

## EVALUATION OF EICOSAPENTAENOIC AND DOCOSAHEXAENOIC ACID VALUES OF TROUT SPECIES DISTRIBUTED IN TURKEY

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### ABSTRACT

This study aims to evaluate and compare the rate of eicosapentaenoic acid (20:5, EPA), docosaheptaenoic acid (22:6, DHA) and  $\Sigma n3/\Sigma n6$  ratios of trout species which are important for human diet and highly preferred in Turkey. *Salmo platycephalus* specimens were sampled by an electro-shocker and fatty acid composition of muscle tissue was determined by a GC-MS. The findings were compared with those reported in the literature for *Salmo trutta macrostigma*, *Salmo trutta labrax*, *Salmo trutta forma fario* and *Oncorhynchus mykiss*. According to the literature and determined fatty acid compositions in *S. platycephalus*, the highest DHA and EPA were found in *O. mykiss* and *S. t. macrostigma* respectively. Fatty acid compositions of fish species, especially EPA and DHA, are important for fishes as well as for humans. It is known that EPA and DHA deficiencies may cause many serious illnesses as they have preventive and remedial effects. As a result, our study shows that EPA, DHA and  $\Sigma n3/\Sigma n6$  ratios of trout species are relatively higher than other fish species. Therefore consumption of trout species can be recommended.

**Key words:** Trout, fatty acid, EPA and DHA

## TÜRKİYE’ DE DAĞILIM GÖSTEREN ALABALIK TÜRLERİNİN EIKOSAPENTAENOİK VE DOKOSAHEKSAENOİK ASİT AÇISINDAN DEĞERLENDİRİLMESİ

### ÖZET

Bu çalışmada Türkiye’de yaygın tüketimi bulunan alabalık türlerinden insan diyeti açısından oldukça önemli olan kas dokusu yağ asidi bileşiklerinden eikosapentaenoik asit (20:5, EPA), dokosaheksaenoik asit (22:6, DHA) ve  $\Sigma n3/\Sigma n6$  değerlerinin karşılaştırılıp değerlendirilmesi amaçlanmıştır. Elektroşok cihazı ile yakalanan *Salmo platycephalus* örneklerinin kas dokusu alınmış ve GC-MS cihazında yağ asidi kompozisyonu analiz edilmiştir. *Salmo trutta macrostigma*, *Salmo trutta labrax*, *Salmo trutta forma fario* ve *Oncorhynchus mykiss* ile ilgili bulgular literatürden temin edilerek karşılaştırma yapılmıştır. *S. platycephalus* ile yapılan analizler ve literatür taraması neticesinde elde edilen verilere göre, en yüksek DHA oranı *O. mykiss*, en yüksek EPA ise *S.t. macrostigma* türünde bulunmuştur. Yağ asitleri ve özellikle EPA ve DHA oranları balıklar için olduğu kadar insan diyeti açısından da oldukça önemlidir, EPA ve DHA eksikliklerinden kaynaklanan birçok hastalık bulunduğu gibi bu yağ asitlerinin önlediği ve tedavi edici etkisinin belirtildiği birçok hastalık da bildirilmektedir. Çalışmamızın sonucunda değerlendirilmesi yapılan alabalık türlerinin EPA, DHA ve  $\Sigma n3/\Sigma n6$  oranlarının diğer balık türlerine göre yüksek olduğu ve bu bağlamda diyetlerde tüketilmelerinin faydalı olacağı ifade edilebilir.

**Anahtar kelimeler:** Alabalık, yağ asidi, EPA ve DHA

## INTRODUCTION

Fats are important for structure of cell membrane besides being used as a source of energy for human and animals. The importance of fats for organisms are partly attributed to their functional unsaturated fatty acids (Gibson 1988, Ackman and Ratnayake 1989). Fish are significant sources of fatty acids such as C20:5 n-3 (EPA, Eicosapentaenoic acid) and C22:6n-3 (DHA, Docosahexaenoic acid) (Suziki et al. 1986). Due to inability to synthesize these essential fatty acids, humans must take these acids from other sources. Freshwater fish in general species cannot synthesize linoleic and linolenic essential fatty acids in their cells (Gurr and Harwood 1991) and must take them via food. Fish species obtain essential fatty acids from their preys but original sources are phytoplanktons which constitutes the first circle of food chain (Konar et al. 1999, Alasalvar et al. 2002, Sushchik et al. 2007). Fish species are rich in terms of polyunsaturated fatty acids (PUFA) and especially EPA and DHA. However, the rate of fatty acids can change between species, organs and environmental conditions. These are generally related with a variety of factors such as catch season, location, temperature, physicochemical properties of water, age and sex of fish (Christiansen et al. 1989, Yılmaz 1995, Buzzi et al. 1997, Inhamuns and Franco 2008). In addition, there is a huge variation between wild and cultured fish in terms of fatty acid profiles. Fatty acid composition of freshwater and marine fish species are determined by the abundance of zooplankton and phytoplankton within their diets (Halver 1988, Haliloğlu et al. 2002). Research conducted shows that generally freshwater fish species include less PUFA especially EPA and DHA in proportion to marine fish species (Ackman 1999). Recently, healthy life has gained importance and people have begun to pay attention to their diets in order to live a healthy life. At this point, fish, because of their rich contents of fatty acids, are suggested by the experts. According to American Heart Association, fish species (especially fatty fish) should be consumed at least twice a week. Many studies suggested that inferiority of low quality food substances and bad eating habits could cause illnesses in humans. For this reason, the consumption of healthy foods rich in unsaturated fatty acid and beneficent for human diet are strongly suggested (Mol 2008). The n-3 unsaturated fatty acids such as EPA and DHA found commonly in fish species

have preventive and remedial effects on heart attack (Breslow 2006), cancer (Kinsella 1988), brain and nervous system disease (Bourre 2007), asthma (Broughton et al. 1997), heart diseases (Kinsella et al. 1990), tooth diseases (Hamazaki 2006), arthritis (Galarrage et al. 2008) and diabetes (Kris-Etherton et al. 2000). The aim of this study was to evaluate the fatty acid compositions of the trout species distributed and commonly consumed in Turkey.

## MATERIALS AND METHODS

### Sampling and Tissue Extraction

*Salmo platycephalus* Behnke, 1968 specimens were sampled in April 2006 by an electro-shocker. Samples were kept at +4°C until length-weight measurements were taken. After cleaning, approximately four grams of muscle tissue was sampled from underneath of the dorsal fin of fish. The samples were labeled by covering aluminum foil and stored at -20°C in a deepfreeze until analysis.

### Extraction of Lipids

Extraction of lipids from muscle samples was carried out according to Hara and Radin (1978). According to the method, one gram of sample was homogenized for 30 seconds in 5 ml hexane isopropanol mixture (3/2). Homogenization container was cleaned with 2 ml hexane isopropanol solution and centrifuged for 10 minutes at 4500 rpm. Supernatant was transformed to closed test tubes (Wang et al. 1990).

### Preparation of Fatty Acid Methyl Ester

Transformation of methyl ester derivatives from fatty acids was done according to Christie (1990) method. Lipid extract in hexane/isopropanol phase were taken to 20 ml test tubes and 5 ml 2% methanolic sulfuric acid was added on and mixed with a vortex. This mixture was incubated for 15 hours at 55°C in an incubator. Tubes were removed from the incubator and cooled at room temperature and then mixed with 5 ml 5% sodium chloride. The ready fatty acid methyl ester was extracted with 5 ml hexane and the phase was taken with a pipet. Extract was treated with 5 ml 2% KHCO<sub>3</sub> and waited for five hours for separation of phases. Just after the separation of phases, the mixture including methyl esters was dissolved with 1 ml hexane and taken in to the 2 ml autosampler vials and analyzed with a GC-MS. The specification of fatty

acid composition of the lipids was carried out with gas chromatography/mass spectrometer device (Agilent GC-6890II) at, Department of Biology, Faculty of Arts and Sciences, University of Kahramanmaraş Sütçü İmam.

## RESULTS AND DISCUSSION

The findings of the present study and the data acquired from the literature are summarized in Table 1. Fish species are generous in polyunsaturated fatty acids such as EPA and DHA and hence they are

is considered as an important index (Piggott and Tucker 1990, Güler et al. 2007). Highest ratio of n3/n6 was seen in freshwater form of *S. t. labrax* (6.27) followed by *S. platycephalus* (4.74). On the other hand the lowest n3/n6 ratio was observed in marine form of *S.t. labrax* (1.37). The n3/n6 ratio calculated in *S. t. forma fario*, *S. t. macrostigma* and *O. mykiss* were 3.28, 2.59 and 1.48, respectively. It is generally known that freshwater fish species include less PUFA especially EPA and DHA when compared with marine fish species (Ackman 1999). However, our findings showed some

**Table 1.** Main fatty acid compositions of *S. t. macrostigma*, *S. t. labrax*, *S. t. forma fario*, *O. mykiss* and *S. platycephalus* (%).

Species	n	C 20:5	C 22:6	ΣPUFA	Σn3	Σn6	Σn3/Σn6	References
<i>S. platycephalus</i>	6	7.70±0.43	12.76±0.96	32.19	26.58	5.61	4.74	In this study
<i>S. t. macrostigma</i>	6	7.88±0.59	8.42±0.27	35.18	25.40	9.78	2.59	Akpınar et al. 2009
<i>S. t. labrax (marine)</i>	10	3.19±0.42	14.25±0.66	34.97	19.62	14.28	1.37	Şahin et al. 2011
<i>S. t. labrax (freshwater)</i>	5	*	21.42±1.61	32.83	28.85	3.98	6.27	Aras et al. 2003
<i>S. t. forma fario</i>	**	7.02±0.01	12.02±0.04	36.00	27.58	8.42	3.28	Kaya and Erdem 2009
<i>Oncorhynchus mykiss</i>	5	3.07±0.28	19.17±1.65	36.88	22.41	14.47	1.58	Haliloğlu et al. 2001

\* Undetermined

\*\*Unknown

accepted as an important human diet (Suzuki et al. 1986). As mentioned previously, EPA and DHA have significant roles in many physiological activities in humans and fish species, and their levels are accepted as an important quality criterion for evaluation of fish oils (Navarro and Sargent 1992). From the compared species, highest rate of DHA (21.42%) was seen in *S. t. labrax* Pallas, 1811 whereas the lowest (8.42%) in *S. t. macrostigma* (Duméril 1858). DHA content in *S. platycephalus*, *S. t. labrax* (marine form), *S. t. forma fario* Linnaeus, 1758 and *O. mykiss* (Walbaum 1792) were observed as follow 12.76%, 14.25%, 12.02% and 19.17%, respectively. EPA was seen at the highest rate in *S. t. macrostigma* (7.88%) and lowest in *O. mykiss* (3.07%). EPA rates in *S. platycephalus*, *S. t. labrax* (Marine form), *S. t. forma fario* were reported as 7.70%, 3.19% and 7.02% respectively. Total PUFA levels were comparable among *S. platycephalus*, *S. t. labrax* (Marine form), *S. t. labrax* (Freshwater form), *S. t. forma fario*, *S. t. macrostigma* and *O. mykiss* with 32.19%, 34.97%, 32.83%, 36.00%, 35.18% and 36.88%, respectively. When comparing

the dietary value of fish fatty acids, total ratio of n3/n6 contradictions to this e.g. marine and freshwater forms of *S. t. labrax*. These differences may be explained by possible effects of environmental factors on fatty acid profiles. The fatty acid composition of fish is highly affected by water temperature and diet compositions (Sargent 1996). It is known that some of n-3 PUFA fatty acids especially EPA and DHA have influence on cell membrane permeability and flexibility (Brenner et al. 1984). Physicochemical properties such as temperature and oxygen contents of sampling localities of *S. t. labrax* vary greatly between freshwater and marine. In addition to this, food web varies greatly too. Because of the different habitats n3/n6 ratios may be remarkably different. The same situation could be the case here in *S. platycephalus* sampled at 1768 meter of altitude. Water temperature in this locality varied between 7.1°C in winter and 19.2°C in summer (Kara et al. 2011). In order to tolerate low temperatures, n-3 PUFA (especially EPA and DHA) compositions could increase, resulting in a higher n3/n6 ratio.

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