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Proximate and Fatty Acid Composition in Muscle Tissues of Rainbow Trout (*Oncorhynchus mykiss*) Cultured in Yazd Province of Iran

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Abstract

Iran is the number one producer of cultured coldwater fish in Asia since 2005. Rainbow trout, *(Oncorhynchus mykiss)* is the most common and important fish produced by Iranian fisheries. There is not enough information about carcass composition of cultured fish in Iran. Rainbow trout muscle samples were collected from six fish farms of Yazd province during February 2008. Muscle samples were frozen at -30 ^{o}C after being homogenized. Proximate composition of samples was measured. Saturated fatty acids including palmitic, stearic, myristic, lauric acids, also unsaturated fatty acids oleic and linoleic were extracted from muscle tissue of fish at different farms, using gas chromatograghy (GC). Other unsaturated fatty acids including α-linolenic acid, Eicosa Pentaenoic Acid (EPA) and Docosa Hexaenoic Acid (DHA) had low concentrations (up to 3 %) in samples. Vitamin E levels were 4.33 - 94.34 µg/100 g.

Keywords: Proximate, fatty acid, muscle, *Oncorhynchus mykiss*, Iran

Introduction

Rainbow trout is one of the most important cultured fish in the world and the number one cultured fish in Iran from 1960 [1]. Iran has one of the highest rates of coldwater fish culture in the world since 2005 [2]. Rainbow trout (*Oncorhynchus mykiss)* is the most important fish for aquatic fish cultures in Iran. Fish culture has been developed in Yazd province of Iran for the last twenty years. Rainbow trout is the most important fisheries product in the area. It is the only cultured fish during cold seasons in the most areas of Iran. It is cultured in underground brackish water, because of limited fresh water resources in Yazd province.

Some aspects of *O. mykiss* culture and feeding [3-9] and breeding [10,11] in underground brackish water of Bafq were studied.

Proximate analysis of muscle tissue has been studied in some marine and cultured fish of Iran such as tuna species [12,13], rainbow trout [14], carp [15] and *Salmo trutta caspius* [16] especially correlated with fish nutrition. Studies of fatty acid composition of fish edible muscle in Iran are limited to some recent studies mainly on non cultured marine species such as sturgeons [17], tuna [18], pike perch [19], golden mullet [20], some canned fish [21], some fish species from the Persian Gulf [22] and some cultured and non cultured marketed fish [23].

Fish is one of the most important sources of animal protein, good fats and other elements for health. Food scientists recommend people consume fish for prevention of cardiovascular disorders, some cancers and autoimmune diseases mainly because of their fatty acids.

Consumption of fish foods at least two times per week is recommended by the American Heart Association (AHA) to achieve cardio protective effects [24]. There is little information about carcass composition and fatty acids of cultured fish in Iran. Also some people believe that cultured fish have lower quality than naturally growing fish. In the present study, proximate analysis and fatty acid composition of muscle tissue of cultured *O. mykiss* in Yazd province of Iran are examined.

Materials and methods

Six farms of *O. mykiss* with similar cultural conditions were selected in different areas of Yazd province at: (1) Eslamieh in a suburb of Taft, (2&3) Chah-Beigi in a suburb of Abar-Kooh, fresh water (f) and brackish water (b) farms, (4) Chah-Afzal in a Suburb of Ardakan, (5) Research Station in a suburb of Bafq, and (6) Behabad. Cultural conditions were similar from the viewpoint of fish nutrition and water management in the selected farms with a mean water temperature between 13.1 - 14.9 °C, pH 7.62 - 8.3 and dissolved oxygen 6.2 - 8.3 mg/Lit. Fries were stocked in early November 2007. The main factors of culture such as fish nutrition and water management were considered during the culture period. Fish food used in all farms was prepared from the same factory.

Fish samples were collected during February 2008 with farmers catching fish with gill nets to demand. Nine cultured *O. mykiss* market size fish (161 - 311 g) were collected from each of the six farms to obtain at least 500 g muscle tissue [25]. Samples were transited alive to the laboratory. Total length and body weight of each sample were measured. Fish were dissected and the muscle sample was removed from the left side of the fish between the dorsal fin and the operculum. Muscle samples were homogenized using a 20,000 rpm grinder. One hundred gram packages of the samples were frozen at –30 °C till examinations for about 4 wk. [26,27].

Wet, crude protein, total fat, ash and carbohydrates percents of fish muscle tissues were measured using standard methods of [28].

Analysis of methyl esters was performed on a gas chromatograph (Dani) using a capillary column (AT-1000), with 25 m length, 0.32 mm diameter and 2 μm thickness. The carrier gas used for the FID detector was nitrogen at a fixed flow rate of 1 ml/min. Gas pressure on the detector were 0.8, 1.1 and 0.9 bars for nitrogen, air and hydrogen, respectively. The injector and detector temperatures were both maintained at 250 and 280 °C, respectively. The oven temperature was set at 180 °C.

Half a gram of the oil sample was mixed with 5 ml alcoholic potash solution 50 mg/ml and put in boiled in a water bath for 30 min. Isopropanol was evaporated after soap formation. 0.35 ml sulfuric acid was added to soap layers slowly and then agitated until neutralization of soap. A thin layer of free fatty acids on the potassium sulfate dreg was extracted using 10 ml of a hexane-butanol solution. The latter solution was filtered by 0.45 μm Millipore filter paper. One μl of filtered solution was used for injection into the gas chromatograph [26,29-30]. Fatty acid percentages and their amounts are expressed as g/100 g muscle tissue of fish.

Vitamin E was measured using HPLC (Knaver, Germany) with a UV detector 292 nm. 0.2 g of the oil sample was poured into a dark bottle. Ten ml of 2 M potassium hydroxide solution and 5 ml of 0.1 M ascorbic acid were added. Then the samples were saponified shaking the mixture at 125 rpm in an incubation system at 60 °C for 45 min. After cooling at room temperature, the resulting mixture was filtered and treated with 5ml of saturated sodium chloride solution and 5 ml of 5 mg/l n-hexane solution. Samples were then stirred for 1 min in the Vortex mixer. The hexane phase was collected by passing through anhydrous sodium sulfate. The aqueous layer re-extracted with 5 ml of n-hexane. The hexane solution was evaporated at 50 °C. The resulting residue was dissolved in 1ml of methanol and then filtered. The resultant solution was used for manual injection at a flow rate of 1.5 cc/mhn. The mobile phase consisted of a mixture of methanol:n-butanol (95:5). Vitamin E amounts are expressed as IU/100 g (International Unit per 100 grams) and μg/100 g fish muscle tissue.

Data analysis was performed using excel (2007) and SPSS (11.5). Means of wet, crude protein, total fat, ash and carbohydrates of fish muscle from different farms were analyzed using one way ANOVA and were compared by HSD Tukey test ($p < 0.05$). Also, results of the proximate analysis of fish muscles were compared between fresh and brackish water farms, by t-student test ($p < 0.05$).

Results

Fish were cultured for 4.5 months in different farms. *O. mykiss* samples were collected from three fresh water farms at Eslamieh, Chah-Beigi (f) and Behabad and three brackish water (8.7 - 9.5 ppt) farms at Chah-Afzal, Bafq and Chah-Beigi (b).

Results of proximate analysis of muscle tissue of cultured *O. mykiss* samples are wet 74.18 - 77.05 %, crude protein 17.05 - 18.53 %, total fat 2.35 - 5.13 %, ash 1.31 - 1.7 % and carbohydrates 0.51 - 2.32 % (**Table 1**).

Table 1 Averages of wet, crude protein, total fat, ash and carbohydrate percents (±SE) in fish food and muscle tissue of cultured *O. mykiss* collected from farms at different areas of Yazd province, winter 2008. (f: fresh water, b: brackish water).

Farm	Wet ± SE	Protein ± SE	Fat ± SE	Ash ± SE	Carbohyd. ± SE
Eslamieh	76.88 ± 0.09	17.91 ± 0.1	2.48 ± 0.11	1.38 ± 0.03	1.35 ± 0.06
Chah-Beigi (b)	74.37 ± 0.1	17.40 ± 0.32	4.88 ± 0.16	1.59 ± 0.09	1.75 ± 0.38
Chah-Beigi (f)	75.32 ± 0.08	17.60 ± 0.06	4.39 ± 0.08	1.44 ± 0.07	1.25 ± 0.14
Chah-Afzal	75.24 ± 0.06	17.84 ± 0.35	3.71 ± 0.02	1.48 ± 0.05	1.72 ± 0.36
Bafq	75.98 ± 0.16	17.74 ± 0.27	3.49 ± 0.02	1.51 ± 0.05	1.28 ± 0.04
Behabad	75.20 ± 0.14	17.86 ± 0.34	3.78 ± 0.19	1.47 ± 0.07	1.69 ± 0.59
Fish food	9.6 ± 0.03	30.77 ± 0.15	10.23 ± 0.17	11.67 ± 0.05	37.72 ± 0.05

One way ANOVA showed differences among wet ($F = 57.16$, $p < 0.0005$) and total fat ($F = 50.68$, $p < 0.0005$) of Eslamieh and Chah-Beigi samples with other samples, however, there were no significant differences among crude protein, ash and carbohydrates of studied farms' samples. HSD Tukey test showed significant differences of mean wet and total fat between different farms ($p < 0.05$). There was no significant difference of wet ($t = 1.64$, $p = 0.12$), total fat ($t = -1.32$, $p = 0.21$), crude protein ($t = 0.62$, $p = 0.54$), ash ($t = -1.98$, $p = 0.06$) and carbohydrates ($t = -0.6$, $p = 0.56$) in muscles between fish cultured in fresh water (Eslamieh, Chah-Beigi, Behabad) with fish cultured in brackish water (Chah-Beigi, Chah-Afzal, Bafq) ($p > 0.05$), by t-student test.

Fatty acids including lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids, eicosa pentaenoic acid (EPA) and docosa hexaenoic acid (DHA) were measured in fish muscles. More abundant saturated fatty acids were palmitic (14.06 - 19.62 %) and stearic (4.44 - 6.1 %) acids. Myristic (1.29 - 5.66 %) and lauric (0.13 - 2.76 %) acids were the other measured saturated fatty acids. Monounsaturated oleic acid was the most abundant (30.24 - 40.14 %) fatty acid in fish muscle samples. Linoleic acid was the most abundant (3.04 - 4.21 %) polyunsaturated fatty acid. EPA and DHA varied from incommensurable amounts to 1.73 and 1.03 %, respectively. The most abundant fatty acids in fish food were linoleic, oleic and palmitic acids (**Table 2**).

Total saturated fats of fish muscle were 24.93 and 24.53 % in brackish and fresh water studied farms, respectively. However, total unsaturated fats were prominently higher in brackish water fish with but no significant, by t-student test ($t = -2$, $p = 0.062$).

Vitamin E contents varied between 4.33 - 96.36 μg/100 g in fish muscle of different farms (**Table 3**).

Table 2 Mean weight (± SE) and percent of fatty acids in fish food and muscle tissue of cultured *O. mykiss* collected from farms at different areas of Yazd province, winter 2008. (f: fresh water, b: brackish water)

Farm	Fatty acid	Lauric acid	Myristic acid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	EPA	DHA
Eslamieh	(%)	0.16 ± 0.03	1.73 ± 0.04	16.88 ± 0.27	5.01 ± 0.04	37.6 ± 0.14	25.2 ± 0.27	3.62 ± 0.04	1.7 ± 0.02	0.96 ± 0.07
	g/100g	0.003 ± 0.00	0.047 ± 0.004	0.376 ± 0.04	0.184 ± 0.02	0.555 ± 0.06	0.473 ± 0.05	-	-	-
Chah-Beigi (b)	(%)	2.53 ± 0.18	5.41 ± 0.19	14.4 ± 0.24	4.51 ± 0.05	30.92 ± 0.31	29.93 ± 0.23	3.9 ± 0.08	0.91 ± 0.04	-
	g/100g	0.05 ± 0.003	0.17 ± 0.02	0.372 ± 0.04	0.193 ± 0.03	0.53 ± 0.07	0.571 ± 0.07	-	-	-
Chah-Beigi (f)	(%)	0.73 ± 0.23	2.6 ± 0.09	16.33 ± 0.34	5.26 ± 0.04	39.66 ± 0.39	26.84 ± 0.11	3.378	-	-
	g/100g	0.018 ± 0.002	0.124 ± 0.009	0.637 ± 0.05	0.355 ± 0.002	1.023 ± 0.06	0.735 ± 0.05	-	-	-
Chah-Afzal	(%)	-	0.869 ± 0.0	18.78 ± 0.59	5.72 ± 0.27	35.12 ± 2.38	35.42 ± 0.66	4.04 ± 0.1	-	-
	g/100g	-	-	0.043 ± 0.03	0.022 ± 0.001	0.052 ± 0.003	0.077 ± 0.005	-	-	-
Bafq	(%)	0.62 ± 0.01	1.31 ± 0.02	16.06 ± 0.1	5.46 ± 0.03	34.72 ± 0.13	30.51 ± 0.11	4.21 ± 0.02	1.3 ± 0.03	-
	g/100g	0.008 ± 0.00	0.025 ± 0.00	0.253 ± 0.003	0.142 ± 0.002	0.362 ± 0.004	0.425 ± 0.005	-	-	-
Behabad	(%)	-	1.52 ± 0.07	19.43 ± 0.18	5.78 ± 0.03	30.55 ± 0.24	25.6 ± 2.67	3.04 ± 0.11	1.24 ± 0.03	0.6 ± 0.07
	g/100g	-	0.207 ± 0.02	2.18 ± 0.26	1.069 ± 0.12	2.268 ± 0.27	2.498 ± 0.28	-	-	-
Fish food	(%)	-	1.75 ± 0.19	18.27 ± 0.08	4.94 ± 0.27	28.34 ± 3.3	42.12 ± 2.23	4.56 ± 0.37	-	-
	g/100g	-	0.1 ± 0.01	0.01	0.29	0.56 ± 0.1	1.63 ± 0.01	-	-	-

Table 3 Vitamin E contents (IU/100g, µg/100g) in muscle tissue of cultured *O. mykiss* collected from farms at different areas of Yazd province, winter 2008.

Farm	µg	IU
Eslamieh	21.26	0.03
Chah-Beigi (b)	14.76	0.02
Chah-Beigi (f)	11.18	0.017
Chah-Afzal	4.33	0.006
Bafq	7.18	0.01
Behabad	94.36	0.14

Discussion

General composition of fish muscle

Crude protein contents in muscle of *Oncorhynchus mykiss* from different studied farms were adjacent but total fat in fish muscle of Chah-Beigi farms were higher than other farms with lower amounts of wet. Proximate composition of salmonids is much influenced by food. Feed composition has a major influence on the proximate composition of salmonids. In particular, whole body fat as well as the lipid content in the edible fillet is directly related to dietary fat content, while the fatty acid composition of the fish flesh is also strongly influenced by the dietary fatty acid profile. While fish body composition appears to be largely influenced by feed composition, an increase in other parameters such as feed ration and fish size also results in enhanced adipose deposition and decreased water content in the fish body. The protein content, however, remains more or less stable [31]. Protein content and composition are showed to be stable during development of carps [32]. Also the muscle essential amino acid compositions of 20 Indian

fish species were the same [33]. In a similar study in Turkey about proximate composition of cultured *O. mykiss* [34], the moisture, protein, lipid and ash contents of the rainbow trout were 71.65, 19.60, 4.43 and 1.36%, respectively that are very similar to the results of the present study.

It is very difficult to ascertain the optimum level of fat in a fish carcass. Generally, the fat percentage of 16 - 18 % in a fillet is too high. Excessive fat deposits reduce the quality of the fish [32].

Tactics for the rearing of salmonids for specific purposes should therefore take the following into consideration. The level of proximate constituents in the whole body as well as the fillet are readily manipulated by feed composition and feeding strategies, whereas its taste, color and odor are less affected by these variables [31]. Cultured fish often have higher amounts of energy and fats with lower protein contents compared with wild samples of the same species as compared in some fish [35-37] that may be attributed to the constituents of the diet of the fish and economic aims of the culturists.

Fish food must have at least 5 - 8 % fats and 28 % proteins. Higher quality fish foods for *O. mykiss* culture have 12 % fats and 38 - 42 % proteins [38,39]. Results of fish food analysis in the presents study showed high fat and medium protein contents.

Fatty acids

Muscle tissue of studied cultured *O. mykiss* included 23.12 - 27.16 % saturated and 60.49-71.13% unsaturated fatty acids. More abundant saturated fatty acids were palmitic (C16), stearic (C18), myristic (C14) and lauric (C12) acids, respectively. The ω_9 oleic acid [C18:1(n9)] was highest (30.24 - 40.14 %) and the only monoenoic acid extracted from muscle of the fish. High amounts (21.18 - 38.85 %) of the ω_6 linoleic acid [C18:2(n6)] was measured in studied fish. Other polyenoics were ω_3 fatty acids. Linolenic acid [18:3(n3)] was the most abundant (1.99 - 2.87 %) ω_3 fatty acid. EPA [C:20(5n3)] and DHA [C:22(6n3)] had insignificant to few amounts up to 1.73 and 1.03 %, respectively.

A similar study of muscle tissue of cultured *O. mykiss* in Turkey showed total monoenoics were 35.565, saturated fatty acids 27.65 % and polyenoics 23.09 % of fatty acids [34]. Higher amounts of unsaturated fatty acids in the present study might be assumed as an advantage of these fish.

Total amounts of saturated, monoenoic and polyenoic fatty acids in 79 g fillet of cultured rainbow trout are 1.2, 1.2 and 1.4 g, respectively. Favorite amounts of stearic, palmitic, myristic and linoleic acids in 100 g fillet of cultured rainbow trout are 0.019, 0.98, 0.28, 1.06 and 0.71 g, respectively [40]. In the present study, total fatty acids were 1.22 - 8.22 g in 100 g fillet. Fatty acid ranges were 0.43 - 3.46, 0.14 - 1.07, 0.03 - 0.21, 0.36 - 2.27 and 0.43 - 2.5 g in 100 g fillet for stearic, palmitic, myristic and linoleic acids, respectively, therefore different with total amounts.

The composition and nutritional value of market size rainbow trout fillets from intensive Italian fish farms were determined. Fish were rich in oleic acid (18.2 - 29 %), and in ω_6 and ω_3. Analytical results confirmed the higher lipid content in reared fish compared to wild ones must however be considered in dietetic regimes because of the higher energy content of the fish flesh [37].

The fatty acid composition of some tissues of *Salmo trutta* labrax, Pallas, 1811 freshwater wild salmon in Turkey was investigated. There was a significant amount of palmitic acid in all of the tissues compared with other saturated fatty acids like lauric, myristic, stearic and arachidic acid. The highest amount of palmitic acid (25.39 %) was in the muscle. The total saturated fatty acid contents were significantly different between tissues, and the highest values (37.20 %) were in the muscle [41]. Analysis of fatty acids composition of 7 marine fish species in Easter Island revealed the highest was saturated fatty acids (35.1 - 54 %) followed by the polyunsaturated fatty acids (22 - 42.5 %).

Palmitic acid was the most important between saturated fatty acids, while oleic acid was the main fatty acid between the monoenoics. In ω_3 family fatty acids, EPA and DHA were the most important [42].

Salmonid flesh quality appears to be under the strong influence of feed composition and feed amount [31]. Changes in the diet of a fish can result in a significant change in its fatty acid constituents in less than 3 wk [43]. Demir [44] reported higher ω_3 levels in the muscle and liver of *O. mykiss* that feed with a high fatty acid composition. Therefore, fish farmers are able to

alter fish lipid composition a month before marketing.

Variation in the results of the lipids analysis may be caused by several other variable environmental, dietary, and physiological factors and significantly by the portion of fish analyzed [45]. Researchers reported that the lipid and fatty acid compositions of fish differ depending on a variety of factors such as the species, maturity period, sex, size and age of the fish, environmental and rearing conditions and geographical location [35,46-48]. Besides aquaculture and nutritional value of fatty acid composition in fish, their environmental aspects are important for ecological studies. Fatty acids have considerable potential for use as biomarkers. Analyses of fish fatty acids as monitors of habitat conditions could prove to be significant tools for establishing environmental policy. Since fish accumulate pollutants preferentially in fatty tissues, bioaccumulation may be a function of the fatty acid composition [29]. Despite individual variation within species, fatty acid signatures provide a powerful tool for identifying fish and invertebrate species [49].

Nutrition and food are the most important effective agents on the final composition of cultured *O. mykiss*. In the present study fatty acid composition of fish food (stearic, palmitic, myristic, oleic, linoleic, and linolenic acids 4.9, 18.3, 1.75, 28.3, 42.12 and 4.56 %, EPA and DHA incommensurable amounts) is different from fish food. In the examined high quality fish food for culture of *O. mykiss*, stearic, palmitic, myristic, oleic, linoleic, and linolenic acids were measured 1.5 - 1.8, 12.2 - 12.4, 4.9 - 5.8, 10.1 - 11.7, 3.9 - 11.7 and 0.9 - 1.8 %, EPA 6.6 - 7.8 %, and DHA 6.3 - 7.5 %, respectively [50].

Vitamin E

Vitamin E is present in fish only in very small quantities; a typical value is 12 μg/100 g vitamin E [51]. Studied samples had often noteworthy amounts of Vitamin E.

Various storage temperatures and storage periods have obvious effects on vitamin E levels of fish muscle. Vitamin E loss in muscle tissue of *Oncorhynchus mykiss* was more in samples stored at −12 °C compared with −18 °C. The decrease in vitamin E levels in samples frozen for 17 wk was statistically significant at the beginning of the 6th wk [52]. Vitamin E in fish is destroyed as polyunsaturated fish oils undergo peroxidation [51]. Increasing the concentration of vitamin E from 300 to 1,500 mg/kg in a fish food containing 30 % lipid can reduce the rate of lipid oxidation in fish fillets and reduce the formation of off-flavors [53].

Conclusions

Results of the present study revealed that attention must be paid to produce the desired cultured *O. mykiss* flesh in Iran in view of fatty acid composition. Making and using higher quality fish food is probably the most important effective agent. Composition and balance of fatty acids in feeding of cultured fish might be considered. However, economic aspects and excessive costs of a good nutrition program and their influence on fish fillet production have to been assayed.

Low or high amounts of some saturated and unsaturated fatty acids especially the high level of ω_6 linoleic acid and very low levels of EPA and DHA in fish food of the present study must be considered further.

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