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Full Length Research Paper

Seasonal changes on total fatty acid composition of carp (*Cyprinus carpio* L.), in İvriz Dam Lake, Turkey

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The effects of seasonal variation on the fatty acid composition of carps *Cyprinus carpio* were determined. A total of 38 different fatty acids were determined in the composition of carps. There were quantitative differences between fatty acids in muscle tissue investigated, depending on the season. Oleic acid C18:1 ω9 was the major monounsaturated fatty acid (MUFA) in all seasons. Palmitic acid C16:0 was identified as the major saturated fatty acid (SFA) in four seasons. Palmitoleic acid C16:1 was the third highest fatty acid in total fatty acids. MUFAs were found to be higher than SFAs and polyunsaturated fatty acids (PUFAs) in all seasons. Docosahexaenoic acid C22:6 ω3, linoleic acid C18:2 ω6 and eicosapentaenoic acid C20:5 ω3 were the highest levels among the PUFAs. The percentages of ω3 fatty acid were higher than those of total ω6 fatty acid in the fatty acid composition of carp. In the present study, ω3/ω6 ratios were found to be 1.08, 1.43, 1.64 and 1.60 in spring, summer, autumn and winter, respectively. *C. carpio* may be a valuable food for human consumption in terms of fatty acids.

Key words: Fatty acid composition, seasonal changes, freshwater fish, Turkey.

INTRODUCTION

Fish lipids are well known to be rich in long-chain ω3 (omega-3) polyunsaturated fatty acids (LC ω3 PUFA), especially eicosapentaenoic (EPA) and docosahexaenoic (DHA). LC ω3 PUFA cannot be synthesized by humans and thus, has to be obtained from the diet (Alasalvar et al., 2002). Linoleic acid (LA) and linolenic acid are the parent essential fatty acids (EFAs). They are desaturated and elongated to form long chain PUFAs. For example, arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid are important structural components of cell membranes (Bezard et al., 1994; Innis, 1991). During recent years, fish lipids have been focused on as being beneficial for human health. Today it is known that ω3 fatty acids, or a balanced ω3/ω6 ratio in the diet are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, diabets, hypertension and cancer. They also affect neurodevelopment in infants, fat glycemic control, learning ability and visual function (Kinsella et al., 1990). Recent studies have shown that an increased intake of long chain polyunsaturated fatty acids during pregnancy increases the length of gestation and birth size. This suggests that maternal long chain polyunsaturated fatty acid status may be critical in the development of the fetus (Muskiet et al., 2006). Major depression is associated with lowered ω3 PUFA levels (Hibbeln, 1998; Maes et al., 1999).

The ω3 fatty acids are always present in fish flesh even in lean fish (Ackman, 2002). The ω3 and ω6 PUFAs are also considered essential to the growth and development of children and they are precursors of composite hormones known as eicosanoids, involved in several metabolic processes of great importance to the human body, mainly related to cardiovascular activity (Eder, 1995; Inhamuns and Franco, 2008).

The amount of long chain ω3 PUFAs differs among and can be influenced by a number of factors. The fatty acids composition of fish tissue can be affected by diet, size, age, reproductive cycle, salinity, temperature, season and geographical location (Henderson and Tocher, 1987; Inhamuns and Franco, 2008; Zlatanos and Laskaridis,

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Abbreviations: LC ω3 PUFA, Long-chain omega-3 polyunsaturated fatty acids; **MUFA,** monounsaturated fatty acid; **SFA,** saturated fatty acid; **PUFAs,** polyunsaturated fatty acids; **EPA,** eicosapentaenoic; **DHA,** docosahexaenoic; **EFAs,** essential fatty acids; **LA,** linoleic acid; **FAMEs,** fatty acid methyl esters; **GC,** gas chromatograph.

2007).

Research indicates that fresh water fish generally have lower levels of ω3 PUFA than marine fish. Fish need PUFA to provide the lower water temperature adaptation. Fatty acids of cold and deep sea fish are abundant and the melting temperatures of ω3 fatty acids are lower than ω6 fatty acids (Rahman et al., 1995; Celik et al., 2005). Various environmental conditions and the different diets of wild and reared fish affect their chemical composition, including their fatty acid profile (Suzuki et al., 1986; Jankowska et al., 2010).

EPA is the most important essential fatty acid of ω3 series in the human diet because it is the precursor to the 3-series eicosanoids (Chen et al., 1995). Compared with freshwater fish, marine fish have higher levels of PUFAs especially DHA and EPA. Arachidonic acid (AA), EPA and DHA are important structural components of cell membranes (Innis, 1991).

Cyprinus carpio as a freshwater fish species have been one of the most widely cultured species all over the world. This species is one of the most abundant freshwater fish in İvriz Dam Lake. The main objective of this study was to determine the seasonal fluctuations of fatty acid compositions of muscle and ω3/ω6 fatty acids ratio of this fish species.

MATERIALS AND METHODS

C. carpio used in this study were obtained from İvriz Dam Lake, Turkey. In the present study, the seasons chosen for analysis were summer, winter, spring and autumn. The samples were collected in the middle month of each season during 2008 - 2009. After being caught, they were transported on ice to the laboratories, filleted and frozen. At the beginning of each analysis, the samples were allowed to equilibrate to room temperature, ground and homogenized in chloroform/methanol mixture (2/1, v/v).

The total lipids obtained were saponified by refluxing with methanol (50%) containing 6% KOH for 1 h. Samples of fillets were extracted (Folch et al., 1957) and lipid samples obtained were transesterified with boron tri-fluoride-methanol (BF_3) (Moss et al., 1974). The saponifiable lipids were converted to their methyl esters by using the standard BF_3 method. Fatty acid methyl esters (FAMEs) were analyzed on a HP (Hewlett Packard) Agilent 6890N model gas chromatograph (GC), equipped with a flame ionization detector (FID) and fitted to a HP-88 capillary column (100 m, 0.25 mm i.d. and 0.2 µm). Injector and detector temperatures were 240 and 250°C, respectively. The oven was programmed at 160°C initial temperature and 2 min initial time. Thereafter, the temperature was increased up to 185°C at a rate of 4°C/min, then increased to 200°C at a rate of 1°C/min and held at 200°C for 46.75 min. Total run time was 70 min. Carrier gas used was helium (1 ml/min).

Identification of fatty acids was carried out by comparing sample FAME peak relative retention times with those obtained for Alltech and Accu standards. Results were expressed as FID response area in relative percentages. Each reported result is given in the average value of three GC analyses. The results are offered as means ± SD.

RESULTS AND DISCUSSION

Seasonal variations on total fatty acid composition of *C. carpio* are presented in Table 1. We found 38 fatty acids in muscle lipids of carp. The highest ratio of fatty acids in the fish in all seasons were 18:1ω9 (oleic acid), 16:0 (palmitic acid), 16:1ω7 (palmitoleic acid), (all seasons), 18:2ω6 (linoleic acid) (spring, summer), 20:5ω3 (EPA) (autumn, winter) and 20:4ω6 (AA) (autumn).

Oleic acid, C18:1 ω9, was identified as a primary monounsaturated fatty acid (MUFA) in the carp for all seasons. This fatty acid in muscle tissue of carp was found to be 25.01, 29.28, 22.55 and 26.37% in spring, summer, autumn and winter, respectively. The highest level of oleic acid was in summer (Guler et al., 2008). Kolakowska et al. (2000) found similar results in carp. Similarly, Guler et al. (2007) found that C18:1 ω9 was the major MUFA in muscle in tissue of zander, *Sander lucioperca*, living in freshwater in Turkey. Guler et al. (2008) determined seasonal changes in the fatty acid composition of carp. Oleic acid was identified as the major fatty acid in MUFA (15.1 - 20.3%). The high levels of oleic, palmitoleic and arachidonic acids had been reported as a characteristic property of freshwater fish oils (Andrade et al., 1995). In the present study, these fatty acids were found to be at higher levels than linoleic and linolenic acids. In carp, palmitoleic acid C16:1 ω7 was the second major MUFA (11.52 - 13.70%). Similar results for carp have also been reported in the literature (Kolakowska et al., 2000). C14:1 ω5, C17.1ω8, C22:1 ω9 and C24:1 ω9 were found to be of low amounts in the MUFA fractions of the muscle investigated. On the other hand, MUFA content was higher than the saturated fatty acid (SFAs) and PUFAs in all season. In summer, a high ratio of C18:1 ω9 (%29.28) increased the MUFA content and a high ratio of C18:3 ω3, C20:5 ω3 and C22:6 ω3 increased the PUFA content in autumn. Variations in the fatty acid composition might be related to changes in the nutritional habits of the fish (Norrobin et al., 1990) and fat content is influenced by species, season, geographical regions, age and maturity (Piggot and Tucker, 1990). In the present study, variations in MUFA and PUFA content in the carp may be attributed to the reason.

In the present study, SFAs were lower than total MUFAs. SFA contents ranged between 26.87 and 29.14% in all seasons. Similar results were reported for SFAs of total lipid of *C. carpio* in Beyşehir Lake (Guler et al., 2008). Palmitic acid was the primary saturated fatty acid, 18.09 - 19.07% for carp in all seasons. Guler et al. (2008) determined seasonal changes in the fatty acid composition of carp. In general, fish are relatively low in SFA (< 30%) except for certain species (Ackman, 1989). This result is in agreement with our study.

The principal fatty acids of both fractions (neutral and phospholipids) were palmitic acid in SFA, oleic acid in MUFA (Bayır et al., 2010). The major fatty acid identified in the carp was 18:1 ω9, while C16:0 was the second highest fatty acids. C8:0 was only found in spring, summer and winter in low amounts (0.01 - 0.17%). C10:0, C11:0, C12:0 and C13:0 were found to be low amounts in the

Table 1. Seasonal variations in total fatty acid composition of fillets of carp (*C. carpio*) from İvriz Dam Lake (% of total FA).

Fatty acids	Spring	Summer	Autumn	Winter
C 6:0*	---------	--------	--------	0.01 ± 0.01
C 8:0	0.02±0.02**	0.01±0.01	--------	0.17 ± 0.10
C10:0	0.08±0.08	0.01±0.01	0.01±0.00	0.13 ± 0.09
C 11:0	0.05±0.05	0.02±0.03	0.01±0.00	0.14 ± 0.10
C 12:0	0.17±0.02	0.14±0.04	0.17±0.02	0.16 ± 0.02
C 13:0	0.05±0.02	0.06±0.02	0.16±0.02	0.09 ± 0.01
C 14:0	2.18±0.29	1.96±0.19	1.56±0.19	1.52 ± 0.05
C 15:0	0.76±0.25	0.79±0.19	0.92±0.07	0.75 ± 0.19
C 16:0	18.77±0.59	18.09±1.46	19.07±0.36	18.87 ± 1.63
C 17:0	0.68±0.26	1,01±0.16	0.89±0.32	0.58 ± 0.17
C 18:0	4.48±0.15	4.30±0.48	5.66±0.53	5.03 ± 0.50
C 19:0	0.01±0.01	--------	--------	0.02 ± 0.01
C 20:0	0.16±0.06	0.18±0.12	0.22±0.03	0.15 ± 0.09
C 21:0	0.04±0.02	0.03±0.01	0.05±0.02	0.04 ± 0.01
C 22:0	0.44±0.23	0.26±0.04	0.41±0.05	0.29 ± 0.04
C 24:0	----------	0.01±0.01	0.01±0.01	--------
Σ SFA	27.89	26.87	29.14	27.95
C 14:1 ω5	0.28±0.07	0.26± 0.11	0.45±0.16	0.45 ± 0.10
C 15:1 ω5	1.33±0.50	1.12±0.41	1.73±0.31	2.66 ± 0.21
C 16:1 ω7	13.27±2.72	13.70±0.89	11.52±0.94	11.52 ± 2.07
C 17:1 ω8	1.47±0.70	2.00±0.33	1.53±0.10	1.26 ± 0.16
C 18:1 ω9	25.01±2.59	29.28±3.34	22.55±1.16	26.37 ± 2.47
C 20:1 ω9	1.51±0.43	1.50±0.21	0.77±0.12	1.15 ± 0.60
C 22:1 ω9	0.01±0.01	0.20±0.02	0.03±0.01	0.02 ± 0.01
C 24:1 ω9	0.01±0.01	0.03±0.02	0.01±0.01	0.0 2 ± 0.01
Σ MUFA	42.89	48.09	38.59	43.45
C 18:2 ω6	7.06±0.14	6.46±2.96	3.89±0.29	4.39 ± 0.92
C 18:3 ω6	0.04±0.03	0.08±0.03	0.12±0.02	0.04 ± 0.03
C 18:3 ω3	3.40±1,09	3.61±0.53	5.34±0.77	4.61 ± 0.72
C 20:2 ω6	0.98±0.64	0.61±0.10	0.79±0.12	0.77 ± 0.17
C 20:3 ω6	0.02±0.01	0.02±0.01	0.07±0.03	0.03 ± 0.02
C 20:3 ω3	0.01±0.01	0.01±0.01	0.02±0.01	0.02 ± 0.01
C 20:4 ω6	5.08±2.57	2.64±0.61	6.15±0.65	4.87 ± 0.90
C 20:5 ω3	5.87±0.77	5.86±0.95	6.38±0.21	6.25 ± 1.60
C 22:2 ω6	0.02±0.01	0.03±0.01	0.03±0.02	0.03 ± 0.02
C 22:3 ω3	0.05±0.04	0.03±0.02	0.07±0.03	0.04 ± 0.01
C 22:4 ω6	0.64±0.49	0.29±0.17	0.65±0.07	0.49 ± 0.04
C 22:5 ω6	0.22±0.17	0.16±0.08	0.52±0.07	0.37 ± 0.15
C 22:5 ω3	2.26±0.73	2.12±0.35	2.67±0.32	2.32 ± 0.41
C 22:6 ω3	3.55±1.31	3.08±0.71	5.58±0.81	4.38 ± 0.85
Σ PUFA	29.20	25.00	32.28	28.61
Σ ω3	15.14	14.71	20.06	17.62
Σ ω6	14.06	10.29	12.22	10.99
ω3/ω6	1,08	1.43	1.64	1.60

*Average of three lots analyzed; **values reported are means ± S.D.

SFA fractions of the muscle investigated. Stearic acid C18:0 was the second highest SFA (4.30 - 5.66%). The ω3/ ω6 ratio is a good index for comparing relative nutritional value of fish oils (Pigott and Tucker, 1990). Muscles of common carp reared in warm water showed a higher ω3/ ω6 PUFA ratio with 1.52 in comparison with carp of same age reared in water of natural temperature with 0.47 (Geri et al., 1995). Guler et al. (2008) found that the average of the carp ratio is near 1 in winter, spring and summer and decreased until 0.5 in autumn. In our study, data show that ω3/ω6 ratio was 1.08 in spring, 1.43 in summer, 1.64 in autumn and 1.60 in winter. The ratio of ω3 /ω6 in total lipids of freshwater fishes changes mostly between 0.5 and 3.8, whereas for marine fishes in 4.7-14.4 (Henderson and Tocher, 1987). Hence, our findings are in accordance with earlier studies.

An increase in the human dietary ω3/ ω6 fatty acid ratio is essential in the diet to help prevent coronary heart disease by reducing plasma lipids and to reduce cancer risk (Kinsella et al., 1990). According to Guler et al. (2007), ω3/ω6 fatty acids ratio was 1.49 in spring, 1.45 in autumn, 1.22 in winter and the lowest value, 0.72, was in summer in *S. lucioperca*. A high level of ω6 fatty acids lowered the ω3/ ω6 ratio in summer in *S. lucioperca* which was the freshwater fish. Our study has revealed that carp is a freshwater fish species having a high nutritional value for human consumption due to its high ω3/ ω6.

The present data showed that DHA (22:6ω3) was predominant fatty acid in muscle lipids of carp. In autumn, a high ratio of DHA (5.58%) increased the PUFA content. Sargent (1996) reported that ω3 PUFA, principally DHA, has a role in maintaining the structure and functional integrity of fish cells. The percentages of EPA and DHA were between 5.86 - 6.38% and 3.08 - 5.58% in all seasons, respectively. The percentages of PUFAs, such as EPA and DHA, in fish muscle are dependent on diet (Sargent, 1997). Nutritionists believe that the ratio of ω6:ω3 should be 5:1 and that the addition of ω3 PUFAs to food could improve the nutritional picture and help in the prevention of diseases. Inhamuns and Franco (2008) determined EPA and DHA in two species of freshwater fish from Central Amazonia (*Hypophthalmus* sp. and *Cichla* sp.). The authors reported that relatively, high amounts for freshwater fish were found for DHA in the species. Gokce et al. (2004) reported that the percentages of EPA and DHA which have a vital role in human nutrition were between 3.36 - 4.26 and 16.8 - 20.2, respectively, in the *Solea solea.*

Freshwater fish normally contain n-6 PUFAs, whereas marine fish are rich in n-3 fatty acids, especially DHA and EPA (Wang et al., 1990). In our study, PUFAs levels were found to be 25.00 and 32.28%. The content of PUFA was generally much higher than the SFA in spring, summer and autumn 37.8, 42.8 and 35.9% ,respectively, (Guler et al., 2008). Guler et al. (2008) reported that LA ω6 (3.64 - 10.5%), EPA (4.10 - 5.69%), AA (4.38 - 6.99%) and DHA (4.32 - 11.0%) were the most obvious PUFA in the carp for all seasons. In our study, LA ω6 (3.89 - 7.06%), EPA (5.86 - 6.38%), AA(2.64 - 6.15%) and DHA (3.08 - 5.58%) were also the most obvious PUFA in carp for all seasons. In PUFAs, AA level was quite important. It was found to be 2.49 - 6.15%. Guler et al. (2008) found that the carp have higher contents of AA in spring, summer, autumn and winter, 5.38, 6.99, 5.57 and 4.38%, respectively. AA is a precursor for prostaglandin and thromboxane which will influence blood clot formation and its attachment to the endothelial tissue during wound healing (Bowman and Rand, 1980). EPA, DHA and AA were found to have high ratios in pikeperch (Uysal and Aksoylar, 2005). C18:3 ω6 and C20:2 ω6 were found to be at low amounts in the ω6 PUFA fractions of tissues investigated. Tokur et al. (2006) found that mirror carp fillets in Seyhan Dam Lake in Turkey had high amounts of 18:2 ω6. In our study, the high amounts of 18:2 ω6 decreased the ω3/ω6 ratio in the spring.

This study has shown that carp is a desirable item in the human diet in the İvriz Dam Lake of Turkey when the levels of EPA, DHA and ω3/ω6 ratio are considered. The fish identified in this study was found to be good sources of ω3 fatty acids.

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