Hindawi Publishing Corporation
Journal of Chemistry
Volume 2015, Article ID 696303, 7 pages
http://dx.doi.org/10.1155/2015/696303

Research Article

Female *Leuciscus lepidus* May Be a New Alternative for Fish Oil Supplements

Ozlem Cakmak,[1] Aynur Altuntas,[2] Veli Ugurcu,[3] Hacı Kemal Erdemli,[4] and Sumeyya Akyol[5]

[1]*Department of Biology Education, Faculty of Education, Gazi University, 06560 Ankara, Turkey*
[2]*Department of Chemistry, Ankara Regional Office of Council of Forensic Medicine, 06300 Ankara, Turkey*
[3]*Department of Medical Biochemistry, Faculty of Medicine, Dumlupınar University, 43050 Kütahya, Turkey*
[4]*Department of Biochemistry Laboratory, Çorum Training and Research Hospital, 19000 Çorum, Turkey*
[5]*Department of Medical Biology, Faculty of Medicine, Turgut Özal University, 06010 Ankara, Turkey*

Correspondence should be addressed to Ozlem Cakmak; ocakmak21@gmail.com

Received 5 May 2015; Accepted 3 August 2015

Academic Editor: Nivia Coelho

The proximate composition of male and female *Leuciscus lepidus* in Beyşehir Lake was investigated. The fatty acid profiles of total lipid, phospholipid, and triacylglycerol in muscle and liver of male and female *L. lepidus* were evaluated by gas chromatography. Proximate analyses showed that meat of male and female *L. lepidus* had 15.13 ± 0.04 and 18.75 ± 0.11% fat, 20.42 ± 0.45 and 22.21 ± 0.56% protein, 65.47 ± 1.37 and 61.28 ± 1.03% moisture, and 1.51 ± 0.05 and 1.50 ± 0.03% ash, respectively. The percentage of total saturated fatty acids was higher in liver than in muscle, whereas the total polyunsaturated fatty acid (PUFA) content was the lowest in all fatty acid profiles. The phospholipids contained more PUFAs than triacylglycerol. Analysis of variance indicated significant differences ($P < 0.05$) between male (47.51%) and female (49.98%) muscle PUFAs in total lipid. The proportion of omega 3 ($\omega 3$) to omega 6 ($\omega 6$) fatty acids of total lipid was 3.15 in male and 3.68 in female. The ratio is an important indicator for comparing the value of fish oil. Therefore, it was concluded that *L. lepidus* was considered to be a high quality product for healthy food choice. Additionally, female *L. lepidus* may especially be used to produce fish oil supplements from freshwater fish combined with vegetable oils.

1. Introduction

Human body cannot synthesize omega 3 ($\omega 3$) polyunsaturated fatty acids (PUFAs) [1]; for this reason, it has to be taken with nutrition [2]. Fish oil is especially rich in $\omega 3$ PUFAs. Two of the most important $\omega 3$ PUFAs contained in high percentage in fish oil are eicosapentaenoic acid (C20:5$\omega 3$, EPA) and docosahexaenoic acid (C22:6$\omega 3$, DHA) [3]. EPA and DHA are characteristic PUFAs of all kinds of fishes. These are either not present or found in very small amounts in foods except for water products. Fish species vary in their capacity to biosynthesize $\omega 3$ long-chain PUFAs, EPA and DHA, which are crucial to the health of higher vertebrates. The synthesis of long-chain PUFAs involves enzyme-mediated fatty acyl desaturation and elongation. Studies show strong evidence that EPA and DHA fatty acids from fish oil can reduce the incidence of blood pressure, triglyceride, cancer, allergy, cardiovascular diseases, depression, Alzheimer's disease, dyslipidemia, insulin resistance, adiposity, psoriasis, inflammatory bowel diseases, and eye diseases [4–7]. There are also good lines of evidence that diets rich in $\omega 3$ PUFAs help with rheumatoid arthritis and have positive effects on bone formation [8], treatment of autoimmune diseases [9], and attention deficit/hyperactivity disorders [10]. The other studies suggested that $\omega 3$ PUFAs reduce the risk of heart attacks, strokes, and death from heart disease [11].

Fish oil supplements are used for a wide range of conditions, and they are usually extracted from marine fish such as white tuna, mackerel, sardines, herring, halibut, salmon, cod liver, or blubber [12]. Fatty acid composition of marine and freshwater fishes is significantly different. That is, marine fish generally contain higher levels of $\omega 3$ PUFAs than freshwater fish. However, freshwater fish have a greater capacity than marine fish to elongate and desaturate the shorter fatty acid

(synthesized by algae or plants) into longer EPA and DHA. In other words, they convert food of poor nutritional value into food of rich nutritional value [13]. In addition, freshwater fish serve as a major source of protein and lipid for a large portion of the world population who live around the rivers and lakes and do not have access to marine fish.

On the whole, the fatty acid composition of fish lipids is affected by age, seasonal change, nutrition, sex, reproductive cycle, salinity, and geographical location [14–20].

Fatty acid profiles of phospholipids are different than that of triacylglycerol, which contains PUFA. The lipid content of diet affects PUFA absorption [21]. In particular, the fatty acid composition of the triacylglycerol strongly reflects that of the diet, implying that triacylglycerol acts as a nutritional storage site in the fish body [22]. Hence, it is necessary to figure out the fatty acid composition of phospholipids and triacylglycerol in order to estimate the nutritive value of fish.

Beyşehir Lake is the third largest lake in Turkey. It is a freshwater lake spanning over the borders of Isparta and Konya provinces in the southwestern part of Turkey. It is located at around $37^{\circ}47'0''$N, $31^{\circ}33'0''$E. It has an area of 650.00 km^2 and is 45 km long and 20 km wide. It carries the same name as the principal urban centre of its region, Beyşehir. The water level in the lake often fluctuates by year and by season of the year. The maximum depth is 10 meters. Beyşehir Lake is mostly harnessed for watering. Besides, it is also a national park. There are thirty-two islets in varying sizes on the lake. The lake has various water sources. Among the major ones are the streams and creeks of the Sultan and Animas mountains. Besides, precipitation is another contributing water source to the lake. In total, 27 streams feed the lake. The most important fish populations of the lake are *Tinca tinca*, *Sander lucioperca*, *Cyprinus carpio*, *L. lepidus*, and *Carassius carassius* [23].

Leuciscus lepidus differs from all other *Leuciscus* species in its elongated, long, rather pointed head with projecting lower jaw [24]. This species has great economic value and people who live in southwestern Anatolia consume this fish abundantly [25].

Research on *Leuciscus lepidus* (Cypriniformes: Cyprinidae) (Heckel, 1843) in Beyşehir Lake has focused on its growth features [25], but no research is available on the nutritional quality of *L. lepidus*. Therefore, the goal of this study is to investigate the proximate composition and fatty acid profiles of total lipid, phospholipid, and triacylglycerol in male and female *L. lepidus*. We expect that the determination of the composition and fatty acid profiles of these species will provide significant information on the nutrient value of this food for consumers.

2. Material and Methods

2.1. Sample Preparation. *L. lepidus* were caught from Beyşehir Lake, in September 2014. The samples were kept in ice after capturing and transported to the laboratory immediately. Male ($n = 3$) and female ($n = 3$) fishes of the same age were used for each analysis. The total length and weight of all samples were measured. The average fork lengths and weights of male and female individuals were 32.4 ± 4.2 cm and 528 ± 81.90 g, respectively.

2.2. Biochemical Analysis. Proximate composition analysis of lipid content was carried out by Bligh and Dyer [26] method. Protein content was determined by Kjeldahl method [27]. Moisture content was determined by oven drying at 105°C to constant weight [28]. Ash content was determined by combustion at 550°C for 3–5 hours [28].

2.3. Fatty Acids Analysis. The fishes were processed for lipid extraction and analysis following the methods described in Bligh and Dyer [26]. For analysis, three groups of three male and female *L. lepidus* were used and each sample was analyzed 3 times. Tissues of fishes (approximately 3 g) were homogenized in glass tubes and extracted three times with chloroform/methanol (2 : 1, v/v). Autoxidation of unsaturated components was minimized by adding 50 μL of 2% butylated hydroxytoluene in chloroform to each sample during the extraction process.

The total lipid extracts were dried under a stream of N_2 and then phospholipid and triacylglycerol fractions were isolated by thin-layer chromatography (TLC), using Silica Gel TLC plates (20 by 20 cm, 0.25 mm thick). After applying the total lipid extracts, the TLC plates were developed in petroleum ether : diethyl ether : acetic acid (80 : 20 : 1, v/v). Lipid fractions were made visible by spraying the TLC plates with 2′,7′-dichlorofluorescein (Supelco, Supelco Park, PA, USA), and phospholipid and triacylglycerol fractions were identified by corresponding standards.

The phospholipid and triacylglycerol fractions were scraped into reaction vials, and the associated fatty acids were transmethylated by refluxing the fractions in acidified methanol for 90 min at 85°C. The fatty acid methyl esters (FAMEs) were extracted from the reaction vials three times with hexane and then concentrated [29].

2.4. Gas Chromatography. The FAMEs were analyzed by gas chromatography using an Ati Unicam 610 gas chromatograph equipped with SP-2330 capillary column (30 m by 0.25 mm i.d., 0.2 μm film thickness, Supelco), a flame ionization detector, and Unicam 4815 recording integrator. A split injection of 0.5 μL was used. The temperature condition detector was set at 250°C. The oven temperature was set at 180°C for 5 min and then reached 200°C with a ramp rate of 2°C/min and then was held for 15 min. The carrier gas was nitrogen (flow rate 1 mL/min) and split ratio was 40 : 1. FAMEs were identified by comparisons of retention times with authentic standards (Sigma Chemical Co., St. Louis, MO, USA). Individual FAMEs were identified by comparisons with the chromatographic behaviors of authentic standards.

2.5. Gas Chromatography-Chemical Ionization Mass Spectrometry. The chemical structures of the FAMEs were confirmed by capillary gas chromatography-mass spectrometry (GC-MS) (HP 5890-E series GC-System, *Hewlett-Packard*, Palo Alto, CA, USA) with mass-selective detection. An *Innowax column* (30 m by 0.25 mm i.d., 0.25 μm film thickness) was used and the temperature was programmed from

Table 1: Proximate composition (%) of males and females of *Leuciscus lepidus*.

Chemical composition	Male	Female
Fat content (%)	15.13 ± 0.04a	18.75 ± 0.11b
Protein content (%)	20.42 ± 0.45a	22.21 ± 0.56b
Moisture content (%)	65.47 ± 1.37a	61.28 ± 1.03b
Ash content (%)	1.51 ± 0.05a	1.50 ± 0.03a

The values are shown as mean ± SD. Mean values are averages of three replicates.
Different letters in the same row represent significant statistical differences, $P < 0.05$.

150 to 230°C at a 2°C/min increase with an initial hold of 36 min. The carrier gas was helium (1 mL/min) and the split ratio was 1 : 50. The injection port and the detector temperatures were 250°C and 300°C, respectively. The mass spectrometer was operated in the electron impact ionization mode (70 eV). Chemical structures of the FAMEs were determined by comparison of the spectra with the Wiley 275 and NIST 98 databank and by comparing obtained spectra with that of authentic standards. The presence of mono- and polyunsaturated fatty acids containing 20 carbon atoms was illuminated using GC-MS. Analysis was carried out at TUBITAK Ankara Test and Analysis Laboratory (ATAL).

2.6. Statistical Analysis. The statistical analysis was performed using a commercial statistical program (SPSS 13.0). Statistical analysis of percentages of fatty acid was tested by *t*-test. Differences between mean values were evaluated as significant when $P < 0.05$. The results were shown as mean ± standard deviation.

3. Results and Discussion

The results of proximate composition of male and female *L. lepidus* are given in Table 1. The contents of fat, protein, moisture, and ash of male *L. lepidus* were 15.13, 20.42, 65.47, and 1.51%, respectively; and those values in female were 18.75, 22.21, 61.28, and 1.50%, respectively. Statistical analysis results showed that significant differences were observed between males and females in terms of fat, protein, and moisture. Proximate composition of females was found to have higher quality content than males. Arslan [30] reported that fat content in fillets of *Cyprinus carpio* is 2.60% in males and 2.20% in females. In females, fat quantity is higher than those of males as shown in a study by Karaton and Gürel İnanlı [31] which reported that fat, protein, moisture, and ash in male *Squalius cephalus* were 12.65, 20.87, 64.18, and 1.51%, respectively, while in females those values were 15.82, 19.40, 62.53, and 1.54%, respectively.

A comparison of males and females in terms of muscle and liver fatty acid composition of total lipid in *L. lepidus* is presented in Table 2. Twenty different fatty acids were detected. The major fatty acids identified in both sexes were palmitic (C16:0), palmitoleic (C16:1ω-7), stearic (C18:0), oleic (C18:1ω-9), linoleic (C18:2ω-6), arachidonic (C20:4ω-6, AA), eicosapentaenoic (C20:5ω-3), docosapentaenoic (C22:5ω-3, DPA), and docosahexanoic (C22:6-ω3) acids. The total

Table 2: Comparison of males and females in terms of muscle and liver fatty acid composition of total lipid in *L. lepidus*.

Fatty acids	Muscle		Liver	
	Male	Female	Male	Female
C12:0	0.02 ± 0.01a	0.12 ± 0.02b	0.15 ± 0.04y	0.10 ± 0.02z
C13:0	0.82 ± 0.07a	1.02 ± 0.09a	0.32 ± 0.05y	0.08 ± 0.01z
C14:0	1.30 ± 0.02a	1.32 ± 0.02a	1.38 ± 0.13y	1.12 ± 0.17z
C15:0	0.40 ± 0.05a	0.53 ± 0.06b	0.28 ± 0.06y	0.87 ± 0.08z
C16:0	14.84 ± 0.30a	10.42 ± 0.41b	15.30 ± 0.39y	13.20 ± 0.75z
C17:0	1.30 ± 0.08a	0.32 ± 0.01b	0.80 ± 0.07y	0.82 ± 0.08y
C18:0	6.15 ± 0.13a	8.06 ± 0.21b	9.01 ± 0.31y	11.21 ± 0.43z
ΣSFA	24.83 ± 0.92a	21.79 ± 1.02b	27.24 ± 1.65y	27.40 ± 1.85y
C16:1ω-7	7.26 ± 0.25a	6.07 ± 0.11a	7.87 ± 0.09y	5.63 ± 0.28z
C18:1ω-9	17.86 ± 0.76a	19.22 ± 0.99b	16.72 ± 0.42y	18.03 ± 0.72z
C20:1ω-9	2.54 ± 0.49a	2.94 ± 0.45a	3.89 ± 0.53y	3.14 ± 0.48y
ΣMUFA	27.66 ± 1.13a	28.23 ± 1.83a	28.48 ± 1.91y	26.80 ± 1.77z
C18:2ω-6	4.65 ± 0.19a	4.18 ± 0.14a	5.39 ± 0.87y	6.95 ± 0.74z
C18:3ω-6	1.02 ± 0.11a	1.50 ± 0.18b	0.70 ± 0.13y	0.81 ± 0.10y
C18:3ω-3	0.82 ± 0.11a	0.53 ± 0.18b	2.70 ± 0.31y	2.83 ± 0.35y
C20:2ω-6	0.27 ± 0.17a	0.51 ± 0.20b	0.42 ± 0.08y	0.56 ± 0.15z
C20:3ω-6	2.42 ± 0.13a	2.40 ± 0.10a	0.20 ± 0.12y	0.24 ± 0.20y
C20:4ω-6	3.07 ± 0.03a	2.10 ± 0.07b	6.14 ± 0.16y	7.14 ± 0.11z
C20:5ω-3	8.16 ± 0.48a	8.06 ± 0.65a	5.10 ± 0.15y	5.07 ± 0.10y
C22:3ω-3	0.06 ± 0.03a	0.12 ± 0.05b	0.10 ± 0.04y	0.04 ± 0.04z
C22:5ω-3	4.02 ± 0.11a	4.57 ± 0.24b	2.70 ± 0.31y	2.02 ± 0.33z
C22:6ω-3	23.02 ± 1.14a	26.01 ± 1.25b	20.83 ± 1.10y	20.14 ± 1.28y
ΣPUFA	47.51 ± 1.68a	49.98 ± 1.71b	44.28 ± 0.68y	45.80 ± 0.68y
Σω3	36.08	39.29	31.43	30.10
Σω6	11.43	10.69	12.85	15.70
Σω3/ω6	3.15	3.68	2.44	1.91

The values are shown as mean ± SD. Mean values are averages of three replicates.
Different letters in the same row and values in male and female represent significant statistical differences, $P < 0.05$.
SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

saturated fatty acids (SFA) were measured as 24.83% in male and 21.79% in female muscle. The percentages of total monounsaturated fatty acids (MUFA) compared to total lipid extracted from muscle males and females were 27.66% and 28.23%, respectively. C18:1ω-9 was a predominant fatty acid to take the form of MUFA and the acid is attributed as healthy due to the monounsaturated status [32]. Analysis of variance indicated significant differences ($P < 0.05$) between male (47.51%) and female (49.98%) muscle PUFA compositions where EPA and DHA acids had the highest levels among others. Similar results have also been reported for other freshwater fish [19, 22, 33], except for ω3/ω6 ratio. In this study, the first major finding is the observation of high level ω3/ω6 ratio (i.e., 3.15 in male and 3.68 in female muscle total lipid). The ratio of ω3/ω6 is an important indicator for comparing the value of fish oil [34]. The UK Department of Health advises an ideal ratio of ω3/ω6 of 4.0 at maximum

[35]. This ratio is very important in order to reduce coronary heart diseases, plasma lipid levels, and risk of cancer [36].

Cengiz et al. [37] investigated total fatty acids in the muscle of female *L. lepidus* from the Tigris river (Turkey), in January. The ratio of $\omega 3/\omega 6$ was detected as 2.31 by Cengiz et al. A similar study was carried out by Özparlak [38] to measure total fatty acids in female *L. lepidus* muscle in Apa Dam Lake (Turkey), in January. He reported the ratio of $\omega 3/\omega 6$ as 2.08. We also carried out our studies with the same fish and in the same month as previous studies, but we observed a higher ratio (3.68 in female) during our study. Hence, in this study, the second major finding is the effect of regional differences on fatty acid composition. The reason for this difference might be the feeding properties of Beyşehir Lake by 27 streams, which may have led to highly nutritious freshwater habitat.

Analysis of variance showed significant differences between total fatty acid compositions in the liver of males and females. The percentage of ΣMUFA was significantly ($P < 0.05$) higher in males (28.48%) than in females (26.89%). However, in the liver, total SFA level (27.24% in males and 27.40% in females) was higher and total PUFA level (44.28% in males and 45.80% in females) was lower than muscle (47.51% in males and 49.98% in females) fatty acid composition (Table 2). Similar results for *Salmo trutta macrostigma* [39] and *Vimba vimba* [40] were also reported in the literature. The most dramatic change in fatty acid composition of fish is observed during their reproduction period. Most of the lipid that would be mobilized for later use in the formation and growth of gonads is stored in muscle, liver, and abdominal regions before the reproduction period [41]. More specifically, the liver stores lipids that will be used to produce the energy required for growth of gonads and gamete. On the other hand, the energy required for reproductive activity is provided by the lipids stored in muscle [42].

A comparison of males and females in terms of muscle and liver fatty acid composition of phospholipid in *L. lepidus* is shown in Table 3. A similar comparison in terms of triacylglycerols is shown in Table 4.

In particular, phospholipids in muscle and liver of *L. lepidus* had higher rate of C20:4ω-6 and C22:6ω-3, but lower rate of C18:1ω-9 compared to triacylglycerol. In addition, phospholipids differed from total fatty acid and triacylglycerol with higher proportion of ΣPUFA and lower proportions of ΣSFA and ΣMUFA. In triacylglycerol fraction, C16:0, C16:1, and C18:1 had the highest and C20:4ω-6 and C22:6ω-3 had the lowest level. The accumulation of ΣSFA and ΣMUFA in triacylglycerol fraction is probably related to energy requirement and the aim of this accumulation may be storing energy-rich material for subsequent consumption during metabolic activity and hard conditions.

Phospholipids function as structural components of membranes, being incorporated to a larger extent into the membrane structure of cellular and subcellular particles. Triacylglycerols are often considered as storage lipids and reflect the fatty acid composition of the food to a greater extent than phospholipids do.

Table 3: Comparison of males and females in terms of muscle and liver fatty acid composition of phospholipid in *L. lepidus*.

Fatty acids	Muscle		Liver	
	Male	Female	Male	Female
C12:0	0.18 ± 0.03a	0.13 ± 0.02b	0.85 ± 0.07y	0.93 ± 0.02z
C13:0	1.02 ± 0.07a	0.42 ± 0.07b	0.35 ± 0.05y	0.08 ± 0.01z
C14:0	1.32 ± 0.09a	1.30 ± 0.03a	1.43 ± 0.13y	1.12 ± 0.17z
C15:0	0.53 ± 0.05a	0.40 ± 0.05b	0.42 ± 0.06y	0.87 ± 0.08z
C16:0	11.42 ± 1.80a	12.68 ± 1.74a	13.11 ± 1.15y	12.20 ± 0.25z
C17:0	1.32 ± 0.08a	1.02 ± 0.05b	1.89 ± 0.07y	0.82 ± 0.08y
C18:0	4.78 ± 0.23a	3.12 ± 0.20b	7.20 ± 0.31y	6.38 ± 0.43z
ΣSFA	20.57 ± 1.22a	19.07 ± 1.62a	25.25 ± 1.65y	24.40 ± 1.85y
C16:1ω-7	2.34 ± 0.22a	1.80 ± 0.13b	3.88 ± 0.09y	3.69 ± 0.28z
C18:1ω-9	14.01 ± 0.96a	12.86 ± 1.04b	14.70 ± 1.42y	14.07 ± 0.72z
C20:1ω-9	2.14 ± 0.45a	1.12 ± 0.05a	0.87 ± 0.53y	1.03 ± 0.48y
ΣMUFA	18.49 ± 1.13a	15.78 ± 1.83b	19.45 ± 1.91y	18.79 ± 1.77z
C18:2ω-6	2.35 ± 0.13a	2.15 ± 0.14a	3.31 ± 0.42y	4.99 ± 0.24z
C18:3ω-6	1.55 ± 0.10a	1.25 ± 0.09b	1.78 ± 0.11y	1.82 ± 0.10y
C18:3ω-3	1.45 ± 0.11a	1.82 ± 0.17b	2.74 ± 0.28y	2.82 ± 0.33z
C20:2ω-6	0.22 ± 0.07a	0.14 ± 0.08b	0.41 ± 0.08y	0.59 ± 0.15y
C20:3ω-6	1.07 ± 0.09a	0.48 ± 0.11b	1.23 ± 0.10y	1.28 ± 0.05y
C20:4ω-6	11.10 ± 0.73a	11.07 ± 0.68a	10.14 ± 0.14y	11.03 ± 0.16y
C20:5ω-3	7.02 ± 0.43a	13.10 ± 0.75b	7.06 ± 0.11y	8.08 ± 0.13z
C22:3ω-3	0.12 ± 0.03a	1.06 ± 0.05b	0.16 ± 0.06y	0.08 ± 0.05z
C22:5ω-3	4.15 ± 0.31a	5.14 ± 0.44b	3.73 ± 0.21y	2.03 ± 0.18z
C22:6ω-3	31.91 ± 1.84a	28.94 ± 1.25b	24.74 ± 1.19y	24.09 ± 1.22y
ΣPUFA	60.94 ± 1.93a	65.15 ± 2.11b	55.30 ± 1.46y	56.81 ± 1.57y
$\Sigma\omega 3$	44.65	50.06	38.43	37.10
$\Sigma\omega 6$	16.29	15.09	16.87	19.71
$\Sigma\omega 3/\omega 6$	2.74	3.31	2.27	1.88

The values are shown as mean ± SD. Mean values are averages of three replicates.
Different letters in the same row and values in male and female represent significant statistical differences, $P < 0.05$.
SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

Logue et al. [43] found increased proportions of C20:4ω-6 and C22:6ω-3 as a result of cold adaptation while the proportion of saturated fatty acids fell in phospholipid. The fatty acid composition correlates well with the temperature-adaptive interspecies differences in membrane physical structure. The increases in the PUFA content of phospholipids may be due to cold weather. Phospholipids, particularly their fatty acid composition, are the principle factors that define the physical properties of the membrane. Fluidity of the membrane is essential to establish an appropriate setting for the membrane to perform its functions. The fluidity is in part dependent on the degree of unsaturation of fatty acids. Furthermore, the adaptation capabilities of fish and other poikilotherms to various environmental temperatures are to a large extent shaped by the same factor, that is, the degree of unsaturation of fatty acids [44]. The ratio of unsaturated fatty acids changes depends on seasonal temperature changes. For instance, as the weather gets colder in winter, an increase in unsaturation

Table 4: Comparison of males and females in terms of muscle and liver fatty acid composition of triacylglycerol in *L. lepidus*.

Fatty acids	Muscle		Liver	
	Male	Female	Male	Female
C12:0	0.04 ± 0.01a	0.18 ± 0.03b	0.22 ± 0.04y	0.19 ± 0.02z
C13:0	1.42 ± 0.06a	1.83 ± 0.10a	1.68 ± 0.05y	1.96 ± 0.01z
C14:0	1.68 ± 0.05a	1.44 ± 0.02a	2.44 ± 0.13y	2.42 ± 0.17z
C15:0	0.45 ± 0.05a	0.57 ± 0.06b	1.25 ± 0.06y	1.22 ± 0.08z
C16:0	20.41 ± 0.30a	17.65 ± 0.41b	22.15 ± 0.39y	23.47 ± 0.25z
C17:0	1.33 ± 0.08a	1.47 ± 0.01b	1.43 ± 0.07y	1.42 ± 0.08y
C18:0	7.15 ± 0.13a	8.43 ± 0.21b	13.01 ± 0.31y	13.05 ± 0.43z
ΣSFA	32.48 ± 0.92a	31.57 ± 1.02b	42.18 ± 1.65y	43.73 ± 1.85y
C16:1ω-7	13.26 ± 0.25a	13.06 ± 0.11a	11.23 ± 0.09y	10.45 ± 0.28z
C18:1ω-9	23.12 ± 0.76a	23.04 ± 0.99b	20.11 ± 0.42y	21.03 ± 0.72z
C20:1ω-9	3.12 ± 0.49a	3.15 ± 0.45a	3.03 ± 0.53y	3.02 ± 0.48y
ΣMUFA	39.50 ± 1.13a	39.25 ± 1.83a	34.37 ± 1.91y	34.50 ± 1.77z
C18:2ω-6	4.89 ± 0.19a	4.49 ± 0.14a	3.39 ± 0.87y	2.51 ± 0.74z
C18:3ω-6	1.24 ± 0.11a	1.59 ± 0.18b	0.81 ± 0.13y	0.88 ± 0.10y
C18:3ω-3	0.85 ± 0.11a	0.64 ± 0.18b	1.24 ± 0.31y	1.13 ± 0.35z
C20:2ω-6	0.25 ± 0.17a	0.47 ± 0.20b	0.38 ± 0.08y	0.44 ± 0.15z
C20:3ω-6	2.10 ± 0.13a	2.12 ± 0.10a	0.46 ± 0.12y	0.34 ± 0.20y
C20:4ω-6	1.14 ± 0.03a	1.16 ± 0.07a	2.61 ± 0.16y	2.13 ± 0.11z
C20:5ω-3	6.93 ± 0.48a	6.14 ± 0.65a	4.83 ± 0.15y	5.05 ± 0.011y
C22:3ω-3	0.11 ± 0.03a	0.19 ± 0.05b	0.09 ± 0.04y	0.02 ± 0.04z
C22:5ω-3	2.02 ± 0.11a	2.14 ± 0.24a	1.51 ± 0.31y	1.17 ± 0.33z
C22:6ω-3	8.49 ± 1.14a	10.24 ± 1.25b	8.13 ± 1.10y	8.10 ± 1.28y
ΣPUFA	28.02 ± 1.68a	29.18 ± 1.71b	23.45 ± 1.12y	21.77 ± 1.03z
Σω3	18.40	19.35	15.80	15.47
Σω6	9.62	9.83	7.65	6.30
Σω3/ω6	1.91	1.96	2.06	2.45

The values are shown as mean ± SD. Mean values are averages of three replicates.
Different letters in the same row and values in male and female represent significant statistical differences, $P < 0.05$.
SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

degree in body fat is often observed. A common way that fish uses to achieve such increase is through the conversion of saturated fatty acids of phospholipids in biological membrane into their corresponding mono- and dienic fatty acids. This mechanism is vital for fish to survive. Through this mechanism, regardless of the environmental temperature, biological membranes can consistently carry out their regular functions [20].

Fish oil can be obtained from eating fish or by taking supplements. Fish oil supplements are usually made from marine fishes. This is because marine fish are especially richer in omega 3 than those of freshwater. On the other hand, Ahlgren et al. [45] reported high ω3/ω6 ratio (5.83%) in the freshwater whitefish (*Coregonus* spp.). Our study suggests that female *L. lepidus* especially may also be used to produce fish oil supplements from freshwater fish combined with vegetable oils (e.g., sunflower, soybean, canola, walnut, flaxseed, and olive) and food sources (e.g., walnuts, flaxseeds, and olives) that are rich in C18:3ω-3 (α-linolenic acid) in a healthy diet for the general population [46]. In addition, many marine fish types require 20:5ω-3 and 22:6ω-3 as part of their diet, as they do not have Δ^5 desaturase enzyme and cannot perform de novo 20:5ω-3 and 22:6ω-3 synthesis [44]. Therefore, fatty acids such as AA, EPA, and DHA are essential in diets of marine fish in order to establish a healthy development and growth [47]. In comparison, freshwater fish are superior to marine fish, as they have Δ^5 desaturase enzyme and are capable of de novo 20:5ω-3 and 22:6ω-3 synthesis. These results showed that fatty acid profiles of some freshwater fish are basically comparable to marine water fish in terms of sources of PUFAs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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