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Comparison of Key Nutrient Content of Commercial Puppy Foods with Canine Dietary Requirements

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Abstract: A balanced diet significantly impacts a dog's development with regards to energy, growth, immunity, and overall health. Customizing a dog's diet according to its age, size, and activity level is imperative for its welfare. Unbalanced diets can lead to nutritional deficiencies. This study assesses the key nutrient content of puppy diets that display information on EPA and DHA fatty acids. The diets fulfilled nutritional requirements for protein and fat according to the European Pet Food Industry Federation (FEDIAF) but varied in terms of levels of essential fatty acids. The nutrient levels in certain diets did not correspond to the label claims. None of the diets fulfilled the EPA and DHA claims, indicating an inconsistent ratio of $n-6$ to $n-3$. Additionally, trans fat such as C18:1 elaidic acid was present in all diets.

Keywords: balanced diet; canine nutrition; docosahexaenoic acid; eicosapentaenoic acid; essential nutrients; complete pet foods; fatty acids; labeling; nutritional adequacy; puppy

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1. Introduction

The annual report from the European Pet Food Industry Federation [1] showed that pet ownership across Europe remained at a high level, with an estimated 90 million households in the European Union (46% of all households) owning at least one pet, of which 25% owned at least one dog. European pet owners spent more than EUR 20 billion on pet food, supplies and services in 2021. Sales growth in the European pet food market was largely influenced by increased awareness of ingredients, customized food products and grain–free and organic foods.

The dog food industry has undergone a significant transformation. Recognition of the critical role that a balanced diet plays in promoting the health and well–being of dogs has been the catalyst for this change. Dogs are now considered as not just pets, but integral members of the household in the current environment [2]. The increased awareness of animal well–being has led to a wider variety of pet food products becoming available. The pet food market has grown dramatically in the last decades and offers a huge number of products that differ for dogs' physical form, composition of nutrients needed and physiological and pathological conditions. But the most popular are maintenance (complete) pet foods, referred to as over the counter diets (OTC). Complete and balanced pet food means pet food which, by reason of its composition, is sufficient for a daily ration [3]. The nutritional quality of commercial pet foods is of paramount importance to the animal's health as, nowadays, these foods are the sole source of nutrients and energy for most dogs [4–6]. In European countries, recommended nutrient profiles are published by the European Pet Food Industry Federation (FEDIAF). The FEDIAF [7] is a crucial contributor to the regulatory framework for the formulation, production, labeling and safety of complete pet food products. The oversight ensures that complete products provide the minimum recommended levels (MRL) of key nutrients. The nutritional guidelines focus on the dietary needs of both adult dogs and puppies. Nutritional deficiencies in puppies are crucial and can affect their overall development. For growing dogs, the FEDIAF nutritional guidelines specify that in addition to major nutrients, five polyunsaturated fatty acids (PUFAs) should be included in their diet: alpha–linolenic acid (ALA, C18:3 $n-3$), linoleic acid (LA, C18:2 $n-6$), arachidonic acid (ARA, C20:4 $n-6$), docosahexaenoic acid (DHA, C22:6 $n-3$) and eicosapentaenoic acid (EPA, C20:5 $n-3$). According to nutritional guidelines [7], after reaching maturity, only linoleic acid remains essential. Several health benefits, like the reduction in cardiovascular diseases and the development of visual and cognitive functions in growing puppies, are associated with $n-3$ PUFA [8–10]. DHA is crucial during fetal and growing body development because it is a component of cell membranes, but it also contributes to brain structure [11,12]. Its impact extends to the nature and number of receptors present in cell membranes. A study on rats suggests that a low intake of $n-3$ fatty acids during pregnancy and lactation can lead to anxiety in these animals [13]. Long–chain (LC) $n-3$ PUFAs–EPA and DHA–appear to have a much stronger effect than the shorter chain $n-3$ PUFA ALA. The conversion of polyunsaturated fatty acids into longer–chain fatty acids occurs through the involvement of specific enzymes. The formation of fatty acids such as EPA and DHA are achieved through desaturation, elongation and β–oxidation processes (Figure 1). Nonetheless, in dogs, the enzymatic activity involved in these processes is inadequate, requiring the provision of EPA and DHA fatty acids through the diet [14].

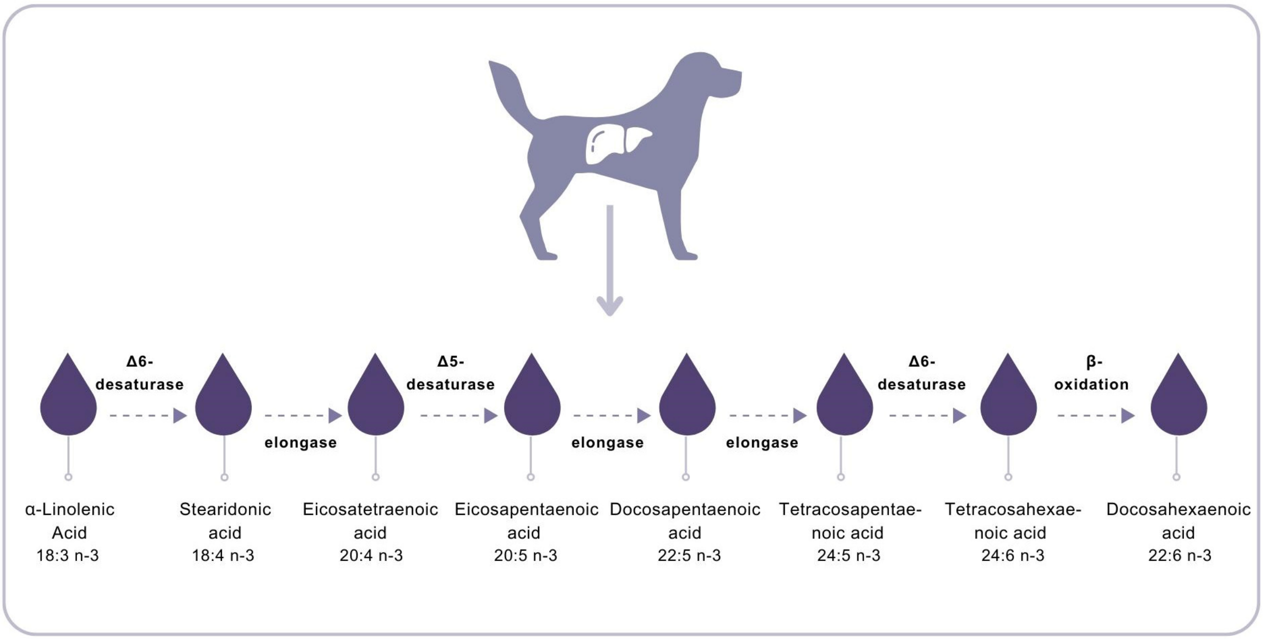

Figure 1. Conversion of $n-3$ fatty acids into LC–PUFAs, EPA and DHA, from their precursors based on [15,16].

Hence, DHA and EPA fatty acids are essential fatty acids (EFAs) in the diet of growing dogs. Research studies have shown the crucial functions of DHA and EPA in numerous physiological processes in dogs [10,17–20]. Throughout life, unsaturated fatty acids (UFAs) are present in cell membranes, influencing their flexibility. The higher their concentration, the more flexible the membrane becomes. However, increased amounts of saturated fatty acids (SFAs) lead to the opposite effect, causing cell walls to become more compact [21]. Consuming DHA can have beneficial effects on the immune system during its development. This is particularly relevant for puppies that do not receive breast milk. DHA is believed to possess anti-inflammatory properties by reducing inflammation markers and increasing the number of phagocytes, albeit with decreased activity, as per one perspective [22]. During early growth stages, puppies mainly receive nutrients from their mother's milk, which contains these fatty acids [23]. Milk replacers can be used if necessary. However, some of them may lack or have a very low content of EPA and DHA, which are essential for this stage of life [24]. After transitioning to commercial food, puppies mainly rely on it for their LC–PUFA needs. According to the available literature, these LC–UFAs, including DHA and EPA, have been found to have beneficial effects on various systems, such as the nervous, immune, reproductive and digestive systems, as presented in Figure 2 [19,25–38].

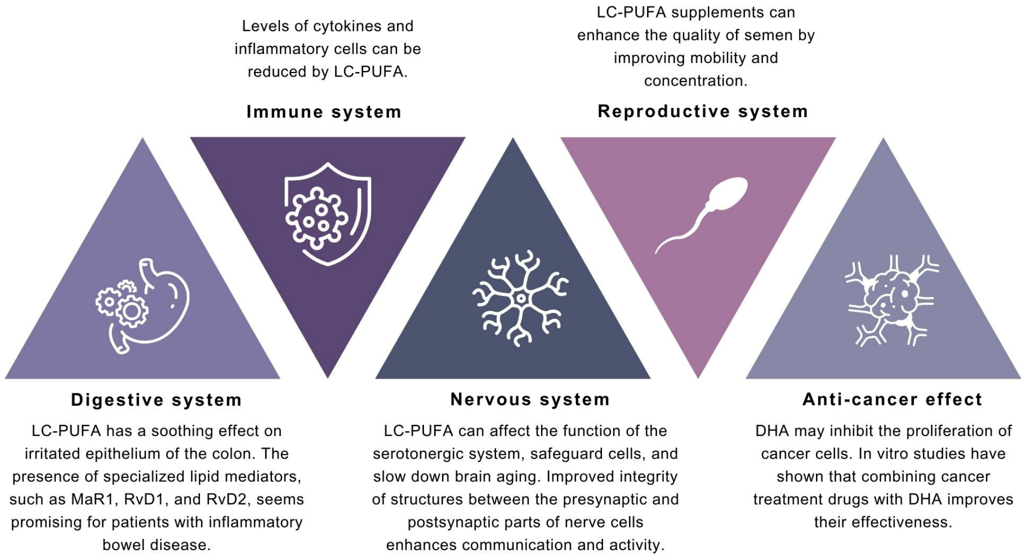

Figure 2. Potential effects of long-chain polyunsaturated fatty acids on various systems in growing and adult dogs based on [20,32,36,39–52].

Although important, the amount of these fatty acids is not required to be declared on the label by manufacturers of complete feeds. Analytical constituents such as crude protein, crude fiber, crude fat, and crude ash are subject to mandatory declaration according to the EU regulation [3]. Other analytical constituents can be labeled on a voluntary basis.

A complete pet food product labeled as such is, by law, balanced in such a way that it can serve as the animal's only source of nutrition without leading to nutrient deficiencies [3,7]. Any unsuitable diet may have harmful health effects. The nutrition products industry is now facing the important challenge of identifying and reducing risks of unbalanced diets [53]. Therefore, it is necessary to evaluate the quality of the pet food, and a number of studies have been conducted to test dog food [54–57]. One major issue is the imbalance in the manufactured products, including the lack of compliance of the composition with the FEDIAF nutritional guidelines [58]. The FEDIAF nutritional guidelines provide MRLs and maximum limits for nutrients. The levels given in the FEDIAF guide reflect the amounts of essential nutrients in commercial pet foods that are required to ensure sufficient and safe nutrition in healthy dogs when consumed over time. Minimum recommended levels include a safety margin to prevent deficiencies due to animal variations and nutrient interactions. For commercial dog and cat foods, it is recommended that the nutrient levels are at or above the levels listed in the tables and do not exceed the nutritional or legal maximum [7]. It is important that along with the amounts of protein and fat, commercial dog foods must provide enough essential amino acids and fatty acids. Kazimierska et al. [59] reported that 60% of commercial dry adult dog foods failed to meet at least one of the FEDIAF nutritional guidelines. Sgorlon et al.'s [60] study that aimed to determine the elemental content of commercial adult dog foods found that all analyzed foods provided levels of macro- and micronutrients exceeding the recommended daily intake. Currently, it is difficult to choose the right food for puppies, because manufacturers usually do not declare the fatty acid content of a given food on the label. Furthermore, there is a lack of studies on puppies' food quality, especially when it comes to the nutritional adequacy of fatty acids.

The hypothesis was that commercial complete extruded dry pet food formulated for the growth of dogs would conform to industry labeling regulations and meet or exceed industry nutrient recommendations, particularly those for essential fatty acids. Therefore, the objective of this study was to evaluate the macronutrient composition and fatty acids profile, with particular emphasis on the levels of the long–chain polyunsaturated fatty acids DHA and EPA, of complete extruded diets for growing dogs and to assess their compliance with the recommended allowances for puppies. The results were also compared to the FEDIAF nutritional guidelines [7] and compared to the data on the label.

2. Materials and Methods

The study involved five different types of commercially available dry extruded puppy foods (DEPFs) in Poland in 2022. All products were bought at once, from a combination of physical and online pet stores. The chosen puppy foods for the study were exclusively selected from those available commercially and with a label declaration of the content of EPA and DHA acids. The purchased packages had sizes ranging from 0.3 to 2.5 kg. In the laboratory, bags were opened, and samples were immediately collected from multiple parts of each bag. Samples from three packages (different production batches) of the same diet were combined, pooled, mixed thoroughly and used for analysis. The samples were ground in a laboratory mill type KNIFETEC 1095 (Foss Tecator, Höganäs, Sweden), passed through a 1 mm sieve and stored in sterile containers for subsequent chemical analysis. The samples were labeled with the symbols DEPF_1–DEPF_5.

2.1. Proximate Analyses

In the product samples, the proximate composition was analyzed following AOAC [61] guidelines. Dry matter (DM), crude protein (CP), crude fiber (CF), crude fat (EE–ether extract) and crude ash (CA) were analyzed, while metabolizable energy (ME) and nitrogen–free extract (NFE–an approximation of carbohydrates) were calculated. To determine dry matter, samples were dried at 105 °C to constant weight (method 945.15). The Kjeldahl method was used to assess the sample crude protein content (method 954.01) using a Büchi B–324 distillation unit (Büchi Labortechnik AG, Flawil, Switzerland). Once the total nitrogen content in the samples was determined, the crude protein content was calculated using a conversion factor of 6.25, since most meat proteins contain about 16% nitrogen [62]. Crude fat was determined by means of the Soxhlet method with diethyl ether (method 920.39) and crude ash (method 920.153) by incineration in a muffle furnace at 580 °C. Crude fiber was determined using an ANKOM220 Fiber Analyser (ANKOM Technology, New York, NY, USA). Nitrogen–free extract was calculated via the subtraction of crude protein, crude fat, crude fiber and ash from the total dry matter.

2.2. Energy Value

Gross energy (GE) was calculated by the equation [7,63]:

$$\text{GE} = (5.7 \times \text{protein}) + (9.4 \times \text{fat}) + 4.1(\text{NFE} + \text{crude fiber}) \quad (1)$$

Energy digestibility (ED) was calculated by the equation:

$$\text{ED} = 91.2 - (1.43 \times \%\ \text{crude fiber in dry matter}) \quad (2)$$

In the next step, digestible energy was calculated by the equation:

$$\text{DE (kcal)} = (\text{GE (kcal)} \times \text{digestibility of energy})/100 \quad (3)$$

Metabolizable energy (ME) for dogs, as the final step of calculation, was calculated by the equation:

$$\text{ME} = \text{DE (kcal)} - (1.04 \times \%\ \text{crude protein}) \quad (4)$$

2.3. Fatty Acid Analyses

For analyzing the fatty acid (FA) composition, 20 µL of fat extract was added to 1 mL of Folch solution [64] chloroform (Merck KGaA): methanol (Merck KGaA, 2:1; *v:v;*), 100 µL of Butylated hydroxytoluene (Merck KGaA) and 100 µL of internal standard C21:0 (Merck KGaA, 2 mg/mL). The solution was vortexed for 20 min and centrifuged at 15,000 rpm for 5 min. Supernatant was saponified with 1 mL of 2 M potassium hydroxide (Merck KGaA) methanol solution at 70 °C for 20 min and then methylated with 2 mL of a 14% boron trifluoride methanol solution (Merck KGaA) under the same conditions. A total of 1 mL hexane (Merck KGaA) and 5 mL of saturated NaCl (Merck KGaA) solution were added. For gas chromatography analysis (GC), 0.5 mL of hexane phase was collected. GC was performed using Agilent Technologies 7890A GC System Agilent (Technologies, Santa Clara, CA, USA) with a SUPELCOWAX 10 Capillary GC Column (15 m × 0.10 mm, 0.10 µm; Supelco). The temperature conditions were as follows: initiating temperature, 40 °C for 0.5 min, then increased by 25 °C/min up to 195 °C for 0 min, by 3 °C/min to 205 °C for 0 min and by 8 °C/min to 250 °C for 0.5 min (total analysis time was 16.158 min). Hydrogen was the carrier gas with gas flow of 1 mL/min. FAs were identified by comparing their retention times with standards (Food Industry FAME Mix, Restek) [65]. The list of fatty acids analyzed in this study is shown in Table 1.

Table 1. Fatty acids included in tested complete commercial dry extruded puppy foods.

Common Name	Structural Formula	IUPAC *	*n*−x **
Saturated Fatty Acids (SFAs)			
Myristic Acid	$C_{13}H_{27}COOH$	C14:0	-
Palmitic Acid	$C_{15}H_{31}COOH$	C16:0	-
Stearic Acid	$C_{17}H_{35}COOH$	C18:0	-
Arachidic Acid	$C_{19}H_{39}COOH$	C20:0	-
Behenic Acid	$C_{21}H_{43}COOH$	C22:0	-
Tricosylic Acid	$C_{22}H_{45}COOH$	C23:0	-
Monounsaturated Fatty Acids (MUFAs)			
Palmitoleic Acid	$C_{15}H_{29}COOH$	C16:1 (9)	*n*−7
Oleic Acid	$C_{17}H_{33}COOH$	C18:1 (9)	*n*−9
Elaidic Acid	$C_{17}H_{33}COOH$	C18:1 (9t)	*n*−9
Polyunsaturated Fatty Acids (PUFAs)			
Linoleic Acid	$C_{17}H_{31}COOH$	C18:2 (9, 12)	*n*−6
γ−Linolenic Acid	$C_{17}H_{29}COOH$	C18:3 (6, 9, 12)	*n*−6
Arachidonic Acid	$C_{19}H_{31}COOH$	C20:4 (5, 8, 11, 14)	*n*−6
α−Linolenic Acid	$C_{17}H_{29}COOH$	C18:3 (9, 12, 15)	*n*−3
Eicosapentaenoic Acid	$C_{19}H_{29}COOH$	C20:5 (5, 8, 11, 14, 17)	*n*−3
Docosahexaenoic Acid	$C_{21}H_{31}COOH$	C22:6 (4, 7, 10, 13, 16, 19)	*n*−3

* IUPAC–International Union of Pure and Applied Chemistry; ** *n*−x-in n minus x nomenclature, a double–bond of the fatty acid is located on the xth carbon–carbon bond, counting from the terminal methyl carbon (designated as *n*) toward the carbonyl carbon.

2.4. Label Evaluation

Each product was visually inspected to ensure compliance with the regulatory requirements specified by the FEDIAF [66], with reference to the main ingredient claims for the presence of a specific list of feed material ingredients as specified in the Code of Good Labeling Practice for Pet Food [66]. Additionally, the feeds underwent evaluation for analytical ingredient tolerances in accordance with Tolerances for analytical constituents and additives [66]. Calorie content was evaluated based on the Guaranteed Analysis [7]. The analyzed values were compared with the guaranteed analysis printed on the label. The food products were assessed following the FEDIAF nutrient profiles, which are expressed in units per 100 g of dry matter [7]. The study compared products labeled for puppy growth with the recommended growth guidelines. FEDIAF profiles were analyzed to assess puppy growth based on early growth (less than 14 weeks of age) and late growth (14 weeks of age and older) for CP, EE and fatty acid analysis.

2.5. Statistical Analyses

All samples were examined twice, and the experiment was repeated three times. The information was represented as grams of CP, CF, EE, CA, NFE and FAs per 100 g of DM; ME was reported in kilocalories per 100 g of dry sample.

One factorial analysis of variance (ANOVA) and principal component analysis (PCA) were carried out using the STATISTICA v. 13.0 software (TIBCO Software Inc., Palo Alto, CA, USA). The Tukey's Honestly Significant Difference (HSD) at $p = 0.05$ was used to find the differences between means. The means denoted by different letters differ statistically (for all rows separately–Table 3).

In order to compare the nutritional value of the dog foods, the authors determined their composition profile (CP, EE, CF, CA, NFE, ME) and the profiles of fifteen fatty acids shown in Table 1. The percentage of a given nutrient or metabolic energy in the profile is expressed by an arithmetic mean converted into units on a 9–point scale. For profile comparison, Cohen's profile similarity coefficient r_c was used, calculated according to Cohen's formula [67]. This coefficient value was measured in the range of 1.00 to 1.00, and its interpretation depends on the following values: $x \geq +0.75$ (high similarity); $+0.75 > x > +0.30$ (moderate similarity); $+0.30 \geq x \geq -0.30$ (no similarity); $-0.30 > x > -0.75$ (moderate dissimilarity); $x \leq -0.75$ (high dissimilarity). The closer the values of r_c were to the boundary values ($1/-1$), the stronger the evaluated similarity/dissimilarity was. Inter–profile analysis was conducted using MS Office 2017.

3. Results

3.1. Composition–Ingredients List

Three out of the five analyzed samples (DEPF_1 to DEPF_3) used different genera of fish as the primary source of animal protein. The DEPF_1 was "with" salmon (Table 2). The use of the term 'with' indicates at least 4% of the ingredients listed on the label, or at least 4% of each ingredient [3,66]. DEPF_2 was labeled as "salmon", indicating that it should contain 26% of this ingredient [66]. The DEPF_3 label indicated that it was made with herring, mackerel, flounder, hake and rockfish. The two remaining diets (DEPF_4 and DEPF_5) were based on chicken, which included animal derivatives, as shown in Table 2. According to the DEPF_4 label, the product includes chicken and rice, with a minimum content of 26% of each. DEPF_5 contained fresh chicken with potatoes, with a minimum of 26% fresh chicken and a minimum of 4% potatoes.

According to its label, the DEPF_1 diet was only one diet marketed as grain–free. Both DEPF_4 and DEPF_5 have been labeled as gluten–free diets. In addition to potatoes, the DEPF_1 diet contained peas, sweet potatoes and tomatoes. DEPF_2 and DEPF_5 contained beets as a plant material. The plant material used in DEPF_2 and DEPF_4 was rice and sorghum. The major plant components in the DEPF_3 food included chickpeas, chicory and beans.

Table 2. The main ingredients declared in the analyzed dry extruded puppy foods (DEPF).

Item	Labelling/Component Claims	Animal Sources of Nutrients	Plant Sources of Nutrients	Source of Fat/Oil	Other Ingredients
DEPF_1	with salmon	sea fish, salmon	potatoes, peas, sweet potatoes, tomatoes	salmon oil, canola oil	chicory, yucca schidigera
DEPF_2	salmon	salmon	beetroot, potatoes, rice	animal fat	ginger, flaxseed, yucca schidigera
DEPF_3	with herring, mackerel, flounder, hake and rockfish	herring, mackerel, flounder, blue whiting, hake, rockfish	chickpeas, chicory, beans	sunflower oil	apples, beets, blueberries, burdock root, carrots, chicory, cranberries, holy thistle, kale, lavender, pears, pumpkin, rosehips, seaweed, spinach, turmeric, turnip greens, valerian root, zucchini
DEPF_4	chicken	chicken, eggs	rice, sorghum	animal fat (poultry and pork), krill, salmon oil	algae, calendula, flaxseed, grape seeds, green tea, lecithin, rosemary
DEPF_5	chicken	chicken	apples buckwheat, beetroot, oats, potatoes	chicken fat, salmon oil	algae, black currant, calendula, collagen, dandelion, flaxseed, green–lipped mussel, parsley, rosemary, thyme

All analyzed samples (DEPF_1–DEPF_5) were found to contain animal fat or vegetable oil. The manufacturer listed the ingredients on the corresponding labels. DEPF_3 was the only sample that contained exclusively plant–based oil (sunflower oil) without any added animal fat. DEPF_2 included animal fats that were categorized by type but not specified. Table 2 lists minor ingredients such as herbal raw materials (e.g., burdock root, calendula, chicory, cranberries, dandelion, ginger, green tea, holy thistle, kale, lavender, parsley, rosehips, rosemary, thyme, turmeric, valerian root and yucca schidigera). The diets contain fruits, such as apples, pears, berries and cranberries. Additionally, they contain vegetables like carrots, pumpkin and zucchini, as well as seeds, including grape seeds and flaxseeds. Algae, lecithin or collagen (DEPF_4, DEPF_5) are also included. Algae and green–lipped mussels were used in DEPF_4 and DEPF_5.

3.2. Proximate Composition and Energy Value

The range of dry matter (DM) in the diets tested was from 92.7 g/100 g of fresh diet to a maximum of 96.6 g/100 g of fresh diet (Table 3). Significant differences were found in the proportions of the nutrients evaluated, depending on the diet tested. The lowest protein content was found in DEPF_1 (30.1 g/100 g DM) and the highest in DEPF_3 (35.3 g/100 g DM). Crude fat values were significantly lowest in DEPF_1 (11.7 g/100 g DM) and highest in DEPF_4 (19.5 g/100 g DM). Crude fiber values obtained from the analysis ranged from the lowest in DEPF_3 (4.5 g/100 g DM) to the highest in DEPF_4 (7.8 g/100 g DM), with no statistical difference. Crude ash was significantly lowest in both the DEPF_2 and DEPF_5 diets (6.4 g/100 g DM) and highest in the DEPF_1 diet (7.7 g/100 g DM).

Nitrogen–free extract as total carbohydrates was calculated according to the equation. Significant statistical differences were present in DEPF_4 with the lowest carbohydrate level (27.7 g/100 g DM). The highest carbohydrate supplied was DEPF_1 (39.8 g/100 g DM). The significantly lowest metabolic energy level per 100 g DM was found in DEPF_1 with 355.9 kcal, while the highest was found in DEPF_5 with 407.8 kcal per 100 g DM.

Table 3. Macronutrient content in dry extruded puppy food analyzed and labeled for growth, compared to the recommended essential nutrient concentrations for growth maintenance.

Item	DEPF_1	DEPF_2	DEPF_3	DEPF_4	DEPF_5	Recommendations *	
						Early Growth	Late Growth
Dry matter (g/100 g fresh food)	95.4 [b]	95.8 [b]	92.7 [a]	95.4 [b]	96.6 [b]	NR **	NR
Nutrient (unit/100 g DM)							
Crude protein (g)	30.1 [a]	31.7 [ab]	35.3 [c]	32.8 [bc]	30.8 [ab]	25.0	20.0
Crude fat (g)	11.7 [a]	19.2 [c]	14.5 [b]	19.5 [c]	19.1 [c]	8.5	8.5
Crude fiber (g)	6.1 [a]	6.2 [a]	4.5 [a]	7.8 [a]	4.7 [a]	NR	NR
Crude ash (g)	7.7 [c]	6.4 [a]	7.2 [b]	7.6 [c]	6.4 [a]	NR	NR
NFE (g)	39.8 [c]	32.3 [ab]	31.3 [ab]	27.7 [a]	35.7 [bc]	NR	NR
ME (kcal)	355.9 [a]	397.6 [b]	373.6 [ab]	379.0 [ab]	407.8 [b]	NR	NR

* Minimum recommended level (MRL) for growth of puppies up to 14 weeks of age (early growth) and for puppies over 14 weeks of age (late growth) [7], ** means with at least one some letter in the superscript ([a], [b], [c]) not differ statistically at $p = 0.05$ (for all columns separately), *** NR–No Recommendation; NFE–Nitrogen–Free Extract; ME–Metabolic Energy.

The differences in the levels of the individual components were evaluated (ANOVA), but a comparative analysis of the nutritional profiles of the tested foods as a whole (Cohen's coefficient of profile similarity) was also performed (Table 4). From this analysis, it is clear that the profiles of the basic composition of the foods DEPF_2 and DEPF_5 are very similar, as shown by the Cohen's coefficient of 0.816. These foods are located in the first quadrant of the coordinate system, which is consistent with the relationship expressed by Cohen's coefficient (Figure 3B). Their shared characteristic is a higher metabolic energy content than other feeds (see Figure 3A). The figure also shows no correlation between NFE and ME or between ME and CP and a negative correlation between ME and CA. A moderately positive correlation was observed between metabolizable energy (ME) and gross energy (EE). The factor coordinates of the other feeds (DEPF_1, DEPF_3, DEPF_4) locate them in the other quadrants of the periodic table (see Figure 3B), which clearly indicates their different basic compositional profiles, as confirmed by the negative values of Cohen's coefficient (Table 4). Feed DEPF_4 contained the most CF, which, despite its high CP content, did not allow for a high ME value.

Table 4. Comparative analysis of the nutritional profiles (Cohen's profile similarity coefficient) for analyzed dry extruded puppy foods.

Item	DEPF_1	DEPF_2	DEPF_3	DEPF_4
DEPF_2	−0.682	–	–	–
DEPF_3	−0.145	−0.356	–	–
DEPF_4	−0.255	0.062	−0.250	–
DEPF_5	−0.374	0.816	−0.155	−0.347

—x ≥ +0.75 (high similarity); —+0.75 > x > +0.30 (moderate similarity); —+0.30 ≥ x ≥ −0.30 (no similarity); —−0.30 > x > −0.75 (moderate dissimilarity); —x ≤ −0.75 (high dissimilarity).

3.3. Fatty Acids

The fatty acid profiles of the tested feeds were compared using Cohen's profile similarity coefficient (Table 5) and PCA analysis (Figure 4A,B). The results indicate a markedly different grouping of feeds based on their similarity with respect to their sub–basic composition profile.

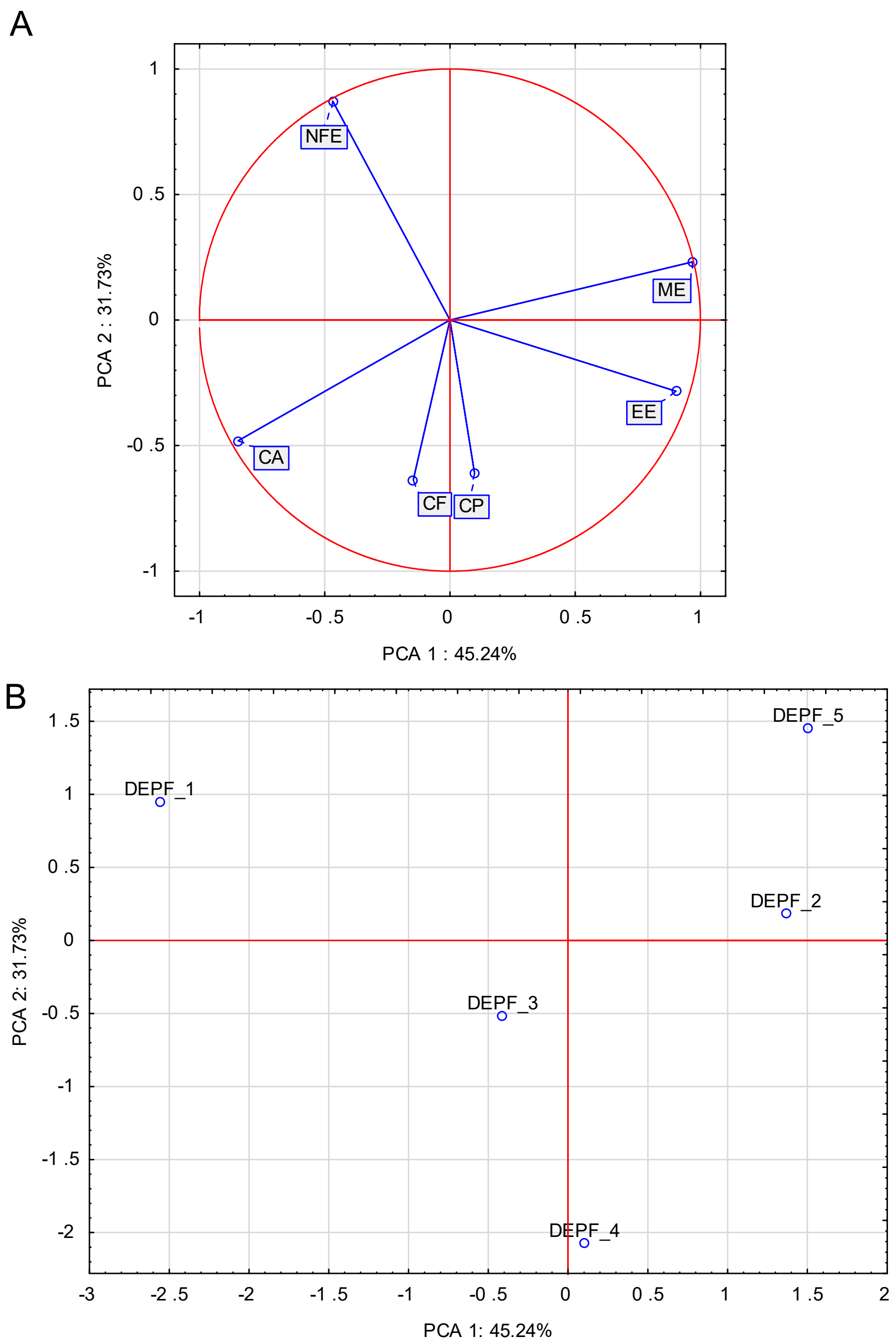

Figure 3. Biplot based on the first two principal component axes for nutritional value and metabolic energy of dog foods (**A**) and distribution of tested puppy foods based on the first two components obtained from principal component analysis (**B**).

Table 5. Comparative analysis of the fatty acids profiles (Cohen's profile similarity coefficient) for analyzed dry extruded puppy foods.

Item	DEPF_1	DEPF_2	DEPF_3	DEPF_4
DEPF_2	−0.516	–	–	–
DEPF_3	0.352	0.175	–	–
DEPF_4	0.167	−0.401	−0.046	–
DEPF_5	−0.045	−0.666	−0.105	0.657

—x ≥ +0.75 (high similarity); —+0.75 > x > +0.30 (moderate similarity); —+0.30 ≥ x ≥ −0.30 (no similarity); —−0.30 > x > −0.75 (moderate dissimilarity); —x ≤ −0.75 (high dissimilarity).

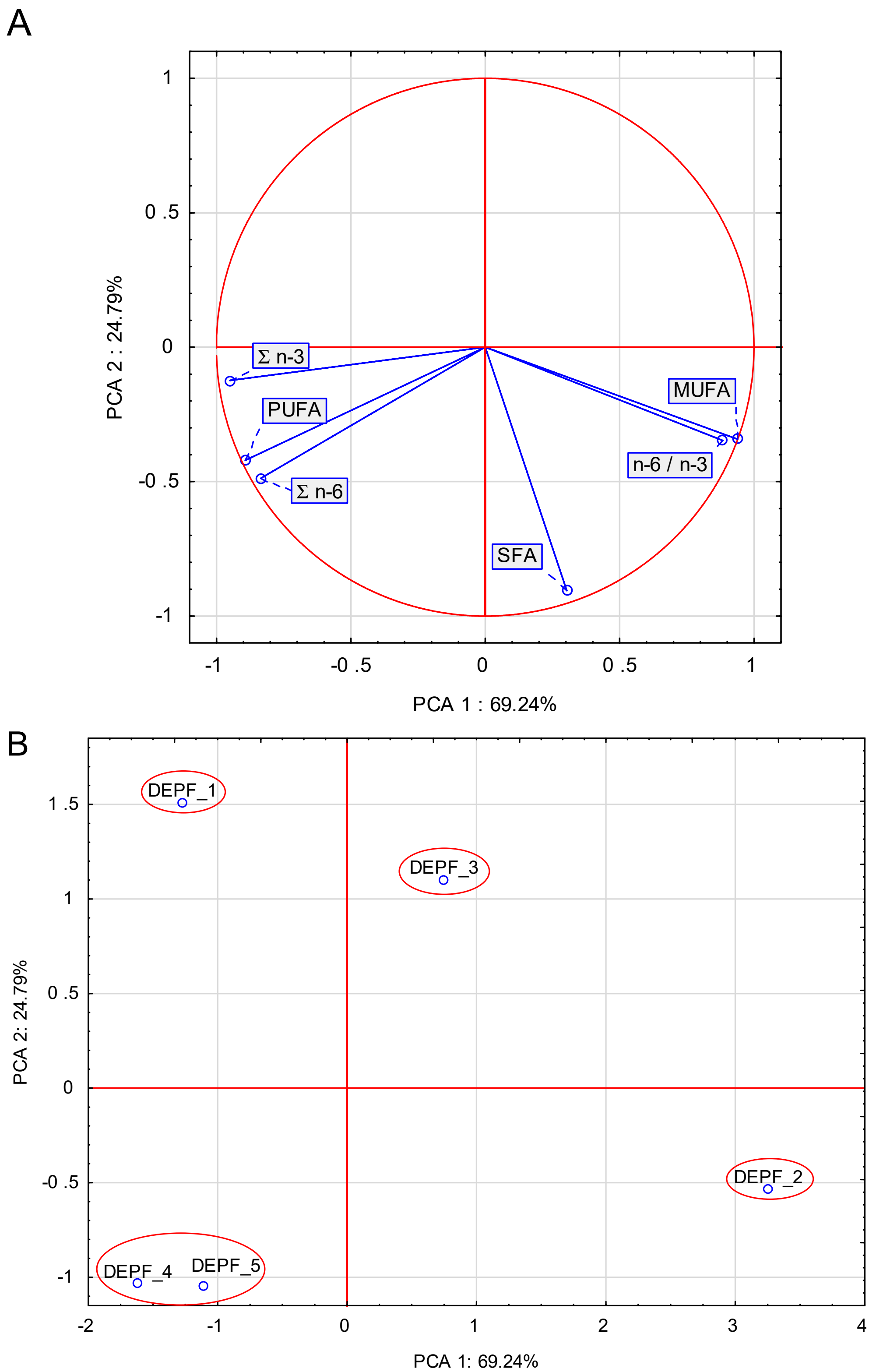

Figure 4. Biplot based on the first two principal component axes for fatty acids composition of dog foods (**A**) and distribution of tested puppy foods based on the first two components obtained from principal component analysis (**B**).

Symptomatic is the similarity of the primary composition profiles of DEPF_2 and DEPF_5 feeds, as mentioned earlier, and the lack of similarity in the fatty acid profiles of these feeds (−0.666). The fatty acid profiles of DEPF_4 and DEPF_5 feeds exhibit high similarity (0.657), despite their significantly different basal composition profiles (as indicated in Table 4, Figure 3A,B). PCA analysis indirectly supports these observations (as shown in Figure 4A,B). The DEPF_4 and DEPF_5 feeds have high levels of $n-3$ and $n-6$ acids and PUFAs, in contrast to the other feeds, and particularly the DEPF_2 feed, which has high $n-3/n-6$ and MUFA quotient values. The quotient was 15.23, which is 2–3 times higher than that found in the other feeds, ranging from 3.35 to 6.34. The food also had a high SFA content.

Based on a study of five puppy foods, PCA analysis showed a high positive correlation between the quotient of $n-6$ and $n-3$ fatty acids with MUFA content, as shown in Figure 4A. Additionally, it can be confirmed that there is no correlation between SFAs and the other parameters used in the analysis.

Table 6 displays laboratory analysis results for fatty acids in dry extruded puppy diets and the corresponding FEDIAF recommendations per 100 g of dry matter. The DEPF_2 feed had the lowest amount of LA (0.55 g/100 g DM), and this value was 57.69% lower than the MRL for this fatty acid in puppies (early and late growth). The DEPF_3 feed had the highest amount of this fatty acid (3.37 g/100 g DM), which met the established MRL. Among the tested feeds, DEPF_1 had the lowest AA (0.01 g/100 g DM), which was 66.67% below the recommended MRL, whereas DEPF_2 had the highest level of AA at 0.06 g/100 g DM, which exceeded the MRL. DEPF_2's ALA level was the lowest (0.03 g/100 g DM) and did not meet the required MRL, being 62.50% below the MRL. The DEPF_4 diet had the highest ALA content (0.74 g/100 g DM) that met the MRL for this acid. The DEPF_2 diet had the lowest concentrations of EPA (0.01 g/100 g DM) and DHA (0.02 g/100 g DM). The total yield of EPA + DHA was 0.03 g/100 g DM, which is 40% lower than the MRL. The DEPF_5 diet had the highest EPA content (0.07 g/100 g DM of EPA and 0.09 g/100 g DM of DHA). When combined, the total EPA + DHA content was 0.16 g/100 g DM. This value is compliant with the MRL for the combined sum of eicosapentaenoic acid and docosahexaenoic acid.

Table 6. Fatty acids (per 100 g DM) content in analyzed dry extruded puppy foods compared to the FEDIAF recommendations for fatty acids for growth of puppies and adult dogs.

Nutrients	Unit	Content					Recommendations *		
		DEPF_1	DEPF_2	DEPF_3	DEPF_4	DEPF_5	Early Growth	Late Growth	Adult
$n-6$:									
LA	g	2.10	0.55	1.18	2.85	3.37	1.30	1.30	1.32
AA	g	0.01	0.06	0.05	0.04	0.03	0.03	0.03	NR *
$n-3$:									
ALA	g	0.58	0.03	0.23	0.74	0.38	0.08	0.08	NR
EPA	g	0.03	0.01	0.02	0.05	0.07	NR	NR	NR
DHA	g	0.03	0.02	0.04	0.05	0.09	NR	NR	NR
EPA + DHA	g	0.06	0.03	0.06	0.10	0.16	0.05 **	0.05 **	NR
label EPA + DHA	g	0.1	0.24	1.82	0.22	0.94	–	–	–

* Ref. [7], NR = No Recommendation, ** recommendation for both EPA and DHA as a total. LA–Linolenic Acid; AA–Arachidonic Acid; ALA–α−Linolenic Acid; EPA–Eicosapentaenoic Acid; DHA–Docosahexaenoic Acid.

According to the analysis of EPA and DHA levels in the diets, their actual levels were lower than the levels claimed on the label (Table 6). The DEPF_1 feed showed the smallest difference between the label and actual values at 40%. However, in the DEPF_3 feed, the actual levels of EPA and DHA were 96.71% lower than the label data. All analyzed samples contained elaidic acid C18:1, trans. The DEPF_2 diet exhibited the highest content of elaidic acid (0.93 g/100 g DM). In the other samples, the amount of C18:1 trans was lower compared to DEPF_2 by 59.14% (DEPF_1), 46.24% (DEPF_3), 49.47% (DEPF_4) and 51.62% (DEPF_5).

3.4. Labelling

Data from our own analyses were compared to the list of the analytical constituents on the label. The percentage of the labeled value measured for each nutrient and for metabolizable energy of foods has been shown in Table 7. No product met or exceeded all labeled maxima as compared to the labelled guaranteed analysis.

Table 7. Percentage (%) of label guaranteed fulfilled for each nutrient of the guaranteed analysis in tested dry extruded puppy foods.

Item	Moisture		Crude Protein		Crude Fat		Crude Fiber		Energy	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
DEPF_1	↓ 48.7%	9–11	WPT	27–33	↓ 19.2%	14.5–18.9	WPT	4.7–6.4	↓ 6.3%	380–420
DEPF_2	↓ 48.1%	8.1–9.9	WPT	31.1–37.1	WPT	19.8–24.2	↑ 63.7%	2.17–3.8	↓ 7.2%	428.4–473.6
DEPF_3	↓ 32%	10.8–13.2	↓ 3.9%	36.8–38.8	↓ 15.5%	17.1–21.5	↓ 23%	5.8–7.8	↓ 10.2%	416.1–459.9
DEPF_4	↓ 36.4%	7.2–8.8	WPT	31.8–37.8	↓ 10%	21.7–26.1	↑ 93%	2.5–4.1	↓ 16.6%	454.1–501.9
DEPF_5	↓ 62.7%	9–11	↓ 2.1%	31.4–37.4	↓ 9.7%	21.1–25.5	↑ 54.3%	1.4 – 3.0	↓ 5.2%	430.4–475.7

↑ the value obtained from our own analyses above the marked value of the maximum tolerance for analytical constituents [3,66]; ↓ the value obtained from our own analyses below the marked value of the maximum tolerance for analytical constituents [3,66]; WPT–Within the Permitted Tolerance.

The moisture level was below tolerance, resulting in the higher dry matter of food. The differences between the moisture levels and the minimum acceptable level ranged from 32% to 62.7% below the minimum. The crude protein contents of three diets, DEPF_1, DEPF_2 and DEPF_4, were within the tolerance range. The diets which met the tolerance range for crude protein were DEPF_1, DEPF_2 and DEPF_4. For the other two diets, DEPF_3 and DEPF_5, the crude protein content was lower than the minimum level established by the Regulation by 3.9% and 2.1%, respectively. The crude fat content was found within the tolerance range only in the DEPF_2 diet. The results of the other diets indicated an underestimation of fat content in relation to the label data. In the DEPF_5 diet, the crude fat content had the lowest value, which was 9.7% below the minimum. In the DEPF_1 diet, it had the highest value, which was 19.2% below the established minimum. One out of five samples for crude fiber were found within the tolerance range for this component. There was not only an undersupply below the minimum, but also an oversupply above the maximum for that nutrient. Only the DEPF_1 diet was within the accepted range of tolerance. The crude fiber content of only the DEPF_3 diet was lower than the accepted minimum, while the other diets exceeded the permitted range. The crude fiber levels for the DEPF_2, DEPF_4 and DEPF_5 diets exceeded the acceptable maximum by 63.7%, 93% and 54.3%, respectively. The calculated energy values (ME) for each analyzed sample were lower than the tolerance range. In the DEPF_4, ME was 16.6% below the minimum acceptable level. The energy value of the DEPF_5 diet had the least deviation from the minimum allowed value, with a difference of 5.2% below the tolerance range.

The fatty acid content of the analyzed dry extruded puppy foods was compared with the FEDIAF recommendations [7] of fatty acids for puppy and adult dog in Table 6. The analysis revealed that the EPA and DHA values in the examined foods were lower than those listed on the product packaging. The EPA and DHA contents of the DEPF_1 diet were found to be 25% and 50% lower, respectively, than their respective declared values. As only the total value was declared, it is not possible to determine the extent to which the EPA and DHA levels in the DEPF_2 diet were lower than claimed by the manufacturer. The DEPF_3 diet displayed the largest discrepancy between the actual levels and the manufacturer's declared levels of these fatty acids. The diet's EPA and DHA contents were 99.98% and 99.96% lower than the respective levels declared on the package. Both the EPA and DHA levels of the DEPF_4 diet were 55% lower than the claimed levels. The EPA and DHA levels of the DEPF_5 diet significantly deviated from the declared levels. The declared EPA level was 87.5% higher and the declared DHA level was 77% higher than their actual levels. The packaging of the product is not obligated to present the ratio of $n-6$ to $n-3$. However, it could be calculated if the content of $n-6$ and $n-3$ fatty acids is provided by the manufacturer. The studied diets showed a variation in the $n-6/n-3$ fatty acids ratio ranging from 3.35 to 15.23. The DEPF_1 diet had the lowest ratio of these fatty acids, while the DEPF_2 diet had the highest ratio. None of the studied diets met the manufacturer's ratio of $n-6$ to $n-3$ fatty acids.

4. Discussion

Compliance with the FEDIAF nutritional guidelines assists manufacturers in ensuring that their products supply dogs with the necessary nutrients for optimal health and vitality. The FEDIAF nutritional guidelines take into account the different dietary requirements of dogs at different life stages, allowing manufacturers to develop specific diets that promote growth, energy levels and overall health. Pet owners aim to make the right nutritional choices for their pets, and products that meet recognized industry standards provide them with a sense of confidence and reliability. Failure to comply with the FEDIAF regulations may cause an imbalance in the nutritional composition of pet foods, leading to deficiencies or excessive amounts of key nutrients. Unbalanced diets can result in nutritional deficiencies or health issues for dogs [59,68–73].

Several studies aim to evaluate the nutritional value of dry and moist foods for adult and growing dogs. These studies reveal inconsistencies in the actual contents of certain ingredients when compared to FEDIAF or AAFCO nutritional guidelines. Rolinec et al. [55] analyzed the results of selected dog foods and compared them to the nutrient values declared on the product packaging. The study found that none of the samples were within ±5% of the stated crude fat concentration. Furthermore, certain samples only partially matched the declared crude protein and crude fiber values.

Proteins are crucial for tissue, organ, muscle and cell growth, making them especially important during the growth phase of a puppy's life for the formation of new tissues and the repair of damaged tissues. Insufficient intake leads to reduced protein turnover and progressive reduction in lean body mass, especially in older dogs [74,75]. Fat serves crucial functions, not only providing energy, but also constructing cell membranes and contributing to diverse cellular processes. It plays a role in cell signaling, which can impact the immune system and inflammatory response [10,14,17].

Gagné et al. [76] and Olivindo et al. [77] found that some feeds analyzed did not contain adequate levels of minerals such as calcium and phosphorus. Sgorlon et al. [60] reported inadequate levels of macronutrients such as selenium, copper, potassium and magnesium in the feeds they investigated. The imbalance problem may be more common in diets containing multiple sources of protein than in diets containing a single source of animal protein [78].

Failing to meet minimum requirements for micronutrients and trace elements in dietary intake can lead to a variety of health problems in the future. Insufficient amounts of calcium and phosphorus, as well as an abnormal ratio during the body's growth stage, can result in abnormal bone and cartilage tissue development [79]. They can also cause abnormal development of the musculoskeletal system or increase susceptibility to damage [80].

It is not just the balance of the diet that is important for the proper development of a dog, but also the safety of the nutritional products used. Shao et al. [81] demonstrated the presence of mycotoxins in both adult and puppy dog food. Out of 32 samples, only 1 was found to be uncontaminated. Kazimierska et al. [82] showed that in addition to the presence of mold in one food, staphylococci were detected in others. Geicu [83] found microbial contamination in feeds with key ingredients such as corn, beets, oils of different origins, rice and wheat. Brazis et al. [84] discovered that dry dog food is a suitable habitat for mites during storage. Furthermore, the team detected mites in one sample right after opening, suggesting that the originally unopened food was already contaminated with mites. In Witaszak et al.'s [85] study, the EU's permissible limits for microbiological contaminants in veterinary feeds were not exceeded, despite their presence.

While there is literature pertaining to the evaluation of major nutrients in food for adult dogs, only a minor portion of it is dedicated to determining levels of other crucial elements, such as fatty acid composition [86–88]. Dietary fats serve not only as an energy reserve, but also play a crucial role in multiple stages of growth. Specifically, lipids are a pivotal component of cell membranes during cellular development. The fatty acid balance in a puppy's diet can impact membrane structure and integrity, which in turn affects cellular communication, function, brain development and immune system support through their anti–inflammatory properties [19,26,27,34,89]. A reduced fat level in the diet could also negatively affect the condition of the skin and coat [18,90]. As demonstrated by Dodd [91], none of the seven plant–based dog foods labeled for puppies or all life stages met the MRLs for DHA and EPA regulated by the FEDIAF nutritional guidelines. In the canine body, the activity of enzymes Δ5–desaturase and Δ6–desaturase is limited, leading to inadequate conversion of precursors into EPA and DHA. Therefore, it is necessary for dogs to obtain EPA and DHA through their diet [14]. The primary source of EPA and DHA acids are marine fish and the oils derived from them (Figure 5) [92–95].

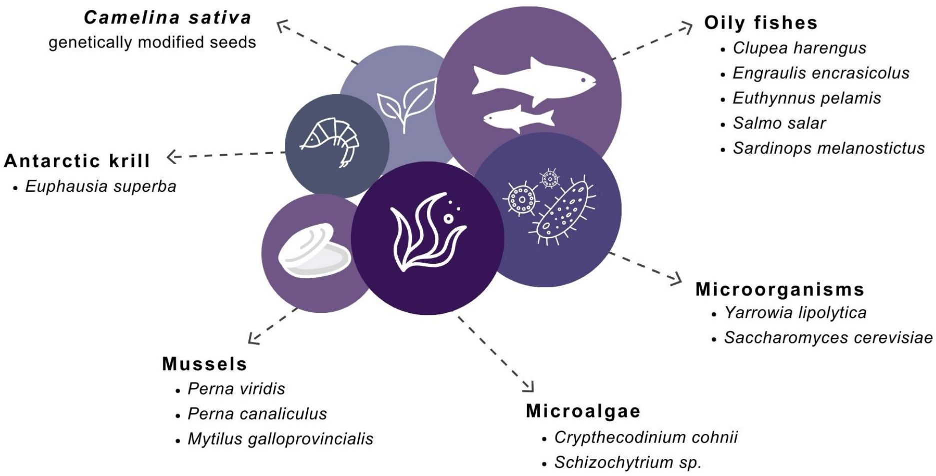

Figure 5. Potential sources of dietary LC–PUFAs EPA and DHA based on [92,96–130].

Uncommon but effective sources of EPA and DHA include genetically modified *Camelina sativa* seeds [96–99,131–133] or microorganisms that efficiently synthesize LC–PUFAs after genetic modification and optimal cultivation conditions [100,101,114,120,134]. Herring, mackerel and salmon, along with green–lipped mussel, algae and krill, were utilized as raw materials in the examined formulas. Even though these are deemed as good sources of fatty acids, the amounts used in the diets did not meet the manufacturer's claims on the food package. The issue with utilizing EPA and DHA fatty acids in dry dog food commercially is that LC–PUFAs are highly susceptible to oxidation due to their high level of unsaturation. Consuming products high in fatty acid oxidation products can have adverse effects on the body. Ingesting malondialdehyde (MDA), a byproduct of lipid oxidation, may cause the formation of extra nucleotide linkages in DNA [135].

An imbalanced ratio of $n-6$ to $n-3$ fatty acids in the diet primarily reduces the level of $n-3$ fatty acids in the body [136]. In contrast, a high consumption of $n-3$ acids can decrease the level of metabolic products of $n-6$ acids [137]. This, in turn, can reduce the likelihood of developing inflammation, allergies or skin diseases. With regards to commercial granulated diets, Popović et al. [138] showed that the content of certain fatty acids supplied with the diet can be well reflected in blood parameters by influencing the lipid composition of plasma membranes or erythrocytes. This finding can be used to determine the intake of fatty acids, particularly $n-3$, which our study shows is present in modest concentrations in certain commercial diets. Fish oils that are rich in the LC–PUFAs EPA and DHA were found to lower levels of blood triglycerides in dogs [139,140].

An excess of $n-6$ fatty acids is a threat to the proper utilization of $n-3$ fatty acids supplied with food because fatty acids from the $n-3$ and $n-6$ families compete for the same enzymes required to convert these acids into a usable form [141–143]. Additionally, dogs that received menhaden oil and flaxseed oil in their diet with $n-6/n-3$ ratios of 0.34:1 and 0.38:1, respectively, exhibited lower superoxide production, which has been associated with an increase in reactive oxygen species in the body [144]. These findings were compared to dogs fed diets with $n-6/n-3$ ratios such as 100:1 (safflower oil) and 9.7:1 (beef tallow), both of which had higher superoxide production. Hall et al. [145] recommend a ratio of $n-6$ to $n-3$ of 1.4:1 or even 1:1 for dogs' optimal fatty acid intake from both families in the proper proportion. According to a study by Kearns et al. [89] and Vaughn et al. [146], the ratios of these acids were suggested to be 5:1 and 10:1. The analyzed samples exhibited a range of $n-6/n-3$ fatty acid ratios from 3.35 to even 15.23.

Dry foods, as processed foods, may contain trans fats derived from technological processes, as shown in our study. There has been a dearth of research concentrating on their effects on dogs' bodies, which can be different from those of humans in many ways. Trans fatty acids, such as the C18:1 acid isomer, have the potential to be generated during the industrial process. The application of vegetable oils, such as those high in polyunsaturated fatty acids, which may be hydrogenated during subsequent processing, resulting in the formation of a trans configuration [147]. Fat changes take place during the production of extruded animal feed as a result of high temperatures and technological processes. These processes can significantly reduce the biological value of fatty acids [148,149]. Trans isomers are also thought to negatively impact inflammation and increase oxidative stress [150]. Ohmori et al. [151] found that this acid, as compared to its cis form oleic acid (C18:1 $n-9$), was associated with the development of colon cancer. It does so by increasing the proliferation of tumor cells and contributing to the development of liver metastases. However, studies on mice reported increased oxidative stress as the acid reduced vitamin E concentration [152]. Ma et al. [153] investigated the effect of this acid on neuroblastoma cells (SH–SY5Y). The obtained results suggest that C18:1 trans decrease cell viability and induces cell death by enhancing oxidative stress. This oxidative stress caused the accumulation of superoxide and malondialdehyde. Plötz [154] observed toxicity against pancreatic β–cells in another model consisting of both rat and human samples.

5. Conclusions

For young puppies in the early growth stage (under 14 weeks old), the minimum recommended protein level is 25 g per 100 g of dry matter. For late–growth–phase puppies (over 14 weeks old), a minimum of 20 g of protein per 100 g of dry matter is advised. Even though two of the five feeds contained a protein content lower than what was declared on their packaging and did not meet the permitted tolerance calculated according to Regulation 767/2009 [3], they still fulfilled the recommended dietary minimums recommended by the FEDIAF [7].

Puppies in both early and late growth stages should consume a minimum of 8.5 g of fat per 100 g of dry food matter, as recommended. All of the evaluated feeds fulfilled these requirements, but only one met the manufacturer's fat level, which was within the permitted tolerance. The levels of certain essential fatty acids were below the values recommended by the FEDIAF [7]. Out of the five feeds, two had all essential fatty acids at levels above the recommended minimum. The remaining three feeds had insufficient levels of at least one fatty acid that is considered essential during these developmental stages. The analysis showed that each of the diets in the study had lower levels of EPA and DHA acids than the levels claimed on their packaging. None of the diets evaluated met the ratio of $n-6$ to $n-3$ fatty acids as recommended by the manufacturer. Dietary requirements for the minimum level of crude fiber are not mandatory. Its levels could only be compared with the label data, which demonstrated that only one diet had a crude fiber content that was similar to the label data. None of the diets matched the metabolic energy amount stated on the product label, and therefore did not comply with the allowed tolerance according to Regulation 767/2009 [3].

As previously discussed, there is a lack of research on the nutritional adequacy of growing dog foods, particularly regarding levels of fatty acids such as DHA and EPA. Therefore, it is reasonable to conclude that this study demonstrates that these acids are essential dietary components essential for proper body development and are present at very low levels, in some cases not even meeting the FEDIAF nutritional guidelines. Although some foods meet the nutritional requirements, inaccuracies in labeling were observed. It is crucial to pay attention to the levels of essential fatty acids and the potential health risks associated with byproducts produced during the manufacturing process. These findings highlight the necessity for better control over the composition of dry puppy foods to support optimal development.

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