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The Fatty Acid Composition and Cholesterol and Vitamin Contents of Different Muscles of *Esox lucius* (Linnaeus, 1758) Living in Lake Ladik

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Abstract: This study examines the fatty acid composition and cholesterol and vitamin contents of the muscles, liver, gonad and kidneys of pike living in Lake Ladik. Of the total Saturated Fatty Acids (SFA), the most predominant fatty acids present in both males and females are palmitic acid (16:0), stearic acid (18:0) and myristic acid. The difference between the muscles are insignificant ($p>0.05$). With regard to the Monounsaturated Fatty Acids (MUFA), 18:1n-9, 18:1n-7 and 16:1 are predominantly found in the liver. The difference between the kidneys and the gonads are significant ($p<0.05$) but there is no significant difference between other muscles ($p>0.05$). Of the polyunsaturated fatty acids, the amount of n-3 PUFA is significantly different in muscle tissues compared with those of other tissues. Similar results were obtained for n-6 PUFA in kidney and for n-3/n-6 proportion in muscle tissues. The examination of the general compositions of the fatty acids reveal an intensification on 16:0, 16:1, 18:0, 18:1n-9, 18:1n-7, 20:4n-6, 20:5n-3 and 22:6n-3. EPA + DHA amount and PUFA/SFA proportion are the highest in muscle tissue. The least amount of cholesterol on the other hand is found in muscle tissue and there is a significant difference with those of other tissues ($p<0.05$). The highest amount of K1 and K2 vitamins are found in gonads and D2, D3 and α-tocopherol were highest in liver. These analyses are conducted with GC and HPLC.

Key words: Fatty acids, cholesterol, K, D, E vitamin, *Esox lucius*, Lake Ladik

INTRODUCTION

A healthy life, growth, mental and physical activities can only be sustained with a sufficient and balanced diet. Fish, among other water products are the foremost sources to provide fat, protein, vitamin and minerals required for a sufficient and balanced diet.

The potential health benefits related with fish consumption are related with unsaturated fatty acids, fat-soluble vitamins, essential amino acids and minerals. Fish fats are major sources of n-3 Polyunsaturated Fatty Acids (n-3 PUFA). EPA (Eicosapentaenoic Acid) and DHA (Docosahexaenoic Acid) are particularly essential for human health due to their fatty acid contents (Carlson and Salem, 1990). Moreover, n-3 PUFA has been shown to have beneficial effects on hypertension, inflammation, arrhythmias, aggression, depression, fever and conditions associated with the immune system (Balik *et al*., 2006; Hu *et al*., 2003).

Therefore, it is recommended to consume fish at least twice or three times a week (Gama *et al*., 2002; He *et al*., 2002). Fish and fish products are the most important sources of protein for human diet worldwide. This protein, in comparison with other proteins is highly digestible and contains all the essential amino acids at levels required for human diet (Srivastava, 1999). In general, the overall biochemical composition of the fish determines the quality of the fish meat. Therefore, the comparison of the biochemical composition of a certain species with those of the others reveals its dietary and edibility values.

The differences in the biochemical composition of the fish meat may be seen even among the members of the same species, depending on the location, season of catch, age, sex, season of reproduction, part of the body and size. Spawning cycle and nutrition habits are the main factors responsible for the differences. In general, fish contain 66-81% water, 16-21% crude protein, 0.2-2.55% crude fat and 1.2-1.5% mineral (Huss, 1995).

It is vital to study the nutritional values of the fish in order to gain the maximum benefit from fish and fish products. Moreover, if the consumer is informed about the content of the fish he or she buys, this fish may become more popular. In addition, if the nutritional and biochemical characteristics of the fish to be used as dietary source and to be cultured is are revealed, it will be easier to identify their biological structures. There are numerous studies in the literature which explain the biochemical components of most of the fish of commercial value (Puangkaew *et al*., 2005; Tang *et al*., 2008). In Turkey, the biochemical compositions of freshwater fish such as *Capoeta capoeta capoeta*, *Anguilla anguilla*, *Salvelinus alpinus*, *Salmo trutta fario*, *Tilapia zilli*, *Tilapia rendalli*, *Salmo trutta macrostigma*, *Sander lucioperca*, *Cyrinus carpio* and *Oncorhynchus mykiss* have been studied (Halilogluet *et al*., 2002; Aras *et al*., 2003; Celik *et al*., 2005; Uysal *et al*., 2006; Kandemir and Polat, 2007). However, there are few studies on the biochemical compositions of saltwater fish (Bayir *et al*., 2006; Erkan and Ozden, 2007; Çelik, 2008). Farkas and Herodek (1967) reported that the fatty acid composition of pike, which is at the top of the freshwater food chain was not different from those of other predators.

Another study examined the fluctuations in the levels of polyunsaturated and saturated fatty acids in eggs and larvae and changes occurring in the lipid class during pike growth (Desvilettes *et al*., 1997). In addition, Kluytmans and Zandee (1973), Kucska *et al*. (2005) and Jankowska *et al*. (2008) studied the fatty acids of pike and the factors affecting them. However, although there are studies on the nutritional content and reproduction of pike, which lives in many lakes and rivers of Turkey, there are no studies conducted on the subject of this current study (Yilmaz and Polat, 2005; Cubuk *et al*., 2005).

Pike is a carnivorous fish of high economic importance (Nilsson and Bronmark, 1999). This fish species, which can tolerate a wide range of environmental conditions prefer temperate or cold waters with a depth of <21 m (Casselman and Lewis, 1996). Pike, which lives in many lakes and rivers of Turkey is an important species in Lake Ladik both for commercial fishing and general fishing activities. The interviews conducted with local fishermen revealed that the annual amount of fish caught in the lake was 100-150 tons and that the majority of this fish was *Esox lucius*. Unfortunately, the population of this fish, which is an important source of income for the local fishermen has dramatically decreased in the recent years (Ugurlu *et al*., 2009). Works on increasing the population with semi and full controlled fry production have been in progress. If these works succeed, the biological efficiency of the lake will increase and *Esox lucius* will regain its former population.

The aim of this study is to determine the basic biochemical nutrition composition of pike, which is of economic importance. We also aim to determine the PUFA requirements of the stock to be produced within the framework of the abovesaid works on increasing the population and to acquire preliminary information on diets to be provided during growth and culturing of pike.

MATERIALS AND METHODS

Region of study: The region studied in this study is situated in the southwest of Samsun (35°40´-36°05´ eastern latitude -40°50’-41°00’ northern longitude). Lake Ladik has a drainage basin of 141.40 km² and is 867 m above the sea level. It is 2.5-6.0 m deep (DSI, 1997). Küpecik and Çakirgümüþ brooks maintain water to the lake. Tersakan brook on the other hand brings away water and helps to control the water level of the lake (Fig. 1).

Sampling: The fish samples used in this study were caught in February, 2008 in Lake Ladik. The samples provided were immediately brought to the laboratory and were kept in a deep freeze at -30°C until they were studied (Kluytmans and Zandee, 1973). Bones were removed from the muscles and muscles were then cut into smaller pieces. These pieces were placed in a blender which had been sterilized and subsequently blended for 30 sec for homogenization.

Lipid exraction: Lipid extraction was conducted according to Hara and Radin (1978) method. For the purpose, 0.5-1 g tissue sample was homogened for 30 sec in the mixture of 5 mL hexan-isopropanol with the ratio 3:2 (v/v). Then, the homogeneration container was washed with 2 mL disrupted solution and taken into the sanrafuge tube. Later, the supernatant part taken from the santrafuged tissue samples were placed into closed experiment tubes in 4500 rpm in 10 min.

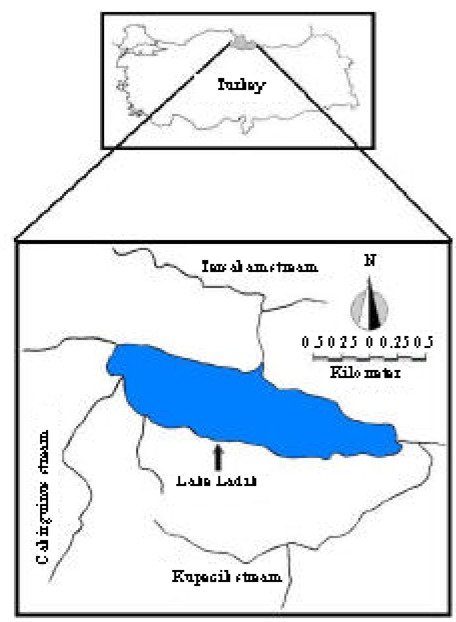

Fig. 1: Map ofLake Ladik

Preparation and analysis of fatty acid methyl esters: In order to conduct the gas chromatographic analysis of the fatty acids found in lipids, they were transformed into methyl esters which are non-polar and stable. Afterwards, the mixture containing methyl esters was placed under a nitrogen flow of 45°C and was dissolved with 1 mL hexane (Christie, 1990). After transforming the fatty acids present in the lipid extract into methyl esters, they were analyzed with SHIMADZU GC 17 Ver. 3. Calculations were made using Class VP software (6.12 SP 5).

Vitamin D, E and K analyses: About 0.3 g of tissue sample was weighed and homogenized for 1 min with 2 mL of acetonitrile/methanol (3/1, v/v). A mixture of 75% acetonitrile and 25% methanol was used as mobile phase in the HPLC analysis. The flow rate for the mobile phase was set at 1 mL min^{-1}. The temperature of the analytical column was fixed at 40°C. The detection wave length was measured at 215 nm for E, D2, D3, K1 and K2 vitamins. The amount of D, E and K vitamins were measured as μg g^{-1} tissue..

Analyses of cholesterol in lipid compound: About 0.3 g of tissue sample was weighed and homogenized for 1 min with 2 mL of acetonitrile/isopropanol (70:30, v/v). About 1 mL of the supernatant part was transferred to the auto sampler vials to conduct HPLC analyses.

Column temperature was fixed at 40°C . Supelcosil LC 18 DB (250×4.6 mm, 5 μm) was used for cholesterol analysis. The analysis was conducted in a UV detector and the detection wave length was measured at 202 nm. The cholesterol amount was calculated as mg g^{-1} tissue (Bragagnolo and Amaya, 2003; Katsanidis and Addis, 1999).

Statistical analysis: Statistical analysis was carried out by using the SPSS for Windows, Ver.13 (SPSS Inc. Chicago, IL, USA). The description of the data are given as mean±Standart Deviation (SD) in the Table 1 and 2. Then, ANOVA Mann-Whitney U model ($p<0.05$) was run to test data.

RESULTS AND DISCUSSION

The pike used in the study had an average length of 64.75 cm and weight of 1457 g. The study involved 10 samples in total. Table 1 and 2 show the fatty acid profiles, w-3/w-6 proportions and cholesterol and vitamin

Table 1: Fatty acid profiles in different tissues of *Esox lucius* (%)

Fatty acids	Tissue	Liver	Gonad	Kidney
14:00	0.79±0.84^{a}	1.02±0.56^{a}	1.19±0.24^{a}	0.88±0.18^{a}
15:00	-	0.43±0.13^{a}	0.64±0.15^{b}	0.48±0.05^{a}
16:00	20.02±1.32^{a}	17.97±1.23^{ab}	18.08±2.33^{ab}	13.91±0.60^{b}
17:00	-	0.43±0.09^{a}	0.58±0.04^{b}	0.78±0.09^{c}
18:00	6.63±0.70^{a}	3.18±0.46^{ab}	3.98±1.05^{ab}	7.69±0.54^{b}
∑SFA	27.45±1.32^{a}	23.05±1.03^{b}	24.49±1.28^{b}	23.76±1.12^{b}
16:01	3.02±0.66^{a}	8.06±1.04^{b}	5.60±0.58^{ab}	5.12±0.73^{ab}
17:01	-	0.92±0.20^{a}	0.39±0.07^{b}	0.69±0.07^{c}
18:1n-7	3.67±0.39^{a}	2.80±0.18^{b}	2.36±0.54^{b}	4.10±0.06^{ab}
18.1n-9	8.89±0.90^{a}	15.41±1.78^{b}	8.56±2.13^{a}	13.04±0.48^{b}
20:1n-9	-	0.16±0.07^{a}	0.14±0.09^{a}	0.72±0.06^{b}
20:1n-11	-	0.14±0.10^{a}	0.32±0.11^{b}	0.16±0.10^{a}
22:1n-9	-	-	0.78±0.23	-
24:1n-9	-	0.45±0.08^{a}	0.16±0.13^{b}	0.22±0.06^{ab}
∑MUFA	15.59±0.98^{a}	27.82±0.89^{b}	18.19±0.36^{ab}	24.07±0.10^{c}
18:2n-6	2.30±0.50^{a}	2.03±0.45^{a}	1.83±0.78^{a}	1.57±0.25^{a}
20:2n-6	-	0.30±0.12^{a}	0.28±0.08^{a}	0.39±0.04^{a}
20:4n-6	5.79±0.44^{a}	7.24±0.13^{b}	9.71±0.21^{ab}	10.09±0.96^{ab}
22:2n-6	0.92±0.12^{a}	1.08±0.71^{a}	1.27±0.31^{a}	1.34±0.07^{a}
22:3n-6	-	-	0.19±0.05	-
22:4n-6	-	1.36±0.55^{a}	1.50±0.23^{a}	1.27±0.29^{a}
22:5n-6	2.05±0.44^{a}	1.01±0.34^{b}	1.17±0.22^{b}	1.10±0.22^{b}
∑n-6	11.08±0.49^{a}	5.95±0.23^{b}	15.62±0.63^{ab}	15.78±0.32^{ab}
18:3n-3	2.04±0.31^{a}	1.71±0.68^{b}	1.42±0.77^{b}	1.23±0.32^{b}
18:4n-3	-	1.36±0.57^{a}	0.65±0.15^{b}	0.44±0.05^{ab}
20:5n-3	9.58±1.11^{a}	6.67±1.22^{b}	11.35±1.25^{b}	8.52±0.72^{b}
22:5n-3	3.38±0.62^{a}	2.05±0.37^{b}	2.55±0.63^{b}	2.32±0.22^{b}
22:6n-3	40.19±2.47^{a}	24.69±2.27^{b}	21.25±2.35^{b}	24.27±1.03^{b}
∑n-3	55.20±1.44^{a}	36.50±0.89^{b}	37.2463±1.32^{b}	36.81±1.02^{b}
PUFA	66.29±2.03^{a}	42.45±1.26^{b}	52.594±1.68^{ab}	52.87±1.96^{ab}
n3/n6	4.98	6.13	2.33	2.38
EPA/DHA	0.23	0.27	0.53	0.35
EPA+DHA	49.77	31.36	32.60	32.79
MUFA/PUFA	0.24	0.66	0.35	0.46
PUFA/SFA	2.01	1.58	1.52	1.54
EPA/AA	1.65	0.92	1.16	0.84

Table 2: Amounts of vitamins ($\mu g\ g^{-1}$) and cholesterol ($mg\ g^{-1}$) in different tissues of *Esox lucius*

Vitamins and cholesterol	Tissue	Liver	Gonad	Kidney
K1	9.048±0.56[a]	5.30±0.23[b]	15.58±0.92[ab]	4.06±0.12[b]
K2	3.86±0.83[a]	12.78±0.79[b]	31.70±0.90[ab]	2.70±0.02[c]
D2	2.56±0.02[a]	13.10±0.74[b]	19.10±0.91[ab]	4.14±0.37[a]
D3	7.32±0.50[a]	26.25±1.16[b]	23.08±1.09[b]	9.27±0.78[a]
α-tokoferol	24.11±0.87[a]	130.62±1.19[b]	212.34±1.24[ab]	121.72±1.29[c]
Total cholestrol	1464.30±1.25[a]	3200.20±1.32[b]	4721.71±1.29[ab]	4708.45±1.38[ab]

values of these samples. We detected and evaluated a total of 25 fatty acids in the muscles, liver, gonad and kidneys of the pike we studied. As shown by Table 1, the dominant fatty acids found in the tissues of pike are 22:6n-3 (DHA), 16:0, 18:1n-9, 18:0, 20:5n-3 (EPA), 20:4n-6 (AA), 16:1, 18:1n-7, 18:2n-6 and 18:3n-3, respectively.

Palmitic acid (16:0), the predominant SFA (Saturated Fatty Acid) had a percentage of 77% and the difference between the tissues was significant ($p<0.05$). Stearic acid (18:0) was the second important SFA with a percentage of 13% and was followed by a 4% myristic acid (14:0). The comparison of these two saturated fatty acids revealed that the difference between the tissues was insignificant ($p>0.05$) for myristic acid and that the difference for stearic acid was significant ($p<0.05$). Another result shown in Table 1 is that 14:0 is found in more amounts in muscles and liver. Also, there are more 18:0 in muscles and kidneys compared with other saturated fatty acids. However, there is no significant difference ($p<0.05$) between the muscle tissue and other tissues with regard to the total SFA values.

It is known that in most bony fish, palmitic acid is the dominant total SFA in all tissues (Ashton *et al*., 1993). It is also reported that palmitic acid has an average 60% share among SFAs (Chen *et al*., 1995). The findings indicate a 77% share, which complies with the literature. In a study conducted by Haliloglu *et al*. (2002) to compare the muscle tissues of three different varieties of trout (*Salvelinus alpinus*, *Salmo trutta fario* and *O. mykiss*) a significant difference was found between the species. This study revealed that palmitic acid was the dominant total SFA with 60% followed by stearic and myristic acids, respectively.

In the study conducted by Aras *et al*. (2003) to compare fatty acid compositions of different tissues of *S. trutta macrostigma*, it was found that of all the SFA, palmitic, stearic and myristic acids had the highest amounts, respectively and that there were significant differences between the tissues. Similar results were also reported for zander and other freshwater fish. It is stressed in these studies that palmitic acid is the key metabolite in fish and that its level is affected by the diet of the fish (Celik *et al*., 2005; Jankowska *et al*., 2008). From these studies, it can be deduced that palmitic acid is dominant in freshwater fish. The study confirms these results as well (Table 1). Of all the MUFA (Monounsaturated Fatty Acid), oleic acid (18:1n-9 fatty acid) has a percentage of 55.

The difference between the muscles and gonad was insignificant ($p>0.05$) but the difference between these tissues and other tissues was significant ($p<0.05$). Liver had the highest value with 15.4±1.78% and gonad had the lowest value with 8.56±2.13%. Thus, it is apparent that oleic acid is the major MUFA in pike.

Similarly, Haliloglu *et al*. (2004) reported that 18:1n-9 was the dominant MUFA in rainbow trout. It is reported that high levels of oleic, palmitoleic and arachidonic acids is characteristics of the fats of freshwater fish (Osman *et al*., 2001). Results of numerous studies conducted on the subject are parallel to the results (Harel and Woods, 1995; Aras *et al*., 2003). Phospholipids of many freshwater species contain high levels of PUFA and SFA; whereas, they contain less MUFA compared with neutral lipids (Henderson and Tocher, 1987). Jankowska *et al*. (2008) report that 18:1n-9 is dominant in muscle tissues of *Esox lucius* with a percentage of 8.62±0.37. In the study, we found similar results with theirs in the gonad and muscle tissues but we found higher levels in liver and kidneys (Table 1). These results confirm that the elongation and desaturation effective in freshwater fish are more active in these tissues (Skuladottir *et al*., 1990). Regarding the fact that the sampling coincides with the reproduction season, it is apparent that MUFA is used for energy purposes.

It was found that PUFAs were lowest in liver with 42.45% and highest in muscle tissue with 66.29%. DHA, EPA, LA (Linoleic acid) and AA (arachidonic acid) are the dominant PUFAs. Data indicate that DHA comprises 21.25-40.19% in all tissues and that muscle tissue has almost twice as much DHA. Similarly, Jankowska *et al*. (2008) found that DHA was the predominant fatty acid in muscle lipids of wild zander. Sargent (1996) reported that n-3 PUFA essentially served for the structural and functional integrity of DHA in fish cells.

Since DHA is the main component in brain, eye retina and heart muscles, it is reported that DHA plays a crucial role in eye and brain development and cardiovascular well-being. EPA on the other hand is reported to play an important role in brain diseases and cancer treatment (Fenton *et al*., 2000). The main characteristic difference between the freshwater and salt water fish is that the former have high levels of C-16 and C-18 acids and low levels of C-20 and C-22 acids. The main reason for this difference is the diet fats (Ackman, 1967). However, freshwater fish contain high amounts of EPA and DHA (Wang *et al*., 1990). The PUFA content of the pike living in Lake Ladik was higher than those reported by Uysal and Aksoylar (2005) for zanders livin in Lake Eğirdir, the second biggest freshwater lake of Turkey. This difference primarily springs from DHA content. The PUFA content of the fish we studied is similar to those found in zanders living in Lake Beyşehir, one of the biggest freshwater lakes of Turkey (Guler *et al*., 2007).

The data we collected shows that the short chain n-3 fatty acid present in the diet of pike was elongated and that it was desaturated. By doing so, it plays a crucial role in forming PUFAs, particularly DHA. They are as effective as 18:3 n-3, 20:5 n-3 (EPA) or 22:6 ω-3 (DHA) which are essential for freshwater fish such as *Oncorhynchus mykiss*. This results from the fact that the metabolisms of these fish are able to synthesize the 20:5 n-3 and 22:6 n-3 they need from 18:3 n-3 (Kanazawa, 1985). The fact that the amounts of EPA and DHA are higher than linolenic acid in the study indicates that pike is highly competent in transforming 18:3 n-3 to long chain fatty acids. Moreover, it can be deduced that carnivorous fish like pike feeding on other fish for which elongation and desaturation are completed are rich in long chain n-3 PUFA and poor in linolenic acid (Table 1).

The difference between the proportions of n-3 PUFA was significant ($p<0.05$). With this respect, the muscle had the highest proportion with 55.20% and the liver had the lowest proportion with 36.50%. The results obtained for other tissues were close to that obtained for the liver. There were significant differences between the tissues with regard to n-6-PUFA ($p<0.05$). The kidney and the gonad had the highest values with 15.78 and 15.62%, respectively which were followed by the muscle tissue with 11.08%. The lowest value was detected in the liver with 5.95%. The proportions of n-3 PUFA were approximately three times as much as those of n-6 PUFA present in muscle and liver and twice as much as those found in gonad and muscle (Table 1)

The proportion of n-3/n-6 has been suggested to be a safe indicator to compare the nutritional values of the fish fat (Osman *et al*., 2001). The study of the n-3/n-6 proportion in pike revealed that this proportion was the highest in liver with 6.13%, which was followed by the muscle with 4.98%. There was a significant difference with other tissues ($p<0.05$). These results are in compliance with those found by Zmijewski *et al*. (2006) for *Esox lucius*. For the well-being of human health, the consumption of fish and fish products rich in n-3 PUFAs and poor in n-6 PUFAs is essential. Dieticians are working on developing a diet which has a n-3/n-6 proportion of approximately 1. This finding is important both for human health and dietary preferences of the people. This proportion is 1/15-17 in the diets of the Western societies. The low proportion of n-3/n-6 PUFA increases the pathogens of numerous diseases such as cardiovascular diseases and inflammation (Simopoulos, 2002). Enzyme activity and chain prolongation which enable desaturation reduces n-3 PUFA and increases n-6 PUFA in freshwater fish compared with saltwater fish. This is the reason behind the low proportion of n-3/n-6 in freshwater fish (Aras *et al*., 2003). The proportion of n-3/n-6 indicates that liver and muscle have the highest proportions while gonad and kidney have the lowest proportions. The differences between the tissues are significant ($p<0.05$).

The results revealed that high proportions of n-3/n-6 are particularly found in liver and muscle and that the amounts of n-3 and n-6 are approximately at the level advised (Table 1). Haliloglu *et al*. (2004) in his study of adult rainbow trout found a n-3/n-6 proportion close to 1. In contrast to the general expectations, they explained the high proportion of n-3/n-6 with high altitude and the characteristics of n-3, which plays a role in the adaptation process depending on long periods of winter. Many researchers found similar results which are parallel to ours (Jankowska *et al*., 2008; Zmijewski *et al*., 2006; Kucska *et al*., 2005). The fact that Lake Ladik is found in a region where winters are long may play a role in higher n-3 compared with n-6.

Moreover, numerous studies conducted on the nutritional benefits of the fatty acids underline that maximum growth, protection against diseases and food conversion of the fish depend on the intake of n-3 series of the linolenic acid family (Watanabe, 1982). Castel *et al*. (1972) found out that *Salmo gairdnerii* needs 1% of 18:3 w3. They reported that if 18:3 n-3 is given 1% in average without comparing 18:2 n-6 and 18:3 n-3, an improvement in food conversion and growth can be achieved. In another study, linolenic acid in *Esox lucius* was detected at 1 mg/100g (Fritche *et al*., 1999). The results indicate that in all tissues, 18:3 n-3 is higher than 1% and that this percentage is sufficient for food conversion and growth of pike. Furthermore, higher level of 18:2 n-6 may result from the characteristics of fats found in freshwater fish (Table 1).

Normally, the w-6 fatty acid contents of freshwater fish are higher than those of saltwater fish. However, in the species we studied, the n-3 fatty acids are higher than n-6 fatty acids. These results are in compliance with those found by Jankowska *et al*. (2008), Zmijewski *et al*. (2006)

and Kucska *et al*. (2005) for pike. Fish have adapted themselves to the changes in temperature in the habitats they live in thanks to the regulating role of the lipids In membrane low (Kozlova and Khotimchenko, 2000). Highly unsaturated fatty acids, such as DHA are effective molecules to sustain the constant flow in body temperature thanks to the low levels of melting (Farkas and Herodek, 1967; Hall *et al*., 2002). Therefore, cold water fish need more n-3 fatty acids compared with fish living temperate waters. Accordingly, fish living in cold water contain higher levels of PUFA.

In the study, the highest amount of EPA + DHA (49.77%) was detected in the muscle with 132.5 mg/100g and the difference with the other tissues was significant ($p<0.05$). This difference was detected for EPA and DHA separately as well. Zmijewski *et al*. (2006) detected the total amount of EPA + DHA as 87.48 mg/100g in the carcass of *Esox lucius*, while Jankowska *et al*. (2008) found it as 34.63%. Yet, Kucska *et al*. (2005) found EPA + DHA content at 23.26%. In accordance with these studies and the results we had for *Esox lucius* living in Lake Ladik, we observed that muscle tissue had more EPA + DHA. The significance of EPA and DHA which are crucial both for fish and human health spring from the fact that EPA plays a role in protecting cardiovascular health and that it is responsible for regulating activities concerning the lipid metabolism in plasma (Leaf and Weber, 1988). Researchers stress that DHA, an provitamin A in larvae is important in the development of brain and retina and survival in larval phase.

They also report that deficiency of DHA lead to many conditions such as abnormal behaviour insufficient levels of pigment and impaired eyesight. In humans, it is reported to play a role in regulating blood pressure, stimulating nerves and reducing the risk of cholesterol by decreasing the level of triglyceride (Stoll and Andrew, 1999). Rice (1996) recommends a daily intake of 200-1000 mg of EPA and DHA, while Simopoulos (1991) recommends 800-1100 mg of linolenic acid (18:3 n-3) and 300-400 mg of long n-3 PUFA. Another study recommends a 0.5 g day^{-1} intake for babies to promote growth and 1 g day^{-1} intake for adults to prevent heart diseases (Kris-Etherton *et al*., 2002). The high level of EPA + DHA in muscle tissues of pike presents it as an important species in human diet and health. In the light of the amounts recommended, we believe that daily EPA + DHA can be met by daily consuming 400 g of pike.

As shown in Table 1, the proportion of EPA/AA is higher in muscle and gonad compared with liver and kidney and the difference between the tissues is significant ($p<0.05$). AA always plays a role in synthesizing eicosanoids, prostaglandins and leukotriens. Eicosanoids produced out of AAs have adverse cardiovascular effects such as vasoconstrictions and thrombocyte conglomeration. EPA, on the other hand has a positive effect in preventing vasoconstrictions and thrombocyte conglomeration.

Therefore, researchers recommend decreasing AA and increasing EPA in diets (Nordoy *et al*., 2001). The proportions of EPA/AA in muscle, liver, gonad and kidney range between 0.84-1.65. The fact that the proportions of EPA and PUFA in muscles of pike are higher than those of AA and SFA, respectively proves this species as a healthy nutritional source. As Table 2 shows, there are significant differences between the amounts of vitamins and total cholesterol found in tissues ($p<0.05$).

Vitamin K: Vitamin K, a fat soluble vitamin well-known for its effect in blood clotting plays a role in bone protein metabolism and vascular biology. This vitamin is found in three forms as K1, K2 and K3. Vitamin K1 and K2 are synthesized by green plants and bacteria, respectively. K3 is a synthetic form. In spite of the fact that the micro flora of the lower intestine in land animals can produce Vitamin K2, the micro flora which synthesizes Vitamin K in fish is not isolated. However, all three forms of Vitamin K are biologically active in most animals and fish. Information on requirement, symptoms of deficiency and metabolism of Vitamin K in fish is limited (Lall, 2005). Quantitative Vitamin K requirement has not been set for most fish (NRC, 1993). But, Vitamin K1 and K2 are actively found in pike.

The highest levels for Vitamin K1 are found in gonad and liver (15.58±0.92 μg g^{-1} and 9.04±0.5 μg g^{-1}), respectively and a significant difference is found between them ($p<0.05$). It is found in lower amounts in muscle and kidney and there is no significant difference between them ($p>0.05$). There are differences between the organs with respect to vitamin K_2, as well. The estimates amounts of Vitamin K required for the growth of salmon and Atlantic cod are 10 and 0.2 mg kg^{-1}, respectively (Halver, 2002). In addition, there is little information on the role of vitamin K in the development of abnormalities of skeleton. Roy (2002) reported that dietary vitamin K supplementation did not have any detectable effect on the amount of bone matrix in haddock. He also stressed its effect on bone health. The comparison of such studies with ours reveal that pike has a sufficient vitamin K content. In the light of this information, it can be argued that if bone diseases of cultured fish are minimized, it will be possible to breed larvae and adult fish which have less growth abnormalities and higher rates of survival.

Vitamin D: Vitamin D is a fat soluble vitamin which has an important role in sustaining the normal level of calcium and phosphate in blood and in normal mineralization of bones (Trivedi *et al*., 2003). Moreover, it is very effective in soothing certain mental conditions and in preventing neurological diseases and cancer (Garcion *et al*., 2002; Holick, 2004). Vitamin D is naturally present in few foods. Common sources of vitamin D are fish oil and fish liver oil (Pigott, 1996). In spite of the fact that the synthesis Vitamin D2 has similar biological activities with Vitamin D3, there has been recent evidence which indicate that in humans, Vitamin D2 is less effective in increasing the circulation level of 25-hydroxyvitamin compared with Vitamin D3 (Armas *et al*., 2004).

We observed that both Vitamin D2 and D3 are active in the species we studied (Table 2). It is estimated that Vitamin D3 may play an important role in larval growth, calcium and phosphate metabolism, growth and re-shaping of bones. However, the physiological role of Vitamin D in fish has not been identified. Vitamin D3 is the primary form of stock in livers of sea teleosts and it was reported that 25-hydroxyvitamin can be converted into 25-hydroxyvitamin Vitamin D3 isomers in various tissues (Takeuchi *et al*., 1991). Graff *et al*. (2002) showed that dietary 1.25 hydroxivitamin D3 had no effect on the bone formation of Atlantic salmon. However, despite all these studies, there is little information on the effects of Vitamin D3 in fish. In recent studies, it was reported that vertebral impairment and hypermelanocyte arose when the level of Vitamin D3 in the food of juveniles of Japanese sole was below 5 $\mu g\ g^{-1}$ (Haga *et al*., 2004).

The results show that it is highest in liver (26.25E $\mu g\ g^{-1}$) and gonad (23.08 $\mu g\ g^{-1}$), respectively. There is no significant difference between the liver and the gonad ($p>0.05$). The muscle and the kidney had 7.32 and 9.27 $\mu g\ g^{-1}$, respectively. The comparison of the results with those of previous studies reveal that *Esox lucius* living in Lake Ladik have a great amount of D3 source and that symptoms arising out of this vitamin do not apply to this species. Takeuchi *et al*. (1991) found out that Vitamin D3 was the stock form in the livers of sea teleosts. In view of this finding, we believe that such is also the case for the liver of *Esox lucius* (Table 2).

It is known that 7-DCH, which spontaneously develops in the livers of adult fish, turns into Vitamin D3 both in light and darkness (Holick, 2003). Fish living close to daylight can achieve such conversion. Pike prefer living in shallow and planted parts of the lakes they live in (Chapman and Mackay, 1984), which can contribute to the synthesis of Vitamin D3, which is found in high amounts in this fish. However, it was observed that cultured fish larvae living in cages could not achieve this conversion in the absence of UV-light and thus needed Vitamin D3 (Takeuchi *et al*., 1987). Although, the metabolic ways of Vitamin D isomers, which are analogues of retinoic acid are not known, it was reported that they could be added to the food of the fish (Hamre *et al*., 2007).

Vitamin E: Vitamin E is known to be essential for skeletal growth; particularly for the combat against endogenous and exogenous free radicals which can impair osteoblasts and stimulate osteoclasts (Arjmandi *et al*., 2002). The main role of Vitamin E is to reduce the peroxy radicals in membrane lipids and to prevent the chain reaction pioneering lipid peroxidation. Therefore, it is reported that Vitamin E is essential for the growth of all tissues including bone and cartilage tissues (Lall and Lewis-McCrea, 2007). We observed that α-tocopherol content in all tissues of pike ranges between 80.3 and 707.8 $\mu g\ g^{-1}$. Gonad has the highest amount with 707.8 $\mu g\ g^{-1}$ and muscle tissue has the lowest amount with 80.3 $\mu g\ g^{-1}$.

This difference between the tissues is significant ($p<0.05$). The vital role of Vitamin E in the reproductive physiology of both fish and other living organisms has been set forth. Gupta *et al*. (1987) reported that inclusion of Vitamin E in adult rations of *C. carpio* increased the gonadosomatic index. They also reported that Vitamin E supplementation caused the ovary of *C. carpio* to grow and subsequently to produce more eggs. The results of this study and high amounts of Vitamin E found in the study are parallel to each other. We believe that higher amounts of α-tocopherol, a precursor of Vitamin E, detected in gonad results from the fact that sampling process coincided with the reproduction season of the fish. It has been proved that the α-tocopherol content inhibits the lipid oxidation and has a positive effect on discoloration (Yang *et al*., 2002).

The increase in PUFA fatty acids, which results from excessive intake of lipids, raises the Vitamin E requirement and its effective utility by lipid supplementation to the food. As reported by many researchers, there is a strong correlation between the PUFA and α-tocopherol requirement and α-tocopherol directly necessitates PUFA consumption (Horwitt *et al*., 1961). However, the results do not comply with these findings. The reason is that the least amount of α-tocopherol was detected in muscle tissue in which the highest amount of PUFA was detected. This finding leads us to think that α-tocopherol is mobilized to gonad from other tissues in the reproduction period. Besides, we have a great number of data on this. Watanabe *et al*. (1970) and Watanabe and Takashima (1977) studied whether the decrease in food led to a decrease in α-tocopherol and observed in their experiments, an insufficient level of nutrition in muscles. They named this condition as sekok disease, characterized by a decrease in flesh on the back of the fish. They deduced that this condition resulted from insufficient levels of α-tocopherol. In addition, they showed lack of fats burning as another reason (Watanabe *et al*., 1970). *Salmo gairdnerii* which fed on food lacking sufficient α-tocopherol lost weight and when fed with food lacking α-tocopherol, inadequate growth, a decrease in food conversion and high rate of mortality were observed. This, however was inverted with a 5 mg α-tocopherol supplementation.

Sinnhuber *et al*. (1968) reported that a 5%-addition of highly oxidized trout fat had an adverse and toxic effect on *Salmo gairdnerii* and that this could be prevented by Vitamin E supplementation. Again, reported that α-tocopherol requirement for *C. carpio* was 10 mg g^{-1} and that this amount was to be supplemented to the food of the fish. They also underlined that food used in fish farms should not involve oxidized lipid and be supported with certain amounts of α-tocopherol. Vitamin E deficiency in fish lead to abdominal dropsy, exophthalmos, anemia, impaired growth, agglomeration in gills, decrease in hemoglobin and hemotocrit decrease in erythrocyte, decraesing in erythrocyte, edema in serous liquid and outer of the kidney membrane, spleen and liver. Moreover, immune system is impaired. To prevent these symptoms and diseases, food given to fish should be supplemented with 500 mg kg^{-1} of α-tocopherol (Roem *et al*., 1991).

Cholesterol content: When we examined the cholesterol content of the samples, we observed that it was highest in gonad and kidney and lowest in muscle tissue. While there was no difference between the gonad and the kidney, there were significant differences among other tissues ($p<0.05$). The amount of cholesterol found in tissues ranges between 146.4-472.1 mg/100g. Uysal *et al*. (2008) found out that the difference between three different freshwater fish with respect to the amount of cholesterol found in muscle tissues was insignificant and detected the level of cholesterol between 103.4-150.1 mg/100g.

Celik (2008) on the other hand found the amount of cholesterol as 35.04 mg/100 g for *Oncorhynchus mykiss*. Imre and Saglik (1998) reported the cholesterol amount for saltwater fish as 40.3-75.3 mg/100 g. Numerous studies conducted by Moreira *et al*. (2001) on the cholesterol content of many freshwater fish showed that the values ranged between 40.99 and 52.79 mg/100 g. Romeo detected the cholesterol amount of *Trachurus murphyi, Sardinops sagax, Oncorhynchus kisutch and Thunnus alalunga* as 87 mg/100 g. The comparison of these results and those of the study reveal that *Esox lucius* has higher cholesterol content. It was deduced that the cholesterol content of the muscle of fish was affected by the PUFA content and that the increase in PUFA content led to a decrease in cholesterol level (Kinsella, 1986). This report confirms the correlation we found between the w-3-PUFA and cholesterol contents of the muscle tissues we studied. High levels of cholesterol detected in other organs can be explained with low levels of n-3-PUFA (Table 2). Luzia *et al*. (2003) reported that freshwater fish had less cholesterol content compared with saltwater fish and that freshwater fish are more beneficial for human health. WHO (1987) recommends a daily allowance of maximum 300 mg cholesterol. Thus, it can be argued that many fish species mentioned in this study including the species we studied are convenient sources of cholesterol.

CONCLUSION

Consequently, pike, an important item of income and a commonly consumed food for local people is a significant species as it contains EPA, DHA, Colesterol amount, D, E, K Vitamins and n-3/n-6 ratio and it is existed in different tissues with an advised amount. Moreover, the fact that its muscle tissue contains less cholesterol compared with those of other tissues highlights it as a healthier alternative to the saltwater fish.

RECOMMENDATIONS

Modern production methods should be provided for *Esox lucius*, which is found in many lakes of Turkey in order to increase its population. To this end, qualified fish rich in w-3 should be bred. Nutritional requirements of the population, such as PUFA requirement should be considered in these efforts. Chemical contents of the food given to fish should be determined in order to introduce many fish species as economically important ones.

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