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DISTRIBUTION OF PHOSPHOLIPID FATTY ACID CONTENT IN THE MUSCLE TISSUE OF FEMALE AND MALE INDIVIDUALS OF *ALBURNUS MOSSULENSIS* DURING THE REPRODUCTION PERIOD

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ABSTRACT

For living systems, fatty acids are very important for their functional functions. Since these fatty acids change depending on ecological factors, they are very important for living things. Therefore, in our study, it was aimed to investigate the change of fatty acids in phospholipid subclasses in muscle tissue in the reproduction period.

In our study, the sexes of *Alburnus mossulensis* fish caught in Batman Dam Lake was determined. After the total lipid extraction from muscle tissue of both individuals, it was separated into phospholipid subclasses by thin-paper chromatography. Samples were analyzed qualitatively as % fatty acid with Gas Chromatography. When the fatty acid distribution was considered, out of the saturated ones, 16:0 in PC and PE; 18:0 in PI; 16:0 and 18:0 in PS; out of monounsaturated ones, 18:1n-9 in all subclasses; out of polyunsaturated ones, 20:5n-3 and 22:6n-3 in PC and PE; 20:4n-6, 20:5n-3 and 22:6n-3 in PI; and 22:6n-3 in PS were found as major fatty acids. When the phospholipid subclasses were taken into account, it was observed that the most one was PUFA, then SFA and the least one was monounsaturated fatty acids (MUFA). Depending on 20:4n-6, the ratio of n-3/n-6 was two times lower in PI than the other subclasses.

Key words: Fatty Acid, Phosphatidylcholin, Phosphatidylethanolamin, Phosphatidylinositol, Phosphatidylserine, *Alburnus mossulensis*

1. INTRODUCTION

Lipids are composed of fatty acids that are their main components (Castell, Lee and Sinnhuber). It has been shown that these fatty acids act as the source of energy in the form of triacylglycerol and as the main component of the cell membrane as phospholipids (Sargent et al.; Kiessling et al.). The phospholipid component in cell membranes is very important for normal cell functions. These phospholipids play an important role in living things. Establishing a permeability gradient is especially important for cells and cell organelles. It also provides a matrix environment for the function of enzymatic processes(Yamaji-Hasegawa and Tsujimoto). Furthermore, phospholipids lead the transport of fat-soluble nutrients, many hormones and eicosanoids (Higgs and Dong).

There is little information about phospholipid subclass components of fish and important metabolic functions in the conducted studies. It is known that especially the changes in water temperature are very important in terms of maintaining the balance of saturated, monounsaturated and polyunsaturated fatty acids and continuation of the life of the living thing (Farkas et al.). It was reported that Atlantic salmon needs n-3 and n-6 polyunsaturated fatty acids (Ruyter et al.; Menoyo et al.). However, more information is needed about the organs and phospholipid subclasses that are sensitive to the deficiency of these fatty acids. In a similar study, it was found that both Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) content decreased in

phosphatidylcholine (PC) and phosphatidylethanolamine (PE), while DHA remained the same in phosphatidylserin (PS) (Ghioni et al.).

In conclusion, studies on phospholipid subclasses are mostly concentrated on marine fish. In our study, it was aimed to reveal changes in the reproduction period of PC, PE, PS and PI fatty acids, which are the phospholipid subclasses, in the female and male individuals of the genus Chalcalburnus (*Alburnus*), belonging to the Cyprinidae family living in the inland waters of our country.

2. MATERIALS AND METHODS

2.1. Collecting Samples and Sex Determination

Sampling station was decided on Yayık Village Batman Dam Lake near the entrance of Diyarbakır Kulp District (38°14' 49. 05"N, 41°06' 04. 25"E, 653 m). The fish were caught by local fishermen in the region. Gonads taken by dissection in captured fish were determined under a light microscope.

2.2. Separation and Analysis of Phospholipid Subclasses

Total lipids extracted that it was used method Folch et al. (1957) (chloroform / methanol (2/1 v/v)). For the separation proses, was used thin-layer chromatography (TLC) plates (Silica gel 60G (Merck)). The method used by Vaden et. all. (2005) (chloroform / ethanol / water / triethylamine (30:35:7:35, v/v)) was used for the separation process.

Fatty acid analysis of fatty acid samples converted into methyl esters was done with flame-ionization detector (FID) and DB-23 (Bonded 50% cyanopropyl) (J & W Scientific, Folsom, CA, USA), using capillary column (30m x 0.25mm inside bore x 0.25µm film thickness) on the SHIMADZU GC 2010 PLUS model Gas Chromatography. The temperature program used was as follows. Detector temperature: 250 °C; injector temperature: 250 °C; The split ratio was 10 / 1. Velocity of gas flow: Carrier gas: helium 0.5 ml / min for 30 m column; hydrogen: 30 ml / min; dry air: 400 ml / min. The initial temperature (170 °C) was kept for 2 min, raised to 210 °C, waiting period 20 minutes; total analysis period: 42 minutes. The injection and the detector temperatures were 250 °C. In the diagnosis of fatty acids, a mixture of methyl esters of fatty acids (Sigma - Aldrich Chemicals) was used as standard. Chromatograms of fatty acid methyl esters and total amounts of fatty acids were obtained by GC Solution (Versiyon 2.4) computer program. The results were given as qualitative value on % fatty acid. Comparing of the percentages of fatty acids was done with SPSS 16 computer program and one-way analysis of variance (ANOVA). The differences were determined by TUKEY HSD test. As a result of the statistics, the differences were accepted to be significant when the data was at $P < 0.05$ level.

3. RESULTS AND DISCUSSION

A total of 16 fatty acids were determined as a result of the analysis. The main fatty acids were determined as Palmitic Acid, Palmitoleic Acid, Stearic acid, Oleic Acid, Arachidonic Acid, Eicosapentaenoic acid, Docosapentaenoic acid and Docosahexaenoic acid. Other fatty acids such as myristic, pentadecanoic and stearic acid were present only in trace proportions (Table1.).

In the reproductive period, 16:0 in PC and depending on this, total saturated fatty acids (SFA) was approximately 3% higher in males. In our study, this component was major, which was 26.15% in females and 32.31% in males (Table1.). Out of monounsaturated fatty acids, 16:1n-7 was 3 times and 18:1n-9 was 1.65 times higher in female individuals. Depending on them, total MUFA was nearly 2 times higher in female individuals. Out of polyunsaturated fatty acids 20:5n-3 was 1.32 times, 22:6n-3 was 1.12 times, depending on them, total polyunsaturated fatty acids (PUFA) and n-3 were 1.16 times more in the male individuals than those in females. On the other hand, n-6 and n-3/n-6 were similar (Table1., Figure 1.and 2.).

In this study, out of saturated ones, 16:0; out of monounsaturated ones, 18:1n-9; and out of polyunsaturated ones, EPA and DHA were major in the muscle PC fraction of females and males of *A. mossulensis*.

As in the PC fraction, in the muscle PE fatty acid content of *A. mossulensis*, out of saturated ones, 16:0; out of monounsaturated ones, 18:1n-9; and out of polyunsaturated ones, EPA and DHA were major (Table1.). Depending on 16:0 in PE, ΣSFA was higher in males, while total PUFA and n-3 were higher in female individuals, depending on 20:5n-3, 22:5n-3 and 22:6n-3. n-6 and n-3/n-6 exhibited a similar distribution. In both individuals, the order of fatty acids was ΣPUFA>ΣSFA>ΣMUFA as percent in the decreasing order (Figure 2.).

In our study, PI, 18:0 (26.05% in females, 22.03% in males), 20:4n-6 (17.05% in females, 13.90% in males) and 22:6n-3 (18.71% in females, 21.90% in males) were major fatty acids. In PI, 18:0 was 1.18 times higher in female individuals than males. A similar distribution was observed in monounsaturated fatty acids. Out of the polyunsaturated fatty acids, 20:4n-6 was 1.23 times higher in females than male individuals. The percentage of n-3 was 1.12 times higher in males than females, depending on 20:5n-3 and 22:6n-3. Since n-6 is higher in females depending on 20:4n-6, n-3/n-6 ratio was lower than males (Table1.). Another finding of the study is that the ratio of n-3/n-6 is rather lower than PC and PE. This ratio was 1.95 for females and 2.55 for males (Table1., Figure 2.). ΣPUFA was the most, then ΣSFA and the ΣMUFA was lowest in *A. mossulensis*.

In our studed that PS has a high percentage in terms of ΣSFA and that 16:0 and 18:0 were close to each other. 16:0 (16.46% in females, 19.85% in males) and 18:0 (17.93% in females, 18.98% in males) were found to be high and similar. 16:0 was 1.21 times more in PS and, depending on this; total SFA was 1.13 times more in male individuals. Monounsaturated fatty acids showed a similar distribution. When polyunsaturated fatty acids are considered, it was observed that 22:5n-3 was 1.57 times; 22:6n-3 was 1.24 times, and depending on them, total PUFA was 1.11 and n-3 was 1.19 times more in female individuals than male individuals (Table1.).

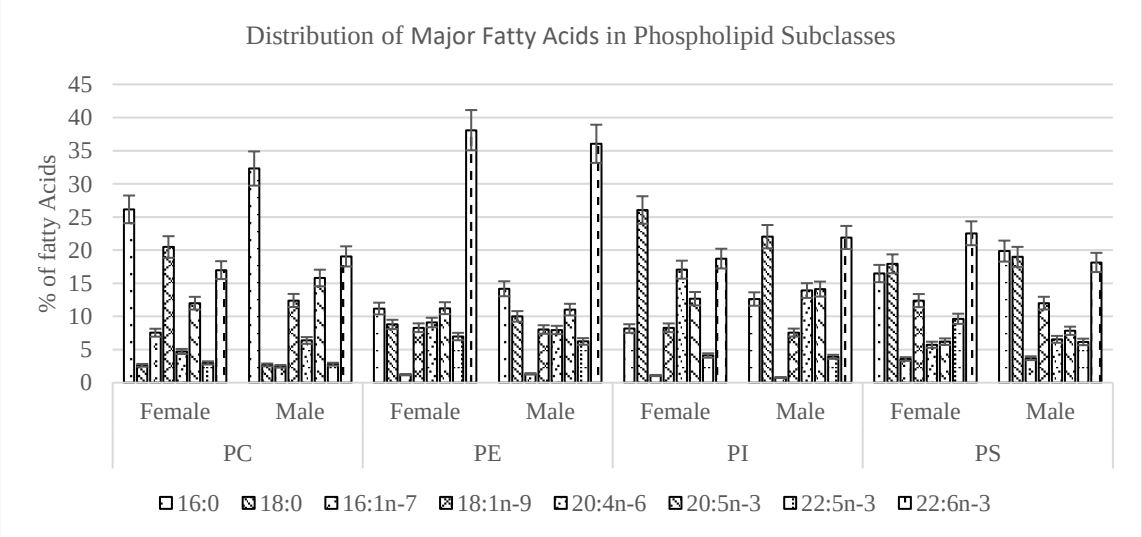

Figure 1. Distribution of Major Fatty Acids in Phospholipid Subclasses

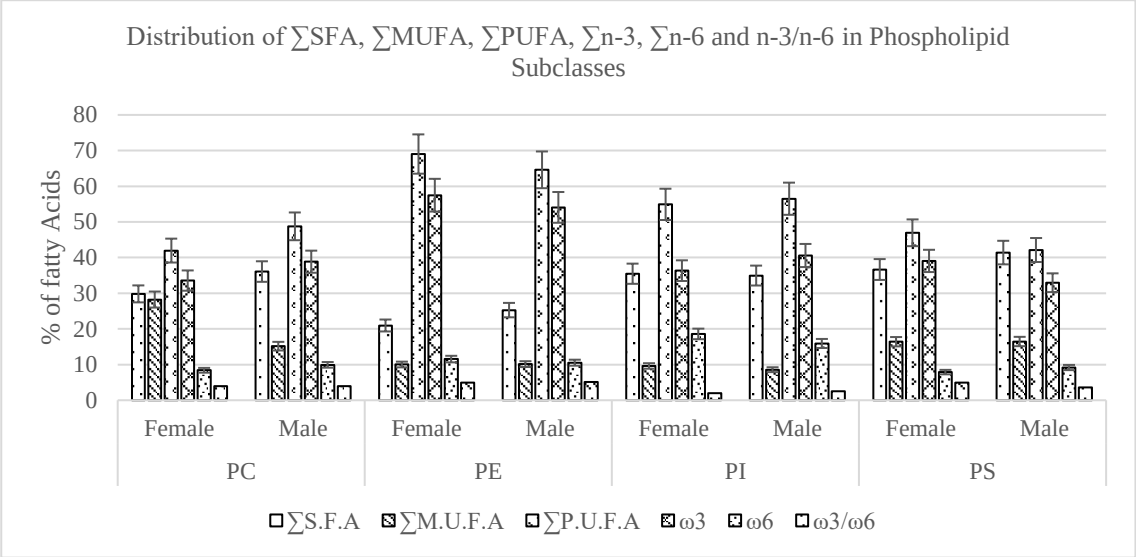

Figure 2. Distribution of ∑SFA, ∑MUFA, ∑PUFA, ∑n-3, ∑n-6 and n-3/n-6 in Phospholipid Subclasses

Table 1. Distribution of phospholipid subclasses in muscle tissue in male and female individuals during the reproduction period of *A. mossulensis*

	PC		PE		PI		PS	
	Female	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
14:0	0.53±0.104a	0.55±0.04a	0.41±0.03a	0.43±0.03a	0.42±0.03a	0.21±0.02b	1.24±0.10a	1.96±0.16a
15:0	0.24±0.02a	0.23±0.02a	0.06±0.00a	0.06±0.00a	0.08±0.01a	0.02±0.00b	0.25±0.02a	0.20±0.02a
16:0	26.15±2.09a	32.31 ± 2.58b	11.18±0.89a	14.17±1.13a	8.17±0.65a	12.62±1.01b	16.46±1.31a	19.85±1.58a
17:0	0.29±0.02a	0.31±0.02a	0.52±0.04a	0.61±0.05a	0.75±0.06a	0.08 ± 0.01a	0.77±0.06a	0.41±0.03b
18:0	2.62±0.21a	2.68±0.21a	8.79±0.70a	10.00 ± 0.80a	26.05±2.08a	22.03±1.76b	17.93±1.43a	18.98±1.51a
∑S.F.A	**29.82±2.38a**	**36.08±2.88b**	**20.96±1.67a**	**25.27±2.02b**	**35.47±2.83a**	**34.96±2.79a**	**36.65±2.92a**	**41.40±3.30b**
16:1n-7	7.54±0.60a	2.47±0.20b	1.21±0.10a	1.33±0.11a	1.08±0.09a	0.77±0.06a	3.57±0.28a	3.69±0.29a
18:1n-9	20.47±1.63a	12.40±0.99b	8.29 ± 0.66a	8.03±0.64a	8.28±0.66a	7.56±0.60a	12.38±0.99a	12.00 ± 0.96a
20:1n-9	0.22±0.02a	0.29±0.02a	0.52±0.04a	0.77±0.06a	0.25 ± 0.02a	0.23 ± 0.02a	0.46±0.04a	0.78±0.06b
∑M.U.F.A	**28.23±2.25a**	**15.16±1.21b**	**10.02±0.80a**	**10.13±0.81a**	**9.61±0.77a**	**8.56±0.68a**	**16.41±1.31a**	**16.47±1.31a**
18:2n-6	3.08±0.25a	2.97±0.24a	1.60±0.13a	1.60±0.13a	1.17±0.09a	1.54±0.12a	1.49±0.12a	1.88±0.15a
18:3n-3	1.61±0.13a	1.21±0.10a	1.20±0.10a	0.79±0.06b	0.84±0.07a	0.66±0.05a	0.69±0.06a	0.83±0.07a
20:2n-6	0.29±0.02a	0.32±0.03a	0.38 ± 0.03a	0.53±0.04b	0.15±0.01a	0.18±0.01a	0.36±0.03a	0.42±0.03a
20:3n-6	0.32±0.03a	0.26±0.02a	0.48±0.04a	0.45±0.04a	0.22±0.02a	0.30±0.02a	0.32±0.03a	0.33±0.03a
20:4n-6	4.71 ± 0.38a	6.37±0.51b	9.08±0.72a	7.95±0.63a	17.05±1.36a	13.90±1.11b	5.72±0.46a	6.54±0.52a
20:5n-3	11.99±0.96a	15.79±1.26b	11.24±0.90a	11.02±0.88a	12.67±1.01a	14.12±1.13a	6.19±0.49a	7.83±0.62a
22:5n-3	2.98±0.24a	2.79±0.22a	6.96±0.56a	6.22±0.50a	4.10±0.33a	3.90±0.31a	9.64±0.77a	6.15±0.49a
22:6n-3	16.98±1.35a	19.05±1.52a	38.09 ± 3.04a	36.04±2.88a	18.72±1.49a	21.90±1.75a	22.54±1.80a	18.14±1.45a
∑P.U.F.A	**41.96±3.35a**	**48.76±3.89b**	**69.03±5.51a**	**64.60±5.15a**	**54.92±4.38a**	**56.50±4.51a**	**46.95±3.75a**	**42.12±3.36a**
ω3	33.55±2.84a	38.84±3.10b	57.49±4.59a	54.07±4.31a	36.33±2.90a	40.58±3.24a	39.06±3.12a	32.95±2.63b
ω6	8.40±0.67a	9.92±0.79a	11.54±0.92a	10.53±0.84a	18.59±1.48a	15.92±1.27a	7.89±0.63a	9.17±0.73a
ω3/ω6	3.99	3.92	4.98	5.13	1.95	2.55	4.95	3.59

Values are provided as mean ± SE of three replicate samples; values with different letters in one line are significantly different (ANOVA, Tukey HSD test, P<0.05)
SFA saturated fatty acid, MUFA monounsaturated fatty acid, PUFA polyunsaturated fatty acid

In the reproductive period, 16:0 in PC and depending on this, total SFA was approximately 3% higher in males. The others were found to be similar. In previous studies, the ratio of 16:0 in the distribution of muscle PC in six tuna fish was between 19.26 and 25.46 (Medina, Aubourg and Martín), average

33.48% in the females of the pearl mullet and 30.20% in the males (Kızmaz). In our study, this component was major, which was 26.15% in females and 32.31% in males (Table1.). Out of monounsaturated fatty acids, 16:1n-7 was 3 times and 18:1n-9 was 1.65 times higher in female individuals.

Depending on them, total MUFA was nearly 2 times higher in female individuals. Out of polyunsaturated fatty acids 20:5n-3 was 1.32 times, 22:6n-3 was 1.12 times, depending on them, total PUFA and n-3 were 1.16 times more in the male individuals than those in females. On the other hand, n-6 and n-3/n-6 were similar (Table1., Figure 1.and 2.). Out of polyunsaturated fatty acids, the ratio of EPA was found to be 7.54 % in *Squaliobarbus curriculus* (Lei et al.), 14.69% on average in males of pearl mullet, while in females it was 11.86 % (Kızmaz). In our study, this component exhibited a similar distribution to *Alburnus tarichi* (11.99% in females, 15.79% in males) (Table1.). Percentage of DHA, a component of n-3, was higher than 25% in nearly thirty species (Takama et al.), 16.58% on average in females, 21.55 in males in pearl mullet (Kızmaz). In our study, this value was found to be 16.98% in females and 19.05% in males, lower than marine fish. It showed a similar distribution to that of freshwater fish, pearl mullet.

In this study, out of saturated ones, 16:0; out of monounsaturated ones, 18:1n-9; and out of polyunsaturated ones, EPA and DHA were major in the muscle PC fraction of females and males of *A. mossulensis*. These fatty acids were similar to the results of Takama et al. (1999) and Kızmaz (2015).

As in the PC fraction, in the muscle PE fatty acid content of *A. Mossulensis*, out of saturated ones, 16:0; out of monounsaturated ones, 18:1n-9; and out of polyunsaturated ones, EPA and DHA were major (Table1.). However, out of these components, DHA was at the highest percentage. Major fatty acid in *Trachurus trachurus* (Bandarra et al.) and in Tuna species (Medina, Aubourg and Martín) was 18:0. However, in our study, the major saturated component was 16:0 as in *A. tarichi*. Depending on 16:0 in PE, total SFA was higher in males, while total PUFA and n-3 were higher in female individuals, depending on 20:5n-3, 22:5n-3 and 22:6n-3. Omega-6 and n-3/n-6 exhibited a similar distribution. In both individuals, the order of fatty acids was ΣPUFA>ΣSFA>ΣMUFA as percent in the decreasing order (Figure 2.). In previous studies, out of total PUFAs, the percentage of AA was found to be 6.72% on average in females and 8.07% in males; EPA was 7.95% for females and 9.57% for males; DHA was 26.36% in females and 33.34% in males in muscle PE of *A. tarichi* (Kızmaz). In our study, the ratio of AA in female was (9.08%), while the ratio of EPA and DHA was higher than that in *A. tarichi* (Table1.). DHA was high in *Hippoglossus hippoglossus* (Lie, Hemre and Bjørnsson) and Tuna fish (Medina, Aubourg and Martín), whereas EPA and DHA were high in *T. trachurus L.* (Bandarra et al.). Similar results were obtained in our study as well. ΣPUFA was the most, while ΣMUFA was the least in five species of lake fish studied in China (Lei et al.) pearl mullet (Kızmaz). The data we obtained in the study were similar. In muscle PE distribution, the ratio of n-3/n-6 was between 0.84 and 1.96 in fish studied in China, 5.08 on average in the females, 5.47 in males of Van Fish (Kızmaz). In our investigation, it was 4.98 in females, 5.13 in males, which was higher than marine fish, while it showed a similar distribution to pearl mullet. Furthermore, as stated in previous studies, since n-6 components were rather low, out of n-3s, DHA was much and the ratio of n-3/n-6 was high.

Depending on the tissue type, the fatty acid contents in the phospholipid subclasses were different. In dorsal muscle analysis of cultured *S. asotus* fish, 18:0 (average 34%), 20:4n-6 (13.1%) and 22:6n-3 (21.30%) were major fatty acids in the PI distribution [19]. 18:0 was major fatty acid in *H. hippoglossus (Lie, Hemre and Bjørnsson)*, Tuna fish (Medina, Aubourg and Martín), *S. asotus* (Shirai et al.) and *A. tarichi* (Kızmaz). In our study, in similar PI, 18:0 (26.05% in females, 22.03% in males), 20:4n-6 (17.05% in females, 13.90% in males) and 22:6n-3 (18.71% in females, 21.90% in males) were major fatty acids. In PI, 18:0 was 1.18 times higher in female individuals than males. A similar distribution was observed in monounsaturated fatty acids. Out of the polyunsaturated fatty acids, 20:4n-6 was 1.23 times higher in females than male individuals. It has been stated that PI is important

because 20:4n-6 balances 22:6n-3 in muscle and 20:5n-3 in liver [19]. The percentage of n-3 was 1.12 times higher in males than females, depending on 20:5n-3 and 22:6n-3. Since n-6 is higher in females depending on 20:4n-6, n-3/n-6 ratio was lower than males (Table1.). Another finding of our study is that the ratio of AA determined in the distribution of PI in the female and male individuals is much higher in PC and PE subclasses. A similar result has been reported in previous studies. AA was reported as 13.10% in *S. asotus* (Shirai et al.), *A. tarichi* was average 12.67% in females and 15.19% in males (Kızmaz). In our study, this component was found high in both sexes.

Another finding of the study is that the ratio of n-3/n-6 is rather lower than PC and PE. This ratio was 1.95 for females and 2.55 for males (Table1., Figure 2.). The reason for this low ratio is that AA is high, out of total n-6 PUFA. It was found to be low as average 2.09 in females, 1.94 in males of pearl mullet (Kızmaz), average 4.42 in females, 3.35 in males of *C. umbla*; 2.88 on average in females and 3.60 in males of *C. regium* (Kayhan). Also, it was found to be similarly the lowest in our study. ΣPUFA was the most, then ΣSFA and the ΣMUFA was lowest in *A. mossulensis*. Our data were similar to those of *A. tarichi* (Kızmaz).

Medina et al. (1995) found as a result of their analysis on a Tuna species that PS has a high percentage in terms of ΣSFA and that 16:0 and 18:0 were close to each other. In our study, 16:0 (16.46% in females, 19.85% in males) and 18:0 (17.93% in females, 18.98% in males) were found to be high and similar. Furthermore, the ratio of 16:0 was found as 19.82% on average, whereas 18:0 was 22.23% in females of pearl mullet; 16:0 was 18.66% and 18:0 was 24.12% in the male individuals (Kızmaz). These results clearly show that, out saturated fatty acids, 18:0 is not only in the PI subclass but also major in PS. Similar results were found in this study. 16:0 was 1.21 times more in PS and, depending on this; total SFA was 1.13 times more in male individuals. Monounsaturated fatty acids showed a similar distribution. When polyunsaturated fatty acids are considered, it was observed that 22:5n-3 was 1.57 times; 22:6n-3 was 1.24 times, and depending on them, total PUFA was 1.11 and n-3 was 1.19 times more in female individuals than male individuals (Table1.). In our investigation, the order of fatty acids in the muscle PS subclass for both sexes was ΣPUFA>ΣSFA>ΣMUFA, while this order became ΣSFA>ΣPUFA>ΣMUFA, in the decreasing order, in the pearl mullet (Kızmaz).

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