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## Comparison of Fatty Acid Profile and Proximate Composition of Three Native Trout Species: Health Benefits and Risk Assessments Associated with Their Consumption

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**Abstract:** Evaluating fish's nutrient content could provide essential guidance for fish consumption and the protection of human health. This study investigated the biochemical composition and fatty acid profiles (FA) of three native trout species: *Salmo rizeensis*, *Salmo ardahanensis*, and *Salmo coruhensis*. This is the first study to characterize FA content and lipid quality indices of *S. rizeensis*, *Salmo ardahanensis*, and *S. coruhensis*. The highest crude protein and crude fat were found in *S. ardahanensis* and *S. rizeensis*, respectively. Although there was no significant difference ( $p < 0.05$ ) among trout species on crude protein, the crude fat content of *S. rizeensis* was significantly higher ( $p < 0.05$ ) than the other species. The FA compositions of trout species ranged from 29.22% to 40.12% saturated FAs (SFA), 27.57% to 37.67% monounsaturated FAs (MUFA), and 26.54% to 28.41% polyunsaturated FAs (PUFA). The most dominant FAs were palmitic acid (C16:0) among SFAs, oleic acid (C18:1n9c) among MUFAs, and linoleic acid (C18:2n6c), eicosapentaenoic acid (EPA; C20:5n3) and docosahexaenoic acid (DHA; C22:6n3) among PUFAs. These species were found to be rich sources of EPA+DHA in the range of 10.49-15.58%. The highest fish lipid quality index (FLQ) and EPA+DHA content were found in *S. ardahanensis*, while the highest h/H value was in *S. coruhensis*. The atherogenic index, thrombogenic index, and  $\sum n-3/n-6$  ratio of all species were within the limit range reported by international organizations. These results showed that all trout species used in this study could be considered as a beneficial and balanced food source for human consumption in terms of rich protein content, FA ratio, and lipid quality indices in future fish farming.

**Keywords:** Fatty acid, chemical composition, *Salmo rizeensis*, *Salmo ardahanensis*, *Salmo coruhensis*

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## INTRODUCTION

The importance of seafood in a balanced diet is increasing day by day due to its high protein and low carbohydrate content, essential amino acids, unsaturated fatty acids, vitamins, minerals, low cholesterol, and low-calorie values. Above all, fish is a virtually uniquely rich source of omega-3 long-chain PUFAs (Çağlak and Karsli, 2017; Bayrakli, 2021; Öğretmen, 2022; Tufan, 2023). The nutritional importance of fish consumption is closely related to each species's  $\omega$ -3 fatty acid content (Çağlak and Karsli, 2020). The PUFA is important for maintaining the integrity of the membranes of all living cells to make prostaglandins, which regulate many body processes such as inflammation and blood clotting. Especially, fish fats contain essential PUFAs such as eicosapentaenoic (EPA, C20:5 n-3), docosahexaenoic (DHA, C22:6 n-3), and arachidonic (C20:4 n-6) acids that are not synthesized in the human body; however, these are essential in human diets (Jabeen and Chaudry, 2011). Variations in lipid and fatty acid composition among and within fish species, depending on nutrient availability, season, location, sex, diet, and age, have been well-reported in previous studies. Furthermore, it is known that lipid and fatty acid composition varies even among fish tissues (Bayir et al., 2010; Kayhan et al., 2015). There are also differences in FA composition between freshwater and marine fish. Freshwater fish are generally considered to contain lower levels of omega-3 PUFAs than marine fish. The chain elongation and desaturation processes are more efficient in freshwater fish. Thus, freshwater fish can be converted into a nutrient-rich food with feed (Akpınar et al., 2009). However, knowing these differences in meat yield and chemical composition of various fish plays an important role in these species' nutritional and economic preferences (Çağlak and Karsli, 2017).

In the industrial sense, aquaculture has made significant progress in the last 30 years in Turkey and worldwide. Today, Salmonids/trouts have become an integral part of the aquaculture sector because of their high economic value (Çankırılıgil and Berik, 2020; Çağlak and Karsli, 2023). According to the World Fishery Production in 2018, Salmons, trouts, and smelts covered 4.33% (82 095 tons) of the total capture and 1.18% of aquaculture in 2018 (FAO, 2020). When Turkey's aquaculture potential is considered, trout constitute 33.68% (146 594 tons) of the total aquaculture amount (TUIK, 2020). Trout is usually consumed fresh. However, in recent years, frozen, fillet, smoked, and marinated trout products have also

contributed to domestic consumption and exports (Çağlak and Karsli, 2015).

Anatolia has a very high diversity and hosts 19 native trout species (Turan et al., 2021). Trout is important in sport fishing and local folk medicine due to its high nutritional content. It is a native and economically important fish species in many parts of Anatolia (Kayhan et al., 2015; Gunlu and Gunlu, 2014). Sympatric *Salmo rizeensis* and *S. coruhensis* were described by Turan et al. (2009) in the Southern Black Sea basin, which had previously known as *S. trutta macrostigma* and *S. trutta labrax*, respectively. *S. coruhensis* is an endemic anadromous fish and is only distributed in the middle and lower parts of the Southern Black Sea rivers and eastern Marmara Sea drainages (Turan and Aksu, 2021). *S. rizeensis* is an endemic resident species known only in the upper part of the rivers of Southern Black Sea drainages (Turan et al., 2009; Yoğurtçuoğlu et al., 2020). Resident Kura trout (*Salmo ardahanensis*) has recently been described in the upper drainages of the Kura River (Caspian Sea basin) in Turkey (Turan et al., 2022), which was previously known as *S. caspius*. There are many studies involving the fatty acid and proximate composition of *S. t. labrax* and *S. t. macrostigma* (Aras et al., 2003a,b; Haliloğlu et al., 2005; Akpınar et al., 2009; Bayir et al., 2010; Şahin et al., 2011; Oz and Dikel, 2015; Çankırılıgil and Berik, 2020; Kaçar et al., 2021a,b). It should not be overlooked that these previous studies partly cover or do not cover the actual distribution of *S. coruhensis* and *S. rizeensis*. At the same time, there is no study on *S. ardahanensis*. However, there are very limited studies on the lipid quality indices of these trout species. To our knowledge, there are no reports about the comparison of proximate composition and fatty acid profile of Çoruh trout (*S. coruhensis*), Anatolian trout (*S. rizeensis*), and Kura trout (*S. ardahanensis*). The species used in this study were sampled from their type localities or native distribution ranges. In this context, the evaluation of the nutritional composition of these samples was presented for the first time in this study. Therefore, this study aims to compare the proximate composition and fatty acid profile in the muscle tissue of three native trout species and to evaluate lipid quality indices.

## MATERIAL AND METHODS

### Fish sampling and measurement

Three freshwater trout species, *Salmo rizeensis*

Turan, Kottelat & Engin, 2010, *S. coruhensis* Turan, Kottelat & Engin, 2009, and *S. ardahanensis* Turan, Kottelat & Kaya, 2022, were used in this study. All fish samples were collected by electrofishing in the spring season. *S. rizeensis* was collected from Ovit Stream (40.5887°N, 40.8583°E), Çoruh River in Rize Province; *S. coruhensis* was collected from Pehlivanlı Stream (40.5176°N, 41.4780°E), Çoruh River in Erzurum Province; *S. ardahanensis* was collected from Toros stream (41.1115°N 42.4468°E), the upper Kura River in Ardahan Province, all in Turkey. The fish were transferred in an insulated box containing ice within 24 hours to the Fish Processing Technology Laboratory, Faculty of Fisheries, RTE University, Rize, Turkey. The body length and weight measurements of fish were carried out using a 0.1 mm sensitive digital caliper (Mahr 16 ER, Germany) and 0.01 sensitive digital scales (And GR-200, Japan), respectively. The average lengths and weights of the *S. rizeensis*, *S. ardahanensis*, and *S. coruhensis* used in the study were  $15.37 \pm 1.23$  cm and  $43.44 \pm 9.72$  g,  $19.53 \pm 6.03$  cm and  $90.30 \pm 18.02$  g, and  $14.03 \pm 0.84$  cm and  $34.50 \pm 6.07$  g, respectively. After body measurement, fish were eviscerated and filleted, and whole edible muscle tissue was obtained for analyzes. Then, these samples were kept at  $-70$  °C until analysis, and 30 fishes in total (10 for each species) were used for proximate composition and fatty acid analyses.

### Chemicals

Analytical grade methanol, chloroform, potassium hydroxide, and chromatographic n-hexane (Supra-Solv) used in this study were purchased from Merck (Darmstadt, Germany).

### Proximate analyses

The moisture content was determined according to AOAC (1995; Method 985.14) by oven drying at  $105$  °C for 24 h until a constant weight was obtained. Crude fat content was determined using a solvent extractor (Velp SER 148/6, Velp Scientifica, Milano, Italy) with petroleum ether ( $130$  °C). Crude protein content was calculated as percent nitrogen by using the factor 6.25 g according to the Kjeldahl method (AOAC, 1990; Method 2.507), and crude ash was determined by a burning method in a muffle furnace at  $550$  °C (AOAC, 1990; Method 7.009). All analyses were conducted in triplicate.

### Fatty acid profile analysis

Total lipid extraction of trout species was carried

out in triplicate according to the procedure described by Bligh and Dyer (1959). Fatty acid methyl esters (FAME %) methyl esters were carried out by transesterification using 2 M potassium hydroxide (KOH) in methanol and n-hexane according to the method described by Ichihara et al. (1996) with minor modifications. For this purpose, ten milligrams of lipid samples were dissolved in 2 mL n-hexane, followed by 4 mL of 2 M methanolic KOH. The tube was then vortexed for 2 min at room temperature. The hexane layer was taken after centrifugation at 4000 rpm for 10 min. Then, the fatty acid content of the samples was determined using gas chromatography-mass spectrometry (GC-MS; QP2010 Ultra with AOC-20i+s model autosampler) using a mass selective detector (GC-MS QP 2010 PLUS) equipped with GC/MS solutions software (Shimadzu, Kyoto, Japan). Afterward, fatty acids were identified by comparing the retention times of FAME with the Supelco TM 37-component. Gas chromatography-mass spectrometry conditions reported by Çağlak and Karsli (2017) were used. Three replicate GC analyses were performed, and the results were expressed in GC area % as mean values  $\pm$  standard deviation.

### Indices of lipid quality

Some indices were used to determine the nutritional quality of lipids using data from fatty acid composition analyses. The lipid quality indices, including atherogenicity index (AI), thrombogenicity index (TI), fish lipid quality (FLQ), hypocholesterolemic/Hypercholesterolemic ratio (h/H) ratio, health-promoting index (HPI), polyene index (PI), and unsaturation index (UI) were calculated using the following formulas:

$$\text{AI: } [\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}] / [\Sigma\text{MUFA} + \text{n-3 PUFA} + \text{n-6 PUFA}] \text{ (Ulbrich and Southgate, 1991)}$$

$$\text{TI: } (\text{C14:0} + \text{C16:0} + \text{C18:0}) / [(0.5 \times \Sigma\text{MUFA}) + (0.5 \times \Sigma\text{n-6 PUFA}) + (3 \times \Sigma\text{n-3 PUFA}) + (\text{n-3 PUFA} / \text{n-6 PUFA})] \text{ (Ulbrich and Southgate, 1991)}$$

$$\text{FLQ: } 100 \times (\text{C20:5 n-3} + \text{C22:6 n-3}) / \Sigma\text{FA} \text{ (Abrami et al., 1992)}$$

$$\text{h/H: } (\text{cis-C18:1} + \Sigma\text{PUFA}) / (\text{C12:0} + \text{C14:0} + \text{C16:0}) \text{ (Chen and Liu, 2020)}$$

$$\text{HPI: } \Sigma\text{UFA} / [\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}] \text{ (Chen and Liu, 2020)}$$

$$\text{PI: } (\text{C20:5} + \text{C22:6}) / \text{C16:0} \text{ (Lubis and Buckle, 1990)}$$

UI: 1 x (% monoenoics) + 2 x (% dienoics) + 3 x (% trienoics) + 4 x (% tetraenoics) + 5 x (% pentaenoics) + 6 x (% hexaenoics) (Chen and Liu, 2020)

### Statistical analysis

The statistical analyses were carried out in triplicate. Data are presented as mean ± standard deviation (SD). The statistical analysis was performed using the JMP 5.0.1 (SAS, Inc., Cary, NC, USA) package program. The percentages of fatty acids were analyzed by one-way analysis of variance (ANOVA), and the significant means were compared with Tukey's test at  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

### Proximate composition

The proximate composition (crude protein, crude fat, moisture, and crude ash) of trout species is given in Table 1. The present study found the highest crude protein content in *Salmo ardahanensis*, and the lowest was found in *S. rizeensis*. However, there were no statistical differences among the protein content of trout species ( $p < 0.05$ ). The crude fat content (2.73%) of *S. rizeensis* differed significantly compared to other trout species ( $p < 0.05$ ). The moisture content of trout species was determined between 77.19% and 77.85% ( $p > 0.05$ ). The crude ash content of *S. rizeensis* was slightly lower than those of *S. coruhensis* and *S. ardahanensis* ( $p < 0.05$ ). The results showed that all trout species used in the present study belonged to low-fat fish but had high protein content. Thus, these results are significant because they inform nutritionists interested in low-fat but high-protein food sources currently available to consumers.

There is no study in the literature on the nutritional composition of *S. rizeensis* and *S. coruhensis*. However, *S. rizeensis* and *S. coruhensis* are known as *S. t. macrostigma* and *S. t. labrax* ecotypes, respectively. Especially previous studies have mainly examined the proximate composition of *S. t. macrostigma* (Bilgin et al., 2007; Duman et al., 2011; Ateş et al., 2013; Gunlu and Gunlu, 2014; Karakaya and Duman, 2016). While there are few studies on the biochemical composition of *S. t. labrax* (Çankırılıgil and Berik, 2020; Şahin

et al., 2011), no study on protein, moisture, and ash contents of *S. ardahanensis* has been found in the literature. Only the total lipid content of *S. caspius* was reported by Bayır et al. (2010). Similarly, Karakaya and Duman (2016) reported that the moisture, protein, lipid, and ash contents of *S. t. macrostigma* were found to be 77.68%, 18.70%, 1.31%, and 1.33%, respectively (Karakaya and Duman, 2016). Gunlu and Gunlu (2014) reported the protein, fat, moisture, and ash contents of *S. t. macrostigma* from different populations in the Mediterranean region of Turkey were found within the ranges of 16.94-19.97%, 1.58-3.75%, 75.49-79.59%, and 1.39-1.56%, respectively. In another study, the protein, lipid, moisture, and ash contents of wild brown trout (*S. t. macrostigma*) from Munzur River, Turkey, in spring were 19.19%, 2.24%, 1.20%, and 77.37%, respectively (Ateş et al., 2013). Duman et al. (2011) reported that the average moisture, protein, fat, and ash contents of *S. t. macrostigma* were 78.87%, 18.45%, 2.65%, and 1.15%, respectively. In another study, the proximate composition of *S. t. macrostigma* was determined as 78.90% for moisture, 16.22% for protein, 2.55% for fat, and 1.33% for ash (Bilgin et al., 2007). Except for the protein content determined by Bilgin et al. (2007), these results are consistent with the proximate composition of *S. rizeensis* determined in the present study. Çankırılıgil and Berik (2020) reported that the proximate composition of wild *S. labrax* obtained from different conditions in spring was between 71.61 and 72.07% for moisture, 17.90 and 17.94% for protein, 7.89 and 8.11% for fat, and 1.26 and 1.35 for ash. These results revealed similar protein and ash, lower moisture, and higher fat content to our values determined in *S. coruhensis*. In the other study, the moisture, protein, fat, and ash contents of *S. t. labrax* were found to be 76.74%, 73.82%, 11.02%, and 8.10% dry weight, respectively (Şahin et al., 2011). Since these results were calculated on dry weight, a comparison with the data in the present study could not be made. Overall, the proximate composition data reported in this study were in agreement with those reported in previous studies. However, some observed differences may be due to factors such as season, regional differences, sex, physiological status, age of the fish, and water

**Table 1.** Proximate composition of *S. rizeensis*, *S. ardahanensis*, and *S. coruhensis* (mean ± SD, n=3, wet weight %)

	Crude protein	Crude fat	Moisture	Crude ash
<i>S. rizeensis</i>	17.97±0.90 <sub>A</sub>	2.73±0.16 <sub>A</sub>	77.85±0.21 <sub>A</sub>	1.01±0.02 <sub>A</sub>
<i>S. ardahanensis</i>	19.56±1.05 <sub>A</sub>	1.93±0.05 <sub>B</sub>	77.19±0.34 <sub>A</sub>	1.22±0.03 <sub>B</sub>
<i>S. coruhensis</i>	18.75±0.79 <sub>A</sub>	1.97±0.01 <sub>B</sub>	77.54±0.56 <sub>A</sub>	1.21±0.05 <sub>B</sub>

Different letters (A-B) in the same column represent significant differences ( $p < 0.05$ ) among fish species.

temperature (Karsli et al., 2020; Öğretmen, 2022).

### Fatty acid composition

Table 2 shows the FA composition in the muscle tissue of *S. rizeensis*, *S. ardahanensis*, and *S. coruhensis*. In the present study, the  $\Sigma$ SFA values of the trout species varied between 29.91% (*S. coruhensis*) and 40.12% (*S. ardahanensis*), and significant differences between the fish species were observed ( $p < 0.05$ ). The  $\Sigma$ SFA value for *S. rizeensis* analyzed in this study was 32.91%, which was also supported by other studies in the literature for *S. t. macrostigma* (Akpınar et al., 2009; Haliloğlu et al., 2005; Kayhan et al., 2015; Kaçar et al. 2021a,b; Ateş et al., 2013; Aras et al., 2003b; Oz and Dikel, 2015), while a higher SFA ranging from 46.40% to 47.58% was reported by Bayir et al. (2010). Compared to the data of *S. coruhensis* in the present study, similar (Aras et al., 2003a), higher (Bayir et al., 2010), and lower (Şahin et al., 2011; Çankırılıgil and Berik, 2020) levels of

EPA and DHA were reported by other studies on *S. t. labrax*. Bayir et al. (2010) reported that SFA content (43.47-46.30%) in the muscle of wild *S. t. caspius* was in agreement with the results of *S. ardahanensis* used in the current study. However, the lower amount of SFA in *S. caspius* fed with different diets was determined by Mohseni et al. (2021). In the present study, palmitic (C16:0), stearic acid (C18:0), and heneicosanoic (C21:0) were the dominating fatty acids among SFAs in all trout species. Palmitic acid had the highest percentage of SFAs in all trout species, which ranged from 17.30% to 18.75% ( $p < 0.05$ ). Many studies also reported that C16:0 was the most abundant SFA in different trout species, including either wild or cultured *S. t. macrostigma* (Aras et al., 2003b, Haliloğlu et al., 2005; Akpınar et al., 2009, Bayir et al., 2010, Ateş et al., 2013; Kayhan et al., 2015; Oz and Dikel, 2015; Kaçar et al., 2021a,b), *S. t. caspius* (Bayir et al., 2010; Mohseni et al., 2021), and *S. t. labrax* (Aras et al., 2003a; Bayir et al., 2010; Şahin et al., 2011;

**Table 2.** Comparison of fatty acid profiles (% total FAME) in the muscle tissue of different trout species (mean  $\pm$  SD, n=3)

Fatty acids	Trout species		
	<i>S. rizeensis</i>	<i>S. ardahanensis</i>	<i>S. coruhensis</i>
C14:0	1.74 $\pm$ 0.01 <sub>A</sub>	3.28 $\pm$ 0.11 <sub>B</sub>	2.11 $\pm$ 0.09 <sub>C</sub>
C15:0	0.25 $\pm$ 0.04 <sub>A</sub>	0.24 $\pm$ 0.01 <sub>A</sub>	0.46 $\pm$ 0.01 <sub>B</sub>
C16:0	17.76 $\pm$ 0.46 <sub>AB</sub>	18.75 $\pm$ 0.15 <sub>A</sub>	17.30 $\pm$ 0.21 <sub>B</sub>
C17:0	0.57 $\pm$ 0.03 <sub>A</sub>	0.57 $\pm$ 0.01 <sub>A</sub>	0.63 $\pm$ 0.08 <sub>A</sub>
C18:0	4.60 $\pm$ 0.04 <sub>A</sub>	6.17 $\pm$ 0.18 <sub>B</sub>	4.74 $\pm$ 0.16 <sub>A</sub>
C20:0	0.38 $\pm$ 0.04 <sub>A</sub>	0.84 $\pm$ 0.02 <sub>B</sub>	0.42 $\pm$ 0.01 <sub>A</sub>
C21:0	6.57 $\pm$ 0.87 <sub>A</sub>	7.95 $\pm$ 0.37 <sub>A</sub>	2.80 $\pm$ 0.04 <sub>B</sub>
C22:0	*	0.62 $\pm$ 0.02 <sub>A</sub>	0.21 $\pm$ 0.04 <sub>B</sub>
C24:0	1.05 $\pm$ 0.06 <sub>A</sub>	1.73 $\pm$ 0.08 <sub>B</sub>	0.58 $\pm$ 0.03 <sub>C</sub>
$\Sigma$ SFA	<b>32.91<math>\pm</math>0.56<sub>A</sub></b>	<b>40.12<math>\pm</math>0.16<sub>B</sub></b>	<b>29.22<math>\pm</math>0.01<sub>C</sub></b>
C14:1	0.06 $\pm$ 0.01 <sub>A</sub>	0.26 $\pm$ 0.01 <sub>B</sub>	0.11 $\pm$ 0.02 <sub>A</sub>
C16:1	7.19 $\pm$ 0.35 <sub>A</sub>	7.60 $\pm$ 0.18 <sub>A</sub>	3.69 $\pm$ 0.13 <sub>B</sub>
C17:1	0.55 $\pm$ 0.04 <sub>A</sub>	0.64 $\pm$ 0.08 <sub>A</sub>	0.55 $\pm$ 0.04 <sub>A</sub>
C18:1n9t	0.17 $\pm$ 0.01 <sub>A</sub>	0.06 $\pm$ 0.01 <sub>A</sub>	0.40 $\pm$ 0.08 <sub>B</sub>
C18:1n9c	26.96 $\pm$ 0.18 <sub>A</sub>	18.51 $\pm$ 0.18 <sub>B</sub>	31.20 $\pm$ 0.73 <sub>C</sub>
C20:1	2.21 $\pm$ 0.03 <sub>A</sub>	0.52 $\pm$ 0.03 <sub>B</sub>	1.75 $\pm$ 0.08 <sub>C</sub>
$\Sigma$ MUFA	<b>37.14<math>\pm</math>0.21<sub>A</sub></b>	<b>27.57<math>\pm</math>0.38<sub>B</sub></b>	<b>37.67<math>\pm</math>0.81<sub>A</sub></b>
C18:2n6c	8.18 $\pm$ 0.01 <sub>A</sub>	3.87 $\pm$ 0.25 <sub>B</sub>	9.38 $\pm$ 0.17 <sub>C</sub>
C18:3n6c	*	0.39 $\pm$ 0.09 <sub>A</sub>	0.39 $\pm$ 0.01 <sub>A</sub>
C20:2cis	2.01 $\pm$ 0.11 <sub>A</sub>	2.57 $\pm$ 0.05 <sub>B</sub>	1.64 $\pm$ 0.06 <sub>C</sub>
C20:3n6	0.85 $\pm$ 0.01 <sub>A</sub>	0.39 $\pm$ 0.02 <sub>B</sub>	1.40 $\pm$ 0.01 <sub>C</sub>
C20:3n3	0.61 $\pm$ 0.01 <sub>A</sub>	0.68 $\pm$ 0.01 <sub>B</sub>	0.53 $\pm$ 0.00 <sub>C</sub>
C20:4n6	1.19 $\pm$ 0.07 <sub>A</sub>	0.97 $\pm$ 0.03 <sub>A</sub>	3.06 $\pm$ 0.25 <sub>B</sub>
C20:5n3	3.15 $\pm$ 0.07 <sub>A</sub>	6.13 $\pm$ 0.30 <sub>B</sub>	3.19 $\pm$ 0.12 <sub>A</sub>
C22:5n3	1.34 $\pm$ 0.06 <sub>A</sub>	2.11 $\pm$ 0.02 <sub>B</sub>	1.53 $\pm$ 0.17 <sub>A</sub>
C22:6n3	9.61 $\pm$ 0.19 <sub>A</sub>	9.45 $\pm$ 0.13 <sub>A</sub>	7.31 $\pm$ 0.02 <sub>B</sub>
$\Sigma$ PUFA	<b>26.93<math>\pm</math>0.29<sub>A</sub></b>	<b>26.54<math>\pm</math>0.16<sub>A</sub></b>	<b>28.41<math>\pm</math>0.32<sub>B</sub></b>
Unidentified	3.04 $\pm$ 0.47 <sub>A</sub>	5.78 $\pm$ 0.06 <sub>B</sub>	4.71 $\pm$ 0.50 <sub>AB</sub>

\*Not detected. Different letters (A-C) in the same row represent significant differences ( $p < 0.05$ ) among fish species.

Çankırılıgil and Berik, 2020).

In the present study, the  $\Sigma$ MUFA content was highest in *S. coruhensis* with 37.67% and the lowest in *S. ardahanensis* with 27.57% ( $p < 0.05$ ). Among MUFAs and FAs in all trout species, the predominant fatty acids were oleic acid (C18:1n9c) in the range of 18.51% (*S. ardahanensis*) to 31.20% (*S. coruhensis*) ( $p < 0.05$ ). The total MUFA content of *S. ardahanensis* was significantly lower ( $p < 0.05$ ) than other species due to its low oleic acid content. The second major MUFA was palmitoleic acid (C16:1), with the highest value in *S. ardahanensis* (7.60%) and the lowest value in *S. coruhensis* (3.69%) ( $p < 0.05$ ). Similarly, many studies conducted on *Salmo trutta* species also reported that oleic and palmitoleic acids were predominant among SFAs (Aras et al., 2003a; Haliloğlu et al., 2005; Bayir et al., 2010; Kayhan et al., 2015; Mohseni et al., 2021; Kaçar et al., 2021a). In addition, these studies reported higher concentrations of MUFAs in trout species than those of PUFAs, which is consistent with the present study.

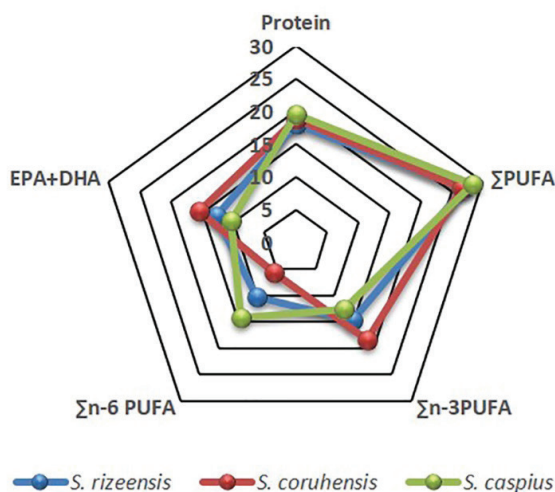
Overall average PUFA composition was 28.41%, 26.93%, and 26.54% for *S. coruhensis*, *S. rizeensis*, and *S. ardahanensis*, respectively.  $\Sigma$ PUFA content of *S. coruhensis* was found to be statistically ( $p < 0.05$ ) higher than those of *S. rizeensis* and *S. ardahanensis* (Figure 1). The freshwater fish's low PUFA content may be because these species are mainly fed on vegetation and plant materials. On the contrary, the high PUFA value of marine fish is due to their feeding on PUFA-rich zooplankton (Jabeen and Chaudhry, 2011). Among PUFAs, linoleic (C18:2n6c), EPA (C20:5n3), and DHA (C22:6n3) were dominant in all trout spe-

cies used in the present study. DHA was present in the highest concentrations in *S. rizeensis* and *S. ardahanensis*, while linoleic acid, essential for humans, was the highest in *S. coruhensis* ( $p < 0.05$ ). Other studies also reported that DHA, EPA, linolenic acid, and algalinolenic acid (C18:3n3) were dominant SFAs in different trout species (Blanchet et al., 2005; Kayhan et al., 2015; Kaçar et al., 2021a,b; Łuczynska et al., 2017; Erdem et al., 2020; Mohseni et al., 2021; Oz and Dikel, 2015; Şahin et al., 2011). However, these researchers reported higher total PUFA values than this study data. On the contrary, Bayir et al. (2010) determined a slightly lower  $\Sigma$ PUFA (24.63% in the spring) in *S. t. caspius*.

When FA results were evaluated, it was seen that there were some differences between the species in this study and the literature studies. It is well known that fatty acid composition in fish muscle varies greatly depending on the species, sex, age, season, geographical location, feeding regime, environmental conditions (such as water temperature, pH, and salinity), the physiological status of the fish (such as gonad maturation, and reproduction), and whether fish are wild or farm-raised (Ateş et al., 2013; Kaçar et al., 2021a). This difference is believed to have arisen from these factors.

### Quality indices of fatty acids

The quality indices of fatty acids in the muscle tissue of *S. rizeensis*, *S. ardahanensis*, and *S. coruhensis* are given in Table 3. PUFA/SFA is the most widely used index to evaluate the effect of diet on cardiovascular health and the nutritional value of dietary foods (Chen and Liu, 2020). The PUFA/SFA ratio of



**Figure 1.** Comparison of trout species in terms of some essential nutritional content

trout species ranged from 0.66 (*S. ardahanensis*) to 0.97 (*S. coruhensis*). Aras et al. (2003a) found PUFA/SFA level (0.88) for wild *S.t. labrax* to be similar to the result of this study. Aras et al. (2003b) reported a slightly higher PUFA/SFA level of 1.35 for wild *S.t. macrostigma* compared to our research. According to the British Department of Health (1994), the PUFA/SFA ratio in the human diet should be a minimum of 0.45. The PUFA/SFA (0.66-0.97) ratios in the present study were higher than the minimum recommendation of 0.45. Similarly, higher PUFA/SFA was reported to be higher than the minimum recommended value for *S. t. macrostigma* (Oz and Dikel, 2015), *S. t. labrax* (Şahin et al., 2011; Çankırılıgil and Berik (2020) and *S. t. caspius* (Mohseni et al., 2021).

The trout species used in this study were found to be very rich in highly beneficial omega-3 long-chain polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA; 20: 5n-3) and docosahexaenoic acid (DHA; 22: 6n-3). DHA and EPA have been reported to have preventive effects on human coronary artery disease (Jabeen and Chaudhry, 211). The total EPA+DHA contents of trout species in the present study were the highest (15.58%) in *S. ardahanensis* and the lowest (10.49%) in *S. coruhensis*. Bayir et al. (2010) reported that the EPA+DHA content of *S. t. macrostigma*, *S. t. caspius*, and *S. t. labrax* obtained in spring was found to be 18.90%, 24.25%, and 20.92%, respectively. Ateş et al. (2013) reported that EPA +DHA content of wild *S. t. macrostigma* varied between 15.65% in spring and 17.81% in Autumn. Şahin et al. (2011) reported that EPA+DHA of *S. t. labrax* was 18.89%. In another study, the EPA + DHA content of muscle of male and female *S. t. macrostigma* was reported as 16.30% and 13.83%, respectively (Akpınar et al.,

2009). The 2015-2020 United States Department of Agriculture guidelines suggest that 224 g of seafood per week or 250 mg/day each of EPA and DHA (500 g total) is enough to provide health benefits (USDA 2015). In the present study, the EPA+DHA content was calculated as an average of 273 mg/100 g in *S. rizeensis*, 303 mg/100 g in *S. ardahanensis*, and 250 mg/100 g in *S. coruhensis*. The recommended weekly intake of EPA+DHA can be met by consuming approximately 183 g of *S. rizeensis*, 165 g of *S. ardahanensis*, and 200 g of *S. coruhensis*. These significant levels of EPA and DHA detected in fish species in this study showed that these species can be used to supplement essential fatty acids in the human diet.

The n-3 and n-6 PUFA content and their ratios are associated with human diseases, including obesity, cardiovascular, cerebrovascular diseases, depression, and cancer, and the lower n-6/n-3 PUFA ratio is more beneficial to human health (Kinsella et al., 1990). Fish muscles typically have a high content of n-3 PUFA and a low n-6/n-3 PUFA ratio. In the present study, the highest content of total n-3 PUFAs was found in *S. ardahanensis*, while the lowest was in *S. coruhensis* ( $p < 0.05$ ). On the contrary, the lowest content of total n-6 PUFAs was found in *S. ardahanensis*, while the highest was in *S. coruhensis* ( $p < 0.05$ ). The n-3/n-6 PUFA ratio is a helpful indicator for comparing the relative nutritional values of fish oils and is recommended to be above 1 for health benefits (Chow, 2008). The n-3/n-6 ratio of *S. rizeensis* and *S. ardahanensis* was greater than the recommended value, while it was slightly lower for *S. coruhensis* ( $p < 0.05$ ). The British Department of Health (1994) reported that the maximum n-6/n-3 PUFA ratio in the human diet should be 4.0, and a higher n-6/n-3 PUFA ratio is associated with an

**Table 3.** Quality indices of fatty acid in the muscle tissue of different trout species (Mean  $\pm$  SD, n=3).

Fatty acids (%)	Trout species		
	<i>S. rizeensis</i>	<i>S. ardahanensis</i>	<i>S. coruhensis</i>
$\Sigma$ PUFA/ $\Sigma$ SFA	0.82 $\pm$ 0.02 <sub>A</sub>	0.66 $\pm$ 0.00 <sub>B</sub>	0.97 $\pm$ 0.01 <sub>C</sub>
EPA+DHA	12.76 $\pm$ 0.26 <sub>A</sub>	15.58 $\pm$ 0.42 <sub>B</sub>	10.49 $\pm$ 0.14 <sub>C</sub>
$\Sigma$ n-3PUFA	14.70 $\pm$ 0.31 <sub>A</sub>	18.36 $\pm$ 0.41 <sub>B</sub>	12.55 $\pm$ 0.31 <sub>C</sub>
$\Sigma$ n-6PUFA	10.22 $\pm$ 0.09 <sub>A</sub>	5.61 $\pm$ 0.30 <sub>B</sub>	14.22 $\pm$ 0.07 <sub>C</sub>
$\Sigma$ n-3 / $\Sigma$ n-6 PUFA	1.44 $\pm$ 0.02 <sub>A</sub>	3.28 $\pm$ 0.25 <sub>B</sub>	0.88 $\pm$ 0.02 <sub>A</sub>
$\Sigma$ n-6 / $\Sigma$ n-3 PUFA	0.69 $\pm$ 0.01 <sub>A</sub>	0.31 $\pm$ 0.02 <sub>B</sub>	1.13 $\pm$ 0.02 <sub>C</sub>
AI	0.40 $\pm$ 0.01 <sub>A</sub>	0.62 $\pm$ 0.01 <sub>B</sub>	0.40 $\pm$ 0.01 <sub>A</sub>
TI	0.35 $\pm$ 0.01 <sub>A</sub>	0.38 $\pm$ 0.01 <sub>B</sub>	0.37 $\pm$ 0.01 <sub>B</sub>
h/H	2.76 $\pm$ 0.04 <sub>A</sub>	2.05 $\pm$ 0.02 <sub>B</sub>	3.07 $\pm$ 0.03 <sub>C</sub>
FLQ	13.16 $\pm$ 0.33 <sub>A</sub>	16.54 $\pm$ 0.46 <sub>B</sub>	11.01 $\pm$ 0.21 <sub>C</sub>
PI	0.72 $\pm$ 0.01 <sub>A</sub>	0.83 $\pm$ 0.02 <sub>B</sub>	0.61 $\pm$ 0.02 <sub>C</sub>
UI	146.71 $\pm$ 1.67 <sub>A</sub>	146.53 $\pm$ 1.27 <sub>A</sub>	146.27 $\pm$ 1.27 <sub>A</sub>

Different letters (A-C) in the same row represent significant differences ( $p < 0.05$ ) among fish species.



increased risk of carcinogenesis. In this study, the ratio of n-6/n-3 PUFA was considerably lower than the recommended value, ranging from 0.31 to 1.13 ( $p < 0.05$ ). A higher percentage of total n-3 PUFAs in trout species was mainly due to the higher sum of DHA and EPA. Bayir et al. (2010) reported that n-3/n-6 contents of *S. t. macrostigma*, *S. t. caspius*, and *S. t. labrax* obtained in spring were 1.46, 2.34, and 1.53, respectively. Şahin et al. (2011) reported higher  $\sum n-3$  (21.23%), n-3/n-6 (1.42), and similar  $\sum n-6$  (15.0%) in farmed *S.t. labrax* when compared with our findings. Ateş et al. (2013) reported higher  $\sum n-3$  (23.78-29.12), n-3/n-6 (2.60-5.20), and lower  $\sum n-6$  (5.60-9.14) in *S. t. macrostigma* from different seasons when compared to those from *S. coruhensis* used in this study. The seasonal variation in the fatty acid composition in the muscle tissue of males (Kaçar et al., 2021a) and females (Kaçar et al., 2021b) *S. t. macrostigma* was investigated. The researchers reported higher n-3/n-6 (in the range of 2.79 to 5.79) than those obtained from *S. rizeensis* and *S. coruhensis* in this study. However, the n-3/n-6 ratio of *S. ardahanensis* determined in the present study was in this range. In another study, lower  $\sum n-3$  (9.65-15.13), n-3/n-6 (0.44-0.88), and higher  $\sum n-6$  (17.20-21.89) in farmed fingerlings *S.t. caspius* was determined (Mohseni et al., 2021). Species, geographical origins, and seasons affect the lipid content of fish (Bayir et al., 2006). Fatty acid patterns in dietary lipids significantly influence fatty acid compositions of tissue lipids in wild fish and reflect the availability of fatty acids in the aquatic food chain (Ateş et al., 2013). The differences may be due to these conditions.

The atherogenic index (AI) and thrombogenicity index (TI) provide indications of the dietetic quality of lipids and their potential effect on the development of coronary disease (Ulbricht and Southgate, 1991). Low values of AI and TI show a better nutritional quality of fatty acids, reducing the potential risk of coronary heart disease, and the values of these indexes should be more than 1.0 in terms of human health (Ouraji et al., 2009). The study found AI (0.40-0.62) and TI values (0.35-0.38) under this established limit. Similarly, Erdem et al. (2020) reported that AI and TI values for wild *S. t. fario*, cultured *S. t. fario*, and cultured *S. salar* ranged from 0.22 to 0.34 and 0.18 to 0.34, respectively. Moreover, AI (0.35-0.81) and TI (0.23-0.36) values of brown trout (*S. trutta* L.) on different dietary treatments reported by Turchini et al. (2003) were in agreement with the current study.

The high h/H ratio in the diet is considered more beneficial for human health and more accurately reflects the effect of the fatty acid composition on cardiovascular diseases than the PUFA/SFA ratio (Chen and Liu, 2020). The h/H ratios of trout species used in this study were found in the range of 2.05 to 3.07, and significant differences were noted among the h/H values of trout species ( $p < 0.05$ ). The h/H values reported by Erdem et al. (2020) in *S. t. fario* (3.46-3.80) and *S. salar* (5.74) were higher than those of the present study. The h/H value (ranging from 2.17 to 2.77) of zander (*Sander lucioperca*), which is a freshwater fish, reported by Çağlak and Karsli (2017) was found to be compatible with the data of this study.

FLQ is used to evaluate the quality of lipids in fish, and a higher FLQ value represents a good quality and nutritious lipid (Abrami et al., 1992). The present study found the highest FLQ value in *S. ardahanensis*, while the lowest was in *S. coruhensis*. There was a statistically significant difference among trout species regarding FLQ value ( $p < 0.05$ ). Łuczynska et al. (2017) reported that the FLQ value of rainbow trout (*Oncorhynchus mykiss*) was in agreement with the results of *S. ardahanensis* in the present study; however, it was higher than *S. rizeensis* and *S. coruhensis* in the present study. On the contrary, Erdem et al. (2020) reported lower FLQ values in wild- and cultured *S. t. fario* and *S. salar*.

The polyene index (PI) is an index usually used to measure the damage of PUFAs (Lubis and Buckle, 1990), and it was found to be 0.72 for *S. rizeensis*, 0.83 for *S. ardahanensis*, and 0.61 for *S. coruhensis* ( $p < 0.05$ ). These findings were consistent with those reported by Ehsani et al. (2017) in rainbow trout.

The unsaturation index (UI), which represents the degree of unsaturation in lipids, was found to be quite similar (ranging between 146.27 and 146.71) in the three different trout species used in this study ( $p > 0.05$ ). Although UI is generally used in FA of macroalgae, it has also been reported in the range of 73-155 in some foods such as crops, meat, and dairy products (Chen et al., 2020). Moreover, Karsli (2021) reported that the UI of the 15 commercial fish oil supplements available in the Turkish market ranged from 139.47 to 434.63. The UI values of this study were found to be within the range of data reported in the literature.

## CONCLUSION

In this study, the proximate composition, fatty ac-

ids, and lipid quality indices of three wild native trout species, including *S. rizeensis*, *S. ardahanensis*, and *S. coruhensis*, were compared. All examined trout species were found to be a good source of protein and essential fatty acids, especially DHA and EPA, which are suitable for health. The highest PUFA, EPA, and DHA values were found in *S. coruhensis*, *S. ardahanensis*, and *S. rizeensis*, respectively. All species contained higher amounts of PUFA/SFA, n-3/n-6, h/H, and FLQ. The AI and TI values of all trout species were within the recommended limit value. According to the calculated lipid quality index data, the consumption of these species does not pose a potential health risk for consumers. Although *S. coruhensis* was the richest species in terms of total PUFAs, *S. ardahanensis* had the highest values in terms of EPA+DHA, n-3/n-6, and FLQ values. In this respect, *S. ardahanensis* and *S. rizeensis* could provide benefits and significant economic value for future fish farming as an alternative to *S. coruhensis* (*S.t labrax*) and *O. mykiss*, which are widely used in aquaculture, and they could be considered as an important protein and  $\omega$ -3 fatty acid source in the human diet.

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## CONFLICTS OF INTEREST

All the authors declared they have no conflict of interest.

## ETHICS APPROVAL

Not applicable.

## AVAILABILITY OF DATA AND MATERIAL

Data will be made available on request.

## AUTHOR CONTRIBUTIONS

BK, EÇ, and CK conceived the study. CK collected the samples. BK performed the laboratory work, analyzed the data, and wrote the original draft. All authors contributed to reviewing and editing the manuscript.

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