9) % composition for the OLA treatment group was greater than the FLX and CAM groups, while FLX and CAM were similar ($P < 0.01$; Table 3). No monounsaturated fatty acids (**MUFAs**) differed across time ($P > 0.05$; Table 4). Oleic acid % composition was greater on weeks 2, 4, and 16 in the OLA group compared to week 0 ($P < 0.01$; Fig. 1). Additionally, % composition of oleic acid was greater in the OLA than the FLX group at weeks 2, 4, 8, and 16, and neither differed from the CAM group. Vaccenic acid (18:1n-7) % composition was greater on weeks 2 and 4 compared to week 0 for the OLA group, while weeks 8 and 16 were similar to all other timepoints ($P = 0.02$; Table 5). Gondoic acid (20:1n-9) % composition was greater at all time points compared to 0 for the CAM group ($P < 0.01$; Table 5). The % composition for summed MUFA was greater at weeks 2, 4, and 16 than week 0 for the OLA group, while week 8 did not differ from any other time points. Additionally, OLA was greater than FLX at weeks 2, 4, 8, and 16. CAM was similar to both FLX and OLA at all time points ($P = 0.02$; Table 5).

Polyunsaturated fatty acids

The % composition of ALA was greater for the FLX group than the OLA group, though neither differed from CAM ($P = 0.01$; Table 3). Eicosadienoic acid (20:2n-6) was greater for CAM than FLX and OLA ($P < 0.01$; Table 3). Arachidonic acid (20:4n-6) % composition was greater in the CAM and FLX groups than in the OLA group ($P < 0.01$; Table 3). The % composition for summed polyunsaturated fatty acids (**PUFAs**) was greater in FLX than OLA, though neither differed from CAM ($P < 0.01$; Table 3). No other PUFA differed among treatment groups ($P > 0.05$; Table 3). The % composition for ALA was greater at weeks 2, 4, and 8 compared to week 0, and week 16 was similar to all time points ($P < 0.01$; Table 4). Arachidonic acid was greater at all time points compared to 0 ($P < 0.01$; Table 4). No other PUFA differed across time ($P > 0.05$; Table 4). The % composition of ALA for the CAM group was greater at week 8 compared to week 0, while weeks 2, 4, and 16 were similar to all other time points. The FLX group was greater at weeks 2, 4, and 16 compared to week 0, but not different from week 8. Finally, ALA was greater in the FLX group than the OLA group at week 16, though neither differed from the CAM group ($P = 0.01$; Fig. 1). Eicosadienoic acid was greater on weeks 4, 8, and 16 compared to week 0 for the CAM group, and week 2 was similar to all other time points. Additionally, the CAM group was greater than the FLX and the OLA groups on weeks 2, 4, 8, and 16 ($P < 0.01$; Table 5). Arachidonic acid increased across time for the FLX and CAM groups, but not the OLA group. Additionally, the CAM group was greater than the OLA group on weeks 4, 8, and 16, though neither differed from the FLX group ($P < 0.01$; Table 5).

Fatty acid groups

The four unsaturated fatty acid groups discussed are listed as follows in order of average proportional representation: $\sum$ n-7 (~2%), $\sum$ n-3 (~7%), $\sum$ n-9 (14%), $\sum$ n-6 (~42%). The % composition of n-3 fatty acids was greater in the FLX treatment group than the OLA treatment group, but neither differed from the CAM treatment group ($P < 0.01$; Table 3). Conversely, the $\sum$ n-9 fatty acids were greater in both the OLA and the CAM treatment groups than the FLX treatment group ($P < 0.01$; Table 3). Other groups did not differ among treatments ($P > 0.05$; Table 3). The $\sum$ n-3 ($P < 0.01$) and $\sum$ n-9 ($P = 0.04$) groups both increased across time when data were pooled across treatment groups (Table 4). Conversely, the $\sum$ n-6:n-3 ratio decreased across time ($P < 0.01$; Table 4). The % composition of $\sum$ n-3 fatty acids for the CAM group was greater on week 10 compared to week 0, but similar to all other time points. The FLX group was greater at weeks 2, 4, and 16 compared to week 0, but not different from week 10. The % composition of ALA for the FLX group was additionally greater than the OLA group at week 16, though both were similar to CAM ($P = 0.01$; Table 5). The $\sum$ n-6:n-3 ratio decreased across time for both the CAM and FLX groups, but did not change for the OLA ($P = 0.01$; Fig. 1). Finally, the $\sum$ n-9 % composition increased across time for both the OLA and CAM groups, but not for the FLX group. The OLA group was greater than the FLX group but similar to the CAM group on weeks 2, 4, and 8. On week 16, the $\sum$ n-9 composition of both the OLA and CAM groups were greater than the FLX group ($P < 0.01$; Table 5).

Discussion

Health parameters

Horses fed camelina, flaxseed, or canola oil have few differences in morphologic, biochemical, or hematological markers of health over a 4-month feeding period, suggesting that the three oils have no negative effects on these health parameters when fed to horses at a dietary inclusion of 370 mg/kg calculated BW/day.

The dietary consumption of FLX and OLA is not associated with any negative health outcomes in horses, and as such, if there were anti-nutritional factors in CAM that have not been adequately characterized previously and affected equine health, we would expect that complete blood counts or plasma biochemistry parameters of horses fed CAM would begin to differ from those of horses fed FLX or OLA (Barrelet and Ricketts, 2002). Additionally, we may expect changes in BW, BCS, or oil intake if feeding CAM were to negatively affect the health of the horse (Hixson et al., 2013). No such changes were observed in the present study, indicating that consumption of camelina oil for 16 weeks did not negatively impact the health of the horses used in this study.

Changes in BW and BCS throughout the study period were not expected and were not observed. It has been previously suggested

Table 2
Biochemical analyte concentrations and complete blood counts of horses (n = 30) fed 370 mg/kg calculated BW/day of camelina (CAM), flaxseed (FLX), or canola oil (OLA) for 16 weeks.

Parameter and Reference Interval[1]	Trt	Mean ± SEM	95% CI	*P*-Values		
				Trt	Wk	Trt × Wk
Calcium	CAM	3.03 ± 0.032	2.98–3.08	0.70	0.35	0.62
2.75–3.35 mmol/L	FLX	2.99 ± 0.035	2.96–3.07			
	OLA	3.02 ± 0.035	2.94–3.03			
Phosphorus	CAM	0.99 ± 0.035	0.93–1.03	0.45	0.32	0.40
0.73–1.71 mmol/L	FLX	0.89 ± 0.035	0.85–0.94			
	OLA	0.93 ± 0.035	0.88–0.98			
Magnesium	CAM	0.72 ± 0.014	0.68–0.74	0.54	0.68	0.50
0.6–1.0 mmol/L	FLX	0.70 ± 0.014	0.69–0.75			
	OLA	0.73 ± 0.014	0.70–0.75			
Sodium	CAM	137 ± 0.347	136–137	0.47	0.12	0.45
136–144 mmol/L	FLX	136 ± 0.347	136–137			
	OLA	136 ± 0.347	136–137			
Potassium	CAM	4.36 ± 0.092	4.18–4.59	0.79	0.21	0.73
3.1–4.3 mmol/L	FLX	4.44 ± 0.092	4.34–4.66			
	OLA	4.44 ± 0.093	4.22–4.49			
Chloride	CAM	100 ± 0.365	100–101	0.84	0.33	0.48
95–104 mmol/L	FLX	100 ± 0.364	99.3–101			
	OLA	100 ± 0.353	99.7–101			
Carbon Dioxide	CAM	25.3 ± 0.287	24.7–26.0	0.31	0.20	0.79
25–36 mmol/L	FLX	25.8 ± 0.279	25.2–26.3			
	OLA	26.0 ± 0.286	25.5–26.7			
Anion Gap	CAM	15.4 ± 0.268	14.7–15.9	0.15	0.38	0.52
6–21 mmol/L	FLX	15.3 ± 0.266	14.7–15.8			
	OLA	14.4 ± 0.273	13.9–14.9			
Sodium:Potassium Ratio	CAM	32.1 ± 0.689	30.4–33.4	0.52	0.14	0.79
–	FLX	31.2 ± 0.693	29.5–32.2			
	OLA	31.0 ± 0.700	30.5–32.6			
Total Protein	CAM	65.6 ± 0.522	63.6–65.7	0.48	0.69	0.21
58–75 g/L	FLX	64.8 ± 0.518	64.5–66.7			
	OLA	64.9 ± 0.511	63.6–66.5			
Albumin	CAM	32.3 ± 0.337	31.7–32.8	0.37	0.97	0.28
30–37 g/L	FLX	31.6 ± 0.343	31.0–32.7			
	OLA	32.3 ± 0.343	31.4–32.5			
Globulin	CAM	33.4 ± 0.500	31.5–33.4	0.42	0.77	0.43
26–41 g/L	FLX	33.3 ± 0.490	32.5–34.9			
	OLA	32.6 ± 0.491	31.9–34.3			
Albumin:Globulin Ratio	CAM	0.98 ± 0.018	0.97–1.04	0.36	0.72	0.38
–	FLX	0.96 ± 0.018	0.91–1.01			
	OLA	1.00 ± 0.018	0.94–1.01			
Urea	CAM	5.49 ± 0.183	5.12–5.80	0.54	0.32	0.62
4.2–8.9 mmol/L	FLX	5.60 ± 0.184	5.46–5.99			
	OLA	5.48 ± 0.183	5.01–5.74			
Creatinine	CAM	92.1 ± 2.522	88.9–95.3	0.53	0.58	0.51
80–130 µmol/L	FLX	91.7 ± 2.500	86.4–97.0			
	OLA	95.6 ± 2.520	91.7–99.5			
Glucose	CAM	5.39 ± 0.075	5.27–5.53	0.34	0.31	0.82
3.7–6.7 mmol/L	FLX	5.33 ± 0.081	5.10–5.29			
	OLA	5.21 ± 0.081	5.19–5.48			
Cholesterol	CAM	2.32 ± 0.050	2.32–2.54	0.39	0.59	0.04
1.70–2.70 mmol/L	FLX	2.34 ± 0.049	2.10–2.42			
	OLA	2.42 ± 0.049	2.29–2.48			
Total Bilirubin	CAM	16.0 ± 0.950	14.0–17.2	0.31	0.76	0.96
21–57 µmol/L	FLX	18.3 ± 0.950	16.2–20.2			
	OLA	17.4 ± 0.950	15.9–19.8			
Conjugated Bilirubin	CAM	5.58 ± 0.329	5.02–6.08	0.27	0.83	0.87
2–10 µmol/L	FLX	6.46 ± 0.337	5.71–6.79			
	OLA	5.93 ± 0.340	5.75–6.60			
Free Bilirubin	CAM	10.4 ± 0.712	8.90–11.3	0.38	0.79	0.98
18–55 µmol/L	FLX	11.8 ± 0.710	10.4–13.5			
	OLA	11.5 ± 0.710	9.96–13.4			
Alkaline Phosphatase	CAM	155 ± 5.111	138–156	0.14	0.11	0.30
119–329 U/L	FLX	140 ± 5.044	127–153			
	OLA	157 ± 5.111	151–179			
Gamma-glutamyl Transferase	CAM	14.2 ± 0.808	12.9–15.7	0.60	<0.01	0.38
7–54 U/L	FLX	15.6 ± 0.819	12.2–15.4			
	OLA	14.9 ± 0.818	12.5–20.6			
Aspartate Aminotransferase	CAM	271 ± 6.538	256–278	0.49	0.03	0.84
259–595 U/L	FLX	272 ± 6.533	259–292			
	OLA	261 ± 6.523	245–278			
Creatine Kinase	CAM	361 ± 28.51	280–484	0.43	0.07	0.60
108–430 U/L	FLX	270 ± 27.93	248–278			
	OLA	274 ± 28.15	239–282			

Table 2 (*continued*)

Parameter and Reference Interval[1]	Trt	Mean ± SEM	95% CI	*P*-Values		
				Trt	Wk	Trt × Wk
Glutamate Dehydrogenase	CAM	7.29 ± 0.669	5.36–6.99	0.16	0.01	0.20
1–7 U/L	FLX	9.08 ± 0.671	6.00–9.55			
	OLA	6.84 ± 0.696	6.49–12.0			
Calculated Osmolarity	CAM	273 ± 0.640	272–275	0.49	0.45	0.49
mmol/L	FLX	273 ± 0.638	272–274			
	OLA	273 ± 0.639	271–274			
White Blood Cell Count	CAM	7.28 ± 0.411	6.77–7.81	0.74	0.17	0.95
5.1–11.0 × 10^9/L	FLX	7.68 ± 0.411	7.06–8.15			
	OLA	7.28 ± 0.411	6.88–7.65			
Red Blood Cell Count	CAM	8.07 ± 0.153	7.65–8.18	0.11	0.43	0.21
6.9–10.7 × 10^{12}/L	FLX	7.65 ± 0.151	7.31–7.92			
	OLA	8.21 ± 0.154	8.06–8.52			
Hemoglobin	CAM	139 ± 2.510	133–141	0.12	0.35	0.17
112–169 g/L	FLX	133 ± 2.462	129–137			
	OLA	140 ± 2.492	137–145			
Hematocrit	CAM	0.42 ± 0.008	0.40–0.43	0.14	0.45	0.33
0.28–0.44 L/L	FLX	0.40 ± 0.008	0.38–0.41			
	OLA	0.42 ± 0.008	0.41–0.43			
Mean Cell Volume	CAM	51.6 ± 0.305	51.4–52.8	0.98	0.75	0.85
42–53 fL	FLX	51.7 ± 0.303	51.1–53.0			
	OLA	51.7 ± 0.310	49.8–51.6			
Mean Cell Hemoglobin	CAM	17.4 ± 0.133	17.2–17.7	0.57	0.22	0.17
14–18 pg	FLX	17.3 ± 0.136	17.2–17.8			
	OLA	17.2 ± 0.135	16.7–17.30			
Mean Corpuscular Hemolobin Concentration	CAM	335 ± 1.604	331–337	0.43	0.07	0.77
328–364 g/L	FLX	335 ± 1.518	335–339			
	OLA	335 ± 1.492	334–339			
Red Cell Distribution Width	CAM	18.1 ± 0.219	17.8–18.4	0.50	0.84	0.61
16–20%	FLX	17.9 ± 0.220	17.5–18.0			
	OLA	18.1 ± 0.217	17.8–18.3			
Platelets	CAM	125 ± 5.480	119–140	0.77	0.95	0.29
83–270 × 10^9/L	FLX	120 ± 5.518	101–121			
	OLA	124 ± 5.435	114–139			
Mean Platelet Volume	CAM	6.71 ± 0.566	6.44–6.73	0.49	0.52	0.47
6–11 fL	FLX	7.34 ± 0.574	6.59–8.31			
	OLA	7.55 ± 0.565	6.23–8.52			
Total Solid Protein	CAM	72.8 ± 0.874	70.9–72.9	0.46	0.49	0.14
57–75 g/L	FLX	71.1 ± 0.887	70.4–73.3			
	OLA	72.1 ± 0.873	70.0–73.1			
Segmented Neutrophil Count	CAM	4.24 ± 0.226	3.63–4.49	0.82	0.37	0.85
2.8–7.7 × 10^9/L	FLX	4.11 ± 0.224	3.87–4.60			
	OLA	4.02 ± 0.222	3.73–4.43			
Lymphocyte Count	CAM	2.65 ± 0.126	2.42–2.96	0.67	0.20	0.29
1.3–4.7 × 10^9/L	FLX	2.80 ± 0.126	2.52–3.15			
	OLA	2.71 ± 0.126	2.44–2.85			
Monocyte Count	CAM	0.29 ± 0.025	0.26–0.34	0.55	0.65	0.91
0.1–0.8 × 10^9/L	FLX	0.28 ± 0.023	0.24–0.31			
	OLA	0.25 ± 0.025	0.21–0.29			
Eosinophil Count	CAM	0.26 ± 0.037	0.22–0.32	0.64	0.79	0.35
0.0–0.7 × 10^9/L	FLX	0.24 ± 0.036	0.22–0.31			
	OLA	0.29 ± 0.035	0.23–0.36			

Abbreviations: CI = Confidence Interval; Trt = treatment; Wk = week.
[1] Animal Health Laboratory, University of Guelph, 419 Gordon Street, Guelph, ON, Canada.

that horses are able to adequately adjust to a high dietary lipid content to maintain BW (Marchello et al., 2000), and since the horses in the present study were provided hay *ad libitum*, it is assumed that they were able to adjust their dietary energy intake to account for the increased calories provided from the oil. No differences in complete blood count or biochemical analytes were observed among treatment groups based on classical hypothesis testing using p-values or based on confidence intervals. Denoting significance based on classical hypothesis testing, which specifies the null hypothesis to be that of no mean treatment difference, has been suggested to be inaccurate when studying feed equivalence (Tempelman, 2004). As such, confidence intervals were also provided to further assess the impacts of these oils on complete blood count and biochemical analytes. The mean values of all biomarkers that were above or below the reference interval set by the Animal Health Laboratory were within the reference intervals determined by Cornell University's Animal Health Diagnostic Center and/or the University of Prince Edward Island Animal Health Diagnostic Center, which use similar analytical techniques to the Animal Health Laboratory. Reference intervals from these laboratories were based on serum from healthy adult horses, rather than heparinized plasma which was used in the current study. Finally, these changes from reference did not occur across time or among treatments and, therefore, are not relevant when assessing the effects of camelina oil on health parameters in adult horses.

Though palatability assessments were not within the scope of this study, the refusal of oil and subsequent need to syringe feed eight horses is unlikely to be of concern when the oil is provided in a more practical application. In the present study, researchers mixed the oil with soaked hay cubes, rather than a more palatable concentrate, since most of the horses had a higher-than-ideal BCS

Table 3
Mean fatty acid % composition (±SEM) of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) identified in plasma total lipids from horses (n = 30) fed 370 mg/kg calculated BW/day of camelina (CAM), flaxseed (FLX), or canola oil (OLA) for 16 weeks.

Fatty acid[1], %	CAM	FLX	OLA	*P*-Value
14:0	0.43 ± 0.05	0.39 ± 0.05	0.38 ± 0.05	0.25
16:0	13.7[a] ± 0.47	13.0[b] ± 0.47	13.1[ab] ± 0.47	0.03
17:0	0.55 ± 0.04	0.56 ± 0.04	0.56 ± 0.04	0.90
18:0	18.1 ± 0.65	18.6 ± 0.65	18.4 ± 0.65	0.84
20:0	0.89 ± 0.07	0.81 ± 0.07	0.80 ± 0.07	0.38
22:0	0.38 ± 0.03	0.40 ± 0.03	0.38 ± 0.03	0.64
24:0	0.45 ± 0.06	0.56 ± 0.06	0.44 ± 0.06	0.06
∑ SFAs	34.7 ± 0.44	34.3 ± 0.44	34.2 ± 0.44	0.73
16:1n-7	0.86 ± 0.21	0.80 ± 0.21	0.92 ± 0.21	0.66
18:1n-9	10.8[a] ± 0.74	9.94[a] ± 0.74	12.4[b] ± 0.74	<0.01
18:1n-7	1.09 ± 0.11	1.03 ± 0.11	1.27 ± 0.11	0.32
20:1n-9	2.08[a] ± 0.12	1.72[b] ± 0.12	1.65[b] ± 0.12	0.09
22:1n-9	0.33 ± 0.02	0.32 ± 0.02	0.30 ± 0.02	0.41
24:1n-9	0.94 ± 0.05	0.83 ± 0.05	0.82 ± 0.05	0.07
∑ MUFAs	16.1[ab] ± 0.92	14.6[a] ± 0.92	17.3[b] ± 0.92	<0.01
18:2n-6	41.1 ± 1.32	42.4 ± 1.32	41.1 ± 1.32	0.21
18:3n-3	5.80[ab] ± 0.36	6.32[a] ± 0.36	5.25[b] ± 0.36	0.01
20:2n-6	0.55[a] ± 0.05	0.43[b] ± 0.05	0.41[b] ± 0.05	<0.01
20:3n-3	0.68 ± 0.06	0.73 ± 0.06	0.65 ± 0.06	0.32
20:4n-6	0.34[a] ± 0.01	0.31[a] ± 0.02	0.27[b] ± 0.02	<0.01
∑ PUFAs	48.4[ab] ± 1.13	50.0[a] ± 1.13	48.4[b] ± 1.13	<0.01
∑ n-6	41.9 ± 1.34	43.0 ± 1.38	41.7 ± 1.34	0.22
∑ n-3	6.49[ab] ± 0.32	7.06[a] ± 0.32	5.92[b] ± 0.32	<0.01
∑ n-6:n-3	6.69 ± 0.48	6.37 ± 0.48	7.23 ± 0.48	0.07
∑ n-9	14.2[a] ± 0.65	12.8[b] ± 0.65	15.2[a] ± 0.65	<0.01
∑ n-7	1.94 ± 0.30	1.84 ± 0.30	2.19 ± 0.30	0.25

Abbreviations: MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; SFAs = saturated fatty acids.
[1] Any fatty acid(s) not detected were removed from the table.
[a,b] Values in a row with different superscripts differ significantly at $P < 0.05$.

Table 4
Mean fatty acid composition (±SEM) and percent contribution of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) for total lipids identified in plasma of horses (n = 30) at weeks 0, 2, 4, 10, and 16 of dietary oil supplementation at 370 mg/kg calculated BW/day (pooled across dietary treatments: flaxseed, canola, and camelina oil).

Fatty acid[1], %	Week 0	Week 2	Week 4	Week 8	Week 16	*P*-Value
14:0	0.41 ± 0.05	0.41 ± 0.05	0.40 ± 0.05	0.39 ± 0.05	0.40 ± 0.05	0.92
16:0	13.5 ± 0.48	13.2 ± 0.48	13.3 ± 0.48	13.2 ± 0.48	13.2 ± 0.48	0.80
17:0	0.52 ± 0.04	0.55 ± 0.04	0.56 ± 0.04	0.56 ± 0.04	0.59 ± 0.04	0.27
18:0	18.6 ± 0.41	18.5 ± 0.41	18.4 ± 0.43	18.2 ± 0.43	18.1 ± 0.41	0.18
20:0	0.79[a] ± 0.06	0.87[b] ± 0.06	0.80[a] ± 0.06	0.87[b] ± 0.06	0.85[b] ± 0.06	<0.01
22:0	0.37 ± 0.03	0.40 ± 0.03	0.36 ± 0.03	0.41 ± 0.03	0.40 ± 0.03	0.46
24:0	0.47 ± 0.05	0.47 ± 0.05	0.48 ± 0.05	0.50 ± 0.06	0.49 ± 0.05	0.94
∑ SFAs	35.5 ± 0.29	34.5 ± 0.29	34.4 ± 0.29	34.2 ± 0.31	34.3 ± 0.29	0.66
16:1n-7	0.96 ± 0.21	0.96 ± 0.21	0.85 ± 0.21	0.76 ± 0.22	0.77 ± 0.21	0.43
18:1n-9	9.76 ± 0.76	11.3 ± 0.76	11.5 ± 0.76	10.9 ± 0.83	11.7 ± 0.76	0.06
18:1n-7	1.06 ± 0.09	1.19 ± 0.09	1.17 ± 0.09	1.11 ± 0.10	1.12 ± 0.09	0.28
20:1n-9	1.61 ± 0.09	1.94 ± 0.09	1.80 ± 0.09	1.89 ± 0.10	1.84 ± 0.09	0.14
22:1n-9	0.30 ± 0.02	0.34 ± 0.02	0.32 ± 0.02	0.30 ± 0.02	0.32 ± 0.02	0.23
24:1n-9	0.76 ± 0.06	0.84 ± 0.06	0.83 ± 0.06	0.93 ± 0.08	0.96 ± 0.06	0.28
∑ MUFAs	14.5 ± 0.95	17.3 ± 0.95	16.5 ± 0.96	15.8 ± 1.04	16.7 ± 0.95	0.08
18:2n-6	43.5 ± 1.36	40.6 ± 1.36	40.6 ± 1.36	41.5 ± 1.45	41.3 ± 1.36	0.07
18:3n-3	5.12[a] ± 0.33	6.03[b] ± 0.33	6.03[b] ± 0.33	6.18[b] ± 0.34	5.62[ab] ± 0.33	<0.01
20:2n-6	0.45 ± 0.06	0.45 ± 0.06	0.47 ± 0.06	0.47 ± 0.06	0.48 ± 0.06	0.87
20:3n-3	0.68 ± 0.05	0.72 ± 0.05	0.69 ± 0.05	0.67 ± 0.05	0.68 ± 0.05	0.65
20:4n-6	0.25[a] ± 0.01	0.32[b] ± 0.01	0.33[b] ± 0.01	0.33[b] ± 0.01	0.32[b] ± 0.01	<0.01
∑ PUFAs	49.9 ± 1.19	48.1 ± 1.18	48.1 ± 1.19	49.0 ± 1.28	48.1 ± 1.18	0.26
∑ n-6	44.1 ± 1.38	41.3 ± 1.38	41.4 ± 1.39	42.3 ± 1.47	42.0 ± 1.38	0.08
∑ n-3	5.82[a] ± 0.29	6.72[b] ± 0.29	6.73[b] ± 0.29	6.87[b] ± 0.31	6.31[ab] ± 0.29	<0.01
∑ n-6:n-3	7.77[a] ± 0.48	6.31[b] ± 0.48	6.34[b] ± 0.48	6.50[ab] ± 0.50	6.88[ab] ± 0.48	0.04
∑ n-9	12.5[a] ± 0.70	14.4[ab] ± 0.70	14.5[ab] ± 0.70	14.0[ab] ± 0.79	14.9[b] ± 0.70	0.04
∑ n-7	2.01 ± 0.30	2.15 ± 0.30	2.01 ± 0.30	1.87 ± 0.31	1.89 ± 0.30	0.56

Abbreviation: SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids.
[1] Any fatty acid(s) not detected within the specific fraction were removed from the table.
[a,b] Values in a row with different superscripts differ significantly at $P < 0.05$.

at the start of the study. Additionally, the dosage of oil in the present study was ~200–250 mL of oil, which is much higher than commonly recommended dosages of ~30–60 mL of oil for many commercial equine oil supplements. The high dosage of oil was needed to assess the effects of the novel camelina oil on health parameters, though this high dosage likely affected the acceptability of the supplements as well. In the literature, there are recommendations for supplementation of larger quantities of oil to

Table 5
Mean fatty acid % composition (±SEM) of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) identified in plasma total lipids from horses (n = 30) fed 370 mg/kg calculated BW/day of camelina (CAM), flaxseed (FLX), or canola oil (OLA) for 16 weeks (Treatment-by-Week interactions).

Fatty acid[1], %	Oil	Mean (Treatment × Week)					*P*-Value
		Week 0	Week 2	Week 4	Week 8	Week 16	
14:0	CAM	0.43 ± 0.05	0.44 ± 0.05	0.42 ± 0.05	0.41 ± 0.06	0.46 ± 0.05	0.27
	FLX	0.39 ± 0.05	0.40 ± 0.05	0.40 ± 0.05	0.37 ± 0.06	0.39 ± 0.05	
	OLA	0.42 ± 0.05	0.39 ± 0.05	0.37 ± 0.05	0.37 ± 0.05	0.35 ± 0.05	
16:0	CAM	13.8 ± 0.515	13.6 ± 0.515	13.7 ± 0.516	13.8 ± 0.546	13.8 ± 0.515	0.56
	FLX	13.3 ± 0.515	13.0 ± 0.515	13.1 ± 0.516	12.7 ± 0.546	13.0 ± 0.515	
	OLA	13.4 ± 0.515	13.0 ± 0.515	13.1 ± 0.523	13.1 ± 0.547	12.8 ± 0.515	
17:0	CAM	0.54 ± 0.04	0.55 ± 0.04	0.57 ± 0.04	0.53 ± 0.05	0.56 ± 0.04	0.01
	FLX	0.53 ± 0.04	0.54 ± 0.04	0.55 ± 0.04	0.57 ± 0.05	0.60 ± 0.04	
	OLA	0.48^{a} ± 0.04	0.56^{ab} ± 0.04	0.57^{ab} ± 0.04	0.58^{ab} ± 0.05	0.60^{b} ± 0.04	
18:0	CAM	18.1 ± 0.69	18.1 ± 0.69	18.4 ± 0.69	17.7 ± 0.71	17.9 ± 0.69	0.05
	FLX	18.6 ± 0.69	18.6 ± 0.69	18.2 ± 0.69	18.7 ± 0.71	18.8 ± 0.69	
	OLA	19.1 ± 0.69	18.6 ± 0.69	18.6 ± 0.70	18.2 ± 0.71	17.7 ± 0.69	
20:0	CAM	0.85 ± 0.07	0.91 ± 0.07	0.88 ± 0.07	0.94 ± 0.07	0.89 ± 0.07	0.66
	FLX	0.76 ± 0.07	0.88 ± 0.07	0.76 ± 0.07	0.82 ± 0.07	0.85 ± 0.07	
	OLA	0.76 ± 0.07	0.83 ± 0.07	0.76 ± 0.07	0.85 ± 0.07	0.81 ± 0.07	
22:0	CAM	0.40 ± 0.03	0.39 ± 0.04	0.35 ± 0.04	0.39 ± 0.04	0.38 ± 0.04	0.26
	FLX	0.37 ± 0.04	0.43 ± 0.04	0.37 ± 0.04	0.42 ± 0.04	0.45 ± 0.04	
	OLA	0.35 ± 0.04	0.37 ± 0.04	0.35 ± 0.04	0.42 ± 0.04	0.37 ± 0.04	
24:0	CAM	0.52 ± 0.07	0.41 ± 0.07	0.45 ± 0.07	0.48 ± 0.07	0.38 ± 0.07	0.06
	FLX	0.49 ± 0.07	0.55 ± 0.07	0.54 ± 0.07	0.58 ± 0.07	0.63 ± 0.07	
	OLA	0.40 ± 0.07	0.45 ± 0.07	0.45 ± 0.07	0.43 ± 0.07	0.46 ± 0.07	
∑ SFAs	CAM	34.6 ± 0.50	34.5 ± 0.50	35.0 ± 0.50	34.5 ± 0.53	34.7 ± 0.50	0.01
	FLX	34.2 ± 0.50	34.4 ± 0.50	34.3 ± 0.50	34.2 ± 0.53	34.8 ± 0.50	
	OLA	34.8^{a} ± 0.50	34.4^{ab} ± 0.50	33.9^{ab} ± 0.52	34.0^{ab} ± 0.54	33.3^{b} ± 0.50	
16:1n-7	CAM	0.93 ± 0.23	0.95 ± 0.23	0.79 ± 0.23	0.75 ± 0.24	0.87 ± 0.23	0.32
	FLX	0.84 ± 0.23	0.87 ± 0.23	0.83 ± 0.23	0.74 ± 0.24	0.71 ± 0.23	
	OLA	1.11 ± 0.23	1.05 ± 0.23	0.93 ± 0.23	0.78 ± 0.24	0.73 ± 0.23	
18:1n-7	CAM	1.06 ± 0.12	1.14 ± 0.12	1.11 ± 0.12	1.03 ± 0.12	1.10 ± 0.12	0.02
	FLX	1.01 ± 0.12	1.06 ± 0.12	1.05 ± 0.12	1.02 ± 0.12	1.00 ± 0.12	
	OLA	1.12^{a} ± 0.12	1.36^{b} ± 0.12	1.35^{b} ± 0.12	1.27^{ab} ± 0.12	1.27^{ab} ± 0.12	
20:1n-9	CAM	1.65^{a} ± 0.14	2.13^{b} ± 0.14	2.20^{b}_{x} ± 0.14	2.32^{b}_{x} ± 0.14	2.15^{b} ± 0.14	<0.01
	FLX	1.62 ± 0.14	1.77 ± 0.14	1.60_{y} ± 0.14	1.60_{y} ± 0.15	1.78 ± 0.14	
	OLA	1.56 ± 0.14	1.91 ± 0.14	1.60_{y} ± 0.14	1.69_{xy} ± 0.14	1.63 ± 0.14	
22:1n-9	CAM	0.29 ± 0.03	0.34 ± 0.03	0.33 ± 0.03	0.36 ± 0.03	0.32 ± 0.03	0.25
	FLX	0.30 ± 0.03	0.34 ± 0.03	0.31 ± 0.03	0.28 ± 0.03	0.36 ± 0.03	
	OLA	0.30 ± 0.03	0.33 ± 0.03	0.32 ± 0.03	0.26 ± 0.03	0.28 ± 0.03	
24:1n-9	CAM	0.78 ± 0.08	0.90 ± 0.08	0.93 ± 0.08	1.03 ± 0.09	1.04 ± 0.08	0.62
	FLX	0.77 ± 0.08	0.82 ± 0.08	0.77 ± 0.08	0.87 ± 0.09	0.95 ± 0.08	
	OLA	0.74 ± 0.08	0.80 ± 0.08	0.79 ± 0.08	0.89 ± 0.09	0.89 ± 0.08	
∑ MUFAs	CAM	14.6 ± 1.05	16.3_{xy} ± 1.05	16.2_{xy} ± 1.05	15.9_{xy} ± 1.14	17.3_{xy} ± 1.05	0.02
	FLX	13.9 ± 1.05	15.2_{x} ± 1.05	15.1_{x} ± 1.05	14.3_{x} ± 1.14	14.7_{x} ± 1.05	
	OLA	14.9^{a} ± 1.05	18.1^{b}_{y} ± 1.05	18.1^{b}_{y} ± 1.07	17.3^{ab}_{y} ± 1.14	18.1^{b}_{y} ± 1.05	
20:2n-6	CAM	0.46^{a} ± 0.06	0.54^{ab}_{x} ± 0.06	0.58^{b}_{x} ± 0.06	0.58^{b}_{x} ± 0.06	0.58^{b}_{x} ± 0.06	<0.01
	FLX	0.46 ± 0.06	0.42_{y} ± 0.06	0.42_{y} ± 0.06	0.41_{y} ± 0.06	0.45_{y} ± 0.06	
	OLA	0.44 ± 0.06	0.38_{y} ± 0.06	0.41_{y} ± 0.06	0.40_{y} ± 0.06	0.41_{y} ± 0.06	
20:3n-3	CAM	0.66 ± 0.06	0.75 ± 0.06	0.68 ± 0.06	0.66 ± 0.06	0.64 ± 0.06	0.16
	FLX	0.74 ± 0.06	0.75 ± 0.06	0.74 ± 0.06	0.72 ± 0.06	0.71 ± 0.06	
	OLA	0.64 ± 0.06	0.65 ± 0.06	0.64 ± 0.06	0.63 ± 0.06	0.68 ± 0.06	
20:4n-6	CAM	0.26^{a} ± 0.02	0.36^{b} ± 0.02	0.37^{b}_{x} ± 0.02	0.38^{b}_{x} ± 0.02	0.35^{b}_{x} ± 0.02	<0.01
	FLX	0.23^{a} ± 0.02	0.32^{b} ± 0.02	0.34^{b}_{xy} ± 0.02	0.33^{b}_{xy} ± 0.02	0.34^{b}_{xy} ± 0.02	
	OLA	0.25 ± 0.02	0.28 ± 0.02	0.28_{y} ± 0.02	0.28_{y} ± 0.02	0.26_{y} ± 0.02	
∑ PUFAs	CAM	49.7 ± 1.29	48.3 ± 1.29	47.8 ± 1.29	48.8 ± 1.40	47.2 ± 1.29	0.24
	FLX	50.7 ± 1.29	49.5 ± 1.29	50.0 ± 1.29	50.5 ± 1.40	49.5 ± 1.29	
	OLA	49.3 ± 1.29	46.5 ± 1.29	46.5 ± 1.29	47.7 ± 1.40	47.7 ± 1.29	
∑ n-6	CAM	44.1 ± 1.50	41.6 ± 1.50	41.2 ± 1.50	41.6 ± 1.60	41.1 ± 1.50	0.18
	FLX	44.8 ± 1.50	42.1 ± 1.50	42.5 ± 1.50	43.4 ± 1.60	42.3 ± 1.50	
	OLA	43.5 ± 1.50	40.3 ± 1.50	40.5 ± 1.50	41.7 ± 1.60	42.5 ± 1.51	
∑ n-3	CAM	5.64^{a} ± 0.38	6.62^{ab} ± 0.38	6.62^{ab}_{xy} ± 0.38	7.27^{b} ± 0.40	6.31^{ab}_{xy} ± 0.38	0.01
	FLX	5.99^{a} ± 0.38	7.34^{b} ± 0.38	7.52^{b}_{x} ± 0.38	7.18^{ab} ± 0.40	7.36^{b}_{x} ± 0.38	
	OLA	5.84 ± 0.38	6.18 ± 0.38	6.04_{y} ± 0.39	6.16 ± 0.40	5.36_{y} ± 0.38	
∑ n-9	CAM	12.7^{a} ± 0.79	14.3^{b}_{xy} ± 0.79	14.4^{b}_{xy} ± 0.79	14.2^{ab}_{xy} ± 0.89	15.4^{b}_{x} ± 0.79	<0.01
	FLX	12.1 ± 0.79	13.3_{y} ± 0.79	13.2_{y} ± 0.79	12.5_{y} ± 0.89	13.1_{y} ± 0.79	
	OLA	12.7^{a} ± 0.79	15.7^{b}_{x} ± 0.79	15.9^{b}_{x} ± 0.81	15.3^{b}_{x} ± 0.89	16.1^{b}_{x} ± 0.79	
∑ n-7	CAM	1.98 ± 0.33	2.08 ± 0.33	1.89 ± 0.33	1.78 ± 0.34	1.96 ± 0.33	0.65
	FLX	1.85 ± 0.33	1.95 ± 0.33	1.88 ± 0.33	1.77 ± 0.34	1.72 ± 0.33	
	OLA	2.21 ± 0.33	2.40 ± 0.33	2.27 ± 0.33	2.05 ± 0.34	1.99 ± 0.33	

Abbreviations: MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids.
SFAs = saturated fatty acids.
[1] Any fatty acid(s) not detected were removed from the table.
[a,b] Values in a row with different superscripts differ significantly across time for a specific treatment at $P < 0.05$.
[x,y] Values in a column with different subscripts differ significantly among treatment groups at a specific time point at $P < 0.05$.

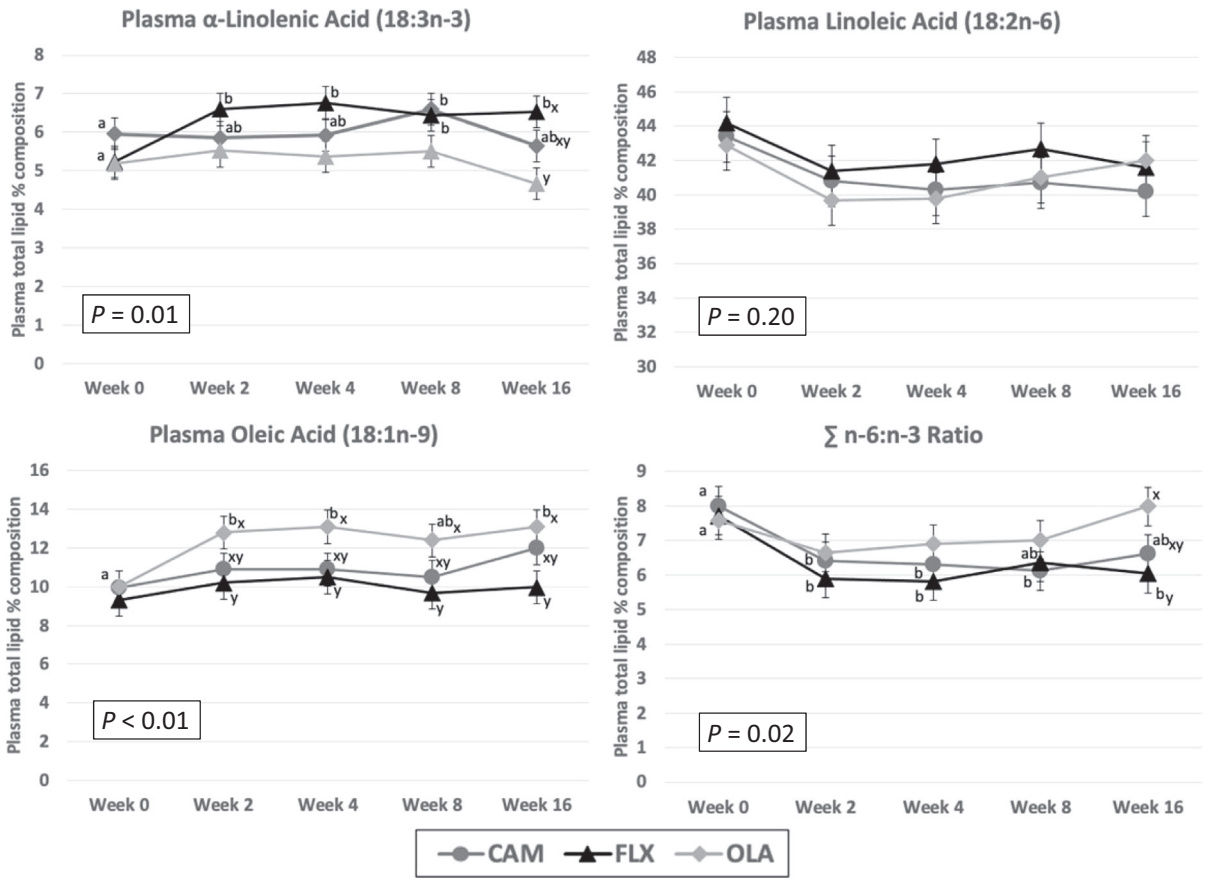

Fig. 1. Mean plasma fatty acid % composition (±SEM) in horses (n = 30) supplemented with camelina oil (CAM), flaxseed oil (FLX), or canola oil (OLA) for 16 weeks. Letters a,b denote changes of a particular treatment group across time ($P < 0.05$). Letters x,y denote differences between treatment groups at a particular time period ($P < 0.05$).

accommodate horses with increased energy requirements or altered metabolic states (Kronfeld et al., 2004; Piccione et al., 2019). Oils can serve as an alternative to high-starch diets, effectively reducing the digestive and metabolic risks commonly associated with diets high in starch and sugar. Furthermore, they may help modulate postprandial glycemic and insulinemic responses (Borgia et al., 2011; Williams et al., 2001; Zeyner et al., 2006). Oils that are high in n-3 fatty acids may also help modulate inflammation associated with exercise, metabolic syndrome, or adiposity (Hall et al., 2004). However, additional work is needed in these areas to better understand the optimal oil inclusion for horses in different metabolic states.

Plasma fatty acids

To the authors' knowledge, no other literature has reported plasma fatty acid profiles of horses fed supplemental canola or camelina oil, although other literature has reported the fatty acid profiles of horses fed supplemental flaxseed oil or milled flaxseed (Hansen et al., 2002; Vineyard et al., 2010; Hess et al., 2012). In the present study, horses on all treatments exhibited changes from baseline (week 0) for many plasma fatty acids. This finding was expected due to the modification to the lipid profiles of the diets after switching from the 4-week wash-in oil (sunflower oil) to the three treatment oils (CAM, FLX, and OLA). Additionally, many differences were seen among treatments and for treatment-by-week interactions, and most of the differences were reflective of the respective fatty acid profiles of the treatment oil provided. However, the relational change in the data did not always represent the proportional differences in fatty acid profiles between the oils. Overall, there was a decrease in the n-6:n-3 ratio across time for both the FLX and CAM treatment groups, though the ratio did not decrease over time for the OLA group. The changes in the n-6:n-3 ratio are reflective of the changes in n-6 and n-3 fatty acids, namely changes in ALA, which are associated with concentrations in the oils and will be discussed below.

Of the two n-3 fatty acids detected in the present study, ALA and eicosatrienoic acid, differences between treatments or across time were only observed in ALA. ALA represented between 5 and 7% of the total fatty acid profile and was observed to both increase across time and differ between dietary treatments. Specifically, the FLX group had a greater ALA concentration than the OLA group and did increase across time, which is reflective of ALA representing more than half of the fatty acid profile of flaxseed oil (~56%). Only minor changes in % ALA composition were observed across time for the CAM group, though % composition for the CAM group remained similar to both the FLX and OLA groups at all time points. Again, this is reflective of the ALA content of the oil provided, where canola oil contains the lowest concentrations of ALA (~12%) and camelina oil has moderate ALA concentrations (~35%). These changes observed in ALA concentrations agree with those of Hansen et al. (2002), who reported an increase in plasma ALA (μmol/L) when horses were fed a supplement containing 10% flaxseed oil (diet: 80% supplement and 20% hay wt/wt). In contrast, other studies that have fed flaxseed meal saw more variability in ALA concentrations. Vineyard et al. (2010) observed an increase in ALA (g/100 g total fatty acids) on day 35 compared to day 0 for horses fed flaxseed meal (6 g ALA/100 kg BW), though this increase in ALA was not sustained and day 70 did not differ from day 0. Hess et al. (2012) found that horses fed supplemental flaxseed meal (38 g ALA/day) had greater mean plasma fatty acids (% fatty acid) in comparison to horses fed a control diet without an

added supplement. Additionally, the flaxseed meal treatment group had greater plasma ALA on day 30 and day 90 but was similar to control on day 0 and day 60. Regardless of these differences from the control group, there was no change in ALA across time for the flaxseed meal treatment group (Hess et al., 2012). Finally, ALA represented only ~1–2% of total fatty acids in the blood, which is much lower than the present study (Hess et al., 2012). These results could indicate that there is a difference between flaxseed oil and meal that impacts the horse's ability to incorporate ALA into circulation. In humans provided either flaxseed meal or oil for consumption at the same ALA inclusion, there was no difference in plasma ALA between the two treatment groups (Taylor et al., 2010). However, humans consuming flaxseed oil, rather than meal, did have increased plasma EPA and docosapentaenoic acid indicating that flaxseed oil was more effective at increasing the n-3 status than flaxseed meal (Taylor et al., 2010). Further research is warranted to further understand the ideal method of delivery and dosage of supplemental ALA on plasma fatty acids in horses.

No eicosapentaenoic acid (**EPA**; 20:5n-3) and docosahexaenoic acid (**DHA**; 22:6n-3) were detected in the present study. Results concerning circulating EPA and DHA status in horses have not been consistent in literature. Similar to the present study, EPA and DHA were not detected in the plasma of horses fed supplemental milled flaxseed for up to 90 days (Vineyard et al., 2010; Hess et al., 2012). Conversely, circulating concentrations of EPA and DHA were detected in horses fed supplemental flaxseed oil (Hansen et al., 2002) and at low concentrations in both horses receiving a corn oil supplement and in a group of horses not receiving a fatty acid supplement (O'Connor et al., 2004; King et al., 2008). Further, concentrations of EPA, but not DHA, increased over time when horses were fed supplemental flaxseed oil for 16 weeks, which would indicate that horses do have an ability to convert ALA to at least EPA and that this conversion may be related to the amount ALA provided in the diet (Hansen et al., 2002). It is unclear why these differences in detection of EPA and DHA have been observed between studies, though it is likely that analytical techniques, diets, or horse demographics between studies had an impact. Further research is warranted to better understand the horse's ability to convert ALA to the longer-chain n-3 fatty acids, EPA and DHA, as this could impact the efficacy of plant-based oils.

An additional factor to consider concerning the changes of fatty acids over time, in particular the n-6 and n-3 fatty acids, is that the horses in the present study were already consuming sunflower oil when baseline samples were collected. Horses tolerate a diet with ~ 20% digestible energy (~0.9 g oil/kg BW) from a supplemental oil (~27% total digestible energy from fat) for 6 months, though it is recommended to slowly introduce fat to the diet to allow the liver sufficient time to increase bile production and subsequent release in the duodenum (Harris et al., 1999). Rapid introduction of fat into equine diets has been associated with decreased fat digestion, indicated by greasy feces (steatorrhea) and increased fecal output (Kronfeld et al., 2004). As such, horses were slowly acclimated to the final lipid inclusion over a 4-week period using sunflower oil, which also served the purpose of standardizing baseline measurements, most notably for the health parameters presented in this paper. However, sunflower oil has a markedly different fatty acid profile than the three oils being investigated in this study, thus potentially accentuating the changes observed across time when compared to baseline.

Plasma fatty acid profiles for horses fed supplemental flaxseed oil or meal have not reported SFA or MUFA values for total lipids (Hansen et al., 2002; Vineyard et al., 2010; Hess et al., 2012). As such, there is little relevant data to compare the current results to pertaining to these groups of fatty acids. However, the changes in plasma fatty acids observed among treatments for MUFAs are not unexpected since they are reflective of the high concentrations of oleic acid and gondoic acid in OLA and CAM, respectively. Oleic acid was the most prominent MUFA in the present study and likely the reason that summed MUFAs observed similar trends to oleic acid. The efficacy of dietary oleic acid sources has not been well studied in horses, though human and mice studies have found that oleic acid may have cardioprotective and immune modulating effects, and may help manage obesity (O'Byrne et al., 1997; Cardoso et al., 2011; Bowen et al., 2019). Little data are available pertaining to the in vivo effects of gondoic acid in horses or other species. However, one in vitro study found that gondoic acid decreased inflammation in liver (Kupffer) cells through the inhibition of reactive oxygen species production and signaling (Fan et al., 2022). As such, the high gondoic acid content in CAM could prove to be beneficial to the inflammatory status of animals; however, further in vivo studies are needed before such claims can be made.

Conclusion

The present study suggests that camelina, canola, and flaxseed oil have no negative effects on health parameters when fed to adult horses. No clinically relevant differences were observed among treatments in health parameters, as determined by BCS, oil intake, calculated BW, complete blood count, and plasma biochemical analytes. These data suggest that feeding camelina oil at an inclusion of 370 mg oil/kg calculated BW/day results in a similar physiological response as feeding flaxseed or canola oil. Additionally, most circulating plasma fatty acids were reflective of the fatty acid profiles of the oils provided, with the n-6:n-3 ratio decreasing over time for horses fed camelina and flaxseed oil. Together, these data suggest that camelina oil is an effective alternative to deliver n-3 fatty acids in equine diets, though further research is warranted to determine the subsequent incorporation into tissues and potential physiological benefits that may be provided.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.101034.

Ethics approval

The use of animals and experimental design were approved by the Animal Care Committee at the University of Guelph (Animal Utilization Protocol #4481) and followed the guidelines set by the Canadian Council on Animal Care.

Data and model availability statement

Original data are available upon reasonable request. Data were not deposited in an official repository.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Declaration of interest

None.

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