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Review Article

Therapeutic Relevance of Dietary Ratio of Polyunsaturated Fatty Acids N-6: N-3 in Canine Atopic Dermatitis

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Abstract

Canine atopic dermatitis (CAD) is an allergic skin condition in dogs characterized by degradation of the skin barrier function and pro-inflammatory responses triggered by injured skin cells. Polyunsaturated fatty acids (PUFAs) that include linoleic acid (LA; omega-6 (n-6)), and alpha linolenic acid (ALA; omega-3(n-3)) have been implicated in the alleviation of clinical features of atopic dermatitis in dogs since 1987. Since these PUFAs are not synthesized in vivo in dogs, they are termed "essential fatty acids (EFAs)" and are obtained via dietary supplementation with n-3 and n-6 rich nutrient sources that include meats, poultry, fish oil, flax seed and vegetable oils. Rebalancing of dietary EFAs exhibits a potential to reorganize the phospholipid membrane and influence the allergic inflammation response by modulating the production of eicosanoids consisting of a series of pro- and anti-inflammatory prostaglandins, thromboxane and leukotrienes (LTBs) that compete for a common enzyme (delta-6-desaturase) in the metabolic pathway. Enrichment with n-3 FAs favors the production of n-3 derived anti-inflammatory metabolites, particularly LTB_5 in neutrophils, over that of the n-6 derived pro-inflammatory metabolites (LTB_4). Despite several clinical trials conducted in dogs over 20 years, lack of standardization in CAD models, trial research design and protocol, age and breed of dogs, supplementary diet specification and control diets of affected dogs has led to extreme variability in outcomes, thereby eluding a clear consensus on the efficacy of EFA dietary supplementation in treating CAD. Achieving the optimal dietary n-6:n-3 ratios is thus a highly controversial and debated issue, further challenged by considerations of pet food ingredient profiles in formulations and ingredient costs. This review summarizes the mechanism of EFA metabolism with key focus on the efficacy of balanced dietary n-6:n-3 ratios on clinical outcomes of CAD.

Keywords: *Canine Atopic Dermatitis; n-6; n-3; Omega-3; Omega-6; Polyunsaturated Fatty-Acids; Linoleic Acid; Alpha-Linolenic-Acid; Eicosanoids; Pet-Food*

Abbreviations

CAD: Canine Atopic Dermatitis; FA: Fatty Acid; PUFA: Poly-Unsaturated Fatty Acid; EFA: Essential Fatty Acid; n-3: Omega-3; n-6: Omega-6; TEWL: Trans-Epidermal Water Loss; LA: Linoleic Acid; ALA: Alpha Linolenic Acid; AA: Arachidonic Acid; EPA: Eicosapentaenoic Acid; DHA: Docosahexaenoic Acid

Introduction

Canine Atopic Dermatitis - Definition, Presentation and Pathology

Canine Atopic Dermatitis (CAD) is primarily defined as a "genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with *enhanced production of immunoglobulin E* (IgE), most commonly directed against environmental allergens" [1] (words in *italics* are author additions). Where allergic flares of CAD are triggered by food, it is termed as "food-induced allergic dermatitis" and clinical presentations similar to CAD, without an associated IgE response are termed as "atopic-like dermatitis". The earliest indications of skin inflammation in canines was identified as 'eczema' in reaction to food allergens [2-4] and as

'spontaneous allergy (atopy)' characterized by rhinitis, conjunctivitis and urticaria in dogs caused by sensitivity to ragweed pollen [5] as well as dust, mold, weeds and grasses [6]. Later studies showed the link between allergen exposure and production of allergen-specific antibodies (IgE), binding to sensitized mast cells and triggering the release of histamine and other pro-inflammatory mediators leading to atopic asthma, conjunctivitis, rhinitis, pruritus and anaphylaxis [7]. Recent research has highlighted the degradation of the epidermal barrier function as a chief contributing factor in the pathogenesis of CAD [8]. The pathogenesis, diagnosis and treatment of CAD is complicated by variations in breed genus [9,10]. Thus, CAD is a complex, multifactorial disease involving multiple genes and complex interactions between skin structure, immune system and the environment resulting in immune dysregulation, allergic sensitization and skin barrier defects [11].

Skin Barrier Function

Skin barrier function is derived from the ultra-structural composition of the epidermis [12,13], consisting of multilamellar lipid sheets or keratinocytes in the stratum corneum and ceramides, one of the main lipid constituents of epidermal lipids [14]. Ceramides, in particular ceramide-1, are functionally important to maintain stability of multilamellar lipid sheets and are instrumental for the maintenance of water homeostasis and inhibition of water loss [15]. Alteration or degradation of the epidermal ultrastructure due to inflammation disrupts water homeostasis and increases pruritus and trans epidermal water loss (TEWL), two of the main clinical features of CAD [16,17]. The significance of skin barrier deficits in CAD [18] is evident from studies showing enhanced TEWL in atopic beagles compared to healthy controls [8,19], changes in stratum corneum and in ceramide profiles in atopic versus healthy dogs [20-24] and altered expression and loss-of-function mutations of filaggrin, the primary protein essential for proper skin barrier function, in atopic disease [25,26]. These studies suggest that restoration of epidermal lipids may reverse skin barrier deficits, reestablish water homeostasis and prevent/reduce pruritus and the incidence of chronic CAD.

Ceramides are membrane sphingolipids consisting of a sphingosine backbone with fatty acid (FA) attachments [15]. FAs are classified as saturated FAs, with hydrogen bonds and no double bonds between main chain carbon atoms, and unsaturated FAs, with one (mono-) or more (poly-) double bonds between main chain carbon atoms [27]. Among polyunsaturated fatty acids (PUFAs), linoleic acid (LA), a prominent omega-6 (n-6) FA, is the most abundant PUFA found in ceramide molecules [15] and subsequently of great importance for maintenance of the epidermal barrier (Figure 1). Arachidonic acid (AA) is the second-most abundant PUFA and is responsible for production of inflammatory mediators such as eicosanoids. A third PUFA called α-linolenic acid (ALA), a prominent omega-3 (n-3) FA, is found in small quantities in the dermis and provides physical and nutritional support to the epidermis. n-3 PUFAs play an immunomodulatory role that affects inflammatory response as well as skin barrier function.

Scope of the review

From a nutrition standpoint, the n-3 and n-6 PUFAs, ALA and LA, respectively are termed as essential fatty acids (EFAs), since they cannot be synthesized *de novo* in mammalian skin and can only be obtained through diet supplementation or enrichment. Sources of n-3 FAs include fresh cold water fish oils and whole fat flax (rich in ALA), whereas n-6 PUFA (LA) is abundant in the oils of corn, safflower, sunflower, cottonseed and soy [28]. In recent years, oral ingestion of LA, ALA and their long-chain derivatives have shown to reverse degradation of epidermal lipid layer [20,24], suggesting dietary essential fatty acid (EFA) supplementation as a viable mode of treating and preventing CAD. Research also suggests that dietary ratio of n-6:n-3 is as important as optimum individual EFA concentration and is used to prevent atopy and flea bite sensitivity and to treat inflammation, cancer and chronic degenerative diseases including colitis and arthritis. Human diet has evolved over the last 100 - 150 years, with current diets being rich in saturated fats (> 10%) and calories, high in n-6 FAs and low in n-3 FAs, leading to n-6:n-3 ratios in the range of 20:1 - 30:1 [29,30], which is significantly different from the apparent 1:1 ratio during the paleolithic era [30,31]. Similar imbalances in n-6:n-3 ratio in canine diets possibly impacts companion animals also, as evident from several recent epidemiological studies and clinical trials. This review aims to revisit knowledge areas of the metabolism and biochemistry of EFAs and the efficacy of dietary n-6: n-3 ratios in preventing and treating CAD. It also highlights typical PUFA ratios found in commercial dry dog diets as specified in product labels and/or nutrient profile.

Biochemical structure of n-3 and n-6 PUFAs

The general structure of FAs consists of a hydrocarbon chain with a methyl group at one end and a carboxyl group at the other. As noted before, FAs vary in their carbon-chain length from 2 to 30 or more carbons, that may be saturated (no double bonds), monounsaturated (one double bond) or polyunsaturated (two or more double bonds). Unsaturated fatty acids are named by identifying the number of double bonds and the position of the first double bond counted from the methyl terminus (with the methyl carbon as ω-1 or n-1). According to this nomenclature, an 18- carbon FA with two double bonds in the acyl chain and the first double bond on the 6th carbon from the methyl terminus will be notated as 18:2n-6, commonly known as linoleic acid (LA) [32] and is the parent FA of the n-6 family (Figure 1). When LA is further desaturated by the addition of an extra double bond between carbons 3 and 4 from the methyl end, it yields 18:3n-3, commonly known as α-linolenic acid (ALA), the parent FA of the n-3 family [33]. The desaturase enzymes required for the synthesis of LA and ALA are abundantly expressed in plants, but not mammals. Hence, LA and ALA, required for the synthesis of downstream PUFA metabolites, are classified as essential FAs (EFAs) and have to be obtained through supplementation or enrichment of diets with n-6 and n-3 FAs.

When it comes to enriching diets with EFAs, an important nutritional aspect to consider is the isomeric configuration of the FA chain, determined by the position of hydrogen atoms attached to the carbons in the acyl chain relative to double bonds. The cis configuration, wherein two hydrogen atoms fall on the same side of a double bond, producing a kink in the FA chain, is the form that occurs naturally and is considered a beneficial fat as it promotes good cholesterol, whereas a trans configuration, wherein two hydrogen atoms fall on opposite sides of a double bond, is commonly introduced during food-manufacturing processes and is considered harmful to cardiovascular health.

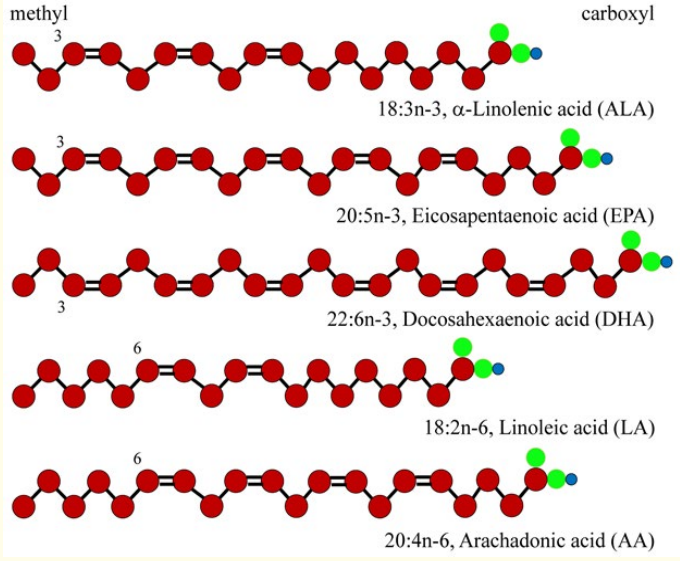

Figure 1: *n-3 and n-6 Fatty Acid Structures.*

Red circles represent main chain Carbon atoms, green circles represent Oxygen atoms, blue circles represent Hydrogen atoms, black lines represent single or double bonds between two Carbon atoms.

(Adapted from http://theinfoscience.blogspot.com/2015/11/omega-3-fatty-acids.html)

Metabolism of n-6 and n-3 PUFAs

Dietary LA and ALA are absorbed by intestinal cells and metabolized into long-chain fatty acids by further desaturation and elongation of the acyl chain through the enzymatic actions of "delta-6 desaturase", "delta-5 desaturase" and elongase in the liver [27]. The actions of

delta-6-saturase are more critical, however, as shown in Figure 2, which shows the most important steps in the transformation of LA and ALA into their principal unsaturated derivates like arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [34]. Thus, LA is converted to γ -linolenic acid (GLA) (18:3n-6) and dihomo- γ -linolenic acid (20:3n-6) and by "delta-5 desaturase" into arachidonic acid (AA) (20:4n-6). The same set of enzymes used to metabolize n-6 PUFAs also metabolize ALA (18:3n-3) into octadecatetraenoic acid (18:4n-3) and eicosapentaenoic acid (EPA) (20:5n-3). EPA undergoes further conversion in peroxisomes to docosapentaenoic acid with the addition of two carbons (22:5n-3), then to 24:5n-3 with the addition of another two carbons, then desaturated to form 24:6n-3, followed by removal of two carbon atoms by limited beta-oxidation to finally yield 22:6n-3, commonly known as docosahexaenoic acid (DHA) [35]. As the same enzymes are involved in the conversion of LA and ALA into other n-6 and n-3 FA derivatives respectively, there is competition between the n-6 and n-3 FAs for metabolism and the proportions of n-6 and n-3 FAs available to the enzyme system determines the quantity and proportions of AA and EPA produced. Furthermore, although ALA is the preferred substrate for the delta-6 saturase enzyme, the amount of ALA required to inhibit formation of GLA by 50% is about 10 times the amount of LA that is present [36]. Hence, given that most modern diets contain far greater amounts of n-6 FAs compared to n-3 FAs, this means that metabolism of n-6 FAs is more prevalent, ultimately resulting in greater quantities of AA relative to EPA [37]. The fact that metabolism and downstream effectors of AA and EPA regulate inflammatory response in cells, forms the basis for supplementation and enrichment of diets with n-3 FAs to adjust for the abnormally high proportions of n-6 FAs in modern diets. Since the synthesis of n-3 metabolite PUFAs is inefficient due to competing enzyme action, EPA and DHA are also classified as dietary EFAs and, in the context of preventing and treating CAD, have been shown to be important for maintenance of a healthy and shiny coat in canines [38].

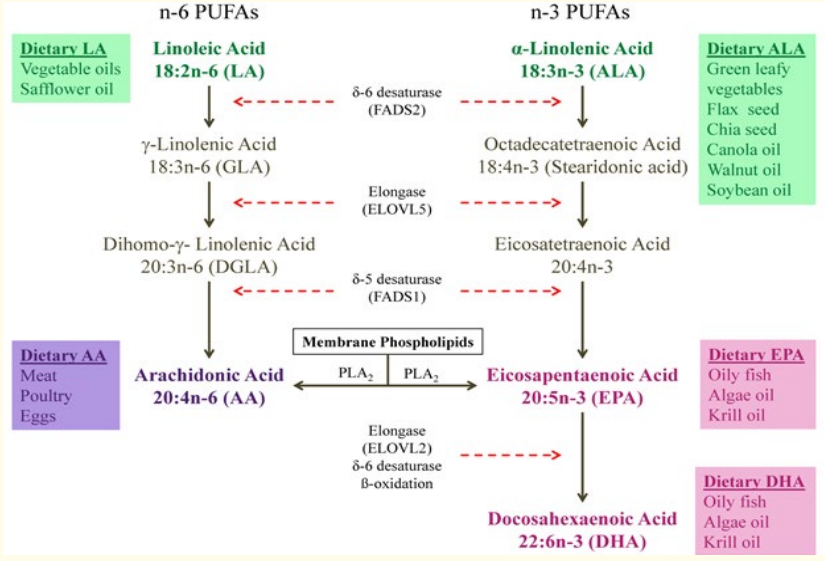

Figure 2: *n-3 and n-6 PUFA metabolism. The dietary sources of essential fatty acids and enzymatic conversion pathway of fatty acids LA and ALA into their downstream metabolites AA, EPA and DHA.*

Downstream inflammatory response of PUFAs – Eicosanoid production

PUFAs play an integral role in cell injury and repair by triggering the inflammation response and synthesizing eicosanoids, which are biologically active downstream metabolites of 20-carbon membrane phospholipids released from cell membranes in response to cell injury. The term 'eicosanoid' encompasses prostaglandins, thromboxanes, leukotrienes and hydroxylated eicosatetraenoic acids, which are produced by enzymatic actions of cycloxygenases (COX-1 and COX-2) and lipoxygenases (LOX) on 20-carbon PUFAs such as dihomo-GLA, AA and EPA following mast cell activation (Figure 3). n-6 eicosanoid derivatives include the COX-derived 2-series prostaglandins

and thromboxanes (PGE_2, PGF_2, PGD_2, TXA_2, TXB_2) and the 5-LOX-derived 4-series leukotrienes (LTA_4, LTB_4, LTC_4, LTD_4, LTE_4), while n-3 eicosanoid derivatives are the 3-series prostaglandins (PGE_3) and thromboxanes and the 5-series leukotrienes (LTB_5, LTE_5). The n-6 and n-3 derived eicosanoids exert opposing effects on inflammation. n-6 derivatives trigger a pro-inflammatory, immunosuppressive and pro-aggregatory response acting as potent mediators in type-1 hypersensitivity reactions [37]. On the other hand, n-3 derivatives diminish the inflammatory response and are vasodilatory, anti-aggregatory and not immunosuppressive in the presence of anti-inflammatory agents such as corticosteroids and non-steroidal drugs [39].

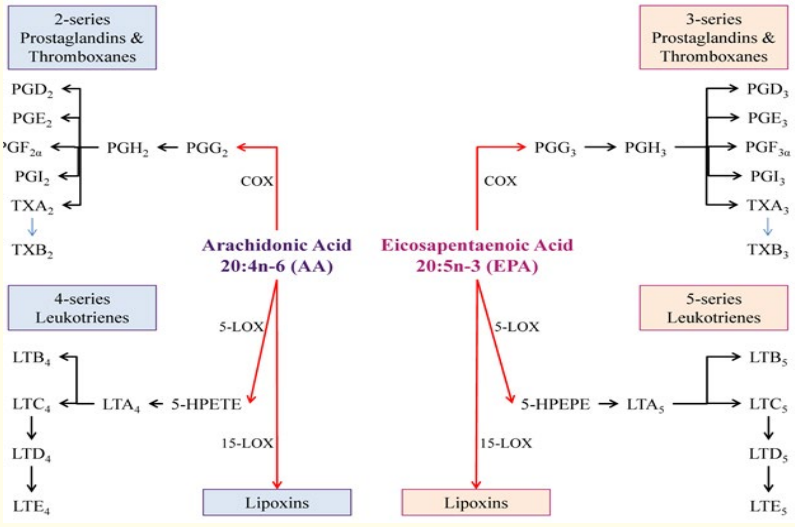

Figure 3: *Downstream inflammatory response of PUFAs: Eicosanoid production. Metabolism of dietary EFAs in response to inflammation leads to generation of eicosanoids encompassing prostaglandins, thromboxanes, leukotrienes and lipoxins - signaling molecules that are either pro-inflammatory (derived from AA) or anti-inflammatory (derived from EPA and DHA).*

The types of eicosanoids synthesized and the resulting inflammatory response cascade depends on the type of FA released by the cell membrane (n-3 or n-6). Since n-6 FAs are typically found in greater proportions compared to n-3 FAs owing to their greater proportions in natural diets, AA is usually the dominant substrate for eicosanoid synthesis leading to, by default, pro-inflammatory cascades upon stimulation. This in turn suggests that an enhancement of dietary n-3 will decrease the proportion of n-6 FAs released from cell membranes relative to release of n-3 FAs and significantly diminish the potential for a pro-inflammatory signaling responses in cells.

However, the potential for n-3 derived eicosanoids to trigger a protective anti-inflammatory response is diminished by the fact that they are believed to be less potent than their n-6 counterparts. For instance, n-3 derivative LTB_5 is 10-100 times less potent as a neutrophil chemotactic agent than the n-6 derivative LTB_4 [37]. Stimulation of LTB_4 receptors on neutrophils is primarily a pro-inflammatory trigger that brings about the cascading effect of neutrophil recruitment, chemotaxis, degranulation and more LTB_4 synthesis, which spreads the inflammatory response [40]. In contrast, n-3 derived LTB_5 is 30-100 times less active in the stimulation of leukotriene B receptors [40-44] that inhibit potential adversities brought about by LTB_4 on inflammation [45]. On the other hand, although LTB_5 is a weak inhibitor of the enzyme 5-lipoxygenase [46] implying lower inhibition rates for the formation of LTB_4, it promotes the synthesizes of EPA in higher amounts by a common enzyme (leukotriene A hydrolase) that also produces AA. Thus, EFAs show potential in guiding the inflammatory response by modulating prostaglandin and leukotriene production, inhibiting cellular cytokine production and altering the composition and function of the epidermal lipid barrier (Figure 4). Hence, with respect to reversing or prevention of CAD, it is vital to manipulate the supplementary n-6:n-3 ratio or the total n-6 to n-3 FA quantity in a manner that has the potential to change tissue FA concentration to a level that will produce favorable outcomes in these measures. It is found that marine oils that are rich in n-3 FA content, increase the

proportion of n-3 FA presence in cell membrane phospholipids of skin and neutrophils [45] and may prove to be an effective n-3 dietary source to regulate the inflammatory response. The clinical relevance and benefits of dietary intake of n-3 and n-6 PUFAs and the n-6:n-3 ratio in treating CAD has been a matter of contentious debate and extensive research.

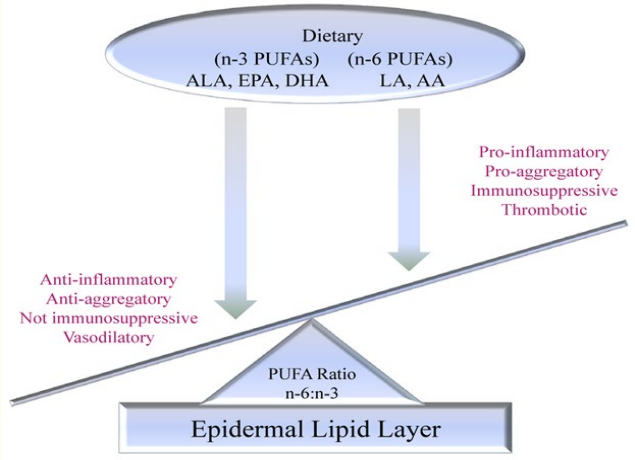

Figure 4: *Dietary Ratio of n-6:n-3 PUFAs - a balancing act regulating the inflammatory response in the canine skin.*

Essential Fatty Acid Supplementation – Do they really work for CAD?

Early studies showed that manipulating the dietary n-6:n-3 FA ratio or diets with high n-3 FA content with specific focus on suppressing LTB_4 and enhancing production of LTB_5 proves beneficial in the management and treatment of canine skin atopy [47]. In a classic study by Vaughn et al [48], the effects of dietary n-6:n-3 fatty acid ratio on leukotriene B synthesis in dog skin and neutrophils were determined. A total of 30 adult beagle dogs were used for the study and were fed with diets containing n-6:n-3 ratios of 5:1, 10:1, 25:1, 50:1 or 100:1 for a period of 12 weeks. Basal diets were formulated with fresh chicken and chicken by-product meal, corn, rice and chicken fat as the lipid source with an overall n-6:n-3 ratio of 28:1. Manipulation of the ratio was achieved by enrichment of the formulation with menhaden oil (concentrated source of EPA and DHA), flax (concentrated sources of ALA) and safflower oil (concentrated source of LA). Leukotriene B concentrations were determined by use of [3H] –LTB_4 RIA kits. Results from this study indicated distinct effects of diets enriched with 5:1 and 10:1 ratios on LTB concentrations. Skin concentrations of n-6 derived LTB_4 decreased while that of n-3 derived LTB_5 increased at the end of 6 and 12 week period of the study. The 5:1 and 10:1 enriched n-3 diets increased skin concentrations of LTB_5 by 79% and 48%, whereas the skin concentrations of LTB_4 decreased by 62% and 48% respectively. At this optimum ratio, neutrophils contained 30 - 33% lower LTB_4 and 370-500% higher LTB_5 concentrations at weeks 6 and 12. Higher n-3 fatty acid proportions also influenced plasma concentrations of LTB_4 and LTB_5 favorably.

EFA-enriched diets with enhanced proportions of n-3 FAs compared to n-6 FAs also impact plasma FA concentrations, severity of pruritus and clinical scores of CAD in atopic dogs. Administration of evening primrose oil, a n-3 enriched source, by itself or along with marine oil in a 4:1 ratio, first worsened pruritus, scaling and edema in atopic dogs but then improved edema and coat conditions over a period of 9 weeks [49]. Dietary supplementation with marine oil containing high-dose n-3 fatty acid EPA showed significant improvement of pruritus and coat character in atopic dogs compared to supplementation with corn oil containing LA and GLA, arguing for a favorable effect of n-3 FA enriched diets on treating CAD [50]. Conversely, supplementation of diets with either sunflower seeds (source of n-6 fatty acid LA) or flax seeds (source of n-3 fatty acid, ALA) for 84 days in mixed breed dogs improved hair coat and skin condition scores, but only temporarily, as no significant improvement in skin condition was observed beyond a period of one month [51]. A single-blinded study of 18 atopic dogs fed on a lamb and rice diet with a n-6:n-3 ratio of 5.5:1 showed a significant 44% decrease in pruritus and altered

plasma and skin FA content within 3 weeks, which was reversed in 3 to 14 days upon withdrawal and again controlled upon reinstation of the diet [52]. High-quality n-3 enriched veterinary diets, such as the Eukanuba Veterinary Diets Dermatosis FP, proved very effective in significantly reducing pruritus and clinical CAD scores [53]. It was suggested that this may be due to the high n-3 content reducing the effective n-6:n-3 ratio to even lower values in the range of 2:1 or 1:1 [54]. Such a supposition is supported by the observation of significant reduction in ex vivo LTB_4 concentrations in canine neutrophils following a six-week regimen of a n-3 rich diet that had a n-6:n-3 ratio less than 1 [55]. Interestingly, a study by Hall., *et al.* [56] showed that plasma FA concentrations in healthy, female geriatric beagles depended on the n-3 FA dose, independent of the n-6:n-3 ratio. Nesbitt., *et al.* [57] extended this further by suggesting that total dietary n-3 content rather than the n-6:n-3 ratio may be more significant, since dietary variations in total n-3 or n-6 FAs and n-6:n-3 ratios proportionally altered plasma FA concentrations reflective of both the n-3 dose as well as the n-6:n-3 ratio, but the extent of decrease in clinical scores of CAD in pruritic dogs did not significantly differ between diets. Subsequently, a double-blinded, placebo-controlled, randomized trial of dogs observed no correlation between the total intake of n-3 or n-6 FAs or n-6:n-3 ratio and clinical improvement of CAD [58].

As is evident, there is a remarkable lack of consistency in the therapeutic outcome of n-3/n-6 enriched diets in the treatment for CAD. A comprehensive review by Olivry., *et al*. [59] highlighted several contributing factors that hinder a consistent interpretation from several randomly controlled clinical trials conducted since the 1980s, such as (a) variability in included subjects due to evolving diagnostic criteria of CAD [60], underlying epigenetic pathophysiology and degree of severity, (b) variability in the age and breed of dogs affecting the response to intervention, (c) inconsistent quality of study design and trial methodology regarding the duration, number of subjects, randomization and control group, (d) lack of standardization of outcome measures leading to heterogeneity in reporting and assessment of treatment efficacy and (e) lack of trial duplications, variability in dosages and timing of outcome measurements. Consequently, the efficacy of EFA-enriched diets and supplementation, tested in over 19 randomly controlled clinical trials over 20 years, has not been universally established to prevent or reverse CAD, owing to lack of standardization of trial protocol, dietary formulation and outcome assessment [59,61]. Recent efforts in establishing canine models of atopic dermatitis that reliably reproduce and closely match the human condition upon allergen stimulation provide hope for better designed trials in future [62].

EFA-enriched diets with low n-6:n-3 ratios may, however, be effective when administered to atopic dogs in conjunction with adjunct therapies. It is reported that EFA supplementation has a steroid sparing effect, improving the skin-healing rate in atopic dogs leading to significantly decreased glucocorticoid administration after a period of 8 weeks [63]. Topical applications of specially formulated lipid mixtures have been effective in restructuring of skin lipid bilayers leading to clinical improvement of CAD [20,24,64]. In a pilot study, administration of specially formulated feed supplement consisting of n-6 fatty acid LA and n-3 fatty acid EPA in a 5:1 proportion for 8 weeks showed marked increase in overall skin lipid content and significantly improved organization of lamellar lipids in the stratum corneum of atopic dogs comparable to healthy dogs [24]. It has long been acknowledged that degradation of the skin epidermal layer acts as a biosensor for disease progression leading to barrier dysfunction and enhanced TEWL in atopic dermatitis [18,65-67]. Thus, even though the benefits of EFA-enriched diets in the treatment of CAD has not been unequivocally proven, it can act as a prophylactic measure to prevent or delay the onset of CAD.

Metabolic relevance of n-3 and n-6 enriched diets

Apart from regulating inflammatory response of the cell and maintaining epidermal lipid content and organization, EFAs also play a role in determining cellular physiology [34]. Incorporation of metabolic products of dietary n-6 (LA) and n-3 (ALA) PUFAs (AA, EPA and DHA) into the cellular membrane regulates fluidity of the cell membrane and, depending of proporation of each derivative, attracts different enzymes, receptors, channels for the formation of unique signaling complexes that enhance downstream cell signaling pathways to evoke specific physiological responses [68-70]. For instance, increase in membrane fluidity due to increased incorporation of PUFAs has been associated with an increase in number of insulin receptors in the cell membrane and their affinity to insulin, leading to a decrease in insulin resistance [71]. Although the exact mechanism underlying this phenomenon needs further elucidation, it is believed that insulin enhances the activity of delta-5 and delta-6 desaturases and amplifies the formation of PUFAs, thereby increasing number of insulin

receptors in the cell membrane [72]. Research indicates that inflammation is a common characteristic of several chronic diseases such as cardiovascular disease, obesity, diabetes, arthritis, mental illness, cancer and autoimmune conditions. Studies highlight the health benefits of incorporating n-3 and n-6 PUFAs in daily food consumption by increasing the probability of managing and potentially reversing such chronic disease states [72-80]. Experimental evidence from several recent studies indicate that an optimal n-6:n-3 ratio of 4:1 to 5:1 but not exceeding 10:1 is necessary to obtain discernable long-term metabolic benefits [81]. Such benefits are also reported for maintenance of healthy skin and prevention or reversal of chronic atopic dermatitis in canines [28,51,82].

n-6:n-3 ratios in commercial canine formulations

To reflect growing evidence of the importance of PUFAs in CAD, the Association of American Feed Control Officials (AAFCO) 2007 Canine Nutrition Expert Subcommittee (CNES) has established minimum (and, where applicable, maximum) concentration requirements for fat/fatty acid content in the AAFCO Dog Food Nutrient Profile [83]. The CNES recommended concentrations (as % of dry matter (DM)) for - (1) minimum total fat is set at 8.5% for Growth and Reproduction (GR) and 5.5% for Adult Maintenance (AM), (2) minimum LA concentration is set at 1.3% for GR and 1.1% for AM and (3) minimum concentrations of ALA and EPA+DHA combination are set at 0.08% and 0.05% for GR. The ALA and EPA+DHA concentrations for AM being set as not determined (ND) was accepted by the AAFCO Pet Food Committee. However, by setting a maximum limit for the n-6:n-3 fatty acid ratio at 30:1, it ensures that sufficient amounts of n-3 FAs are necessarily added to meet this requirement. From a food safety view point, it is recommended that PUFA concentrations be balanced with that of Vitamin E with the recommended ratio of IU of Vitamin E to grams of PUFA be ≥ 0.6:1, since n-3 FAs are unstable and prone to oxidative rancidity, requiring antioxidant protection. Hence, it is necessary that for a diet containing 50 IU of Vitamin E, the PUFA content be ≤ 83 grams and an additional 0.6 IU of Vitamin E be added for every additional gram of PUFA thereafter [83].

Commercial dog foods available from specialty pet food stores adhere to the AAFCO nutritional recommendations with varying n-6:n-3 ratios (Table 1). Although the amount of n-3 FAs in the formulation is important to achieve the modulatory effect on n-6 metabolism, the n-6:n-3 ratio in the diet is equally significant [84]. Inefficient supplementation of n-6 and n-3 FAs in a complete and balanced diet poses risk implications including 1) unbalanced FA ratios, 2) poor client compliance, and 3) inconsistent therapeutic treatment response [85]. In contrast, over supplementation with n-3 FAs in the formulation could lead to decreased platelet aggregation and increased blood clotting time. Maintaining optimal n-6:n-3 FA ratio, while fulfilling nutrient requirements is a challenging process, considering the complex and diverse ingredient portfolio in pet food nutrition. Pet food manufacturers use ingredient sources such as flaxseed and menhaden fish oils for concentrated n-3 FA supplementation, which have a short window of shelf-stability and are very expensive with periodic cost inflations [86]. Considering the ingredient contribution from grain and vegetable oils rich in n-6 FAs compared to the contribution from expensive n-3 rich sources (meat, fish oil and poultry), it is not surprising that the n-6 FA content is invariably higher in proportion to n-3 FAs, making it a difficult and cost-heavy process to achieve optimal n-6:n-3 ratios. It is however evident from Table 1 that pet food manufacturers are making every effort possible to achieve targeted or optimal n-6:n-3 ratio in their diets. Recent advances to induce endogenous conversion of n-6 FAs into n-3 FAs in mice by introducing a fat-1 gene encoding a n-3 FA desaturase enzyme, leading to production of n-3 enriched foods [87] may prove beneficial in reducing the cost margins of production. Such strategies are invaluable to boost the animal's innate immunity to combat skin allergies as well as gratify pet-owners for every dollar spent on their purchases.

Commercial Dog Food Brand	Recipe	Life-stage	n-6	n-3	Ratio
Brand A	Chicken and Brown Rice	Small Breed Puppy	3.72	1.39	2.68:1
Brand B	Chicken	Puppy	3.5	0.6	5.83:1
Brand C	Chicken	Puppy	3.5	0.4	8.75:1
Brand A	Chicken and Brown Rice	Puppy	3.72	1.39	2.68:1
Brand A	Chicken and Brown Rice	Adult	3.39	1.04	3.26:1
Brand A	Chicken and Brown Rice	Large Breed Adult	3.39	1.04	3.26:1
Brand A	Chicken and Brown Rice	Small Breed Adult	3.39	1.04	3.26:1
Brand A	Chicken and Brown Rice	Small Breed Mature Adult	3.1	0.47	6.6:1
Brand A	Lamb and Brown Rice	Adult	3.45	0.57	6.05:1
Brand A	Lamb Meal and Rice	Small bite Adult	3.33	0.67	4.97:1
Brand B	Lamb and Barley	Adult	2.5	0.8	3.13:1
Brand B	Fish and Sweet Potato	Adult	2.7	1.1	2.45:1
Brand D	Fish and Sweet Potato	Adult	1.4	0.75	1.87:1
Brand D	Sweet Potato and Fish	Small Breed Adult	1.4	0.75	1.87:1
Brand B	Chicken and Brown Rice	Large Breed Adult	2.5	0.5	5:1
Brand D	Trout, Salmon Meal, Whitefish	Adult	1.8	0.8	2.25:1
Brand B	White Fish	Adult	2	0.8	2.5:1
Brand B	Turkey	Adult	3.25	0.5	6.5:1
Brand B	Chicken	Large Breed	3.25	1	3.25:1
Brand B	Duck	Adult	4	1	4:1
Brand C	Duck	Adult	4.5	0.4	11.25:1
Brand C	Salmon	Adult	3.5	0.4	8.75:1
Brand C	Buffalo and Sweet Potato	Adult	4	0.4	10:1
Brand D	Sweet Potato and Bison	Adult	1.7	0.5	3.4:1
Brand B	Duck	Adult	4	1	4:1
Brand D	Lamb	Adult	2	0.3	6.6:1
Brand B	Turkey and Oat Meal	Small Breed Adult	0.5	0.6	0.83:1
Brand B	Chicken	Toy Breed Adult	3.5	0.75	4.67:1
Brand B	Chicken	Large Breed Adult	2.5	0.5	5:1
Brand A	Chicken and Brown Rice	Mature Adult	3.1	0.47	6.6:1
Brand B	Beef	Senior	2.2	0.4	5.5:1
Brand C	Beef	Senior	2.4	0.3	8:1
Brand E	Chicken and Brown Rice	Small Breed Senior	3.5	0.15	23.33:1
Brand C	Chicken and Sweet Potato	All life stages	4.8	0.4	12:1
Brand F	Venison and Bison	All life stages	2.8	0.3	9.3:1
Brand C	Beef and Sweet Potato	All life stages	3.5	0.35	10:1
Brand C	Pork	All life stages	2.5	0.4	6.25:1

Table 1: *Dietary n-6:n-3 ratios found in canine diets.*

Source: Pet food manufacturer's nutritional profiles as specified in specialty pet retail store websites in the USA as of 10/2014. Brand names are intentionally unspecified in order to respect copyright and privacy.

Conclusion

Twenty carbon chain n-3 and n-6 PUFAs are essential for the production of eicosanoids and inflammatory mediators and maintenance of the lipid configuration of cellular membranes. In the skin, EFAs regulate inflammation response and maintain structural integrity of the epidermis, which are the two main areas of dysfunction in CAD. Use of EFA supplementation in both human and animal diets is now a reality, with the benefits of n-3 FAs derived from fish oils and flaxseed oils widely advertised and, in some cases, prescribed as a dietary supplement for chronic medical conditions and acute conditions like post-surgical traumatic stress. As CAD is a major concern, significantly improving skin and coat health by reorganizing the epidermal lipid layer of dog skin with a n-6:n-3 FA ratio-balanced diet has tremendous potential to act as a prophylactic measure as well as reverse disease by recovering skin barrier integrity. However, challenges remain in determining the therapeutic efficacy of EFA-enriched dietary interventions over anti-inflammatory drugs on CAD. First, better CAD models and standardized trial protocols are required for more consistent and conclusive evidence. Second, formulation of EFA-enriched therapeutic diets with optimally balanced fatty acid ratios is an on-going process dependent on the ease and cost of procuring ingredients rich in n-3 and n-6 FAs. Despite these limitations, fatty acid supplementation has added health benefits in both human and canines afflicted with inflammation-induced chronic pathologies and hence should be considered as a therapeutic option after expert medical advice and after careful consideration of the regulatory claims published by FDA/USDA for human consumption. Further research accounting for variations in life-cycles, breeds and genetic predisposition of canines is vital to maximize, while avoiding overestimation of, the benefits of PUFA supplementation in canines.

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Conflict of Interest Statement

The authors declare no conflicts of interest.

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