

## PHOSPHOLIPID FATTY ACID PROFILES OF PLASMA AND ERYTHROCYTE MEMBRANES IN DOGS FED WITH COMMERCIAL GRANULATED FOOD

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Intake of long-chain n-3 polyunsaturated fatty acids (PUFA) benefits human and animal health. Our study aimed to analyze the long-chain n-3 PUFA content of two types of food and their effect on plasma and erythrocyte phospholipids of Belgian Shepherd dogs. A total of 10 dogs were fed commercial granulated food (Food 1), and another 10 were provided commercial Premium granulated food of high quality (Food 2). All the analyses were performed using gas-liquid chromatography. Our results showed that Food 1 contained more n-3 PUFA than Food 2, which was reflected in higher n-3 PUFA in plasma and erythrocyte phospholipids. Because long-chain n-3 PUFA in phospholipids are precursors for antioxidative molecules, further studies should investigate the effects of the analyzed commercial granulated food rich in n-3 on oxidative stress parameters in dogs.

**Keywords:** commercial foods, dogs, fatty acids profiles, n-3 fatty acids, phospholipids.

### INTRODUCTION

In recent years, many pet owners have abandoned conventional, veterinary-recommended commercial diets in search of more “natural” and “homemade” choices [1]. Still, it is more common practice nowadays for owners to use commercialized labeled food for pets such as canines. Among other ingredients, polyunsaturated fatty acids (PUFA) are important for human and animal health. The conversion of short chain to long chain PUFA is rate-limiting and varies between species [2]. The balance of n-6/n-3 ratio in phospholipids, as well as the balance between reactive oxygen species and reactive nitric species on one side and antioxidative defense, on the other, is important for the normal physiological function of organisms. In dogs, fewer

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inflammatory mediators were produced when fed diets ratios (n-6/n-3) of 5:1 and 10:1 in comparison with being fed an n-6-rich diet with a fatty acid ratio of 100:1 [3].

Mammals are not able to synthesize fatty acids (FA) with double bonds at C-9, but are able to some extent to elongate and further saturate the aliphatic chain [4]. It is well known that diet could affect fatty acid profiles in plasma and erythrocyte membranes (EM) and that changing its composition causes changes in many parameters, which is confirmed in animal and human studies [3]. Since the FA composition of the EM correlates with that of other cell membranes, the effect of dietary FA supplementation may be analyzed by studying EM. It is well known that plasma phospholipids profiles reflect short-term changes in the diet while changes in erythrocyte membrane phospholipids reflect long-term dietary habits (up to 3 months prior analysis). Comparing those two profiles could lead us to a conclusion about the diet habits of examined animals in general [5]. The FAs profile of the serum phospholipids is related to the average dietary FAs intake during the last 3 to 6 weeks, while the composition of erythrocyte phospholipids depends on the dietary fat intake during the preceding months [5]. The FAs profile in the tissues partly reflects not only the dietary fat intake but also the efficiency of FAs metabolism in the body [6].

The FAs profile of tissues and triglycerides (TG) is known to be influenced by many factors, including dietary intake, age, gender, and endogenous metabolism [7]. Considerable interest exists in the possible health benefits of increasing dietary intake of n-3 PUFA [8]. Three families of long-chain PUFAs with different biological roles exist, n-3, n-6 and n-9 and they are derived from the shortest non-synthesizable precursors: linoleic acid (LA) (18:2, n-6) and alpha-linolenic acid (ALA) (18:3, n-3). Humans can desaturate and elongate ALA, as a precursor of n-3 series, to eicosapentanoic acid (EPA) and docosahexanoic acid (DHA). This process is dependent on aging, presence and type of disease, inflammation processes, and other factors [4]. In rats, which are often used as animal models, the rate of conversion of ALA to DHA is high in the liver, although  $\Delta 5$  and  $\Delta 6$  desaturases are expressed in many other rodent tissues besides the liver [9].

There are few commercial pet dog foods with EPA and DHA concentrations adequate for the treatment of disease or some vulnerable conditions. Target ranges for EPA and DHA vary quite widely for different conditions but typically fall between 50 and 220 mg/kg body weight. Commercial diets with n-3 fatty acids typically provide less EPA and DHA than desirable and may be advertised as containing flaxseed or canola oil (rich in ALA) [10].

In fact, there are adverse effects associated with the use of n-3, and an increase in the concentration of EPA and DHA in commercial pet food (dogs) makes the topic important to revisit. Those effects include altered platelet function, gastrointestinal adverse effects, potential effects for nutrient excess, weight gain, altered immune function, and effects on glycemic control and insulin sensitivity. As far as dogs and specific abnormalities are concerned decreased epithelization of wounds after 5

days (n-6/n-3=0.3:1), increased plasma and urine thiobarbituric reactive substances (n-6/n-3=5.4:1), decreased plasma vitamin E (n-6/n-3=1.4:1), decreased skin and neutrophil leukotriene B4/increased leukotriene B5, lower delayed-type hypersensitivity response (n-6/n-3=1.4:1), decreased CD4+T lymphocyte count (n-6/n-3=1.4:1), decreased lymphocyte proliferation (EPA/DHA=0.8) [11]. Metabolically, however, fatty acid patterns of plasma phospholipid fractions again revealed a sparing effect of ALA on LA. It should be mentioned that a direct effect of ALA on improvements of skin and coat could not completely be ruled out in these studies while long-chain n-3 PUFAs from fish oil or other marine sources appear to be especially capable of modifying inflammatory and immune responses [12].

The aim of the study was to analyze the fatty acid content of two different diets for dogs and to examine the effects of their daily consumption on the erythrocyte membrane and plasma phospholipid profiles.

## MATERIALS AND METHODS

### Animals

This study which lasted for 12 weeks, was approved by the Ethics Commission of the Faculty of Veterinary Medicine are included dogs of the Belgian Shepherd (Malinoa) breed from two kennels (1 and 2). In kennels, we selected 10 dogs, (5 females and 5 males), age categories of 3 to 7 years, with body weight  $30.2 \pm 2.2$  kg. We measured weight gain monthly and it changed up to 1 kg/dog. Male dogs weight gain was slightly higher than females but without statistical difference. By the basic examination of the dogs in both kennels (blood pressure, pulse, temperature, breathing, skin condition, and skin cover), there was a constant absence of disease, otherwise, all dogs had neatly managed health cards. According to the constitution they fell into 3 categories, which means they have an ideal weight corresponding to this breed of dog. The dogs had their activities in the morning and in the evening for 60 minutes (walking, running), otherwise, these dogs are considered as working dogs. In kennel number 1, the dogs were fed commercial granular foods that normally satisfies the standard nutritional needs of dogs (I). In kennel number 2, the dogs were fed Premium granulated food of high quality, this being the most sold dog food in Serbia-(II). The amount of food (400 g/day/dog) was divided into two meals one in the morning and the other in the evening at the same time each day.

### Sample collection and analysis

Granulated foods samples (I and II) (from four representative large markets) were analyzed. The primary sample was generated by mixing an equal portion of four samples taken from different markets. Five replicate samples of the composite sample were analyzed by standard laboratory methods to measure the concentration of

proteins, carbohydrates, lipids, minerals, and water. Fatty acid composition from the lipids was done by standard laboratory procedure, as described below. At the end of the study blood samples were taken from the (*vena cephalica antebrachii*), with the aid of EDTA vacuum blood collection tubes. Erythrocytes and plasma were separated and stored at a temperature of  $-80\text{ }^{\circ}\text{C}$ . After blood sampling by the routine method, analysis of erythrocytes and plasma was done by gas-liquid chromatography (GC).

Nutritional analysis was carried out by an accredited chemical laboratory at the Institute of Public Health in Požarevac. Ash content was determined by the direct gravimetric method which includes ashing of the samples in an oven at  $550\text{ }^{\circ}\text{C}$  until a constant weight was attained. Moisture was determined gravimetrically [13]. Crude protein content was estimated based on the total nitrogen content of the sample determined by the Kjeldahl method (AOAC 955.04D) [13]. Crude fat content was determined gravimetrically (Soxhlet extraction, AOAC method [13]. Total carbohydrate content, crude “by difference”, was calculated by the following formula: total carbohydrate (%) =  $100\% - \% (\text{protein} + \text{ash} + \text{fat} + \text{moisture})$ . The energy content of food was calculated based on determining content by the following formula: Energy value (estimated, kJ/100 g) =  $[4 \times \text{protein} (\%)] + [4 \times \text{carbohydrate} (\%)] + [9 \times \text{fat} (\%)]$ .

## **Fatty acids extraction and analysis**

### **Isolation of lipids**

The method consists of homogenization of plasma with a 2:1 chloroform/methanol mixture. Washing of the mixture with a 5 times smaller volume of water or saline (0.9 g NaCl in 100 ml of water). The resulting mixture was separated into two phases. The lower phase was the total pure lipid extract.

First the lipids present in the volume of 0.5 ml of erythrocytes were extracted with a mixture of chloroform isopropanol (7:11) following the prescribed procedure [14]. After that we isolated the phospholipids from the lipid subclasses by thin-layer chromatography on silica-gel plates using petroleum ether, diethyl ether and glacial acetic acid (87:12:1, by volume). In a modified procedure by Christopherson and Glass [15], we made a direct transesterification of fatty acids. Lipid extracts derived from hexane were evaporated under a nitrogen stream to full vapor and dry bottom of Eppendorf's tubes. After that, the residue was dissolved in 10 $\mu\text{l}$  hexane. We took 1  $\mu\text{l}$  and injected it into a chromatograph. Methyl esters of fatty acids were analyzed by gas-liquid chromatography in a Shimadzu chromatograph GC 2014 (Kyoto, Japan) equipped with a flame ionization detector on an Rtx 2330 column (60mm x 0,25 mmID, film thickness 0,2  $\mu\text{m}$ , Restek, Bellefonte, PA, USA). Adequate separation was obtained over a 50 min period with an initial temperature of  $140^{\circ}\text{C}$  maintained for 5 min. The temperature was then increased to  $220^{\circ}\text{C}$  at a rate of  $3^{\circ}\text{C}/\text{min}$  and kept at the final temperature for 20 min. The identification of fatty acid methyl esters (FAME)

was made by comparing peak retention times with standard mixtures (PUFA-2 and/or 37 FAMES mix, Supelco, Bellefonte, PA, USA). Finally, the content of fatty acids, from 16:0 through 22:6n-3, was expressed as a percentage of the total content of identified fatty acids.

### Statistical analysis

Statistical analysis was done by one-way ANOVA test and Student t-test (significance  $p < 0.05$ ) in SPSS. All results were expressed in percentages of a total of 100%.

## RESULTS

### Biochemical parameters

Analyzed biochemical parameters such as triglycerides and plasma cholesterol did not show statistical significance in the plasma of canines treated with two different diets. Although there were some changes in LDL cholesterol, previously reported by Ravic *et al* [16].

### Analyzed food

There were differences in FA percentage between the examined foods. There was an equal percentage of palmitic (16:0) acid, but stearic acid was more abundant in Food 1 ( $p < 0.01$ ). Monosaturated FA, specially palmitoleic acid, was present at a higher percentage in Food 2 ( $p < 0.01$ ), while oleic acid (18:1 n-9) was detected almost in the same percentage. Linoleic acid (18:2 n-6) was also present in more than 10% in both Food 1 and Food 2. Long-chain PUFAs were present in a very low percentage with statistically significant differences between Foods. ALA (18:3n3) was statistically significant in a higher percentage in Food 1 ( $p < 0.001$ ), as well as EPA ( $p < 0.001$ ) and DHA ( $p < 0.001$ ) compared to Food 2 (Table 1).

**Table 1.** Composition and FA patterns of the diets

Fatty acids (%)	Food 1	Food 2
16 :0 Palmitic acid	23.93± 0.15	23.13 ± 0.1
16 :1 Palmitoleic acid	2.47± 0.26	3.72 ± 0.07**
18 :0 Stearic acid	13.36± 0.26	9.26 ± 0.26**
18: 1,n 9 Oleic acid	36.88± 0.55	40.61± 0.92
18: 1,n 7 Vascenic acid	4.00± 0.21	3.46 ± 0.33
18: 2 Linoleic acid	15.13± 1.34	18.04 ± 0.26*

cont. Table 1.

18: 3 n 3 Linolenic acid	2.99± 0.22	0.95 ± 0.02***
20: 3 Eicosatrienoic acid	0.07± 0.01	0.13± 0.02***
20: 4 Arachidonic acid	0.45± 0.09	0.36± 0.12
20: 5 Eicosapentanoic acid	0.22± 0.09	0.04 ± 0.02***
22 : 4 Docosatetraoic acid	0.09± 0.01	0.13 ± 0.02**
22 : 5 Docosapentanoic acid	0.07± 0.01	0.07 ± 0.01
22: 6 Docosahexanoic acid	0.31± 0.08	0.07 ± 0.01***
Proteins	27.29 (0.05)	25.51(0.04)
Fats	16.44 (0.04)	15.22 (0.04)
Carbohydrates	44.54 (0.22)	46.41 (0.24)
Minerals	6.69 (0.01)	7.67 (0.01)
Water	5.05 (0.02)	5.21 (0.03)

Values (proteins, carbohydrates, fats, minerals and water) are expressed as % by 100g.

Abbreviations SD, Standard deviations. Fatty acids are expressed as % ± st. dev with significance

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

### Dog plasma and erythrocyte membrane phospholipid fatty acids

It is well known that plasma phospholipids fatty acids profiles represent the short-term (up to several days) food intake, while erythrocytes present data composition of fatty acids for long term (last 3 months) food intake. In our study, we compare dogs' FAs profiles in plasma and erythrocytes membrane phospholipids in two kennels (Table 2-4).

In plasma phospholipids FA profiles in palmitic acid (16:0) were increased in Kennel 1 ( $p < 0.001$ ) as well as stearic acid (18:0), results showed that dogs in Kennel 1 had a higher percentage of saturated fatty acids (SFA) in plasma phospholipids compared to Kennel 2. As far as monosaturated fatty acids, palmitoleic (16:1) was increased in Kennel 2 while oleic acid (18:1, n-9) was increased in Kennel 1 ( $p < 0.01$ ) compared to dogs fed Kennel 2. Linoleic acid (18:2) was increased ( $p < 0.001$ ) in dogs in Kennel 2 while polyunsaturated n-3 especially ALA ( $p < 0.01$ ) eicosapentaenoic (EPA) ( $p < 0.001$ ), docosapentaenoic (DPA) ( $p < 0.001$ ) and docosahexaenoic (DHA) ( $p < 0.001$ ) were increased in Kennel 1.

In the erythrocyte membrane, phospholipids' percentage of fatty acids has almost the same distribution and percentages as in plasma phospholipids between Kennels. Saturated fatty acids (PA and SA) ( $p < 0.01$ ) were in higher percentages in Kennel 1-fed dogs as well as monounsaturated FA ( $p < 0.1$ ). Percentage of EPA ( $p < 0.001$ ), DPA ( $p < 0.01$ ), and DHA ( $p < 0.001$ ) were increased in Kennel 1 in plasma phospholipids,

while ALA concentration did not significantly change. These results go along with the plasma phospholipids FA distribution already mentioned.

These results suggest that the differences are negligible between plasma and erythrocytes phospholipids fatty acids percentages.

**Table 2.** Plasma and erythrocyte phospholipids fatty acids profiles of dogs in Kennel 1 and 2.

Fatty Acids (%) (plasma)	Kennel 1	Kennel 2
16 :0	17.09 ± 1.29	14.55 ± 1.48 ***
16 :1	0.39 ± 0.12	0.68 ± 0.33 ***
18 :0	30.12 ± 1.50	29.81 ± 1.81
18: 1 ,n 9	5.76 ± 0.67	4.89 ± 0.45 **
18: 1 ,n 7	1.96 ± 0.14	1.69 ± 0.23 *
18: 2	16.63 ± 2.12	24.52 ± 1.73 ***
18: 3 n 6	0.34 ± 0.06	0.40 ± 0.04 *
18:3 n 3	0.23 ± 0.01	0.15 ± 0.07 **
20: 3	1.27 ± 0.11	1.86 ± 0.51 **
20: 4	17.86 ± 2.27	17.71 ± 2.21
20: 5	2.26 ± 1.18	0.24 ± 0.03 ***
22 : 4	0.43 ± 0.01	0.82 ± 0.02 ***
22 : 5	2.38 ± 0.88	1.91 ± 0.51 ***
22: 6	3.00 ± 0.78	0.49 ± 0.02 ***
Fatty Acids (%) (erythrocytes)	Kennel 1	Kennel 2
16 :0	19.2 ± 0.69	17.15 ± 1.53 **
16 :1	0.24 ± 0.038	0.22 ± 0.012
18 :0	30.20 ± 0.78	29.49 ± 1.68
18: 1 ,n 9	7.87 ± 0.24	7.09 ± 0.53 *
18: 1 ,n 7	2.03 ± 0.11	1.83 ± 0.12 *
18: 2	9.31 ± 1.04	13.82 ± 0.37 ***
18: 3 n 6	0.45 ± 0.06	0.42 ± 0.05
18:3 n 3	0.17 ± 0.04	0.20 ± 0.02
20: 3	2.51 ± 0.25	2.78 ± 0.35
20: 4	24.0 ± 0.96	23.20 ± 2.44
20: 5	1.48 ± 0.26	0.23 ± 0.04***
22 : 4	0.96 ± 0.23	1.64 ± 0.22 ***
22 : 5	± 0.22	0.73 ± 0.18 **
22: 6	1.08 ± 0.21	0.23 ± 0.11 ***

**Table 3.** Estimated desaturase 9,6,5 activities in plasma and erythrocytes in dogs in Kennel 1 and 2.

Estimated desaturase (D) activity	Kennel 1 plasma	Kennel 2 plasma	Kennel 1 erythrocytes	Kennel 2 erythrocytes
D9 desaturase	0.19 ± 0.03	0.17 ± 0.02	0.25 ± 0.01***	0.24 ± 0.02***#
D6 desaturase	6.01 ± 0.20	5.04 ± 1.50	15.65 ± 5.42***	13.43 ± 2.63
D5 desaturase	0.14 ± 0.07	0.01 ± 0.005####	0.06 ± 0.01***	0.01 ± 0.003####

Test significance were used between groups, \*statistical significance between plasma and erythrocytes in both Kennels, # statistical significance between plasma in both Kennels and erythrocytes in both Kennels, with \*#p<0.05, \*\*##p<0.01 and \*\*\*###p<0.001

**Table 4.** Percentage of overall n-3, n-6 and n-6/n-3 ratio in Kennel 1 and Kennel 2 in plasma and erythrocytes of examined dogs.

	Plasma K1	Plasma K2	Erythrocytes K1	Erythrocytes K2
N-3	7.18 ± 0.32	5.95 ±0.55	2.43±0.40	2.52 ± 0.33
N-6	36.50±1.33	33.23 ±0.11	35.48±1.42	34.52 ± 0.88
N6/N3	5.07±1.00	6.05 ±0.95	15.00 ± 3.64	16.7±0.43

K1: Kennel 1; K2: Kennel 2.

## DISCUSSION

Generation of SFA, MUFA and PUFA fatty acids is connected with mediators such as prostaglandins, leukotrienes and others, which are able to influence metabolic changes in dogs in health and disease. The n-6/n-3 balance is important in everyday balanced diet and is recommended to be as low as it could be. Following up on intakes of linoleic acid, as n-6 family fatty acid, and its metabolic pathway to DGLA and arachidonic acid (AA) and cell membrane incorporation is of great importance as information of biochemical or clinical parameters for the evaluation of dog's health [5]. Omega-6 linoleic and its transformation to ARA affect not only from the cell structural point but also affect the membrane response to stimuli, thus membrane fatty acid composition can be useful as information of pro and anti-inflammatory predisposition of the canine organism [17].

By correlating plasma and erythrocyte phospholipids FA composition after the treatment, we investigated if FA from both plasma and erythrocytes could represent markers of dietary n-3 intake.

Our discussion is more based on n-3 PUFA significance and intake in canines. Certain results indicate a beneficial effect of n-3 long-chain polyunsaturated fatty acids (LC PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on canine health. Several studies have examined the effects of fish oil, linseed oil, or combinations on plasma phospholipids fatty acids profile in canine species [18], lymphocyte proliferation, or neutrophil composition in laboratory animals [19,20].



The nutrient requirements of canine athletes who have a greater capacity for fat oxidation are unique concerning that canine metabolism is unique [10]. Relative to body mass size dogs metabolize free fatty acids at twice the rate observed in men. Consequently, the muscles are more adapted to use fat than human muscles [21]. Usual daily food requirements are water, energy, major nutrients, fibers, minerals, and vitamins. High fat diet, fatty acids chain length and saturation affect a variety of issues from inflammation to oxidative stress in animals [22,23]. However, there is very little information regarding optimal dietary fats intake for canine athletes [23].

Medium-chain triglycerides yield 8 carbon to 12 carbon-free fatty acids (coconut, palm oil) when digested and are directly transported through absorption into the blood, bound to albumin, to the liver *via* the portal circulation, leading to sparing of glycogen [24]. The fatty acid composition also influences detection in scent-trained dogs, hunting dogs, and service dogs. It is well known that polyunsaturated fatty acids may modestly improve performance in dogs that require activity as a part of their work [25]. The use of carbohydrates as a major dietary substrate is essential. Soluble fibers may alter the large intestinal microflora which produces short-chain fatty acids. A small amount of soy fiber and fructooligosaccharides are components of gastrointestinal veterinary diets. Deficiencies in major minerals have been observed in dogs fed with non-traditional diets. It is recommended that bones should be ground into the diet to improve calcium and phosphorus balance. Vitamins (fat-soluble or water-soluble) are involved in cellular metabolism or coenzymes in the citric acid cycle. A study by Poel *et al.* (2017) [26] was conducted to determine the stability of two preparations of GAA (granulated and crystallized) and CMH in a moist and a dry dog food formulation during production and storage. Most commercial dog foods contain vitamins above the minimum requirement. Diets high in PUFA (fish-based sled dog diets) should contain more vitamin E to prevent lipid peroxidation. In the study by Vessecchi *et al.*, the study aimed to evaluate the macronutrient composition, fatty acids and amino acids profile, and essential minerals contents of vegan pet foods available in the Brazilian market, and to assess their compliance with recommended allowances for dogs and cats [27].

Omega -3 fatty acids incorporated in cell membranes, especially EPA and DHA, decrease clinical signs of osteoarthritis in dogs. EPA serves as a substrate for the COX and LOX enzymes. Omega-6 FA are involved in physiological processes while inflammation contributes to the formation of “proinflammatory” prostaglandins and leukotrienes. Omega-3 produces less inflammatory prostanoids and 5-leukotrienes. Dogs have limited ability to convert ALA to DHA. That is the reason for diet EPA and DHA consumption directly. Diets that contain EPA and DHA have been recommended for degenerative joint diseases, aging in general, as anti-inflammatory supplements, and in growing puppies [25]. Currently, omega-3 FAs are used in managing many diseases including neoplasia, dermatologic diseases, hyperlipidemia, cardiovascular, gastrointestinal, and orthopedic diseases [11]. There are few commercial pet foods with EPA and DHA adequate for the treatment of disease. Joint diets, renal diets

dermatologic conditions contain more omega-3 fatty acids than maintenance diets. Commercial diets with omega-3 provide less EPA and DHA than desirable and contain flaxseed instead. Because diets with ALA have different effects when compared with diets enriched in EPA and DHA composition information may very much contribute to results. Purushotman et al. study on beagles fed with flaxseed added to a basal diet (57% alfa-linolenic acid, ALA at the rate 100ml/kg for 3 weeks) showed that plasma ALA, EPA, and LA increased steadily and significantly from 0-22 days while arachidonic acid showed no difference. Plasma DHA showed no significant changes over time which agrees with previous studies in dogs [2]. Dunbar et al., concluded that hepatic conversion of DHA is slow in the canine and after its production from ALA it is likely to relocate to neurological tissue [29]. Extensive studies are required for clarifying this issue and to confirm how different breeds metabolize PUFA. Waldron et al. confirm earlier reports in their study on dogs fed a fish oil diet that ALA was converted to EPA and further elongated to DPA but was not converted to DHA in plasma phospholipids [18]. The beneficial effect of n-3 FA due to their competition with n-6 for cellular membrane incorporation leads to the suggestion that n-6: n-3 ratio could be used as a dietary index to modulate cell composition and cellular function [30]. Still, the type and amount of n-3 PUFA, but not the n-6:n-3 ratio in diets, contribute to membrane n-3's highly unsaturated fatty acid composition and further changes in cell function [18]. Stoeckel et al. (2011) concluded that in dogs an increase of dietary n-3 FA content leads to a rapid inclusion of n-3 into the erythrocyte membrane, regardless of whether the n-3 FA is offered as an enriched diet or as a normal diet supplemented with an n-3 FA additive [31].

The cardiovascular benefits of n-3 PUFA could originate from their ability to improve lipid metabolism and reduce the synthesis of proinflammatory eicosanoids derived from n-6 PUFA. At an adequate level of incorporation, EPA and DHA influence the membrane fluidity as well as membrane protein-mediated reactions, generation of lipid-mediators, cell signaling, and gene expression in different cells [32]. Data on cats, together with the results of lipoprotein analysis, indicate possible disturbances in the hepatic transformation of LDL and VLDL, and a high risk of atherogenic events [33]. Dogs have the capacity to metabolize n-3 fatty acids and the effects of omega-3 fatty acids on the skin and coat, inflammatory responses, and neurologic development in puppies are quite visible [34].

Concerning specific beneficial effects that fish oil or EPA and DHA have on canines, it would be useful to add it to the examined food or as an everyday supplement to those dogs. Diagnostic tests performed in dogs provide valuable information [35] and our data can contribute to it.

Our further examination and studies will address it, and we planned a study on the treatment of PUFA in canines (EPA +DHA). That study could be useful and give us a more complete conclusion about the significance of EPA and DHA in canine feeding.

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## Ethical approval

The research related to the use of animals has been complied with all the relevant national regulations and institutional policies for the care and use of animals (Ministry of Agriculture and Forestry and Water Management - Veterinary Administration, number 323-07-00364/2017-05/6 date 13. 07. 2017., and approved by the Ethical comity of the Faculty of Veterinary Medicine, 17 June 2017 number 01-592).

## Authors' contributions

TP was involved in concept and design, drafting, and supervision. JDM, BP, and SR participated methodology and data collection. BR was involved in methodology, analysis, and interpretation of data. MG participated as project administration and funding acquisition. PS was involved in conceptualization funding acquisition.

## Declaration of conflicting interests

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publication of this article.

## Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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## **PROFILI FOSFOLIPIDA MASNIH KISELINA PLAZME I MEMBRANE ERITROCITA KOD PASA HRANJENIH KOMERCIJALNOM GRANULIRANOM HRANOM**

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Unos dugolančanih n-3 polinezasićenih masnih kiselina (PUFA) koristi zdravlju ljudi i životinja. Naša studija je imala za cilj da analizira sadržaj dugolančanog n-3 PUFA u dve vrste hrane i njihov uticaj na fosfolipide plazme i eritrocita belgijskih ovčara. Ukupno 10 pasa je hranjeno komercijalnom granuliranom hranom (hrana 1), a još 10 je obezbeđeno komercijalnom premijum granuliranom hranom visokog kvaliteta (hrana 2). Sve analize su obavljene gasno-tečnom hromatografijom. Naši rezultati su pokazali da hrana 1 sadrži više n-3 PUFA nego hrana 2, što se odrazilo na više n-3 PUFA u plazmi i eritrocitnim fosfolipidima pasa iz grupe 1. Pošto su dugolančani n-3

PUFA u fosfolipidima prekursori za antioksidativne molekule, dalje studije bi trebalo da istraže efekte analizirane komercijalne granularne hrane bogate n-3 na parametre oksidativnog stresa kod pasa.