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Factors affecting nutritional quality in terms of the fatty acid composition of *Cyprinion macrostomus*

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SUMMARY: This study aimed to evaluate the effect of different factors (season, gender, location, total lipid, weight and length) on the fatty acid composition and nutritional quality of *Cyprinion macrostomus.* The results were evaluated through PERMANOVA, principal coordinates (PCO), and cluster analysis for similarity ranges. An analysis of similarity (ANOSIM) was performed on the distance matrix using multiple permutations within a significant fixed effect ($p < 0.05$). C18:1ω9, EPA and DHA were the most important fatty acids which had an effect on the nutritional quality in all the factor groups. Total lipid amount, season and length factors were the most influential on the fatty acid compositions of *C. macrostomus*. Summer and Spring were the best the periods for the good nutritional quality of *C. macrostomus* in terms of AI (Atherogenicity index), TI (Thrombogenicity index) and h/H (Σhypocholesterolemic/Σhypercholesterolemic fatty acid index). In addition, station, gender and weight had no effect on nutritional quality. The study indicated that *C. macrostomus* is a potential fish meat for human nutrition with high nutritional value in terms of fatty acid composition.

KEYWORDS: *AI;* Cyprinion macrostomus*; EPA; Fatty acids; h/H; PERMANOVA*

RESUMEN: ***Factores que afectan la calidad nutricional en términos de composición de ácidos grasos de*** **Cyprinion macrostomus.** El estudio tuvo como objetivo evaluar el efecto de diferentes factores (estación, género, ubicación, lípidos totales, peso y talla) que afectan la composición de ácidos grasos sobre la calidad nutricional de *Cyprinion macrostomus*. Los resultados se evaluaron mediante PERMANOVA, coordenadas principales (COP) y análisis de cluster para rangos de similitud. Se realizó un análisis de similitud (ANDSIM) en la matriz de distancias utilizando múltiples permutaciones dentro de un efecto fijo significativo ($p<0,05$). C18:1ω9, EPA y DHA fueron los ácidos grasos más importantes que tuvieron efecto sobre la calidad nutricional para todos los grupos de factores. Los factores más influyentes fueron la cantidad total de lípidos, la estación y la longitud, en la composición de ácidos grasos de *C. macrostomus*. El verano y la primavera fueron los mejores períodos para la buena calidad nutricional de *C. macrostomus* en términos de IA (Índice de aterogenicidad), IT (Índice de trombogenicidad) y h/H (Índice de ácidos grasos Σhipocolesterolémico/Σhipercolesterolémico). Asimismo, la estación, el sexo y el peso de los grupos de factores no tuvieron efecto sobre la calidad nutricional. El estudio indicó que *C. macrostomus* es una carne de pescado potencial en la nutrición humana con un alto valor nutricional en términos de composición de ácidos grasos.

PALABRAS CLAVE: *Ácidos grasos;* Cyprinion macrostomo*; EPA; h/S; AI; PERMANOVA*

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1. INTRODUCTION

Cyprinion macrostomus (HECKEL, 1843), belonging to Cyprinidae family, is a widely distributed fish species between the Tigris Euphrates Rivers and the Asi Basin (Coad, 1995). The Murat River is between the Tigris Euphrates Rivers and Asi Basin in Turkey. Despite the aquaculture fishery of *C. macrostomus* in some inland regions such as Northern Iraq (Langroudi and Mousavi, 2018), its catch is very limited in countries such as Turkey, where it is commonly found in its natural waters, yet has not been farmed for consumption.

Marine fishery is more common than freshwater fishery around the World for both aquaculture and captured fish. Carp species are in the highest percentage and include Grass carp, Silver carp, and Common carp (29%, 2016), among the freshwater fish. Every day, natural stocks are decreasing and cultural fisheries are increasing (FAO, 2018). Although its importance as a commercial species is well-known, its ecological and biochemical characteristics have not been fully investigated. It is critical to gain a better understanding of its energy storage, diet and nutrition quality of lipids, especially fatty acids (FAs) as they are considered the most important energy source in aquatic ecosystems. FA precursors of anti-inflamatory eicosanoids have vital functions in a living metabolism for physiological processes, including maintenance of cell membranes and their functions, as well as energy storage (Parrish, 2009).

Aquatic ecosystems are the primary source of ω3 FAs in the environment, thus supporting both aquatic and terrestrial heterotrophs through trophic transfer of these important essential fatty acids (EFAs) through food webs (Gladyshev *et al.,* 2013). EFAs support multiple physiological processes and cannot be synthesized at all or in sufficient proportions to meet the demand. Therefore, these compounds must be obtained through diet by aquatic organisms for optimal growth, reproduction and survival (Parrish, 2009). There is a large body of literature reporting that the reproduction and development of various consumers in aquatic ecosystems is limited by certain essential fatty acids. Therefore, fatty acids are promising biochemical components to be used in determining the food quality of organisms in an ecosystem basin (Galloway and Winder, 2015). Moreover, certain sources of lipids, such as some long-chain polyunsaturated fatty acids (LC-PUFAs), provide consumers with essential nutrients. LC-PUFAs such as 20:5ω3 (eicosapentaenoic acid, EPA), 22:6ω3 (docosahexaenoic, DHA) and 20:4ω6 (arachidonic, ARA) are the most important nutrients in aquatic ecosystems (Parrish, 2009). Specific FAs are also considered dietary biomarkers (Napolitano, 1999) and consumers' fatty acid composition typically reflects that of the fish's diet (Parrish, 2009) and feeding habitat (Parzanini *et al.*, 2020). Over the past 150 years, an increased intake of ω6 LC-PUFA has been associated with an increase in heart disease, which has contributed to the development of a healthy diet concept that balances ω3 to ω6 LC-PUFA (Simopoulos, 2008). To date, multiple lines of scientific evidence have confirmed the beneficial effects of dietary ω3 LC-PUFA, EPA and DHA on human health (Calder, 2018). It is known that marine fish species have more quality lipid and fatty acid contents than freshwater fish species. However, it is more difficult to reach marine fish in interior regions where freshwater resources are more abundant. In the inner regions where *C. macrostom*us is found, it is preferable and often consumed by local people as it is more flavorful and boneless than other Cyprinidae species. *C. macrostomus* can be added to cyprinidae species as an alternative nutrition source in addition to species such as Common carp, Grass carp, and Silver carp, which play important roles in freshwater fish. If *C. macrostomus* has a high omega fatty acid content in its natural environment, we think that it would be appropriate to culture it under suitable conditions all over the world. Thus, a quality food source would be provided for human consumption. Firstly, we need first know the food quality in its natural environment if it is to be consumed as a cultured fish or by hunting. Nutrition quality indicators such as omega 3 (ω3), DHA/EPA, ω6/ω3, which reveal food quality should be investigated. Consumers prefer natural products rather than synthetic products, and the market demand for natural ω3 LC-PUFA, especially from the natural environment is increasing. Therefore, there is an urgent need to find and to extend cultured alternative sources of natural ω3 LC-PUFA. In this study, the differences in fatty acid composition of the edible muscle of *C. macrostomus* collected from the Murat River, Bingöl province, Turkey were investigated by quantifying variations in fatty acid composition according to season, loca-

tion, gender, weight and length. In particular, two different stations with hotter and colder water temperatures were selected. The objective of the study is to reveal the effects of different factors (season, gender, location, total lipid, station, weight and length) on the nutritional quality of *C. macrostomus* in terms of fatty acids.

2. MATERIALS AND METHODS

2.1. Sampling area and samplings

Fish samples were procured alive by hunting from the Murat River, Turkey. Wild fish samples were collected monthly from the stations (Garip and Ilıcalar). Nets with different eye apertures were used for catching the fish. Two stations on the Garip Stream of the Murat River 111 were determined in the Bingöl Province, Turkey. One of the stations was chosen from the Ilıcalar location (Ilıcalar Station; 36º59'01.5'' N, 40º40''58.9''E), which has water temperatures above seasonal norms. The other station was the Garip location (Garip Station; 30º47'10.7''N, 40º32'58.7''E), which has water with colder temperature. Water temperature was measured randomly at the same location in both stations between March 2017-February 2018. Nets with different eye apertures were used for catching the fish. Individual fish weight ranged from 8 to 87 g, while length ranged from 8.5 to 18.5 cm.

2.2. Laboratory studies

2.2.1. Preliminary preparations

Wild-caught fish were kept on ice and delivered to the laboratory in 1 h. Wild *C. macrostomus* samples were kept on ice before being slaughtered, since ice has an anesthetic effect on small fish. 30 fish samples from the Garip Station and 17 fish samples from the Ilıcalar Station were used in the study. Length and weight measurements were made for each fish sample. Fish samples were divided into groups according to length and weight. Length was divided into two groups: Group 1, 8.5 -14.5 cm and Group 2, 15 -18.5. Weight was divided into three groups: Group 1, 8-40 g; Group 2, 42.5-58 g; and Group 3, 60.5-87 g. The samples were chosen from sexually mature fish. Transport and slaughter procedures were applied as established in the European Commission (EU) report on the welfare of fish and a mechanism was applied for using effective stunning and slaughter equipment, in accordance with the European Food Safety Authority (EFSA), 2013 guidelines. The mechanism was established in accordance with the Bingol University Animal Experiments Local Ethics Committee Directive (2016/06-5). Fish samples were cut following the butterfly fillet technique. The edible muscle tissue (raw form) of the fish samples was separated from the inedible parts of the fish. The internal organs were removed by hand. Gender determination was made macroscopically from the gonads. This procedure was performed to evaluate to total lipid and fatty acid in the muscle tissue of the fish according to gender discrimination. Every fish muscle was cut into uniform pieces of (2.0 131 × 2 × 1 cm; ~1-2 g) using a scalpel from the non-posterior part. Also, every sample was sealed in plastic bags. All the fish muscle samples were stored at -80 ˚C for further analysis.

2.2.2. Lipid extraction and fatty acid derivatization

Lipid extraction was performed on the separated muscle tissue samples. The weight of each sample was determined with a precision of 0.001 mg wet weight (WW). A hexane/isopropanol mixture (3/2) was used for lipid extraction. The homogenate was centrifuged (5000 rpm, 5 min, 4 ºC) and the supernatant phase was used for the fatty acid analysis (Hara and Radin, 1978).

20 g methanolic sulfuric acid were mixed into 1000 mL of pure water and a 2% methanolic sulfuric acid solution was prepared and a 5 mL methanolic sulfuric acid solution (20%) was added. The mixture was left to be methylated in an oven at 55 ºC for 15 hours. At the end the period, 5 mL of 5% NaCl were added. 5 mL hexane was added to the fatty acid methyl esters (FAMEs) formed in the tubes and the tubes were turned over. After waiting for 3 hours at room temperature, the hexane phase formed was taken from the top, and 5 mL of 2% $KHCO_3$ solution were added to the tubes, and the nitrogen (N_2) was left to evaporate with the help of the nitrogen evaporator (Allsheng WD-12). To determine the amount of dry lipid remaining after voiding occurred, the samples were weighed on a precision scale and the average total lipid amount (%) per individual was calculated as given in the formula below. After adding 1 mL of hexane to the dry lipid layer, they were vortexed (Christie, 1992) and the samples were taken

into 2 mL capped autosampler vials and analyzed in a mass spectrometer gas chromatograph (GC/MS).

Total Lipid (%) = (Wet Weight/ Dry Weight) x100

Wet Weight = Weight of wet fish sample (g)

Dry Weight = Weight of lipid remaining after evaporation (g)

2.3. GC-MS analysis

GC-MS (7890A-Agilent 5975C) was used for the FAME analysis. MS and FID detectors were used simultaneously. The injection volume was 1 µL and the splitless mode was selected. The GC column was a BPx90 capillary column. The column length was 100 m with an internal diameter 0.25 mm. The column temperatures started from 120 °C and reached 252 °C at a rate of 3 °C/min and was held there for 8 minutes. The injector temperature started at 150 ºC and was ramped up to a final temperature of 250 ºC at a rate of 120 ºC/min. The detector temperature was held at 260 °C. He (1 mL/min) was used as carrier gas. Total analysis time was 52 minutes. FAME analysis of the samples was made by injecting a standard of fatty acid methyl esters (Supelco component FAME Mix) and the retention times of each fatty acid were determined.

After the analysis, wsearch32 software (Wsearch 2008; version 1.6 2005, Sidney, Australia) was used for integration of the peaks of each fatty acid. Quantification was done by interpolation of peak areas with a calibration curve of the fatty acid standards. The total concentration of identified FAME in the sample (mg/mL) was considered as 100%, and an individual FAME was calculated as a proportion of the total identified FAME.

2.4. Statistical analysis

Multivariate statistics were used to analyze differences in total lipid amount and total fatty acid composition for PRIMER-e 2017. Total lipid amount and all fatty acids were used in the multivariate analyses of all samples at the stations. The Bray Curtis similarity coefficient was used for PERMANOVA, principal coordinates (PCO), and CLUSTER analysis for similarity ranges. In the analyses, the fatty acid data from *C. macrostomus* were factored by weight and length groups, total lipid groups, season, gender and stations. The average total lipid, weight and length were calculated during the sampling period. Data were divided into groups according to above and below the almost-average values. The averages for the factor groups were 3.65% for total lipid, 41.36 g for weight and 14.68 cm for length in all the sampling seasons and the stations. Group 1 (TL1, n=30): ≤ 3.8; Group 2 (TL2, n=17): ≥ 3.9 for the total lipid factor; Group 1 (L1, n=24): ≤ 14.5; Group 2 (L2, n=23): ≥ 15 for the length factor; Group 1 (W1, n=12): ≤ 40; Group 2 (W2, n=21): > 40-58 for the weight factor; Group 3 (W3, n=14): > 58. The fatty acids that showed the greatest differences in all samples were investigated in the factor groups. SIMPER (Cut off for low contributions: 70%) was used to identify the fatty acids which contributed the most to the similarities between or within factor groups. Analysis of similarity (ANOSIM) was performed on the distance matrix using multiple permutations within a significant fixed effect ($p < 0.05$). The ANOSIM-R value indicated the extent to which the groups differed (R > 0.75: highly different; R= 0.50–0.75: different; 0.25–0.50: slightly different; R< 0.25: similar with some differences) (Pethybridge *et al.,* 2010). ANOVA tested for significant ($P < 0.05$) main effects of the factors (station, season, month) and their interactions on FA composition and total lipid amount. Variations and significant differences between the groups were investigated with the TUKEY HSD test using STATISTICA software.

3. RESULTS AND DISCUSSION

3.1. Effect of factor groups on the nutritional quality of *C. macrostomus*

In the present study, total lipid fatty acid composition was determined under the influence of the different factors as fatty acid composition of the edible muscle of *C. macrostomus* changed depending on various factors.

Table 1 shows the average seasonally total lipid amount, weight and length of *C. macrostomus* during the sampling season from independent stations. The most significant differences were between autumn and winter for total lipid ($p=0.0005$) and summer and winter for length ($p=0.005$), and weight ($p=0.03$) (Table 2). There was no difference between the Garip and Ilıcalar Stations for total lipid (3.98%, 3.06%, respectively) weight (42.30 g, 39.71 g, respectively) or length (14.86, 14.36, respectively) of *C. macrostomus* ($p < 0.05$, Tukey HSD), although the water temperature of the Ilıcalar Station was, on average, annually 5 ºC higher than the Garip Station, (Table 2). Henderson and Tocher (1987)

Table 1. Seasonal averages of the factor groups (total lipid, weight, length)

Factors	Spring (n=11)	Summer (n=5)	Autumn (n=16)	Winter (n=15)
Total lipid (%)	2.90±1.23^{a}	3.67±2.62^{ab}	5.48±2.93^{b}	2.24±0.83^{a}
Weight (g)	40.77±20.12^{ab}	66.10±6.04^{a}	46.47±24.88^{ab}	28.1±14.10^{b}
Length (cm)	15.26±2.65^{ab}	17.76±0.64^{a}	14.68±3.03^{ab}	13.23±1.94^{b}

Table 2. Seasonal water temperatures (ºC) at the Stations (Garip and Ilıcalar) during the sampling period

SPRING	SUMMER	AUTUMN	WINTER	ANNUAL
GARIP				
10.93	21.91	12.67	8.03	13.39
ILICALAR				
12.63	23.57	21.73	16.67	18.65

reported that temperature had no direct effect on body lipid content. It was emphasized by Kheriji *et al.* (2003) that when the temperature rises, an indirect effect can be seen related to the increased appetite of the fish.

All fatty acids were used in the multivariate analysis of the 47 samples. In PERMANOVA analyses of the fatty acid, data were factored by season, station, gender, total lipid, length and weight groups.

The total lipid groups gave the highest Pseudo-F (5.53) for all the factors and the lowest *P(perm)* value (0.001). Thus, changes in fatty acids in total lipid groups for all the factor groups were significantly different from each other. Season gave the second lowest *P(perm)* value (0.001, with the highest Pseudo-F (4.76). Autumn-winter and spring-autumn were significantly different from the other seasons *P(perm)*=0.001. However, autumn-winter presented the most significant difference (t=3.83) among seasons. The length groups were statistically the third most important factor group. The Pseudo-F value was 3.37, *P(perm)*=0.02. Station difference was in fourth place, with (*P(perm)*:0.02, Pseudo-F:2.74). The station factor was followed by gender with (*P(perm)*:0.05, Pseudo-F:2.25) and weight with (*P(perm)*:0.13, Pseudo-F:1.55). Therefore, the weight and gender were statistically the least important factor group for the fatty acid composition of *C. macrostomus;* whereas total lipid, season and station were statistically important factor groups for *C. macrostomus.* For this reason, we only used the statistically most effective factor groups (season, total lipid) in the study.

3.2. The most effective factors on nutritional quality of *C. macrostomus*

Evaluation of the stations revealed that the seasonal difference in fatty acids in *C. macrostomus* was very different at both the Garip (ANOSIM-R: 0.64, Pseudo-F: 4.68, *P(perm)*:0.001) and the Ilıcalar Stations (ANOSIM-R: 0.47, Pseudo-F: 4.13, *P(perm)*=0.001). However, locational differences in fatty acid composition within the station were higher in the Garip than the Ilıcalar Station. Although *P(perm)* values for the stations were the same (0.001), they had different ANOSIM-R values and the ANOSIM-R value was significantly different for the Garip Station.

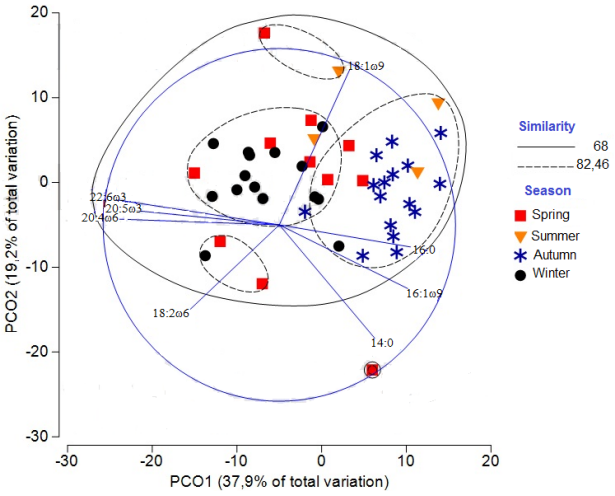

Figure 1. Two-dimensional configuration plot of a PCO analysis of a resemblance matrix of fatty acids in seasons. The lower tringular matrix was created using Bray-Curtis similarity coeffients. Pearson correlation > 0.65.

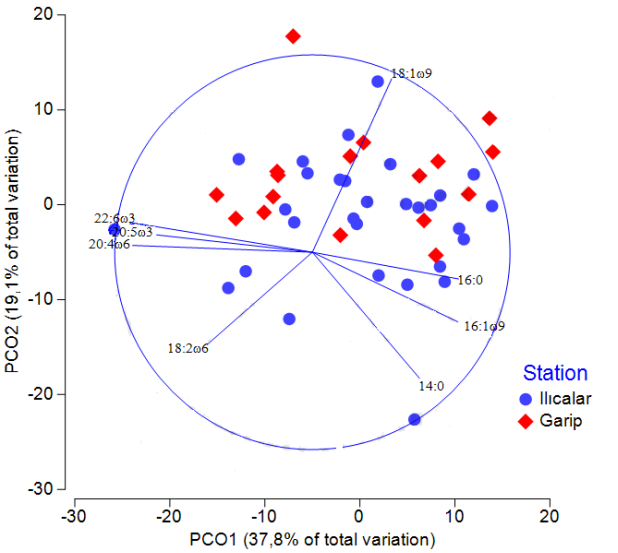

Figure 2. Two-dimensional configuration plot of a PCO analysis of a resemblance matrix of fatty acids in the stations. The lower tringular matrix was created using Bray-Curtis similarity coeffients. Pearson correlation > 0.65.

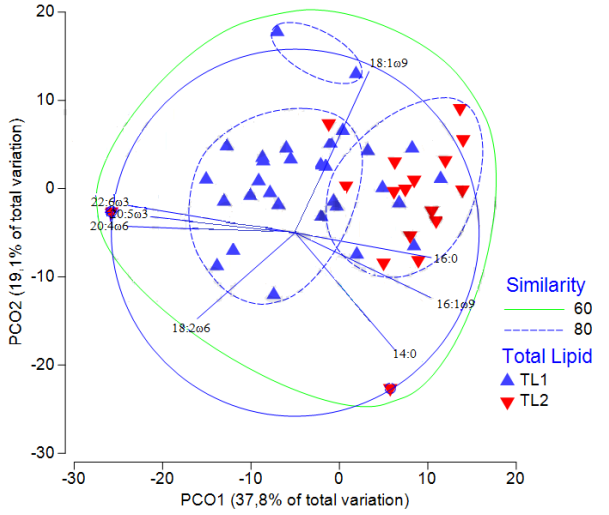

Figure 3. Two-dimensional configuration plot of a PCO analysis of a resemblance matrix of fatty acids in the total lipid groups (TL1, TL2). The lower tringular matrix was created using Bray-Curtis similarity coeffients. Pearson correlation > 0.65.

The figures show a two-dimensional configuration plot of a PCO analysis of resemblance matrix for total fatty acid data. 18:1ω9 was the major fatty acid in all the factor groups. Figure 1 shows that autumn was characterized mostly by 16:0; whereas spring, and especially winter, were characterized mostly by 18:1ω9 with 82.46% similarity. 14:0, 18:2ω6, 18:1ω9, EPA, DHA, ARA, 16:0 and 16:1ω9 were the main fatty acids for all seasons with 68% similarity. 18:1ω9 and 16:0 was the main fatty acid at both stations. However, 16:0 was more in the foreground at the Ilıcalar Station than at the Garip Station (Figure 2).

Figure 3 shows that TL1 was characterized mostly by 18:1ω9 and EPA, DHA and ARA. The fatty acids presented in TL1 are higher than TL2 with 80%. 16:0 was the most characteristic fatty acid for TL2 with 80% similarity. Also, seasonal differences for fatty acid composition were significantly important within the total lipid groups. TL1 factor group gave more seasonal differences (Pseudo-F: 2.45, *P(perm)*:0.002) than TL2 (Pseudo-F: 1.73, *P(perm)*:0.11). However, the most important difference was between summer and winter *(P(perm)*:0.002, t:2.15) in the TL2 factor group. Additionally, all the factor groups were characterized by ARA, 18:2ω6, EPA and DHA at between 60-65% similarity. Based on these results, this study showed that the most influential factors were firstly lipid amount and secondly season factors on the fatty acid composition of *C. macrostomus*. Therefore, it was decided that it would be most appropriate to examine the fatty acids that reveal the nutritional quality of *C. macrostomus* depending on these factors. The weight, especially the length and gender were not important factors in determining the fatty acid composition of *C. macrostomus*.

It was found that total MUFA was higher than total PUFA and total SFA in both stations. Only ΣPUFAs were higher than ΣMUFAs in winter at the Garip Station (Table 2). Güler *et al.* (2008) indicated that 16:0 was the primary SFA, with 14.6–16.6% for carp in all seasons. Similar results were reported by Kolakowska *et al.* (2000) for carp. Generally, fish species are relatively low in SFA (< 30%) except for some species (Güler *et al.,* 2008). Similar results were identified in this study for all seasons for *C. macrostomus* and ΣSFA varied from 24.62-32.38% (spring-autumn) at the Garip Station, with 28.85-31.76% (winter-autumn) at the Ilıcalar Station. 16:0 was the main SFA and higher in summer (20% and 25%, respectively) and autumn (22% and 23%, respectively) than the other seasons at both the Ilıcalar (Table 1) and Garip Stations (Table 1).

The ΣPUFA/ΣSFA ratio (P/S) is an index used to express the nutritional quality of dietary lipids. Generally, foods with a P/S ratio of less than 0.45 are considered undesirable for the human diet because of their potential to induce hypercholesterolemia (Fer-

nandes *et al.,* 2014). Matos *et al*. (2019) found that the P/S ratios were as > 0.45 for the Grass, Common and Bighead carps (0.50-0.60); while the P/S was < 0.45 for Nile tilapia (cage and pond) and Silver carp (0.10 - 0.44). Ramos-Filho *et al.* (2008) reported P/S < 0.45 for freshwater fish fillets of cachara (0.44) and pacu (0.13). The P/S ratio was < 0.45 in all seasons and at both stations for *C. macrostomus* in the study. The lowest P/S ratios were 0.88 (summer), 0.73 (autumn) at the Garip Station and 0.71 (autumn) at the Ilıcalar Station. Furthermore, P/S was >1 for *C. macrostomus* in the other season at the stations. The highest ratio was 1.47 (winter) at the Garip Station and 1.22 (summer) at the Ilıcalar Station. However, the P/S ratio alone may not be sufficient to determine the nutritional quality of lipids, as it does not consider the metabolic effect of MUFAs (e.g 18:1ω9). 18:1ω9 was generally the main fatty acid in *C. macrostomus* in all the factor groups. 18:1ω9 was the highest value in summer and autumn (23%) at the Ilıcalar Station (Table 2); whereas it was the highest value in spring and summer (27%) at the Garip Station (Table 3). 18:1ω9 is the predominant MUFA in *Cyrinus carpio* from Cyprinidae (Güler *et al*., 2008).

Cengiz *et al.* (2010) researched the fatty acid compositions of total lipids in the muscle tissues of nine freshwater fish from the River Tigris, Turkey. They found that *C. macrostomus* had the low ΣMUFA (15%) and 18:1ω9 was the main MUFA. In addition, 18:1ω9 was significantly higher in summer than in winter for *Carassius gibelio* and *Sander lucioperca,* but it was higher in winter than in summer for *Cyrinus carpio* and *Leuciscus lepidus*. Also, they explained that ARA, DHA and EPA were the major fatty acids. The fact that *C. macrostomus* had both high 18:1ω9 and long-chain ω3 in all the factor groups suggested that consuming this fish fillet would be beneficial to human health. 18:1ω9 was not only the main fatty acid in ΣMUFA, but also the main fatty acid among all the fatty acids of *C. macrostomus*. 18:1ω9 has several health benefits, such as increasing HDL (high-density lipoprotein) content and lowering blood pressure (Hlais *et al.,* 2013).

18:1ω9 is used to estimate the nutritional quality of the lipids along with other hypocholesterolemic fatty acids (18:3ω6, C18:3ω3, EPA, DHA). 12:0, hypercholesterolemic fatty acids (14:0, 16:0), and 18:0 are also used to estimate the nutritional quality of lipids. The Σhypocholesterolemic/Σhypercholesterolemic fatty acid index (h/H) is an important additional index for determining the effect of individual fatty acids on cholesterol metabolism (Santos-Silva *et al.,* 2002). In terms of nutritional value, a higher h/H ratio is directly proportional to the higher PUFA content, which is considered more beneficial for human health. Carp, Nile tilapia (cage) and Grass carp have high h/H values which range between 2.15 and 2.94. These freshwater fish have a nutritional quality which is comparable to the h/H values of marine fish such as mackerel or sardines with an average of 2.46 h/H. (Fernandes *et al.*, 2014). Conversely, h/H values were reported at 1.84 for Pintado and 1.49 for Dourado from Brazilian freshwater fish fillets by Ramos-Filho *et al.* (2008). In this study, it was between 1.15-2.39 (summer-spring) at the Garip Station (Table 2) and 1.34-2.20 (autumn-summer) at the Ilıcalar Station (Table 2) for *C. macrostomus*. Therefore, summer at the Ilıcalar Station and spring at the Garip Station were the best the periods for the nutritional quality of *C. macrostomus* in terms of h/H. Also, the atherogenicity (AI) and thrombogenicity (TI) indexes are two other frequently used indexes to show the potential to stimulate platelet aggregation. [(12:0 + (4 x 14:0) + 16:0)] / (ΣMUFA + Σω6 + Σω3) is given for AI. (14:0 + 16:0 + 18:0) / [(0.5 x ΣMUFA) + (0.5 x Σω-6 + (3 x Σω3) + (Σω3/ Σω6)] is given for TI (Santos-Silva *et al.,* 2002). Foods with low AI and TI values have a greater potential to protect against coronary disease. Matos *et al.* (2019) indicated that AI values ranged between 0.34 and 0.88 in the Common carp with 0.34 and Nile tilapia (cage) with 0.42, showing the lowest AI values. The Pintado and Pacu fish species were reported to have comparable AI values (0.49, 0.86, respectively) by Ramos-Filho *et al.* (2008). The TI value was lower in the Bighead carp fillet (0.47) than the freshwater fish Cachara (0.59). However, the TI value was the lowest in the marine fish White needle (0.44) (Fernandes *et al*., 2014). AI was found between 0.38-0.49 (summer-winter) at Ilıcalar (Table 2) and 0.31-0.43 (spring-winter) at the Garip Station (Table 3) for *C. macrostomus*. TI was between 0.26-0.39 (summer-autumn) at the Ilıcalar Station and 0.22-0.40 (spring-autumn) at the Garip Station. Therefore, summer at the Ilıcalar Station and spring at the Garip Station were the best the periods for the nutritional quality of *C. macrostomus* in terms of AI and TI, similar to h/H.

Table 3. Fatty acid composition of *C. macrostomus* at Ilıcalar Station during the sampling period (% Total FAME).

Fatty Acids	SPRING (n=9)	SUMMER (n=2)	AUTUMN (n=10)	WINTER (n=9)
14:0	3.78±0.90	1.38±0.15	5.36±0.45	3.59±1.19
15:0	-	-	0.68±0.55	-
16:0	19.14±2.87	20.03±3.21	21.82±1.30	19.14±1.46
16:1ω11	1.32±1.04	0.82±0.91	0.50±0.70	1.24±0.75
16:1ω9	11.10±2.50	3.74±1.83	15.78±2.66	10.17±2.71
16:1 ω7	0.91±0.56	0.76±0.62	1.81±1.01	1.29±0.63
ι17:0	0.71±0.25	1.10±0.44	0.61±0.24	0.68±0.14
16:2ω4	0.72±0.24	-	1.07±0.54	0.52±0.31
17:0	0.94±0.23	1.13±0.44	0.94±0.23	0.67±0.51
17:1	1.13±0.56	-	0.62±0.50	0.85±0.54
16:3ω3	0.73±0.68	-	-	-
18:0	4.29±2.55	6.46±3.96	2.77±1.53	4.79±1.97
18: ω11	0.62±0.46	-	-	-
18:1ω9	19.42±9.33	22.91±13.79	22.50±2.39	19.96±3.14
18:1ω7	1.43±1.58	1.14±1.28	1.35±1.20	2.14±1.52
18:1ω6	-	0.74±1.05	-	-
18:2ω 6	2.93±0.54	2.36±0.77	2.61±0.54	2.93±0.80
18:3ω4	0.52±0.44	0.68±0.03	0.55±0.36	0.57±0.27
ALA	8.29±1.76	9.06±0.81	6.88±1.82	7.36±2.07
20:1ω9	1.22±0.73	0.75±1.06	0.83±0.82	1.05±0.83
20:2α	-	0.56±0.79	-	-
20:3ω6	0.86±0.45	1.66±0.91	0.34±0.20	0.46±0.36
ARA	1.64±0.33	2.64±1.53	0.74±0.22	1.62±0.41
20:3ω3	0.99±0.44	0.60±0.85	0.66±0.33	0.98±0.53
20:4ω3	0.78±0.24	-	-	0.65±0.47
EPA	4.27±1.32	5.14±0.95	3.31±0.95	5.54±1.09
21:5ω3	0.64±0.38	0.88±0.88	0.28±0.16	0.53±0.24
22:5ω3	1.54±1/74	1.44±0.60	0.50±0.24	0.95±0.22
DHA	6.03±2.56	10.62±3.98	3.83±0.97	8.39±1.82
24:1	-	0.54±0.15	-	-
ΣMFA*	3.69±0.98	2.72±0.78	4.80±1.76	4.42±1.45
ΣSFA	29.13±4.76	29.34±1.38	31.76±2.29	28.85±2.33
ΣMUFA	38.11±7.22	32.73±12.47	44.47±2.46	38.13±4.66
ΣPUFA	31.36±4.48	36.04±10.33	22.31±1.92	31.50±5.07
P/S	1.10±0.22	1.22±0.29	0.71±0.09	1.10±0.25
ω3	23.31±3.36	27.81±7.79	16.17±1.33	24.53±4.00
DHA/EPA	1.46±0.58	1.97±1.01	1.17±0.11	1.53±0.27
Bacterial	4.33±1.12	4.62±1.47	3.66±0.68	3.75±0.97
Zooplankton	1.26±0.74	1.17±1.12	0.83±0.82	1.05±0.83
Terrestial	11.22±2.16	11.42±0.03	9.48±1.97	10.29±2.73
ω6	6.53±1.78	7.63±4.58	4.35±0.80	5.86±0.94
ω6/ω3	0.28±0.80	0.27±0.23	0.27±0.21	0.34±0.26
16:1ω7/16:0	0.05±0.03	0.08±0.03	0.09±0.05	0.05±0.03
h/H	1.67±0.45	2.20±0.98	1.34±0.85	1.81±0.87
AI	0.50±0.12	0.38±0.09	0.67±0.12	0.49±0.05
TI	0.28±0.08	0.26±0.05	0.39±0.09	0.28±0.07

*: Minor FAs (MFAs) with mean proportion < 0.5 in all the sampling periods. Values are mean 95% confidence interval. ΣSFAs, total saturated fatty acids; ΣMUFAs, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid; ALA, alpha linoleic acid; AI, atherogenicity index; TI, thrombogenicity index; h/H, The Σhypocholesterolemic/Σhypercholesterolemic fatty acid index; ι, iso-branched FAs; ω, omega fatty acids; P/S, Polyunsaturated fatty acids/Saturated fatty acids

18:3ω3 (Alpha Linolenic Acid, ALA) is the metabolic precursor to ω3 long-chain polyunsaturated fatty acids (LC-PUFA) such as EPA and DHA (Brenna, 2002). 18:2ω6 (Linoleic acid, LA) is the precursor to ARA, one of the precursors to the biosynthesis of eicosanoids, which perform important functions in the human body (Aguiar *et al.*, 2007). EPA and DHA provide human health, early development and prevention of some diseases. Therefore, dietitians have been increasingly recommending the consumption of foods containing these fatty acids (Jobling and Leknes, 2010) as ω3 LC-PUFA cannot be adequately biosynthesized by humans. Thus, the fatty acids must be obtained from the diet (Williams and Burdge, 2006). Also, fish cannot synthesize these fatty acids and obtain them from the food they consume such as algae and plankton (Falk-Petersen *et al.,* 1998). Freshwater fish lack the ability to produce certain fatty acids, especially C18 acids such as 18:2ω6 and 18:3ω6, and can directly take many long-chain polyunsaturated fatty acids such as ARA, DHA, EPA from their prey (Tocher, 2010). They are essential to the overall health of organisms and most consumers synthesize them inefficiently from their precursors (eg, 18:3ω3 and 18:2ω6). Many studies highlighted that terrestrial plants synthesize 18:3ω3+18:2ω6 fatty acids in abundance and they are used as a dietary marker in the fatty acid composition of aquatic organisms. Additionally, 18:1ω9 and 18:2ω6 are available from primary producers only. Two essential fatty acids are obtained from the diets of animals such as EPA and DHA (Parrish, 2009). Brown and red algae, vascular plants and dinoflagellates are their main sources (Kelly and Scheibling, 2012). Also, 18:1ω9 is used as a characteristic fatty acid marker for cryptophyceae along with dinophyceae and chlorophyta (Napolitano, 1999). EPAs are fatty acid markers of diatoms from Bacillariophyceae; while DHAs are fatty acid markers of dinoflagellates from Dinophyceae (Viso, and Marty, 1993). This study showed that *C. macrostomus* contain a substantial amount of PUFAs ω3 with carbon chains with C20 and C22 in all the factor groups.

Increasing water temperature increases food intake, and reduces food efficiency. The growth of fish growth can also be adversely affected by this (Norambuena *et al.*, 2016). When aquatic organisms are exposed to high water temperatures, PUFAs increase and SFAs decrease (Şen Özdemir *et al.,* 2017; Wijekoon *et al.,* 2021). 18:3ω3, ALA is lost more with the increase in temperature (Turchini and Francis, 2009). However, this higher disappearance was not associated with the higher appearance of ω3 fatty acid bioconversion products. Regarding the apparent in vivo enzymatic activities, the apparent elongations in in vivo activity were not affected by temperature considering both the ω6 and ω3 pathways (Mellery *et al.*, 2016). In the study, ALA was generally lower at the Ilıcalar Station, where the temperature was higher than at the Garip Station. It was only slightly higher at the Ilıcalar Station (8.23%) than at the Garip Station (7.92%) in spring. Here, the temperature difference between the stations was very low in spring compared to the other seasons (Table 2). PUFAs were higher at the Garip Station except for summer than at the Ilıcalar Station throughout the year (Table 1, Table 3). Omega 3 LC-PUFA comprised at least 40% of the ΣPUFA in *C. macrostomu*s in the present study. The ω3 LC-PUFA content of the plant lipids which the fish are fed was affected because of the change in the lipid bioconversion capacity (Mellery *et al.*, 2016). *C. macrostomus* can use the food taken at a high temperature (35 ºC in Sivas) to a minimal extent and the high temperature significantly affects metabolic activity. Water temperature varied between 12.63 ºC (spring) and 23.57 ºC (summer) at the Ilıcalar Station, 8.03 ºC (winter) and 21.91 ºC (summer) at the Garip Station (Table 2). Similarly, the highest ΣPUFAs (36%) of *C. macrostomus* were in low temperatures in winter (8 °C) at the Garip Station. The temperature at Ilıcalar Station was higher than at Garip Station throughout the year (Table 1). It is thought that the water temperature was especially effective in this difference.

Several researchers have suggested ω6/ω3 as a useful indicator of fish lipids' nutritional value, and a lower ratio is more effective in preventing cardiovascular diseases associated with plasma lipid levels (Rhee *et al.,* 2017). According to nutritional recommendations (FAO, 2014), the ω6/ω3 ratio should not exceed 5.0 in the human diet. Matos *et al.* (2019) reported that the ω6/ω3 ratio was 8.16 for Nile tilapia (cage), 5.40 for Carp (5.40) and 5.27 for Grass carp. High ω6/ω3 in the edible muscle of fish may be due to the high 18:2ω6 levels in terrestrial plant-based feed products used in modern aquaculture (Simat *et al.,* 2015). Also, consumption of low ω3 PUFAs and excess ω6 PUFAs is highly associated with the

Table 4. Fatty acid composition of *C. macrostomus* at Garip Station during the sampling period (% Total FAME).

Fatty Acids	SPRING (n=2)	SUMMER (n=3)	AUTUMN (n=6)	WINTER (n=6)
14:0	1.36±1.69	2.82±0.69	3.36±1.05	2.70±0.68
16:0	16.91±12.50	25.12±1.25	23.28±1.77	19.03±1.75
16:1ω11	1.41±0.35	0.53±0.57	1.28±1.07	0.62±0.43
16:1ω9	5.83±5.28	10.97±2.96	11.60±1.30	9.94±1.31
16:1ω7	-	-	-	0.66±0.43
ι17:0	0.60±0.38	0.61±0.04	0.58±0.14	-
16:2ω4	0.92±0.15	1.69±0.34	0.52±0.47	0.59±0.22
17:0	0.74±0.11	0.70±0.05	0.84±0.20	0.63±0.38
16:3ω4	-	-	0.81±1.26	-
17:1	-	0.75±0.04	0.95±0.65	0.85±0.16
18:0	5.39±1.99	2.25±2.01	4.21±0.85	4.91±0.44
18:1ω11	0.85±0.11	0.54±0.20	-	0.51±0.46
18:1ω9	26.88±11.90	26.45±4.78	24.39±4.36	19.64±3.53
18:1ω7	0.87±0.44	0.83±0.80	1.31±1.37	0.54±0.53
18:2α	0.57±0.02	-	-	-
18:2ω6	2.70±1.51	2.18±0.38	2.33±1.06	3.05±0.49
18:3ω6	0.53±0.11	-	-	-
ALA	-	-	0.65±0.49	0.53±0.29
20:1ω9	7.92±3.74	10.09±0.17	8.39±1.68	8.89±1.22
20:2ω6	-	-	1.87±1.00	-
20:3ω6	0.55±0.07	-	0.68±0.36	0.96±0.40
ARA	2.35±0.85	1.15±0.97	0.93±0.46	2.12±0.46
20:3ω3	0.85±0.32	0.94±0.24	0.86±0.30	0.87±0.12
20:4ω3	0.76±0.13	0.70±0.14	0.59±0.26	0.59±0.36
EPA	6.29±3.15	2.52±1.93	2.43±1.41	7.47±1.84
21:5ω3	0.64±0.20	-	-	-
22:5ω3	1.21±0.51	0.88±0.36	0.56±0.25	0.62±0.49
DHA	9.88±4.64	3.93±3.47	3.16±1.57	10.57±1.48
ΣMFA*	4.67±0.97	4.33±1.02	5.17±1.65	2.44±0.23
ΣSFA	24.62±18.75	31.37±4.03	32.38±1.91	27.75±0.96
ΣMUFA	37.74±23.67	40.87±4.41	42.94±3.26	33.35±2.79
ΣPUFA	36.35±18.69	26.69±7.99	23.56±3.13	38.09±3.68
P/S	1.47±0.61	0.88±0.38	0.73±0.12	1.38±0.17
ω3	27.69±14.51	19.71±6.51	16.60±2.62	29.67±2.93
DHA/EPA	1.57±0.89	1.50±0.22	1.38±0.38	1.45±0.18
Bacterial	2.77±1.97	2.94±0.16	3.48±0.74	2.75±0.49
Zooplankton	-	-	1.93±0.96	-
Terrestial	10.62±5.35	12.27±0.42	10.72±2.15	11.94±1.45
ω6	6.46±3.48	4.29±1.64	4.74±0.71	6.73±0.95
ω6/ω3	0.23±2.65	0.22±0.52	0.29±0.48	0.23±0.51
16:1ω7/16:0	0.02±0.02	0.02±0.02	0.03±0.02	0.02±0.02
h/H	2.39±1.09	1.18±0.98	1.15±0.34	1.76±0.97
AI	0.31±0.09	0.56±0.12	0.57±0.10	0.43±0.12
TI	0.22±0.05	0.35±0.07	0.40±0.04	0.24±0.03

*: Minor FAs (MFAs) with mean proportion < 0.5 in all the sampling periods. Values are mean 95% confidence interval. ΣSFAs, total saturated fatty acids; ΣMUFAs, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid; ALA, alpha linoleic acid; AI, atherogenicity index; TI, thrombogenicity index; h/H, The Σhypocholesterolemic/Σhypercholesterolemic fatty acid index; ι, iso-branched FAs; ω, omega fatty acids; P/S, Polyunsaturated fatty acids/Saturated fatty acids

pathogenesis of many modern diet chronic diseases (Simopoulos, 2008). Therefore, the ω6/ω3 ratio is an important factor for food quality. The highest ω6/ω3 was 0.34 in winter (Ilıcalar Station) (Table 2) and 0.29 in autumn (Garip Station) (Table 3) for *C. macrostomus* in the study. The values did not exceed 5.0 and Σω3 fatty acids were higher than Σω6 fatty acids. *C. macrostomus* had good fatty acid nutritional quality in its natural environment because high ω3, low ω6 improves the nutritional quality of the diet.

CONCLUSIONS

Season, total lipid amount, length, station, weight and gender were used as factor groups affecting the nutritional quality of *C. macrostomus.* It was observed that the effect of fatty acids on the nutritional quality of *C. macrostomus* varied depending on the factors. While total lipid amount, season and length were found to be the most effective factors on these changes, station, gender and weight from the factor groups did not have any effect on the nutritional quality of *C. macrostomus*. Unsaturated fatty acids such as 18:1ω9, EPA, DHA were the most important fatty acids which had an effect on nutritional quality for all the factor groups. The study indicated that *C. macrostomus* had high nutritional value for humans. It would be beneficial to introduce *C. macrostomus*, which was seen to have good nutritional value, like the other freshwater fish that are widely grown and consumed. However, continued studies are needed in order to collect more information and increase our understanding of the nutritional value and health benefits of *C. macrostomus* for human consumption in different regions and aquaculture experiments. It should be kept in mind that the fish diets have a significant effect on the change in fatty acid composition, especially in aquacultural studies.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in the paper.

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