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Salmonids as Natural Functional Food Rich in Omega-3 PUFA

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Abstract: Salmonids are valuable fish in the human diet due to their high content of bioactive omega-3 very long-chain polyunsaturated fatty acid (VLC PUFA). The aim of this study was to assess the omega-3 VLC PUFA content in selected salmonid fish present on the food market regarding whether they were farm-raised or wild. It was assumed that farm-raised fish, by eating well-balanced feed enriched with omega-3 PUFA, might contain omega-3 VLC PUFA in levels similar to that of wild fish. Fat content, fatty acid composition and omega-3 VLC PUFA content in fish fillets were measured. Farm-raised salmon from Norway, wild Baltic salmon, farm-raised rainbow trout and brown trout were bought from a food market whereas wild trout (rainbow and brown) were caught alive. The fat content in fish ranged from 3.3 to 8.0 g/100 g of fillet. It was confirmed that although wild salmonid fish contain 10–25% more omega-3 VLC PUFA in lipid fraction, the farm-raised ones, due to the 60–100% higher fat content, are an equally rich source of these desirable fatty acids in the human diet. One serving (130 g) of salmonid fish fillets might provide a significant dose of omega-3 VLC PUFA, from 1.2 to 2.5 g. Thus, due to very high content of bioactive fatty acids eicosapentaenoic (EPA), docosapentaenoic (DPA) and docosahexaenoic (DHA) in their meat, salmonid fish currently present on the food market, both sea and freshwater as well as wild and farm-raised, should be considered as natural functional food.

Keywords: functional food; omega-3 VLC PUFA; salmon; trout

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1. Introduction

The omega-3 (n-3) very long-chain polyunsaturated fatty acids (VLC PUFAs) eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) are important components of the human diet, having desirable effects on brain development and cardiovascular health [1,2]. The main dietary sources of omega-3 VLC PUFAs are sea fish and supplements containing fish oil [3,4]. However, due to overfishing, the global supply of omega-3 VLC PUFA from natural sources is rapidly decreasing [3,5]. Alternatives to the main natural sources of omega-3 VLC-PUFAs are predominantly in the aquaculture sector [3,6].

EPA and DHA are essential nutrients due to their beneficial effects on the functioning of the central nervous system, as well as decreasing the risk of coronary heart disease (CHD) [6–10]. Although usually only EPA and DHA are indicated as health-promoting fatty acids, docosapentaenoic acid (DPA, C22:5n-3), too, plays an important role, especially in brain function [9]. DPA increases EPA and DHA levels in tissues of the major metabolic organs. EPA and DHA, as well as DPA, are also precursors of mediators involved in the resolution of inflammation, e.g., resolvins, protectins, maresins and isoprostanes [11]. It has been shown that EPA, DPA and DHA improve the function of the cardiovascular system and decrease markers of metabolic diseases risk, such as plasma lipid parameters, platelet aggregation, blood pressure, insulin sensitivity and cellular plasticity. Moreover, it has been shown that DPA is the most abundant fatty acid in the central nervous system, just after DHA. This indicates that DPA might be especially beneficial for early-life development, as well as elderly neuroprotection, and decrease the risk of depression [9,11,12].

Salmonids are one of the most valuable fish in the human diet in term of high omega-3 VLC PUFA content. Although trout is usually a freshwater fish, it contains similar omega-3 VLC PUFA levels as salmon [13,14]. It has been found that trout prefer diets rich in omega-3 VLC PUFAs, e.g., wild rainbow trout and gammarids [15,16]. Thus, salmonids can be treated as a functional food providing significant amounts of desirable omega-3 VLC PUFAs. The amount and composition of fish fat depends on the fish's diet, the year season and whether the fish is living in cold or warm waters [15]. Aquaculture, by using well-balanced feed enriched with omega-3 PUFAs, can elevate the content of these fatty acids in tissues of farm-raised fish [13,17,18]. Due to the high content of omega-3 VLC PUFAs in some fish species, they are considered as health-promoting functional foods [14,18].

The aim of this study was to assess the omega-3 VLC PUFA content in selected salmonid fish present on the food market regarding whether they were farm-raised or wild. It was assumed that farm-raised fish, by eating a well-balanced feed enriched with omega-3 PUFAs, might contain omega-3 VLC PUFA in levels similar to that of wild fish.

2. Materials and Methods

2.1. Sample Preparation

Salmonid fish including Norwegian farm-raised salmon (*Salmo salar*), Baltic wild salmon (*Salmo salar*), farm-raised rainbow trout (*Oncorhynchus mykiss*) and farm-raised brown trout (*Salmo trutta*) were purchased in supermarkets. The fish were fresh, gutted and displayed in stalls on crushed ice. After purchasing, the fish were subjected to laboratory treatments on the day they were collected. Wild rainbow trout (*Oncorhynchus mykiss*) and wild brown trout (*Salmo trutta*) were caught with a fishing rod in the Skotawa river (Pomerania, Poland) and used as references to farm-raised ones. Immediately after being caught, the fish were gutted, frozen and transported to the laboratory in this condition. All fish samples were obtained in April. Three fish of each species were used.

Before the samples were taken for testing, the fish were filleted and skinned. To eliminate foreign materials, fillets were rinsed with fresh water and the rest of the external water was eliminated by drying on a paper towel. For further analysis, fish meat was ground in a meat grinder and homogenized. Samples of homogeneous mass were taken for analytical tests. The results were the mean value of 3 parallel determinations.

2.2. Determination of Fat Content

To determine the oil content in the meat of fish, a Soxhlet extraction was conducted. Petroleum ether was used as a solvent and the extraction was completed for 4 h. Then, the solvent was removed under vacuum at 50 °C. The flask containing the extracted fat was stored under a hood for 15 min, and then, the determination of fat content was done gravimetrically.

2.3. Fatty Acid Analysis

The omega-3 VLC PUFA content in fish fillets was determined by gas chromatography (GC). To prepare samples for methylation, the lipid fraction was extracted from fillets according to the Folch method [19]. Folch extraction avoids high temperature and long duration, which are typical for Soxhlet extraction. One gram of homogenized meat sample was further homogenized in a solvent, which was a cold mixture of chloroform and methanol in the proportion 2:1. The mixture was filtered and the solid residue was washed with a mixture of chloroform–methanol and filtered again. Both filtrates were combined and transferred to the measuring cylinder. One quarter of the volume of the filtrates mixture of deionized water was added. Then, the mixture was shaken and left to separate the two phases. The lower phase of chloroform was drained and the rest of the water was removed by passing it through anhydrous Na_2SO_4. The dried chloroform phase was then roto-evaporated at 40 °C under vacuum. The remaining lipid fraction was covered by nitrogen, weighed and washed out by hexane, again dried by anhydrous Na_2SO_4 and closed in a vial under nitrogen.

Fatty acid methyl esters (FAMEs) were prepared according to the AOCS method Ce 1b-89 with some modification [16,20]. Hexane was evaporated from previously prepared samples by nitrogen flow. The obtained dry lipid fraction was saponified using 0.5 N NaOH solution with methanol, covered with nitrogen and then mixed under heating at boiling point for 40 min. Transmethylation of the saponified sample was conducted using 14% BF_3 in methanol reagent, under nitrogen, at boiling point for 3 min. After cooling, 3 mL hexane was added, covered with nitrogen and shaken for 30 s. Then, 40 mL of saturated NaCl water solution of was added, shaken and left to separate the two phases. The upper layer of hexane was removed using a syringe and transferred to a thin glass tube, dried by anhydrous Na_2SO_4 and decanted to a small vial and capped under nitrogen.

2.4. Chromatography

For the GC analysis, 1 µL of prepared FAME was injected into the chromatograph under appropriate conditions. The internal standard of tricosanoic acid (C23:0) methyl ester was used (Sigma-Aldrich, Steinheim, Germany). The Agilent 6890 N chromatograph (Agilent, Böblingen, Germany) was used with a 100-m length silica capillary column Rtx 2330 of internal diameter (ID) 0.25 mm and 0.1 µm d_f (Restek Corp, Bellefonte, PA, USA). As the carrier gas, hydrogen was used at a flow rate of 0.9 mL/s. A split-splitless (50:1) injector at 235 °C and a flame-ionization detector (FID) at 250 °C were used. The column temperature was as follows: initial 155 °C, 55 min, then increased at 1.5 °C/min to maximum 210 °C. Results were elaborated using the software HP-Chem (Hewlett Packard, Palo Alto, CA, USA). Peaks reflecting particular fatty acids were identified by comparison with menhaden oil standard and FAME Mix Supelco 37 standard (both Supelco, Germany). The fatty acids composition of the samples was calculated from the area of the peaks corresponding to particular fatty acids according to the AOCS Ce 1b-89 method [20]. Each sample was analyzed in triplicate.

2.5. Data Analysis

The obtained results were analyzed using a one-way ANOVA. The significance of differences between levels of particular fatty acid groups and omega-3 VLC PUFA in samples was assessed. For analysis, the Statistica v. 13.3 software was used (StatSoft, Inc., Krakow, Poland). The calculations were made at a significance level of $\alpha = 0.05$.

3. Results

3.1. Fat Content

The fat content in the analyzed fish ranged from 3.3 to 8.0 g/100 g of meat (fillet). The highest content of fat was found in farm-raised brown trout, while the lowest one in wild rainbow trout (Table 1). A significant difference in fat content between farm-raised fish (Norwegian salmon, farm-raised brown trout and farm-raised rainbow trout) and fish living in natural conditions (Baltic salmon, wild brown trout and wild rainbow trout) was found. Farm-raised fish contained significantly more fat than wild ones. The differences ranged from 60% to 100%. This was probably due to differences in the composition and amount of available food. Taking into account that all fish were caught in the same season (spring), it was most likely that wild fish were forced to search for food which required more physical effort, while farm-raised ones received well-balanced fodder without much effort.

3.2. Fatty Acids Content

Although the lipid fraction of the analyzed samples of fish fillets contained 43 fatty acids, significant levels were found for 15 of them (Figure 1; Table 2). The predominant fatty acids in all fish samples were monounsaturated C18:1 (18.3–26.2%), saturated C16:0 (12.3–21.7%) and polyunsaturated C22:6 DHA (12.1–17.6%). The other fatty acids of high levels were C18:2 n-6 linoleic acid (LA) (4.9–17%), C20:5 n-3 EPA (5.1–8.6%) and C16:1 (4.1–8.0%), C20:1 (2.2–8.4%), 22:1 (2.7–8.6) and C22:5 DPA (1.7–5.7%). The level of the total omega-3 PUFA content ranged from 22.1% to 28.7%, and for omega-3 VLC PUFA, from 19.8% to 31.2% of total fatty acids. The highest level of omega-3 VLC PUFAs was found in wild Baltic salmon (31.2%) and wild rainbow trout (27.9%), which did not differ significantly from each other. Ranges for particular omega-3 VLC PUFAs were as follows: EPA 5.1–8.6%; DPA 1.7–5.7%; DHA 12.1–17.6% of all fatty acids. However, there were some differences in omega-3 VLC PUFA content between farm-raised and wild fish, i.e., salmon 24.4% and 31.2%, brown trout 19.8% and 21.4%, rainbow trout 22.6% and 26.9%, respectively. Each of the particular omega-3 VLC PUFA levels was higher in wild fish compared to farm-raised ones.

Table 1. Fat content in salmonid fish fillets; g/100 g of raw meat.

Fat Content	Sea Fish		Freshwater Fish			
	Salmon Farmed (Norwegian)	Salmon Wild (Baltic)	Brown Trout		Rainbow Trout	
			Farmed	Wild	Farmed	Wild
Mean	7.7	3.8	8.0	4.1	5.3	3.3
±SD	0.2	0.3	0.2	0.1	0.2	0.1

Values are expressed as mean ± standard deviation (SD) (n = 3).

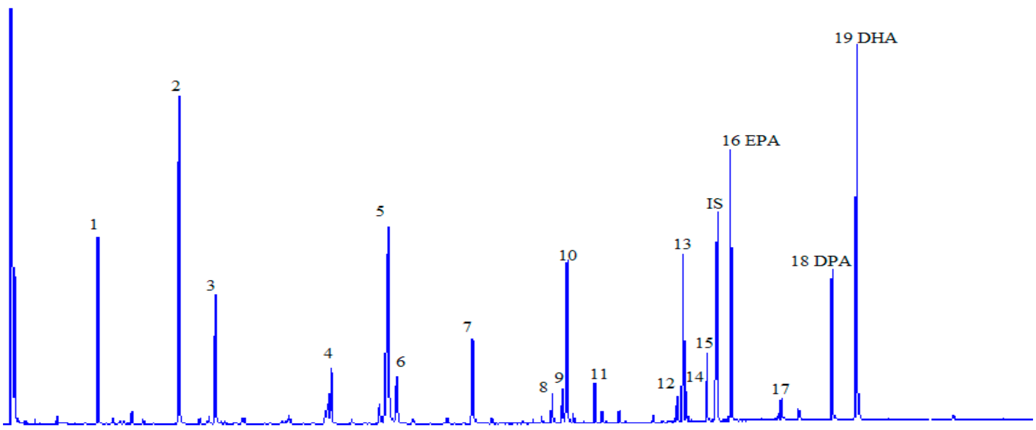

Figure 1. Chromatogram of fatty acid methyl ester (FAME) analysis of lipids extracted from salmonid fish fillets; example of wild salmon (Baltic): 1—C14:0; 2—C16:0; 3—C16:1; 4—C18:0; 5—C18:1n-9; 6—C18:1n-7; 7—C18:2n-6; 8—C18:3n-3; 9—C20:1n-11; 10—C20:1n-9; 11—C18:4n-3; 12—C20:3n-6; 13—C22:1n-11; 14—C20:4n-6; 15—C22:1n-9; 16—C20:5n-3; 17—C24:1c; 18—C22:5n-6; 19—C22:6n-3; IS—internal standard C23:0.

Among PUFAs, the omega-3 family dominated in all fish samples, while omega-6 fatty acid levels were smaller, which is reflected in the data cited in the literature [13,14]. The sum of omega-6 fatty acids ranged from 6.5% to 18.6% and was the highest in farm-raised brown trout and the lowest in wild salmon. All farm-raised fish contained significantly higher amounts of omega-6 fatty acids than wild ones.

The ratio of omega-6 to omega-3 PUFAs ranged from 0.19 (wild salmon) to 0.77 (farm-raised brown trout) and was nutritionally more desirable in wild fish than in farm-raised ones.

3.3. Omega-3 VLC PUFA in Fillets

The level of omega-3 VLC PUFAs in fillets differed between samples and ranged from 0.87 to 1.87 g/100g. It was higher in the meat of farm-raised fish than in wild ones (Figure 2). The level of omega-3 VLC PUFAs increased along with fat content and was the highest in the fattiest fish, which were farm-raised brown trout and farm-raised salmon (1.58% and 1.87%, respectively). The omega-3 VLC PUFA content in meat was high and nutritionally desirable. The omega-3 VLC PUFA content in 100 g of salmonid fish fillets ranged from 0.9 to 1.9 g.

Table 2. Fatty acid composition of fat extracted form fillets of salmonids; % of total fatty acids.

Fatty Acids	Sea Fish		Freshwater Fish			
	Salmon Farmed (Norwegian)	Salmon Wild (Baltic)	Brown Trout		Rainbow Trout	
			Farmed	Wild	Farmed	Wild
C12:0	-	-	0.1 ± 0.0	0.9 ± 0.1	0.2 ± 0.0	0.2± 0.0
C14:0	4.7 ± 0.3	4.1 ± 0.4	3.9 ± 0.2	4.1 ± 0.5	3.9 ± 0.2	3.6 ± 0.4
C16:0	14.2 ± 0.6	12.3 ± 0.8	15.0 ± 0.6	21.7 ± 1.1	15.7 ± 0.7	18.5 ± 0.9
C18:0	2.8 ± 0.2	2.6 ± 0.3	2.5 ± 0.2	4.2 ± 0.5	1.4 ± 0.1	3.5 ± 0.4
Sum saturates	21.7 ± 0.5	19.0 ± 0.7	21.5 ± 0.5	30.9 ± 0.9	21.2 ± 0.6	25.8 ± 0.8
C14:1	-	-	-	0.3 ± 0.0	-	0.2 ± 0.0
C15:1	0.4 ± 0.0	-	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
C16:1	5.9 ± 0.2	5.7 ± 0.3	5.8 ± 0.2	8.0 ± 0.5	4.1 ± 0.2	6.6 ± 0.4
C18:1	19.9 ± 0.9	18.3 ± 1.1	23.4 ± 0.9	20.4 ± 1.2	26.2 ± 1.0	21.6 ± 1.3
C20:1	6.4 ± 0.4	8.4 ± 0.6	2.7 ± 0.3	2.2 ± 0.4	4.5 ± 0.3	2.3 ± 0.3
C22:1	7.6 ± 0.3	8.6 ± 0.5	3.2 ± 0.2	2.7 ± 0.2	5.6 ± 0.4	2.8 ± 0.4
C24:1	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	-	-	0.2 ± 0.0
Sum monoenes	40.7 ± 0.8	41.5 ± 0.9	35.7 ± 0.7	34.0 ± 0.8	40.8 ± 0.8	34.1 ± 0.9
C18:2n-6 LA	8.1 ± 0.4	4.9 ± 0.3	17.0 ± 0.6	8.2 ± 0.5	7.9 ± 0.4	7.7 ± 0.5
C18:3n-3 ALA	1.6 ± 0.1	1.1 ± 0.1	2.9 ± 0.2	2.9 ± 0.2	2.6 ± 0.2	2.6 ± 0.2
C18:4n-3	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	1.1 ± 0.1
C20:2n-6	0.6 ± 0.0	-	0.6 ± 0.0	-	0.4 ± 0.0	-
C20:3n-6	0.7 ± 0.0	0.8 ± 0.1	0.6 ± 0.0	0.7 ± 0.1	0.5 ± 0.0	0.8 ± 0.1
C20:4n-6 AA	0.7 ± 0.1	0.8 ± 0.1	0.4 ± 0.0	-	0.6 ± 0.1	-
C20:5n-3 EPA	7.0 ± 0.2	8.6 ± 0.3	5.7 ± 0.1	6.4 ± 0.2	5.1 ± 0.1	7.4 ± 0.2
C22:5n-3 DPA	4.1 ± 0.2	5.7 ± 0.3	2.0 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	1.9 ± 0.1
C22:6n-3 DHA	13.3 ± 0.5	16.9 ± 0.7	12.1 ± 0.4	13.1 ± 0.6	15.8 ± 0.5	17.6 ± 0.6
Sum PUFA	37.5 ± 0.4	40.3 ± 0.6	42.7 ± 0.5	34.3 ± 0.5	36.0 ± 0.4	39.1 ± 0.5
Sum omega-3	27.4 ± 0.4	33.8 ± 0.6	24.1 ± 0.4	25.4 ± 0.5	26.6 ± 0.4	30.6 ± 0.5
Omega-3 VLC PUFA	24.4 ± 0.4	31.2 ± 0.6	19.8 ± 0.4	21.4 ± 0.5	22.6 ± 0.5	26.9 ± 0.5
Sum omega-6	10.1 ± 0.3	6.5 ± 0.3	18.6 ± 0.5	8.9 ± 0.5	9.4 ± 0.4	8.5 ± 0.5
Omega-6/omega-3 ratio	0.37	0.19	0.77	0.35	0.35	0.27

Values are expressed as mean ± standard deviation (SD) (n = 3). LA—linoleic acid; ALA—alpha linolenic; AA—arachidonic; EPA—eicosapentaenoic; DPA—docosapentaenoic; DHA—docosahexaenoic; VLC PUFA—very long-chain polyunsaturated fatty acid.

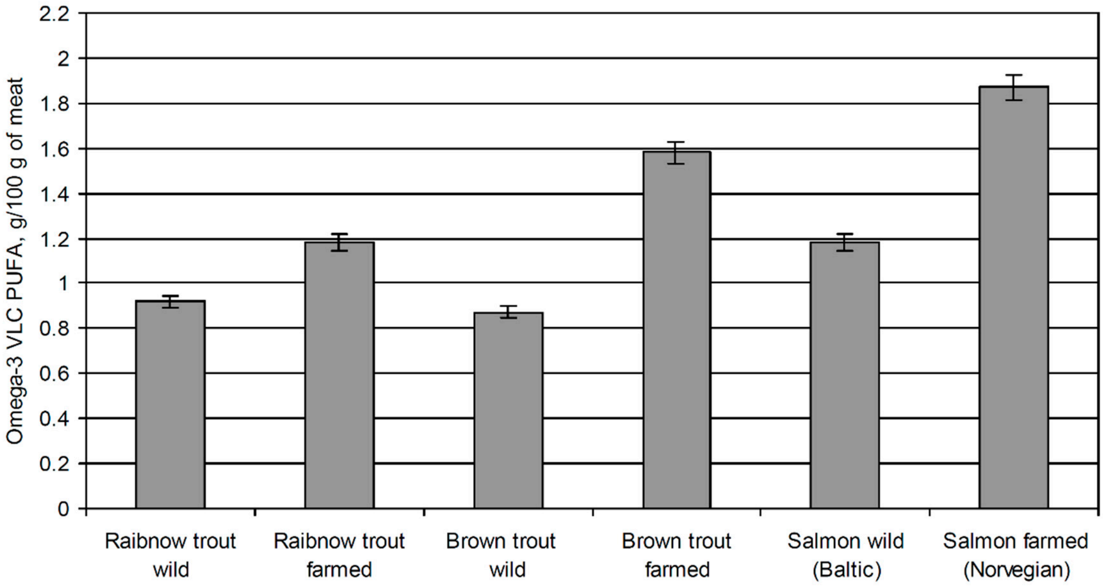

Figure 2. Omega-3 VLC PUFA content in salmonid fish fillets; g/100 g of raw meat. Values are expressed as mean ± standard deviation (SD) (n = 3).

4. Discussion

Among PUFAs, the omega-3 family dominated in all analyzed fish, while omega-6 levels were significantly lower. This has been noted by other authors too [13,14,21]. The omega-3 LC PUFA content in salmon was found to be 1.2 to 1.9 g/100 g of fillets. In other studies, it was from 1.0 to 2.6 g/100 g [14,21]. In the case of rainbow trout, the omega-3 VLC PUFA content in meat was from 0.9 to 1.2 g/100 g and was similar to that reported in other studies (0.6–1.4 g/100 g) [14]. The differences in omega-3 content depend on many factors, especially the fish's diet. However, it is also associated with gene expression of key metabolic processes, as it was shown in the case of salmon [22].

Fish are a uniquely rich source of omega-3 VLC PUFAs in the human diet. Other natural sources of omega-3 VLC PUFA are cultivated marine algae and single cell oils (s.c.o.) obtained from this source, marine mammals and crustaceans as krill. Omega-3 VLC PUFAs are also provided by supplements and enriched foods. These can contain fish oil, s.c.o. or krill oil. The ratios of particular omega-3 VLC PUFAs (EPA, DHA and DPA) differ between the various sources, although DPA is usually in a lower level than EPA and DHA [23]. Nevertheless, in recent years, the importance of DPA in health promotion had increased [9]. The European Food Safety Authority (EFSA) dietary recommendations (DRI) indicate that healthy individuals should consume an amount of omega-3 VLC PUFAs between 250 and 500 mg per day. This has been based on cardiovascular risk considerations for adults [23], whereas for patients with documented CHD, 1 g of omega-3 VLC PUFAs per day is recommended [3].

One portion of fish meal is 130 g, as advised by the EFSA [24]. Such a portion of farm-raised Norway salmon might provide 2.5 g omega-3 VLC PUFA. However, in a study focused on Scottish salmon, this dose was shown to be 1.8 g [21]. In the case of trout, depending on the species, it was found to be from 1.2 to 2.1 g. Other authors have reported this level to be from 1.5 to 2.7 g. The differences were dependent on fat content in fish as well as fish oil content in the fish's feed, as reported by other authors [25–27]. Moreover, the ratio of omega-6 to omega-3 PUFAs was much more nutritionally desirable in wild fish than in farm-raised ones. This has been also shown in other studies [27].

Due to the omega-3 VLC PUFA content, it is recommended that everyone should eat two portions of fish per week [10,28]—especially since most of the Western adult population is at risk of CHD [10]. According to the obtained results, it could be estimated that by following above dietary recommendation by eating two portions of Norwegian salmon per week, 5 g of omega-3 VLC PUFA could be provided, which corresponds to 714 mg per day. This would more than satisfy the daily DRI levels for healthy people and would reach close to the levels recommended for CHD patients. In the case of farm-raised trout, this figure is approximately 3.8 g per week, i.e., 542 mg per day, and slightly less in the case of wild ones.

The strong move towards a healthy diet promotes the consumption of fish. Nevertheless, due to overfishing, fish stocks are declining, which also refers to wild salmon and wild trout [2,5]. Thus, to protect endangered fish species, it is necessary to eat fish from sustainable sources such as aquaculture [29]. Aquaculture production of fish might counterbalance this supply issue. In farm-raised fish, the quality of meat, fatty acid profile as well as the concentration of vitamins and minerals are influenced by fish diet composition [18]. Nowadays, fish feeds contain additions of fish oils, single cell oils and other sources of omega-3 VLC PUFAs. This contributes to increase the omega-3 VLC PUFA level in farm-raised fish [25,30,31]. Moreover, other sustainable sources of omega-3 VLC PUFAs for farm-raised fish feeding are considered. One of the new sources of fish feed could be cultured gammarids (*Amphipoda*, *Crustacea*). In natural conditions, wild trout preferably eats gammarids, which is well known among fly-anglers [16].

Nevertheless, anthropogenic contaminants in farmed and wild fish, e.g., salmon, increase the health risks of fish consumption. At the beginning of this century, many reports showed that salmon, especially that which is farmed, contained high concentrations of organochlorine compounds such as polychlorinated biphenyls (PCBs), dioxins and chlorinated pesticides [32]. It was related to the quality of fish feed as well as the level of pollution of the seas and oceans. However, the latest reports have indicated that contaminant concentrations in Atlantic salmon are well below the maximum levels applicable in the European Union [27]. Thus, constant improvement of fish feed quality and care of the aquatic environment increase the food safety of farm-raised and wild fish.

Salmonids are exquisite fish, highly appreciated for their taste and high amount of omega-3 VLC PUFAs. It is agreed that omega-3 VLC PUFAs are one of the most potent bioactive substances enhancing health. Nowadays, health protection is becoming the most important issue for everyone. Frequent consumption of salmonids, both wild and farm-raised, might provide significant doses of omega-3 VLC PUFAs. This might desirably influence health, of both children and adults, by preventing many so-called lifestyle diseases, as well as improving brain development and function.

5. Conclusions

To generalize the conclusions from this study, more tests should be conducted on fish obtained from different locations at different times of the year. Nevertheless, it was confirmed that, contrary to the existing opinion, not only sea fish may be considered as rich sources of omega-3 VLC PUFAs in the diet but also some freshwater ones too, e.g., trout. Moreover, it was confirmed that although wild salmonids contain 10–25% more omega-3 VLC PUFAs in lipid fraction, farm-raised ones, due to the 60–100% higher fat content, are an equal or even more rich source of these desirable fatty acids. One serving (130 g) of salmonid fillets might provide a significant dose of omega-3 VLC PUFAs, from 1.2 to 2.5 g. Thus, due to the very high content of the bioactive fatty acids EPA, DPA and DHA in their meat, salmonid fish currently present on the food market, both sea and freshwater as well as wild and farm-raised, should be considered as natural functional food. The main health effects of regular salmonid fish consumption might decrease the risk of many so-called lifestyle diseases, as well as improving brain development and function.

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