The comparison of fatty acids, fat-soluble vitamins and cholesterol in the muscle of wild caught, cage and pond reared rainbow trout (*Oncorhynchus mykiss* W., 1792)

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Introduction

Fish meat is a good source of fats, proteins, vitamins and minerals. Therefore, it is an important component of a balanced diet for humans (Stancheva *et al*., 2010). Fish store the lipids in various organs, particularly in muscles and liver. Somatic muscle is the dominant tissue in fish and constitutes more than 60% of the live fish weight. Fatty fish such as salmonid mainly deposit lipids in their muscle (Love, 1980). Lipids are important components of fish diets due to their role in providing energy and essential fatty acids, as they carry of fat-soluble vitamins, and resource of polar lipid including sterols, which are important structural compounds of cell membranes (Görgün and Akpınar, 2007). Recently, there has been heightened interest in the lipid and fatty acid (FA) composition of fish. Fish naturally contain high levels of polyunsaturated fatty acids (PUFA) that are recognized as essential biochemical components of the human diet because of their beneficial effects for human health (Sushchik *et al*., 2007). Recent studies have shown that n-3 PUFAs play a vital role in the prevention and treatment of cardiovascular disease, inflammation, aggression, depression, hypertension, autoimmune disorders and cancer. Along with omega-3 and omega-6 fatty acids play a crucial role in brain function as well as in normal growth and development. Polyunsaturated fatty acids (PUFAs), stimulate skin and hair growth, regulate metabolism, and maintain bone and reproductive health. (Stancheva *et al*., 2010). The lipid content and fatty acid profile of fish vary between and within

species even in dark and white muscle. Because they are affected by many factors such as the temperature, salinity, season, size, age, habitat, life stage, type and abundance of food (Ackman and Takeuchi, 1986). Fish are one of the main sources of vitamins (Cahu *et al*., 2004). The fat-soluble vitamins are essential nutrients controlling a diversity of biologically important processes in human body. Vitamin A, also called retinol, takes part in photoreception and regulates gene expression and cell division, bone growth, teeth development, reproduction etc. Vitamin D_3 (cholecalciferol) plays a crucial role in regulating bone metabolism. The biologically active isomer of vitamin E also called alpha-tocopherol (a-TP) acts as an antioxidant, protecting membrane structures, essential fatty acids, and vitamins A and C from oxidation (Stancheva *et al*., 2010).

Trout, as a freshwater fish species constitutes a great food potential for human. Omega-3 (n-3) and omega-6 (n-6) fatty acids (FA), as well as fat-soluble vitamins are essential compounds of fish lipids and exclusively provided by the diet (Kandemir and Polat, 2007; Özogul *et al*., 2007; Stancheva *et al*., 2010). In Turkey, rainbow trout is the main cultured fish species with approximately 60% of the total fish production obtained from aquaculture (Yılmaz *et al*., 2008). In addition, rainbow trout has been one of the most widely cultured species all over the world (Harlıoğlu, 2011). As the world's fish stocks are limited, consumers are now being proposed farmed fish as an alternative. However there is concern that: is there any nutritional difference between wild caught and farm raised fish? Which one is better than the others (Hossain, 2011)?

Therefore, the aim of present study is to evaluate the composition of the content of fatty acids (FA), fat-soluble vitamins (retinol, D_2, D_3, α-tocopherol, K_1, K_2) and cholesterol in the muscle tissue of wild caught, cage and pond reared rainbow trout.

Materials and methods

Experimental animals

A total of 45 rainbow trout were used in this study. Fifteen fish samples for each group were obtained from a commercial fish farm for pond reared rainbow trout, from a cage fish farm established on Keban Dam Lake for cage reared rainbow trout and from Euphrates River for wild caught rainbow trout.

Muscle samples taken from each fish were homogenized. Fat-soluble vitamins (retinol, D_2, D_3, α-tocopherol, K_1, K_2) and cholesterol were simultaneously analysed using high performance liquid chromatography (HPLC) system. The fatty acid components were analysed by gas chromatography as the methyl esters.

Mean weight and length of fish samples and mean temperature of water from which fish samples were obtained are given in Table 1.

Table 1: Weight and length of fish samples and mean temperature of water from which fish samples were obtained (mean±SD, n=15).

	Wild caught	Cage reared	Pond reared
Mean weight, g	80.1±6.8	80.8±8.2	80.5±4.8
Mean length, cm	35.1±2.8	35.7±4.3	35.3±3.7
Water temperature, ^{o}C	8.0±0.4	16.0±0.4	14.5±0.4

Extraction of lipids

Lipid of tissue samples was extracted with hexane-isopropanol (3:2 v/v) by the method of Hara and Radin (1978). Nearly 1 g tissue sample was homogenized with 10 mL hexane-isopropanol mixture. 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 ADEK, cholesterol and fatty acid analysis.

Preparation 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. After it was cooled to room temperature, 5 mL of 5% sodium chloride was added and then it was vortexed again. Fatty acid methyl esters were extracted with 2x5 mL hexane. Fatty acid methyl esters were treated with 5 mL 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 auto sampler vials.

Gas chromatographic analysis of fatty acid methyl esters: 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 colon 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. 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 made and the Class GC 10 software version 2.01 was used to process the data.

HPLC Analysis of ADEK Vitamins

Five mL supernatant was taken in 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. Lipophilic 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 (50+50%, v v-1) acetonitrile/methanol mixture and then was taken to auto sampler vials and was analyzed. The analysis was made with the Shimadzu brand 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 (15x4.6cm 5µm; Sigma USA) column was used. For vitamin E and cholesterol 202 nm, retinol, 326nm and for vitamin D and K, 265 nm was used (Katsanidis and Addis, 1999; L'opez-Cervantes *et al*., 2006).

Statistical analysis

The SPSS software (SPSS Inc, Chicago, IL, USA) was used for statistical analyses. Results for the groups are expressed as mean±standard error of the mean (SEM). Differences between the groups' means were analyzed for significance using the ANOVA Duncan's Multiple Range Test. Statistical significance was defined as $p<0.05$.

Results and discussion

Fatty acids composition

The fatty acid composition in the muscle of wild caught, cage and pond reared rainbow trout are given in Table 2. The fatty acids were grouped as saturated fatty acid (SFA), mono unsaturated fatty acid (MUFA) and polyenoic fatty acids (PUFA). The results of present study showed that PUFA was the highest followed by MUFA and SFA in the muscle of all groups. Harlioğlu (2012) found same results in the muscle of pond reared rainbow trout. The percentage of MUFAs and PUFAs were higher in the cage reared rainbow trout compared with wild caught and pond reared rainbow trout, whereas SFAs were higher in wild caught rainbow trout. PUFAs were not statistically significant amongst the all groups ($p>0.05$). However, MUFAs were significantly found higher in cage reared trout compared with the other groups ($p<0.05$). This significant difference is probably due to be both natural and artificial foods in the diet of the cage reared trout. Many investigations have reported that assimilation pattern of dietary fatty acids in fish muscle reflect the content of the dietary lipid sources (Wickins and Lee, 2002).

Haliloğlu *et al*. (2004) and Şahin *et al.* (2011) have 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 tissues of rainbow trout living in seawater and freshwater. Blanchet *et al*. (2005) reported that SFAs were the lowest in the muscle tissues of trout.

Table 2: Fatty acid percentage (% of total fatty acids) in the muscle rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) from different habitats.

Fatty acid	Cage reared	Wild caught	Pond reared	α
C12.0	ND	0.63±0.18	ND	ns
C14.0	2.59±0.83	2.88±0.27	2.75±0.22	ns
C15.0	0.15±0.06	0.03±0.03	0.09±0.05	ns
C15.1	ND	0.19±0.13	0.12±0.08	ns
C16.0	15.87±0.46 [a]	18.17±0.44 [b]	17.73±0.21 [b]	*
C16.1n7	3.85±0.14 [a]	7.61±0.97 [b]	4.18±0.44 [a]	*
C17.0	0.04±0.04 [a]	0.89±0.26 [b]	0.03±0.03 [a]	*
C17.1	ND	0.26±0.23	ND	ns
C18.0	3.01±0.75 [a]	4.80±0.31 [b]	4.04±0.16 [a.b]	*
C18.1n9	26.56±1.21	18.83±1.91	24.29±2.82	ns
C18.2n6	17.15±0.99 [a]	5.97±0.80 [b]	8.92±1.03 [c]	*
C18.3n3	2.17±0.06 [a]	3.55±0.44 [b]	1.60±0.10 [a]	*
C18.3n6	0.18±0.18	0.10±0.10	ND	ns
C20.1n9	2.58±0.17	1.98±0.43	2.51±0.63	ns
C20.2n6	0.63±0.16	0.16±0.11	0.48±0.15	ns
C20.3n6	ND	0.46±0.29	ND	ns
C20.4n3	0.29±0.18	ND	ND	ns
C20.4n6	0.46±0.19	0.99±0.31	0.97±0.14	ns
C20.5n3	3.06±0.26 [a]	11.65±1.07 [b]	3.95±0.62 [a]	*
C21.0	0.18±0.07	0.08±0.05	0.13±0.06	ns
C22.0	0.66±0.02	0.55±0.09	0.62±0.12	ns
C22.1	0.71±0.43	0.22±0.11	1.11±0.35	ns
C22.2	ND	0.09±0.09	ND	ns
C22.5n3	2.48±0.4	3.34±0.4	2.79±0.22	ns
C22.6n3	17.02±2.18	15.98±2.52	23.28±4.66	ns
C24.0	0.03±0.03	ND	0.08±0.05	ns
ΣSFA	22.53±0.28 [a]	28.03±0.40 [b]	25.47±0.11 [a]	*
ΣMUFA	33.70±0.48 [a]	29.09±0.63 [b]	28.03±0.86 [b]	*
ΣPUFA	43.44±0.51 [a]	42.2±0.61 [a]	41.99±0.98 [a]	ns
ΣPUFA/SFA	1.92±1.05 [a]	1.50±1.50 [a]	1.64±0.52 [a]	ns
Σn-3	25.02±0.61 [a]	34.52±1.10 [b]	31.62±1.40 [b]	*
Σn-6	18.42±0.38 [a]	7.68±0.64 [b]	10.37±0.44 [b]	*
Σn-3/Σn-6	1.35±0.49 [a]	4.49±0.82 [b]	3.04±0.92 [b]	*

Each value is given as means of 45 samples ± standard error (SE). ND: Not determined.
Level of statistical significance (α): not significant (ns) = $p>0.05$;significant (*)= $p<0.05$

In the present study, it was determined that the total SFA content of lipids was 22.53% in the cage reared trout, 25.47% in the pond reared trout and 28.03% in the wild caught trout. ΣSFA in the muscles of wild caught trout was found the highest and statistically significant from other groups. In addition, palmitic acid (C16:0) was the primary SFA in the all groups followed by stearic acid (C18:0) and these two fatty acids were the major SFAs in the muscle of trout in all groups. These findings seem to agree with other studies conducted on trout (Aras *et al.*, 2003; Haliloğlu *et al.*, 2004; Çelik *et al.*, 2008; Stancheva *et al.*, 2010; Harlioğlu, 2012). Furthermore, the palmitic and stearic

acid content of cage rainbow trout was significantly (p<0.05) lower than that of trout in other groups (Table 2). In the present study it was also determined that oleic acid (C18:1 n-9) was the predominant FA within the MUFAs in the muscles of all groups. These results are in agreement with those of Görgün and Akpınar (2007) for trout. Similarly, Haliloğlu *et al.* (2004) and Harlioğlu (2012) also reported that C18:1 n-9 was the dominant MUFAs in rainbow trout.

Among n-6 series of the fatty acids, Linoleic acid (C18:2 n-6) was the primary n-6 PUFA in all the groups. The amount of C18:2n-6 was significantly higher in the muscle of cage reared rainbow trout than that of other groups. Σn-6 and Σn-3 were found to be the highest in the muscles of cage reared trout and they were also found significantly different from other groups. The primary fatty acid in Σn-3 was found as docosahexaenoic acid (C22:6 n-3, DHA) for all groups. Similarly, Stancheva *et al*. (2010) and Harlioğlu (2012) have found that DHA was the predominant fatty acid in the muscles of rainbow trout. The amount of eicosapentaenoic acid (EPA, 20:5n-3) in the wild caught trout was nearly 3 times higher than that in cage and pond reared tout. The proportion of EPA was substantially high in wild trout but low in cultured fish (Nettleton, 2001). The percentage of n-3 PUFA in cultured fish lipid is often lower than that in wild fish (Aslan, 2007). The results of the present study confirmed the findings of others (Ackman and Takeuchi, 1986; Aslan, 2007). According to Aslan (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, PUFA/SFA ratios in the muscles of cage, wild and pond trout were 1.92, 1.50 and 1.64 respectively. Although the total PUFA contents and PUFA/SFA ratios were the highest in the muscle of cage reared trout, there was no statistically significant different amongst all groups. 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). In the present study, PUFA/SFA ratios were found to be between 1.50 and 1.92 which is higher than the recommended value (0.4).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 in reducing cardiovascular diseases (Cahu *et al*., 2004). That value was found to be 0.62 for cultured rainbow trout by Stancheva *et al*. (2010) and this ratio was high in marine fish lipids (Tanakol *et al*., 1999; Saglik and Imre 2001). The value of 2.20 was found for cultured fish (Hearn *et al.*, 1987). Another study found 2.17 in the cultured fish and additionally 1.58 in the wild (Aslan, 2007). In the present study, n-3/n-6 proportion values

were found to be 1.35 in cage, 3.04 in pond reared trout and 4.49 in wild caught trout. Our lowest value (1.35) would constitute a healthy human diet according to results given by Stancheva *et al*. (2010).

Fat-soluble vitamins and cholesterol content

The values of A (retinol), D_2, D_3, E (α-tocopherol), K_1 and K_2 vitamins are shown in Table 3. Among the vitamins (A, E and D_2, D_3, K_1, K_2) was analyzed in all habitats. Vitamin E content was the highest followed by that of vitamins K_2, K_1, D_3, D_2 and A. Similarly, Harlioğlu (2012) found that vitamin E content was the highest in the muscles of rainbow trout.

The findings showed that there was no statistically significant difference in the A, D_2 and D_3 vitamins amongst cage, wild and pond reared rainbow trout. But there was a statistically significant difference in the values of vitamins K_1, K_2 and vitamin E amongst cage, wild and pond reared rainbow trout.

Table 3: The fat soluble vitamins (µg/g) and cholesterol (mg/100g) in the muscles of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) from different rearing habitats.

Fat soluble vitamins	Cage reared	Wild caught	Pond reared	α
A (Retinol)	0.12±0.02	0.15±0.03	0.2±0.04	ns
D_2	0.35±0.07	0.41±0.24	0.67±0.11	ns
D_3	0.71±0.13	1.10±0.26	1.12±0.47	ns
K_1	1.92 ± 0.39^{a}	0.81 ± 0.13^{b}	2.98 ± 1.06^{c}	*
K_2	7.37 ± 1.59^{a}	5.22 ± 1.19^{a}	13.76 ± 2.89^{b}	*
E (α-Tocopherol)	$22.95\pm4.39^{a,b}$	11.35 ± 2.03^{a}	30.40 ± 5.77^{b}	*
Cholesterol	262.49 ± 9.56^{a}	366.16 ± 36.0^{b}	357.58 ± 40.8^{b}	*

Each value is given as means of 45 samples ± standard error (SE). ND: Not determined.
Level of statistical significance (α): not significant (ns) = $p>0.05$; significant (*)= $p<0.05$

In the present study, the cholesterol content of the muscles in rainbow trout was found to be 262.49±3.36 in cage reared trout, 366.16±36.00 in wild caught trout and 357.58±40.8 in pond reared trout. The results showed that cholesterol level of cage reared rainbow trout was lower than that of pond and wild caught trout and the differences were significant ($p<0.05$).

Generally, in the present study, we investigated the differentiation of fatty acid composition, fat soluble vitamins and cholesterol content of wild, cage and pond reared trout. Σn-3/Σn-6 ratios in the wild caught rainbow trout were

found to be within the range recommended for human health. Furthermore cage reared rainbow trout was also found to be good for human health containing rich ΣPUFA and ΣPUFA/SFA ratios and low levels of cholesterol. The fat-soluble vitamin contents in pond reared rainbow trout were found to be higher than those in the other two groups. This difference may be caused by the addition of vitamins in the diet of pond reared fish. In conclusion, it can be recommended that all of the groups were healthy for human diet in terms of fatty acid composition, fat-soluble vitamins and cholesterol content.

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