

Divergences of Biochemical Features of Three Reared Trouts; Brook Trout (*Salvelinus fontinalis*, Mitchill 1814), Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1972), and Black Sea Trout (*Salmo trutta labrax* Pallas 1811)

Ayşe ÖZYILMAZ¹⁶⁷, Kadriye OCAK², Sevil DEMİRCİ³

^{1,2,3}İskenderun Technical University, Faculty of Marine Sciences and Technology, Iskenderun, Hatay/Türkiye
 ¹https://orcid.org/0000-0002-4376-0873, ²https://orcid.org/0000-0002-0258-0315, ³https://orcid.org/0000-0003-4229-6236
 i ayse.ozyilmaz@iste.edu.tr

ABSTRACT

The objective of this present study is to signify biochemical features of three reared trouts (brook trout, rainbow trout, Black Sea trout) that are economically consequential and reared fish species in the Eastern Black Sea region. The rainbow trout has been reared successfully for a long period of time. However, brook trout and the Black Sea trout have been two new species to be reared in the region with a high potential contribution to the economy. Therefore, there is a strong need to determine and report the differences between the fish (especially the two new species), levels of carbohydrates, energy, carotenes (Vitamin A), fatty acid, and proximate composition (protein, lipid, moisture, and ash). In addition, the lipid indices [Atherogenic Index (AI), Thrombogenic Index (TI), and polyene index (PI)] were also measured and reported for the trouts. Moreover, the color of the fillets of the fish was also measured. The results of this present study show that the average levels of moisture and protein varied while the average levels of lipid and ash were close to each other. The highest levels of carbohydrates, energy, and carotenes were determined in the muscle of brook trout in this study. The highest meat yield was also obtained from brook trout followed by Black Sea trout and rainbow trout. A total of 19 fatty acids were determined for Black Sea trout and brook trout and 17 fatty acids for rainbow trout in the present study. The omega-3 levels of the all trout used for the present study were roughly twice as much as that of the omega-6, except for rainbow trout.

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Yetiştiriciği Yapılan Üç Tür Alabalığın; Kaynak Alabalığı (*Salvelinus fontinalis*, Mitchill 1814), Gökkuşağı Alabalığı (*Oncorhynchus mykiss* Walbaum, 1972) ve Karadeniz Alasının (*Salmo trutta labrax* Pallas 1811) Biyokimyasal Özelliklerinin Farklılıkları

ÖZET

Bu çalışmanın amacı, Doğu Karadeniz Bölgesi'nde ekonomik olar önemli ve yetiştirilen üç alabalık (kaynak alabalığı, gökkuşağı alabal ve Karadeniz alabalığı) türünün bazı biyokimyasal özellikleri uzun belirlemektir. Gökkuşağı alabalığı süredir basarıy yetiştirilmektedir. Ancak kaynak alabalığı ve Karadeniz alabalı bölgede yetiştirilmeye başlanan ve ekonomiye katkısı yüksek iki ye tür olmuştur. Bu nedenle, balıklar (özellikle iki yeni tür) arasında karbonhidrat, enerji, karoten (A Vitamini) ve yağ asidi ve bes bileşenleri (protein, lipit, nem ve kül) seviyelerindeki farklılıkla belirlemeye ve raporlamaya güçlü bir ihtiyaç vardır. Ayrıca, balıklar lipit indeksleri [Aterojenik Indeks (AI), Trombojenik Indeks (TI) polien indeksi (PI)] de hesaplanmıştır. Ek olarak, bu çalışma balıkların fileto renkleri de ölçülmüştür. Balıklarda ortalama nem protein seviyeleri farklılık gösterirken, lipit ve kül seviyeleri birbiri yakın bulunmuştur. Bu çalışmada en yüksek karbonhidrat, enerji karoten seviyeleri kaynak alabalığında belirlenmiştir. En yüksek verimi yine kaynak alabalığından elde edilmiştir, bunu Karadeniz ala ve gökkuşağı alabalığı izlemiştir. Çalışma için Karadeniz alası ve

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kaynak alabalığında toplam 19 yağ asidi ve gökkuşağı alabalığı için 17 yağ asidi belirlenmiştir. Çalışmada kullanılan tüm alabalıkların yaklaşık omega-3 seviyelerinin, gökkuşağı alabalığı hariç, omega-6 seviyelerine oranları kabaca iki katı olduğu belirlenmiştir.

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INTRODUCTION

Brook trout, rainbow trout, and Black Sea trout are three reared fish species in the Eastern Black Sea region. These fish have their places on the counters in fish markets, and they compete with each other to get the attention of the customers. The brook trout and Black Sea trout species have challenges in the rearing field comparing the rainbow trout, which is relatively easy to rear. Although rainbow trout have many advantages in rearing conditions, the brook trout and Black Sea trout seem to be one step ahead in marketing prices. Recently, the brook trout, Black Sea trout, and rainbow trout are all subjected to many scientific studies not only in Turkey but also in many other countries such as the USA, Canada, Sweden, Ukraine, and China (Memiş et al., 2020; Cankiriligil & Berik, 2020; Latiu et al., 2020; Martling et al., 2020; Závorka et al., 2020; Zhang et al., 2021; Turan & Aksu, 2021; Barylo et al., 2021; Bayar et al., 2021; İspir et al., 2021; Baesu et al., 2022).

Fish and fish-related dishes nearly come first as a preferred food in diets for a variety of reasons. The taste and lipid content of the fish that make it a healthy food are among the many reasons. Consumption of fish provides some health benefits because fish contains bioactive constituents that improve nutritional quality (Li et al., 2020; Tacon et al., 2020; Chen et al., 2021). Department of health in many countries encourages fish consumption to develop a healthy population of people.

The main parts in muscle of fish are generally known to provide moisture, protein, lipid, and ash which are known proximate components and may vary from species to species (Öksüz & Özyılmaz, 2010; Kayım et al. 2011; Şahin et al., 2011; Öksüz, 2012; Yeşilayer & Genc, 2013; Ozyilmaz, 2019; Çankiriligil & Berik, 2020; Memiş et al., 2021). Fish consumption may give us an opportunity to get health benefits Individuals should consume fish regularly to get the benefits out of it.

Choosing the right fish for individual consumption, therefore, could be a crucial decision to obtain all beneficial ingredients available in the fish. The aim of this present study is to investigate the amounts of ingredients such as protein, lipid, ash, moisture, meat yield, carbohydrate, energy, carotene (Vitamin A), and fatty acids, and their lipid indices (Atherogenic Index, Thrombogenic Index, Polyene Index) available in three different salmonids (Brook trout, rainbow trout, Black Sea trout) in order for us to evaluate the similarities and differences. The fillet colors of these three reared trouts were also determined instrumentally to measure the color changes. The present study provides data on the biochemical attributes of these three fish species, specifically on fatty acid, protein, lipid, moisture, and ash of reared fish, providing unique knowledge to decide what type of fish should be included in an individual's diet.

MATERIALS and METHODS

Fish Materials

Brook trout (Salvelinus fontinalis, Mitchill 1814), rainbow trout (Oncorhynchus mykiss, Walbaum, 1972), and Black Sea trout (Salmo trutta labrax, Pallas 1811) have a growing importance in the Eastern Black Sea region. The fish used in the present were purchased after regular study harvesting times whenever they were ready for sale by the owner of the fish rearing unit. We did not interfere with any prior steps of our purchase of the fish samples however we asked the sellers of the fish about their life history of the fish and recorded the information in detail. Because we observed three different trouts that were reared in a commercial fish rearing unit in Trabzon/Turkey in this research, we present the information that we gathered in the following section.

The fish were grown from eggs to harvest in the commencal fish rearing units and fed with commercial feed. More specifically, the fish were fed with a meal called "range, nurse-s fish hatchery diet" until fish was 1 g in weight. Later on, the fish were fed with another type of feed (Sürsan, Aquamax, extruded fish meal, Muğla, Turkey) for the trouts from 1 g to harvesting. The fish meals were ordinary fish meals that were regularly used in rearing units in the region. The fish used in this current study was an average of 18-20 months old. The spring water was generally used by the units however the river water was also used whenever the spring water was not adequate.

A total of 14, 18, and 22 fish out of approximately 3000 kg capacity units were randomly selected for brook trout, rainbow trout, and Black Sea trout, respectively for the present study. The trouts were harvested late in March, in the middle of April, and early in May, which are the three months when the fish were sold frequently in the markets. Fish in ice boxes were transported to the laboratory. Their measurements (length and weight) were taken before filleting the fish. Only dorsal parts of the fish were used for the chemical analysis. The inside of the fillets was used for color measurements. The skinless fillets were finely chopped, gathered in a container, and mixed for the biochemical analysis.

Methods

The official methods were performed for the proximate compositions (the crude moisture, ash, protein, and lipid) which were given in detail in Öksüz (2012). The calculations used for carbohydrate contents and energy values were provided by Güner et al. (1998).

Determination of Total Levels of Carotene (vitamin A)

The spectrophotometric determination method described in TS, 1987 (Turkish Standard, number: 5036) and Morello et al. (2004) method were used to determine total carotene levels for all three fish species. A total of 7.5 g muscle lipid of Brook trout, rainbow trout, and Black Sea trout was weighed and dissolved with cyclohexane in a 25 mL volumetric flask. The mixture was made up to 25 mL volume with cyclohexane. The absorbance was read at 470 nm with a spectrophotometer (Shimadzu Hitachi U-1900 model). Its absorbance was measured with a spectrophotometer (Morella et al., 2004). The following equation was used for calculations.

Amount of carotenoids (mg carotenoids/kg lipid) = $(A470 \times 106)/(2000 \times 100 \times L)$

A = Absorbance

L = Light path (cell thickness, mm)

Color measurements

A chroma meter (CR-400, Minolta, Osaka, Japan) was used to measure the color of the fillets of all three fish specimens. Inside of the fillets, places of the anterior, posterior, and caudal locations, were used for colorimetric analysis which was explained in Öksüz (2012).

Fatty Acid Methyl Esters

After obtaining lipids from all three fillets of the trouts, fatty acid methyl esters were carried out by

using the lipid. GC-MS (Gas Chromatography-Mass Spectrometry) was used to determine the fatty acids of the lipids for all three fish muscles. Preparation, conversion, and separation of the fatty acid methyl esters were described in Öksüz and Özyılmaz (2010). The instrument and column conditions were also detailed in the same study, except for the column.

Lipid quality indexes

The data obtained from the fatty acid composition were used to calculate the Atherogenicity Index (AI), and Thrombogenicity Index (TI). The following equations reported by Ulbricht & Southgate (1991) were used to calculate Lipid Quality Indexes (AI and TI).

 $AI=[(4xC14:0)+C12:0+C16:0]/[(\Sigma PUFA-n6+\Sigma PUFA-n3)+\Sigma MUFA]$

IT=[(C14:0+C16:0+C18:0)/(0.5xMUFA+0.5xPUFAn6+3xPUFA-n3+PUFA-n3/PUFA-n6)]

Additionally, the following equation was used the calculate polyene index (PI), which evaluates the PUFAs damage (Lubis & Buckle, 1990).

(PI) = (C20:5 + C22:6)/C16:0

Statistical analysis

Statistical analysis was performed with SPSS (22.0). Significance was established at P<0.05. The data obtained from this study regarding three different trout species were subjected to a one-way analysis of variance (ANOVA), and a mean comparison was carried out by using Duncan's Multiple Range test to see if there are any statistically significant differences between groups. The homogeneity of variances was tested before ANOVA analysis was performed.

RESULTS and DISCUSSION

In this study, the length and the total weight of the brook trout, rainbow trout, and Black Sea trout were tabulated in Table 1. The average lengths were measured in the range of 25.82±2.12 -27.93±1.01 cm for the trouts. The highest weight obtained from rainbow trout was followed by brook trout and Black Sea trout. The highest length was also obtained from rainbow trout.

 Table 1. Total length and total weight of the brook trout, rainbow trout, and Black Sea trout

Çizelge 1. Kaynak alabalığı, gökkuşağı alabalığı ve Karadeniz alasının boy ve ağırlığı

| Fish species | Length (cm) | Total Weight (g) | | |
|-----------------|------------------|--------------------|--|--|
| Brook Trout | 25.82 ± 2.12 | 232.93 ± 43.01 | | |
| Rainbow Trout | 27.93 ± 1.01 | 272.61 ± 13.86 | | |
| Black Sea Trout | 25.89 ± 1.07 | 199.77±10.41 | | |
| | | | | |

n=14 brook trout, n=18 rainbow trout, and n=22 Black Sea trout

The proximate composition, meat yield, carbohydrate, energy, and carotenes levels of the brook trout, rainbow trout, and Black Sea trout were shown in Table 2. The meat yield diverged in trout species. This divergence was measured to be statistically significant (P<0.05). The highest meat yield was obtained from brook trout followed by Black Sea and rainbow trout in this study. Sahin et al. (2011) study reported that the meat yield of brook trout was higher than that of Black Sea trout. The present study obtained similar results regarding meat yield. Additionally, Özyılmaz (2019) reported the meat yield of rainbow trout as 66.23±0.71% which was higher than that of the rainbow trout in the present study.

Table 2. The proximate composition, meat yield, carbohydrate, energy, and carotene (Vitamin A) levels of the brook trout, rainbow trout, and Black Sea trout

Çizelge 2. Kaynak alabalığı, gökkuşağı alabalığı ve Karadeniz alasının besin bileşenleri, et verimi, karbonhidrat, enerji ve karoten (A vitamini) düzeyleri

| Fish species | Brook Trout | Rainbow Trout | Black Sea Trout |
|---|------------------------|------------------------|----------------------------|
| Components | | | |
| Moisture (%) | 73.92±0.68ª | 76.19 ± 0.88^{b} | 77.630 ± 99^{b} |
| Lipid (%) | $1.14{\pm}0.08^{a}$ | 1.31 ± 0.05^{b} | $0.81 \pm 0.04^{\circ}$ |
| Ash (%) | 1.15 ± 0.02^{a} | 1.37 ± 0.05^{b} | $1.51 \pm 0.06^{\circ}$ |
| Protein (%) | $22.29{\pm}0.83^{a}$ | 19.92 ± 0.56^{b} | $18.67 \pm 0.34^{\circ}$ |
| Meat yield (%) | $56{\pm}0.02^{a}$ | 48 ± 0.03^{b} | $50{\pm}0.01^{\circ}$ |
| Carbohydrate (%) | 1.50 ± 0.54^{a} | 1.21 ± 1.03^{a} | $1.38{\pm}0.77^{a}$ |
| Energy | 445.12 ± 11.46^{a} | 406.58 ± 13.93^{b} | $369.30 \pm 14.84^{\circ}$ |
| Total carotenes (µg 100 g ⁻¹) | 94.22 ± 7.90^{a} | 36.00 ± 0.88^{b} | 79.72±1.34° |

n=3 for the chemical analysis

 $_{a,b,c}$ Values within same row with different superscripts diverge significantly at P<0.05

The average moisture levels were calculated in the following order; brook trout<rainbow trout<Black Sea trout (Table 2). Only the amount of moisture in brook trout was statistically different from the two others (P<0.05). The brook trout in this study has got the least moisture levels. Additionally, while the findings relating mean moisture levels of Black Sea trout were found to be higher than that of wild brown trout (74.8±1.1%) and reared rainbow trout (75.6±1.2%) (Yeşilayer & Genc., 2013), they were found to be very closer to the that of wild brown trout (77.80±0.3%) (Kayım et al., 2011).

Ranges of the average lipid levels of the trouts were calculated to be 0.81±0.04-1.31±0.05%. The lipid levels of the trouts varied only in a small amount. Although small changes were observed among the lipid levels in trouts, it was evaluated to be statistically significant (P<0.05). Given that all trout species have less than 2% lipid level, they were all defined as lean fish. The fish were harvested after the spawning period. The reason for having less lipid level could be the result of the period. One other reason could be their diet. Another reason could be the environmental effects. The wild Black Sea trout came from two different places, cultured Black Sea trout came from three different fish farming units, and three filial generations were measured in the range of 6.13±0.18% - 8.11±0.21% in the study of Cankırılıgil & Berik (2020). Lipid levels of the trouts in the present study were found lower than that stated in this previous study (Cankırılıgil & Berik, 2020). On the other hand, Ateş et al. (2013) measured the levels of the lipid in wild brown trout in winter as 1.48%, which was close to the levels of lipids in Black Sea trout in this current study. According to Özyılmaz (2019), the lipid of rainbow trout was $10.61\pm0.05\%$, which was higher than that of the rainbow trout, brook trout, and Black Sea trout in the present study. The differences in lipid levels of the trouts in the present study and that in the previous studies can be attributed to the changes in the feed and farm conditions.

The highest ash levels were measured in the Black Sea trout, followed by rainbow trout and brook trout. The ash levels among trouts differed from each other. Differences in ash content were found to be statistically significant (P<0.05). According to the study of Çakmak et al. (2018), the ash level of the fifth generation of Black Sea trout was $1.51\pm0.12\%$, the results very similar to the one reported in the present study.

The protein levels of the brook trout were the highest among all three trouts in the present study. Similarly, Barylo et al. (2021) reported the protein levels of three salmonids namely brown trout, rainbow trout, and brook trout. Accordingly, the brook trout was the highest one as to their protein levels. Additionally, the protein levels of the brook trout were measured to be $22.29\pm0.83\%$ which was higher than that of rainbow trout, and Black Sea trout. Moreover, Çakmak et al. (2018) reported that the protein level of the fifth generation of Black Sea trout as $15.22\pm0.48\%$ which was lower than that of Black Sea trout as well as all other trouts used in the present study. The protein levels of wild Black Sea trout from two different rivers (Altindere River and Çağlayan River), three different rearing units, and three different filial generations were found to be in the range of 17.94±0.10^{-17.52±0.18%} (Çankırılıgil & Berik, 2020). These values of protein in wild Black Sea trout, reared Black Sea trout, and filial generation of the Black Sea trout in study of Çankırılıgil & Berik (2020) were lower than those of protein in reared Black Sea trout which was 18.67±0.34% in the present study.

The carbohydrate amounts, energy value, and total carotene levels of the brook trout, rainbow trout, and Black Sea trout were shown in Table 2. The carbohydrate amounts of all trouts used in this current study were in the range of 1.21±1.03-1.50±0.54 %. Çakmak et al. (2018) reported the carbohydrate level of the fifth generation of Black Sea trout as 0.89±0.02%. The findings of the carbohydrate level of Black Sea trout as well as the other two salmonids in this study are higher than those of previously published findings (Çakmak et al., 2018). The brook trout also got the highest percentage of energy value compared to the two other salmonids. Similar energy values were measured for three freshwater fish species while lower carbohydrate levels were stated for those three freshwater fish species (Ozyilmaz et al., 2016). The total carotene levels are statistically significant from each other in the present study (P < 0.05).

The average levels of the total carotene (vitamin A) in the brook trout were almost three times higher than those of the total carotene in the rainbow trout. The mean amount of the total carotene in the liver oil of guitarfish, string ray, and eagle ray (249.72±69.6 µg 100g⁻¹, 401.49±4.06 µg 100 g⁻¹, and 104.53±2.10 µg 100 g⁻¹, respectively) were reported to be higher than that of carotene in all trouts under investigation in the present whereas that of carotene levels in bignose shark (29.26±2.83 µg 100g ⁻¹) were lower (Özyılmaz & Öksüz, 2015). The amounts of total carotenes in the livers of the smooth-hound and cownose ray were reported to be $83.78\pm3.53 \ \mu g \ 100 \ g^{-1}$ and $73.22\pm0.35 \ \mu g$ 100 g⁻¹, respectively. Findings of total carotenes in the livers of these two different cartilaginous fish species were in the range of carotene levels of all trouts in this present study (Özyılmaz & Öksüz, 2015).

The color of the muscle in the fishery sector can be considered a crucial issue to many customers as well as retailers. That is why the color of the fish fillets was measured for the present study. We aimed at figuring out whether the flesh color of these three salmonids can be a distinctive character to distinguish the trouts from each other. Although these three salmonids in this study belonging to the same family, their color of the muscle are different from each other visually and instrumentally. The color measurements of fillets' brook trout, rainbow trout, and Black Sea trout were tabulated in Table 3.

| Table 3. Color measurements of fillets' brook trout, rainbow trout, and Black Sea trout |
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| Cizelge 3. Kavnak alabalığı, gökkusağı alabalığı ve Karadeniz alası filetolarının renk ölcümleri |

| | | Lx (Lightness) | C (Chroma) | H (Hue) | |
|-----------|-----------------|------------------|------------------|------------------|--|
| Anterior, | Brook Trout | 47.31 ± 1.05 | 10.85 ± 0.26 | 50.53 ± 0.56 | |
| | Rainbow Trout | 56.86 ± 0.35 | 6.07 ± 0.19 | 66.42 ± 0.44 | |
| | Black Sea Trout | 45.41 ± 1.15 | 3.85 ± 0.18 | 77.38±1.02 | |
| Posterior | Brook Trout | 48.59±0.89 | 10.31±0.33 | 78.52±0.64 | |
| | Rainbow Trout | 48.75 ± 0.57 | 4.57 ± 0.34 | 71.15 ± 1.01 | |
| | Black Sea Trout | 39.46 ± 0.65 | 6.80 ± 0.41 | 47.69 ± 0.64 | |
| Caudal | Brook Trout | 61.64 ± 0.49 | 23.78±0.30 | 73.46±0.51 | |
| | Rainbow Trout | 48.53 ± 0.77 | 7.45 ± 0.46 | 62.08 ± 1.08 | |
| | Black Sea Trout | 47.65 ± 0.70 | 5.55 ± 0.43 | 66.72 ± 0.63 | |

The average means and standard deviations for measurements of fillets (n=3)

The lightness (L*) values in the trouts generally differed in the anterior, posterior, and caudal parts of the muscle, except for, a few minor similarities. Only the average chroma (C) values in the anterior and posterior parts of the brook trout were similar. Other than that all chroma values divert in fillets of the brook trout, rainbow trout, and Black Sea trout. Like the chroma values, Hue (H) values also varied in different parts (anterior, posterior, and caudal) of the same fillets for each fish species. The highest lightness and chroma levels were measured in caudal parts of the brook trout fillet whereas the highest hue values were determined in the fillets of anterior parts of the Black sea trout and posterior parts of the brook trout. All three salmonid species had different fillet colors in anterior, posterior, and caudal parts which can be evaluated to be some possible identifiers for the processed fish fillets in the present study. According to Erikson & Misimi (2008), there are different factors (e.g., perimortem handling stress, rigor mortis, ice storage) that affect skin and fillet color changes in Atlantic salmon. while healthy wild and reared Mediterranean amberjacks were measured to be similar with regard to their fillet colors (Öksüz, 2012), three salmonid species in the present study were not similar in their fillet colors.

A total of 19 fatty acids were determined for Black Sea trout and brook trout, and a total of 17 fatty acids were determined for rainbow trout in the present study. The fatty acid components of the brook trout, rainbow trout, and Black Sea trout were tabulated in Table 4. All of the trouts have less than 24% of total saturated fatty acid and have higher than 26% total fatty monounsaturated acids. Their total monounsaturated fatty acids were determined in the range of 48.18-50.84%. The lowest total saturated fatty acids were measured in rainbow trout. Palmitic acid (C16:0) was the highest fatty acid in total saturated fatty acid followed by stearic acid (C18:0) and myristic acid (C14:0) in the present study. Additionally, Pentadecylic acid (C15:0) and Arachidic acid (C20:0) were not found or found in lower amounts than detection limits in the muscle of rainbow trout.

The mean amount of C16:0 in brook trout and Black Sea trout was found to be different however these differences were not statistically significant (P>0.05). The amount of C16:0 in the muscle of rainbow trout was found to be significantly lower than that of C16:0 in the muscle of brook trout and Black Sea trout (P<0.05). In addition, the average levels of C16:0 in brook trout and Black Sea trout in the present study were found to be higher than those of C16:0 in brook trout and Black Sea trout as reported in the study of Şahin et al. (2011). These differences for the same fish species in two different studies could be attributed to the differences in the environmental conditions of the fish species.

Among monounsaturated fatty acids; palmitoleic acid (C16:1n9), oleic acid (C18:1n9), vaccenic acid and eicosenoic acid (C20:1n9) (C18:1n7), were determined in the muscle of brook trout, rainbow trout, and Black Sea trout in this study. The levels of C18:1n9 in the flesh of all trouts were the highest fatty acid in total monounsaturated fatty acids. The percentages of C18:1n9 out of all monounsaturated fatty acids determined in the present study were calculated to be 70.71%, 77.53%, and 79.20% in the muscle of brook trout, rainbow trout, and Black Sea trout, respectively.

The amount of C18:1n9 in brook trout was found to be similar to that of C18:1n9 in Black Sea trout (P>0.05) whereas the mean level of the same fatty acid was higher in rainbow trout (P<0.05). The average level of C18:1n9 in rainbow trout was found to be 22.44 \pm 0.25% which was higher than that of C18:1n9 in wild caught rainbow (18.83 \pm 1.91%) and lower than that of cage reared rainbow (26.56 \pm 1.21%) and pond reared rainbow (24.29 \pm 2.82%) (Ural et al., 2017). The mean amounts of C18:1n9 in brook trout and Black Sea trout in this study were found to be lower than those of C18:1n9 in male and female brook trout and Black Sea trout and their hybrids (Sahin et al., 2011). The difference can be attributed to diet differences.

We have separated polyunsaturated fatty acids in two groups in Table 4 (omega-6 and omega-3). Linoleic acid (LA, C18:2n6), eicosadienoic acid (C20:2n6), and arachidonic acid (ARA, C20:4n6) have been classified as omega-6 fatty acids. The linolenic acid (ALA, C18:3n3), stearidonic acid (C18:4n3), eicosatrienoic acid (C20:3n3), eicosatetraenoic acid (C20:4n3),eicosapentaenoic acid (EPA, C20:5n3), (DPA, C22:5n3), docosapentaenoic acid and docosahexaenoic acid (DHA, C22:6n3) have been classified as omega-3 fatty acids in all trouts investigated for this study. Therefore, a total of three omega-6 fatty acids and six omega-3 fatty acids were determined in polyunsaturated fatty acids.

The mean levels of DHA in the muscles of brook trout and Black Sea trout were found to be the highest in all polyunsaturated fatty acids. DHA is considered a health promoted fatty acid for consumers (Zhang et al., 2021). On the other hand, the greatest amount of fatty acid for rainbow trout in all polyunsaturated fatty acids were seem to be the LA in the study. The average levels of ALA were higher than that of EPA for all trouts.

The average amounts of LA in the muscle of brook trout and Black Sea trout were found to be the greatest in all omega 6 groups and second greatest in all polyunsaturated fatty acids. On the other hand, rainbow trout had the highest amount of LA followed by DHA and ALA. Studies related to reared fish fatty acid simply showed that LA amounts in reared fish tended to be higher depending on the fish feeding ingredients (Öksüz, 2012; Yeşilayer & Genç, 2013; Yeşilayer et al., 2014; Dernekbaşı et al., 2015; Dernekbaşı et al., 2017; Özyılmaz, 2019; Dernekbaşı & Karatas, 2020; Dernekbaşı & Karayücel, 2021).

Fatty acid related lipid indices namely atherogenic index (AI), thrombogenic index (TI), and polyene index (PI) of the brook trout, rainbow trout, and Black Sea trout showed some differences. Some of these differences were found to be statistically significant (P<0.05). The differences between brook trout and Black Sea trout related to the values of AI, TI, and PI were always statically significant (P<0.05) whereas that of rainbow trout did not (P>0.05). The AI, TI, and PI point out the quality of the lipid for their health benefits. The amounts of AI, TI, and PI in the lipid of the brook trout, rainbow trout, and Black Sea trout investigated in this present study varied from species to species in the same salmonid family. Küçükgülmez et al. (2018) reported that values of AI, TI, and PI for

golden grey mullet and gold band goatfish differed from season to season.

| Table 4. The fatty acid components (% of total fatty acid) and related lipid indices [Atherogenic Index (AI), |
|---|
| Thrombogenic Index (TI), and Polyene Index (PI)] of the brook trout, rainbow trout, and Black Sea trout |
| Çizelge 4. Kaynak alabalığı, gökkuşağı alabalığı ve Karadeniz alasının yağ asidi bileşenleri (toplam yağ asidinin |
| yüzdesi) ve lipit indeksleri [aterojenik indeks (AI), trombojenik indeks (TI) ve polien indeksi (PI)] |

| Fatty Acids | <u>Brook Trout</u> | Rainbow Trout | Black Sea Trout |
|----------------|----------------------|-------------------------------|------------------------------|
| C14:0 | 1.83±0.23ª | 1.76±0.01ª | $1.18\pm0.01^{ m b}$ |
| C15:0 | $0.89{\pm}0.27^{a}$ | ND | $0.84{\pm}0.16^{a}$ |
| C16:0 | 17.26±1.36ª | 13.68 ± 0.04^{b} | 16.22±0.33ª |
| C18:0 | $3.60{\pm}0.25^{a}$ | 4.11 ± 0.06^{b} | 3.96 ± 0.08^{b} |
| C20:0 | 0.32 ± 0.03^{a} | ND | 1.82 ± 0.51^{b} |
| ∑SFA | 23.90ª | 19.54ª | 24.02 ^b |
| C16:1n9 | 2.92±0.43ª | $2.62{\pm}0.01^{a}$ | 1.76 ± 0.03^{b} |
| C18:1n9 | 20.45 ± 1.37^{a} | 22.44 ± 0.25^{b} | 20.60 ± 0.39^{a} |
| C18:1n7 | $2.26{\pm}0.26^{a}$ | $1.90{\pm}0.10^{\rm b}$ | 2.51 ± 0.12^{a} |
| C20:1n9 | $3.30{\pm}0.65^{a}$ | 1.98 ± 0.03^{b} | $1.14{\pm}0.18^{b}$ |
| ∑MUFA | 28.92ª | 28.94ª | 26.01ª |
| C18:2n6 | 12.49 ± 1.38^{a} | 21.78 ± 0.15^{b} | 13.78 ± 0.37^{a} |
| C20:2n6 | $0.94{\pm}0.13^{a}$ | $0.79{\pm}0.03^{a}$ | 0.91 ± 0.04^{a} |
| C20:4n6 | $0.85{\pm}0.02^{a}$ | 0.81 ± 0.03^{a} | 1.11 ± 0.18^{b} |
| n6 | 14.28ª | 23.38 ^b | 15.80ª |
| C18:3n3 | $5.69{\pm}0.54^{a}$ | 6.80 ± 0.08^{b} | $5.22{\pm}0.07^{\mathrm{a}}$ |
| C18:4n3 | $1.51{\pm}0.16^{a}$ | $1.34{\pm}0.03^{a}$ | 0.81 ± 0.07^{b} |
| C20:3n3 | $1.03{\pm}0.16^{a}$ | $0.91{\pm}0.03^{a}$ | $0.59{\pm}0.24^{a}$ |
| C20:4n3 | $0.66{\pm}0.13^{a}$ | $0.52{\pm}0.03^{a}$ | $1.19{\pm}0.27^{ m b}$ |
| C20:5n3 | $4.64{\pm}0.96^{a}$ | $2.94{\pm}0.06^{b}$ | 3.13 ± 0.04^{b} |
| C22:5n3 | $1.44{\pm}0.17^{a}$ | 1.11 ± 0.04^{a} | $2.06\pm0.46^{ m b}$ |
| C22:6n3 | 16.93 ± 1.55^{a} | 13.83 ± 0.23^{b} | $21.24{\pm}0.67^{\circ}$ |
| n3 | 31.90ª | 27.46^{b} | 34.23° |
| ∑PUFA | 46.18 ª | 50.84 ^b | 50.03° |
| AI | $0.33{\pm}0.02^{a}$ | 0.26 ± 0.00^{b} | 0.28 ± 0.01^{b} |
| TI | $0.19{\pm}0.01^{a}$ | $0.18 \pm 0.00^{\mathrm{ab}}$ | $0.17{\pm}0.00^{ m b}$ |
| PI | 1.25 ± 0.06^{a} | 1.23 ± 0.02^{a} | $1.50\pm0.02^{\rm b}$ |
| SFA | 23.90ª | 19.54ª | 24.02 ^b |
| MUFA | 28.92ª | 28.94ª | 26.01ª |
| PUFA | 46.18ª | 50.84 ^b | 50.03° |
| n6 | 14.28^{a} | 23.38^{b} | 15.80ª |
| n3 | 31.90ª | 27.46^{b} | 34.23° |
| n3/n6 | 2.23ª | 1.17 ^b | 2.17ª |
| n6/n3 | 0.45ª | 0.85 ^b | 0.46ª |
| EPA | 4.64ª | 2.94 ^b | 3.13 ^b |
| DHA EPA/DHA | 16.93ª 0.27 | 13.83 ^b 0.21 | 21.24° |
| DHA/EPA | 0.27 3.65 | 0.21 4.70 | 0.15 6.79 |
| PUFA/SFA | 1.93 | 2.60 | 2.08 |
| EPA+DHA | 21.57 | 16.77 | 24.36 |

^{a,b,c} Values within same row with different superscripts diverge significantly at P<0.05

AI (Atherogenic Index), TI (Thrombogenic Index), and PI (Polyene Index)]

CONCLUSIONS

The highest average moisture and ash level were found in the flesh of rainbow trout. In addition, all three fish have lower lipid levels and higher amounts of protein and carotene (vitamin A) which are considered very important for a healthy diet. Palmitic acid (C16:0) and oleic acid (C18:1n9) were the highest level of fatty acids in saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), respectively. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in polyunsaturated fatty acids (PUFA) were found to be different from each other (P<0.05). Although the levels of EPA and DHA in fish flesh are different from each other, they are good for health. All trouts in the present study seem suitable for consumption, and they should be added to diets in order to take full benefits.

Statement of Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Authorship Contribution Statement

The contribution of the authors is equal

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