Effects of dietary camelina, flaxseed, and canola oil supplementation on inflammatory and oxidative markers, transepidermal water loss, and skin and coat health parameters in healthy adult dogs and horses

by

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ABSTRACT

EFFECTS OF DIETARY CAMELINA, FLAXSEED, AND CANOLA OIL SUPPLEMENTATION ON INFLAMMATORY AND OXIDATIVE MARKERS, TRANSEPIDERMAL WATER LOSS, AND SKIN AND COAT HEALTH PARAMETERS IN HEALTHY ADULT DOGS AND HORSES

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Camelina oil provides a rich source of omega-3 fatty acids, which are commonly used to support skin and coat health claims in canine and equine diets. Hence, the focus of this thesis is to investigate the effects of dietary camelina oil on skin and coat health outcomes in dogs and horses, in comparison to flaxseed and canola oil. No differences in inflammatory and oxidative marker concentrations, transepidermal water loss, or skin and coat health scores, were observed in animals fed camelina oil vs. flaxseed or canola oil. These findings suggest that in terms of the skin and coat health outcomes assessed, camelina oil is comparable to flaxseed and canola oil, which are currently used to increase omega-3 inclusion in canine and equine diets. Therefore, this ingredient has the potential to provide an alternative oil source of omega-3 fatty acids for dogs and horses, while supporting skin and coat health claims.
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LIST OF ABBREVIATIONS

AA: Arachidonic acid
ALA: α-linolenic acid
COX: Cyclooxygenase
DE: Digestible energy
DHA: Docosahexaenoic acid
DNFB: Dinitrofluorobenzene
DPA: Docosapentaenoic acid
Dγ-LNA: Dihomo-γ-linolenic acid
EFA: Essential fatty acid(s)
EPA: Eicosapentaenoic acid
FA: Fatty acid(s)
FAS: Fatty acid synthase
FFA: Free fatty acid(s)
FADH2: Flavin adenine dinucleotide
GAG: Glycosaminoglycan
JUP: Junction plakoglobin
KLH: Keyhole limpet hemocyanin
LA: Linoleic acid
LOX: Lipoxygenase
MUFA: Monounsaturated fatty acid(s)
NADH: Nicotinamide adenine dinucleotide
NE: Net energy
NO: Nitric oxide
PG: Prostaglandin
PGE2: Prostaglandin E₂
PUFA: Polyunsaturated fatty acid(s)
RvE1: Resolvin E1
SAFA: Saturated fatty acid(s)
SCFA: Short chain fatty acid(s)
SFA: Saturated fatty acid(s)
TEWL: Transepidermal water loss
VFA: Volatile fatty acid(s)
n-3: Omega 3
n-6: Omega 6
γ-LNA: γ-linolenic acid
Chapter 1: Literature review

1.1 Introduction

Dogs and horses require dietary fat as a vector for fatty acids (FA), which are vital for the execution of numerous physiological functions. Specifically, FAs aid in the absorption of fat-soluble vitamins, modulate inflammation, regulate antioxidant signaling, promote growth and cognitive development, support skin and coat health, and comprise an integral component of the cell membrane (NRC, 2007; Xenoulis & Steiner, 2010; Oppedisano et al., 2020; Calder et al., 2020; Sakai et al., 2017; DiNicolantonio & O’Keefe, 2020; Rabionet et al., 2014; Bernardi et al., 2012).

Omega-6 (n-6) linoleic acid (C18:2n-6; LA) and omega-3 (n-3) α-linolenic acid (C18:3n-3; ALA) are physiologically essential fatty acids (EFAs) for both dogs and horses, as they cannot be produced endogenously. A vast amount of the biological effects of ALA and LA have been attributed to their longer chain metabolites: n-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and n-6 arachidonic acid (AA), respectively (Goyens et al., 2006; Sinclair et al., 2002). In order to be converted into their corresponding longer chain FAs, both ALA and LA require Δ5- and Δ6-desaturase and elongase enzymes. The shared requirement for these enzymes creates a competitive relationship between the n-6 and n-3 pathways (Zivkovic et al., 2011). Therefore, in order to allow for adequate production of longer chain FAs in both pathways, a balanced n-6:n-3 ratio is required.

To achieve a balanced ratio, ingredients rich in both n-6 and n-3 FAs are required; however, the majority of dietary lipid sources currently included in canine and equine diet formulations
have a greater concentration of n-6 FAs than n-3 FAs. Marine oils, like krill and algal, are often used to increase n-3 FA inclusion due to their high EPA and DHA contents. However, ingredient supply from aquatic environments is lower than the demand from the growing human population, much less the growing livestock and companion animal populations (Shepon et al., 2022). Consequently, finding alternative sources of EPA, DHA, and ALA, that are economically and environmentally sustainable, and can support the endogenous synthesis of n-3 EFA metabolites in the growing human and animal populations is paramount.

Camelina oil serves as a source of n-3 FAs as a result of its n-6:n-3 ratio of 1:1.8. Additionally, this ingredient contains high concentrations of tocopherols and polyphenols, which have been associated with improved skin and coat health due to their antioxidant properties (Zubr & Matthäus, 2002). Although camelina oil is a rich source of n-3 FAs (28.6-36.7% ALA), it has a lower concentration compared to flaxseed oil (51.0-58.3% ALA); this, coupled with its naturally high concentration of antioxidants, make its shelf-life stability better by comparison (Eidhin et al., 2003). Camelina oil is the product of a low-input, high-yield crop, Camelina sativa, which has a short growing season, and is resistant to various seasons, climates, soil types, and pests, that would often pose problems for other oilseeds, making it a desirable crop rotation for farmers (Moser, 2010; Berti et al., 2016; Putnam et al., 1993; Vollmann & Eynck, 2015).

This literature review will focus on FA metabolism and its role in the skin and coat health of dogs and horses. An emphasis will be placed on camelina oil as a potential environmentally and economically sustainable ingredient that can supply ALA, and in turn, be employed to support skin and coat health claims in canine and equine diet formulations, while reducing reliance on marine oil production.
1.2 Fatty acid metabolism

1.2.1 Classification of fatty acids

Mammalian FAs, composed of hydrogenated carbons with a carboxyl group at the alpha carbon, can be classified based on the number of double bonds found within their carbon chain. Short-chain fatty acids (SCFAs) or volatile FAs (VFAs) contain 2-5 carbons and are produced by bacteria in the gastrointestinal tract. Saturated fatty acids (SAFAs) are fully hydrogenated, containing no double bonds. Monounsaturated fatty acids (MUFAs) contain one double bond, most commonly located between the 9th and 18th carbons from the alpha carbons, and occasionally between the 7th and 8th carbons. Polyunsaturated fatty acids (PUFAs) contain between 2-6 double bonds (NRC, 2006; Rustan & Drevon, 2001). Once in the body, FAs have various metabolic fates; one of the most notable being contributing to the production of energy via β-oxidation.

1.2.2 β-oxidation: the role of fatty acids in energy production

When glucose supplies are limited, dietary fat and fat mobilized by tissues can be used to produce ATP or cellular energy through the β-oxidation pathway. The process of β-oxidation occurs in the mitochondria and peroxisomes and involves the breakdown of FAs to release energy. More specifically, β-oxidation involves breaking down long FAs that have been converted to acyl-CoA chains into progressively smaller fatty acyl-CoA chains. Through four enzymatic reactions (Figure 1.1), the acyl-CoAs are degraded into acetyl-CoA units. First, an acyl-CoA-ester is dehydrogenated to produce a trans-2-enoyl-CoA. Next, hydration of the double bond results in L-3-hydroxy-acyl-CoA, which is dehydrogenated to 3-keto-acyl-CoA. Lastly, thiolytic cleavage of the 3-keto-acyl-CoA produces a two-carbon chain-shortened acyl-CoA plus
acetyl-CoA (Houten & Wanders, 2010). Mitochondrial β-oxidation yields 4 ATP equivalents per round of oxidation, in the form of one flavin adenine dinucleotide (FADH$_2$) molecule and one nicotinamide adenine dinucleotide (NADH) molecule, as well as one acetyl CoA molecule (Talley & Mohiuddin, 2022). Carbon molecules from FAs undergoing β-oxidation can be incorporated into ketone bodies, cholesterol, or FAs synthesized de novo.
Fatty acid β-oxidation is the process by which fatty acids are broken down to produce energy. There are four main steps: 1) Acyl-CoA-ester is dehydrogenated to yield a trans-2-enoyl-CoA; 2) Hydration of the double bond; 3) The resulting L-3-hydroxy-acyl-CoA is dehydrogenated to 3-keto-acyl-CoA; 4) Thiolytic cleavage of the 3-keto-acyl-CoA produces a two-carbon chain-shortened acyl-CoA plus acetyl-CoA. Each cycle yields an acyl-CoA shortened by two carbon atoms, an acetyl-CoA, and one nicotinamide adenine dinucleotide (NADH) and one flavin adenine dinucleotide (FADH₂). Carbon from fatty acids undergoing β-oxidation can be incorporated into ketone bodies, or cholesterol or fatty acids synthesized de novo. (Adapted from Adeva-Andany et al., 2019).
1.2.3 Fatty acid biosynthesis

Mammals, including dogs and horses, can produce SCFAs in the gut. In addition to this, they have the ability to produce MUFAs and SAFAs endogenously via FA biosynthesis, also referred to as *de novo* lipogenesis (Figure 1.2) (NRC, 2006; Rustan & Drevon, 2001). During this process, acetyl-CoA is converted into malonyl-CoA by acetyl-CoA carboxylase, which is then used as a building block by the enzyme fatty acid synthase (FAS) to produce myristic acid (14:0), and mystic acid can be elongated to produce palmitic acid (16:0) (Figure 1.2). From palmitic acid, FAs can be further elongated and desaturated by enzymes to produce other MUFAs and SAFAs (Steenson et al., 2017).
Figure 1.2 Fatty acid synthesis de novo.

The process of de novo lipogenesis in mammals, where palmitic acid is the major product, which may be further elongated or desaturated to form other monounsaturated of saturated fatty acids. Enzymes are numbered: (1) acetyl-CoA carboxylase; (2) fatty acid synthase; (3) fatty acid elongases; (4) Δ9- desaturase.
1.2.4 Production of polyunsaturated fatty acids

Although mammals can produce SFAs, SAFAs, and MUFAs in the body, they are unable to produce PUFAs endogenously. The inability of animals to produce PUFAs is due to the lack of the enzyme Δ-12 desaturase, which facilitates the conversion of oleic acid (18:1) to LA, and the enzyme Δ-15 desaturase, which inserts a double bond into LA to produce ALA, which are the parent compounds for the n-3 and n-6 PUFAs. Consequently, LA and ALA are required in the diet. Among the ALA and LA derived PUFAs, AA, EPA, and DHA are the most biologically active EFAs in mammals. Arachidonic acid can be synthesized by Δ6 desaturation of LA to produce γ-linolenic acid (γ-LNA; 18:3n-6) that is elongated to dihomo-γ-linolenic acid (Dγ-LNA; 20:3n-6) followed by Δ5 desaturation to AA (Cooke, 2004). Eicosapentaenoic acid is synthesized from ALA, following the same enzymes and pathways as AA, and can be further metabolized into DHA via elongation, Δ6 desaturation, and β-oxidation (Figure 1.3) (Sprecher, 2000; Emken et al., 1994; Pawlosky et al., 2001; Gao et al., 2010).
Fatty acid metabolism including the omega-3, omega-6, omega-7, and omega-9 cascades, de novo synthesis of saturated fatty acids (SFAs), starting with palmitic acid, and enzymatic elongase and desaturase reactions (Adapted from NRC, 2006 & Butler et al., 2017).

Figure 1.3 Fatty acid metabolism overview.
1.2.5 Efficiency of linoleic acid / α-linolenic acid conversion to longer chain metabolites

Although providing ALA and LA in the diet supports the conversion of these FAs into their respective long chain metabolites via Δ-5 and Δ-6 desaturase and elongase enzymes, the rate of this conversion is low in humans (on average, 1-10% of ALA is converted to EPA and 0.5-5% into DHA) and is also believed to be rather limited in dogs (Bauer, 2011; Adas et al., 1999; Bibus et al., 1993; Duda et al., 2009; Brenna, 2002; Gerster, 1998; Plourde and Cunnane, 2007; Burdge and Wootton, 2002; Lennox and Bauer, 2013; ). Horses fed a fish oil supplement (rich in EPA and DHA) had higher plasma EPA, DHA, and AA concentrations than horses fed corn oil (~60% LA), indicating that, similar to humans and dogs, horses have a limited ability to convert LA and ALA into their long chain PUFAs (Hall et al., 2004; Dupont et al., 1990).

1.2.6 The n-6:n-3 ratio

The shared requirement for the Δ5 and Δ6 elongase and desaturase enzymes creates a competitive relationship between the n-6 and n-3 pathways. Both n-3 EPA and DHA, and n-6 AA are parent compounds for the production of inflammatory eicosanoids, however, AA-derived eicosanoids (i.e. Prostaglandin E2, Leukotriene B4, Thromboxane A2, Thromboxane B2, Leukotriene C4, Leukotriene D4, and Leukotriene E4) result in a pro-inflammatory, prothrombotic state, in comparison to EPA and DHA-derived eicosanoids (i.e. Prostaglandin E3, Leukotriene B5, Maresin, Programmed cell death protein I, and Resolwins) which elicit anti-inflammatory and pro-resolving effects (Zivkovic et al., 2011) (Figure 1.4). Thus, an unbalanced n-6:n-3 ratio in favor of n-6 PUFAs is prothrombotic and pro-inflammatory, which contributes to the prevalence of atherosclerosis, obesity, diabetes, and additional co-morbidities (Simopoulos, 2001; Kang, 2003; Simopoulos, 2008; Simopoulos, 2013; Donahue et al., 2011; Kromhout & de...
Goede, 2014). Indeed, regular consumption of diets rich in n-3 PUFAs has been associated with low incidence of these diseases (Kromann & Green, 1980; Adler et al., 1994; Schraer et al., 1999).
Figure 1.4 Metabolic pathways for omega-6 and omega-3 fatty acids that result in a variety of inflammation mediators and cell function effectors.

Proinflammatory (red) and anti-inflammatory (green) molecules are denoted within ellipses. AA, arachidonic acid; ALA, alpha linolenic acid; COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDHA, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LA, linoleic acid; LOX, lipoxygenase; LT, leukotriene; LX, lipoxin; MaR, maresin; PD1, protectin D1; PG, prostaglandin; Rv, resolvin; TX, thromboxane.(Fabian et al., 2015).
1.3 Lipid digestion and absorption

1.3.1 Lipid digestion and absorption in dogs

In dogs, digestion of lipids begins in the stomach and requires the coordination of the liver, gall bladder, pancreas, and small intestine for maximal digestion and absorption. In the stomach, triglycerides are cleaved into free fatty acids (FFAs) via gastric lipase. The FFAs then stimulate the secretion of cholecystokinin in the duodenum, which in turn, stimulate gall bladder and pancreatic contractions. The FFAs, along with cholesterol and fat-soluble vitamins from the consumed fat are further cleaved by pancreatic lipase and colipase and emulsified via bile salts from the gall bladder to produce a mixed micelle, which are then absorbed by enterocytes in the jejunum via passive diffusion or carrier mediated uptake. Once in the enterocyte the FAs, cholesterol, and other phospholipids are packaged into a lipoprotein rich compound, a chylomicron, which is secreted into the intestinal lymphatic system. A smaller portion of dietary fat also passes directly into the portal circulation (NRC, 2006). The presence of soluble fibers and starches decrease fat digestibility, while insoluble fibers do not appear to significantly affect fat digestibility in dogs (Burkhalter et al., 2001; Kienzle et al., 2001; Prola et al., 2010; Niza et al., 2003).

1.3.2 Lipid digestion and absorption in horses

Similar to dogs, horses begin fat digestion in the stomach via gastric lipase; however, the small intestine is the primary site of lipid digestion rather than the stomach. One large difference between dogs and horses is horses do not have a gall bladder and as such bile salts are excreted from the liver constantly, which is likely due to the natural foraging behavior of horses where they spend most of their day grazing. Horses have been shown to tolerate a lipid inclusion of
20% digestible energy for six months though it is recommended to slowly introduce fat to the diet to allow the liver time to upregulate bile and subsequent digestive capacity (Hintz et al., 1989; Harris et al., 1999). Ponies fed supplemental corn oil had a greater digestible energy (DE) to net energy (NE) conversion efficiency when supplemented corn oil (85% efficiency), compared to hay and grain alone (60% efficiency) (Kane et al., 1979; NRC, 2007). Fat and oil ingredients have a greater mean digestibility of lipids compared to concentrates or forages, making them desirable sources of lipids for horses.

**1.4 Biological significance of PUFAs in the skin and coat**

1.4.1 The role of FAs in the skin

Fatty acids are important structural components of triglycerides, phospholipids, glycosylceramides, and ceramides, which are crucial in supporting the function and integrity of the skin barrier (Khnykin et al., 2011). This barrier is organized into three layers: the dermis, hypodermis, and epidermis. The bottom layer of the skin, the hypodermis, is tasked with storing energy, insulating, and protecting the body, and connecting the dermis layer to the muscles and bones. The main function of the dermis, which consists mostly of collagen and elastin, is to provide physical and nutritional support to the epidermis (Brown & Krishnamurthy, 2021). The epidermis is the outer layer of the skin, and as such, provides a protective barrier against mechanical, physical, and thermal assaults, including UV radiation, and prevents the loss of moisture. Furthermore, the epidermis is a heavily active site of lipid synthesis, exceeding the liver, kidney, and gastrointestinal epithelia on a per weight basis (Brown & Krishnamurthy, 2021).
1.4.2 n-6 PUFA in the skin

Linoleic acid is the most abundant PUFA in the epidermis, serving as an important component of ceramides, which comprise 40-50% of the lipid profile in the outer layer (stratum corneum) (Wertz et al., 1992). Ceramides are key lipid components for barrier function and are composed of a sphingosine and a fatty acid. The role of LA in barrier function was first determined by a series of animal experiments carried out by Burr and Burr, who observed that supplementing rats deficient in EFAs with safflower oil (55.1-77% LA), primrose oil (70-74% LA), and purified LA preparations, repaired barrier function, whereas supplementation with n-3’s had no effect (Burr & Burr, 1929; Burr & Burr, 1930; Timoszuk et al., 2018; Matthaus et al., 2015).

Arachidonic acid makes up approximately 9% of the FAs found in the epidermis, making it the second most abundant epidermal PUFA. It is an important building block of phosphatidylinositol and phosphatidylserine, phospholipids found in the membranes of epidermal keratinocytes. Additionally, AA promotes wound healing and acts as the major source of epidermal eicosanoids, which are potent mediators of the inflammatory response (Wertz et al., 1992; Gray et al., 1975).

Another PUFA that has gained interest due to its impact on the skin barrier is γ-LNA. Supplementation of γ-LNA has resulted in beneficial effects on various biophysical skin parameters, including skin moisture, TEWL, firmness, roughness, and elasticity (Brosche et al., 2000; Muggli et al., 2005; Neukam et al., 2011). A clinical study supplementing patients (n=130) with mild atopic dermatitis γ-LNA-rich oil over four weeks (the minimum period for middle-aged epidermis to turn over and FAs to reach new tissue concentrations) observed lower TEWL
and a higher stratum corneum index compared to the control (Kawamura et al., 2011). The generation of anti-inflammatory metabolites from γ-LNA might be a reasonable explanation of the mechanism of skin barrier recovery.

1.4.3 n-3 PUFAs in the skin

Unlike n-6 LA and AA, which play a major structural role in the epidermis, n-3 FAs comprise less than 2% of the epidermis and serve an immune-modulating role. Supplementation of n-3 PUFAs, ALA, and corresponding derivatives, have been associated with numerous benefits regarding the preventative and therapeutic use in individuals with various skin disorders, including atopic dermatitis. Some of these benefits include barrier function support and maintenance, stratum corneum maturation and differentiation, lamellar body formation, lipoxygenase (LOX) and pro-inflammatory eicosanoid inhibition, cytokine suppression, inhibition of mast cell degranulation and modulation of other immune cells (Li et al., 2019; Nishi et al., 2019; Huang et al., 2018). As an example, supplementing fish oil improved dermatitis symptoms due to its anti-inflammatory effects in humans: inhibition of lymphocyte proliferation, cytokine and antibody production, adhesion molecules expression, natural killer cell activity and triggering apoptosis (Kew et al., 2004; Li et al., 2019). Additionally, fish oil supplementation reduced dryness, increased cutaneous hydration by 30%, and the eliminated itch-related scratching behavior in 60 days. Furthermore, following 90 days on the same supplement, uptake of DHA, EPA, and docosapentaenoic acid (DPA) into the skin was increased. Resolvin E1 (RvE1) is an eicosanoid produced within the n-3 pathway from EPA and has been found to reduce inflammatory interleukin 12 production, neutrophil transendothelial migration, and dermal inflammation in complex disease models (Arita et al., 2005; Hasturk et al., 2006; Ariel et
al., 2007). In mice, RvE1 reduced the development of skin lesions in patients with atopic dermatitis induced by 2,4-dinitrofluorobenzene (DNFB) treatment by suppressing the production of inflammatory eicosanoids interleukin 4 and interferon-γ by activated CD4+T cells, and by decreasing lesional infiltration by CD4+, CD8+ T cells, as well as the mast cells and eosinophils, in addition to suppression of total serum immunoglobulin E levels (Kim et al., 2012). In the treatment of atopic dermatitis, the use of DHA and EPA may also ameliorate inflammation by modulation of toll-like receptor-2 and toll-like receptor-4 and subsequent signaling (McCusker et al., 2010). Supplementation of fish oil, a rich source of EPA and DHA, was found to improve skin quality in dogs from baseline based on a clinical score, with maximal improvement occurring after 8 weeks. The same study observed that following supplement withdrawal, skin and coat health clinical scores remained the same for one month and began to deteriorate following the second month (Combarros et al., 2020). Together, chronic supplementation of n-3 may be necessary in individuals with inflammation associated with poor skin and coat.

1.4.4 n-6:n-3 balance in the skin

Overall, both n-3 and n-6 cascades contribute to the function and integrity of the skin barrier. The n-6 cascade has a key role in many structural components of the skin, while the n-3 pathway serves more of an immune modulating role, both of which are vital in the maintenance of the skin barrier. As mentioned previously, without a proper n-6:n-3 ratio an inflammatory state may occur, and as such, it is important to include dietary sources of both of these FAs in order to obtain their associated benefits. There are few rationales for combined n-3 PUFAs, and γ-LNA enriched supplementation: (a) n-3 long chain PUFAs inhibit the conversion of γ-LNA metabolite Dγ-LNA to ARA; (b) this combination inhibits leukotriene production, as well as the
genes for pro-inflammatory cytokines; (c) addition of n-3 long chain PUFAs enriches cells and tissues with EPA, DPA, and DHA and their potent anti-inflammatory metabolites (Sergeant et al., 2016).

1.4.5 The role of fatty acids in the coat

Although lipids make up only 2-6% of the overall weight of hair, they play a crucial role in maintaining hair health and strength. Combarros and colleagues supplemented dogs with fish oil (average dose 20.4 mg/kg of EPA and DHA) and observed a significant improvement in hair coat using a clinical scale (Combarros et al., 2020). Additional work carried out by Rees et al., (2001) supplemented flaxseed oil and sunflower oil to dogs and found an increase in the relative % of ALA concentrations in serum phospholipids in the flaxseed group, but no change in the sunflower group. This is likely a result of the FA profiles of the oils themselves. Supplementation with both sunflower (n-6:n-3 = 1:0) and flaxseed oil (n-6:n-3 = 1:4.19) provides temporary improvement in skin and hair coat. Further, these changes appear to be associated with increased serum 18 carbon PUFAs (Rees et al., 2001). Although uncommon, EFA deficiency has been observed to result in a poor pigmentation of the coat, resulting in a dull coat and hair loss (Davidson et al., 1986). Additionally, FA supplementation is associated with a decrease in alopecia and self-grooming behavior in macaques and humans (Hamel et al., 2017; Le Floc’h et al., 2015). Together, in addition to benefits to skin health, supplementation of EFAs or long chain n-3 PUFA, support hair or coat health in mammals when provided in the form of a balanced ratio.
1.5 Camelina oil

1.5.1 Camelina oil

Camelina oil contains naturally low levels of the anti-nutritional compound (<4%) erucic acid, along with a unique FA composition (Table 1.1): 60% PUFAs, 30% MUFAs, 6-10% SFAs, 15% LA, and 27-39% ALA (Zubr and Matthaus, 2002; McVay and Lamb, 2008; Kirkhus et al., 2013; Jiang et al., 2014; Abramovic & Abram, 2005). Although the high PUFA content of camelina oil makes it susceptible to oxidation, this is counterbalanced by the high antioxidant content (Ergönül and Özbek, 2020). Camelina oil contains high concentrations of tocopherols and polyphenols, which have been associated with improved skin and coat health due to their antioxidant properties (Zubr & Matthäus, 2002). The total tocopherol content in camelina oil is 972.3 mg/kg, compared to flaxseed oil, at 588.7 mg/kg (Grajzer et al., 2020). Additionally, a multiple regression analysis completed by Grajzer and colleagues found significant effects of polyphenols found in camelina oil on 2,2-diphenyl-1-picryl-hydrazyl-hydrate antioxidant activity compared to flaxseed oil (Grajzer et al., 2020).

1.5.2 Camelina sativa

The cold- and drought-tolerant crop, camelina sativa, requires low fertiliser inputs, and when compared to other oilseed crops like canola or sunflower, is better suited to environments with limited water resources (Putnam et al., 1993; Obour et al., 2015). Camelina sativa has a short growing cycle and matures between 75 and 112 days after planting, depending on the production environment and the date of sowing (McVay and Lamb, 2008; Sintim et al., 2016). Furthermore, this crop is resistant to a variety of seasons, climates, soil types, and pests (Moser, 2010; Berti et al., 2016; Putnam et al., 1993; Vollmann & Eynck, 2015; Zubr & Matthäus, 2002).
The amount of oil produced from this oil seed ranges from 30 to 40% on average but has been reported to be as high as 48% (Obour et al., 2015).
### Table 1.1 Fatty acid composition of camelina oil, flaxseed oil, and canola oil as a % of total fatty acids.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Camelina Oil</th>
<th>Flaxseed Oil</th>
<th>Canola Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic (C14:0)</td>
<td>0.1</td>
<td>&lt;0.1-0.14</td>
<td>ND</td>
</tr>
<tr>
<td>Palmitic (C16:0)</td>
<td>5.1-6.1</td>
<td>5.1-16.3</td>
<td>4.3-4.7</td>
</tr>
<tr>
<td>Stearic (C18:0)</td>
<td>2.4-2.6</td>
<td>3.1-5.1</td>
<td>2.0-2.21</td>
</tr>
<tr>
<td>Arachidic (C20:0)</td>
<td>1.3-1.8</td>
<td>0.06-0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Behenic (C22:0)</td>
<td>0.3-.8</td>
<td>ND; 0.05</td>
<td>0.4</td>
</tr>
<tr>
<td>Oleic (C18:1n-9)</td>
<td>15.7</td>
<td>15.8-21.5</td>
<td>60.7-62.5</td>
</tr>
<tr>
<td>Eicosenoic (C20:1n-9)</td>
<td>14.6</td>
<td>0.04-0.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Erucic (C22:1n9)</td>
<td>1.5-4.2</td>
<td>0.3</td>
<td>0.4-1.27</td>
</tr>
<tr>
<td>Linoleic (C18:2n-6)</td>
<td>14.4-20.6</td>
<td>15.0-17.8</td>
<td>17.6-19.6</td>
</tr>
<tr>
<td>α-linolenic (C18:3n-3)</td>
<td>28.6-36.7</td>
<td>51.0-58.3</td>
<td>9.34-10.00</td>
</tr>
</tbody>
</table>

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

(Berti et al., 2016; Jaśkiewicz et al., 2014; Raczyk et al., 2016; Yang et al., 2016; Lewinska et al., 2015; Olomu & Baracos, 1991; Rueda et al., 2014)
1.6 Conclusion

To conclude, FAs have numerous benefits, particularly on skin and coat health. However, due to the competitive relationship between the n-3 and n-6 cascades, a balanced ratio is required to prevent an inflammatory, prothrombotic state, and associated risks. In order to achieve this ratio, sources of both n-3 and n-6 FAs are required, however, the majority of dietary fat sources currently used to formulate canine and equine diets are higher in n-6 FAs, or are not environmentally or economically sustainable, which is essential to consider in order to meet the demands of both the growing human and animal populations. Thus, alternative sources of n-3 FAs that are sustainable are needed to support the pet and equine nutrition sectors. Camelina oil has a desirable n-6:n-3 ratio, is environmentally and economically sustainable, and has the potential to serve as an alternative oil source of n-3 FAs, while supporting skin and coat health claims.

1.7 Thesis objectives and hypotheses

To our knowledge, no research exists directly comparing the effects of camelina oil supplementation to the effects of existing oil supplements commonly used in canine and equine diets on the skin and coat health of the respective species. Therefore, the objective of the first part of this work was to compare the effects of camelina oil to flaxseed oil and canola oil supplementation on the skin and coat health of dogs. This was done by assessing changes in three outcomes: (1) transepidermal water loss, (2) inflammatory and oxidative markers, and (3) skin and coat health quality scores using a 5-point scale, over a 16-week period. We hypothesized that camelina oil is comparable to flaxseed oil and canola oil in terms of its effects on the outcomes measured.
The objective of the second portion of this work was to assess the effects of camelina oil supplementation on the same skin and coat health parameters: transepidermal water loss, inflammatory and oxidative markers, and skin and coat quality scores, in healthy adult horses, compared to flaxseed and canola oil supplementation. Similar to the canine study, we hypothesize that camelina oil is comparable to flaxseed oil and canola oil, in terms of its effects on the outcomes measured.
1.8 References


Chapter 2: Effects of dietary camelina, flaxseed, and canola oil supplementation on inflammatory and oxidative markers, transepidermal water loss, and skin and coat health parameters in healthy adult dogs

1Submitted and formatted for publication in Frontiers in Veterinary Science Special Issue: Nutrition and Management of Animals We Keep as Companions, Volume II.

2.1 Abstract

Camelina oil contains a greater concentration of the omega-3 (n-3) essential fatty acid (EFA) α-linolenic acid (C18:3n-3; ALA) than the omega-6 (n-6) EFA linoleic acid (C18:2n-6; LA), in comparison to alternative fat sources commonly used to formulate canine diets, such as sunflower oil. Omega-3 FAs are frequently used to support canine skin and coat health claims and reduce inflammation and oxidative stress; however, there is a lack of research investigating camelina oil supplementation and its effects on these applications in dogs. The objective of this study was to compare the effects of camelina oil supplementation to flaxseed and canola oil supplementation on skin and coat health characteristics, skin barrier function, and inflammatory and oxidative markers. Thirty (17 females; 13 males; 7.2±3.1 years old; 27.4±14.0 kg body weight (BW)) privately-owned dogs of various breeds were used. After a 4-week wash-in period consuming sunflower oil (n6:n3=1:0) at 8.2 g oil/100g food and a commercial kibble, dogs were blocked by age, breed, and size, and randomly assigned to one of three treatment oils: camelina (n6:n3=1:1.18), canola (n6:n3=1:0.59), flaxseed (n6:n3=1:4.19) at an inclusion of 8.2g oil/100g of total food intake in a randomized block design. Transepidermal water loss (TEWL) was measured using a VapoMeter on the pinna, paw pad, and inner leg. Fasted blood samples were collected to measure serum inflammatory and oxidative marker concentrations. Prostaglandin E2 (PGE2) and junction plakoglobin (JUP) concentrations were measured via enzyme-linked
immunosorbent assay (ELISA) kits, while nitric oxide (NO) and glycosaminoglycan (GAG) concentrations were determined using spectrophotometric assays. A 5-point-Likert scale was used to assess skin and coat characteristics. All data were collected on weeks 0, 2, 4, 10, and 16 and analyzed using PROC GLIMMIX in SAS. No significant changes occurred in TEWL, or inflammatory and oxidative marker concentrations among treatments, across weeks, or for treatment by week interactions. Softness, shine, softness uniformity, color intensity, and follicle density increased, and skin moisture decreased from baseline in all treatment groups (P < 0.05). Outcomes did not differ (P > 0.05) among treatment groups over 16-weeks, indicating that camelina oil is comparable to existing plant-based canine oil supplements, flaxseed, and canola, at supporting skin and coat health and inflammation in dogs, but all may improve skin and coat health in contrast to oils with a greater n-6:n-3 ratio such as sunflower. Further research employing an immune or inflammatory challenge are warranted.

2.2 Introduction

Dogs are unable to produce the omega-6 (n-6) linoleic acid (C18:2n-6; LA) and the omega-3 (n-3) \( \alpha \)-linolenic acid (C18:3n-3; ALA), endogenously, and as such, these must be obtained in the diet [1]. Omega-3 fatty acids (FAs) in particular have been linked to numerous health benefits, including a reduction in inflammation and oxidative stress, and improved skin and coat health properties, which are directly associated [2-7].

There is a competitive relationship between the n-6 and n-3 FA pathways for the use of the \( \Delta 5 \)- and \( \Delta 6 \)-desaturase enzymes needed to convert LA and ALA into longer chain FAs. Consequently, a balanced dietary n-6:n-3 ratio is needed to ensure that neither the n-6 nor the n-3 pathway to allow sufficient conversion to longer chain FAs in both pathways. Specifically, and
most notably, LA is converted into arachidonic acid (AA), and ALA is converted into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [8]. Both AA and EPA and DHA are parent compounds for the production of inflammatory eicosanoids, however, the eicosanoids produced from AA have opposing properties from those produced from EPA and DHA. An increase in endogenous n-6 AA results in a prothrombotic, pro-constructive, and pro-inflammatory state, whereas increased EPA and DHA give rise to resolvins, which are anti-inflammatory and pro-resolving. Greater concentrations of n-6 FAs and a higher n-6:n-3 ratio allow for greater conversion of n-6 FAs to AA and more pro-inflammatory effects. In contrast, greater concentrations of n-3 FAs and a lower n-6:n-3 ratio allow for increased production of EPA and more anti-inflammatory effects [9]. As a result, excessive amounts of n-6 FAs and a high n-6:n-3 ratio promote the pathogenesis of many inflammatory, autoimmune, and dermatological disorders, whereas greater concentrations of n-3 FAs and a low n-6:n-3 ratio exert suppressive effects [10].

In order to formulate canine diets to meet the ideal n-6:n-3 ratio of between 5:1 and 10:1, n-3 rich ingredients are typically required [11]. Two oils commonly used to increase n-3 inclusion in canine diets are fish oil, as a result of its high levels of EPA and DHA (180 mg EPA, 120 mg DHA/1000 mg of oil provided in the most common fish oil capsules in the United States today, however, doses vary widely between supplements), and flaxseed oil, due to its favorable n-6:n-3 ratio of 1:4.19 [12-15]. However, large-scale fish oil production required to meet the demands of the growing pet food industry is not environmentally sustainable long-term, and the high abundance of ALA in flaxseed oil makes it susceptible to oxidation, making its use in commercial diets difficult [12, 15]. Additionally, flaxseed crops are sensitive to various climates,
diseases, and pests, making both of these options less than desirable [12, 14, 15]. Alternative
animal-based (beef, 1:0.05; milk, 1:0.07; eggs, 1:0.05) and plant-based (canola, 1:0.59; corn,
1:0.01; soybean, 1:0.12; and sunflower oil, 1:0.00) lipid sources commonly used in canine diet
formulations all have higher concentrations of n-6 FAs rather than n-3 FAs [15-17]. This leaves
room in the market for an alternative plant-based oil source that is economically and
environmentally sustainable, with good shelf-stability and a favorable concentration of n-3 FAs
that could contribute to achieving the ideal n-6:n-3 ratio in canine diets.

The oil seed camelina (*Camelina sativa*) is considered a low-input, high-yield crop due to its
short growing season and resistance to various seasons, climates, and soil types [18-21]. The
product of this robust crop, camelina oil, provides a rich source of n-3 FAs as a result of its
desirable n-6:n-3 ratio of 1:1.8 [22]. Additionally, camelina oil contains high concentrations of
tocopherols and polyphenols, which have been associated with improved skin and coat health
due to their antioxidant properties [22]. Due to camelina oil being naturally high antioxidants as
well as having a slightly lower concentrations of n-3 FAs in contrast to flaxseed oil, it’s shelf-
stability is better by comparison [23].

Camelina oil has previously been found to be safe for canine consumption by our laboratory
[24]. The inclusion of oil supplements in canine diets is often associated with claims of
maintenance or support of skin and coat health, but currently there is no data directly comparing
the effects of camelina oil supplementation to the effects of other oils approved for use in pet
foods on markers of skin and coat health and inflammation. The objective of this study was to
compare the effects of dietary camelina oil supplementation to those of flaxseed oil and canola
oil supplementation on skin and coat health and inflammatory and oxidative markers in healthy,
adult dogs. Outcomes include changes in oxidative and inflammatory biomarkers, and skin and coat health parameters. Additionally, skin barrier function and integrity were assessed by measuring transepidermal water loss (TEWL). We hypothesize that camelina oil is comparable, if not superior to, flaxseed and canola oil in terms of its effects on oxidative and inflammatory markers, skin and coat health parameters, and TEWL.

### 2.3 Materials and Methods

#### 2.3.1 Animals and housing

This experiment was approved by the University of Guelph’s Animal Care Committee (AUP #4365) and was carried out in accordance with national and institutional guidelines for the care and use of animals. Thirty client-owned, adult (7.2 ± 3.1 years) dogs of mixed sex (17 females: 16 spayed, 1 intact; 13 males: 10 neutered, 3 intact), weight (27.4 ± 14.0 kg) and breed participated in this study (Table 2.1). All dogs were deemed healthy based on their previous medical history as well as a pre-study physical examination performed by a licensed veterinarian, complete blood count (CBC), and serum biochemistry profile. During the recruitment process, dogs were excluded if they had any skin conditions, received any pro- or anti-inflammatory medications two-months prior to baseline samples, had abnormalities on their physical examination, CBC, or serum biochemistry, or were younger than two years of age. Dogs were housed at their owners’ homes for the duration of the study, they followed their usual daily routines.
Table 2.1 Mean age, mean body weight, breeds, and male:female and neutered:spayed:intact ratios of 30 client-owned dogs enrolled in a research trial investigating the effects of three oil supplements (camelina, canola, flaxseed) on transepidermal water loss, inflammatory and oxidative markers, and skin and coat health parameters over a 16-week period.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean age¹</th>
<th>Mean BW²</th>
<th>Breeds</th>
<th>Male:Female</th>
<th>Neutered:Spayed:Intact</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAM</td>
<td>7.8</td>
<td>25</td>
<td>Miniature Dachshund Havanese Mix, Unknown Mix, Australian Shepherd/Collie Mix, Boxer Whippet Standard Poodle Norwegian Elkhound Labrador Retriever (3)</td>
<td>2:8</td>
<td>2:7:1</td>
</tr>
<tr>
<td>FLX</td>
<td>7.7</td>
<td>27</td>
<td>Miniature Dachshund Pekingese Mix, Sled dog/unknown Mix, Border Collie/Sheltie Mix, Husky/Pointer Great Dane Standard Poodle Bernese Labrador Retriever (2)</td>
<td>6:4</td>
<td>5:4:1</td>
</tr>
<tr>
<td>OLA</td>
<td>6.05</td>
<td>28</td>
<td>Mix, Mastiff/Boxer King Charles Cavalier Spaniel Mix, Samoyed/Collie Sheltie German shepherd Barbet Standard Poodle Bernese Labrador Retriever (2)</td>
<td>6:4</td>
<td>4:4:2</td>
</tr>
</tbody>
</table>

¹Mean age and body weight of dogs on week 0 of research trial; units=years.
²Mean body weight of dogs on week 0 of research trial; units=kilograms.

BW, body weight; CAM, camelina oil; FLX, flaxseed oil; OLA, canola oil
2.3.2 Dietary treatments

Over a 4-week wash-in period, all dogs were acclimated to a dry extruded commercial kibble (SUMMIT Three Meat Reduced Calorie Recipe, Petcurean, Chilliwack, BC, Canada; Table 2.2), sunflower oil (SA Kernel-Trade, Kuiv, Ukraine; Table 2.3), and beef-based treats (Beef Tendersticks, The Crump Group, Brampton, ON, Canada; proximate analysis: metabolizable energy 3039 kcal/kg; crude protein minimum 65%; crude fat minimum 5.1%; crude fibre maximum 4.0%; moisture max 9.56%). Oil was included in the diet at 8.2 grams of oil per 100 grams of total food intake, bringing the total dietary lipid content to 20% on an as-fed basis. Treats were included in the diet up to 2.5 grams per 100 grams total intake, and the remaining proportion of the diet was provided as kibble. During the wash-in period and throughout the study, daily portions of food, oil, and treats were pre-weighed by researchers and provided to the owners in two-week intervals to be offered to dogs daily at a frequency determined by the owner. To avoid the occurrence of lipid peroxidation, owners were instructed to mix the oil with the food immediately before feeding. Any leftover kibble, oil, and/or treats were returned to researchers and subsequently weighed and recorded. Dogs were initially fed to meet their estimated maintenance energy requirements (110 kcal ME x kg BW^{0.75}), and BW was recorded every two weeks starting at baseline. Each dog’s food allotment was then adjusted accordingly to maintain baseline BW throughout the study.
Table 2.2 Proximate analysis, metabolizable energy, omega-6 and omega-3, and linoleic and docosahexaenoic acid content of a commercial extruded kibble on an as-fed basis, fed to 30 client-owned dogs during a skin and coat health trial over a 16-week period.

<table>
<thead>
<tr>
<th>Nutrient profile</th>
<th>As fed basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>8.00</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>21.0</td>
</tr>
<tr>
<td>Nitrogen-free extract (%)</td>
<td>52.0</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>2.80</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>9.00</td>
</tr>
<tr>
<td>Omega 6 (%)</td>
<td>2.00</td>
</tr>
<tr>
<td>Omega 3 (%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Linoleic acid (%)</td>
<td>1.90</td>
</tr>
<tr>
<td>Docosahexaenoic acid (%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>7.10</td>
</tr>
<tr>
<td>Metabolizable energy (kcal/kg)</td>
<td>3,324</td>
</tr>
</tbody>
</table>

1Chicken meal, whole brown rice, whole white rice, barley, oatmeal, chicken fat (preserved with mixed tocopherols), peas, lamb meal, salmon meal, natural chicken flavor, whole dried egg, sunflower oil, rice bran, flaxseed, dried kelp, dicalcium phosphate, potassium chloride, choline chloride, sodium chloride, calcium carbonate, vitamins (vitamin A supplement, vitamin D3 supplement, vitamin E supplement, niacin, L-ascorbyl-2-polyphosphate (a source of vitamin C), d-calcium pantothenate, thiamine mononitrate, beta-carotene, riboflavin, pyridoxine hydrochloride, folic acid, biotin, vitamin B12 supplement), minerals (zinc proteinate, iron proteinate, copper proteinate, zinc oxide, manganese proteinate, copper sulphate, ferrous sulphate, calcium iodate, manganous oxide, selenium yeast), DL-methionine, glucosamine hydrochloride, chondroitin sulphate, yeast extract, Yucca schidigera extract, dried rosemary.
Table 2.3 Analyzed fatty acid profiles of camelina oil, canola oil, flax oil, and sunflower oil fed to 30 client-owned dogs top dressed on commercial kibble during a skin and coat health trial over a 16-week feeding period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sunflower(^1)</th>
<th>Canola(^2)</th>
<th>Flaxseed(^2)</th>
<th>Camelina(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated Fatty Acids (%)</td>
<td>9.61</td>
<td>6.50</td>
<td>8.20</td>
<td>9.50</td>
</tr>
<tr>
<td>Monounsaturated Fatty Acids (%)</td>
<td>14.1</td>
<td>63.8</td>
<td>16.6</td>
<td>35.2</td>
</tr>
<tr>
<td>Polyunsaturated Fatty Acids (%)</td>
<td>76.3</td>
<td>29.7</td>
<td>75.2</td>
<td>55.3</td>
</tr>
<tr>
<td>Omega 6 (%)</td>
<td>76.2</td>
<td>18.6</td>
<td>16.5</td>
<td>19.8</td>
</tr>
<tr>
<td>Omega 3 (%)</td>
<td>0.04</td>
<td>11.1</td>
<td>58.6</td>
<td>35.4</td>
</tr>
</tbody>
</table>

\(^1\)Numerical values from Kostik et al. (2013) and only represent generic sunflower oil, not the brand used for this study.

\(^2\)Samples analyzed in duplicate by SGS Canada Inc. (Guelph, ON, Canada), average values reported. Burron et. al (2020).
2.3.3 Study design

This study was conducted using a randomized complete block design (RCBD) with repeated measures. Following the 4-week wash-in period, dogs were blocked by breed, age, and BW and groups were randomly assigned to one of 3 treatment oils: camelina oil (CAM) (n=10; 8 females; 2 males), flaxseed oil (FLX) (n=10; 5 females; 5 males), or canola oil (OLA) (n=10; 4 females; 6 males). The sunflower oil used during the wash-in was replaced with either CAM, FLX, or OLA, and feeding continued as described for 16 weeks.

2.3.4 Blood collection

Dogs were fasted for a minimum of 10 hours overnight and blood was collected via cephalic venipuncture using a syringe (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Of the collected blood, 5 mL was put into a serum vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Blood was allowed to clot and was centrifuged at 7200× g for 15 min using an accuSpin Micro 17 centrifuge (Thermo Fisher Scientific, Waltham, MA, USA). Then, the serum aliquots were frozen at −80 °C until later analysis.

2.3.5 Inflammatory and oxidative markers

Serum samples were analyzed for prostaglandin E₂ (PGE₂) (Canine Prostaglandin E₂ ELISA Kit MBS013017, MyBioSource, Vancouver, BC) and junction plakoglobin (JUP) (Canine Junction Plakoglobin ELISA Kit MBS104997, MyBioSource, Vancouver, BC) using commercially available ELISA (Enzyme-linked immunosorbent assay) kits. Samples were run in duplicate according to the manufacturer’s instructions. Serum glycosaminoglycan (GAG) (dimethyl methylene blue) and nitric oxide (NO) (Griess Reaction; Molecular Probes, Eugene,
OR) concentrations were determined using spectrophotometric assays [26, 27]. Serum NO and GAG samples were analyzed as previously described by MacNicol et al. (2018) [28].

2.3.6 Skin barrier

Skin barrier function and integrity were assessed by measuring TEWL, which is defined as the amount of water that passively evaporates through skin to the external environment due to a water vapor pressure gradient on both sides of the skin barrier and is commonly used to characterize skin barrier function and integrity [29, 30]. On weeks 0, 2, 4, 10, and 16, TEWL was measured using a VapoMeter® SWL-3 (Delfin Technologies Ltd, Kuopio, Finland), according to the manufacturer’s instructions. Since privately-owned dogs were used, it was not feasible to shave multiple patches for TEWL measurements, and as a result, researchers chose three body sites with little hair to measure TEWL, including: the right paw pad, right pinna, and right inner thigh. Ten measurements were taken per body site and the average was used for analyses. Once the averages were calculated, any values above or below the average by 50 g/m²/h or more were considered outliers and removed. All dogs were brought to the University of Guelph by their owners on collection days to ensure environmental conditions during collections remained consistent. All measurements were carried out by a single operator, in the same order of body sites, and in a climate-controlled room to maintain consistency between samples and to avoid variation in VapoMeter® readings due to temperature and humidity fluctuations [29]. Room conditions were stable at 22-23°C ambient temperature and 44-50% ambient relative humidity. The evaporation rate value is calculated in grams of water per square metre per hour (g/m²/h). All dogs were behaviorally acclimated to the use of the VapoMeter®, the researchers involved in
sample collection, and the collection room, prior to the first sample day to minimize stress, thereby reducing variation in measurements.

2.3.7 Skin and coat characteristics

Two researchers blinded to treatment were trained to perform a skin and coat assessment on weeks 0, 2, 4, 10, and 16 using a 5-point Likert scale. A Likert scale was used to measure softness, shedding, dander, shine, spring, softness uniformity, color, color uniformity, follicle density, skin color, and moisture. To increase consistency among dogs given different management practices in each household, all dogs were bathed two weeks prior to each assessment and owners were instructed to keep dogs dry and to not brush or groom them during this period. On data collection days, dogs were brushed by the same assessor immediately before completing the 5-point assessment. Brushing was done on the body of the dog by applying 10 brush strokes along the dorsal median line from the point of the scapula to the root of the tail and 10 brush strokes on each side of the body, by starting behind the front arm spanning over the thorax and flank. Additionally, the chest and neck were brush by applying 10 brush strokes on the chest, from the larynx to the point of the sternum, and 10 brush strokes on the left and right lateral sides of the neck from the point of chin to the beginning of the scapula.

2.3.8 Statistical analysis

Data are presented as mean ± SD unless otherwise stated. All statistical analyses were performed using the PROC GLIMMIX of SAS Studio® software (v.9.4., SAS Institute Inc., Cary, NC, USA). Dog was the experimental unit, and treatment, TEWL site, and sex, and age were treated as fixed effects. Week was treated as a repeated measure. An analysis of variance (ANOVA) was performed to assess the effects of treatment on inflammatory and oxidative
marker concentrations, TEWL, and skin and coat parameters. When the fixed effects were significant, the means were separated using Tukey–Kramer adjustments. Significance was declared at a $P \leq 0.05$. Trends were declared at $P \leq 0.10$.

2.4 Results

2.4.1 Inflammatory and oxidative markers

2.4.1.1 Prostaglandin E$_2$

There were no differences among treatments ($P=0.973$), across weeks ($P=0.397$), or for treatment by week interactions ($P=0.987$) (Table 2.4). Additionally, no differences were observed due to sex ($P=0.937$) or age ($P=0.274$).

2.4.1.2 Junction plakoglobin

There were no differences among treatments ($P=0.969$), across weeks ($P=0.249$), or for treatment by week interactions ($P=0.913$) (Table 2.4). No differences were observed due to sex ($P=0.914$) or age ($P=0.743$).

2.4.1.3 Glycosaminoglycan

There were no differences among treatments ($P=0.208$), across weeks ($P=0.995$), or for treatment by week interactions ($P=0.915$) (Table 2.4). Concentrations of GAG tended to be greater in males compared to females ($P=0.078$). There were no differences observed due to age ($P=0.329$).
2.4.1.4 Nitric oxide

There were no differences among treatments (P=0.648), across weeks (P=0.359), or for treatment by week interactions (P=0.729) (Table 2.4). No differences were observed due to sex (P=0.226) or age (P=0.424).
Table 2.4 Serum prostaglandin E2, junction plakoglobin, glycosaminoglycan, and nitric oxide concentrations of healthy adult dogs supplemented one of three treatment oils1 on weeks 0, 2, 4, 10, and 16 of a skin and coat health trial, presented as lsmeans.

<table>
<thead>
<tr>
<th>Prostaglandin E2 (pg/mL)</th>
<th>Week</th>
<th>P-values</th>
<th></th>
<th></th>
<th>Treatment*Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>CAM</td>
<td>0.88</td>
<td>2.77</td>
<td>3.49</td>
<td>2.35</td>
<td>2.32</td>
</tr>
<tr>
<td>OLA</td>
<td>3.07</td>
<td>3.07</td>
<td>2.41</td>
<td>2.82</td>
<td>2.80</td>
</tr>
<tr>
<td>FLX</td>
<td>2.55</td>
<td>4.07</td>
<td>3.07</td>
<td>3.44</td>
<td>3.15</td>
</tr>
<tr>
<td>Mean ± SEM (Week)</td>
<td>2.17 ± 0.89</td>
<td>3.30 ± 0.90</td>
<td>2.99 ± 0.92</td>
<td>2.87 ± 0.91</td>
<td>2.76 ± 0.92</td>
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<table>
<thead>
<tr>
<th>Junction Plakoglobin (ng/mL)</th>
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<tbody>
<tr>
<td>CAM</td>
<td>8.73</td>
<td>9.38</td>
<td>8.56</td>
<td>8.65</td>
<td>7.82</td>
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</tr>
<tr>
<td>OLA</td>
<td>10.09</td>
<td>9.60</td>
<td>9.51</td>
<td>9.96</td>
<td>7.39</td>
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<tr>
<td>FLX</td>
<td>8.94</td>
<td>10.97</td>
<td>10.78</td>
<td>9.34</td>
<td>8.35</td>
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<tr>
<td>Mean ± SEM (Week)</td>
<td>9.25 ± 0.66</td>
<td>9.98 ± 0.66</td>
<td>9.62 ± 0.67</td>
<td>9.32 ± 0.67</td>
<td>7.85 ± 0.69</td>
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<table>
<thead>
<tr>
<th>Glycosaminoglycan (ug/mL)</th>
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<tbody>
<tr>
<td>CAM</td>
<td>4.43</td>
<td>4.73</td>
<td>4.23</td>
<td>4.91</td>
<td>3.97</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>OLA</td>
<td>3.03</td>
<td>4.34</td>
<td>4.47</td>
<td>4.17</td>
<td>3.74</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>FLX</td>
<td>4.33</td>
<td>4.50</td>
<td>4.82</td>
<td>4.85</td>
<td>4.04</td>
<td></td>
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</tr>
<tr>
<td>Mean ± SEM (Week)</td>
<td>3.93 ± 0.44</td>
<td>4.52 ± 0.44</td>
<td>4.50 ± 0.44</td>
<td>4.65 ± 0.47</td>
<td>3.92 ± 0.46</td>
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<thead>
<tr>
<th>Nitric Oxide (uM/mL)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>CAM</td>
<td>2.20</td>
<td>9.30</td>
<td>4.82</td>
<td>8.34</td>
<td>10.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>OLA</td>
<td>4.31</td>
<td>7.19</td>
<td>5.85</td>
<td>9.26</td>
<td>10.15</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLX</td>
<td>11.70</td>
<td>12.76</td>
<td>19.56</td>
<td>13.74</td>
<td>16.34</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM (Week)</td>
<td>6.07 ± 3.41</td>
<td>9.75 ± 3.41</td>
<td>10.0 ± 3.46</td>
<td>10.4 ± 3.45</td>
<td>12.4 ± 3.48</td>
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</tr>
</tbody>
</table>

1Treatment oils: CAM, Camelina; OLA, Canola; FLX, Flaxseed oil
2.4.2 Transepidermal water loss

Of the 4440 individual TEWL measurements collected throughout the study period, 18 were considered outliers and removed (D=Dog, W=Week; Paw pad: D6W2, D8W16(2 values), D9W16, D17W4, D18W2, D18W4(2 values), D23W10, D23W16; Inner ear: D5W4, D5W10, D12W10; Inner leg: D6W2, D6W10, D12W0, D16W0, D29W0.)

There were no differences among treatments (P=0.726), across weeks (P=0.738), or for treatment by week interactions (P=0.996). Additionally, there were no differences for site by week (P=0.378), or sex (P=0.274) (Table 2.5). However, there were differences observed among sites (P<0.0001), in that TEWL values for the paw pad were greater than those of the pinna or inner thigh (Figure 2.1). Additionally, there was a trend observed in age (P=0.072), in that senior dogs (11-14 years; n=3) tended to have lower mean TEWL values compared to young (2-4 years; n=7), young adult (5-7 years; n=9), and adult dogs (8-10 years; n=9).
Table 2.5 Mean transepidermal water loss (TEWL) values (g/m²/h) of the right paw pad, right inner ear, and right inner thigh of healthy adult dogs supplemented one of three treatment oils\(^1\) on weeks 0, 2, 4, 10, and 16 of a skin and coat health trial.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Site</th>
<th>Week</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Trt</th>
<th>Site</th>
<th>Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAM</td>
<td>Paw Pad</td>
<td>0</td>
<td>92.57</td>
<td>98.97</td>
<td>88.28</td>
<td>83.98</td>
<td>92.70</td>
<td>&lt;0.0001</td>
<td>0.7261</td>
</tr>
<tr>
<td>OLA</td>
<td>Paw Pad</td>
<td>2</td>
<td>88.27</td>
<td>86.95</td>
<td>76.32</td>
<td>71.38</td>
<td>67.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLAX</td>
<td>Paw Pad</td>
<td>4</td>
<td>99.43</td>
<td>109.51</td>
<td>100.37</td>
<td>87.38</td>
<td>88.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SEM (Site*Week)</td>
<td></td>
<td>0</td>
<td>93.42±5.12</td>
<td>98.48±5.16</td>
<td>88.32±5.16</td>
<td>80.92±5.24</td>
<td>82.90±5.24</td>
<td>0.7261</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CAM</td>
<td>Pinna</td>
<td>10</td>
<td>14.03</td>
<td>12.27</td>
<td>18.78</td>
<td>14.47</td>
<td>16.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OLA</td>
<td>Pinna</td>
<td>4</td>
<td>14.43</td>
<td>15.84</td>
<td>16.87</td>
<td>24.43</td>
<td>18.40</td>
<td></td>
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</tr>
<tr>
<td>FLAX</td>
<td>Pinna</td>
<td>10</td>
<td>9.10</td>
<td>12.69</td>
<td>12.13</td>
<td>13.27</td>
<td>9.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SEM (Site*Week)</td>
<td></td>
<td>0</td>
<td>12.52±5.16</td>
<td>13.60±5.16</td>
<td>15.93±5.16</td>
<td>17.39±5.24</td>
<td>15.00±5.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAM</td>
<td>Inner Thigh</td>
<td>4</td>
<td>23.11</td>
<td>23.56</td>
<td>18.20</td>
<td>17.52</td>
<td>22.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OLA</td>
<td>Inner Thigh</td>
<td>10</td>
<td>16.86</td>
<td>15.72</td>
<td>18.18</td>
<td>17.32</td>
<td>21.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLAX</td>
<td>Inner Thigh</td>
<td>4</td>
<td>15.70</td>
<td>13.44</td>
<td>16.36</td>
<td>14.30</td>
<td>16.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SEM (Site*Week)</td>
<td></td>
<td>0</td>
<td>18.56±5.16</td>
<td>17.58±5.16</td>
<td>17.58±5.16</td>
<td>16.38±5.24</td>
<td>20.22±5.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Treatment oils: Camelina (CAM), Canola (OLA), Flaxseed oil (FLAX);
Figure 2.1 Mean pooled transepidermal water loss (g/m²/h) values of three different body sites of 30 healthy client-owned adult dogs supplemented with one of three treatment oils (Camelina, Canola, Flaxseed) during a skin and coat health trial over 16-weeks.

A, B Bars without a common letter differ significantly (P<0.05)
2.4.3 Skin and coat characteristics

2.4.3.1 Softness

There were no differences among treatments (P=0.539), for treatment by week interactions (P=0.757), or due to age (P=0.479), week by age (0.338) or week by sex (P=0.738) interactions. However, there were differences observed across weeks for pooled data (P=0.005) in that softness was greater on week 10 and 16 compared to week 0, and greater on week 10 compared to week 2. Week 4 was not different from any other time points (Figure 2.2). Additionally, softness was greater in females compared to males (P=0.026).

2.4.3.2 Shedding

There were no differences among treatments (P=0.882), due to age (0.894) or sex (P=0.760), or for treatment by week (P=0.444), week by age (P=0.302), or week by sex (P=0.514) interactions. For pooled data across weeks, shedding was greater on weeks 0 and 2 compared to weeks 10 and 16 (P=0.004). Week 4 was not different from any other time points (Figure 2.2).

2.4.3.3 Dander

There were no differences among treatments (P=0.648), due to age (P=0.114) or sex (P=0.349), across weeks (P=0.129), or for treatment by week (P=0.869), week by age (P=0.171), or week by sex (P=0.163) interactions (Figure 2.2).

2.4.3.4 Shine

There were no differences among treatments (P=0.815), due to age (P=0.945), or sex (P=0.191), or treatment by week (P=0.998), week by age (0.992), or week by sex (P=0.375)
interactions. However, there were differences across weeks for pooled data (P<0.0001) in that shine on weeks 2, 4, 10, and 16 was greater than at week 0 (Figure 2.2).

2.4.3.5 Spring

There were no differences among treatments (P=0.918), due to age (P=0.663) or sex (P=0.401), or for treatment by week (P=0.397), week by age (P=0.773), or week by sex (P=0.997) interactions. However, there were differences across weeks for pooled data (P=0.014) in that spring was greater on week 10 compared to week 4 and 0. There were no differences on weeks 2 and 16 (Figure 2.2).

2.4.3.6 Softness uniformity

There were no differences among treatments (P=0.969), due to age (P=0.860) or sex (P=0.132), or for treatment by week (P=0.799), week by age (P=0.996), or week by sex (P=0.142) interactions. However, a trend was observed across weeks for pooled data (P=0.065) in that softness uniformity tended to be greater on week 16 compared to week 0. Weeks 2, 4, and 10 were not different from any other time points (Figure 2.2).

2.4.3.7 Fur color

There were no differences among treatments (P=0.323), due to age (P=0.770) or sex (P=0.546), or for treatment by week (P=0.567), week by age (P=0.345), or week by sex (P=0.954) interactions. However, there were differences across weeks for pooled data (P<0.0001) in that color was higher on weeks 4, 10 and 16 compared to week 0. Additionally, color was greater on week 10 and 16 compared to week 2. Furthermore, color tended to be higher on week 10 compared to week 4 (Figure 2.2).
2.4.3.8 Fur color uniformity

There were no differences among treatments (P=0.541), due to age (P=0.893) or sex (P=0.911), across weeks (P=0.362), or for treatment by week (P=0.291), week by age (P=0.787), or week by sex (P=0.910) interactions (Figure 2.2).

2.4.3.9 Follicle density

There were no differences among treatments (P=0.873), due to age (P=0.795) or sex (P=0.854), or for treatment by week (P=0.670), week by age (P=0.846), or week by sex (P=0.299) interactions. However, there were differences across weeks for pooled data (P=0.027) in that follicle density was greater on week 16 compared to week 0. Weeks 2, 4, and 10 were not different from any other time points (Figure 2.2).

2.4.3.10 Skin color

There were no differences among treatments (P=0.751), due to age (P=0.134) or sex (P=0.317), across weeks (P=0.286), or for treatment by week (P=0.452), week by age (P=0.909), or week by sex (P=0.258) interactions (Figure 2.2).

2.4.3.11 Moisture

There were no differences among treatments (P=0.693), due to sex (P=0.285), or for treatment by week (P=0.810), week by age (P=0.613), or week by sex (P=0.714) interactions. However, there were differences across weeks for pooled data (P≤0.001) in that moisture was scored lower at weeks 10 and 16 compared to weeks 0, 2, and 4 (Figure 2.2). Additionally, moisture tended to be scored lower in senior (11-14 years; n=3) dogs compared to young (2-4 years; n=7), young adult (5-7 years; n=9), and adult dogs (8-10 years; n=11) (P=0.058).
Figure 2.2 Mean skin and coat health parameter scores completed using a 5-point Likert scale on 30 client-owned healthy adult dogs fed one of three treatment oils (Camelina oil, Canola oil, Flaxseed oil) and commercial kibble.

Bars without a common letter differ significantly (P<0.05)
2.5 Discussion

The purpose of this study was to assess the effects of camelina oil supplementation on skin and coat health compared to canola and flaxseed oil, two oils currently used to formulate canine diets. The results presented herein suggest no differences in TEWL, or the inflammatory and oxidative markers, or skin and coat health characteristics assessed due to treatment over the 16-week period.

2.5.1 Inflammatory and oxidative markers

In the current study, concentrations of GAG tended to be higher in males compared to females. Studies in humans by (1) Larking (1989) and (2) Claassen and Werner (2004) found that, similar to the present study, females have lower concentrations of GAG. Claassen and Werner analyzed GAG in thyroid cartilage while Larking measured GAG excretion in the tissue. Since GAG is a marker of cartilage turnover, Claassen and Werner attribute their findings to greater cartilage turnover in males, while Larking accredits their findings to the males in their study having a greater mean height [31, 32]. It is possible that the female dogs in the present experiment had a smaller average height and lower cartilage mineralization than the males, which contributed to the lower concentration of circulating GAGs observed. However, height and cartilage mineralization were not measured in the present study. Furthermore, the observation made in our study was only a tendency; this, combined with the dearth of work carried out in dogs and lack of equal distribution of male/female, intact/neutered/spayed dogs in the current study make it difficult to form any cogent conclusions. Future research should investigate this relationship further using a dog model.
No significant changes were observed in PGE$_2$, JUP, GAG, or NO concentrations over the 16-week study period. It is possible that the stability of these concentrations across time and among treatments is attributed to the lack of exercise or immune challenge experienced by the dogs on the current study. It is well established that both exercise and immune challenges result in a wide range of physiological and biochemical adaptations, the magnitude of which is directly related to the intensity and duration of the exercise or immune challenge encountered [33-36]. This wide range of physiological and biochemical adaptations include changes in inflammatory and oxidative biomarker concentrations [28, 33].

Dogs and horses both experience increased PGE$_2$ concentrations following exercise. In horses, NO and GAG concentrations increase following exercise and compared to baseline, but no change was observed in dogs [28, 33]. Pearson et. al (2020) attribute these results, similar to previous findings, to variations in NO production depending on exercise intensity, suggesting that it is possible that the lack of changes observed in NO concentration in the current study is due to the low intensity of the exercise experienced by the dogs [33]. Markers like PGE$_2$, NO, GAG, and JUP are often upregulated during times of immune challenge/disease [37-40]. A myriad of studies completed in humans suggest no effects of n-3 PUFA supplementation on inflammatory or immune markers in healthy individuals [41-43]. As an example, Pot et. al found that supplementing fish oil and sunflower oil to healthy individuals had no effect on chemokine, cytokine, or cell adhesion molecule concentration compared to baseline [41]. Healthy individuals, similar to the canine subjects of our study, generally have low levels of circulating inflammatory markers. Thus, the chance that low levels of inflammation are reduced even further by an intervention with oil is very small and difficult to measure. The dogs of the present study
were healthy upon recruitment and on every sample period based on a veterinary examination, as well as CBC and biochemistry analysis, indicating a lack of immune response that would elicit an inflammatory response. Additionally, the dogs did not participate in any intense exercise prior to or on sample days, and thus had no known reason to elicit any exercise stress induced response impacting markers of inflammatory or oxidative stress. For safety and animal care purposes, no procedures with the potential to cause harm to the animals, like an inflammatory or immune challenge, can be carried out in client-owned dogs. Additionally, the objective of the present study was to determine how these three oils compare to one another in terms of their effects on these biomarkers to gauge their use in dog food formulations for typical pets, not to evaluate their performance following an exercise or immune challenge. Future studies should compare the effects of these three oils and their performance following exercise and immune challenge.

2.5.2 Transepidermal water loss

In the present study, mean TEWL values were significantly greater when measured on the paw pad compared to the inner leg and inner ear. This is likely the result of the tubular, unbranched eccrine glands that open directly onto the skin of the paw pads and noses of canines. These glands allow sweat to be released from these areas, contributing to the water-loss detected by the VapoMeter, and thereby likely contributing to greater TEWL values compared to the inner leg and pinna [44]. Additionally, TEWL values were found to be lower in senior dogs compared to young, young adult, and adult dogs. Similar findings have been observed in other canine and human studies and although the exact mechanism behind these observations is unclear, there are various theories [45, 46]. The thickness of the stratum corneum and flattening
of corneocytes increases with age, while natural moisturizing factors, stratum corneum hydration, and epidermal lipid synthesis are reduced [47-53]. Additionally, the density of dermal capillaries decreases with age, which may lower skin temperature and in turn decrease water diffusion [51, 54]. All of these findings provide examples of mechanisms that increase the path length and resistance of a water molecule and subsequently contribute to lower TEWL in older individuals, and in agreement with the present study. These findings likely contribute to the decreased moisture observed in senior dogs compared to young, young adults, and adult dogs.

2.5.3 Skin and coat health characteristics

Spring and follicle density increased significantly from baseline. This is likely due, at least in part, to the growth of winter coats as the study began at the end of summer and went into the winter (September-January). Dogs have a light summer undercoat that is shed before a thick winter undercoat grows in, which could explain the increase in spring and follicle density. This further supports the observation of the present study in that shedding was greater in all dogs at the beginning of the study at weeks 0 and 2, compared to weeks 10 and 16. Skin moisture decreased from baseline, which authors also attribute to seasonality changes, as skin moisture is often reduced entering colder seasons. With a drop in dew point in the winter, the air becomes drier, causing water to evaporate from the skin surface. This rise in barometric pressure experienced in the winter forces water out of the epidermis, reducing the amount of moisture [55].

Softness, shine, and color of the dogs’ coats increased from baseline. This is likely a result of the dogs consuming an increased amount of n-3 FAs following baseline, which can be further metabolized into EPA and DHA, though with limited efficiency in comparison to fish oil.
Supplementation of fish oil, a rich source of EPA and DHA, was found to improve skin and hair coat quality in dogs from baseline based on a clinical score, with maximal improvement occurring after 8 weeks [56]. The positive effects on skin and coat health are thought to be due to an increase in EPA and DHA in the erythrocyte membrane, along with increased total lipids in the hair shaft [56]. The same study observed that following supplement withdrawal, skin and coat health clinical scores remained the same for one month and began to deteriorate following the second month [56]. Although we did not take measurements on week 8, we did take measurements on week 10, and this is where we saw the largest improvement (i.e., softness, shedding, shine, spring and color). This is most likely due to the increase in ALA, which is the parent compound of EPA and DHA, the dogs received from their treatment oil (CAM 1:1.8, FLX 1:4.19, OLA 1:0.59) in comparison to the wash-in sunflower oil (1:0).

All dogs in the current study were considered healthy, with no dermatological conditions or skin disorders. The coats of these dogs were already in relatively good condition at baseline, and future research should investigate these oil supplements and their effects on skin and coat health in dogs with poor skin and coat quality as a result of conditions like atopic dermatitis. Future studies should also compare the effects of different withdrawal times of these supplements.
2.6 Conclusion

In conclusion, camelina oil is comparable to canola and flaxseed oil in terms of its effects on skin barrier function, skin and coat health parameters, and inflammatory and oxidative markers measured in the current study when fed and observed for 16-weeks. Canola and flax are already commonly used in canine food formulations. Flaxseed oil specifically has the ability to support skin and coat health claims, making camelina oil a potential alternative plant-based oil source has superior economic and environmental sustainability, with high concentrations of ALA that could contribute to achieving the ideal n-6:n-3 ratio in canine diets, while supporting skin and coat health claims.

2.7 Acknowledgements

Authors would like to thank the undergraduate and graduate students who assisted with this project, along with all of the dogs and their owners for their commitment and cooperation during this study.
2.8 References


Chapter 3: Effects of dietary camelina, flaxseed, and canola oil supplementation on inflammatory and oxidative markers, transepidermal water loss, and skin and coat health parameters in healthy adult horses

3.1 Abstract

Camelina oil is derived from a low-input, high-yield crop and provides a greater amount of the n-3 ALA than the n-6 LA, in comparison to other dietary fat sources currently used to formulate equine diets. Despite this, no research exists assessing the effects of feeding camelina oil in contrast to commonly used oils. Thus, the objective of this study was to compare the effects of supplementing camelina oil vs. flaxseed and canola oil, on outcomes related to skin and coat health in horses. Thirty adult horses (23 mares, 7 geldings; 14.9 years ± 5.3 years; 544 ± 66 kg BW (Mean ± SD)) underwent a four-week wash in period consuming hay and sunflower oil. Following the wash in period, horses were blocked by location, age, sex, weight, and breed, and were assigned to one of three treatment oils: Camelina, Canola, Flaxseed oil. Feeding (0.37 g oil/kg BW) continued for 16 weeks. On weeks 10 and 12, KLH was administered intramuscularly on the left side of the neck to elicit an immune response. Fasted blood samples were collected to measure plasma prostaglandin E₂ (PGE₂), nitric oxide (NO), and glycosaminoglycan (GAG) concentrations on weeks 0, 14, and 16, using ELISA kits and spectrophotometric assays. On weeks 0, 2, 4, 8, and 16, transepidermal water loss (TEWL) was measured using a VapoMeter pre- and post-acetone application, and a 5-point-Likert scale was used to assess skin and coat characteristics. All data were analyzed with ANOVA using PROC GLIMMIX in SAS. Color and shine increased from baseline on the side of the horses, and plasma GAG was greater on week 0 compared to weeks 14 and 16. Pre- and post-acetone TEWL
values were greater on weeks 4 and 8 compared to weeks 0, 2, and 16. There were no differences
in the outcomes assessed between the horses supplemented camelina oil and those supplemented
canola or flaxseed oil, suggesting that camelina oil is comparable to existing plant-based oil
supplements, flaxseed, and canola, at supporting skin and coat health and inflammation in
horses.

3.2 Introduction

Dietary fat provides approximately 2.25 times more energy than an equal weight of
digested carbohydrates. Standard horse diets consisting of pasture or hay, or hay supplemented
with concentrates, provide low amounts of fat (2-4%). Oils are energy dense fat sources
commonly included in equine diets to increase energy content and subsequently reduce reliance
on grains to meet energy demands. Providing oil supplements in the diet has numerous
physiological benefits for the horse, including decreasing thermal load and enhancing metabolic
adaptations, both of which have the potential to improve athletic performance (Potter et al.,
1990). One of the most notable benefits of including dietary fat sources, like oils, is the provision
of essential fatty acids (EFAs) (O’Connor et al., 2007; Goh et al., 2004; Harris et al., 1999).

Horses have a dietary requirement for the cis-polyunsaturated 18-carbon (C18:2; n-6 and
C18:3; n-3) fatty acids (FAs), linoleic acid (LA) and α-linolenic acid (ALA). While these FAs
help maintain cell membrane integrity, neural and retinal development, EFAs also modulate
immunity and inflammation and support skin and coat health (Bazan et al., 2011; Goh et al.,
2004; Harris et al., 1999). The n-6 and n-3 FAs compete for the same Δ5- and Δ6-desaturase
enzymes required to convert LA and ALA into their respective long chain FAs (Hansen et al.,
Due to this competitive relationship, an excess of n-6 fatty acids in the diet has been shown to inhibit metabolism of n-3 fatty acids, which can disturb the homeostasis of the eicosanoids produced from both cascades (Sinnhuber 1969). The eicosanoids produced from the n-6 derived AA have a different effect than the eicosanoids produced from n-3 derived EPA and DHA. More AA results in pro-inflammatory effects, whereas more EPA and DHA result in resolvins, which are anti-inflammatory and pro-resolving. Therefore, enrichment of the diet with n-3 FAs may help to suppress inflammatory and allergic reactions, improve the blood lipid profile, and increase tissue sensitivity to insulin (Shahidi et al., 2018). Thus, a balanced n-6:n-3 ratio is required to allow sufficient conversion to longer chain FAs in both the n-3 and n-6 pathways and avoid disruption of homeostasis.

Oil supplements commonly used in equine diets have a higher concentration of n-6 FAs than n-3 FAs (canola, 10:5.9; corn, 10:0.1; soybean, 10:1.2; and sunflower oil, 10:0) (Sarker et al., 2013; AAFCO, 2020; Simopoulos and Robinson, 1999). In order to obtain a balanced ratio, ingredients rich in n-3 FAs are required. Flaxseed oil and fish oil have been used to increase n-3 inclusion; however, long-term fish oil production is not environmentally sustainable to continue to meet the demands of the growing population, and fish oil is not always palatable to horses. Furthermore, the high levels of ALA in flaxseed oil make its shelf-life stability poor, and flaxseed crops are vulnerable to different climates, seasons, and pests (Sarker et al., 2013; Muir et al., 2003). As a result, there is room in the market for an alternative oil supplement that is environmentally and economically sustainable and can provide a rich source of n-3 FAs.

*Camelina sativa* is a low-input, high-yield crop, with a short growing season and resistance to various seasons, climates, and soil types (Moser 2010; Berti et al., 2016; Putnam et
The derivative of this crop, camelina oil, has a desirable n6:n3 ratio of 1:1.8, making it a rich source of n-3 FAs (Zubr and Matthäus, 2002). This oil ingredient contains naturally high levels of tocopherols and polyphenols, which have been associated with improved skin and coat health. Furthermore, camelina oil has slightly lower levels of n-3 FAs than flaxseed oil, making its shelf-life better by comparison (Eudhin et al., 2003).

Supplementing oil in equine diets is often associated with claims of improved skin and coat health, but currently there is no data directly comparing the effects of camelina oil supplementation to the effects of other oils being used in equine diet formulations on skin and coat health and inflammation. Therefore, the objective of this study was to compare the effects of dietary camelina oil supplementation to those of flaxseed oil and canola oil supplementation on skin and coat health and inflammatory and oxidative markers in healthy adult horses. Outcomes include changes in oxidative and inflammatory biomarkers, and skin and coat health parameters. Additionally, skin barrier function and integrity were assessed by measuring TEWL. We hypothesize that camelina oil is comparable, if not superior to, flaxseed and canola oil in terms of its effects on oxidative and inflammatory markers, skin and coat health parameters, and TEWL.

### 3.3 Materials and methods

#### 3.3.1 Animals and housing

This experiment was approved by the University of Guelph’s Animal Care Committee (Animal Use Protocol #4481) and was carried out in accordance with national and institutional guidelines for the care and use of animals. Thirty adult horses (23 mares, 7 geldings; 14.9 years ± 5.3 years; 544 ± 66 kg BW (Mean ± SD)) were enrolled in this study based on the following inclusion criteria: (1) Horses were deemed clinically healthy upon assessment, (2) Horses were
not receiving anti-inflammatory medications, and (3) Horses had no abnormalities in routine blood biochemistry and hematology. Three herds of horses from three separate locations were used to complete this study: The Arkell Research Station Herd (Arkell, Ontario; 19 mares, 4 geldings), CJ Equestrian Centre (Rockwood, Ontario; 3 mares, 3 geldings), and the Equine Sports Medicine and Reproduction Centre (ESMRC) (University of Guelph, Guelph, Ontario; 3 mares). All horses were housed outdoors, with access to shelters, in mixed herd of up to 9 horses per pasture or paddock. Due to availability of horses, study start dates were staggered for each location, however, all treatments were equally dispersed across all locations and start dates. The study periods for the Arkell Research, CJ Equestrian, and ESRMC Herds, were June to December, July to December, and August to January, respectively.

3.3.2 Dietary treatments

Horses housed at all locations had *ad libitum* access to hay, although there were minor variations in management techniques between these locations that must be disclosed. From June to the beginning of October, the horses at the Arkell Research Station were on pasture; thereafter, hay was made available to them to supplement pasture until mid-November, when horses were re-located to winter housing and provided *ad libitum* access to hay for the remainder of the study period. The EMSRC horses were provided *ad libitum* access to hay, along with pasture, while the horses at CJ Equestrian had sporadic access to electrolytes, in addition to pasture and *ad libitum* hay with 1-inch slow feed netting. Neither EMSRC nor CJ Equestrian horses were moved to winter housing during the study period.
3.3.3 Study design

This study was completed using a randomized complete block design (RCBD) where the oil inclusion rate was gradually increased from 0.05g oil/kg BW to the final inclusion level of 0.37g oil/kg BW over a 4-week wash-in period using sunflower oil (Table 1). Following the wash-in period, horses were blocked by location, age, sex, weight, and breed, and randomly assigned to one of three treatment oils: flaxseed oil (FLX), camelina oil (CAM), or canola oil (OLA). During the entire study period (4-week wash-in, 16-week treatment), oil was mixed with soaked hay cubes (Premium TimothyTM Hay Cubes, Ontario Dehy, Goderich, ON, Canada) to form a mash, and fed once or twice daily, depending on the location and start date. During the 16-week treatment period, oil was provided at the final inclusion level of 0.37g oil/kg BW reached during the wash-in period.
Table 3.1 Fatty acid profile of camelina oil, canola oil, flaxseed oil, and sunflower oil.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sunflower ¹</th>
<th>Canola ²</th>
<th>Flax ²</th>
<th>Camelina ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated Fatty Acids (%)</td>
<td>9.61</td>
<td>6.50</td>
<td>8.20</td>
<td>9.50</td>
</tr>
<tr>
<td>Monounsaturated Fatty Acids (%)</td>
<td>14.1</td>
<td>63.8</td>
<td>16.6</td>
<td>35.2</td>
</tr>
<tr>
<td>Polyunsaturated Fatty Acids (%)</td>
<td>76.3</td>
<td>29.7</td>
<td>75.2</td>
<td>55.3</td>
</tr>
<tr>
<td>Omega 6 (%)</td>
<td>76.2</td>
<td>18.6</td>
<td>16.5</td>
<td>19.8</td>
</tr>
<tr>
<td>Omega 3 (%)</td>
<td>0.04</td>
<td>11.1</td>
<td>58.6</td>
<td>35.4</td>
</tr>
<tr>
<td>Trans fat (%)</td>
<td>N/A²</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Total Fat (%)</td>
<td>N/A²</td>
<td>99.9</td>
<td>100</td>
<td>99.9</td>
</tr>
</tbody>
</table>

¹ Numerical values are adapted from Kostik et al. (2013) and only represent generic sunflower oil and not the specific brand used for this study (Kostik et al., 2013).
² Samples run in duplicate by SGS Canada Inc., average values reported.
³ Abbreviation: N/A, Not Available.
Burron et al., (2020)
3.3.4 Blood collection

Blood collection was performed via jugular venipuncture using a 20- or 21-gauge x 1 ½ inch VACUETTE™ Multi-Drawing Blood Collection Needle (Greiner Bio-One, Kremsmünster, Upper Austria, Austria) and a Multi-Sample Needle Holder (Globe Scientific, Mahwah, NJ, USA). Approximately 5 mL of blood was collected into a sodium heparin plasma vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and gently inverted 8-10 times to ensure proper mixing of blood with the anticoagulant. Plasma 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 frozen at -80°C until later analysis of inflammatory and oxidative markers.

3.3.5 Inflammatory and oxidative markers

Horses were injected intramuscularly with 500ug of keyhole limpet hemocyanin vaccine and 1g of Quil-A adjuvant (mixed into syringe as cocktail) on weeks 10 and 12 to elicit an immune response. Plasma samples from weeks 0, 14, and 16 were analyzed for prostaglandin E₂ (PGE₂) (DetectX Prostaglandin E2 Enzyme Immunoassay; Arbor Assays, Ann Arbor, MI) using a commercially available ELISA (Enzyme-linked immunosorbent assay) kit. Samples were run in duplicate according to the manufacturer’s instructions. Plasma glycosaminoglycan (GAG) (dimethyl methylene blue) and nitric oxide (NO) (Griess Reaction; Molecular Probes, Eugene, OR) concentrations were determined using spectrophotometric assays. Serum NO and GAG samples were analyzed as previously described by MacNicol et al. (2018) et al., also on weeks 0, 14, and 16. Plasma samples for GAG analysis were diluted 1:10.
3.3.6 Skin barrier function

Transepidermal water loss (TEWL) is commonly used to assess skin barrier function and integrity and is defined as the amount of water that passively evaporates through the skin into the external environment as a result of a water vapor pressure gradient on both sides of the skin barrier. All horses were acclimated to use of the VapoMeter®️, the researchers involved in sample collection, and the collection room, prior to the first sample day to minimize stress, thereby reducing variation in values measured. Subsequently, on weeks 0, 2, 4, 8, and 16, TEWL was measured using a VapoMeter®️ SWL-3 (Delfin Technologies Ltd, Kuopio, Finland). All body sites were shaved 24 hours prior to measurements being taken. Measurements were taken according to the manufacturer’s instructions, on the chest, inner arm, rump, and withers of the horses, before and after application of acetone, to assess barrier disruption (10 pre-acetone, 10 post-acetone, per body site). Ten measurements were taken per body site and the average was used for analyses. Once the averages were calculated, any values above or below the average by 50 g/m²/h or more were considered outliers and removed. All measurements were carried out by a single operator, in the same order of body sites. The horses at the Arkell research station had TEWL measurements taken in an enclosed room, with two dehumidifiers, to allow more control over temperature and humidity fluctuations. The two other barns did not have access to an enclosed room for TEWL measurements, therefore, researchers created a vapor barrier using transparent poly drop sheets and tuck tape to seal around a horse stall and continued to use two dehumidifiers to reduce temperature and humidity fluctuations. The evaporation rate value is calculated in grams of water per square metre per hour (g/m²/h).
3.3.7 **Skin and coat characteristics**

Two researchers blinded to treatment were trained to perform a skin and coat assessment on weeks 0, 2, 4, 8, and 16 using a 5-point Likert scale. A Likert scale was used to measure shine, softness, hair quality, color intensity, and moisture of the skin and coat on the rump and side. To increase consistency among horses, all horses were brushed 10 strokes on the rump and side, using the same brush immediately prior to each assessment.

3.3.8 **Statistical analysis**

Data are presented as mean ± SE unless otherwise indicated. All statistical analyses were performed using the PROC GLIMMIX of SAS Studio® software (v.9.4., SAS Institute Inc., Cary, NC, USA). Horse was treated as the experimental unit, treatment oils and TEWL site were fixed effects, and week was a repeated measure. An analysis of variance (ANOVA) was performed to assess effects of treatment on TEWL, inflammatory and oxidative marker concentrations, and skin and coat health parameters. Least-square means were used to assess differences in means of treatment, week, TEWL site, pre- and post-acetone values, and treatment by week interactions. When fixed effects were significant, means were separated using Tukey-Kramer Adjustments. Significance was declared at a P ≤ 0.05.

3.4 **Results**

3.4.1 **Inflammatory and oxidative markers**

3.4.1.1 **Prostaglandin E₂**

No differences were observed in PGE₂ concentrations among treatments (P=0.4385), between weeks (P=0.1468), or for treatment by week interactions (P=0.9357).
3.4.1.2 Glycosaminoglycan

No differences were observed in GAG concentrations among treatments (P=0.3758), or for treatment by week interactions (P=0.5981). However, there were differences between weeks (P=0.0326) in that GAG on week 0 was greater than on weeks 14 and 16.

3.4.1.3 Nitric Oxide

No differences were observed in NO concentrations among treatments (P=0.2077), between weeks (P=0.7704), or for treatment by week interactions (P=0.3540).
Table 3.2 Plasma prostaglandin E₂, glycosaminoglycan, and nitric oxide concentrations of healthy adult horses supplemented one of three treatment oils¹ on weeks 0, 14*, and 16 of a skin and coat health trial, presented as lsmeans.

<table>
<thead>
<tr>
<th>Prostaglandin E₂ (pg/mL)</th>
<th>0</th>
<th>14*</th>
<th>16</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAM</td>
<td>23.8008</td>
<td>26.1218</td>
<td>29.0855</td>
<td>Trt: 0.4385, Week: 0.1468, Trt*Week: 0.9357</td>
</tr>
<tr>
<td>OLA</td>
<td>19.7653</td>
<td>20.716</td>
<td>22.6017</td>
<td></td>
</tr>
<tr>
<td>FLX</td>
<td>20.7818</td>
<td>25.5461</td>
<td>24.6336</td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM (Week)</td>
<td>21.45±1.98</td>
<td>24.12±2.24</td>
<td>25.44±1.98</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Glycosaminoglycan (µg/mL)</th>
<th>0</th>
<th>14*</th>
<th>16</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAM</td>
<td>6.7311</td>
<td>6.2128</td>
<td>4.6241</td>
<td>Trt: 0.3758, Week: 0.0326, Trt*Week: 0.5981</td>
</tr>
<tr>
<td>OLA</td>
<td>6.4100</td>
<td>5.5372</td>
<td>5.8086</td>
<td></td>
</tr>
<tr>
<td>FLX</td>
<td>5.8702</td>
<td>5.2465</td>
<td>4.6477</td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM (Week)</td>
<td>6.34±0.57</td>
<td>5.67±0.65</td>
<td>5.03±4.62</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nitric Oxide (µM/mL)</th>
<th>0</th>
<th>14*</th>
<th>16</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAM</td>
<td>11.6170</td>
<td>11.8281</td>
<td>14.9596</td>
<td>Trt: 0.2077, Week: 0.7704, Trt*Week: 0.3540</td>
</tr>
<tr>
<td>OLA</td>
<td>12.1291</td>
<td>15.0128</td>
<td>14.4382</td>
<td></td>
</tr>
<tr>
<td>FLX</td>
<td>26.1511</td>
<td>16.3029</td>
<td>14.2344</td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM (Week)</td>
<td>16.63±2.36</td>
<td>14.38±2.66</td>
<td>14.54±2.46</td>
<td></td>
</tr>
</tbody>
</table>

¹Treatment oils: CAM, Camelina; OLA, Canola; FLX, Flaxseed oil.
*Prior to week 14 sample collection, on weeks 10 and 12, horses were administered 500µg of keyhole limpet hemocyanin (KLH) and 1g of Quil-A adjuvant intramuscularly on the left side of the neck to elicit an immune response.
3.4.2 Transepidermal water loss

Of the 10,360 TEWL measurements collected throughout the study period, 11 were considered outliers and removed (H=Horse, W=Week; Withers: H114W0(post), H118W4(post), H118W8(post) (2 values); Inner arm: H118W8(post) (3 values), H120W4(post); Chest: H120W8(post) (2 values); Rump: H130W0(post)). Two horses were excluded from TEWL measurements as they did not acclimate to the enclosed room and any stress alters respiration and TEWL. This left 28 horses in total for TEWL analysis.

There were no differences among treatments (P=0.5828, 0.9132), for treatment by week interactions (P=0.6421, 0.0666), or for treatment by site (P=0.2545, 0.3793) interactions for pre- or post-acetone TEWL values, respectively. However, TEWL values were greater on weeks 4 and 8 compared to weeks 0, 2, and 16 (P<0.0001). Additionally, pre-acetone TEWL values were higher on the inner arm compared to the withers, rump, and chest (P<0.0001).

Post-acetone values were higher on the inner arm than the withers and chest (P=0.0231). Additionally, there were differences across weeks in that post-acetone TEWL values were greater on weeks 4 and 8 compared to weeks 0, 2, and 16, and TEWL values on week 16 were greater than on weeks 0 and 2 (P<0.0001). Furthermore, post-acetone TEWL values were significantly greater than pre-acetone TEWL values (P<0.0001).
Table 3.3 Mean transepidermal water loss (TEWL) values (g/m²/h) of the chest, withers, rump, and inner arm of 28 healthy adult horses supplemented one of three treatment oils¹ on weeks 0, 2, 4, 8, and 16 of a skin and coat health trial.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Site</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAM</td>
<td>Inner</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>OLA</td>
<td>Inner</td>
<td>18.47</td>
<td>30.77</td>
<td>19.15</td>
<td>23.79</td>
<td>41.15</td>
<td>40.57</td>
</tr>
<tr>
<td>FLX</td>
<td>Inner</td>
<td>17.30</td>
<td>23.08</td>
<td>14.96</td>
<td>21.47</td>
<td>39.95</td>
<td>47.15</td>
</tr>
<tr>
<td>Mean SEM</td>
<td>(Week)</td>
<td>19.4±2.56</td>
<td>26.4±2.91</td>
<td>17.8±2.91</td>
<td>21.8±2.91</td>
<td>36.8±2.56</td>
<td>43.8±2.91</td>
</tr>
<tr>
<td>CAM</td>
<td>Chest</td>
<td>12.58</td>
<td>18.23</td>
<td>13.34</td>
<td>20.17</td>
<td>31.04</td>
<td>38.91</td>
</tr>
<tr>
<td>OLA</td>
<td>Chest</td>
<td>12.68</td>
<td>22.59</td>
<td>13.46</td>
<td>19.86</td>
<td>24.11</td>
<td>35.58</td>
</tr>
<tr>
<td>FLX</td>
<td>Chest</td>
<td>12.44</td>
<td>15.56</td>
<td>11.68</td>
<td>19.93</td>
<td>23.37</td>
<td>47.31</td>
</tr>
<tr>
<td>Mean SEM</td>
<td>(Week)</td>
<td>12.6±2.56</td>
<td>18.8±2.91</td>
<td>12.8±2.56</td>
<td>19.9±2.91</td>
<td>26.2±2.56</td>
<td>40.6±2.91</td>
</tr>
<tr>
<td>CAM</td>
<td>Withers</td>
<td>16.09</td>
<td>24.50</td>
<td>18.57</td>
<td>23.63</td>
<td>31.04</td>
<td>41.65</td>
</tr>
<tr>
<td>OLA</td>
<td>Withers</td>
<td>13.59</td>
<td>26.73</td>
<td>19.83</td>
<td>23.99</td>
<td>25.47</td>
<td>37.09</td>
</tr>
<tr>
<td>FLX</td>
<td>Withers</td>
<td>13.59</td>
<td>24.05</td>
<td>13.84</td>
<td>24.38</td>
<td>22.69</td>
<td>42.04</td>
</tr>
<tr>
<td>Mean SEM</td>
<td>(Week)</td>
<td>14.4±2.56</td>
<td>25.1±2.91</td>
<td>17.4±2.56</td>
<td>24.0±2.91</td>
<td>26.4±2.56</td>
<td>40.3±2.91</td>
</tr>
<tr>
<td>CAM</td>
<td>Rump</td>
<td>15.88</td>
<td>25.25</td>
<td>12.83</td>
<td>24.68</td>
<td>23.09</td>
<td>38.03</td>
</tr>
<tr>
<td>OLA</td>
<td>Rump</td>
<td>14.56</td>
<td>27.34</td>
<td>12.97</td>
<td>26.63</td>
<td>18.54</td>
<td>36.74</td>
</tr>
<tr>
<td>FLX</td>
<td>Rump</td>
<td>11.83</td>
<td>25.17</td>
<td>11.22</td>
<td>23.82</td>
<td>22.36</td>
<td>45.15</td>
</tr>
<tr>
<td>Mean SEM</td>
<td>(Week)</td>
<td>14.1±2.56</td>
<td>25.9±2.91</td>
<td>12.3±2.56</td>
<td>25.0±2.91</td>
<td>21.3±2.56</td>
<td>39.9±2.91</td>
</tr>
</tbody>
</table>

¹Treatment oils: CAM, Camelina; OLA, Canola; FLX, Flaxseed oil.
3.4.3 Skin and coat health characteristics

3.4.3.1 Shine

There were no differences among treatments (\(P=0.1215\)), across weeks (\(P=0.1268\)) or for treatment by week interactions (\(P=0.7155\)) for shine (rump). Additionally, there was no effect of treatment (\(P=0.4949\)) or treatment by week interaction (\(0.5623\)) on shine (side). Shine (side) was greater (\(P=0.0132\)) on week 16 compared to weeks 0 and 8 and all were similar to weeks 2 and 4.

3.4.3.2 Softness

No differences were observed among treatments (\(P=0.3221\)), across weeks (\(P=0.4576\)) or for treatment by week interactions (\(P=0.1267\)) for softness (side). Additionally, no effect of treatment (\(P=0.3806\)) or treatment by week interaction (\(P=0.5046\)) was observed in softness (rump); however, softness(rump) was greater (\(P=0.0135\)) on weeks 0 and 16 compared to week 2. No differences were observed on weeks 4 and 8.

3.4.3.3 Hair

There were no differences among treatments (\(P=0.4865\)), or for treatment by week interactions (\(P=0.4478\)) in hair(side). However, hair(side) quality was lower on weeks 0 and 2 compared to weeks 4 and 8, and greater on weeks 4 and 8 compared to week 16 (\(P=0.0006\)). No differences among treatments (\(P=0.5709\)), between weeks (\(P=0.2979\)), or for treatment by week interactions (\(P=0.0922\)) were observed for hair(rump).

3.4.3.4 Colour

Colour (rump) was greater in the FLX treatment group compared to the OLA treatment group (\(P=0.0436\)). Additionally, colour(rump) was greater on week 16 compared to weeks 2 and
4 (P=0.0155). No differences occurred on weeks 0 and 8. Colour(rump) tended to be greater in the camelina group on week 8 and the flaxseed group on week 0 compared to the canola group on week 2 (P=0.0811). Colour(side) values tended (P=0.0963) to be greater in the FLX group compared to the OLA group. Furthermore, colour(side) was greater on week 16 compared to weeks 0, 2, and 4 (P=0.0009). No changes were observed on week 8. No treatment by week interactions were observed (P=0.4278).

3.4.3.5 Moisture

No differences were observed among treatments (P=0.4005), across weeks (P=0.9958) or for treatment by week interactions (P=0.2674) for moisture(side). Additionally, no differences due to treatment (P=0.9079) or treatment by week interaction (P=0.4535) were observed in moisture (rump). However, moisture(rump) was greater on week 0 compared to week 2, and greater on week 16 compared to week 2 (P=0.0376). No differences occurred on weeks 4 and 8.
Figure 3.1 Mean skin and coat health parameter scores of the side (S) and rump (R) completed using a 5-point Likert scale on 30 adult horses fed one of three treatment oils (Camelina oil, Canola oil, Flaxseed oil) and soaked hay cubes.

A, B Bars without a common letter differ significantly (P<0.05)
3.5 Discussion

3.5.1 Inflammatory and oxidative markers

The results presented herein indicate plasma GAG concentrations were greater on week 0 compared to weeks 14 and 16, which may be a result of the amount of exercise performed by the horses on weeks 14 and 16 relative to week 0. Additionally, no changes were observed in PGE$_2$ or NO concentrations following KLH injection, in opposition to our hypothesis, in which injecting KLH would elicit an inflammatory response, thereby increasing PGE$_2$ and NO concentrations. This opposition may be the result of the lack of difference between the omega 6:3 ratios of our treatment oil, along with the type of oil provided (plant vs. marine).

Glycosaminoglycan is a marker of cartilage turnover that increases due to exercise in horses (MacNicol et al., 2018). On week 0, all horses were housed outdoors with free access to pasture. Mid-way through the study period, moving into the winter season, 13 of the 30 horses were moved off pasture to a winter barn as a result of the cold weather. It is possible that the horses on this study were exercising less due to the change in location and weather, resulting in lower circulating GAG by week 14 and 16 compared to baseline, when they were on pasture, in the summer season.

Studies carried out by Hall and O’Hara et al. compared fish oil and corn oil supplementation in horses and attribute their observed differences in PGE$_2$, LTB$_4$, and LTB$_5$ concentrations to the n6:n3 ratios of the treatment oils and the associated long-chain FAs produced (Hall et al., 2001; O’Hara et al., 2001). Hall et al found that PGE$_2$, a metabolite produced within the n-6 pathway, significantly increased from baseline in horses fed corn oil but
not in those fed fish oil. Additionally, O’Hara et al., observed that concentrations of LTB₅ and LTB₄, along with the ratio of LTB₅:LTB₄ was greater in the horses supplemented fish oil compared to those supplemented corn oil. In both studies, authors found a correlation between these findings and the ratio of EPA:AA in the treatment oils. Hall et al., found a negative correlation between EPA:AA and PGE₂, in that the higher the EPA:AA ratio, the less PGE₂ produced. O’Hara et al., found the higher EPA:AA, the more LTB₄ and LTB₅ produced and the higher the LTB₅:LTB₄ ratio (O’Hara et al., 2001; Hall et al., 2001). Overall, it is suggested that the ratio of n-6:n-3 FAs in the treatment oils can contribute to an increase or decrease in these marker concentrations through the production of their respective eicosanoids. This can be further supported by a study carried out by Wander et al., who fed dogs diets with n-6:n-3 FA ratios of 31:1, 5.4:1, and 1.4:1. These researchers observed increased PGE₂ concentrations in the 31:1 group compared to the 1.4:1 group. However, no differences were observed in PGE₂ concentrations in the 5.4:1 and 1.4:1 diets (Wander et al., 1997).

In summary, differences in inflammatory marker concentrations were observed as a result of treatment in studies comparing fish oil, an n-3 supplement, to corn oil, an n-6 supplement. Wander and colleagues observed difference between the diets with n-6:n-3 ratios of 31:1 and 1.4:1. It is important to note that the 31:1 and 5.4:1 diets were supplemented with fish oil, whereas the 1.4:1 diet was supplemented with corn oil. Therefore, all three studies observed differences in inflammatory markers between animals fed fish oil (a marine source) and corn oil (a plant source) supplements.

The stability of inflammatory marker concentrations observed in our study could be attributed to the fact that we are comparing all plant oils, rather than plant oils and marine oils.
Marine oils have a greater conversion efficiency of n-3 ALA and n-6 LA into their respective long chain derivatives, EPA, DHA, and AA, which are all parent compounds for inflammatory eicosanoids, including PGE\textsubscript{2}, that would induce an inflammatory state and subsequently increase NO concentrations (Hall et al., 2004; Dupont et al., 1990). It is possible that if our study employed a group of horses supplemented fish oil, or a control group fed no oil, differences in inflammatory markers may have been observed in the plant oil vs. marine oil vs. control groups due the differences in their n6:n3 ratios and the efficiency with which they can be further metabolized into their longer chain derivatives. However, this is outside of the scope of the present study, which was to compare camelina oil to existing plant oil alternatives, like canola and flaxseed oil. In terms of the inflammatory markers assessed in this study, camelina oil was comparable to flaxseed and canola, which are currently used in equine diet formulations.

### 3.5.2 Transepidermal water loss

There is an abundant amount of literature addressing the direct impact that temperature, environment, season, and climate has on TEWL in humans and mice (Grubauer et al., 1989; Grice et al., 1971; Green et al., 2022). An increase in skin temperature by 7-8°C doubles TEWL in human skin, and individuals exposed to an environment with more particulate matter or NO\textsubscript{2} had greater TEWL (Grice et al., 1971; Green et al., 2022). Conflicting results have been found on the effects of season and climate, with no consensus reached (Firooz et al., 2016; Song et al., 2015; Kim et al., 2019; Wan et al., 2014; Yang et al., 2020; Doleckova et al., 2021). Although the researchers of the present study attempted to control fluctuations in temperature on sample days, due to changes in seasonality and lack of an enclosed space, fluctuations still occurred (31.3°C in July, 10°C in December). Additionally, outside of sample days researchers had no
control over temperature. The horses were housed at different locations, in different environments, in changing seasons. Furthermore, horses started the study at different times (some horses baseline samples occurred in July, others in September) and some had access to a winter barn mid-way through while others did not. Similar to the skin and coat assessment data, these differences and variability in the management are practical and real but make it difficult to determine the actual effect of these oils over time with the current sample size. However, the goal of the present study was not to determine changes due to oil supplementation overtime, it was to assess the effects of camelina oil supplementation versus canola and flaxseed oil supplementation in terms of the skin and coat health outcomes measured. Camelina oil is comparable to flaxseed and canola oil in terms of its impact on TEWL.

As expected, TEWL following barrier disruption via acetone application was greater than pre-acetone values. This is a common method of barrier disruption, however, to the authors knowledge, this is the first study to use this method in horses. The differences between pre- and post-acetone values, representing barrier recovery as a marker of integrity, was greater on weeks 4, 8, and 16 compared to weeks 0 and 2, in all horses. This could be due to a combination of the colder weather, and the multiple applications of acetone over the 16-week period, however, similar to above, it becomes difficult to confidently make any conclusions based on changes due to week with all of the inconsistencies at play. However, there were no differences due to treatment, suggesting that camelina oil is comparable to flaxseed and canola oil in terms of its effects on barrier integrity. Most horses are housed outdoors, and in very few situations would they be housed in a controlled environment. Therefore, it is important to be able to confidently unravel data of this manner, and as such, future studies should investigate the effects of
environment, season, climate, and temperature on TEWL in horses to make informed interpretations in future work.

3.5.3 Skin and coat health assessment

To the authors' knowledge, there are no studies to date that have investigated the effects of oil supplementation on skin and coat health characteristics in horses using a scoring system. Color intensity increased from baseline over the 16-week treatment period on both the rump and side of all horses and was observed to be greater in the horses provided flaxseed oil compared to the horses provided canola oil, while camelina oil was intermediate. In a study carried out by Combarros and colleagues, dogs supplemented fish oil exhibited improved skin and coat characteristics, including reduced coat dullness, with maximal improvements occurring at week 8. They attribute these findings to the increased EPA and DHA provided by the fish oil supplement (Combarros et al., 2020). It is possible that the greater colour intensity observed in the horses provided flaxseed oil in our study is in part due to the higher content of ALA, which can be converted to EPA and DHA, compared to canola. This could be why the horses consuming camelina oil were not different from either flaxseed or canola oil, as the n-3 content of camelina oil is intermediate to canola and flaxseed oil. However, it is important to note that the dogs on the Combarros et al. study were considered to have poor quality coats at baseline, and the horses in our study were provided a source of ALA, which is not equally as efficient at providing EPA and DHA as fish oil (Swanson et al., 2012). Overall, these results do support the fact that oil addition to a diet improves skin and coat colour intensity as is commonly believed in the equine industry. Similarly, shine(side) increased from baseline in all treatments, likely due to the increase in n-3 FAs compared to the wash-in sunflower oil. Due to availability of horses, this
study was carried out in a staggered design in that study start and end dates ranged from July-January. Additionally, these horses were housed at three different locations, with one location providing horses access to a winter barn in November. It is well accepted that environment impacts skin and coat quality, and because we enrolled horses from three sites and had a rolling enrollment process, these management differences and seasonality changes may have created variability in the data, making it difficult to form any solid conclusions regarding the actual effect of oil supplementation over time (Parrado et al., 2019; Burke et al., 2019; English et al., 2003). However, the aim of this study was to determine if there were any differences between how camelina oil vs. canola and flaxseed oil impact skin and coat health. Since there were no differences between camelina oil supplementation and canola and flaxseed supplementation, this suggests that camelina oil is comparable to flaxseed and canola oil in terms of its effects on skin and coat health characteristics. Similar to the TEWL data, there is merit is further investigating the effects of season, climate, temperature, and environment on skin and coat health in horses, in order to better interpret similar data in the future, as it accurately reflects how most horses are normally kept year-round.

3.6 Conclusion

Additional work should investigate the effects of season, climate, environment, and temperature on these outcomes in horses in order to informatively analyze data of this kind that reflects the natural habitat of most horses year-round. Furthermore, the horses in this study were deemed healthy upon recruitment; future research should explore the impact of supplementing these oils in horses with conditions like atopic dermatitis. There is merit in conducting dose response studies as well, due to the horses limited ability to consume fat. In conclusion, despite
the changes in season, temperature, and horse management, no treatment differences were observed between camelina oil, and canola and flaxseed oil supplementation, in terms of their effects on TEWL, skin and coat characteristics, and the inflammatory and oxidative markers measured in this study. Thus, camelina oil is comparable to these oils commonly used in equine diets when it comes to their effects on the measured outcomes and should be considered as a potential alternative oil source to increase n3 FAs in equine diet formulations.
3.7 References

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Chapter 4: General discussion

The world population has tripled in less than a century, from approximately 2.5 billion people in 1950 to an estimated 8 billion by the end of 2022 (UNDESA, 2022). As a result of this immense population growth, food demand has increased, causing a subsequent decrease in food security. This is further compounded by the simultaneous growth of companion animal and livestock populations, as well as the associated overlap in the human and animal food chains (Swanson et al., 2013). The pet population, specifically, has tripled since the 1970’s, to approximately 157 million, with over 50% of US households owning a cat or dog (WWF, 2016; GfK, 2016). The amount of money spent by consumers annually on their pets has also increased considerably, from $17 billion in 1994 to $58 billion in 2014; the majority of this money being spent on pet food (American Pet Products Association, 2019; Statista, 2019). In addition, there is a growing trend among pet owners to anthropomorphize their animals, which leads them to desire pet foods made with high-quality or “human-grade” components. This phenomenon has also built the foundation for human health food trends to bleed into the pet food industry (i.e. vegan, vegetarian, grain-free diets, and supplements). The equine industry is also seeing a rise in the need for supplements, whether that be for health, sporting, or performance purposes (reviewed in Swanson et al., 2013; Murray et al., 2018). There is competition for resources in order to meet the demands of both the growing human and companion animal populations. As a result, finding ingredient alternatives in order to sustain both the animal and human food chains, reduce food security risk, and support the growing populations is paramount.

Omega-3 (n-3) and omega-6 (n-6) fatty acid (FA) supplementation, in particular, is advantageous to both animals and humans, for a variety of reasons, including energy provision,
fat-soluble vitamin absorption, inflammatory management, cardio-protective benefits, and support for the health of the skin and hair/coat (NRC, 2007; Xenoulis & Steiner, 2010; Oppedisano et al., 2020; Calder et al., 2020; Sakai et al., 2017; DiNicolantonio & O’Keefe, 2020; Rabionet et al., 2014; Bernardi et al., 2012). In order to obtain these benefits, an optimal ratio of n-6:n-3 FAs is required. However, the majority of n FA sources are greater in n-6 FAs, rather than n-3 FAs. The most efficient way to obtain the long-chain derivatives of n-3 FAs, EPA and DHA, is through marine sources (Shepon et al., 2022). Unfortunately, present fishery practices cannot scale to meet the food demand of the increased human population, much less the increased companion animal and livestock populations. The present rate of production has already taken a significant toll on fish populations and their risk for extinction (Dulvey et al., 2003). In order to provide a source of n-3 FAs to companion animals and livestock, in addition to the human food chain, while protecting fish species and the oceans’ ecosystems, alternative sources for long-chain polyunsaturated FAs (PUFAs) from the n-3 pathway are required. The purpose of this thesis was to propose camelina oil as an alternative ingredient to support this need for additional sources of n-3 FAs.

_Camelina sativa_, the robust crop from which camelina oil is derived, has a number of qualities that make it appealing to farmers, including: (1) low input requirements, (2) high yield, (3) tolerance to environmental conditions and temperatures that would pose problems for some other oil seed crops, (4) a short growing season, making it a desirable rotation option, and (5) resistance to various pests. This crop seems to be particularly adapted to cold, dry climates, and is widely grown or cultivated in almost all regions of Europe, North America, South America, Asia, Australia, and New Zealand. In addition to the numerous agricultural benefits associated
with *Camelina sativa*, its oil product has several nutritional benefits to be noted as well. Camelina oil has a higher oxidative stability than flaxseed oil, increasing its shelf-life, which is an important factor when considering incorporating this ingredient into a commercial feed product for consumers. In addition its desirable shelf-life, camelina oil contains high concentrations of polyphenols and tocopherols, and a low concentration of the undesirable FA erucic acid. Furthermore, unlike many other crops, camelina is not genetically modified, increasing its attraction to consumers. All together, these attributes make *camelina sativa* a desirable crop to producers with high product demand. Its oil product, camelina oil, can be used to increase n-3 inclusion, providing space to support both human and animal food chains.

Additional outcomes of the trials outlined in chapters 2 and 3 include proving the safety of camelina oil by investigating complete blood count and biochemistry analytes, body weight, and feed intake, in dogs and horses, which was carried out with success (Burron et al., 2020). Once deemed safe, it is important to ensure the efficacy of camelina oil, i.e. that feeding camelina produces similar effects on metabolism and skin and coat health, a consumer noticeable set of claims that are commonly employed when supplemental oils are added to dog food and equine feed. To further support the importance of targeted nutrition to consumers, a survey asked 2181 people how important it was that their pet food provides some benefit to their pet, and the mean score was 6.47 out of 7 (Schleicher et al., 2019). Consumers no longer want their choice of pet food to be a simple vector of nutrients, they want to be feeding something to their pet that provides additional noticeable benefits, like skin and coat support, for example. Furthermore, skin and coat health is a gauge of general health, and as such, consumers tend to place even greater value upon it (Marchegiana et al., 2020).
Oil supplements in the equine space have numerous roles outside of skin and coat support, especially for horses used for exercise or sport. Providing n-3 FAs decreases thermal load and enhances metabolic adaptations, both of which have the potential to improve athletic performance (Potter et al., 1990). Additionally, providing n-3 FAs provides a further benefit to a low-dust diet in the management of horses with chronic lower airway inflammatory disease (Nogradi et al., 2015). Moreover, oils provide a dense source of energy and fat which can reduce reliance on grains or protein as a source of energy.

There are limitations from both the canine and equine trials that must be discussed, one being the dietary inclusion level of camelina oil. The amount used in these studies is considerably higher than would be used in a home or barn, and as such, future studies should investigate the impact of various doses of camelina oil supplementation on skin and coat health in dogs and horses, as this will be important not only for the animal but possibly for the processing and palatability of formulations. In addition to this, other oils commonly used in the pet food industry are often incorporated into a final product (i.e. kibble) via processing, rather than being top-dressed, like in the study carried out in chapter 2. As a result, work should be done to determine the impact of processing on camelina oil as an ingredient (i.e. bioavailability, palatability, etc.) to ensure that the same benefits are made available to the animal in this form. An additional limitation in the canine trial was the use of client owned dogs, which introduced greater variability as all dogs were housed in different environments, with different owners and routines. There were also differences in age, breed, and sex, however, this was partially remedied by the block design. Although the inclusion of client-owned dogs creates more variability in the sample population, it better depicts how commercial dog diets are sold, and better represents how dogs
in different households will respond to camelina oil as an ingredient. One of the largest
limitations during these two trials was seasonality changes. The dog study began the trial in
September and went until February, while the horse study began in June and went until January.
Additionally, due to lack of availability, horses were used from three different locations and their
start dates were staggered by 3 months. Copious amount of research has shown the effects of
season, temperature, humidity, and overall environment on the skin and coat outcomes assessed
in chapters 2 and 3 (Grubauer et al., 1989; Grice et al., 1971; Green et al., 2022). As a result, it
becomes difficult to make any confident conclusions based on week, as environmental
conditions were not constant. This was accounted for as best as possible by evenly blocking
treatment oils, however, differences in seasons and locations may have still had an effect.
Despite these limitations, in combination with the blocking, the lack of significant differences
among oils enables us to conclude with confidence that camelina oil can be supplemented to
support skin and coat health to a similar extent as flaxseed and canola, which are currently used
in dog and horse feed formulations.

Fatty acid analysis will be carried out on equine skin punch biopsies collected during this
trial to further understand how camelina oil is incorporated into the skin and provide support for
the skin and coat claims made to consumers. Future research should investigate the impact of
camelina oil supplementation in dogs and horses following exercise. This could be especially
prudent for sport horses. Furthermore, there are numerous studies supporting the therapeutic use
of oil supplements in those with dermatological disorders, which have paved the way for future
research to determine the impact that camelina oil supplementation has on individuals with poor
hair/coat health and/or skin disorders.
The focus of this thesis, specifically, was to elucidate the potential role of camelina oil as an ingredient in canine and equine nutrition. However, this ingredient should be further investigated as a vector of n-3 FAs in other species in order to support the tremendous amount of population growth and associated food demand from the human and animal sectors. Although more research must be done to determine this species-specific information, providing a mixture of marine oils, as a source of EPA and DHA, along with plant oils, like camelina oil, poses an environmentally and economically sustainable support that reduces the current reliance on the marine production industry. There is a vast amount of research to be carried out on camelina oil as an ingredient in dogs and horses but ensuring that this supplement is analogous to existing supplements in terms of its claims to consumers is paramount in its success.
4.1 References


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