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SEASONAL VARIATIONS OF FATTY ACID CONTENT OF PHOSPHOLIPID SUBCLASSES IN MUSCLE TISSUE OF FEMALE AND MALE INDIVIDUALS OF *CAPOETTA TRUTTA*

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ABSTRACT

Fish and other seafood, which are energy sources for living systems, it has been located in our lives as a main food source throughout human history. Therefore, the analysis to be carried out in fish can be executed both by determining the fatty acids that will be necessary for health and by analyzing the fatty acids that have a functional property for the fish. As a result of these analyses, the distribution of fatty acids used for basic energy and the determination of physiological symptoms in growth, development and reproduction of living things are also important. Furthermore, fat and fatty acids are the most changing biochemical compounds depending on ecological factors. Thus, in the studies on fish biology, it is very important to know the fatty acid composition and essential fatty acid requirement of fish in the events such as reproduction, adaptation, growth and development. In the present study, it was aimed to determine seasonal variations of fatty acids in phosphatidylcholin, phosphatidylethanolamine and phosphatidylinositol, which are phospholipid subclasses, to better understand changes in fatty acid in phospholipid fractions.

Capoetta trutta, which belongs to the Cyprinidae family, is a species that widely exists and is consumed as a basic food source. In our study, sexes of the samples caught from Batman Dam Lake were determined in the laboratory. Then, phospholipid (PL) subclasses in muscle tissue of these individuals were separated from each other by Thin Layer Chromatography (TLC) using Vaden et al. (2005) method. Fatty acids that were converted to their methyl esters were analyzed by Gas Chromatography. The results were given as % fatty acids quantitatively.

A total of 16 fatty acids were determined as a result of the analysis. According to the results, out of saturated fatty acids, 16:0 and 18:0; out of monounsaturated ones, 18:1n-9 and out of polyunsaturated ones, 22:6n-3 was found as predominant fatty acids. Other fatty acids were present onlyin trace proportions. When phospholipid subclasses were considered, it was observed that 16:0 was high in Phosphatidylcholin (PC) and Phosphatidylethanolamin (PE), 18:0 was high in Phosphatidylinositol (PI) and Phosphatidylserine (PS), 20:4n-6 was higher in PI, 22:6n-3 was higher in PC and PE, n-6 was higher in PI, and ∑SFA was higher in PI and PS, while 22:6:n-3, ∑PUFA and n-3 were lower in PI, and the ratio of n-3/n-6 was lower in PS than the other subclasses. From these data, it was determined that PS and PI were rather high in ∑SFA, whereas PC and PE were high in ∑PUFA. When the seasonal changes are considered, it was determined that ∑SFA and ∑PUFA were the most influenced, while ∑MUFA's were hardly ever influenced.

Keywords: Fatty Acid, Phosphatidylcholin, Phosphatidylethanolamin, Phosphatidylinositol, Phosphatidylserine, *Capoetta trutta*

1. INTRODUCTION

People have made fish and other seafood a major food source since ancient times. It is known that fish and other seafood are one of the easiest supplied and the most consumed foods in the period

before the cultivation of agricultural products and domestication of animals (Besler, 2015). Although fish are similar to red meat such as beef, sheep, pork and poultry meat in terms of protein content, they differ from these in terms of fat, some mineral and vitamin contents. Fish, like other meats, do not contain carbohydrates, and the energy of fish is due to fat and protein. While protein content does not differ significantly among fish species, they differ considerably in terms of fat content. Thus, it has been determined that the energy values of fish change according to the amount of oil in their composition and the energy of oily fish is higher than that of lean fish (Baysal, 2002). It is known that the fat content in fish varies not only according to the fish type but also depending on the seasons, nutritional characteristics, the salt ratio of water and other factors. Hence, it has been determined that the fish have a wide variation between 1-14% in terms of fat content (Baysal, 2002). Unlike mammals that store lipids in fat tissue, fish store them more in skeletal muscle and liver tissue (Watanabe, 1982). Most of these lipids are mobilized to different parts of the body for use in different physiological events, and a significant part of them are used as energy sources (Rodriguez et al. 2004). Lipids are a source of energy for fish, a structural component of the cell membrane, transport of fat-soluble nutrients, and numerous hormones and eicosanoid precursors (Higgs ve Dong, 2000). Furthermore, fish need Eicosapentaenoic acid (EPA), Docosapentaenoic acid (DHA) and Arachidonic Acid (AA) for normal growth, development and reproduction (Rodriguez et al. 2004). In fish fed with nutrients lacking fatty acids, which is the basic component of lipids, growth slows down, there is an abrasion in tail fin, pigmentation is not sufficient, and fatty acid composition and lipid metabolism are inadequate (Castell et al. 1972).

Lipids in animals contain many different classes of lipids and out of basic lipid classes, triacylglycerol (TAG), phospholipid (PL), wax esters and sterols are some of them (Tocher 2003; Turchini et al. 2009). TAG which consists of three fatty acids and one glycerol, and wax esters consisting of fatty acid esters are neutral lipids (Tocher, 2003). Phospholipids, which are one of the most important components of cellular membranes, are found in high concentrations in the glands, blood plasma, egg yolk, seeds of legumes, brain, liver, kidney, pancreas, lung and heart muscle. Phospholipids, which are approximately 40% in the structure of erythrocyte membrane lipids, make up more than 95% of the inner membrane of the mitochondria (Reddy et al. 1991).

All fats in living things consist of chains of fatty acids. Fat molecules in the structures of fatty acids are of different lengths and different numbers and contain different bond structures. According to these bonds they contain; fatty acids which do not contain double bonds are called saturated fatty acids (SFA), fatty acids having one double bond are called monounsaturated fatty acids (MUFA), those containing more than one double bond are called polyunsaturated fatty acids (PUFA), fatty acids containing more than twenty carbon atoms and more than four double bonds are called highly unsaturated fatty acids (HUFA). Chain length, number and position of the double bond determine the biological properties of the fat (Voet and Voet 1990). The ratio of saturated fatty acid in fish is 20-30%, ratio of unsaturated fatty acid is 70-80%, and amount of PUFA is 25-30% (Ackman 1988).

In a study carried out by Tocher and Sargent (1984), the amounts of PL subclasses of the fish were determined as phosphatidylcholine (PC) > phosphatidylethanolamine (PE) > phosphatidylinositol (PI) > phosphatidylserine (PS), in the decreasing order. When the structure of the cell membrane is considered, it can be seen that PC comes first (40-50%), PE is the second (20-50%), and PS and PI (2-10%) are the third in terms of the phospholipid it contains. Different mammalian cells and tissues characteristically have different phospholipid compositions. For example, it was determined that 50-68% of the total phospholipids in the erythrocyte, gill, spleen, heart, liver and muscle tissue of the flounder (*Hippoglossus hippoglossus*) was PC, 20-39% was PE, 6-15% was PI and 3-14% was PS (Lie et al. 1992a). Due to the methyl group ($-CH_3$) in its structure, phosphatidylcholine is essential for numerous biochemical processes, such as protein and nucleic acid synthesis and regulation, and phase-2 hepatic detoxification. PC has been shown to increase serum acetylcholine levels, which play an important role in many brain processes (Canty and Zeisel 1994). Phosphatidylethanolamine is known as cephalin because it is abundant in the brain, spinal cord and other nervous tissues. About 45% of brain phospholipids contain cephalin. It is known to play an

important role in perception and memory events. Phosphatidylserine is predominantly found in the white matter of the brain and is particularly important in the transmission of impulses between nerve cells. It plays an important role in the development of learning and memory ability. In the case of its deficiency, negative effects occur on memory functions and ability to concentrate. It is known that phosphatidylserine plays a vital role in the process of apoptosis. It enables apoptotic cells to be recognized by macrophages. Phosphatidylinositol is very important in the regulation of proteins at the cell interface and intracellular signal transmission. These molecules rapidly change to transform into secondary messengers such as diacylglycerol, inositol 1,4,5-triphosphate, phosphatidylinositol 3,4-bisphosphate (PtdIns 3,4P2) and phosphatidylinositol 3,4,5-triphosphate (PtdIns 3,4,5-P3) (Canty and Zeisel 1994).

Capoetta trutta genus belonging to the Cyprinidae family is a species widely found in rivers in Iran, Iraq, Syria and Turkey, and used as a food source. The fatty acid content of PLs in this fish is affected by season, environment, temperature, nutrient and tissue type (Wodtke 1981, Lie et al. 1992a, Shirai and Wada 2001). Furthermore, fish oil and fatty acid composition are the biochemical compounds that change the most according to the ecological factors and the physiological condition of the fish. Therefore, it is very important to know the fatty acid composition and essential fatty acid requirement of the fish while working on issues related to feeding and fish biology such as reproduction, adaptation, growth and development. In order to better understand the fatty acid changes in the PL fractions, it was aimed to investigate the seasonal change of the fatty acids in phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and Phosphatidylserine (PS), which are the subclasses of this fraction.

2. MATERIAL AND METHOD

2.1.Collection of Samples

Yayık Village (38°14' 49. 05"K, 41°06' 04. 25"D, 653m) Batman Dam Lake, which is close to the entrance of the Diyarbakır Kulp district, was selected as sampling stations. Fish were caught using blind (pulling) nets through local fishermen in the region.

2.2. Sex Determination

The abdominal areas of the caught fish were opened with the help of dissection scissors and their sexes were determined by morphological observation of the gonads. In young samples, sex was determined by examining a piece taken from gonads under a light microscope.

2.3. Lipid Extraction and Separation of Phospholipid Subclasses

Total lipids extracted that it was used method Folch et al. (1957) (chloroform/methanol (2/1 v/v)). For the separation of phospholipid subclasses, method of Vaden et al. (2005) was used; thin-layer chromatography (TLC) plates (Silica gel 60G (Merck) were placed in a propelling tank in which chloroform/ethanol/water/triethylamine (30:35:7:35, v/v) mixture is present so that the PL subclasses were separated from each other.

2.4. Gas Chromatography Conditions

Fatty acids of fat samples converted into methyl esters were analyzed using a SHIMADZU GC 2010 PLUS model Gas Chromatography instrument, flame ionization detector (FID) ve DB–23 (Bonded 50 % cyanopropyl) (J & W Scientific, Folsom, CA, USA) capillary column (30m x 0.25mm inner diameter x 0.25µm film thickness). Detector temperature: 250 ºC; injector temperature: 250 ºC; injection: Split–model 1/10. Gas flow rates: carrier gas: helium 0.5 ml/min for 30 m column; hydrogen: 30 ml / min; dry air: 400 ml/min. Column (oven) temperature: 170 ºC, standby time, 2 min; 2 ºC/min to 210 ºC, standby time 20 min; total analysis time süresi: 42 min. In the diagnosis of fatty acids, a mixture of methyl esters of fatty acids (Sigma-Aldrich Chemicals) was used as standard. Chromatograms for methyl esters of fatty acids and total amounts of fatty acids were obtained using the GC Solution (Version 2.4)

computer software. The peaks in the chromatogram of the analyzed samples were identified by comparing the retention times of the methyl esters of all fatty acids in the standard. The results are given as qualitative value as % fatty acid. Percentages of fatty acid were compared with SPSS 16 computer program by using one-way analysis of variance (ANOVA). The differences were determined by the 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

3.1.PC Group

Most of the studies on fatty acid analysis of phospholipid subclasses were carried out with marine fish abroad. In our country, it was first performed by Kızmaz (2015) on pearl mullet. In our study, out of saturated fatty acids (SFA), palmitic acid (average 34.23% in females, 30.19% in males); out of monounsaturated (MUFA) ones, oleic acid (average 14.34% in females, 15.05% in males); out of polyunsaturated (PUFA), docosapentaenoic acid (average 25.53% in females, 24.76% in males) were determined as major fatty acids in the muscle tissue of female and male individuals analyzed every season (Table 1.).

The palmitoleic acid (16:1n-7) was the lowest in November (1.49%) in the female individual, while it increased towards the summer period and reached its highest value in June (4.71%). These values were close to each other in the male individual. Oleic acid (18: 1n-9) was the highest in January (11.30%), June (13.46%), April (15.25%) and November (17.33%) in the female individual, and it was highest in November (20.79%) in male individuals, while it was similar in other months. Depending on them, MUFA was the lowest in January (14.10%) in female individual and it was the highest in November (25.04%) in male individual, whereas they were similar to each other in other months (Figure 1.). When the data among the seasons were analyzed, 20:4n-6 was the lowest in November (4.40%) and 20:5n-3 was the highest in June (11.59%) in female, while both females and males were close to each other in other months. Total PUFA fatty acids were the lowest in November (40.95%) in females and the highest in April (50.77%) in men, while they were similar to each other in the other months. On the other hand, N-3 fatty acids were close to each other in both female and male individuals; n-6 was found to be similar in female and male individuals among themselves. N-3/n-6 ratio showed a similar distribution in both individuals (Table 1.)

According to the data; the highest one was ∑PUFA, followed by ∑SFA and the lowest one was ∑MUFA for male and female individuals in all months (Figure 1.). It has been reported that PC and PE are similarly affected by dietary fatty acids (Shirai and Wada, 2001). In the study carried out on 27 types of bony fish, 16:0, 18:1n-9, 20: 5n-3 and 22: 6n-3 were the main components in the PC subclass of fish muscles. The ratio of DHA was different depending on the species and was found more than 25% in migrant, beach and surface fish, and slightly lower than this ratio in bottom fish. The bottom fish contained higher EPA. Total amount of SFA in muscle PC was determined as 35-38% in tissues of migratory fish. Total PUFA in PC fatty acids was determined as 40-55% in the tissues of tested fish except for the testicles of migratory fish. Another important finding is that the n-3/n-6 ratio in PUFA was high since the n-6 PUFAs were low in all tissues of migratory fish (Takama 1999). Also, Lin et al. (2012) found the ratio of ∑PUFAs, which are the richest components in PL fractions, between 40.94 - 47.81% in PC. In our study, n-6 PUFA (average 45.01% in females, 42.78% in males), n-3 PUFA (average 38.94% in females, 48.14% in males) and n-3/n-6 (average 5.10% in females (4.95% in males) were found to be similar (Figure 1.). In our study, n-6 PUFA (average 45.01% in females, 42.78% in males), n-3 PUFA (average 38.94% in females, 48.14% in males) and n-3/n-6 (average 5.10% in females, 4.95% in males) were found to be similar (Table 1.).

In a study conducted on six kinds of tuna fish, out of PC, 16:0 (19.26 - 25.46%) and out of PUFA, 22:6n-3 (28.70 - 41.63%) was determined as the major saturated fatty acid in the muscle PL fraction (Medina et al. 1995). In *D. sargus*, 16:0 and 22:6n-3 were found in a high ratio in PC in muscle tissue (Cejas et al. 2004). As a result of the evaluations of the analysis, it was observed that 16:0 (average 34.23% in females, 30.19% in males), 22:6n-3 (average 25.53% in females, 24.76% in males) were similarly high. MUFA (average 18.02% in females, 19.26% in males) was high in contrast to these studies (Table 1., Figure 1.).

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Table 1. Seasonal variations of Phosphatidylcholine and Phosphatidylethanolamine in muscle tissue in male and female of *Capoetta trutta*

	Phosphatidylcholine (PC)								Phosphatidylethanolamine (PE)							
	Female				Male				Female				Male			
Fatty Acids	**November**	**January**	**April**	**June**	**November**	**January**	**April**	**June**	**November**	**January**	**April**	**June**	**November**	**January**	**April**	**June**
14:0	0.56±0.04	0.64±0.05	0.70±0.06	0.87±0.07	0.36±0.03	0.64±0.05	0.65±0.05	1.14±0.09	0.40±0.03	0.78±0.06	0.39±0.03	1.04±0.08	0.19±0.02	0.99±0.08	0.38±0.03	1.82±0.15
15:0	0.26±0.02	0.82±0.07	0.38±0.03	0.86±0.07	0.20±0.02	0.62±0.05	0.40±0.03	0.76±0.06	0.08±0.01	0.57±0.05	0.13±0.01	0.22±0.02	0.09±0.01	0.10±0.01	0.10±0.01	0.27±0.02
16:0	36.72±2.93	38.07±3.04	32.24±2.57	29.87±2.38	30.19±2.41	32.93±2.63	27.88±2.22	29.76±2.37	26.50±2.11	19.48±1.55	15.55±1.24	13.51±1.08	18.90±1.51	15.84±1.26	15.33±1.22	23.19±1.85
17:0	0.28±0.02	0.32±0.03	0.32±0.03	0.03±0.00	0.23±0.02	0.70±0.06	0.54±0.04	0.11±0.01	0.09±0.01	0.04±0.00	0.59±0.05	0.07±0.01	1.20±0.10	0.26±0.02	0.89±0.07	0.07±0.01
18:0	1.77±0.14	1.88±0.15	2.23±0.18	4.11±0.33	2.41±0.19	2.81±0.22	2.77±0.22	4.99±0.40	18.17±1.45	10.79±0.86	8.62±0.69	15.91±1.27	14.12±1.13	10.11±0.81	11.03±0.88	16.36±1.31
∑S.F.A	**39.59±3.16**	**41.73±3.33**	**35.88±2.86**	**35.74±2.85**	**33.38±2.66**	**37.70±3.01**	**32.24±2.57**	**36.76±2.93**	**45.24±3.61**	**31.66±2.53**	**25.27±2.02**	**30.75±2.45**	**34.49±2.75**	**27.30±2.18**	**27.73±2.21**	**41.71±3.33**
16:1n-7	1.49±0.12	2.47±0.20	2.97±0.24	4.71±0.38	4.04±0.32	3.95±0.32	2.73±0.22	3.02±0.24	0.44±0.04	1.31±0.10	0.69±0.06	2.12±0.17	0.51±0.04	2.32±0.19	2.06±0.16	3.14±0.25
18:1n-9	17.33±1.38	11.30±0.90	15.25±1.22	13.46±1.07	20.79±1.66	12.75±1.02	12.77±1.02	13.89±1.11	9.07±0.72	11.16±0.89	9.04±0.72	10.36±0.83	10.07±0.80	10.22±0.82	10.52±0.84	16.42±1.31
20:1n-9	0.65±0.05	0.33±0.03	1.25±0.10	0.87±0.07	0.21±0.02	0.72±0.06	1.48±0.12	0.69±0.06	1.15±0.09	0.86±0.07	2.99±0.24	1.21±0.10	1.25±0.10	1.46±0.12	2.87±0.23	1.03±0.08
∑M.U.F.A	**19.47±1.55**	**14.10±1.12**	**19.47±1.55**	**19.04±1.52**	**25.04±2.00**	**17.42±1.39**	**16.99±1.36**	**17.60±1.40**	**10.66±0.85**	**13.34±1.06**	**12.71±1.01**	**13.68±1.09**	**11.83±0.94**	**14.00±1.12**	**15.46±1.23**	**20.59±1.64**
18:2n-6	0.83±0.07	1.31±0.10	1.03±0.08	0.88±0.07	1.60±0.13	1.94±0.15	1.04±0.08	0.87±0.07	0.48±0.04	1.32±0.11	0.80±0.06	0.58±0.05	0.69±0.06	1.39±0.11	1.10±0.09	0.47±0.04
18:3n-3	0.09±0.01	0.80±0.06	0.34±0.03	0.42±0.03	0.63±0.05	1.08±0.09	0.30±0.02	0.20±0.02	0.10±0.01	0.64±0.05	0.30±0.02	0.22±0.02	0.16±0.01	0.72±0.06	0.54±0.04	0.12±0.01
20:2n-6	0.12±0.01	0.29±0.02	0.20±0.02	0.11±0.01	0.21±0.02	0.33±0.03	0.14±0.01	0.06±0.00	0.09±0.01	0.50±0.04	0.22±0.02	0.18±0.01	0.98±0.08	0.53±0.04	0.27±0.02	0.13±0.01
20:3n-6	0.27±0.02	0.25±0.02	0.20±0.02	0.18±0.01	0.11±0.01	0.28±0.02	0.15±0.01	0.15±0.01	0.23±0.02	0.23±0.02	0.22±0.02	0.23±0.02	0.26±0.02	0.27±0.02	0.27±0.02	0.15±0.01
20:4n-6	4.40±0.35	6.08±0.49	6.09±0.49	6.38±0.51	6.15±0.49	5.92±0.47	6.48±0.52	7.26±0.58	5.25±0.42	8.91±0.71	8.17±0.65	7.96±0.64	8.02±0.64	9.65±0.77	6.61±0.53	4.98±0.40
20:5n-3	7.85±0.63	7.53±0.60	7.61±0.61	11.59±0.92	9.02±0.72	9.78±0.78	11.54±0.92	10.96±0.87	4.20±0.34	6.59±0.53	6.26±0.50	8.79±0.70	6.23±0.50	8.42±0.67	8.58±0.68	5.29±0.42
22:5n-3	1.06±0.08	2.36±0.19	1.36±0.11	3.24±0.26	0.74±0.06	2.91±0.23	2.22±0.18	1.79±0.14	1.92±0.15	4.04±0.32	2.82±0.22	5.28±0.40	1.98±0.16	4.78±0.38	3.74±0.30	1.90±0.15
22:6n-3	26.32±2.10	25.55±2.04	27.83±2.22	22.42±1.79	23.13±1.85	22.65±1.81	28.92±2.31	24.35±1.94	31.84±2.54	32.77±2.61	43.21±3.45	32.32±2.58	35.36±2.82	32.94±2.63	35.72±2.85	24.65±1.97
∑P.U.F.A	**40.95±3.27**	**44.17±3.52**	**44.65±3.56**	**45.22±3.61**	**41.58±3.32**	**44.88±3.58**	**50.77±4.05**	**45.64±3.64**	**44.10±3.52**	**55.00±4.39**	**62.01±4.95**	**55.57±4.43**	**53.68±4.28**	**58.70±4.68**	**56.82±4.53**	**37.70±3.01**
ω3	35.33±2.82	36.24±2.89	37.13±2.96	37.67±3.01	33.51±2.67	36.41±2.90	42.97±3.43	37.30±2.98	38.06±3.04	44.04±3.51	52.60±4.20	46.62±3.72	43.74±3.49	46.86±3.90	48.57±3.87	31.96±2.55
ω6	5.62±0.45	7.93±0.63	7.52±0.60	7.55±0.60	8.07±0.64	8.47±0.68	7.80±0.62	8.34±0.67	6.05±0.48	10.96±0.87	9.41±0.75	8.95±0.71	9.94±0.79	11.84±0.94	8.25±0.66	5.74±0.46
ω3/ω6	6.28	4.57	4.94	4.99	4.15	4.3	5.51	4.47	6.29	4.02	5.59	5.21	4.4	3.96	5.89	5.57

Values are provided as mean ± SE

SFA saturated fatty acid, MUFA monounsaturated fatty acid, PUFA polyunsaturated fatty acid

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Table 2. Seasonal variations of Phosphatidylinositol and Phosphatidylserine in muscle tissue in male and female of *Capoetta trutta*

	Phosphatidylinositol (PI)								**Phosphatidylserine (PS)**							
	Female				**Male**				**Female**				**Male**			
Fatty Acids	**November**	**January**	**April**	**June**	**November**	**January**	**April**	**June**	**November**	**January**	**April**	**June**	**November**	**January**	**April**	**June**
14:00	0.01±0.00	0.95±0.08	2.06±0.16	2.17±0.17	0.22±0.02	0.97±0.08	8.82±0.70	1.45±0.12	1.47±0.12	3.69±0.29	1.48±0.12	1.66±0.13	1.18±0.09	2.25±0.18	1.35±0.11	3.17±0.25
15:00	0.04±0.00	0.56±0.04	0.68±0.05	0.08±0.01	0.11±0.01	0.24±0.02	0.15±0.01	0.6±0.05	0.48±0.04	2.89±0.23	0.13±0.01	0.37±0.03	0.22±0.02	0.34±0.03	0.51±0.04	0.83±0.07
16:00	10.61±0.85	11.18±0.89	14.88±1.19	14.96±1.19	9.18±0.73	16.4±1.31	8.95±0.71	14.73±1.18	26.33±2.10	34.62±2.76	14.98±1.20	16.57±1.32	26.23±2.09	21.57±1.72	22.56±1.80	20.00±1.60
17:00	0.12±0.01	0.23±0.02	0.85±0.07	0.07±0.01	1.25±0.10	0.28±0.02	0.59±0.05	0.05±0.00	0.90±0.07	0.28±0.02	0.62±0.051.06	0.09±0.01	0.74±0.06	0.18±0.01	1.34±0.11	0.09±0.01
18:00	26.23±2.09	31.52±2.51	13.03±1.04	25.24±2.01	23.96±1.91	23.15±1.85	23.05±1.84	33.12±2.64	24.71±1.97	20.16±1.61	13.34±2.44	23.36±1.86	39.11±3.12	15.65±1.25	38.07±3.04	26.5±2.11
∑S.F.A	**37.00**±2.95	**44.43**±3.54	**31.5**±2.51	**42.52**±3.39	**34.71**±2.77	**41.03**±3.27	**41.55**±3.31	**49.94**±3.98	**53.88**±4.30	**61.64**±4.92	**30.56**±2.44	**42.05**±3.35	**67.48**±5.38	**39.99**±3.19	**63.83**±5.09	**50.59**±4.04
16:1n-7	0.79±0.06	2.27±0.18	6.63±0.53	4.52±0.36	1.58±0.13	3.01±0.24	2.39±0.19	2.18±0.17	3.22±0.26	6.11±0.49	4.21±0.34	3.32±0.26	0.81±0.06	5.32±0.42	1.23±0.10	4.28±0.34
18:1n-9	12.18±0.97	10.85±0.87	20.43±1.63	11.16±0.89	12.70±1.01	12.45±0.98	8.74±0.70	13.33±1.06	17.80±1.42	14.01±1.12	16.72±1.33	13.35±1.07	20.71±1.65	12.30±0.98	14.43±1.15	11.73±0.94
20:1n-9	0.14±0.01	0.84±0.07	1.17±0.09	0.48±0.04	0.55±0.04	1.88±0.15	1.12±0.09	0.72±0.06	1.23±0.10	0.30±0.02	1.33±0.11	0.59±0.05	0.44±0.04	1.77±0.14	1.01±0.08	0.47±0.04
∑M.U.F.A	**13.11**±1.05	**13.96**±1.11	**28.22**±2.25	**16.16**±1.29	**14.83**±1.18	**17.34**±1.38	**12.25**±0.98	**16.23**±1.29	**22.26**±1.78	**20.42**±1.63	**22.25**±1.78	**17.26**±1.38	**21.96**±1.75	**19.39**±1.55	**16.66**±1.33	**16.48**±1.31
18:2n-6	0.60±0.05	0.59±0.05	2.31±0.18	1.50±0.12	0.36±0.03	0.73±0.06	0.95±0.08	0.53±0.04	0.07±0.01	0.77±0.06	1.59±0.13	0.82±0.07	0.27±0.02	1.21±0.10	1.51±0.12	0.46±0.04
18:3n-3	0.03±0.00	0.43±0.03	1.18±0.09	0.60±0.05	0.12±0.01	0.17±0.01	0.33±0.03	0.09±0.01	0.54±0.04	0.82±0.07	0.44±0.04	0.17±0.01	0.52±0.04	0.40±0.03	0.08±0.01	0.03±0.00
20:2n-6	0.10±0.01	0.19±0.02	0.32±0.03	0.16±0.01	0.13±0.01	0.22±0.02	0.11±0.01	0.07±0.01	0.38±0.03	0.15±0.01	0.48±0.04	0.32±0.03	0.08±0.01	0.35±0.03	0.07±0.01	0.22±0.02
20:3n-6	0.11±0.01	0.13±0.01	0.44±0.04	0.26±0.02	1.41±0.11	0.62±0.05	0.14±0.01	0.11±0.01	2.69±0.21	0.08±0.01	0.62±0.05	0.3±0.03	0.65±0.05	0.15±0.01	0.31±0.02	0.17±0.01
20:4n-6	13.74±1.10	16.33±1.30	4.76±0.38	10.45±0.83	15.38±1.23	11.7±0.93	11.18±0.89	10.69±0.85	1.46±0.12	1.85±0.15	15.67±1.25	3.77±0.30	1.19±0.09	12.56±1.00	3.87±0.31	2.69±0.21
20:5n-3	8.17±0.65	5.78±0.46	11.62±0.93	9.10±0.73	7.81±0.62	7.53±0.60	10.12±0.81	7.28±0.58	1.25±0.10	1.43±0.11	6.56±0.52	8.07±0.64	0.38±0.03	3.6±0.29	1.72±0.14	6.61±0.53
22:5n-3	1.55±0.12	2.36±0.19	4.96±0.40	3.62±0.29	1.62±0.13	2.96±0.24	3.31±0.26	1.77±0.14	1.71±0.14	2.52±0.20	3.81±0.30	6.14±0.49	0.68±0.05	3.30±0.26	2.26±0.18	3.50±0.28
22:6n-3	25.58±2.04	15.8±1.26	14.69±0.93	15.64±1.25	23.64±1.89	17.71±1.41	20.06±1.60	13.30±1.06	15.76±1.26	10.34±0.82	18.02±1.44	21.10±1.68	6.78±0.54	19.07±1.52	9.69±0.77	19.24±1.53
∑P.U.F.A	**49.89**±3.98	**41.61**±3.32	**40.28**±3.21	**41.32**±3.30	**50.46**±4.03	**41.63**±3.32	**46.2**±3.69	**33.83**±2.70	**23.87**±1.91	**17.95**±1.43	**47.19**±3.76	**40.69**±3.25	**10.56**±0.84	**40.63**±3.24	**19.51**±1.56	**32.93**±2.63
ω3	35.34±2.82	24.37±1.94	32.45±2.59	28.96±2.31	33.19±2.65	28.36±2.26	33.81±2.70	22.43±1.79	19.26±1.54	15.11±1.21	28.83±2.30	35.48±2.83	8.36±0.67	26.37±2.10	13.75±1.10	29.38±2.34
ω6	14.56±1.16	17.24±1.38	7.83±0.62	12.37±0.99	17.27±1.38	13.26±1.06	12.39±0.99	11.40±0.91	4.60±0.37	2.84±0.23	18.36±1.46	5.21±0.42	2.20±0.18	14.26±1.14	5.75±0.46	3.54±0.28
ω3/ω6	2.43	1.41	4.14	2.34	1.92	2.14	2.73	1.97	4.19	5.32	1.57	6.81	3.8	1.85	2.39	8.3

Values are provided as mean ± SE

SFA saturated fatty acid, MUFA monounsaturated fatty acid, PUFA polyunsaturated fatty acid

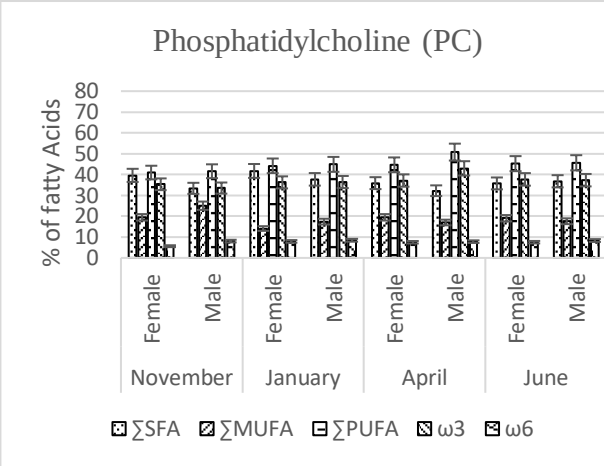

Figure 1. Distribution of ∑SFA, ∑MUFA, ∑PUFA, ∑n-3 and ∑n-6 in PC

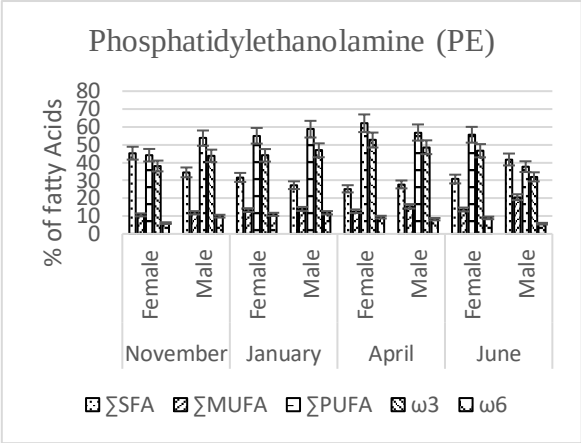

Figure 2. Distribution of ∑SFA, ∑MUFA, ∑PUFA, ∑n-3 and ∑n-6 in PE

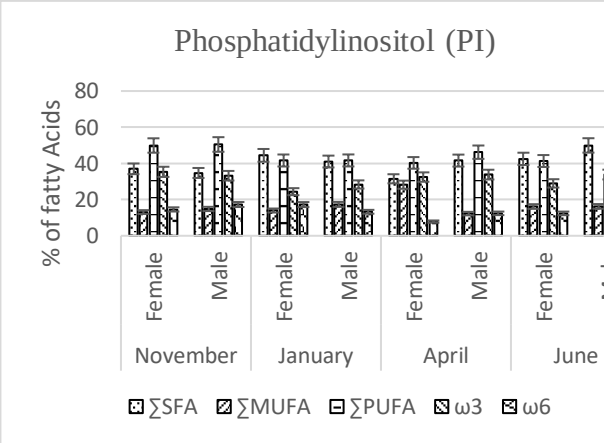

Figure 3. Distribution of ∑SFA, ∑MUFA, ∑PUFA, ∑n-3 and ∑n-6 in PI

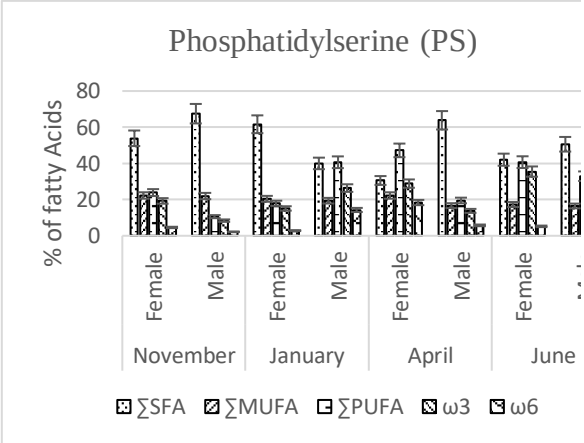

Figure 4. Distribution of ∑SFA, ∑MUFA, ∑PUFA, ∑n-3 and ∑n-6 in PS

3.2. PE Group

When seasonal variation of PE fatty acids, one of the PL basic subclasses of the muscle tissue of *Capoetta turutta* was considered, it was observed that, out of SFA, 16:0 and stearic acid (18:0); out of MUFA, 18:1n-9 and out of PUFA, 22:6n-3 were major. Out of the saturated fatty acids, 16:0 was the highest in November (26.50%) for female individual, while it was the highest in June (23.19%) for male individuals. Total SFA showed a similar distribution depending on 16:0. Out of monosaturated fatty acids, 16:1n-7 was the highest in June for females (2.12%) and males (3.14%). Distribution of 18:1n-9 fatty acid was similar in female and male individuals. On the other hand, 20:1n-9 was found to be two times in April for both sexes. While the ∑MUFA was close to each other in females, it was the lowest in November (11.83%) in males, which gradually increased in other months and became approximately 2 times and the highest in June (20.59%). Out of the polyunsaturated fatty acids, 20:4n-6 was the lowest in November (5.25%) for female individuals, while it was the lowest in June (4.98%) for the male individual, and it was similar in other months. 20:5n-3 was the lowest in November (1.92%), whereas it was the highest in June (5.28%) in females. It was two times lower in November (1.98%) and June (1.90%) for the male individual than those in other months. While the amount of 22:6n-3 was the lowest (43.21%) in April in female, it was lowest (24.65%) in June in male and similar in both individuals in the other months. Total PUFA was the lowest in November in females (44.10%) and in June in males (37.70%), while it was similar in both sexes in other months. N-3s showed a similar distribution as ∑PUFA depending on 22:6n-3. N-6 was found to be lowest in November (6.05%) in females and in June (5.74%) in males depending on 20:4n-6. The n-3/n-6 distribution was similar in both individuals. According to the data; ∑PUFA (50.07% on average in females, 51.72% in males) was the highest, followed by ∑SFA (on average 33.48% for females, 32.81% for males), ∑MUFA (12.45% on average in females, 15.47% in males) was at a lower ratio in all months except November for females, and in all months for males (Table 1., Figure 2.).

PUFA was at the highest (56.55%) and the ∑MUFA was at the lowest (22.80%) in muscle PE of five fish species (*S. curriculus, E. ilishaeformis, P. f ulvidraco, B. sinensis* and *S. kneri* Garman) collected from Poyand Lake, China's largest freshwater lake. The lowest and highest values of ∑SFA (27.12-37.46%) were determined in PE. The ratio of ∑PUFAs, which were the richest components in PL fractions, was between 48.21 - 56.55% in PE. In all species except for the S. kneri Garman species (6.04% 20:4n-6 in PE), 20:4n-6 and 22:6n-3 were found more than 10% in PL fractions. 20:5n-3 was found to be 3.15% in PE in S. curriculus. The n-3/n-6 ratio in PE subclass was between 0.84-1.96 (Lin et al. 2012). In our study on the muscle PE of both sexes of *Capoetta trutta*, PUFA was the most, whereas MUFA was the least in the majority of the months analyzed (Figure 2). Arachidonic acid ratio was average 7.66% in females and 7.32% in males. However, the n-3/n-6 ratio was rather higher (average 5.10 in females and 4.95 in males) than the fish studied in China (Lin et al. 2012). This is because capoetta trutta is rich in n-3 fatty acids, while it is poor in n-6 ones. Muscle PE fraction of flounder (*H. hippoglossus*) is also rich in long-chain PUFA, such as PC, especially 22:6n-3 (Lie et al. 1992a). A similar result was found in our study. PC and PE were identified as the basic phospholipid subclasses in horse mackerel (*Trachurus trachurus* L.) studied in Portugal. In these subclasses, the ratio of 22:6n-3 was high. PE was rich in 22:6n-3 and 18:0 (Nadcisa et al. 2001). In our study, it was richer in 16:0 and 22:6n-3.

In a study carried out on six kinds of Tuna Fish, major SFA in PC in the muscle PE fraction was 18:0 (17.85 - 23.25%), not 16:0 as in PC. It was also found with a high rate (21.61 - 30.89%) in DHA PE (Medina et al. 1995). Also, in Capoetta trutta fish, the content of 18:0 in muscle PE was rather higher than PC. However, the ratio of this component was less than 16:0 in all months except June in females and in all months in males in the analyzed months. This

data shows that the ratio of 18:0 in our fish is lower than the perch fish (Nadcisa et al. 2001) and tuna fish (Medina et al. 1995). As in the other studies (Nadcisa et al. 2001, Medina et al. 1995), 22:6n-3 was found at a very high rate in muscle PE (Table 1.). This finding indicates that there is DHA in the sn-2 position in the PE fraction of capoetta trutta.

3.3. PI Group

As a result of evaluations, out of saturated fatty acids, 16:0 and 18:0; out of monosaturated fatty acids, 18:1n-9; and out of polyunsaturated fatty acids, 20:4n-6 and 22:6n-3 were found as major fatty acids. When the distribution of individual fatty acids was considered, it was found that, out of the saturated fatty acids, 14:0 was significantly high in April and June for female and male individuals. Especially, this ratio in the male individual in April was nearly four times different from that in the female individual. 14:0 was high, while 16:0 decreased in male individuals. When the distribution in ∑SFA was considered, it was observed that it was the highest in females in January, whereas it was the highest in June in males. On the other hand, 18:1n-9 was high in April (20.43%) in females, while it was close to each other in the other months. It was the lowest in male individuals in April (8.74%), which was similar in other months. Depending on these fatty acids, the ∑MUFA distribution was the highest in April (28.22%) in females and the lowest in males (12.25%), however, the other months were similar. Out of the major PUFAs, 20:4n-6 was the lowest in April (4.76%) in female individuals, whereas its levels were close to each other in other months. Its level was high in November (15.38%) in male individuals and similar in other months. 22:6n-3 was found to be high in November (Female 25.58%, Male 23.64%) in both individuals. The other months were similar in female individuals, while the lowest value was observed in June (13.30%) in male individuals. Accordingly, ∑PUFA was higher in female (49.89%) and male (50.46%) individuals in November, the other months were similar in females, while it was the lowest in males in June (33.83%). Since the ∑n-3s increased in November and April in female and male individuals depending on DHA, they were higher in November and April than those in other months in both individuals. The ratio of n-3/n-6, which was 2.58 on average in females, decreased to 1.41 in January; while it was 2.19 on average for males, which increased to 2.73 in April. In both sexes in all analyzed months, out of SFA, 18:0 was major; out of MUFA, 18:1n-9 was major; out of PUFA, 20:4n-6 and 22:6n-3 were major (Figure). According to the data, ∑PUFA was the highest (43.27% in females, 43.03% in males) in both individuals in all months, then came ∑SFA (average 33.48% in females, 32.81% in males), and ∑MUFA was lowest (on average, 17.86% in females and 15.16% in males) than the other two groups of fatty acids (Figure). The reason for the higher ratio of 18:0 and 20:4n-6 compared to other subclasses is that these components are in the *sn-1* and *sn-2* positions, respectively, in the PI (Table 2., Figure 3.).

Phosphatidylinositol constitutes approximately 10% of phospholipids and has an important biochemical role since it acts as the second messenger in cells (Ikezawa, 1991). In the analysis of the summer and winter seasons of the cultured Japanese Catfish (*S. asotus*), major components of the PI subclass in the dorsal muscle was 18:0 (average 34%), 20:4n-6 (13.1%) and 22:6n-3 (21.30%) (Shirai and Wada, 2001). The ratio of 18:0 determined in our study was lower, whereas ratios of 20:4n-6 and 22: 6n-3 were similar to *S. asotus*. 22:6n-3 was also found as major in the dorsal muscle of Morina (Lie et al. 1992b) and Tuna fish (Medina et al. 1995), as was the case with the cultured Japanese Catfish *S. asotus*. Similarly, it was found high in our study. The quantitative fatty acid content of the same PL subclass may differ in close species. For example, the PI subclass in the dorsal muscle of the Japanese Catfish contained 18:1n-9 at a lower rate and 22:6n-3 at a higher rate than that of the Channel Catfish (Shirai and Wada, 2001).

Shirai and Wada (2001) have suggested that 20:4n-6 is important for PI, balancing changes in 22:6n-3 in muscle and 20:5n-3 in liver. For example, in the winter, when 22:6n-3 increases in muscle, 20:4n-6 decreases, while in summer, when 22:6n-3 decreases, 20:4n-6 increases. The ratio of n-3/n-6 in muscle, liver and ovaries of *S. asotus* was higher in winter than that in summer. As a result, it has been suggested that season and temperature affect the PI fatty acid content in various tissues of the cultured Japanese Catfish (Shirai and Wada, 2001). In Capoetta turrutta fish, 16:0 increased in June in females and males, as in the other subclasses, 18:0 increased in January in females and in June in male fish, 20:4n-6 increased in January in females (16.33%) and in males (11.70 %) (Table 2.).

In a study carried out on six types of Tuna Fish, 18:0 was determined as major SFA (14.70- 25.60%) in PI in muscle PL fraction. The ratios of 18:1n-9 out of MUFA were similar in all subclasses. The ratio of 22:6n-3 was low, while that of saturated fatty acids was high in PI and PS (Medina et al. 1995). Although it was emphasized that PI was rich in 20:4n-6 in some studies carried out on marine fish (Bell et al. 1985), in our study, the ratio of 20:4n-6 in muscle PI was very high as in marine fish.

As Phosphatidylcholine and PI contained a high rate of ∑SFA in tissues of the flounder (*H. hippoglossus*), however, major SFA was 18:0 (17–31%), not 16:0. Similar results were found in our study. Furthermore, PI was richer (6-21%) in terms of 20:4n-6 compared to other subclasses. This component is the main PUFA in fish, especially in its gill, spleen, heart and liver. Therefore, the subclass whose n-3/n-6 ratio was the lowest was PI. In our study, 20:4n-6 (average 11.32% in females, 12.24% in males) was high, while n-3/n-6 (average 2.58% in females, 2.19% in males) was found to be the lowest. At the same time, in marine fish, PI is also rich in usually 18:0 out of saturated fatty acids and 20:4n-6 out of PUFAs. The major SFA in *Capoetta turutta* was 18:0, which was found to be two or even three times this component in most of the analyzed months, and was 22:6n-3 instead of 20:4n-6 out of PUFAs (Table 2., Figure 3.). This finding was a feature of PI subclass.

3.4. PS Group

Palmitic Acid and 18:0 were major fatty acids out of saturated fatty acids, 18:1n-9 was major one out of monosaturated ones, and 22:6n-3 was major one out of polyunsaturated fatty acids in female and male individuals. 14:0 was at the lowest level in females (1.47%) and males (1.18%) in November, while it was three times higher in January in females (3.69%) and in June in males (3.17%). 16:0 was between 14.98 and 34.62, and was the highest in January in female individuals, while it varied between 20 and 26.23 in the males and was the highest in November. Out of the saturated fatty acids, 18:0 was found to be the lowest in April in females (13.34%), while it was high in males (38.07%). Accordingly, the highest value of ∑SFA was observed in January (61.64%) in females and in November (67.48%) in males, while the lowest percentage was obtained in April (30.56%) in the females and in January (39.99%) in the males. The percentage of 18:1n-9 was the highest in November in both individuals (17.80% in females, 20.71% in males), and was the lowest in June (13.35% in females, 11.73% in males). In the male individual, other months except November showed a similar distribution. On the other hand, in the female individual, November and April, January and June had a similar percentage. Total MUFA showed a similar distribution in the female individual except for June. In male individuals, November and January, April and June periods were similar. The distribution of 20:5n-3 was rather high in the female individual in April and June compared to other months. It was higher in January and June than that in other months for male individuals. 22:5n-3 was significantly higher in April and June in the females, while it was higher in January and June in male individuals. In June, 22:6n-3 was found to be high in both individuals (Female 21.10%, Male 19.24%). It was at the lowest value in November (6.78%) in male individuals and in January (10.34%) in female individuals. Depending on these fatty acids, ∑PUFA was at the

highest percentage in females in April (47.19%) and in males in January (40.63%). Similarly, 22:6n-3 was at the lowest percentage in January (17.95%) in females and in November (10.56%) in males. Depending on 22:6n-3, the ratio of N-3 was the lowest in the female in January and in the male in November, whereas it was the highest in both individuals in June. The percentage of N-6 was the highest in April in females and in January in males, depending on 20:4n-6. It was at the lowest level in January in females and in November in males. The ratio of n-3/n-6 was high in both individuals in June, when n-3 was high. According to the data, unlike other subclasses, the highest one was ∑SFA (47.03% in females, 55.47% in males), then comes ∑PUFA (average 32.42% in females, 25.90% in males) and lowest one was ∑MUFA (20.55% in females, 18.62% in males) (Table 2., Figure 4.).

In a study carried out on six types of tuna fish, the richest subclass in muscle PL in terms of ∑SFA was PS, which contained 16:0 and 18:0 acids in a similar percentage. The ratio of 22:6n-3 was low, whereas saturated fatty acids were found in a high ratio in PS (Medina et al. 1995). The data that we obtained from muscle PS of *Capoetta trutta* are generally compatible with this study. Because, in our analysis, 16:0 and 18:0 ratios were close to each other in both sexes, while 22:6n-3 ratios were lower than those of the other subclasses (Table 2.). These findings are specific to the PS subclass. Furthermore, the majority of 18:0 in PS, as in PI, is the common feature of these two acidic subclasses. As in the phosphatidylinositol, we can say that the PS fatty acid content is also affected by the season (Table 2., Figure 4.)

4. Conclusion

In some studies in which our propellent mixture can effectively separate PL subclasses, since acidic lipids, PI and PS, do not separate well from each other, fatty acid contents are given together (Baticic et al. 2011). Medina et al. (1995) found that the ratio of 22:6n-3 was low and that of saturated fatty acids was high in PI and PS. Similar results were observed in our study.

EPA and DHA values, which are considered as important for human health as well as for fish and have a healing role in the treatment of many diseases, and the ratios of these components to each other are considered as one of the important indicators. The same researchers draw attention to behavioral disorders, nervous abnormalities and visual impairments in DHA deficiency (Navarro and Sargent 1992). The high value of this in our study shows that the fish is valuable.

The fatty acid content of PLs in fish is affected by season, environment, temperature, food and tissue type (Wodtke 1981, Lie et al. 1992b, Shirai and Wada 2001). PC and PE were similarly affected by nutritional fatty acids, however, fatty acid content of PI was least affected by nutritional lipids. For instance, 18:0, 20:4n-6 and 22:6n-3 contents were found to be 34.00, 13.00 and 21.13%, respectively, in the Muscle PI subclass of Japanese Catfish which was fed with food whose 18:0, 20:4n-6 and 22:6n-3 contents were 1.00, 0.75 and 9.01%, respectively, (Shirai and Wada 2001). In our study, 16:0 and 18:0 and depending on these, the ∑SFA was the highest, while 22:6n-3 was the lowest in PS; the n-3/n-6 ratio was lower than the other subclasses, whereas 20:4n-6 was the highest in PI; and 22:6n-3, ∑PUFA and n-3 were higher than the other subclasses in PC and PE (Table 2., Figure 4.).

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