

Seasonal and Sexual Variations of Total Protein, Fat and Fatty Acid Composition of an Endemic Freshwater Fish Species (*Capoeta antalyensis*)

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ABSTRACT

In this study, the total protein and lipid contents and fatty acid composition of endemic freshwater fish *Capoeta antalyensis* was investigated according to the variation in seasons and gender. Total protein content (%) of *C. antalyensis* varied from 63.80% to 78.15% and total fat content from 4.57% to 21.29% in different seasons. The palmitic, stearic, palmitoleic, oleic, eicosapentaenoic and docosahexaenoic acids were the most abundant fatty acids in the muscles of *C. antalyensis*. The ratio of eicosapentaenoic acid (EPA) in muscles of both genders was higher in spring and autumn, while docosahexaenoic acid (DHA) ratio was higher in winter. The ratio of omega 3 polyunsaturated fatty acids (n3 PUFAs) content in the muscles reached the highest level in spring in males and in summer in females. The ratios of n-3 PUFAs to omega 6 polyunsaturated fatty acids (n6 PUFAs) in muscles of *C. antalyensis* of both genders changed from 3.29 to 5.44 as the seasons changed. Total fat and fatty acid contents were found to be at the lowest level in both genders during winter. This shows that nutritional content of *C. antalyensis* species is quite affected by variation in seasonal conditions.

Keywords: *Capoeta antalyensis*, fatty acid, gender, protein, season

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INTRODUCTION

Fish constitutes the most important part of the products and also makes up a considerable part of animal food resources for human with rich protein and lipid contents. Also, fishes have essential fatty acid content that cannot be produced in the human body. Fatty acid composition in muscle of fish is affected by feeding status and the fatty acid composition of fish feeding. Therefore, the chemical composition of fish is not stable and it depends on species, sex, environment, diet, season, and age of capture (Emre et al., 2015; FAO, 1995).

Polyunsaturated fatty acids (PUFAs) are classified as omega 3 (n3) and omega 6 (n6) fatty acids in which the first double bond occur either three or six carbon atoms from the methyl terminus of the fatty acid (FA) molecule, respectively (Dayhuff and Wells, 2005). n3 and n6 fatty acids play an important role in human heart diseases, brain development, cancer, infancy mortality,

anemia, skin diseases, hypertension and diabetes (Çelik, 2008; Mahaffey, 2004; Sidhu, 2003).

Omega 3 polyunsaturated fatty acids (n3 PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and omega 6 polyunsaturated fatty acids (n6 PUFAs), especially arachidonic acid, have significant physiological roles in both fish and human as components of membrane phospholipids and biologically active hormones as eicosanoids (Sargent et al., 1999). Some researchers indicated that fatty acids are modulators of nuclear transcription factors affecting genes and gene products (Clarke and Jump, 1993; Clarke and Jump, 1994; Jump and Clarke, 1999; Sessler and Ntambi, 1998). Such valuable roles have shown that determination of fish lipid composition is very important for fish physiology.

C. antalyensis is an endemic freshwater fish species living in Antalya basin in Turkey. The taxonomic features of *C. antalyensis* are D IV 8-9; A III 5-6; LL 49-55, pharyngeal teeth 2.3.5-

5.3.2. The body is thick and flattened from the sides. *C. antalyensis* has a pair of mustaches in upper jaw (Küçük and Güçlü, 2017). No studies are available about fatty acid composition of *C. antalyensis*. Therefore, the aim of this study is to provide information about the seasonal and sexual variations of total lipid and protein contents and the fatty acid compositions of *C. antalyensis*.

MATERIAL AND METHOD

The specimens of *C. antalyensis* used in this experiment were caught from the streams surrounding Antalya city. Mean weights and lengths of the representative fishes were 28.90±4.17 g and 12.70±1.42 cm in female, 34.9±3.98 g and 14.94±0.39 cm in male, respectively. A total of 40 fishes (20 male and 20 female and n=5 at each season) were used in experiments. The dorsal muscle specimens from each gender were taken by excision for the analysis.

Measurements

Total lipid extraction procedure based on methyl ester preparation was carried out by the method of Bligh and Dyer (1959). Methyl esters of fatty acids were prepared by transmethylation using 2M KOH in methanol and hexane (Ichihara et al., 1996). Extracted lipids (10 mg) were dissolved in 2 ml hexane and then in 4 mL of 2 M methanolic KOH. After these procedures, the samples were centrifugated at 4000 rpm for 10 min, and then analyzed.

Gas Chromatographic Conditions

The fatty acid profiles were analyzed by gas chromatography method. The samples were held 140°C in the oven for 5 min. After, the temperature was raised to 200°C at a rate of 4°C/min and then to 220°C at a rate of 1°C/min. The carrier gas was controlled at 16 psi and the split used was 1:40. Fatty acids were defined by comparing the holding times of fatty acid methyl esters mixture (SUPELCO). Also the amounts (g/100g wet weight) of total SFAs, UFAs, MUFAs, PUFAs, n3 PUFAs and n6 PUFAs in both genders were worked out according to the formulae indicated in Tufan and Köse (2014) and Balçık Mısır et al. (2014):

Fatty Acid content (g/100 g wet weight) = Fatty acid methyl esters% x Fatty acid conversion factor x lipid content %/100.

Statistical Analyses

The results are listed as means±standard error (SEM). The data was statistically analyzed with the Statistical Package for the Social Sciences 22 Programme (IBM Corp.; Armonk, NY, USA). The One-Way ANOVA Tukey's multiple comparison test was used for the determination the relations between seasonal variations and fatty acid compositions. The sexual comparison was determined by student t- Test. The level of significance was identified as p<0.05.

RESULTS AND DISCUSSION

The total fat ratios of muscle tissue of *Capoeta antalyensis* (%) was found at the lowest level during winter (4.57%), at the highest level in summer (21.29%) (Table 1). The total protein content of muscle was found at the lowest level during spring (63.80%), at the highest level in winter (78.15%) (Table 1). The summer and autumn seasons during which the total fat content is at the highest level, is the period corresponding to the intense feeding period following the reproduction of *C. antalyensis* species. It was reported that the food quality of

Table 1. The body compositions of *C. antalyensis*

%	Spring	Summer	Autumn	Winter
Moisture	72.11	72.48	74.08	76.45
Drymatter	27.89	27.52	25.92	23.55
Ash	12.89	12.35	11.89	16.16
Fat (as dryweight)	20.00	21.29	17.72	4.57
Protein (as dryweight)	63.80	67.98	70.77	78.15

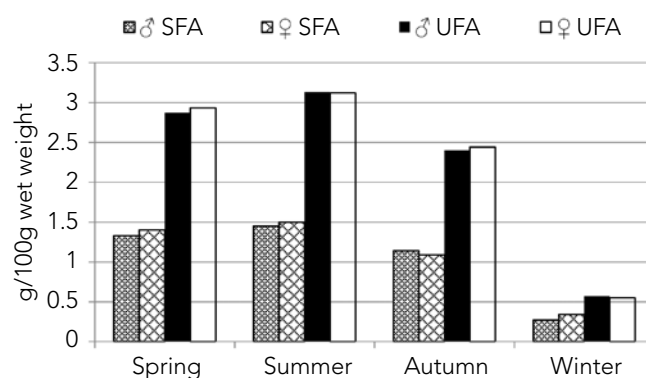


Figure 1. Seasonal and sexual changes in the levels of SFAs and UFAs in the muscle of *C. antalyensis*

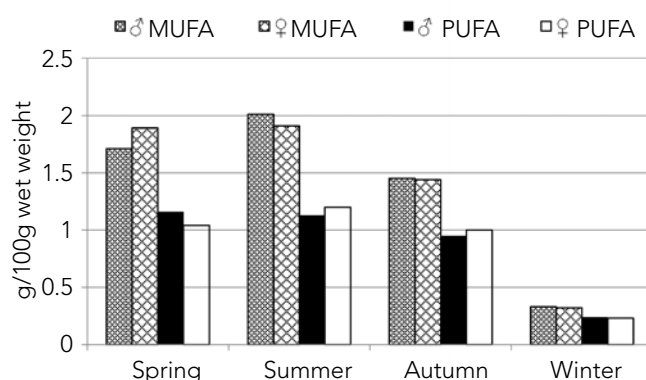


Figure 2. Seasonal and sexual changes in the levels of MUFAs and PUFAs in the muscle of *C. antalyensis*

fish was generally higher in the feeding period of summer (mostly) and autumn for *Capoeta angorae* (Emre et al., 2015). Searching the effect of climate on lipid content variations, Krzynowek (1985) reported that the fat content of fishes might differ by approximately 10% according to the season. At the same time, the fatty acids composition and lipids contents of fish tissues were effected by species, gender, water temperature, age, and nutritional conditions (Christiansen et al., 1989; Dal Bosco et al., 2012; Turon et al., 2005).

The seasonal variations of fatty acid composition in muscle of *C. antalyensis* were shown in Table 2. Also the amounts of the total fatty acid groups were presented in Figure 1, 2. In both genders, the saturated fatty acid (SFA) values were highest in summer, while the lowest values were found in winter and the difference

Table 2. Seasonal variations of fatty acid composition in muscle of male and female *C. antalyensis* (% of total fatty acids)

Fattyacids	Spring		Summer		Autumn		Winter	
	Male	Female	Male	Female	Male	Female	Male	Female
C12:0	0.36±0.03	0.47±0.23	0.61±0.05	0.55±0.01	0.82±0.33	0.39±0.07	0.48±0.03	0.30±0.12
C13:0	0.02±0.00	0.30±0.10	-	-	0.03±0.00	-	-	-
C14:0	2.98±0.18	3.12±0.17	3.38±0.20	3.12±0.09	3.17±0.17	2.93±0.01	2.11±0.13	1.96±0.26
C15:0	0.28±0.00	0.30±0.11	0.29±0.01	0.27±0.01	0.32±0.07	0.27±0.00	0.28±0.01	0.29±0.14
C16:0	18.28±0.12	19.05±0.90	18.98±0.62	19.49±0.49	17.84±0.49	18.29±0.10	17.56±0.62	22.33±0.14
C17:0	0.30±0.03	0.34±0.00	0.34±0.04	0.39±0.03	0.36±0.01	0.32±0.00	0.35±0.01	0.64±0.60
C18:0	3.57±0.27	3.94±0.23	3.28±0.26	3.79±0.22	3.18±0.17	3.52±1.18	6.00±0.27	8.96±0.75
C20:0	0.25±0.01	0.29±0.13	0.21±0.00	0.23±0.02	0.22±0.02	0.24±0.03	0.39±0.01	0.46±0.45
C21:0	0.44±0.04	0.31±0.03	0.38±0.01	0.41±0.03	0.41±0.03	0.43±0.04	0.36±0.03	-
C22:0	0.06±0.00	0.08±0.00	0.09±0.01	0.09±0.00	0.10±0.01	0.07±0.00	0.18±0.02	-
Σ SFA	26.54±0.38	27.94±0.91	27.58±0.62	28.36±0.56	26.47±0.61	26.47±0.38	27.68±0.82	34.85±0.48
C14:1	0.07±0.01	0.10±0.00	0.14±0.00	0.11±0.00	0.14±0.01	0.10±0.01	0.09±0.00	-
C15:1	-	0.20±0.00	-	-	-	-	-	-
C16:1	15.00±0.80	13.07±0.28	14.39±1.26	13.20 ±0.33	13.57±0.73	13.76±0.06	10.89±0.63	7.95±0.95
C17:1	0.50±0.05	0.45±0.04	0.49±0.06	0.45±0.01	0.71±0.10	0.62±0.10	0.30±0.01	0.67±0.00
C18:1n9	10.82±1.48	16.51±0.27	16.36±1.08	15.17±0.52	14.58±1.60	14.10±0.94	14.12±0.58	16.26±0.53
C18:1n7	6.18±0.61	5.96±0.29	5.42±0.25	5.85±0.20	4.64±0.27	5.34±0.38	6.81±0.10	7.06±0.57
C20:1	1.32±0.12	1.54±0.05	1.30±0.07	1.44±0.12	1.34±0.03	0.87±0.01	1.65±0.12	1.28±0.20
C22:1n9	0.06±0.01	0.06±0.00	-	0.15±0.00	0.17±0.06	0.13±0.00	-	-
Σ MUFA	33.98±0.87	37.72±0.29	38.10±0.61	36.27±0.56	35.09±1.53	34.95±1.13	33.80±0.67	32.78±1.43
C18:2n6	2.40±0.25	3.76±0.45	3.13±0.26	3.12±0.24	3.34±0.38	3.55±0.59	2.94±0.34	2.20±0.10
C18:3n3	6.10±0.59	5.77±0.39	7.44±0.37	7.45±0.60	6.37±0.97	6.07±0.69	4.84±0.41	2.67±0.15
C20:3n6	0.09±0.00	0.09±0.00	0.09±0.00	0.10±0.00	0.14±0.01	0.16±0.04	0.16±0.00	-
C20:4n6	0.20±0.01	0.14±0.03	0.10±0.01	0.08±0.00	0.12±0.01	0.18±0.03	0.10±0.00	-
C20:2n6	0.83±0.03	0.83±0.11	0.85±0.05	0.95±0.07	1.19±0.10	1.41±0.21	1.94±0.15	2.78±0.33
C20:5n3	10.03±0.41	7.43±0.36	7.10±0.36	7.85±0.28	7.85±0.91	8.43±0.02	8.11±0.34	7.70±0.35
C22:6n3	3.45±0.23	2.55±0.11	2.69±0.17	3.30±0.23	3.91±0.23	4.29±0.36	6.62±0.52	8.09±0.35
Σ PUFA	23.12±0.61	20.61±0.58	21.41±0.81	22.84±0.42	22.96±1.10	24.12±0.20	24.66±0.63	23.45±0.42
n3/n6	5.53	3.26	4.14	4.39	3.77	3.54	3.85	3.71
Σ Other	16.30±0.83	13.72±0.62	12.89±0.38	12.51±0.52	15.46±1.05	14.45±1.33	13.85±0.80	8.90±0.50

between genders is statistically insignificant ($p > 0.05$). Palmitic acid is the SFA with the highest ratio in muscle tissue and ratios differed significantly according to the seasons ($p < 0.05$). The palmitic acid level of female was the highest in winter (22.33%) and the lowest in autumn (18.29%) while these values are the lowest in winter (17.85%) and highest in summer (18.98%) in male. Aras et al. (2003) reported that palmitic acid was the primary SFA for carp in all seasons and there were significantly high palmitic acid ratios in all tissues compared to the other SFAs like lauric

(12:0), myristic (14:0), stearic (18:0) and arachidic (20:0) acids. Luczynska et al. (2016) and also Rasoarahaona et al. (2005) noted that palmitic acid was the highest FA during their studies for some fresh water fish species. In this study, myristic (C14:0) and stearic (C18:0) acids in the muscle of *C. antalyensis* were also relatively high among the SFAs (Table 2). The myristic acid content in muscle of male *C. antalyensis* in winter was minimum while stearic acid content was maximum.

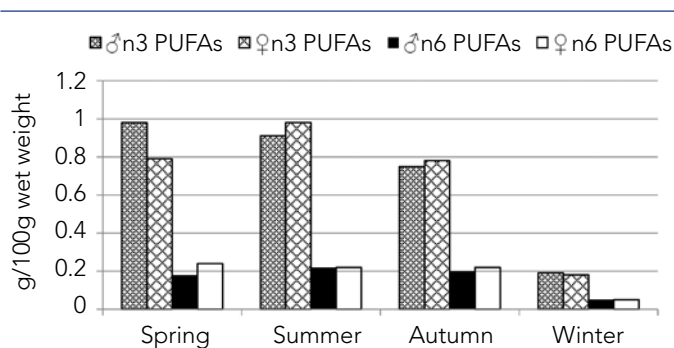


Figure 3. Seasonal and sexual changes in the levels of n3 and n6 PUFAs in the muscle of *C. antalyensis*

Monounsaturated fatty acid (MUFA) amounts of *C. antalyensis* species were the highest during summer and the lowest in winter in both genders. The levels of oleic acid (C18:1n9), which is a primary MUFA, changed from 14.10% to 16.51% in female and from 10.82% to 16.36% in male, respectively. Oleic acid content of female was high in spring (16.51%) and low in autumn (14.10%). Muscle oleic acid content of male *C. antalyensis* was minimum in spring (10.82%) and maximum in summer (16.36%). It was clearly shown that the oleic acid contents were significantly different between genders in spring ($p < 0.05$). Cengiz et al. (2010) reported that oleic acid was the main MUFA in freshwater fish species such as *Barbus rajonorum*, *Leuciscus lepidus*, *Carasobarbus luteus*, *Chondrostoma regium*, *Liza abu*, *Alburnus mossulensis*, *Cyprinion macrostomus*, *Acanthobrama marmid*, *Silurus triostegus*. Kolakowska et al. (2000) also reported that oleic acid was primary MUFA in the carp in all seasons. In this study, palmitoleic acid (C16:1) was the second most abundant MUFA in the muscle of *C. antalyensis*. Palmitoleic acid content was the highest in spring (15.00%) and the lowest in winter (10.9%) in male. The high oleic, palmitoleic and arachidonic acids levels have been often seen in freshwater fish oils. (Andrade et al., 1995; Aggelousis and Lazos, 1991). In this study, it was also seen that the vaccenic acid (C18:1n7) content in muscle of *C. antalyensis* was also high in all seasons, ranging from 4.64% to 7.06%. However, vaccenic acid content was at minimum level in autumn while at maximum level in winter in both genders. The ratios of vaccenic acid significantly varied between female and male in winter ($p < 0.05$).

Polyunsaturated fatty acid (PUFA) amounts of *C. antalyensis* species (g/100g wet weight) changed from 0.23 to 1.20 in female and from 0.24 to 1.16 in male. The amounts of n3 PUFAs and the ratios of n3 PUFAs to n6 PUFAs in the edible portions of fish are quite important for human health. The amounts (g/100g wet weight) of total n3 PUFAs changed from 0.19 to 0.98 in male and from 0.18 to 0.98 in female (Figure 3). The ratio of EPA (C20:5n3) in the muscles of male *C. antalyensis* significantly decreased to a minimum level in summer (7.10%) and increased to a maximum level in spring (10.03%) ($p < 0.05$). DHA (C22:6n3) was also high (6.62%) in male in winter. DHA content of female *C. antalyensis* was minimum in autumn (4.29%) and maximum in winter (8.09%). The ratios of total EPA and DHA were the highest (13.48%) in autumn and the lowest (9.79%) in summer in male. It was reported that EPA and DHA were the dominant PUFAs in muscle of a lot

fish species such as *Barbus rajonorum*, *Chondrostoma regium*, *Leuciscus lepidus*, *Carasobarbus luteus*, *Liza abu*, *Alburnus mossulensis*, *Cyprinion macrostomus*, *Acanthobrama marmid*, *Silurus triostegus* (Cengiz et al., 2010).

The n3 PUFAs/n6 PUFAs ratio has been suggested as a useful indicator for evaluating the nutritional values of fish oils. An increase of n3 PUFAs/n6 PUFAs ratio in the human diet is essential to help prevent coronary heart diseases and to reduce the risk of cancer (Kinsella et al., 1990). The n3 PUFAs/n6 PUFAs ratio was found to be the highest in spring for muscle tissue of *C. antalyensis* species (5.44). In both genders of *C. antalyensis* species, the n3 PUFAs/n6 PUFAs rate was found to be higher than 3.29 in all seasons. This result about the ratios of n3 PUFAs/n6 PUFAs found in the present study was parallel to the result reported by Geri et al. (1995).

CONCLUSIONS

As a result, it was observed that the total fat composition of muscle tissue of *C. antalyensis* decreased in winter importantly, and that the protein content reached the highest level. In parallel to the decrease of total fat content in winter, total SFAs, UFAs, PUFAs rates were observed to be at the lowest levels. This situation shows that *C. antalyensis* species is quite affected by cold winter conditions.

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