Original Paper

Fatty Acid Profile of Commercial Dry Puppies' Food

Matúš Džima[1], Boglárka Bodon[2], Daniel Bíro[1], Milan Šimko[1], Branislav Gálik[1], Michal Rolinec[1], Ondrej Hanušovský[1], Mária Kapusniaková[1], Stanislava Drotárová[1], Andrej Duchoň[1], Štefánia Buschbacher[1], Ela Tarišková[1], Miroslav Juráček[1]*

[1]*Slovak University of Agriculture in Nitra, Slovakia*
[2]*P.G. Trade, spol. s r.o. Komárno, Slovakia*

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The aim of this study was to determine the fat content and fatty acid profile of 5 commercial dry granulated dog foods purchased in Slovakia and to compare the analyzed content with the minimum requirements for fat and fatty acid content according to FEDIAF (2021), and also to compare the fat content with the manufacturer's declared fat content on the packaging. The samples were analyzed in the Laboratory of Quality and Nutritional Value of Feeds, Slovak University of Agriculture in Nitra, according to standard laboratory procedures and techniques. The results confirmed that four out of the five puppy compound feeds tested, met the requirements for fatty acid content according to FEDIAF (2021). All samples met the requirements for a minimum fat content of 85.00 $g.kg^{-1}$ DM, a minimum linoleic acid content of 13.00 $g.kg^{-1}$ DM, a minimum arachidonic acid content of 0.30 $g.kg^{-1}$ DM, and an α-linolenic acid content of 0.80 $g.kg^{-1}$ DM. Four samples met the minimum EPA + DHA content requirement, while sample E did not meet the minimum limit (0.50 $g.kg^{-1}$ DM). When comparing the declared fat content on the packaging and the determined total fat content of the tested foods, we found a lower fat content of 0.23% for sample B and 4.78% for sample C.

Keywords: puppy, nutrition, complete food, fat quality

1 Introduction

Feeding and proper nutrition of puppies is focused on providing proper growth, long term health with subsequent achievement of the highest possible age of the dogs. It is therefore important that a balanced puppy food contains all nutrients in the right proportion and composition. Fats are an essential component of the diet of dogs, not only to provide energy, through beta-oxidation of fatty acids, but also as a source of n-3 essential fatty acids: α-linolenic, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and also the n-6 fatty acids: linoleic acid and arachidonic acid (Han and Ye, 2021). Moreover, to being a primary source of energy, they are part of cell membranes, play a role as cofactors of enzymes, they are part of hormones and many other molecules (Gaylord et al., 2018). On the other hand, the amount of fat and fatty acid composition can influence oxidative stress in dogs (Mihok at al., 2023). EPA and DHA acids are irreplaceable (essential) in the diet of dogs as polyunsaturated long-chain n-3 FAs because of the limited ability of dogs to synthesize them. Deficiency of unsaturated fatty acids has been associated with various health disorders such as infertility, inflammatory diseases, cardiovascular diseases, cognitive dysfunction, visual disorders, or slow growth in young dogs (Gaylord et al., 2018; Alonge et al., 2019; Santos et al., 2020). The European Federation of Pet Food Manufacturers (FEDIAF) lists recommended minimum requirements for fat and fatty acid content in puppy diets (FEDIAF, 2021). The aim of this study was to determine the fat and fatty acid content of dry granulated puppy foods formulas available on the Slovak market and to compare the analyzed content with the manufacturer's declared content and also with the minimum requirements for fat and some fatty acid content according to FEDIAF (2021).

***Corresponding Author:** Miroslav Juráček, Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Nutrition and Genomic, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovakia
miroslav.juracek@uniag.sk https://orcid.org/0000-0003-1260-2319

2 Material and methods

Five dry granulated puppy feeds (three repetitions from each sample) purchased in Slovakia were analyzed for fat and fatty acid content. Each sample was labelled with the letter A, B, C, D or E. Samples of the granulated feeds were from different manufacturers and were designed for puppy nutrition. Samples were analyzed in Laboratory of Quality and Nutritional Value of Feeds (Department of Animal Nutrition, Institute of Nutrition and Genomics, FAFR, Slovak University of Agriculture in Nitra). The dry dog food samples were stored at -40 °C in a deep-freeze cabinet (EVERmed, Italy) until analyzed. The dry matter (DM) content was determined by drying the sample until a constant weight was achieved at a temperature of 103 ±2 °C. The crude fat content was analyzed according to the regulation of the Slovak Ministry of Agriculture no.2145/2004-100. The crude fat content after acid hydrolysis was determined by extraction using the Soxhlet principle. For the determination of FAMEs (FA methyl esters), the GC system Agilent 6890A was utilized, equipped with a split injection autosampler, DB 23 analytical column, and flame ionization detector (FID). The manufacturer's declared nutrient concentration in the dry dog food was verified from the batch of dry dog food. The total fatty acid content in g.kg^{-1} DM was calculated from the determined fat content using a conversion factor of 0.95 for the conversion of fatty acid content. Subsequently, the amount of individual fatty acids was calculated from the total fatty acid content and the ratio of fatty acids determined in the samples. Concentration of analyzed nutrients is shown in grams per kilogram of dry matter. The results were statistically analyzed using the statistical program SPSS 26.0 (IBM) using one-way ANOVA. Tukey test was used for evaluation of statistical significance between variables (FA, fat and DM content).

3 Results and discussion

Table 1 shows the values of declared and analyzed fat. The following feed materials were declared as significant sources of fats/oils in the analyzed puppy foods: Sample A – animal fat, fish oil, soybean oil; Sample B – animal fat, fish oil, coconut oil; Sample C – chicken fat, salmon oil; Sample D – chicken fat, salmon oil; Sample E – chicken fat, salmon oil. From the above, all samples contained animal sources of fats, each sample contained fish oil as a source of polyunsaturated fatty acids (PUFA), mainly EPA and DHA (Shahidi et Ambigaipalan, 2018; Molversmyr et al., 2022). Only in sample A: soybean oil and in sample B: coconut oil were declared as significant sources of plant-derived fats. Coconut oil can be identified as a source of medium-chain fatty acids (MCFAs), mainly lauric acid, while soybean oil is a source of PUFAs, mainly linoleic acid (O'keefe et al., 2015; Mat et al., 2022). Sample A had the total highest analyzed fat content followed by samples B, D, E and C. All 5 samples of dry granulated puppy foods exceeded 85.00 g.kg^{-1} DM, which is the minimum amount of fat according to the requirements for the both – early and late growth (FEDIAF, 2021) as shown in Table 3. Samples A, D and E contained the amount of fat declared on the packaging. Samples B and C were found to be 0.23% (B) and 4.78% (C) lower in fat content than declared on the packaging. Rolinec et al. (2016) analyzed 15 dry granulated dog foods on the Slovak market, and in twelve of the analyzed foods the fat content was more than 10% lower than the declared content.

The fatty acid contents determined in the five commercial puppy feeds are listed in Table 2. Among the fatty acids, the highest oleic acid content (C18:1cis n9) was recorded, ranging from 60.739 (C) to 82.203 (D) g.kg^{-1} DM. Statistically significant differences (P <0.05) were observed between sample D with the highest oleic acid content and sample A with a difference of 10.29%, sample E with a difference of 13.67%, and sample C with 26.12% difference. Oleic acid belongs to MUFAs which are typically found in various oils and are considered healthier than SFAs. SFAs are beneficial for dogs when included in their diet in appropriate amounts. SFAs are found in various animal fats and some plant oils. High levels of SFAs, such as palmitic, myristic or lauric acid, can be associated with health issues. In the context of dog diets, it's important to monitor and control the intake of SFAs to maintain optimal health (Crisi et al., 2021; Hall

Table 1 Declared and analyzed fat and DM content of granulated compound feed for puppies

	A	B	C	D	E
	g. kg^{-1} DM				
Declared fat content	220.00	230.00	180.00	200.00	170.00
Fat (analyzed)	231.43^{a} ±0.98	229.48^{a} ±1.95	171.39^{b} ±1.94	222.76^{a} ±1.58	205.81^{c} ±0.58
Declared DM	max. 950.00	920.00	900.00	900.00	900.00
DM (analyzed)	929.90^{a} ±5.26	946.50^{b} ±8.03	949.00^{b} ±4.02	970.55^{c} ±6.86	947.50^{b} ±2.68

A, B, C, D, E – dry granulated compound feed; DM – dry matter; ^{abc} – means within a row with different et Jewell, 2012). The second most represented acid was palmitic acid (C16:0). Its content ranged from 30.198 (C) to 49.669 (B) g.kg^{-1} DM. Statistically significant (P <0.05) lower content of palmitic acid compared to sample B was found in sample A by 6.50%, sample E by 8.96%, sample D by 12.99% and sample C by 39.20%. The third most abundant fatty acid in the samples was linoleic acid (C18:2cis n6), which content varied from 25.227 (C) to 53.788 (A) g.kg^{-1} DM. Sample A in comparison to sample B, sample E and sample D and sample C had statistically significant (P <0.05) lower linoleic acid content by 22.91%, 37%, 41.92% and 53.10%, respectively. All tested samples had linoleic acid content higher than the minimum recommended amount of 13.00 g.kg^{-1} DM according to FEDIAF (2021) and at the same time none of the samples exceeded the maximum nutritional limit of 65.00 g.kg^{-1} DM for early growth.

Other fatty acids present in samples were α-linolenic acid (ALA, C18:3 n3), arachidonic acid (C20:4 n6), eicosapentaenoic acid (EPA, C20:5 n3) and docosahexaenoic acid (DHA, C22:6 n3). N-3 fatty acids, including ALA, EPA, and DHA, are essential components of a dog's diet for proper growth, development, and

Table 2 Fatty acid content of granulated puppy foods

Fatty acid	A	B	C	D	E
	g.kg^{-1} DM				
C8:0	ND	0.478 ±0.001	ND	ND	ND
C10:0	ND	0.424 ±0.000	ND	ND	ND
C12:0	3.083^{a} ±0.015	3.470^{b} ±0.012	0.586^{cd} ±0.001	0.691^{d} ±0.014	0.398^{c} ±0.113
C14:0	3.958^{a} ±0.048	3.194^{ab} ±0.021	3.137^{ab} ±0.005	3.616^{ab} ±0.070	2.706^{b} ±0.602
C14:1	0.509 ±0.003	ND	ND	ND	ND
C15:0	ND	ND	0.286 ±0.000	ND	0.435^{b} ±0.074
C16:0	46.440^{a} ±0.341	49.669^{b} ±0.488	30.198^{c} ±0.039	43.218^{d} ±0.632	45.215^{a} ±0.016
C16:1	10.005^{a} ±0.046	10.679^{b} 0.084	5.225^{c} ±0.004	6.903^{d} ±0.114	7.404^{d} ±0.326
C17:0	ND	0.332^{a} ±0.008	0.517^{b} ±0.004	0.703^{c} ±0.005	0.851^{d} 0.014
C18:0	12.287^{a} ±0.114	12.216^{a} ±0.133	13.418^{b} ±0.009	19.458^{c} ±0.225	19.136^{c} ±0.133
C18:1cis n9	73.752^{a} ±0.378	81.417^{b} ±0.707	60.739^{c} ±0.061	82.203^{b} ±0.707	70.963^{a} ±2.211
C18:2cis n6	53.788^{a} ±0.245	41.464^{b} ±0.343	25.227^{c} ±0.076	31.236^{d} ±0.475	33.900^{d} ±1.623
C18:3 n6	0.324 ±0.004	ND	ND	ND	ND
C18:3 n3	4.304^{a} ±0.016	3.298^{b} ±0.031	4.802^{a} ±0.024	4.788^{a} ±0.085	6.604^{c} ±0.319
C20:0	ND	ND	0.372^{a} ±0.001	0.470^{b} ±0.000	0.305^{c} ±0.001
C20:1 n9	1.017^{a} ±0.008	1.295^{b} ±0.006	2.630^{c} ±0.004	2.661^{c} ±0.024	0.827^{d} ±0.039
C20:2 n6	0.420^{a} ±0.003	0.429^{a} ±0.000	0.807^{b} ±0.004	0.949^{c} ±0.011	0.377^{d} ±0.019
C20:4 n6	0.669^{a} ±0.002	0.514^{b} ±0.009	0.346^{c} ±0.004	0.500^{b} ±0.012	0.499^{b} ±0.023
C20:3 n3	ND	ND	0.314 ±0.004	ND	ND
C20:5 n3 (EPA)	1.976^{a} ±0.020	0.909^{b} ±0.003	1.828^{c} ±0.017	1.532^{d} ±0.020	ND
C22:1 n9	ND	ND	0.325 ±0.004	ND	ND
C24:1 n9	0.876^{a} ±0.001	1.558^{b} ±0.028	2.341^{c} ±0.020	2.034^{d} ±0.060	0.360^{e} ±0.020
C22:6 n3 (DHA)	ND	ND	0.290 ±0.008	ND	ND
Unidentified fatty acids	6.447^{a} ±0.021	6.661^{a} ±0.095	9.433^{b} ±0.058	10.671^{c} ±0.127	5.536^{d} ±0.129
PUFA	61.481^{a} ±0.281	46.614^{b} ±0.379	33.613^{c} ±0.129	38.994^{d} ±0.603	41.380^{d} ±1.984
MUFA	86.159^{a} ±0.436	94.948^{b} ±0.824	71.261^{c} ±0.077	93.801^{b} ±0.905	79.553^{d} ±2.596
SFA	65.767^{a} ±0.519	69.783^{b} ±0.663	48.514^{c} ±0.059	68.156^{ab} ±0.945	69.045^{b} ±0.685
EPA + DHA	1.976^{a} ±0.021	0.909^{b} ±0.004	2.118^{c} ±0.025	1.532^{d} ±0.020	ND
$\sum$n3/$\sum$n6	0.250^{a} ±0.002	0.216^{b} ±0.002	0.446^{c} ±0.003	0.408^{d} ±0.006	0.371^{e} ±0.008
$\sum$n6/$\sum$n3	19.325^{a} ±0.085	21.978^{b} ±0.237	5.938^{c} ±0.015	10.964^{d} ±0.119	10.295^{e} ±0.208

A, B, C, D, E – dry granulated compound feed; EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid; PUFA – polyunsaturated fatty acids; MUFA – monounsaturated fatty acids; SFA – saturated fatty acids; ND – not detected overall health (Gaylord et al., 2018). The occurrence of α-linolenic acid ranged from 3.298 (B) to 6.604 (E) $g.kg^{-1}$ DM. The highest α-linolenic acid content was observed in sample E, and the differences were statistically significant ($P < 0.05$) compared to the other samples, ranging from 27.38% (C) to 50.08% (B). ALA is a type of essential polyunsaturated fatty acid (PUFA) that contributes to the n-3 fatty acid pool and has limited anti-inflammatory properties. Although ALA can be converted to EPA or DHA, the conversion rates are inefficient in dogs. Therefore, the level of EPA and DHA is more important for dogs (Mehler et al., 2016). All analyzed samples of complete dry granulated puppy foods met the requirements for a minimum α-linolenic acid content, 0.80 $g.kg^{-1}$ DM, for both early and late growth (FEDIAF, 2021). The arachidonic acid content ranged from 0.346 (C) to 0.669 (A) $g.kg^{-1}$ DM. Sample A had highest arachidonic acid content with statistically significant differences ($P < 0.05$) of 22.39% (sample B), 25.37% (both sample D and E) and 47.76% (sample C). The recommended minimum arachidonic acid content of 0.30 $g.kg^{-1}$ DM according to FEDIAF (2021) was met by all the analyzed samples. According to FEDIAF (2021), puppy food should contain a minimum of 0.50 $g.kg^{-1}$ DM EPA + DHA. From the analyzed samples, samples A, B, C, D achieved this content. Samples A, B, D and E did not contain DHA. Out of the tested samples, only sample C contained both EPA and DHA. Sample E contained none of these acids (EPA and DHA) and therefore did not meet the minimum recommended value. From the MUFAs, which ranged from 71.261 (C) to 94.948 (B) $g.kg^{-1}$ DM, oleic acid and palmitoleic acid were the most abundant. Among the saturated fatty acids (SFA), palmitic acid, stearic acid and myristic acid were mainly represented. The SFA content was lowest in sample C (48.514 $g.kg^{-1}$ DM) and highest in sample B (69.783 $g.kg^{-1}$ DM). The PUFA content varied from 33.613 (C) to 61.481 (A) $g.kg^{-1}$ DM. The most represented PUFAs in the dry granulated puppy foods were mainly linoleic acid, α-linolenic acid, and EPA. Important long-chain polyunsaturated fatty acids in the metabolism of dogs are EPA and DHA. Since their bioconversion from α-linolenic acid is limited, due to low enzymatic activity, their dietary intake is essential (Bauer, 2016). Therefore, polyunsaturated n-3 and n-6 fatty acids such as linoleic acid, arachidonic acid, α-linolenic acid and especially EPA and DHA are essential fatty acids in the diet of growing dogs. They play an important role in the growth of puppies, especially in the process of vision formation, cognitive function, also for the healthy development of the cardiovascular and musculoskeletal systems (Dillon et al., 2018; Hadley et al., 2017, Barbeau-Grégoire et al., 2022). They are part of nervous tissue, also involved in the formation of cell membranes, have a positive effect on the cardiovascular system, skin and coat, and also play an important role as anti-inflammatory agents, due to their inhibition of the formation of prostaglandins, thromboxanes and leukotrienes from arachidonic acid (Buddhachat et al., 2017; Combarros et al.,2020; Watson et al., 2021). Also, the therapeutic effect of n-3 fatty acids has been reported in allergic dermatitis, valvular disease, osteoarthritis, keratoconjunctivitis sicca and haircoat disorder (Magalhães et al., 2021). The balance between n-6 and n-3 polyunsaturated fatty acids (PUFAs) is crucial for regulating lipid metabolism in animals. The previous studies have shown (Jiang et al., 2010; Ebrahimi et al., 2018) that this ratio can influence the deposition of body fat and the composition of fatty acids in tissues such as the liver, intramuscular fat, and subcutaneous fat. The n-6/n-3 ratio ranged from 21.978 (B) to 5.938 (C) $g.kg^{-1}$ DM in the samples. Samples A, C, D, and E had statistically significant differences ($P < 0.05$) from sample B. These differences ranged from 12.07% to 72.98%. For dogs, an n-6/n-3 ratio lower than 5 : 1 is considered ideal, and it is recommended not to exceed a ratio of 10 : 1, as it helps maintain a healthy balance of fatty acids, reducing inflammation and the risk of metabolic diseases (Vastolo et al., 2021). On the other hand, a diet with an n-6/n-3 PUFA ratio of 18 : 1 has been found to be beneficial for lipid decomposition and PUFA transport for supplying

Table 3 Comparison of the minimum recommended content of certain fatty acids with the content in granulated puppy foods

Fatty acid	Min. recommended level (FEDIAF, 2021)	Difference				
	EG and LG	A	B	C	D	E
	$g.kg^{-1}$ DM	$g.kg^{-1}$ DM				
Linoleic acid (C18:2cis n6)	13.00	+40.788	+28.46	+12.22	+18.23	+53.96
Arachidonic acid (C20:4 n6)	0.30	+0.36	+0.21	+0.04	+0.20	+0.19
α-linolenic acid (C18:3 n3)	0.80	+3.50	+2.49	+4.00	+3.98	+5.80
EPA + DHA (C20:5 n3 + C22:6 n3)	0.50	+1.47	+0.40	+1.61	+1.03	-0.50

EG – early growth (<14 weeks), LG – late growth (≥14 weeks), A, B, C, D, E – dry granulated compound feed, EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid energy in silver foxes to withstand the low temperatures, which could be extrapolated to suggest similar benefits in dogs (Zhong et al., 2022).

4 Conclusions

The results confirmed that 4 of the 5 commercial puppy foods formulas analyzed in this study, met the FEDIAF (2021) requirements for minimum specific fatty acid content. All samples met the requirement for a minimum fat content of 85.00 g, minimum linoleic acid content of 13.00 g, minimum arachidonic acid content of 0.30 g and alpha linolenic acid content of 0.80 g in kg of DM. The requirement for the recommended minimum EPA + DHA content (0.50 $g.kg^{-1}$ DM) was met by 4 samples (A, B, C, D), except for sample E. When comparing the manufacturer's declared fat content on the packaging and the determined total fat content of the tested feeds, we found a lower fat content of 0.23% for sample B and 4.78% for sample C. However, it can be concluded that despite this low deviation from the declared fat content, the minimum fat content requirements according to FEDIAF were exceeded.

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