



Seasonal differences in the muscle fatty acid profiles of two freshwater fish species (*Scardinius erythrophthalmus*, *Squalius cephalus*)

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Abstract

Fatty acid compositions of the muscle lipids and its seasonal variations in *Scardinius erythrophthalmus* (rudd) and *Squalius cephalus* (chub) in Sapanca and Terkos Lake, were examined using a gas chromatographic method. Palmitic acid is saturated fatty acid and the other dominant fatty acid is stearic acid in both species. SFA contents in rudd were between 26.79% and 41.54% in all seasons. Oleic acid C18:1 ω9 was identified as the main monounsaturated fatty acid (MUFA) in both fishes for four seasons. It was found that chub had a high amount of C18:1 (15.09% to 28.56 %) in all seasons compared to rudd. It was noticed from the present data that docosahexaenoic acid (DHA) (22:6ω3) was predominant PUFA in muscle lipids of rudd. According to these data, it can be concluded that both freshwater fishes are a good sources for eicosapentaenoic acid (EPA) and DHA.

Key words: muscle, fatty acid, seasonal changes, fish

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İki tatlusu balık türünün (*Scardinius erythrophthalmus*, *Squalius cephalus*) kas yağ asidi bileşiminde mevsimsel farklılıklar

Özet

Sapanca ve Terkos göllerinde bulunan *Scardinius erythrophthalmus* (kızılkanat) ve *Squalius cephalus* (kefal)'un kas lipidlerinin yağ asit kompozisyonu ve mevsimsel değişimleri gaz kromatografik yöntemle incelenmiştir. Palmitik asit doymuş yağ asitidir ve diğer dominant yağ asidi her iki türde de stearik asitdir. Kızılkanattaki doymuş yağ asidi içeriği bütün mevsimlerde %26.79 ile % 41.54 arasındadır. Oleik asit C18:1 ω9 her iki balık türünde, bütün mevsimlerde primer doymamış yağ asidi olarak bulunmuştur. Kefalde, oleik asit C18:1(%15.09-%28.56) kızılkanata nazaran bütün mevsimlerde yüksek miktara sahiptir. Kızılkanat kas lipidlerinde dokosahexaenoik asit (DHA) (22:6ω3) dominant aşırı doymamış yağ asidi olarak tespit edilmiştir. Bu verilere göre her iki tatlı su balığının DHA ve eikosapentaenoik asit (EPA) yönünden iyi bir kaynak olduğu sonucuna varılmıştır.

Anahtar kelimeler: kas dokusu, yağ asidi, mevsimsel değişiklik, balık

1. Introduction

Fish use lipids rather than carbohydrates as energy source. They accumulate important amounts of lipids in liver, adipose tissues or their muscles "(Sheridan, 1988)". Fish lipids are quite rich in long-chain n-3 PUFAs, specially DHA and EPA "(Polak Juszcak and Komar Szymczk, 2009)".

Fish lipids have been recognized as a beneficial material for human health, during recent years. The omega 3 fatty acids are always present in fish flesh even in lean fish "(Ackman, 2002)". The omega 3 and omega 6 PUFAs are considered to be basic to the growth of children. These fatty acids are precursors for composite hormones known as eicosanoids, involved in a lot of metabolic processes of high importance for the human body, mainly related to cardiovascular activity "(Inhamuns and Franco, 2008)". n-3 PUFA cannot be synthesized by humans, these fatty acids absolutely must be taken with diet "Alasalvar et al., 2002". PUFA content, in particular, has been shown to be beneficial

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in the reduction of coronary artery disease, respiratory distress in asthmatics and rheumatoid arthritis "Leaf and Weber, 1988; Broughton et al., 1997". DHA is essential for normal fetal brain and cognitive development as the formation of neuron synapses in the brain strongly depends on the integration of this fatty acid into growing neurons "(Jensen, 2006)". The fatty acid composition of fish lipids is affected by temperature, reproductive cycle, spawning, diet, geographical location and season "(Henderson and Tocher, 1987)". The fatty acid profiles is influenced by water temperature. There is a marked increase in unsaturated FA composition at low temperatures "(Henderson and Tocher, 1987)". At low temperatures in the water a higher level of unsaturation in cell membrane phospholipids is needed to maintain permeability and flexibility "(Lovell, 1991)". Marine fish have higher quantity of PUFAs specially EPA and DHA, compared with freshwater fish "(Özogul and Özogul, 2007)". Docosahexaenoic acid, eicosapentaenoic acid, and arachidonic acid (AA) are basic structural components of cell membranes "(Innis, 1991)". DHA and eicosapentaenoic acid, found only in fish and seafoods, have extremely useful properties for, in particular, the prevention of human coronary artery disease "(Leaf and Weber, 1988)".

Although there are a lot of studies that have published fatty acid composition of different fish species from various geographical regions, there is a small amount of information on the fatty acid composition of rudd and chub. The aim of this study was to determine the muscle fatty acid profile and $\omega 3/\omega 6$ fatty acids levels of rudd and chub.

2. Materials and methods

Scardinius erythrophthalmus (Linnaeus 1758) (rudd) samples investigated in this study have been taken from the Sapanca Lake which has a surface area of 46.9 km² located 12 km away from Sakarya city in the west of Sakarya. *Squalius cephalus* (Linnaeus 1758) (chub) have been taken from Terkos Lake which has 39 km² surface area and located about 40-50 km away from Istanbul in the north-west direction. In the present study, samples collected all four seasons were analysed. Fish species were caught in the middle of each month for each season during 2012-2013. They were obtained from local fisherman in four seasons. Gender differences were ignored. They were transported to the laboratories an ice in the frozen form, filleted and frozen. The fillets were homogenized in chloroform/methanol mixture (2:1, v/v).

Fish muscles were extracted by the method described by "Folch et al., 1957". BF₃ methanol was used for methylation "Moss et al., 1974".

HP Agilent 6890N model gas chromatography was used for fatty acids methyl esters. Detector and injector temperatures were 280°C and 270°C, respectively. Column temperature program was 190°C for 35 min and then increased at a ratio of 30°C/min up to 220°C where it was maintained for 5 min. Helium was used as a carrier gas (2 ml/min). Identification of normal fatty acids was carried out by comparing sample FAME peak relative retention times with those obtained for Alltech standards. The data were represented as means±SD. The data were subjected to an analysis of variance at 0.05 significance level, using LSD.

3. Results

Fatty acid profiles of rudd and chub in 4 seasons is presented in Table 1 and Table 2. 29 fatty acids were found in muscle lipids of *S. erythrophthalmus* and 36 fatty acids were determined in muscles lipids of *S. cephalus*. The most abundant fatty acids were detected to be C16:0 (palmitic), C18:1 ω 9 (oleic), C16:1 ω 7 (palmitoleic), C18:2 ω 6 (linoleic), C20:5 ω 3(EPA), C20:4 ω 6 (AA), C22:6 ω 3 in the fishes in four seasons. Oleic acid in fillet of rudd was determined to be 13.03% (spring), 16.87% (summer), 9.94% (autumn) and 8.56% (winter). In this study, the highest ratio of C 18:1 was determined in summer. Chub had a high amount of C18:1 (15.09% to 28.56 %) in all seasons compared to rudd. It was followed by C16:1 in two species.

Table 1. Total fatty acid profile of muscles of *Scardinius erythrophthalmus* from Sapanca Lake (%)

Fatty acids	spring	summer	autumn	winter
C14:0	1.32±0.01a	1.25± 0.03a	0.61±0.05b	1.01±0.07c
C15:0	0.31 ±0.02a	0.42±0.01a	0.28±0.01a	0.25±0.02a
C16:0	30.41±0.41a	20.41±0.03b	18.89±0.17b	24.21±0.99a
C17:0	0.59±0.01a	0.72±0.04b	0.52±0.01a	0.43±0.01c
C18:0	6.55±0.04a	6.27±0.09a	4.83±0.05b	4.98±0.13b
C20:0	0.14±0.03a	0.11±0.01a	0.24±0.01b	0.42±0.01c
C21:0	0.64±0.03a	0.44±0.02b	0.41±0.01b	0.45±0.02b
C22:0	0.82±0.54a	0.30±0.02b	0.77±0.04a	1.33±0.14c
C24:0	0.46±0.17a	0.27±0.25b	0.23±0.17b	0.72±0.19c
ΣSFA*	41.54±0.65	30.30±0.14	26.79±0.20	33.79±1.22
C14:1 ω5	0.11±0.01a	0.06±0.02b	0.17±0.00a	0.03±0.01b

Table 1. continued

C15:1 ω5	0.29±0.01a	1.23±0.03b	1.41±0.05b	0.49±0.03c
C16:1 ω7	5.28±0.07a	6.11±0.18a	4.81±0.11a	5.14±0.23a
C17:1 ω8	0.25±0.02a	0.73±0.03b	0.36±0.01a	0.27±0.01a
C18:1 ω9	13.03±0.03a	16.87±1.41a	9.94±0.02b	8.56±0.18b
C20:1 ω9	0.09±0.03a	0.83±0.72b	0.06±0.01a	0.13±0.10a
C22:1 ω9	0.45±0.77a	0.08±0.04b	—	0.07±0.06b
C24:1 ω9	—	0.23±0.34a	—	1.32±0.12b
ΣMUFA*	19.50±0.69	26.50±2.21	16.75±0.17	16.00±0.02
C18:2 ω6	8.04±0.75a	7.77±1.06a	9.74±0.02b	7.91±0.26a
C18:3 ω6	0.36±0.06a	0.20±0.01b	0.17±0.00b	0.01±0.01c
C18:3 ω3	3.24±0.17a	3.60±0.69a	5.75±0.05b	5.12±0.11b
C20:2 ω6	1.46±0.40a	0.58±0.01b	0.81±0.01b	1.46±0.04a
C20:3 ω6	0.82±0.54a	0.30±0.02b	0.77±0.04a	1.33±0.14c
C20:3 ω3	0.88±0.89a	2.61±0.42b	1.85±0.15c	1.56±0.23c
C20:4 ω6	7.16±0.12a	6.26±0.27b	7.78±0.02a	10.52±0.15c
C20:5 ω3	5.00±0.08a	5.86±0.57b	7.51±0.46c	6.25±0.09b
C22:2 ω6	0.16±0.28a	0.41±0.30a	—	0.45±0.74a
C22:5 ω3	—	0.72±1.24a	3.03±0.12b	2.93±0.41c
C22:5 ω6	0.71±1.24a	—	—	0.04±0.07b
C22:6 ω3	11.13±0.73a	14.88±0.79b	19.05±0.06c	12.63±0.46b
ΣPUFA*	38.96±1.34	43.20±2.88	56.46±0.80	50.20±1.59
Σω3	20.24±0.84a	27.68±3.35b	37.19±0.73c	28.49±1.02b
Σω6	18.72±0.80a	15.52±0.92b	19.27±0.08a	21.71±0.79c
Σ ω3/6	1.08±0.05a	1.79±0.28b	1.92±1.09b	1.31±0.05c

^aaverage of analysed.

^bmeans±S.D.

^{c abc}within the lines, values in four seasons in a species of fish are significantly different at p<0.05.

Table 2. Total fatty acid profile of muscles of *Squalius cephalus* from Terkos (Durusu) Lake (%)

Fatty acids	spring	summer	autumn	winter
C10:0	—	—	—	0.01±0.00
C11:0	—	—	—	0.01±0.01
C12:0	—	0.02±0.02a	—	0.10±0.11b
C13:0	—	0.01±0.01a	—	0.13±0.09b
C14:0	0.61±0.22a	0.56±0.03a	0.92±0.15b	0.81±0.26b
C15:0	0.32±0.08a	0.62±0.11b	0.45±0.10c	0.47±0.10c
C16:0	14.88±2.61a	19.80±1.36b	19.07±2.16b	19.19±2.76b
C17:0	0.96±0.09a	0.60±0.05b	0.66±0.07b	0.80±0.10a
C18:0	3.77±0.30a	6.22±0.78b	4.10±0.53c	4.26±0.63c
C19:0	—	—	0.02±0.04a	0.06±0.12a
C20:0	0.77±0.63a	0.13±0.14b	0.06±0.07b	0.22±0.07c
C21:0	0.72±0.62a	0.32±0.15b	0.39±0.23b	0.73±0.01a
C22:0	0.53±0.44a	0.30±0.07b	0.29±0.03b	0.69±0.31c
C24:0	0.77±0.33a	0.55±0.37b	0.71±0.50a	0.71±0.20a
ΣSFA*	23.33±1.28	29.12±1.66	26.66±2.56	28.18±3.96
C14:1 ω5	0.20±0.07a	0.08±0.04b	0.10±0.06b	0.18±0.07a
C15:1 ω5	0.34±0.12a	0.22±0.13b	0.33±0.05a	0.47±0.21c
C16:1 ω7	5.19±1.24a	5.29±0.15a	9.86±2.37b	6.91±2.18c
C17:1 ω8	1.16±0.36a	0.20±0.02b	0.97±0.15c	0.31±0.12b
C18:1 ω9	28.56±7.44a	17.71±0.71b	19.80±4.09c	15.09±0.75b
C20:1 ω9	1.00±0.64a	0.94±0.10a	1.59±0.47b	1.37±0.11c

Table 2. continued

C22:1 ω9	—	0.10±0.10a	0.60±0.69b	0.02±0.02c
C24:1 ω9	—	0.68±0.10a	0.43±0.48b	0.82±0.95c
ΣMUFA*	36.44±5.14	25.22±0.81	33.68±6.67	25.16±1.53
C18:2 ω6	21.17±8.20a	5.66±0.81b	3.19±0.59c	4.48±1.26c
C18:3 ω6	0.77±0.93a	0.19±0.20b	0.10±0.03b	0.18±0.05b
C18:3 ω3	1.33±0.22a	0.95±0.23b	1.72±0.63c	1.67±0.49c
C20:2 ω6	0.21±0.13a	0.46±0.05b	0.44±0.04b	0.91±0.80c
C20:3 ω6	0.53±0.44a	0.40±0.41a	0.29±0.03b	0.07±0.03c
C20:3 ω3	1.76±0.35a	3.67±0.64b	3.14±0.52c	3.85±0.31b
C20:4 ω6	2.53±1.52a	8.93±0.49b	6.66±1.28c	7.84±1.61b
C20:5 ω3	4.93±4.98a	7.49±0.29b	5.83±0.83c	6.35±1.39b
C22:2 ω6	—	0.47±0.38a	0.12±0.08b	0.36±0.68a
C22:3 ω3	—	0.02±0.02a	—	0.07±0.03b
C22:4 ω6	—	0.23±0.16a	—	0.65±0.07b
C22:5 ω3	1.22±0.01a	1.93±0.12b	2.52±0.18c	2.90±0.53c
C22:5 ω6	—	0.51±0.75a	—	0.52±0.07a
C22:6 ω3	2.72±0.45a	14.78±1.18b	15.15±3.06b	14.61±4.26b
ΣPUFA*	37.17±0.78	45.68±0.58	39.16±4.98	44.46±6.31
Σ ω3	11.95±5.28a	28.83±0.57b	28.35±3.92b	29.45±6.62b
Σ ω6	25.20±6.06a	16.85±0.66b	10.79±1.13c	15.00±1.68b
Σ ω3/6	0.47±0.33a	1.71±0.09b	2.63±0.16c	1.96±0.52b

^a average of analysed.

^b means±S.D.

^c abc within the lines, values in four seasons in a species of fish are significantly different at p<0.05.

4. Conclusions and discussion

Among saturated fatty acid and MUFA identified in present study the highest was palmitic acid and it was followed by oleic acid which is comparable with the findings of other studies. Similar results to this study were determined for MUFAs of total lipid of *S. cephalus* in Serban Dam Lake. Oleic acid (16.82%) and palmitoleic acid (13.60%) were the major MUFAs "(Bulut and Mert 2014)". Total monounsaturated fatty acid was found to be 31.27-34.56% in *Carassius gibelio* "(Bulut, 2010)", and 13.80%-21.36% in *Sander lucioperca* "Celik et al. 2005; Ozogul et al. 2007". Monounsaturated fatty acids was 22.40-23.87% in *S. lucioperca*, 36.10% and 37.15% in carp from Turkey "(Ozparlak, 2013)".

"Kalyoncu et al., 2010a,b" have reported seasonal differences in the fatty acid profile of carp and rainbow trout and identified oleic acid as major MUFA (25,01-29,28%) in carp, (23,65-34,06%) in rainbow trout. Similarly "Citel et al. 2014" have reported that fatty acid composition of *S. erythrophthalmus* from Isikli Dam Lake and identified oleic acid as major MUFA (16.07%). Study has reported high levels of 18:1. According to "Steffens (1997)" C18:1 is a typical MUFA in fish tissues. The high amounts of C18:1, C16:1, and AA had been identified as a typical content of freshwater fish species oils "Andrade et al., 1995". In our study, the level of oleic acid, palmitoleic acid and arachidonic acids were determined higher than other fatty acids. C16:1 ω7 was the second high monounsaturated fatty acid (4.81-6.11%) in rudd. There were no differences between four seasons in terms of palmitoleic acid content (p<0.05). Although *S. erythrophthalmus* has the most constant fatty acid profile, there is quantitative differences. "Ackman (1989)" defined that highest values of palmitoleic acid is one of the typical of freshwater fish species. PUFA fractions were higher than the MUFAs and SFAs and in three seasons (winter, summer, autumn). A very high quantity of C16:0 (30.41%) increased the saturated fatty acid (SFA) content in spring, and a high quantity of, and C22:6, C20:5, C20:4 ω6 raised the PUFA amount in other seasons except spring. In this study, whereas total PUFA in chub was higher than total MUFA and SFA in four seasons, PUFA was higher in autumn, winter and summer in rudd. The levels of SFA, MUFA and PUFA were found to be 28.12%, 32.52%, 38.19% of lipid respectively in chub"(Bulut and Mert, 2014)".

Variations in fatty acids of freshwater and marine fishes should not only be considered with respects to species environment but also based on their diet specially whether a species is carnivorous, omnivorous, or herbivorous "Sargent et al., 1995; Ozogul and Ozogul, 2007". Rudd individuals of which it is stated that herbivores fed and

generally showed widespread in tropical regions. Chub is supplied with a variety of fish fry. In this study, differences in PUFA and MUFAs content in the chub and rudd may be attributed to this reason.

C 16:0 (palmitic acid) was main SFA in two species. Another heavy SFA was C18:0 (stearic acid). SFA ingredient ranges between 26.79% and 41.54% in rudd in four seasons. "Ozogul et al., 2007", "Donmez (2009)" and "Kalyoncu et al., 2010a" have also reported that prime saturated fatty acid was C 16:0 and the other was stearic acid in *C. carpio*. "Ackman et al., 1975" described that the levels of palmitic acid of fish was not affected by diet. However a lot of factors could influence fatty acid metabolism in fish, these: life mode and water of temperature. In general fishes have comparatively low in SFA (<30%) except for some species "(Ackman, 1989)". Results of in this study are in agreement with the other literature in data. Total saturated fatty acid was considerably higher in summer than in other seasons in chub. C 16:0 was the basic SFA, 18.89-30.41% for *S. erythrophthalmus* and 14.88-19.80% for chub in all seasons. Similar results to this study for different fish species have also been determined in other studies "Celik et al., 2005; Guler et al., 2008". Stearic acid was the second highest SFA (4.83-6.55%) in rudd and in chub (3.77-6.22%).

In this study, data demonstrated that $\omega 3/\omega 6$ rate was 1.08 (in spring), 1.79 (in summer), 1.92 (in autumn) and 1.31 (in winter) in rudd, 0.47 (in spring), 1.71 (in summer), 2.63 (in autumn) and 1.96 (in winter) in chub. According to "Valfre et al., (2003)" the $\omega 3/\omega 6$ ratio varies between 1 and 4 in freshwater fish. $\omega 3/\omega 6$ rate in muscle tissues in chub were determined to be 1.28 in winter (February 2010) "(Bulut and Mert, 2014)". $\omega 6$ fatty acids were lower than $\omega 3$ fatty acids in all seasons in rudd. $\omega 3/\omega 6$ rate in rudd were found to be 2.15 "Citil et al., 2014". "Ozparlak (2013)" have determined this ratio in winter to be 3.19 (*S. lucioperca*), 2.36 (*C. gibelio*), 2.08 (*L. lepidus*) and 1.06 (*C. carpio*). Hence, our results are in accordance with other former studies "Kalyoncu et al., 2009; Cakmak et al., 2012; Gorgun et al., 2013". Our study has revealed that *S. erythrophthalmus* is a well source due to its superior $\omega 3/\omega 6$ rate. In this study, PUFA levels have been found to be 38.96%-56.46% for rudd. The most abundant PUFA in rudd was DHA (17.94%) from Isıklı Dam Lake "Citil et al., 2014". AA level is fairly important. AA was found to be the third highest fatty acid in samples collected in winter (10.52%). Total opinion all of these percentage, rudd (38.96%, 43.20%, 56.46%, 50.20%) and chub (37.17%, 45.68%, 39.16%, 44.46% respectively) seems to be two fish species rich in point of the PUFAs. "Bowman and Rand (1980)" reported that AA is a vanguard for prostaglandin and tromboxan.

The present results shows that DHA (22:6 $\omega 3$) is predominant PUFA in muscle lipids of rudd. The high level DHA (19.05%) increases the PUFA content in rudd in autumn. In spring, a high rate C18:2 $\omega 6$ (21.17%) raises in the PUFA amount in chub. All fish species were rich in C 18:2 n-6 which is fundamental in human nutrition as DHA, C 18:2 n-6 are not synthesised in the body but they are necessary for tissue development. The level of EPA and DHA were between 4.93-7.51% and 2.72-19.05% in both species and four seasons respectively. It is known that, in seawater fish, the EPA and DHA amounts are higher than in freshwater fish "Czesny et al., 1999". EPA and DHA, have extremely helpful properties for the avoid of human coronary artery disease "(Leaf and Weber 1988)". Thus among the n 3 series the chub and rudd are well sources of EPA and DHA and $\omega 3/\omega 6$ rate observed in the recommended values (1-4) in the species that we work with. DHA is basic for the development the eye retina and of the foetal brain "Birch et al., 2000" and it was identified that DHA decreases the concentration of LDL cholesterol in plasma "Childs et al., 1990". According to "Simopoulos (1991)", because n 3 fatty acids are fundamental in growth throughout the life cycle and development, omega 3 should be included in the diets of all humans. n3 and n6 fatty acids are not interconvertible in the human body and are significant components of practically their cell membranes. Whereas cellular proteins are genetically determined, the polyunsaturated fatty acid (PUFA) composition of cell membranes is to a great extent dependent on the dietary intake. *Scardinius erythrophthalmus* and *Squalius cephalus* were good nutrient of PUFA, particularly total ω -3 fatty acids.

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