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Fatty Acid Composition, Fat Soluble Vitamins and Cholesterol Content of Farmed Rainbow Trout (*Oncorhynchus mykiss*)

Ayşe Gül Harlioğlu*
Fisheries Faculty, Fırat University, 23119, Elazığ, Turkey

Abstract.- In this study, fatty acid composition, fat soluble vitamins (retinol, D_2, D_3, δ-tocopherol, α-tocopherol, K_1, K_2) and cholesterol content of farmed rainbow trout (*Oncorhynchus mykiss*) were investigated. The results show that there were significant differences in saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) contents between trout muscle and diet ($P<0.01$ and $P<0.05$, respectively), and in SFA, PUFA, and monounsaturated fatty acids (MUFAs) contents between the trout liver and diet ($P<0.05$, $P<0.001$, $P<0.001$, respectively). However, no significant differences were found in the MUFAs between the muscle and diet. Docosahexaenoic acid (22:6 n-3, DHA) was the predominant fatty acid in the muscle and liver of trout. The ratio of n-3/n6 PUFA at 1.1 indicates the availability of n-3 PUFA that is beneficial for human health. Among the vitamins analyzed, vitamin E content was the highest in the muscle. It can be concluded that the farmed *O. mykiss* are good to human body containing rich PUFA (38.69 %) and vitamins (retinol, D_2, D_3, δ-tocopherol, α-tocopherol, K_1, K_2, 12.4, 1, 13,2, 58, 714, 626, 392 µg/ 100 g, respectively) with low-level cholesterol (40.2 mg/100 g).

Key words: *Oncorhynchus mykiss*; rainbow trout; fatty acid; cholesterol; fat-soluble vitamins; muscle; liver.

INTRODUCTION

Lipids are important components of fish diets due to their role in providing energy and essential fatty acids, as carries of fat-soluble vitamins, and resource of polar lipid including sterols, which are important structural compounds of cell membranes (Görgün and Akpınar, 2007). Fish lipids are well known to be rich in long-chain n-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Fish do not synthesize the long chain (C_{20} and C_{22}) PUFA and they, or their precursors, must be provided in the diet (Bell, 1998). Thus, essential n-3 and n-6 fatty acids (FA), as well as fat-soluble vitamins are essential compounds of fish lipids and exclusively provided by the diet.

Fish is one of the main sources of vitamins (Cahu *et al*., 2004). Therefore, fish is valuable source of essential fatty acids, vitamins and low levels on saturated fatty acids and cholesterol (Stancheva *et al*., 2010).

Liver and muscle in fish are fat depots, whereby the liver is the main lipid storage organ in the body of many fish species (Kozlova, 1998). Fatty acid composition of fish is highly variable between and within species. Various biotic and abiotic factors have an impact on the quantity and composition of animal's lipids. So, the qualitative composition of lipids differs among species and various organs, tissues and lipid classes within individual animals (Ackman *et al.*, 1980). In culture conditions, diets also represent the major determining factor influencing fatty acid composition with which the aquaculture industry possesses a great tool to beneficially modify the fatty-acid profile of fish (Gonzalez *et al.*, 2006).

Aquaculture is the third component of fisheries production in Turkey (Yılmaz *et al.*, 2008). In recent years, it developed very fast. This sector initiated with rainbow trout culture in the early 1970s. At present, trouts are the main cultured freshwater fish species in Turkey. Trout production is approximately 60% of the total fish production obtained from aquaculture.

Raceways and floating cages are employed in culture of trout (Harlıoğlu, 2011). In addition, minor attempts were carried out in terms of sea farming until 1985 beginning with gilthead sea bream and sea bass culture in Aegean Sea (Aydın *et al.*, 2005). However, in recent years both freshwater and sea farming have an increasingly important role in the production of fishery products (Harlıoğlu, 2011).

* Corresponding author: aharlioglu@firat.edu.tr
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The first rainbow trout farm was set up in the Marmara region in 1971. The total production of trout in Turkey was approximately 58,000 tons in 2007 (Anonymous, 2007) from a total of 880 rainbow trout farms; about 6,000 tons (10.3%) was cultivated in 94 of the farms (10.7%) (Yıldız *et al*., 2009). This fish is preferred because of its good taste and suitability to water temperatures of the rivers and reservoirs in Turkey. Thus, trout production was increased to 65 928 tons in 2008 (Harlıoğlu, 2011). Producers of trout are mainly concentrated in the Aegean region where approximately 25% of the total production is obtained.

The Keban Dam Lake reservoir is the fourth largest lake in Turkey. It has a very good potential to produce fish species, such as trouts. The yearly farmed trout production in Elazığ city has increased from 70 ton in 1999 to 8360 tons in 2009 (Gökhan, 2010). Moreover, an increase in trout production is expected because of the capacity increments of trout farms in the city.

The quality of cultured rainbow trout. Therefore, the aim of present study is to evaluate the composition and content of the fatty acids in the liver and muscle and fat-soluble vitamins (retinol, D_2, D_3, δ-tocopherol, α-tocopherol, K_1, K_2) and cholesterol in the muscle of farmed rainbow trout.

MATERIALS AND METHODS

Cultured rainbow trout obtained in the spring of 2011 from a commercial fish farm in Elazığ, Turkey. The water temperature of the fish farm is 12.2±0.4°C. A commercial fish diet has been used for the farmed rainbow trout. The proximate analysis of the pellet contains 44.18% protein, 13.81% fat, 13.14% ashes, and 89.86% dry matter.

Ten specimens of *O. mykiss* an average weight and total length of 300±15 g and 29±1.3cm were transported in ice to laboratory where they were weighted and immediately processed. The muscle and liver of samples were cut out and homogenized separately.

Proximate analysis

Proximate analysis of triplicate samples food were made as follows (Official Methods of Analysis (AOAC), 1995): dry matter, after drying at 105°C for 24 h to constant weight in an oven; proteins were determined by Kjeldahl analyses (nitrogen x 6.25); fat was determined by soxhlet apparatus with petroleum ether; ash content was determined by weighing after burning at 550°C.

Lipid extraction: Fatty acid analysis and the fat soluble vitamins

Lipid extraction was conducted according to Hara and Radin (1978). For the purpose, 1g tissue sample was homogenized with 10 ml hexane-isopropanol mixture with the ratio 3:2 v/v. The homogenate was centrifuged at 5000 rpm for 5 min at 4°C and parts of tissue remnants were precipitated. The supernatant part was used in the A, D, E and K vitamins, cholesterol and fatty acid analysis.

Preparation and analysis of fatty acid methyl esters

Fatty acids in the lipid extracts were converted into methyl esters including 2% sulfuric acid (v/v) in methanol (Christie, 1992). The mixture was vortexed and then kept at 50°C for 12 h. Then, after being cooled to room temperature, 5 ml of 5% sodium chloride was added and then it was vortexed. Fatty acid methyl esters were extracted with 2x5 ml hexane. Fatty acid methyl esters were treated with 5ml 2% $KHCO_3$ solution and then the hexane phase was evaporated by the nitrogen flow and then by dissolving in 0.5 ml fresh hexane (Christie, 1992), they were taken to autosampler vials.

Methyl esters were analyzed with the Shimadzu GC-17 Ver. 3 gas chromatography (Kyoto, Japan). For this analysis, 25 m of long Machery-Nagel (Germany) capillary column with an inner diameter of 0.25 µm and a thickness of 25 micron film was used. During the analysis, the colon temperature was kept at 120-220°C, injection temperature was kept at 240°C and the detector temperature was kept at 280°C. The nitrogen carrier gas flow was 1 mL/min. During the analysis, the colon temperature was kept at 120-220°C and the increment of temperature was 3°C/min. The methyl esters of fatty acids were identified by comparison with authentic external standard mixtures analyzed under the same conditions. After this process, the

necessary programming was done and the Class GC 10 software version 2.01 was used to process the data.

Vitamin A, D, E and K and cholesterol

Five ml supernatant was taken to 25 ml tubes with caps and 5% KOH solution was added and immediately vortexed for 20 s. The tubes were placed in a water bath at 85°C for 15 min. The tubes were then taken and cooled to room temperature and 5 ml of distilled water was added and mixed. Lypophilic molecules that did not saponify were extracted with 2x5 ml hexane. The hexane phase was evaporated with nitrogen flow. It was dissolved in 1 mL acetonitrile/methanol mixture (50+50%, v/v) and then was taken to autosampler vials and analyzed.

The analysis was done with the Shimadzu HPLC device. HPLC conditions were as follows: mobile phase 60:38:2 (v/v/v): acetonitrile/ methanol/water; The mobile phase flow rate was determined to be 1mL A UV detector was used for the analysis and as a column the Supelcosil LC 18 (15×4.6cm 5µm; Sigma USA) column was used. For vitamin E and cholesterol 202nm, retinol, 326nm and for vitamin D and K, 265 nm was used (Katsanidis and Addis, 1999; L'opez-Cervantes *et al.*, 2006).

The analyses were performed using a statistical program (SPSS 15.0). Data are presented as mean ± standard deviation and subjected to correlation test and ANOVA independent-samples t-test for determining significant differences between feed and muscle and between feed and liver fatty acids means.

RESULTS

Fatty acids composition

The fatty acids composition of rainbow trout muscle, liver and feed is given in Table I. The fatty acids analyzed were grouped as SFA and MUFA, while polyenoic fatty acids were grouped as PUFA. The results of present study showed that PUFA was the highest followed by MUFA and SFA in the muscle, but PUFA was the highest followed by SFA and MUFA in the liver.

The results revealed that among the SFAs, palmitic (C16:0) and stearic (C 18:0) acids were the major SFAs in the muscle and liver of trout. In addition, oleic acid (C18:1 n-9) was the predominant FA within the MUFAs in the muscle and liver of trout.

The results also revealed that PUFAs were the highest in the liver with 49.19% and the lowest in the diet with 32.2%. Linoleic acid (C18:2n-6) was the major n-6 PUFA in both liver and muscle. The muscle of trout had significantly higher content of C18:2n-6 than that of the diet, but the amount of C18:2n-6 was significantly lower in the liver than the diet ($P<0.05$). Furthermore, the muscle and liver of trout had significantly higher content of docosahexaenoic acid (C22:6 n-3, DHA) than the diet ($P<0.05$ and $P<0.001$, respectively).

The n-3/n-6 ratio in farmed rainbow trout was the highest in the liver with 4.58, which was followed by the diet with 2.04. It was the lowest in the muscle with 1.10. Therefore, the n-3/n-6 proportion of liver was significantly higher than that of diet ($P<0.01$) and the n-3/n-6 proportion of diet was significantly higher that that of muscle ($P<0.05$).

In addition, the ratio of PUFA to SFA was found to be 1.59 for the muscles of trout.

Fat-soluble vitamins and cholesterol content

The retinol, D_2, D_3, δ-tocopherol, α-tocopherol, K_1, K_2 vitamin contents of rainbow trout muscle are given in Table II.

The cholesterol content of rainbow trout muscle was 40.2±3.36 mg/100 g (Table II).

DISCUSSION

In the present study, PUFAs were the highest followed by MUFAs and SFAs in the muscle. On the other hand, PUFAs were the highest followed by SFAs and MUFAs in the liver. Haliloğlu *et al.* (2004) found similar results for the total PUFAs content in the muscle and liver of rainbow trout in a study on the comparison of fatty acid composition in some tissue of rainbow trout living in seawater and freshwater. Blanchet *et al.* (2005) reported that SFAs were the lowest in the muscle of trout.

In the present study it was found that among the SFAs, palmitic (C16:0) and stearic acid (C 18:0)

Table I.- The fatty acid composition (% of total fatty acids) of diet, muscle and liver of farmed *O. mykiss* [a]

Fatty acids	Diet	Muscle	Correlation (diet and muscle)	Liver	Correlation (diet and liver)
C14:0	4.67±0.13	2.38±0.23***	0.508	1.13±0.21***	0.023
C14:1	0.09±0.01	nd[b]		nd	
C15:0	0.38±0.08	nd		nd	
C16:0	14.13±0.46	15.25±0.70ns	-0.429	17.37±2.52*	-0.308
C16:1 n-7	6.33±0.26	3.99±0.52***	0.236	2.64±0.78***	0.722
C17:0	0.53±0.02	nd		nd	
C17:1	0.51±0.19	nd		nd	
C18:0	3.01±0.15	3.88±0.29***	-0.427	7.38±0.96***	-0.294
C18:1 n-9	22.38±0.25	27.09±2.48*	0.393	16.10±2.44**	0.874
C18:2 n-6	10.26±0.20	17,44±4,6*	0.051	7.60±2.60*	0.452
C18:3 n-3	2.42±0.01	2.48±0.39ns	-0,281	1.35±0.21*	-0.784
C20:0	0.12±0.07	nd		nd	
C20:1, n-9	5.63±0.08	3.37±0.34***	-0.470	1.49±0.76***	-0.846
C20:2 n-6	0.31±0.01	0.95±0.21**	0.947 [c]	1.21±0.34**	-0.189
C20:4 n-3	0.65±0.04	0.77±0.09ns	-0.78	3.66±0.69***	0.728
C22:0	0.94±0.01	0.77±0.04**	0.231	0.7±0.14ns	0.408
C22:1	4.56±0.15	2.09±0.18***	-0,917 [c]	nd	
C20:5 n-3	8.02±0.07	3.12±0.43***	0.310	4.88±0.59***	0.782
C22:2	0.36±0.01	nd		nd	
C24:0	3.66±0.11	1.97±0.32***	-0.736	3.99±0.33*	-0.235
C22:6 n-3	10.54±0.11	13.93±2.8*	-0.064	30.49±3.84***	0.642
ΣSFA	27.44±0.49	24.25±0.89**	-0.202	30.57±2.96*	-0.495
ΣMUFA	39.5±0.14	36.54±3.23ns	0.009	20.23±3.41***	0.355
ΣPUFA	32.2±0.31	38.69±3.23*	0.728	49.19±3.92***	0.555
PUFA/SFA	1.17±0.03	1.59±0.14***	0.635	1.60±0.09***	0.409
Σn-3	21.63±0.18	20.3±2.98ns	0.167	40.38±5.67**	0.609
Σn-6	10.57±0.19	18.39±4.8*	0.123	8.81±2.47ns	0.300
n-3/n-6	2.04±0.03	1.10±0.41**	-0.304	4.58±2.11*	0.413

[a] Data are expressed as mean ± SD (n=10)
[b] Not detected
Level of statistical significance (ANOVA, T test) (p value): ns=p>0.05, * p<0.05, **p<0.01, ***p<0.001
[c] correlation is significant at the 0.05 level

Table II.- The fat soluble vitamins (µg/100 g) and cholesterol (mg/100 g) content in the muscle of farmed *O. mykiss* [a]

Vitamin and cholesterol	Muscle
A	12.4±0.9
D_2	1±0.1
D_3	13.2±1.1
δ-Tocopherol	58±1.9
α- Tocopherol	714±9.44
K_1	626±54
K_2	392±4.68
Cholesterol	40.2±3.36

[a] Data are expressed as mean ± SD (n=10)

were the major SFAs in the muscle and liver of trout. This finding seems to agree with other studies conducted on trout (Aras *et al*., 2003; Haliloğlu *et al.*, 2004; Çelik *et al.*, 2008; Stancheva *et al.*, 2010). In addition, the amount of C16:0 in the liver was significantly higher than that of the diet in the present study (P<0.05). However, in this study the amount of palmitic acid was not significantly different between the diet and muscle (14.43, 15.25%, respectively). Similarly, Haliloğlu *et al.* (2002) reported that palmitic acid was the dominant SFA in *Salvelinus alpilus*, *Salmo trutta fario* and *O. mykis* raised under the same conditions. It has been

shown in these studies that palmitic acid level is affected by the diet of fish (Ackman *et al*., 1975)

The present study indicated that oleic acid (C18:1 n-9) was the predominant FA within the MUFAs in the muscle and liver of trout. These results are in agreement with those of Görgün and Akpınar (2007) for trout. Similarly, Haliloglu *et al.* (2004) also reported that C18:1 n-9 was the dominant MUFAs in rainbow trout. In this study, the muscle tissue of rainbow trout also contained significantly higher amounts of C18:1 n-9 than the diet ($P<0.05$). On the other hand, liver tissue contained significantly lower amount of C18:1 n-9 and MUFAs than the diet ($P<0.01$ and $P<0.001$ respectively). Ackman (1989) and Gonzalez *et al.* (2006) stated that the FA composition of fish might differ depending on a variety of factors including species, age, freshwater or marine origin, and diet.

In this study, it was found that PUFAs were the highest in liver (49.19%) and the lowest in the diet (32.2%). Linoleic acid (C18:2n-6) was the major n-6 PUFA in both liver and muscle. The amount of C18:2n-6 was significantly higher in the muscle than in diet, but the amount of C18:2n-6 was significantly lower in the liver than in diet ($P<0.05$). Moreover, the amount of docosahexaenoic acid (C22:6 n-3, DHA) was significantly higher in the muscle and liver than in diet ($P<0.05$ and $P<0.001$, respectively. Similarly, Stancheva *et al.* (2010) found that DHA was the predominant fatty acid in the muscle of *O. mykiss*. The results revealed that the short chain n-3 fatty acid present in the diet of rainbow trout was elongated and that it was desaturated. Most freshwater fish can desaturate and elongate C18:2n-6 and C18:3n-3 to their C_{20} and C_{22} homologs (Henderson, 1996).

The n-3/ n-6 ratio is a better index in comparing relative nutritional value of fish (Piggott and Tucker, 1990). Stancheva *et al.* (2010) reported that n-3/ n-6 ratio between 0.2-1.6 would constitute a healthy human diet. Furthermore, a high ratio of n-3/ n-6 has important role to reduce cardiovascular diseases (Cahu *et al.*, 2004). That value was found to be 0.62 for cultured rainbow trout by Stancheva *et al.* (2010).

In the present study, n-3/n-6 proportion in farmed rainbow trout revealed that this value was the highest in the liver and there was a significant difference in the n-3/n-6 proportion between the liver and the diet. In addition, a significant difference was also observed between the diet and muscle. According to Aslan *et al.* (2007), fatty acid composition of the cultured fish does not always depend on that of feed because of the fish metabolism.

In the present study, the ratio of PUFA to SFA was found to be 1.59 for the muscle of trout. According to the general nutritional guidelines of the Department of Health (1994) of the United Kingdom, a ratio of 0.4 or more is recommended as a balanced fatty acid intake on a healthy diet (Wood *et al.*, 2003). Therefore, the results obtained in the present study revealed a PUFA/SFA ratio of 1.59 which is within the recommended range.

Fat-soluble vitamins and cholesterol content

The values of retinol, D_2, D_3, δ-tocopherol, α-tocopherol, K_1 and K_2 vitamins are shown in Table II. In a similar type of study from Bulgaria on rainbow trout muscle farmed in Bulgaria retinol, D and α-tocopherol were found to be 22.3, 6 and 809.1 µg/100 g, respectively (Stancheva *et al.* (2010). In another study from Portugal these values for rainbow trout were found to be 8.8, 19 and 130 µg/100 g, respectively (Dias *et al.*, 2003).

In the present study, the cholesterol content of the rainbow trout muscle was found to be 40.2±3.36 mg/100 g. Çelik *et al.* (2008) determined slightly lower cholesterol content for wild *O. mykiss* as 35.04 mg/100 g. However, National Nutrient Data Base (USDA) reported higher cholesterol content than these studies for the muscle of *S. gairdneri* (59 mg/100 g) (USDA, 2003). Nevertheless, higher cholesterol content was found (52.60±4.36 mg per 100 g) in wild freshwater spiny eel (*Mastacembelus simack*) by Harlıoğlu and Yılmaz (2011). İmre and Sağlık (1998) have reported that the cholesterol amount for saltwater fish species ranged between 40.3 and 75.3 mg/100 g. Moreira *et al.* (2001) reported the cholesterol values between 40.99 and 52.79 mg/100 g. Moreover, Luzia *et al.* (2003) concluded that freshwater fish had less cholesterol content compared with saltwater fish.

CONCLUSIONS

In the present study, fatty acids profile, fat-soluble vitamins and cholesterol content of rainbow trout farmed in Turkey were defined. In comparison to SFA and MUFA, PUFAs were the highest level in *O. mykiss* muscle and liver. The highest PUFA values associated to the high level concentration of DHA. The n-3/n-6 ratio in muscle was found to be 1.1. It can be concluded that rainbow trout farmed in Turkey provides an important income and a commonly consumed food for people is a significant species as it contains DHA, n-3/n-6 ratio, PUFA/ SFA, cholesterol amount, A, D, E, K vitamins within recommended limits.

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