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SEASONAL VARIATION OF FATTY ACID COMPOSITION OF PHOSPHOLIPID SUBCLASSES IN MUSCLE TISSUE IN MALE, *ALBURNUS MUSSULENSIS*

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ABSTRACT

Fatty acids are quite important both as an energy source and in terms of functional tasks for living systems. In addition, these compounds are the structures that have the greatest change depending ecological factors in terms of biochemical. These biochemical compounds are located in the cell structure. So, Analysis of these fatty acids, which have very important membrane integrity and permeability in life, is very important for living things. Therefore, fatty acid analysis at the phospholipid level is quite important. In this study, it was aimed to determine the seasonal variation of the fatty acids of phospholipid subclasses in muscle tissue.

In our study, sexes of the samples caught from Batman Dam Lake were determined in the laboratory. After the total lipid extraction of the muscle tissue belonging to the male one was made, it was separated into phospholipid subclasses by thin layer chromatography. After the methylation process, samples were analyzed qualitatively as % fatty acid with Gas Chromatography. As a result of analysis, a total of 16 fatty acids were determined. As a results of analyses, out of saturated fatty acids, 16:0 and 18:0; out of monosaturated ones, 16:1n-7 and 18:1n-9; out of polyunsaturated ones, 20:4n-6, 20:5n-3 22:5n-322:5n-3 and 22:6n-6 were identified as the major fatty acids. When the fatty acid distribution is examined, 16:0 in PC; 18:0 in PI and PS; 18:1n-9 in PC and PS; in 20:4n-6 PI; 20:5n-3 in PC and PI; 22:6n-3 and n-3 in PE and n-6 were found to be higher in PI than others. It is detected that in April, total PUFA and n-3 in PC, PE and PI; in November and January, total SFA in PSs are higher than the other months. In our study, the PI subclasses n-3/n-6 rate of the fish has been at the lowest level according to PC, PE and PS.

Keywords: Fatty Acid, Phosphatidylcholin, Phosphatidylethanolamin, Phosphatidylinositol, Phosphatidylserine, *Alburnus Mussulensis*

1. INTRODUCTION

Lipids are quite important in terms of structural and functional with basic energy source for various metabolic events in living beings. In this respect, it is important to distinguish between the structural lipids of the cell membrane and the lipids stored in the tissues. It is found that storage lipids were used for the egg and sperm maturation and that they significantly decreased in the body weight in the post-ovulation period (Danneving and Noeum, 1982). While in particular lipids are stored in mammals in fatty tissue, they are stored in fish in skeletal muscle and liver (Watanabe, 1982). Most of these stored lipids are mobilized in different parts of the body to be used as an energy source and in physiological events (Rodriguez et al. 2004). Besides all of these, lipids lead to cell membrane's structural component, many hormones and eicosanoids (Higgs and Dong, 2000). Components such as EPA, DHA and AA, which have high unsaturation and number of carbon in fish, protect cell and organelle integrity and the fluidity and structure of the membranes. They are also paracrine that is biologically active and known as eicosanoids is the precursor of the hormone groups (Sargent et al. 1999).

Lipids are composed of fatty acids, which are the main components. It has been found that in case of the lack of these in fish nutrition, the physiological symptoms occur such as the slowdown in fish growth, abrasion on tail fin, insufficient pigmentation, and inadequacy of the acid components and lipid metabolism (Castell et al. 1972). Shirai et al. (2002) stated that fatty acid components in phospholipid (PL) and Triacilroglycerol (TAG) fractions, the main lipid classes, should be revealed in order to understand the fatty content in muscle tissue and determine its nutritional value. In addition, it is found that TAG as energy source and PL as basic component of cell membrane structure are assigned (Sargent et al.1995, Kiessling et al. 2001). It has been determined that neutral lipids used for energy purposes stored temporarily physiologically important polyunsaturated fatty acids (Tocher et al. 1985; Napolitano et al. 1988).

Phospholipids, one of the most important components of cellular membranes have a role as synthesis of structural elements, regulation of permeability processes of membranes and energy source (Reddy et al. 1991). In the studies done, it has been detected that PL subclasses' phosphatdylcholine (PC), phosphatidylethanolamine (PE), phsphatidylserine (PS) and phosphatidylinositol (PI) is majör and PC, PE, PI and PS is in the order from the most extra to the least (Tocher and Sargent 1984). Lie et. all. (1992) stated in their analysis that in terms of phospholipid, firstly phosphatidylcholine (PC) (40-50%) is the highest, second is phosphatidylethanolamine (20-50%) and the last one is Phosphatidylserine and Phosphatidylinositol (2-10%). It is also stated that these ratios have different mammalian cells and tissues characteristically different phospholipid composition. Phosphatidylcholine is quite important in biochemical process because of the methyl groups (-CH3) in its structure (Canty ve Zeisel 1994). Phosphatidylethanolamine is known to be present in the brain, spinal cord and other nervous tissues and play an important role in perception and memory events. Phosphatidylserine plays a role especially in the transmission of impulses between nerve cells and the development of learning and memory ability. Phosphatidylinositol is known to act as a secondary messenger in the regulation of proteins at the cell interface and intracellular signal transduction (Canty ve Zeisel 1994).

It was reported that the Chalcalburnus (*Alburnus*), which belongs to the Cyprinidae family, is distributed in the Tigris and Euphrates River systems, Southern Iran, Van and Aral Lakes and Black Sea and Caspian Sea basins, and there are many species and subspecies in these basins, but they are represented only by four species in our country's inland waters (Balık ve Geldiay 1999). Fatty acids in the fish are affected by season, environment, temperature, food and tissue type (Wodtke 1981, Lie et al. 1992, Shirai et. all., 2001). Besides, these compounds are the structures to biochemically undergo the changes the most according to ecological factors and the physiological state of the fish. That is the reason why it is quite important to know the fatty acid compounds and essential fatty acid need of the fish. In order to understand better the fatty acid changes in the PL fractions, it is intended to investigate the seasonal variation of the subclasses of this fraction, PC, PE, PS and PI fatty acids.

2. Material and Method

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) ve DB–23 (Bonded 50 % cyanopropyl) (J & W Scientific, Folsom, CA, USA), using capillary column (30m x 0.25 mm inside bore x 0.25µm film thickness) on the SHIMADZU GC 2010 PLUS model Gas Chromatography. Detector temperature: 250 ^{o}C; injector temperature: 250 ^{o}C; injection: Split–model 1/10. Velocity of gas flow: Carrier gas: helium 0.5 ml/min for 30 m column; hydrogen: 30 ml/min; dry air: 400 ml/min. Column (oven) temperature: 170 ^{0}C, waiting period: 2 minutes; 2^{0}C/minute for 210^{0}C, waiting period 20 minutes; total analysis period: 42 minutes. 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 (Version 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

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 (Table 1.).

3.1. PC GROUP

When fatty acid distribution was examined, it was determined that saturated fatty acid 16:0, monousaturated fatty acid 18:1n-9, polyusaturated 20:5n-3 and 22:6n-3 were major. It was founded that 16:0 was similar in November (38.04%) and January (%38.87); its lowest level was in April (32.31%). It has been seen that while 16:1n-7 was at the highest level in June (4.74%); 18:1n-9 was identified similar in November (16.15%), in June (15.40%), in January (13.38%) and in April (12.40%). While 20:4n-6 was the highest in November (8.46%), it was determined to decrease to the lowest level in June (5.65%). 22:6n-3, on the other hand, was found to be higher in April (19.05%) that other months (Table 1.). It was found that Total SFA was at the lowest percentage in April (36.08%) depending on 16:0, and total MUFA was close to each other. Total PUFA was found determined higher in April (48.76%) than in other months due to 20:5n-3 and 22:5n-3. N-3 has been found to be at the lowest level in November (27.70%) and the highest level in April (38.84%). N-6 was observed to be close to each other. N-3/n-6 rate was marked to be lower in November (2.53%) than in other months. Total SFA in November (43.71%) and total PUFA in April (48.76%) were the highest but they were found to be similar in the other months. Total MUFA had a similar distribution and was found to be at the lowest level among them.

PC amount was determined as 50-85% in different tissues of twenty-seven fish species (Takama and Ark. 1999). The amount of PC in *C. baicalensis* and *C. dybowski's* muscle, liver and ovaries was determined in the range of 60.7-75.1% (Kozlova and Khotimchenko, 2000). In the previous study, the rate of 16: 0 in the muscle PC fraction of six tuna fish was in the range of 19.26-25.46 (Medina et al. 1995), and it was approximately 33.48% in the females of, and 30.20% in the males of Van Fish, *A. tarichi* (Kızmaz, 2015). In our study, this component was found to be dominant and the average rate of *A. Mussulensis* in muscle tissue was determined as 36.07%. EPA rate among polyunsaturated polymers with 27 carbon was found to be 7.54% in *Squaliobarbus curriculus* (Lin and ark. 2012), 8-17% in 27 species of bony fish (Takama et al. 1999), on average 14.69% in males and 11.86% in females of *A. tarichi* (Kızmaz, 2015). In our study, this component (EPA approximately 13.81%) was found to be similar to *A. tarichi*. As mentioned earlier, it shows that this fish collected from Batman Dam Lake is rich in EPA. Takama and ark. (1999) in their analysis, found that 20-40 % of total fatty acid of DHA among twenty carbon n-3 components was between 14-39%, approximately 25% in bottom fish; Medina et. all (1995) found tuna fish in the range of 28.70-41.63%

and Kızmaz (2015) found 16.58% in females and 21.55% in males of Van Fish. In our study, the rate of DHA was determined as an average of 14.26% in *A. Mussulensis* muscle tissue. This component has been detected at a lower rate than marine fish. The high content of EPA caused the increase of n-3 / n-6 ratio in these fish. As a matter of fact, we can say that this rate we obtained from the study is similar to *A. tarichi*. In this study, 16:0 from saturated, 18:1n-9 from monounsaturated, EPA and DHA from polyunsaturated was determined major in *A. Mussulensis* muscle PC fraction. These components have been identified as dominant in various marine fish (Takama et al. 1999) and *A. tarichi* (Kızmaz 2015) studied before. It was detected that five fish species studied in China and *A. Tarichi* had ∑PUFA the most and ∑MUFA the least. In our study, while total SFA in November (43.71%) and total PUFA in April (48.76%) were the highest and similar in other months, total MUFA showed similar distribution and was the lowest among them. Factors such as season, environment, nutritional fatty acids (Lie et al. 1992a) and temperature (Wodtke 1981) have been reported to affect phospholipid fatty acid content. For example, in Van Fish, it was emphasized that 16:0 and 18:1n-9 increased in August and EPA and DHA decreased in the same period (Kızmaz, 2015). In our study, it was determined that 16:0 and 18:1n-9 decreased and EPA and DHA increased in April, the spring term. These data show that the muscle PC fatty acid content may vary depending on the season.

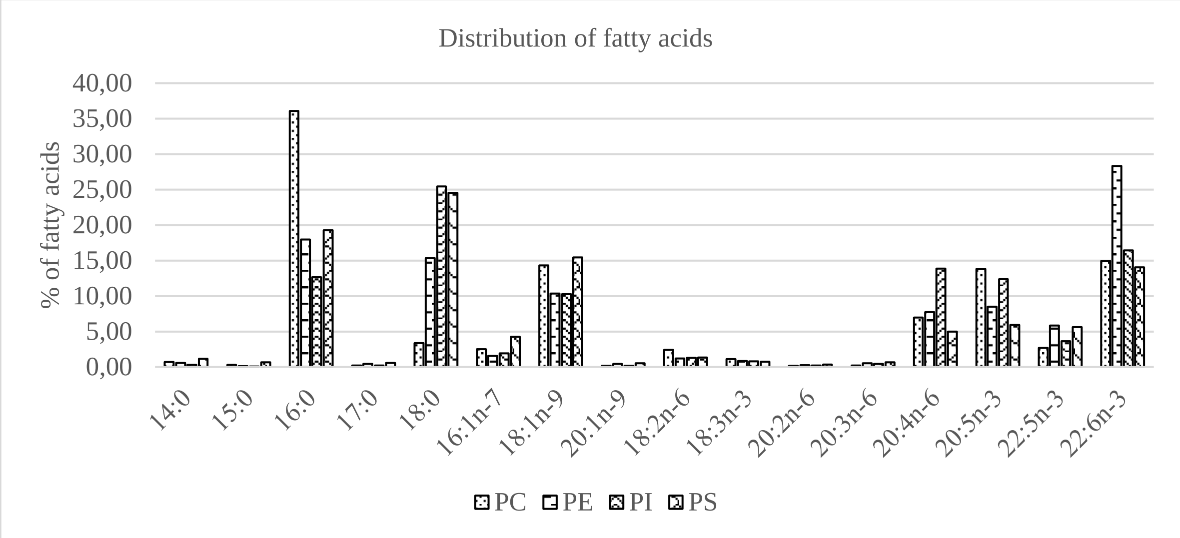

Figure 1. Distribution of fatty acids

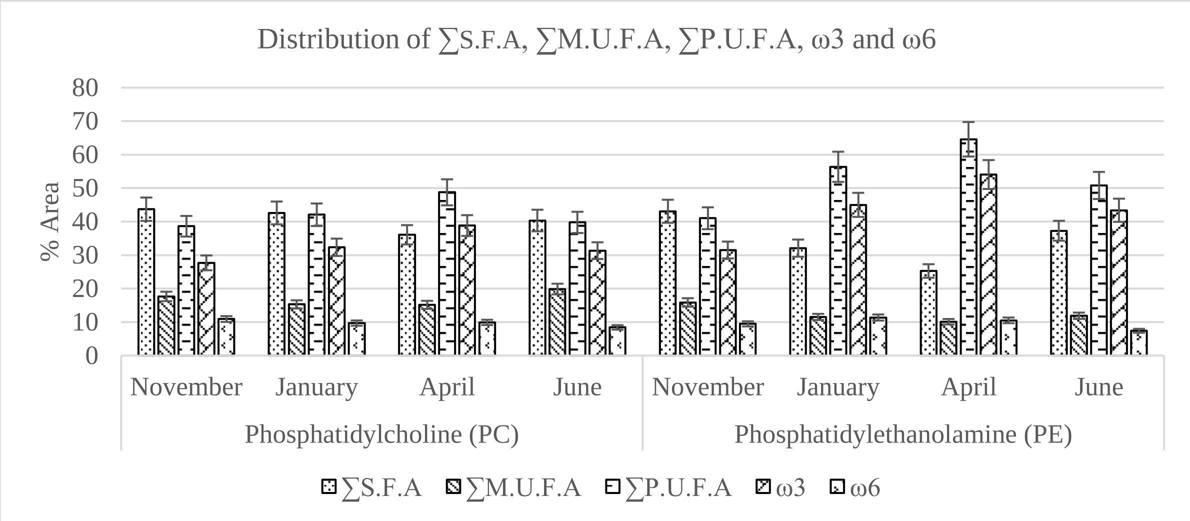

Figure 2. Distribution of ∑S.F.A, ∑M.U.F.A, ∑P.U.F.A, ω3 and ω6 in PC and PE

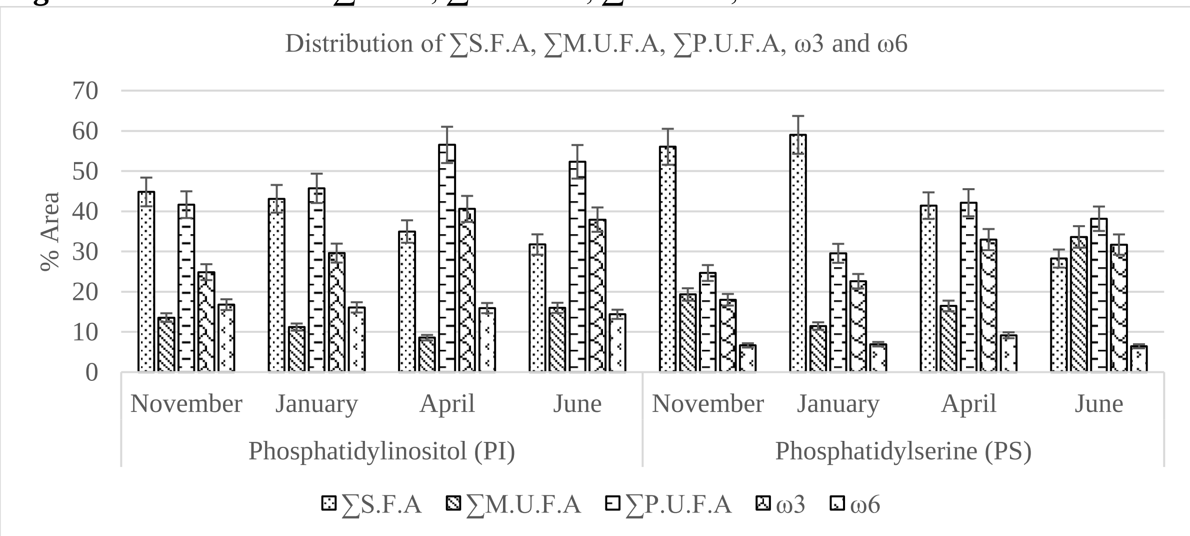

Figure 3. Distribution of ∑S.F.A, ∑M.U.F.A, ∑P.U.F.A, ω3 and ω6 in PI and PS

Table 1. Seasonal Variation of phospholipid subclasses in muscle tissue in male, *Alburnus mussulensis*

	Phosphatidylcholine (PC)				Phosphatidylethanolamine (PE)				Phosphatidylinositol (PI)				Phosphatidylserine (PS)			
Fatty Acids	**November**	**January**	**April**	**June**	**November**	**January**	**April**	**June**	**November**	**January**	**April**	**June**	**November**	**January**	**April**	**June**
14:0	0.51±0.04	0.59±0.05	0.55±0.04	1.19±0.09	0.16±0.01	0.52±0.04	0.43±0.03	1.11±0.09	0.07±0.01	0.21±0.02	0.21±0.02	0.72±0.06	0.28±0.02	0.82±0.07	1.96±0.16	1.55±0.12
15:0	0.25±0.02	0.38±0.03	0.23±0.02	0.42±0.03	0.11±0.01	0.22±0.02	0.06±0.00	0.05±0.00	0.07±0.01	0.12±0.01	0.02±0.00	0.06±0.00	0.59±0.05	1.62±0.13	0.20±0.02	0.23±0.02
16:0	38.04±3.03	38.87±3.10	32.31±2.58	35.05±2.80	18.00±1.44	18.91±1.51	14.17±1.13	20.72±1.65	13.79±1.10	10.68±0.85	12.62±1.01	13.52±1.08	16.83±1.34	25.88±2.06	19.85±1.58	14.46±1.15
17:0	0.28±0.02	0.23±0.02	0.31±0.02	0.02±0.00	0.74±0.06	0.43±0.03	0.61±0.05	0.02±0.00	0.50±0.04	0.14±1.01	0.08±0.01	0.07±0.01	0.90±0.07	0.63±0.05	0.41±0.03	0.29±0.02
18:0	4.63±0.37	2.54±0.20	2.68±0.21	3.66±0.29	24.09±1.92	12.01±0.96	10.00±0.80	15.38±1.23	30.37±2.42	31.95±2.55	22.03±1.76	17.36±1.38	37.43±2.99	30.04±2.40	18.98±1.51	11.71±0.93
∑S.F.A	**43.71±3.49**	**42.61±3.40**	**36.08±2.88**	**40.34±3.22**	**43.10±3.44**	**32.09±2.56**	**25.27±2.02**	**37.28±2.97**	**44.80±3.57**	**43.10±3.44**	**34.96±2.79**	**31.73±2.53**	**56.03±4.47**	**58.99±4.71**	**41.40±3.30**	**28.24±2.25**
16:1n-7	1.47±0.12	1.79±0.14	2.47±0.20	4.24±0.34	1.81±0.14	1.31±0.10	1.33±0.11	1.82±0.15	2.27±0.18	1.13±0.09	0.77±0.06	3.45±0.28	3.18±0.25	1.45±0.12	3.69±0.29	8.67±0.69
18:1n-9	16.15±1.29	13.38±1.07	12.40±0.99	15.40±1.23	13.88±1.11	9.71±0.77	8.03±0.64	9.78±0.78	11.22±0.90	9.97±0.80	7.56±0.60	12.26±0.98	15.80±1.26	9.69±0.77	12.00±0.96	24.32±1.94
20:1n-9	0.05±0.00	0.14±0.01	0.29±0.02	0.24±0.02	0.19±0.02	0.53±0.04	0.77±0.06	0.30±0.02	0.07±0.01	0.10±0.01	0.23±0.02	0.27±0.02	0.33±0.03	0.33±0.03	0.78±0.06	0.63±0.05
∑M.U.F.A	**17.67±1.41**	**15.31±1.22**	**15.16±1.21**	**19.88±1.59**	**15.88±1.27**	**11.55±0.92**	**10.13±0.81**	**11.90±0.95**	**13.56±1.08**	**11.20±0.89**	**8.56±0.68**	**15.98±1.27**	**19.31±1.54**	**11.47±0.92**	**16.47±1.31**	**33.62±2.68**
18:2n-6	2.24±0.18	2.01±0.16	2.97±0.24	2.45±0.20	1.01±0.08	1.27±0.10	1.60±0.13	0.84±0.07	0.84±0.07	1.02±0.08	1.54±0.12	1.79±0.14	0.78±0.06	0.63±0.05	1.88±0.15	1.99±0.16
18:3n-3	0.75±0.06	1.01±0.08	1.21±0.10	1.42±0.11	0.72±0.06	0.99±0.08	0.79±0.06	0.88±0.07	1.13±0.09	0.49±0.04	0.66±0.05	0.92±0.07	1.16±0.09	0.22±0.02	0.83±0.07	0.71±0.06
20:2n-6	0.06±0.00	0.12±0.01	0.32±0.03	0.11±0.01	0.13±0.01	0.25±0.02	0.53±0.04	0.19±0.02	0.25±0.02	0.16±0.01	0.18±0.01	0.20±0.02	0.23±0.02	0.29±0.02	0.42±0.03	0.43±0.03
20:3n-6	0.17±0.01	0.13±0.01	0.26±0.02	0.22±0.02	0.95±0.08	0.42±0.03	0.45±0.04	0.24±0.02	1.08±0.09	0.17±0.01	0.30±0.02	0.26±0.02	1.67±0.13	0.14±0.01	0.33±0.03	0.51±0.04
20:4n-6	8.46±0.67	7.46±0.60	6.37±0.51	5.65±0.45	7.40±0.59	9.43±0.75	7.95±0.63	6.14±0.49	14.61±1.17	14.75±1.18	13.90±1.11	12.13±0.97	3.97±0.32	5.88±0.47	6.54±0.52	3.50±0.28
20:5n-3	12.74±1.02	13.54±1.08	15.79±1.26	13.18±1.05	5.89±0.47	9.04±0.72	11.02±0.88	8.09±0.65	9.92±0.79	11.28±0.90	14.12±1.13	14.22±1.13	2.22±0.18	3.55±0.28	7.83±0.62	10.08±0.80
22:5n-3	2.22±0.18	3.19±0.25	2.79±0.22	2.56±0.20	4.61±0.37	7.27±0.58	6.22±0.50	5.25±0.42	2.56±0.20	3.92±0.31	3.90±0.31	4.19±0.33	4.92±0.39	5.70±0.45	6.15±0.49	5.64±0.45
22:6n-3	11.99±0.96	14.61±1.17	19.05±1.52	14.19±1.13	20.31±1.62	27.71±2.21	36.04±2.72	29.18±2.33	11.25±0.90	13.91±1.11	21.90±1.75	18.59±1.48	9.70±0.77	13.13±1.05	18.14±1.45	15.28±1.22
∑P.U.F.A	**38.63±3.08**	**42.07±3.36**	**48.76±3.89**	**39.78±3.17**	**41.02±3.27**	**56.38±4.50**	**64.60±5.15**	**50.81±4.05**	**41.64±3.32**	**45.70±3.65**	**56.50±4.51**	**52.30±4.17**	**24.65±1.97**	**29.54±2.36**	**42.12±3.37**	**38.14±3.04**
ω3	27.70±2.21	32.35±2.58	38.84±3.10	31.35±2.50	31.53±2.52	45.01±3.59	54.07±4.31	43.40±3.46	24.86±1.98	29.60±2.36	40.58±3.24	37.92±3.03	18.00±1.44	22.60±1.80	32.95±2.63	31.71±2.53
ω6	10.93±0.87	9.72±0.78	9.92±0.79	8.43±0.67	9.49±0.76	11.37±0.91	10.53±0.84	7.41±0.59	16.78±1.34	16.10±1.28	15.92±1.27	14.38±1.15	6.65±0.53	6.94±0.55	9.17±0.73	6.43±0.51
ω3/ω6	2.53	3.33	3.92	3.72	3.32	3.96	5.13	5.86	1.48	1.84	2.55	2.64	2.71	3.26	3.59	4.93

3.2. PE GROUP

When the major fatty acid distribution in PE was examined, 16:0 and 18:0 of saturated, 18:1n-9 of monounsaturated and 22:6n-3 of polyunsaturated fatty acid were determined. When the individual fatty acid distribution was examined, it was determined that 16:0 (14.17%) was the lowest in April, while similar results were found for the other months as well. 18:0 was the highest in November (24.09%) and low in January (12.01%) and April (10.00%). 18:1n-9 was found to be slightly higher in November (13.88%) compared to other months. Among the polyunsaturated fatty acids, 20:5n-3 (11.02%) and 22:6n-3 (36.04%) were determined to be higher in April in comparison to other months. N-3 was observed to the lowest in November (31.53%) and the highest in April (54.07%), while n-6 was the lowest in June (7.41%) and had similar values in other months. N-3/n-6 was the lowest in November (3.32%) and the highest in June (5.86%) (Table 1). It was also determined that the total SFA was the lowest in April (25.27%) while the total PUFA was the highest in the same month (64.60%), and that while the total PUFA was the lowest in November (41.02%), the total SFA was found to be the highest (43.10%). In the other months apart from November, PUFA was the highest in PE, followed by SFA and MUFA with the lowest percentage (Figure 2.).

In different tissues of 27 fish species, PE amount was determined to be in the range of 6.3% - 35.3% (Takama et al., 1999), and PE ratio in the muscles, liver and ovarium of *C. baicalensis* and *C. dybowski* was found to be within the range of 14.5% - 25.7% (Kozlova and Khotimchenko, 2000). In a previous study conducted, AA ratio, which is one of the polyunsaturated fatty acids, in the muscle fraction of Van Fish, *A. tarichi* was found to be an average of 6.72% in females and 8.07% in males, EPA ratio as 7.95% in females and 9.57% in males, and DHA ratio as 26.36% in females and 33.34% in males (Kızmaz, 2015). In our study, AA, EPA and DHA ratios in *A. Mussulensis* species were found to be similar to the ratios in *A. tarichi*. DHA was found to be high in *H. hippoglossus* (Lee et al., 1992b) and in various tunas fish (Medina et al.,1995), while EPA and DHA were determined to be high in *T. trachurus L.* (Nadcisa et al., 2001). We obtained similar results in our study.

The dominant saturated fatty acid was determined to be 18:0 in scad (*T. trachurus*) (Nadcisa et al., 2001) and 6 tunas fish species (Medina et al., 1995), while it was 16:0 in *A. tarichi* (Kızmaz, 2015). However, in our study, major saturated component was determined to be 16:0 (17.95% on average) and 18:0 (15.37% on average). In a previous study, it was determined to be 16:0 in *A. tarichi* and ∑SFA rate was found to increase in April (Kızmaz, 2015), while in our study, 16:0 was found in the muscle tissue of *A. Mussulensis* and ∑SFA percentage was determined to decrease in April. Again, it was determined in female Van fish that DHA and consequent ∑PUFA decreased in August (Kızmaz, 2015), while in our study, EPA, ∑PUFA and n-3/n-6 ratio were determined to increase (Table 1.). In five lake fish species studied in China (Lin et al., 2012) and in *A. tarichi* (Kızmaz, 2015), ∑PUFA was determined to be the highest and ∑MUFA as the lowest. The results obtained in our studies are consistent with this result. N-3/n-6 ratio in muscle PE fraction was determined to be between 0.84 -1.96 in five fish species in China, while it was found to be an average of 5.08 in female Van fish and 5.47 in male Van fish (Kızmaz, 2015). It was found that n-3/n-6 ratio was high since n-6 components, as was discussed earlier, was extremely low in fish, our subject-matter, whereas n-3 polyunsaturated fatty acids were high. In our study, it was also found that the amount of PE fatty acids in muscles in fish increased and decreased over time depending on the season. These data demonstrate that changes in terms of PE fraction in muscles in fish depending on the season may vary.

3.3. PI GROUP

It was determined that 16:0 and 18:0 from saturated fatty acids, 18:1n-9 from monosaturated fatty acids, and 20:4n-6, 20:5n-3 and 22:6n-3 from polyunsaturated fatty acids were predominant fatty acids. 16:0, an individual fatty acid type, was observed to have similar distribution over the months. It was observed that there were significantly decreases in 18:0 and therefore in total SFA in April (18:0 22.03%, SFA 34.96%) and in June (18:0 17.36%, SFA 31.73%).

It was determined at a low level that 18:1n-9 (7.56%) and total MUFA (8.56) were lower in April compared to other months. It was found that 20:4n-6, a polyunsaturated fatty acid, had similar percentages over time. It was also found that the amount of PI in muscles, liver and ovarian tissues in *S. asotus* did not change in summer and winter; however, the amount of PI in liver was found to be higher than in ovaries (Shirai et. all., 2001). It was discovered that 20:5n-3 was lower in November (9.92%) compared to other months. 22:5n-3 was found to be at the lowest level in November (11.25%) and at the highest level in April (21.90%). Total PUFA and n-3 had similar distribution related to 20:5n-3 and 22:6n-3. The percentage distributions of N-6 were similar over the months. The N-3/n-6 ratio increased approximately twice more in April (2.55%) and in June (2.62%) compared to other months (Table 1.). While Total SFA was at the lowest in April (34.96%) and in June (31.73%), total PUFA was at the highest in April (56.50%), and Total MUFA values were similar and at the lowest percentage (Figure 3).

The amount of PI in different tissues of 27 fish types was found to be 1.11-7% (Takama et al., 1999). It was found that the amount of PI and PS in muscles, liver, and ovaries in *C. baicalensis* and *C. dybowski* was lower than the PC and PE (Kozlova and Khotimchenko, 2000). The dominant components that we identified in PI fatty acid analysis in muscles in *A. mussulensis* in the present study were observed to be similar to those of *H. hippoglossus* (Lie et al., 1992b), six different types of thunnus (Medina et al., 1995), *S. asotus* (Shirai et. all., 2001), and *A. tarichi* (Kızmaz, 2015) in previous studies. In *A. mussulensis* that we analysed in the study, it was found that 18:0 was identified as the major saturated fatty acid (as in other fish types), rather than 16:0, in the PI subclass compared to PC and PE subclasses. It was discovered that 18:0 was between the range of 17-31% in *H. Hippoglossus* (Lie et al., 1992b), 14.70-25.60% in thunnus (Medina et. al., 1995), 34% in *S. asotus* (Shirai et. all, 2001), a mean of 28.37% in A. tarichi females, and 32.49% in males (Kızmaz, 2015). All these data demonstrate that 18:0 acid is present in the PI subclass at sn-1 position. We have observed similar results in our study.

In *S. asotus*, AA was found as 13.10% (Shirai et. all. 2001), on average, *A. tarichi* was 12.67% in females and 15.19% in males (Kızmaz 2015). Total saturated fatty acids were found to be as high as 41.81% in *A. tarichi* females and 47.33% in males (Kızmaz 2015). Medina et al. (1995) reported that tuna fish are rich in saturated. A similar result has been previously found in other fish species. For this reason, the PI subclass has been reported to be rich in 18:0 from saturated and AA from polyunsaturated. In our study, these components were found to be higher in *A. mussulensis* fish species. The reason for the high percentage of ∑SFA in the PI subclass of fish is that this subclass is rich in saturated component 18:0.

In previous studies, fatty acids in the muscle PI fraction of *S. asotus* were analysed in the summer and winter seasons, with an average of 18:0, 34%, AA 13.1% and DHA 21.30% (Shirai et. all. 2001). In *A. tarichi*, which was analysed every two months for a year, the ratio of 16:0 was 11.39% in females, 8.75% in males, 28.37% in females, 32.49% in males; 18:1n-9 percentage was 9.43% in females, 8.65% in males; AA percentage was 12.61% in females, 15.19% in males; EPA percentage was 12.74% in females and 12.12% in males; DHA percentage was 12.66% in females and 14.48% in males; ∑SFA percentage was 41.81% in females and 41.94% in males; ∑MUFA percentage was 12.32% in females and 10.73% in males; ∑PUFA percentage was 45.87% in females and 47.33% in males; the n-3/n-6 ratio was 2.09 in females and 1.94 in males (Kızmaz 2015). The results we obtained in our studies are generally compatible with these data. In *A. mussulensis*, as in *S. asotus* (Shirai et. all. 2001) and *A. tarichi* (Kızmaz 2015), it was found to be higher than saturated 18:0, polyunsaturated AA, and the other dominant subclasses PC and PE. This content is a unique feature of PI subclass. In *S. asotus*, which was analysed in summer and winter, 16:0, 18:0, 18:1n-9 and DHA increased in winter and AA increased in summer (Shirai et. all. 2001). In our study, it was determined that 18:0 of the dominant components increased in *A. mussulensis* in November and January, and the AA ratio did not increase in *A. mussulensis*, as in *S. asotus*, but were close to each other. In females of *A. tarichi*, 16:0 increased in August, and 18:0 in April, and in male and female, ∑SFA increased in summer, AA and DHA increased in December. In our study, as mentioned above, it was determined that DHA percentage increased in the spring season. In our study, the n-3/n-6 ratio (mean 2.13) determined in PI subclass of fish was lower than the PC and PE subclass (Table 1.). The ratio of n-3/n-6 was 2.09 in *A. tarichi* females and 1.94 in males (Kızmaz 2015). The reason for the low rate of this rate in PI subclass in fish is the high AA rate among major n-6 components. In *A. mussulensis*, ∑PUFA was identified as the highest, then ∑SFA and ∑MUFA was the lowest on average. On both sexes of A. tarichi, a descending order was seen as in ∑PUFA> ∑SFA > ∑MUFA (Kızmaz 2015). *A. mussulensis* fatty acids in muscle tissue PI fraction varied depending on the season, as in other subclasses PC and PE.

3.4. PS GROUP

When the distribution in PS is examined, 16: 0 and 18: 0 from saturated fatty acids; 18:1n-9 from monounsaturated and 22: 6n-3 from polyunsaturated were determined as major compared to other fatty acids. The amount of PS was found to be 0.4-6.2% in different tissues of twenty-seven fish species (Takama et al. 1999). It has been determined that PI and PS amount is less in the tissues of *C. baicalensis* and *C. dybowski's* fish muscle, liver and ovary compared to PC and PE (Kozlova and Khotimchenko 2000). Of the saturated fatty acids, 16:0 in January (25.88%), 18:0 in November (37.43%) and in Total SFA in November (56.03%) and January (58.99%) due to these major fatty acids, were determined to be higher percentage compared to the other months. In the previous study, it was suggested that the subclass with the highest percentage in terms of ∑SFA among the subclasses in six different tuna species was PS, and in this subclass 16:0 and 18:0 fatty acids were found in close ratios and DHA was in low rates (Medina et al. 1995)

A. tarichi average; females 19.82% in 16:0; 22.23% in 18:0, males 18.66% in 16:0, 24.12% in 18:0, ∑SFA percentage on females, 44.13%; on males, 44.93%; DHA percentage on females 15.60%; 13.83% on males was found (Kızmaz 2015). Similar results were found in this study. *A. mussulensis* muscle was detected in the PS subclass, especially at 16:0 and 18:0 at close values (average 16:0; 19.26%, 18:0; 24.54%). These results show that 18:0 acid from saturated fatty acids is dominant not only in PI subclass, but also in acidic PS fraction like this subclass. In addition, in our study, the percentage of DHA was found to be lower than that of the PE subclass, as in tuna fish (Table 1). It was observed that the ∑SFA rates we determined in *A. mussulensis* type were quite close to *A. tarichi*.

Oleic Acid, and accordingly, total MUFA the lowest in January (9.69% in 18:1n-9, 11.47% in ∑MUFA) was highest in June (124.32% in 8:1n-9, 33.62% in ∑MUFA). In polyunsaturated fatty acids, 20:4n-6 was close to each other; 22:6n-3 and accordingly, total PUFA and n-3 were higher in April and June than in other months. n-6, on the other hand, was found to be 27% higher in April (9.17%) than in other months. N-3/n-6 ratio was determined highest in June (4.93%) compared to other months (Table 1.). In November and January, Total SFA which was followed by ∑PUFA and at least ∑MUFA; In June, ∑PUFA, ∑MUFA and

∑SFA were the lowest, while in April, ∑PUFA and ∑SFA were similar with the lowest ∑MUFA (Figure 3.). In this study, *A. mussulensis* fish was found to be rich in 18:0, 18:1n-9 and ∑MUFA, and poorer in ∑PUFA and DHA (Table 1.). *A. mussulensis* PS fatty acids were noted to vary depending on the season. For example, the percentage of 18:1n-9 and ∑MUFA in January; EPA and ∑PUFA in November; ∑SFA in June were identified to decrease.

4. CONCLUSION

As a result of the analysis, the fatty acid distribution is given in table 1. When examined fatty acid’s 4-months average, it was indicated that 16:0 in PC, 18:0 in PI and PS, 18:1n9 in PC and PS, 20:4n-6 in PI, 20:5n-3 in the PC and PI, 22:6n-3 in PE, n-3 in PE and n-6 in PI was higher than others (Figure 1). It was indicated that Palmitik acid and 18:1n-9 in PC, 18:0 and AA in PI, and DHA in PE was higher compared to the others. It was observed that the richest subclass in terms of n-3/n-6 ratio was PE. Hiratsuka et. all. (2004) didn’t identify PI and PS in the ovaries of the same kind of fish and they found that 16:0 in PC, 18:0 and DHA in PE was higher.

In recent years, especially in marine fish, fatty acid analyses of PL subclasses such as PC, PE, PS and PI have been carried out. PC and PE are the most abundant of these subclasses. PI and PS follow them. It was determined that the amounts of PL subclasses change according to the tissue. *C. baicalensis* and *C. dybowski* studied the amount of PL subclasses in ovarian, muscle and liver tissues of fish. According to the results of the research, the amount of PC in both fish was found mostly in the ovaries, PE was high in the liver, PI and PS were found in the muscle more than other tissues (Kozlova ve Khotimchenko 2000). The amount of PE and PC in the gonads of the Euthynnus pelamis fish species was determined differently. PE in fish's testicle and PC in ovaries were determined to be more (Hiratsuka et. all. 2004). In another study, it was determined that PI percentage in *S. asotus* liver was higher than ovarian (Shirai et. all. 2001). PC and PE, which were determined as the basic PL classes in *Trachurus trachurus L*. were quite rich in terms of EPA and DHA. In addition, it was determined that PE had high DHA and 18:0; and PC had high 16:0 content (Nadcisa et. all. 2001). It was found that in six different Tuna species, the dominant saturated component in PC was 16:0, it was 18:0 in PE and dominant PUFA in both subclasses was DHA. Another subclass, PI, has a high ratio of 18:0. With this subclass, the saturated was high in PI but DHA is low. Compared to other subclasses, the lowest n-3/n-6 ratio was seen in PI (Medina et. all. 1995). In our study, the PI rate was found at the lowest percentage with an average of 2.13. In some studies, AA was found at a high rate in the PI subclass (Bell et. all. 1985). However; Medina et. all. (1995) did not find such a finding in their research. In our study, it was determined that the percentage of PI in terms of AA with %13.85 was higher compared to the other subclasses.

In April, total PUFA in PC; total PUFA and n-3 in PE and PI; total SFA in PS, total SFA in November and January was higher than in other months (Figure 2 ve 3). Cejas ve ark. (2003), PE and PC fatty acid content in *D. Saugus* was compared with TAG and according to the results taken from that, it was determined that DHA, EPA and AA were found high in PL subclasses and MUFA was high in TAG. In PE, DHA in than to the PC, SFA and MUFA in PC has determined higher. PI was rich in terms of AA in *Callichrous pabda*, which is a freshwater fish, and EPA and DHA ratios have found close to each other in the PC and PE (Ghosh 1997).

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