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PROOF

Seasonal Variations of Fatty Acid Profiles in the Muscle of *Capoeta angorae*

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Abstract

In this study, the total lipid, total protein and fatty acid compositions of *Capoetaangorae* were investigated. Total lipid content ratios (as wet weight) were found to be as 1.44% in spring, 6.41% in summer, 4.25% in autumn and 4.29% in winter. Total protein ratios in the muscle of *C. angorae* also varied from16.55% to19.24% during the year. The major fatty acids in the muscles of both sexes were palmitic, oleic, palmitoleic, eicosapentaenoic and docosahexaenoicacids. The proportions of n3 PUFAs were higher than those of n6 PUFAs through the year. The ratios of total n6 PUFAs to n3 PUFAs in the muscle of *C. angorae* changed from 0.14 to 0.19 in female and from 0.12 to 0.31 in males, respectively. The levels of EPA+DHA in the muscles of both genders changed from 0.19 to 0.91g/100g wet weight. The results showed that the ratios of total lipid and fatty acids in the muscle of *C. angorae* significantly varied by the year.

Keywords: *Capoeta angorae*, fatty acids, season, nutritional value

***Capoeta angorae* Türünün Kas Dokusu Yağ Asidi Profillerinin Mevsimsel Değişimleri**

Özet

Bu çalışmada *Capoeta angorae* türünün toplam lipid, toplam protein ve yağ asidi bileşimi incelenmiştir. Toplam yağ içerikleri (yaş ağırlık olarak) ilkbaharda %1,44, yazın %6,41, sonbaharda %4,25 ve kışın %4,29 oranlarında bulunmuştur. *C. angorae* türünün kas dokusundaki toplam protein oranları ise yıl içerisinde %16,55 ile %19,25 arası değişmiştir. Her iki cinsiyetin kas dokusundaki en çok bulunan yağ asitleri palmitik, oleik, palmitoleik, ökosapentaenoik ve dokosaheksaenoik asitlerdir. Yıl boyunca n3 PUFA oranları n6 PUFA oranlarından daha yüksek bulunmuştur. *C. angorae* türünün kas dokusundaki toplam n6 PUFA değerlerinin n3 PUFA değerlerine oranı sırasıyla dişilerde 0,14 ile 0,19, erkeklerde 0,12 ile 0,31 arası değişmiştir. Her iki cinsiyetin kas dokusundaki EPA+DHA seviyeleri 0,19 ile 0,91 g/100g yaş ağırlık arasında değişmiştir. Elde edilen sonuçlar, *C. angorae* türünün kas dokusundaki toplam lipid ve yağ asitleri oranlarının yıl içerisinde önemli derecede değiştiğini göstermiştir.

Anahtar Kelimeler: *Capoeta angorae*, yağ asitleri, mevsim, besin değeri.

Introduction

Fish is one of the major food sources for humans in terms of providing a significant amount of the animal protein in diet (Martinez *et al*., 2012; Katselis, 2007). When compared to red meat, fish meat is easily digestible (Njinkoue *et al*., 2002). Fish is also used as a raw material in food, pharmaceutical, chemical, agricultural and aquaculture industries (Diraman and Dibeklioğlu, 2009). Some nutritional components in fish such as fatty acids and trace elements also have therapeutic effects towards to the prevention of particular diseases (Güner *et al*., 1998). Fatty acids are important for cell growth, differentiation, metabolism and protection against stress (Villenueve *et al*., 2005). The significance of the long chain polyunsaturated fatty acids (PUFAs) has gained attention lately because of their involvement in prevention of human coronary arterial disease (Balk *et al*., 2006; Harris, 2007), visual function (Harris, 2007), cardiovascular diseases (Iso *et al*., 2001; Marichamy *et al*., 2012), improvement of retina and brain development and also decreased incidence of breast cancer, rheumatoid arthritis, multiple scelerosis, psoriasis and inflammation (Ozoğul *et al.*,2007).

The long chain PUFAs, especially eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) are mostly found in fish. Besides, the fatty acid composition of fish also varies according to the season, diet and feeding, sex, and the state of their reproductive cycle. Therefore, the positive health effect of fish with regard to the n3 polyunsaturated fatty acids (n3 PUFAs) varies with these factors (Shirai *et al*., 2001; Shirai *et al*., 2002).

Capoeta angorae is a freshwater fish species found in Ceyhan River drainage. It lives in benthopelagic zone of subtropical water. Currently, the effect of seasonal changes on the fatty acid composition of *C.angorae* is not described in the available literature. Therefore the aim of this study is to determine the seasonal variations of muscle fatty acid composition of *C. angorae.*

Materials and Methods

The *Capoeta angorae* used for this research were captured from the Fırnız Stream of Ceyhan River, Turkey. After the total weight and length measurements, the fish specimens were transported to the laboratory on ice. The mean weight and mean length of the fishes were 88.44±10.08 g and 21.3±0.9cm in female; 73.00±6.04 g and 20.9±0.8cm in male, respectively. 40 fish (5 male and 5 female fish at each season) were used in experiments. The dorsal muscle specimens of the fishes were excised and homogenized in a warring blender for lipid extraction. Total lipid extraction was performed according to the procedure of Bligh and Dyer method (Bligh and Dyer, 1959).Methyl esters were prepared by trans methylation using 2M KOH in methanol and hexane according to the method as described by Ichihara *et.al*. (1996). Extracted lipids were dissolved in 2 ml hexane followed by 4 ml of 2 M methanolic KOH. The lipid extractin the test tubes were then vortexed at room temperature. After centrifugation, the hexane layer was taken for analyses.

The fatty acids were analyzed by gas chromatography (GC) with auto sampler (Thermo Scientific Focus) equipped with a flame ionization detector and a fused silica capillary column (30m x 0.32mm, ID x 0.25 µm film). The oven temperature was 140 ^{o}C, held 5 min, raised to 200°C at rate of 4°C/min and to 220°C at a rate of 1 ^{o}C/min, while the injector and the detector temperature were set at 220°C and 280°C, respectively. Fatty acids were identified by comparing the retention times of fatty acid methyl esters (FAME) mixture (SUPELCO). The GC data were expressed as percent ratios of total fatty acids. Also the amounts of total SFAs, UFAs, PUFAs, n3 PUFAs, n6 PUFAs and EPA+DHA in both gender were calculated as the light of the following formulae indicated in Weihrauch *et al.* (1977), Tufan *et al.* (2014) and Balçık-Mısır *et al.* (2014):

FA content (g FA per 100 g edible fish muscle) = (FAME% x FACF x lipid content%) / 100,

where FAME is fatty acid methyl esters, FACF: the lipid conversion factor (fatty acid conversion factor, gFA/gram lipid). FACF is reported as 0.90 for fatty fish muscle (Greenfield and Southgate, 2003), however, Weihrauch *et al.* (1977) suggest that if the fat content of fish is below 5%, the following formulae must be used to calculate FACF in fish muscle: 0:933 – (0.143 /TL); where TL refers to total lipid of fish muscle.

The data of the present study were statistically analyzed by using the SPSS Package Programme, version 12. The relations between seasonal variations and fatty acid profiles were determined by the method of One-Way ANOVA followed Tukey's multiple comparison test. The comparisons among sexes were analyzed by student's t test. The results were considered as significant at $P<0.05$.

Results and Discussion

Total protein and lipid contents (% of the wet weight) of *Capoeta angorae* have changed with seasons from 16.55 to 19.24 and from 1.44 to 6.41, respectively. Total lipid level was highest in summer and lowest in spring, while total protein level was highest in spring and the lowest in summer (Figure. 1). High lipid level in summer and low lipid level in winter have also been noted for a variety of fish (Dal Bosco *et al*., 2012; Gökçe *et al*., 2004). It was also reported that total lipid content of fish was affected by the factors such as species, sex, age, water temperature, nutritional condition (Bandarra *et al*., 1997; Gills and Weatherley, 1984; Skuladottir *et al*., 1990; Uysal *et al*., 2011).

Seasonal changes in muscle fatty acid compositions of *C. angorae* in both sexes are shown in Table 1 and 2.Total SFAs levels (% of total fatty acids) of male and female were highest in summer and lowest in winter. However, the change in the amounts of total SFAs in the muscle of *C. angorae* in summer was statistically important in male ($P<0.05$), but not important in female ($P>0.05$) as shown in Tables 1 and 2. On the contrary, the amounts of SFAs of some fishes were not seasonally affected (Bulut*et al*., 2012).

The ratios of palmitic acid (C16:0) in the muscle of both male and female *C. angorae* were highest among the SFAs as shown in Tables 1 and 2. It was also reported that palmitic acid was found to be the major SFA in *Epinephelus aeneus, Cephalopholis taeniops, Serranus scriba* (Louly *et al*., 2011), and in *Thymallus arcticus* (Sushchik *et al*., 2007). In this study, the levels of myristic (C14:0) and pentadecanoic acids (C15:0) in the muscle of male *C. angorae* were higher in winter compared to the other seasons (Table 2). Myristic acid content of female was high in springand low in autumn (Table 1). Also, the lauric acid (C12:0) and pentadecanoic acid contents in the muscle of female *C. angorae* were increased in summer. Stearic acid (C18:0) content of female was high in autumnand low in winter.The muscular stearic acid content of male *C. angorae* was increased in summer, decreased in winter (Table 1).It was reported that the predominant SFAs were palmitic, stearic and myristic acidsin *Trachurus trachurus* (Rittenschober *et al*., 2013). Palmitic, myristicand stearic acids contents in the fillets of Atlantic blue fin tuna, crevalle jack and Atlantic Spanish mackerel were also changed by seasons from 61.25% to 27.80%, from 48.68% to 31.17% , and from 26.60% to 10.08%, respectively (Martinez *et al*., 2012). However, our data show that palmitic, myristic and stearic acid contents of *C. angorae* were seasonally changed from 16.05 to18.16%, from 1.84 to 4.66%, and from 2.30 to 5.48%, respectively.

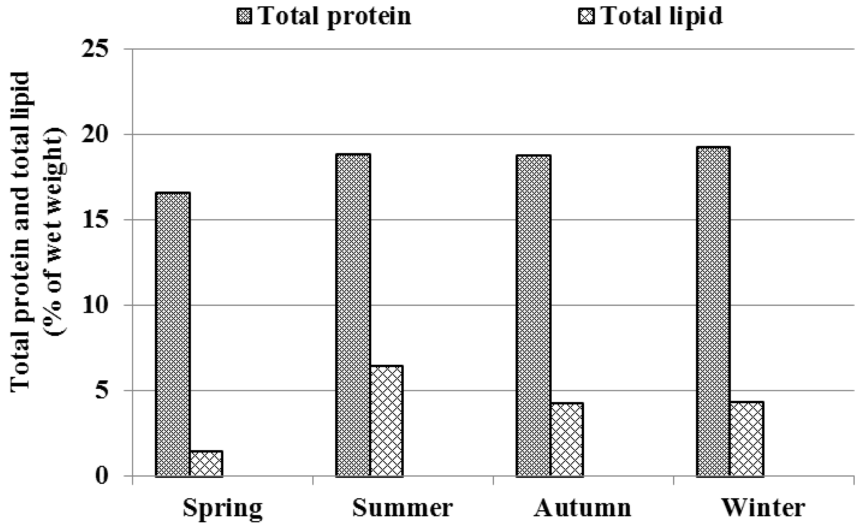

Figure 1. Seasonal variations of mean total protein and total lipid in muscle of *C. angorae.*

Table 1. Seasonal variations of fatty acid ratios in the muscle of female *C.angorae*(as % of total fatty acids)

Fatty Acids	Spring Mean±SEM	Summer Mean±SEM	Autumn Mean±SEM	Winter Mean±SEM
C12:0	0.15±0.02	0.42±0.13	0.15±0.03	0.20 ±0.04
C14:0	4.66±0.22	4.04±0.47	1.84±0.27	3.86±0.29
C15:0	0.36±0.03	0.60±0.15	0.32±0.04	0.24±0.01
C16:0	16.76±0.43	17.15±0.45	16.96±1.13	16.34±0.39
C17:0	0.22±0.03	0.81±0.17	0.73±0.11	0.36±0.04
C18:0	3.15±0.11	2.99±0.25	5.48±1.11	2.85±0.17
C20:0	0.21±0.01	0.20±0.02	0.26±0.04	0.19±0.02
C21:0	0.31±0.04	0.40±0.12	0.68±0.15	0.77±0.06
$\bar{x}$ SFA	25.75±0.46	26.69±0.97	26.27±1.79	24.81 ±0.45
C14:1	0.10±0.01	0.12±0.01	0.11±0.04	0.12±0.01
C16:1	15.87±0.48^{b}	13.36±1.32^{bc}	7.30±1.05^{a}	12.16±0.82^{c}
C17:1	0.66±0.10	0.84±0.25	0.58±0.18	1.75±0.20
C18:1n9	7.75±0.33	10.67±0.86	12.07±2.74	8.67±0.65
C18:1n7	4.74±0.17	5.77±1.08	4.32±0.26	4.45±0.14
C20:1	0.99±0.07	1.19±0.09	1.45±0.19	1.12±0.09
C22:1n9	0.13±0.02	0.10±0.02	0.24±0.04	0.19±0.02
$\bar{x}$ MUFA	30.11 ±0.68	32.08 ±2.80	25.92 ±3.28	28.34 ±1.12
C18:2n6	1.93±0.05	2.63±0.49	3.48±0.85	2.11±0.09
C18:3n3	3.22±0.27	4.39±1.53	4.90±1.51	6.90±0.45
C20:2n6	1.29±0.06	1.24±0.17	3.53±1.42	0.79±0.07
C20:3n6	0.11±0.01	0.14±0.05	0.28±0.09	0.08±0.01
C20:4n6	0.12±0.01	0.12±0.01	0.14±0.03	0.09±0.01
C20:5n3	11.35±0.19^{b}	6.79±1.19^{a}	8.59±1.52^{ab}	10.52±0.6^{ab}
C22:6n3	6.13±0.2^{a}	6.67±0.49^{a}	11.76±2.13^{b}	6.73±0.51^{a}
$\bar{x}$ PUFA	24.03 ±0.31^{a}	21.94±2.63^{a}	32.63 ±1.98^{b}	27.18 ±0.77^{ab}
Unidentified fatty acids	20.11±0.79	19.29±1.33	15.18±1.49	19.66±0.61

* Means labeled by the same lettershow no statistical differences (P <0.05).

Table 2. Seasonal variations of fatty acid ratios in the muscle of male *C.angorae*(as % of total fatty acids)

Fatty Acids	Spring Mean±SEM	Summer Mean±SEM	Autumn Mean±SEM	Winter Mean±SEM
C12:0	0.22±0.06	0.13±0.02	0.12±0.03	0.34±0.02
C14:0	4.27±0.47	2.92±0.25	3.04±0.10	4.31±0.29
C15:0	0.62±0.24	0.27±0.04	0.25±0.02	0.23±0.01
C16:0	16.24±0.65[a]	17.61±0.66[a]	18.16±0.28[b]	16.05±0.31[a]
C17:0	0.35±0.06	0.62±0.08	0.34±0.04	0.29±0.02
C18:0	3.21±0.52	4.51±0.27	3.30±0.27	2.30±0.12
C20:0	0.17±0.02	0.18±0.01	0.18±0.01	0.17±0.01
C21:0	0.48±0.09	0.78±0.13	0.61±0.04	0.78±0.07
$\bar{x}$ SFA	25.50±0.66[ab]	27.02±0.56[b]	25.88±0.21[ab]	24.48 ±0.23[a]
C14:1	0.14±0.01	0.11±0.03	0.13±0.02	0.16±0.03
C16:1	14.91±1.68	10.81±1.32	11.42±0.70	12.41±0.42
C17:1	1.12±0.24	0,.72±0.14	1.01±0.10	1.90±0.26
C18:1n9	9.16±0.63[a]	12.09±1.08[ab]	14.40±0.90[b]	9.41±0.47[a]
C18:1n7	4.89±0.39	4.62±0.13	4.46±0.17	4.18±0.12
C20:1	1.28±0.13	1.57±0.06	1.74±0.12	1.25±0.08
C22:1n9	0.15±0.00	0.15±0.00	0.16±0.03	0.15±0.01
$\bar{x}$ MUFA	31.49 ±1.94	29.88 ±2.17	33.37 ±1.37	29.47 ±0.87
C18:2n6	2.16±0.14	2.30±0.24	2.50±0.21	2.27±0.17
C18:3n3	4.68±0.96	6.02±1.09	6.65±0.34	7.59±0.48
C20:2n6	1.47±0.17	1.51±0.20	1.14±0.22	0.55±0.04
C20:3n6	0.14±0.01	0.22±0.08	0.11±0.03	0.09±0.01
C20:4n6	0.14±0.01	0.14±0.01	0.27±0.17	0.09±0.01
C20:5n3	9.23±0.56	8.05±0.65	8.61±0.57	10.17±0.48
C22:6n3	6.59±1.01[ab]	7.73±0.74[b]	5.52±1.00[ab]	4.35±0.31[a]
$\bar{x}$ PUFA	24.35 ±1.62	25.94±1.88	24.74 ±1.40	25.09 ±0.46
Unidentified fatty acids	18.67±1.37	17.16±0.97	16.00±0.49	20.96±0.42

* Means labeled by the same lettershow no statistical differences (P<0.05).

Although the seasonal changes of all SFAs in the muscle of female *C. angorae* were not statistically important (P>0.05), the amount of palmitic acid in the muscle of male *C. angorae* was remarkably high in autumn (P<0.05). The amounts (g/100g wet weight) of the saturated (SFA) and unsaturated (UFA) fatty acids in the muscle of *C. angorae* were also highest in the summer and lowest in the spring in both genders (Figure 2).

The levels of total monounsaturated fatty acids (MUFAs) were changed from 29.47% to 33.37% in female and from 25.92% to 32.08% in male and it was found that the seasonal differences were not statistically important (Tables 1 and 2).Researchers have reported that the amount of total MUFAs ranged from 12% to 14% in the fillet of Atlantic cod (Gruger *et al*., 1964). In our study, palmitoleic acid (C16:1) content was increased in the autumn, decreased at spring for both genders (Table 1 and 2). The levels of oleic acid (C18:1n9), primary MUFA in the muscles of the male and female *C. angorae,* were significantly dropped to minimum level in spring and increased to maximum in autumn in male only (Tables 1 and 2).It was informed that the MUFAs were mainly composed of oleic and palmitoleic acids in some fish (Martinez *et al*., 2012 and Rittenschober *et al*., 2013). Oleic and palmitoleic acids are also two specific fatty acids for freshwater fish and their amounts in freshwater fish were higher than that of sea fish (Bulut *et al*., 2012; Louly *et al*., 2011).

As shown in Figure 3, total polyunsaturated fatty acids (PUFAs) contents (g/100g wet weight) of male *C. angorae* were high in summer (1.50) and low in spring (0.29). The muscle PUFAs contents of female *C. angorae* were higher in summer and autumn compared to other seasons.The ratio of EPA (C20:5n3), an important PUFA, in the muscle of *C. angorae* was highest in spring and the lowest in summer in female (P<0.05). The EPA ratio of the male fish was also highest in the winter butlowest in the summer (Tables 1 and 2). It was reported that the EPA content varied not to much among some fish species, ranging from 8% to 13% in the lean fishes and from 8% to 11% in the fat fishes (Huynh and Kitts, 2009).

In this study, DHA (C22:6n3) in the muscle of female *C. angorae* was significantly higher in autumn (11.76% of total fatty acids) compared to the other seasons (P<0.05) (Table 1). It was reported that DHA was the major n3 PUFA with the levels of 5–9% for *Cephalopholis taeniops*, 13–17% for *Serranus scriba*, and 10–16% for *Epinephelus aeneu*s (Louly *et al*., 2011). Marichamy *et al*. (2012) also reported that the levels of EPA and DHA in the muscle of *Congresox talabon, Arius dussumieri, Dussumieria acuta and Opisthopterus tardoore* were changed from 5.43 to 12.35% and from 5.92 to 12.56%, respectively. Our findings of the ratios of EPA and DHA in the muscle

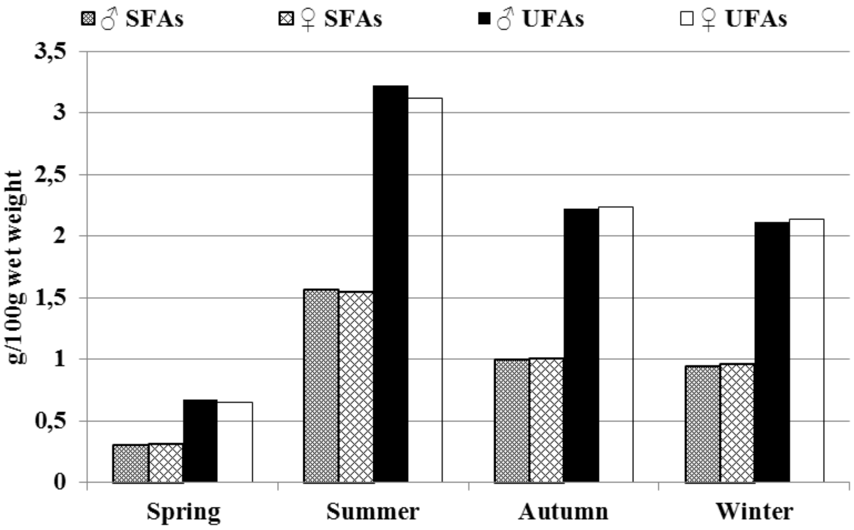

Figure 2. Seasonal variations in the ratios of total SFAs and UFAs in the muscle of *C. angorae.*

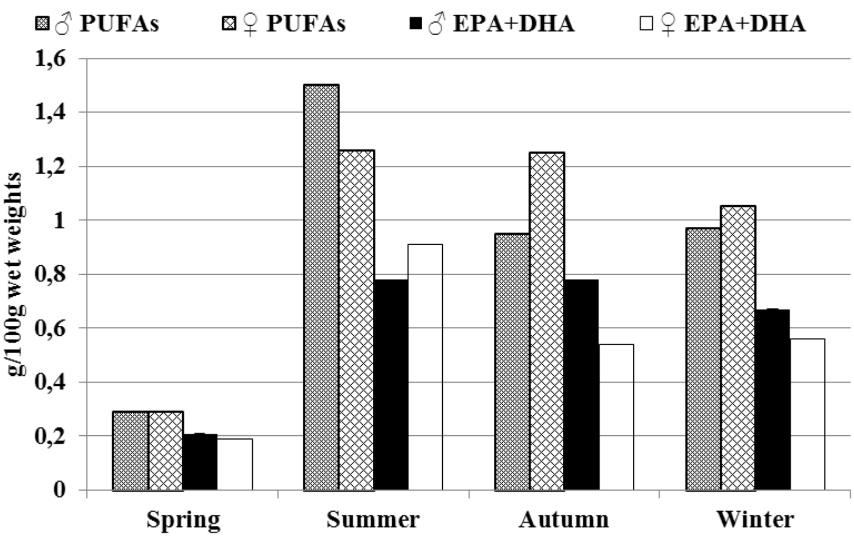

Figure 3. Seasonal variations in the ratios of total PUFAs and EPA+DHA in the muscle of *C. angorae.*

of *C. angorae* were similar to the findings in the literature. In addition, the amount (0.91g/100g wet weight) of EPA+DHA in the muscle of *C. angorae* reached the maximum level in summer (Figure 3).

The present study demonstrated that the amounts (g/100g as wet weights) of n3 PUFAs in the muscle of *C. angorae* ranged from 0.24 to 1.26. The amounts of n6 PUFAs also ranged from 0.04 to 0.28 during the year (Figure 4). Murillo *et al*. (2014) indicated that the amounts of total n3 and n6 PUFAs in 100 g wet weights for four Pacific native fishes ranged from 388 mg to 1007 mg and from 67 mg to 124 mg, respectively. It was informed that the amonuts of n3 PUFAs of fish are generally higher than the amount of n6 PUFAs and this characteristic of fish meat is very importantas a healthy nutritionin human diet. The amonuts of n3 PUFAs in the muscle of *C. angorae* were also higher than the amonuts of n6 PUFAs for four seasons (Figure 4).

Conclusion

The present study demonstrates that *C. angorae* is a good source of polyunsaturated fatty acids during the whole seasons. However, it can be said that *C. angorae* captured in the months of summer is healthier than other seasons due to its higher amounts (g/100 g wet weight) of n3 PUFAs (Figure 4).

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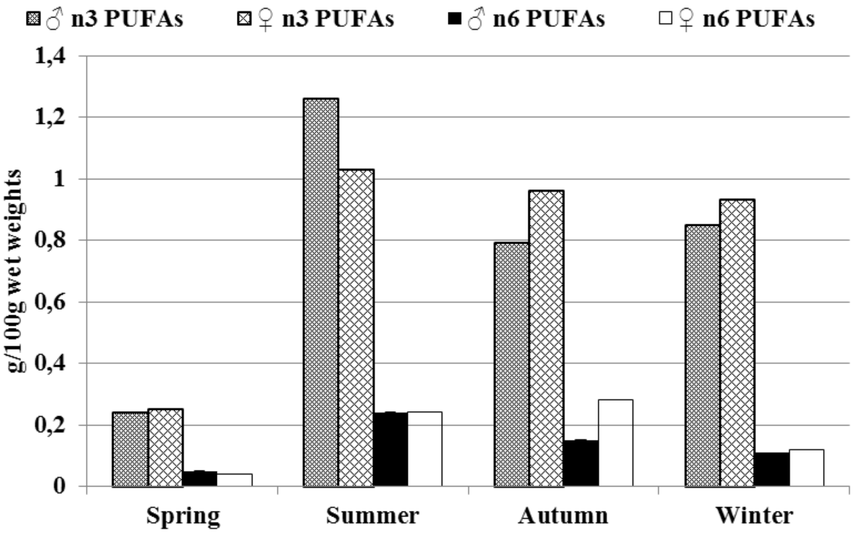

Figure 4. Seasonal variations in the amounts of total n3 and n6 PUFAs in the muscle of *C. angorae.*

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