Seasonal variations of ω3 polyunsaturated fatty acids in muscle of *Capoeta caelestis*

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Abstract

In this study, seasonal variations of fatty acid amounts in muscle tissues of *Capoeta caelestis* were investigated. We have found that the ω3 contents of *C. caelestis* in spring, summer, autumn and winter were 17.43%, 15.29%, 17.54%, 13.63% in female and 16.96%, 19.25%, 18.66%, 14.76% in male, respectively. The amounts of ω3 polyunsaturated fatty acids were higher than those of ω6 polyunsaturated fatty acids. The eicosapentaenoic acid amounts were always higher than the docosahexaenoic acid amounts in *C. caelestis*. Female *C. caelestis* presented a higher amount of eicosapentaenoic acid and docosahexaenoic acid than males. The amounts of docosahexaenoic acid to eicosapentaenoic acid varied from 0.44% to 0.56% in females and from 0.45% to 0.59% in males in different seasons. The levels of eicosapentaenoic acid + docosahexaenoic acid in female muscles changed from 10.34% to12.51% and in male muscles from 11.16% to 15.41%, respectively. It was seen that the ω3 polyunsaturated fatty acid amounts of *C. caelestis* reached the highest level in summer and autumn in both sexes. Therefore, it can be concluded that the nutritional value of this fish is better in the both seasons with respect to the ω3 polyunsaturated fatty acid amounts.

Keywords: *Capoeta caelestis*, ω3 fatty acid, Sex, Season.

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Introduction

The consumption of fish oil has increasedin view of the wide range of health benefits it provides (Guerrero *et al*., 2011; Güler *et al*., 2007; Lovegrove *et al*., 1997) because fish and fish oils contain omega 3 polyunsaturated fatty acids (ω3 PUFAs); in particular, eicosapentaenoic acid (C20:5 ω3; EPA) and docosahexaenoic acid (C22:6ω3; DHA) (Huynh *et al*., 2007; Ayas, 2012; Taheri *et al*., 2012). The amount of ω3 PUFAs differs among species and can be influenced by a number of factors such as diet, size, age, reproductive cycle, salinity, temperature, season and geographical location (Görgün and Akpınar, 2007; Le Nechet *et al*., 2007; Mnari *et al*., 2007; Akpınar *et al*., 2009; Gladyshev *et al*., 2009; Inhamuns *et al*., 2009; Usydus *et al*., 2012; Zakipour Rahimabadi and Dad, 2012). Some ω3 and ω6 PUFAs have been regarded as essential fatty acids. ω3 PUFAs cannot be synthesized effectively by humans and therefore they must be obtained by diet (Jankowska *et al*., 2003; Sushchik *et al*., 2007; Akpınar *et al*., 2009).

Increased intakes of fish oil reduces total blood triacylglyceride concentrations and biochemical risk factors associated with cardiovascular diseases, neurodevelopmental diseases in infants, fat glycemic control, arthritis, psoriasis, asthma, inflammatory bowel disease and cancer, and human growth and development (Conner, 2000; Mozaffarian *et al*., 2005; Eilander *et al*., 2007). Fish lipids are a good source of EPA and DHA. Since DHA is a major component of brain, eye retina and heart muscle, DHA has been considered as important for brain and eye development and also good for cardiovascular health (Özoğul *et al*., 2007).

C. caelestis belongs to the *Cyprinidae* family and lives in the spring water flowing into the Göksu River (Schöter *et al*., 2009). *C. caelestis* prefers the benthopelagic zone of subtropical waters. No report has been found concerning the fatty acid composition of this fish. Therefore, the aim of this study was to determine the seasonal and sexual variations in the fatty acid compositions of *C. caelestis.*

Material and methods

The specimens of *C. caelestis* used in this experiment were caught from spring waters such as Dedemli, Balkusansu in Karaman (Turkey). Mean weights and lengths of the representative fish were 53,37±13,29 g and 15,90±1,54 cm in females, 44,62±7,88 g and 16,00±0,99 cm in males, respectively. A total of 40 fish (n=5 at each determination) were used in experiments. The skinless muscle specimens from each sex were taken and homogenized in a warring blender.

Total lipid extraction

Total lipid extraction was performed according to the method of Bligh and Dyer (1959). Methyl esters were prepared by transmethylation using 2M KOH in methanol and hexane according to the method of Ichihara *et al.* (1996). Extracted lipids (10 mg) were dissolved

in 2 ml hexane and followed by 4 ml of 2 M methanolic KOH. After centrifugation at 4000 rpm for 10 min, the hexane layer was taken for GC analyses application.

Gas chromatographic analyses

The fatty acid profiles were analyzed by GC with a flame ionization detector and a fused silica capillary column. The oven temperature was kept at 140°C for 5 min, raised to 200°C at rate of 4°C/min and to 220°C at a rate of 1°C/min, while the injector and the detector temperature were set at 220°C and 280°C, respectively. The carrier gas was controlled at 16 psi. The split used was 1:40. Fatty acids were identified by comparing the retention times of fatty acid methyl ester mixture.

Results

Seasonal variations of the fatty acids in muscle of females and males of *C. caelestis* are given in Tables 1 and 2.

Table 1: Seasonal variation of fatty acid amounts in muscle of female *Capoeta caelestis* (% of total fatty acids).

Fatty Acids	Spring Mean±SE	Summer Mean±SE	Autumn Mean±SE	Winter Mean±SE
C12:0	0.38±0.05	0.31±0.03	0.21±0.05	0.10 ±0.01
C13:0	0.05 ±0.00	0.03 ±0.01	-	0.04 ±0.00
C14:0	3.16±0.22	3.11±0.31	3.14±0.05	5.44±0.27
C14:1	0.13±0.00	0.09±0.02	0.22±0.00	0.26±0.02
C15:0	0.35±0.03	0.35±0.02	0.29±0.00	0.58±0.05
C16:0	15.41±0.37	19.51±0.49	17.27±0.00	17.29±0.31
C16:1	12.20±0.64	15.02±1.19	11.89±0.03	13.61±0.67
C17:0	0.53±0.05	0.48±0.03	0.40±0.00	0.29±0.01
C17:1	0.50±0.08	0.32±0.05	0.34±0.01	0.26±0.02
C18:0	3.16±0.05	4.09±0.42	3.31±0.01	2.55±0.32
C18:1ω9	13.22±1.62	14.48±2.32	17.95±0.04	15.30±0.83
C18:1ω7	6.68±0.72	5.89±0.11	7.68±0.00	5.76±0.39
C18:2ω6	4.25±0,25	3.27±0.23	3.81±0.01	5.10±0.76
C18:3ω3	4.93±0,81	3.42±0.53	5.77±0.02	3.29±0.23
C20:0	0.24±0,02	0.21±0.01	0.23±0.00	0.15±0.01
C20:1	1.25±0,23	0.91±0.09	1.28±0.01	1.05±0.09
C20:3ω6	0.31±0,00	0.30±0.05	0.18±0.01	0.19±0.03
C20:4ω6	0.19±0.02	0.21±0.02	0,12±0.00	0.22±0.03
C20:2ω6	1.31±0.02	1.38±0.15	1.40±0.00	2.86±0.35
C20:5ω3	8.89±1.00	8.08±0.46	7.99±0.04	6.55±0.78
C22:1ω9	0.07±0.01	-	-	0.07±0.00
C21:0	0.44±0.10	0.34±0.14	0.42±0.01	0.19±0.01
Unidentified fatty acids	18.79±0.59	14.40±0.76	12.35±0.07	15.09±0.37
∑ SFA	23.71±0.28	28.43±0.79	25.26±0.00	26.61 ±0.47
∑ PUFA	23.48 ±0.25	20.45±0.88	23.04 ±0.05	22.00 ±1.49
∑UFA	57.50±0.32	57.17±1.23	62.39±0.07	58.29±0.42
PUFA/SFA	0.99 ±0.02	0.72±0.02	0.91±0.00	0.83 ±0.06
∑ ω3 PUFA	17.43 ±0.62	15.29 ±0.92	17.54 ±0.05	13.63 ±1.68
∑ ω6 PUFA	6.05 ±0.24	5.17 ±0.18	5.51 ±0.05	8.37 ±0.56
ω6/ω3 PUFA	0.35 ±0.01	0.34 ±0.03	0.31 ±0.01	0.66 ±0.11
EPA+DHA	12.51±0.83	11.65±1.33	12.27±0.54	10.34±1.59

Table 2: Seasonal variation of fatty acid amounts in muscle of male *Capoeta caelestis* (% of total fatty acids).

Fatty Acids	Spring Mean±SE	Summer Mean±SE	Autumn Mean±SE	Winter Mean±SE
C12:0	0.70±0.13	0.30±0.08	0.24±0.01	0.13±0.02
C13:0	0.06±0.00	0.02±0,.00	0.05±0.01	0.06±0.00
C14:0	3.25±0.21	2.61±0.26	3.37±0.12	4.40±0.32
C14:1	0.16±0.02	0.07±0.01	0.18±0.02	0.21±0.03
C15:0	0.32±0.01	0.41±0.06	0.53±0.08	0.56±0.04
C16:0	14.73±0.36	21.19±0.87	16.04±0.52	15.93±0.53
C16:1	11.90±0.44	15.06±1.20	11.90±1.17	11.60±1.12
C17:0	0.48±0.05	0.56±0.05	0.55±0.04	0.37±0.06
C17:1	0.46±0.01	0.57±0.23	0.61±0.08	0.28±0.02
C18:0	3.25±0.17	5.10±0.46	3.37±0.09	2.70±0.18
C18:1ω9	14.30±0.89	10.32±0.86	14.55±1.77	15.92±1.44
C18:1ω7	5.75±0.02	5.51±0.63	6.51±0.39	5.39±0.20
C18:2ω6	5.24±0.46	2.66±0.50	4.06±0.68	5.59±0.35
C18:3ω3	4.81±0.26	3.68±1.06	7.23±1.71	3.60±0.44
C20:0	0.23±0.01	0.27±0.02	0.23±0.02	0.18±0.02
C20:1	1.37±0.07	0.95±0.17	1.21±0.13	1.10±0.04
C20:3ω6	0.40±0.03	0.22±0.03	0.19±0.03	0.24±0.02
C20:4ω6	0.25±0.02	0.18±0.04	0.19±0.03	0.30±0.02
C20:2ω6	1.46±0.13	1.89±0.27	1.52±0.18	2.90±0.34
C20:5ω3	8.36±0.27	10.09±0.61	7.82±1.18	7.03±0.78
C22:1ω9	0.07±0.00	-	0.09±0.01	0.06±0.00
C21:0	0.37±0.04	0.32±0.05	0.57±0.13	0.27±006
Unidentified fatty acids	18.29±0.53	12.57±0.77	15.34±0.80	17.05±0.61
∑ SFA	23.41±0.33	30.76±1.01	24.94±0.40	24.59 ±0.69
∑ PUFA	24.31 ±0.74	24.19±0.85	24.62 ±1.39	23.78 ±1.69
∑UFA	58.30±0.78	56.67±0.42	59.72±1.09	58.35±1.01
PUFA/SFA	1.03 ±0.03	0.79 ±0.01	0.99 ±0.07	0.97 ±0.07
∑ ω3 PUFA	16.96 ±0.75	19.25 ±1.05	18.66 ±1.85	14.76±1.71
∑ ω6 PUFA	7.35 ±0.36	4.95 ±0.24	5.96 ±0.82	9.01 ±0.23
ω6/ω3 PUFA	0.44 ±0.03	0.26 ±0.03	0.36 ±0.09	0.65±0.08
EPA+DHA	12.15±0.50	15.41±1.27	11.41±1.66	11.16±1.29

Twenty-three fatty acids were identified from the muscle of males and females. The maximum and minimum amounts of total lipid and protein in muscle ranged from 10.36% and 64.73% to 20.98% and 79.18% by seasonal and sexual variations, respectively. Total lipid content was maximum in winter and minimum in summer. Contrary to the lipid amount, protein content was also maximum in summer and minimum in winter. In this study, palmitic acid (C16:0) was found to be the major saturated fatty acid (SFA) in both sexes. Stearic acid (C18:0) and myristic acid (C14:0) levels were also the mostly found saturated fatty acids in the muscles of both sexes of *C. caelestis*. Oleic acid (C18:1ω9) was also identified as the major

monounsaturated fatty acid (MUFA) in the muscle of both sexes.

In the present study, the highest level of EPA (C20:5ω3) was found in the ω3 PUFA fraction in the muscle of females and males. Male muscles had substantial levels of DHA with a value of 10.09% in summer. The ratio of EPA in the muscles of males decreased to minimum level in winter (7.03%) and increased to maximum in summer (10.09%). EPA was also high (8.89%) in females in spring.

Discussion

As seen in Tables 1 and 2, palmitic acid (C16:0) was the major saturated fatty acid (SFA) in both sexes of *C. caelestis*. The amount of palmitic acid was determined in the range of 15.41%-19.51% and 14.73%-21.19% in males and females, respectively. The palmitic acid levels were significantly highest in summer (p<0.05) in both sexes. Similar results were reported by Jankowska *et al*. (2003) and Güler *et al*. (2007) for *Sander lucioperca*, Haliloglu *et al*. (2004) for *Oncorhynchus mykiss*, Ibarz *et al*. (2007) for *Sparus aurata*, Inhamuns *et al*. (2009) for *Cichla ocellaris*, Pacetti *et al*. (2010) for *Merluccius merluccius, Trachurus trachurus, Solea solea, Engralius encrasicolus, Scomber scombrus,* and *Sardina pilchardus.*

Myristic acid content in the muscle of female *C. caelestis* was the maximum while stearic acid content was at the minimum in winter. The levels of myristic acid were significantly different between females (5.44%) and males (4.40%) in winter. As can be seen in Tables 1 and 2, significant differences were determined between the ratios of palmitic, myristic, and stearic acids in muscles of males and females. According to Luczynska *et al*. (2008), the most abundant saturated fatty acids in freshwater fish were palmitic and stearic acid which are in good agreement with the present findings.

Oleic acid (C18:1ω9) was identified as the major monounsaturated fatty acid (MUFA) in muscles of both sexes. Oleic acid content of females was highest in autumn (17.95%) and lowest in spring (13.22%) and in males it was the minimum in summer (10.32%) and the maximum in winter (15.92%). Huynh *et al*. (2007) reported that oleic acid was the most abundant MUFA in herring (23.48%), but was minimal in capelin (4.97%) and sardine (4.16%). Güler *et al*. (2007) also reported that oleic acid in muscle tissues of zander changed from 8.30% to 11.90% in different seasons. Vaccenic acid (C18:1ω7) was found at high levels in the MUFA fraction changing from 5.76% to 7.68% in females and from 5.39% to 6.51% in males. Palmitoleic acid (C16:1) was another notable fatty acid in the MUFA fraction. This acid decreased to its lowest level in autumn and reached the highest level in summer in both sexes (Tables 1 and 2).

The level of total PUFAs of *C. caelestis* changed from 20.45% to 23.48% in females and from 23.78% to 24.62% in males. ω3 PUFAs content of females was the minimum in winter

(13.63%) and the maximum in autumn (17.54%). ω3 PUFAs levels of males were the highest in summer (19.25%) and the lowest in winter (14.76%). It was reported that the ratio of ω3 PUFAs in total fatty acids was 38.70% in common sole and 51.20% in European Anchovy (Pacetti *et al*., 2010). Linoleic acid (C18:2ω6) was significantly low (2.66%) in summer in males. This fatty acid was also at its highest level in winter both in females and males. The level of alfa-linoleic acid (C18:3ω3) in muscles of *C. caelestis* changed from 3.29% to 5.77% in females and from 3.60% to 7.23% in males (Tables 1 and 2).

In this study, ω3 PUFAs EPA amounts were the highest in the muscles of both sexes. The EPA amount in the muscles of males decreased to a minimum level in winter and increased to a maximum in summer (Table 1 and 2). DHA (C22:6ω3) was the second highest amount of fatty acid among the ω3 PUFAs in both sexes. The levels of DHA changed from 3.62% to 3.79% in females and from 3.61% to 5.48% in males. It was reported that EPA and DHA are important structural components of the membranes (Bezard *et al*., 1994). The ratios of EPA and DHA sums were highest (12.51%) in spring and lowest (10.34%) in winter in females; highest (15.41%) in summer and lowest (11.16%) in winter in males. According to some reports, EPA and DHA were the dominant PUFAs in the muscles of a lot of fish species such as *Gadus morhua* (Bell and Dick, 1991), *Psetta maxima* (Regost *et al*., 2003), *Abramis brama*, *Tinca tinca*, *Perca fluviatilis* and *Lota lota* (Ahlgren *et al*., 1994). It was reported that EPA+DHA deficiencies cause coronary heart disease (Kris-Etherton *et al*., 2002), Crohn's disease, multiple sclerosis, lupus erythematosus, psoriasis (Simopoulos, 2002) and cardiovascular diseases (Larsen *et al*., 2011). Because of the relatively high level of ω3 PUFAs, especially EPA and DHA, it may be said that *C. caelestis* is a healthy food for human.

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