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The effects of season on fatty acid composition and ω3/ω6 ratios of northern pike (*Esox lucius* L., 1758) muscle lipids

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Abstract In the present study, the effects of season on fatty acid composition, total lipids, and ω3/ω6 ratios of northern pike muscle lipids in Kızılırmak River (Kırıkkale, Turkey) were investigated. A total of 35 different fatty acids were determined in gas chromatography. Among these, palmitic, oleic, and palmitoleic acids had the highest proportion. The main polyunsaturated fatty acids (PUFAs) were found to be docosahexaenoic acid, eicosapentaenoic acid, and arachidonic acid. There were more PUFAs than monounsaturated fatty acids (MUFA) in all seasons. Similarly, the percentages of ω3 fatty acids were higher than those of total ω6 fatty acids in the fatty acid composition. ω3/ω6 ratios were calculated as 1.53, 1.32, 1.97, and 1.71 in spring, summer, autumn and winter, respectively. Overall, we found that the fatty acid composition and ω3/ω6 fatty acid ratio in the muscle of northern pike were significantly influenced by season.

Keyword: freshwater fish; fatty acid composition; omega-3 PUFA; lipid content; season

1 INTRODUCTION

Esox lucius L. (northern pike) is one of important freshwater piscivorous fishes. It feeds on a wide variety of prey from invertebrates to fishes (Craig, 2008). Northern pike are distributed throughout the northern hemisphere. In addition, it is the most widely distributed member of the Esocidae family (Lenhardt and Cakıc, 2002; Vine et al., 2005). It generally inhabits in lakes, but also in rivers and streams. This species is a commonly caught fish because of its taste and importance in sport fishing. Northern pike prefer intermediate vegetation densities, and clean and calm waters, which provide better visibility that allows for more efficient prey capture.

Freshwater fish are an important source of high-quality protein, minerals, vitamin D, and essential fatty acids such as ω3 (Médale et al., 2003). All fats of fish species contain polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA; C20:6 ω3), docosahexaenoic acid (DHA; C22:6 ω3), α-linolenic acid (ALA; 18:3 ω3) and linoleic acid (LOA; 18:2 ω6). There are essential dietary nutrients in all vertebrates because these compounds cannot be synthesized de novo and, hence, must be obtained from the diet (Tocher, 2010).

Long chain ω3 PUFAs have been associated with numerous health benefits. These include providing tolerance to low water temperature in fish (Bolgova et al., 1983; Dominic and Castell, 1999); maintaining the integrity of membranes of all living cells; regulating body cholesterol metabolism (Connor, 2000); improving learning ability (Yonekubo et al., 1992) and visual function (Birch et al., 2000); and therapeutic effects on human physiology (Ackman, 1989) including reducing supplementation suicidality (Paudel-Tandukor et al., 2011) and supporting cardiovascular benefits (Mozaffarian and Wu, 2012).

DHA has a crucial role maintaining the structure and function of the retina and brain cell membranes (Lauritzen et al., 2001). EPA is responsible for a wide range of physiological actions such as blood clotting, the immune response, the inflammatory response,

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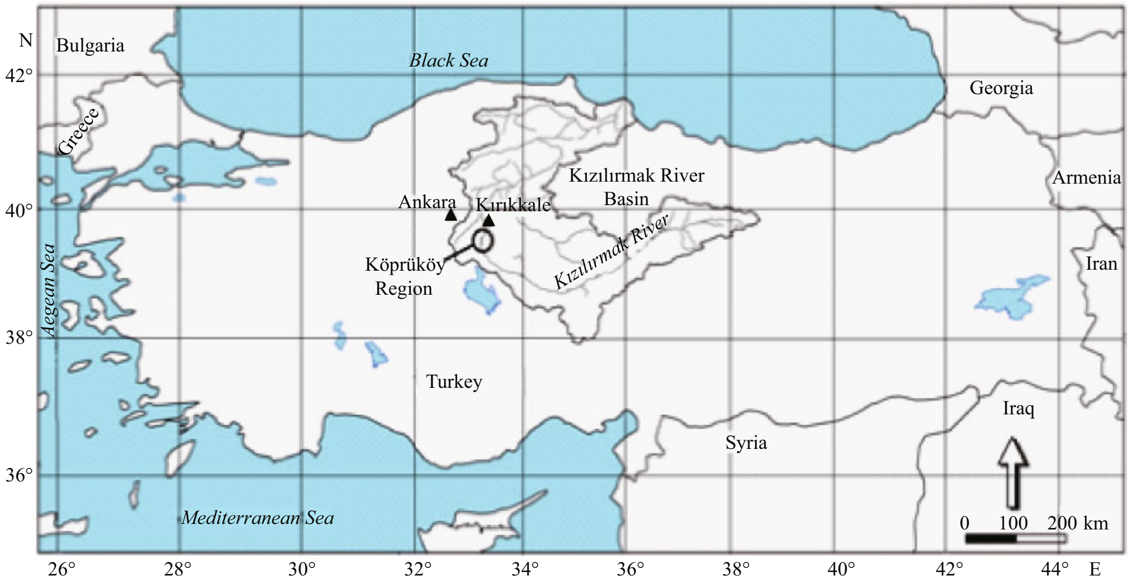

Fig.1 Map of the capture region (Köprüköy Region-Kızılırmak River)

cardiovascular tone, renal and neural function, and reproduction. Very long chain and polyunsaturated fatty acids such as EPA and DHA are abundant in fish, shellfish, and sea mammals and are synthesized by phytoplankton (Connor, 2000).

Omega-3 fatty acids or a balanced ω3/ω6 ratio in the diet play an important role not only in normal growth and development, but also the prevention and treatment of several diseases (Kinsella, 1987). The ω3/ω6 ratio has been suggested to be a reliable index for comparing the relative nutritional value of different fish species (Pigott and Tucker, 1990). It was pointed out that the ratio of ω3/ω6 fatty acids was usually between 1 and 4 in freshwater fish and between 5 and 14 in marine fish (Aras et al., 2003). Therefore, the American Heart Association recommends eating fish, especially oily fish, at least twice a week (Kriss-Etherton et al., 2002).

The quantity and composition of fatty acids from lipids are not only associated with the species, but also depend on diet, temperature, seasonality, age, size, reproductive cycle, salinity, wild or cultured fish, habitat and sex of the fish (Henderson and Tocher, 1987; Ackman, 1989; Kriss-Etherton et al., 2002).

Only a few studies have reported the fatty acid composition (Schwalme, 1992; Zmijewski et al., 2006; Jankowska et al., 2008; Kandemir, 2010; Grahl-Nielsen et al., 2011) and seasonal variations in fatty acid compositions of wild pike in lakes (Schwalme et al., 1993; Bulut, 2010; Bulut et al., 2010). However, no data were found in the seasonal variations of fatty acid composition of pike in river systems. In the present study, we aimed to determine the seasonal variations in total fatty acid composition and ω3/ω6 fatty acid ratio of muscle of pike in the river ecosystem for the first time, and to compare the results with previous results that were obtained from the lake ecosystems.

2 MATERIAL AND METHOD

2.1 Study area and samples collection

The Kızılırmak River is the longest river in Turkey (1 355 km). It is of great importance because of both its fish population and potential as a fresh water source in Turkey (Fig.1). The current study was carried out in the Kızılırmak River between the locations of 38°32′54.21″N and 34°55′24.66″E (Köprüköy Region, Kırıkkale). The fish samples, 10 specimens for each season, were collected between November 2009 and October 2010. These specimens were caught using a professional trammel net. The muscle samples were taken from under the dorsal fin. Samples were kept at -25°C until analyses. Before analysis, muscle specimens were warmed to 4°C to facilitate saponification. The water temperature measured 3.9, 13.3, 18.9, and 14.9°C in winter, spring, summer, and autumn, respectively.

2.2 Fatty acid analysis

Ten grams of muscle tissue was weighed and homogenized in a chloroform:methanol mixture (2:1, v:v) (Bligh and Dyer, 1959). The amount of the lipid was determined by gravimetric methods, then a 25-mg sample was used for esterification with 14% standard boron trifluoride-methanol (BF3) (AOAC, 1995). Methylated fatty acids were subjected to analyses using an Agilent 7890N model gas chromatograph fitted with a FID detector and HP-88 capillary column (100 m in length, 0.25 mm inner diameter and 0.20 μm film thickness) (Aras et al., 2009). The temperature of the injector and detector was 250°C and the beginning temperature of the column was 180°C; later the temperature was increased to 220°C at 2°C/min, and left at 220°C for 30 min. Helium was used as carrier gas (2 mL/min) with a split ratio of 30:1. The fatty acids in the specimens were determined as percent areas of the peaks and compared retention times of the standard pre-run fatty acids (Supelco-37 mix).

2.3 Statistical analysis

Analysis for each specimen was run in triplicate and the averages and standard deviations were calculated. One-way ANOVA and variant analyses were performed in the statistical comparisons. Statistical analyses were performed using SPSS 16.0 software (SPSS, Chicago, IL, USA).

3 RESULT AND DISCUSSION

Total lipid content and fatty acid composition of *Esox lucius* are given in Table 1. In the study, 35 different fatty acids were identified in the muscle lipids of northern pike. The minimum total lipid content (2.57%) was measured in spring samples and the maximum value (2.97%) in winter samples. The highest percentages of the fatty acid ratios were found in muscle C16:0 (palmitic acid) in SFA (all season), C18:1 ω9 (oleic acid) in monounsaturated fatty acids (MUFAs) (all season) and C22:6 ω3 in PUFAs (except summer) (Table 1).

The percentage composition of SFA ranged from 21.70% to 28.77%, and there were significant differences ($P<0.05$) between the seasons. Among the SFAs, the most abundant fatty acid was palmitic acid (from 15.19% to 18.95%), which is the predominant source of potential metabolic energy during growth and the roe formation stage in fish (Henderson et al., 1984). Similar results in northern pike (Bulut, 2010; Bulut et al., 2010) and other carnivorous freshwater fishes (Güler et al., 2007; Kalyoncu et al., 2010; Görgün and Akpınar, 2012) were reported.

Among the MUFAs, the highest concentration was found in oleic acid (from 12.51% to 18.35%), then palmitoleic acid (from 10.34% to 13.20%) in all seasons. Similarly, Bulut (2010) and Bulut et al. (2010) found that the ratio of oleic acid concentration was the highest among the studied MUFAs in muscle tissue of northern pike. However, the oleic acid ratio was lower than our findings. In addition, the high levels of oleic acid and palmitoleic acids were reported as a characteristic property of freshwater fish oils (Paaver et al., 2002; Haliloğlu et al., 2004; Çelik et al., 2005).

In the present study, we found that the PUFA contents of northern pike were higher than MUFA contents in summer, winter, spring and autumn, by 35.09%, 40.12%, 43.89%, and 52.23%, respectively. The principal PUFAs were DHA (22:6 ω3), DPA (22:5 ω3), EPA (20:5 ω3), arachidonic acid (AA) (20:4 ω6) and LA (18:2 ω6). Similarly, high levels of DHA, EPA, AA and DPA were reported by Kucska et al. (2006), Jankowska et al. (2008) and Grahl-Nielsen et al. (2011) for northern pike, and by Uysal and Aksoylar (2005), Uysal et al. (2006), Güler et al. (2008), Çakmak et al. (2012) for pike perch (*Sander lucioperca* L.) in different lakes. In fish muscle, the high proportion of PUFAs depends on the diet (Sargent, 1997) and changes of the nutritional habits (Norrobin et al., 1990; Kriss-Etherton et al., 2002). According to our results, the high ratio of DHA, EPA, AA, and DPA increased the PUFA content, particularly in autumn.

DHA and EPA levels in fish muscle play an important role in the valuation of the nutritional value in human health (Loukasa et al., 2010). In particular, EPA and DHA are important contributors in prostaglandin metabolism, thrombosis and atherosclerosis, immunology and inflammation, and membrane function (Galli and Simopoulos, 1989). Among the ω3 series, northern pike are good sources of EPA and DHA. In the present study, the percentages of EPA+DHA in the northern pike were calculated as 19.27%, 20.61%, 15.28%, and 27.57% in winter, spring, summer and autumn, respectively. Similar results were reported from Karamık and the Eber Lakes for the northern pike as good sources of EPA+DHA. The EPA+DHA in the northern pike in the Karamık Lake was reported as 32.34%, 28.16%, 29.20%, and 32.67% in spring, summer, autumn and winter, respectively (Bulut et al., 2010). The percentages of EPA+DHA in the Eber Lake were reported as 29.26%, 31.37%, 31.50%, and 21.09% in spring, summer, autumn and winter, respectively

Table 1 Seasonal variation of the fatty acids in muscle tissue of *E. lucius* (%)*

Fatty acids	Winter (mean±SD)	Spring (mean±SD)	Summer (mean±SD)	Autumn (mean±SD)
C6:0***	0.019±0.004 ^{a**}	0.030±0.004 ^{a}	0.043±0.022 ^{a}	0.094±0.029 ^{b}
C8:0	0.023±0.007 ^{a}	0.029±0.012 ^{a}	0.044±0.009 ^{a}	0.099±0.020 ^{b}
C10:0	0.023±0.002 ^{a}	0.037±0.005 ^{a}	0.046±0.007 ^{a}	0.113±0.010 ^{b}
C11:0	0.024±0.002 ^{a}	0.033±0.007 ^{a}	0.034±0.005 ^{a}	0.154±0.037 ^{b}
C12:0	0.038±0.006 ^{a}	0.039±0.006 ^{a}	0.133±0.023 ^{b}	0.140±0.016 ^{b}
C13:0	0.018±0.004 ^{a}	0.024±0.005 ^{a}	0.116±0.009 ^{b}	0.093±0.010 ^{b}
C14:0	0.644±0.052 ^{a}	0.678±0.113 ^{a}	0.966±0.083 ^{a}	0.719±0.030 ^{a}
C15:0	0.237±0.032 ^{a}	0.196±0.067 ^{a}	0.272±0.023 ^{a}	0.215±0.018 ^{a}
C16:0	18.950±0.747 ^{a}	16.384±0.487 ^{ab}	18.691±0.668 ^{a}	15.188±0.495 ^{b}
C17:0	0.275±0.051 ^{a}	0.269±0.032 ^{a}	0.852±0.201 ^{b}	0.389±0.075 ^{a}
C18:0	4.106±0.428 ^{a}	5.372±0.594 ^{b}	6.816±0.320 ^{c}	3.234±0.283 ^{a}
C20:0	0.126±0.027 ^{a}	0.097±0.006 ^{a}	0.133±0.059 ^{a}	0.308±0.100 ^{a}
C21:0	0.268±0.008 ^{a}	0.275±0.034 ^{a}	0.284±0.046 ^{a}	0.400±0.052 ^{a}
C22:0	0.119±0.013 ^{a}	0.047±0.002 ^{a}	0.166±0.021 ^{a}	0.142±0.005 ^{a}
C23:0	0.041±0.031 ^{a}	0.044±0.016 ^{a}	0.053±0.020 ^{a}	0.232±0.106 ^{b}
C24:0	0.043±0.035 ^{a}	0.057±0.031 ^{a}	0.119±0.036 ^{a}	0.184±0.074 ^{a}
∑SFA	24.954 ^{a}	23.611 ^{a}	28.768 ^{b}	21.704 ^{c}
C14:1 ω5	0.029±0.004 ^{a}	0.040±0.002 ^{a}	0.235±0.062 ^{b}	0.142±0.039 ^{a}
C15:1 ω6	1.367±0.332 ^{a}	2.011±0.239 ^{b}	3.19±0.386 ^{c}	1.892±0.199 ^{b}
C16:1 ω7	13.080±0.96 ^{a}	12.682±0.260 ^{a}	13.196±0.411 ^{a}	10.339±0.262 ^{b}
C17:1 ω8	0.239±0.030 ^{a}	0.324±0.076 ^{a}	0.644±0.121 ^{a}	0.168±0.036 ^{a}
C18:1 ω9	18.345±0.663 ^{a}	16.696±0.408 ^{a}	17.680±0.432 ^{a}	12.511±0.469 ^{b}
C20:1 ω9	0.193±0.017 ^{a}	0.271±0.058 ^{a}	0.163±0.007 ^{a}	0.212±0.006 ^{a}
C22:1 ω9	0.199±0.036 ^{a}	0.244±0.008 ^{a}	0.261±0.041 ^{a}	0.315±0.032 ^{a}
C24:1 ω9	0.141±0.154 ^{a}	0.107±0.081 ^{a}	0.165±0.042 ^{a}	0.345±0.091 ^{b}
∑MUFA	33.593 ^{a}	32.375 ^{a}	35.534 ^{a}	25.924 ^{b}
C18:2 ω6	4.244±0.410 ^{a}	3.616±0.505 ^{a}	4.816±0.394 ^{b}	4.542±0.182 ^{ab}
C18:3 ω6	3.026±0.305 ^{a}	5.838±0.560 ^{b}	2.492±0.133 ^{a}	3.883±0.739 ^{a}
C18:3 ω3	1.683±0.186 ^{a}	2.264±0.211 ^{ab}	1.146±0.084 ^{a}	2.715±0.307 ^{b}
C20:2 ω6	0.039±0.006 ^{a}	0.043±0.001 ^{a}	0.056±0.012 ^{a}	0.111±0.01 ^{a}
C20:3 ω6	0.414±0.025 ^{a}	0.337±0.025 ^{a}	0.406±0.059 ^{a}	0.501±0.026 ^{a}
C20:4 ω6	6.796±0.555 ^{a}	7.232±0.369 ^{a}	7.062±0.605 ^{a}	7.974±0.742 ^{b}
C22:2 ω6	0.274±0.063 ^{a}	0.209±0.061 ^{a}	0.226±0.038 ^{a}	0.440±0.115 ^{b}
C20:5 ω3	7.447±1.059 ^{a}	7.522±0.661 ^{a}	8.518±0.673 ^{a}	12.432±1.359 ^{b}
C22:4 ω6	0.016±0.009 ^{a}	0.057±0.062 ^{a}	0.023±0.011 ^{a}	0.126±0.061 ^{b}
C22:5 ω3	4.360±0.786 ^{a}	3.682±0.467 ^{b}	3.587±0.174 ^{b}	4.365±0.332 ^{a}
C22:6 ω3	11.824±1.155 ^{a}	13.092±1.548 ^{a}	6.761±0.493 ^{b}	15.136±0.685 ^{c}
∑PUFA	40.123 ^{a}	43.892 ^{a}	35.093 ^{b}	52.225 ^{c}
PUFA/SFA	1.62	1.87	1.23	2.46
Lipids	2.973±0.160 ^{a}	2.566±0.156 ^{a}	2.678±0.140 ^{a}	2.647±0.090 ^{a}
ω3	25.314	26.560	20.012	34.648
ω6	14.809	17.332	15.081	17.577
ω3/ ω6	1.710	1.532	1.327	1.971

* The average of the three lots analyzed; ^{abc} values for each sample with different superscript letters in the same fraction are significantly different at $P<0.05$.

(Bulut, 2010). For the other carnivorous fishes, Güler et al. (2007) found that EPA+DHA were between 21.30% and 29.20% in *S. lucioperca*, and Kalyoncu et al. (2010) found that EPA+DHA were between 10.09% and 22.74% in *Oncorhynchus mykiss* (Walbaum, 1792). According to Rueda et al. (1997), the differences in EPA+DHA percentages among the seasons could be explained as a result of the lack of lipid originating from phytoplankton and other aquatic organisms.

The ω3/ω6 PUFA ratio has been suggested to be a useful index for comparing relative nutritional values of fish oils (Pigott and Tucker, 1987). The ω3/ω6 ratio typically ranges from 0.5 to 3.8 in freshwater fish and 4.7 to 14.4 in marine fish (Henderson and Tocher, 1987). In our study, the total ω3 fatty acid concentrations were found higher than the total ω6 fatty acid concentrations, and the ω3/ω6 ratio with the highest value was found in autumn (1.97), followed by winter (1.71) and spring (1.53), and the lowest value was found in summer (1.33). These results can be explained by an increasing value of ω3 PUFAs from 20.012 to 30.648 in autumn. Our results agree with the recommendation for the ω3/ω6 ratio in all seasons.

When compared with results from other species in stream ecosystems, our results are lower than *Salmo trutta macrostima* (Dumeril, 1858), which live in the Munzur Creek (Ateş et al., 2013). In a previous study (Ateş et al., 2013), the highest ω3/ω6 ratio was recorded in the autumn (5.26) and the lowest values in the spring (2.60). Conversely, Aggelousis and Lazos (1991) found similar ω3/ω6 ratios (2.0) for *S. lucioperca* in the Evros River (Greece). However, when compared with our results of other species in lake ecosystems, our ω3/ω6 ratios are usually lower than those reported previously (Jankowska et al., 2008; Bulut, 2010; Bulut et al., 2010).

The PUFA/SFA ratio is another factor for the evaluation of fish nutrition quality. Fish generally need PUFAs to tolerate low water temperatures; therefore, higher PUFA (especially DHA) concentrations are expected in fish that live in cold environments (Haliloğlu et al., 2004). The highest PUFA/SFA ratio was observed in autumn (2.46), followed by in spring (1.87), winter (1.62), and summer (1.23). The most balanced PUFA/SFA ratio was obtained in summer for northern pike. According to Her Majesty's Stationery Office (Culyer, 1994), a minimum value of the PUFA/SFA ratio should be greater than 0.45. Our results are higher than 0.45 in all seasons. Similar results were reported in the Dgal Wielki Lake (2.27) (Jankowska et al., 2008), in Eber Lake (1.09–1.82) (Bulut, 2010) and in Karamık Lake (1.38–1.93) (Bulut et al., 2010) for wild northern pike.

4 CONCLUSION

In conclusion, total SFAs, total MUFAs, total PUFAs, and ω3/ω6 ratios were found different in season in northern pike filets. Though there is no statistical difference of total lipid content ($P>0.05$), seasonal differences were observed in the amount of some fatty acids ($P<0.05$). The northern pike filets are valuable sources of the PUFAs (particularly EPA, DHA, AA, and DPA), total lipids and the ω3/ω6 fatty acid ratio in the diet (especially in autumn and winter). In addition, our results agree with some of the other aforementioned studies in different localities of the same species (lake and river) and some other carnivorous fish species. When human health is taken into account, the northern pike from the Kızılırmak River appears to be quite nutritious in terms of fatty acid composition, total lipids, and the ω3/ω6 ratio.

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