

BLACK SEA SHAD AND RED MULLET AS SOURCES OF OMEGA 3 FATTY ACIDS

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ABSTRACT

PURPOSE: The purpose of this work was to study the seasonal changes of the quantity of omega-3 polyunsaturated fatty acids (n-3 PUFA) in two commonly consumed Black Sea fish species shad (*Alosa pontica*) and red mullet (*Mullus barbatus ponticus*). These fish species appear as one of the best sources of omega-3 polyunsaturated fatty acids.

MATERIAL AND METHODS: The total lipids were extracted by the method of Blight and Dyer. The fatty acid composition was analysed by GC/MS.

RESULTS: Very-Long Chain n-3PUFA (VLCPUFA) eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) were presented in the highest concentration in shad in both season, whereas red mullet show an increased n-3 VLCPUFA levels in autumn. The spring shad total content of n-3 FA was significantly higher than the total content of omega 6 (n-6) PUFA, whereas red mullet showed opposite trend. The n-6/n-3 FA ratio was within the recommended range for the analyzed Black Sea fish species.

CONCLUSION: Shad and red mullet can be classified as fatty fish species. A 100 g portion of fillet delivers between 350 and 2690 mg of sum of EPA and DHA, depending on the season. This is over than 100% of daily recommended amounts of these FAs. Obtained results for omega-3 PUFA content and n-6/n-3 ratio indicates that these Black Sea fish species are an excellent source of these PUFA and would be recommended as part of a healthy or therapeutic diet for adults in Bulgaria during all year.

Key words: omega-3, EPA, DHA, Human health, Black Sea shad, red mullet

INTRODUCTION

Several health agencies and professional organizations as the American Heart Association (16), the European Society for Cardiology (5), the Scientific Advisory Committee on Nutrition (UK) (12), the European Food Safety Authority (6), the Australian Health and Medical Research Council (13) have issued recommendations for increased intakes of n-3

fatty acids (FAs). These recommendations are based on strong evidence derived from a variety of scientific approaches linking dietary deficiency of long chain n-3FAs with risk for cardiovascular events, notably sudden death. Fish lipids are the richest in valuable n-3 LCPUFA decreasing the cardiovascular risk. Thus a more frequent consumption of fish is recommended. Few randomized controlled trials on fish in relation to coronary and all-cause mortality have been conducted in cardiac patients. He et al (11) estimated in a meta-analysis of prospective cohort studies, that eating fish once per week was associated with a 15% lower risk of coronary death compared with a fish intake of less than once per month. Each 20-g/d increase in fish consumption was related to a 7% lower risk of Coronary Heart Diseases (CHD) mortality. The marine-derived VLCPUFA n-3 PUFA

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Received: September 25, 2013

Accepted: October 7, 2013

EPA and DHA are assumed to be primarily responsible for these health effects of fish. The fundamental mechanism by which n-3 FA appear to mitigate risk for CHD begins with the enrichment of membrane phospholipids with EPA and DHA. Mozaffarian and Rimm (18) combined data from prospective cohort studies estimated that a reduction of CHD mortality may be achieved with relatively low intakes of EPA and DHA. Modest consumption of fish (1–2 servings/wk, which is 100–200 g fish/wk) was associated with a 36% lower risk of coronary death. They suggested that for the general population an intake of 250 mg/d of EPA+DHA (1 serving of fatty fish/wk) would be sufficient. Others have recommended target intakes of about 500 mg/d (5, 6, 7, 9, 12, 13). Most studies have mainly focused on fish consumption as the main source of EPA and DHA (4, 8, 10, 11, 14, 15). High fish consumers in Western countries also tend to have a higher socioeconomic position and lower prevalence of depressive symptoms both significant independent risk factors for CHD. Considering all these facts the importance of consuming fish rich in n-3 PUFA can be taken into consideration.

According to Food Based Dietary Guidelines for Adults in Bulgaria (8) fish consumption is particularly recommendable, because in Bulgaria the consumption of fish is very low (4.5kg annual per capita) compared with the average European levels (23 kg annual per capita) and people have a relatively low intake of n-3 PUFA.

The Pontic shad (*Alosa pontica*) is anadromous fish species. This species is native for Bulgaria, Romania, Russia, Turkey and Ukraine. It occurs in Black sea and Sea of Azov and for spawning, migrates in Danube, Dnepr, etc. *A. pontica* is a commercial fish species. Its catchments in the last decade vary significantly with a maximum in 2004 (21.95t) and minimum in 2007 (0.2 t) (14). Red mullet is distributed all over the shelf of Black Sea. From the Black Sea fisheries perspective in the waters of Bulgaria and Romania, demersal fish species red mullet (*Mullus barbatus*) is not important target for fisheries but due to its taste it is preferred for consumption. However information about the LCPUFA content of these Bulgarian Black Sea fish species is lacking.

The aim of this study was to determinate and compared seasonal changes of total lipids and

quantity of n-3 PUFA in commonly consumed Black Sea shad (*Alosa pontica*) and red mullet (*Mullus barbatus ponticus*) in fish edible tissues.

MATERIAL AND METHODS

Sampling of fish species

The samples of two Bulgarian fish species pontic shad (*Alosa pontica*) and red mullet (*Mullus barbatus ponticus*) were purchased from Varna local fish market during 2010. The samples were immediately frozen at -20°C and whole stored in a fridge. Biometrical characteristics and total lipids content were determined and noted (see Table 1). Prior to analysis the edible fish tissue was filleted with the skin left on and homogenized using kitchen homogenizer. Their total lipids (TL) and fatty acid content in two seasons (spring and autumn) were analyzed and compared.

Lipid extraction

Prior to analysis, the head, tail, fins, and viscera of the fish were removed. The raw edible fish tissue was extracted by the method of Blight and Dyer (3) using chloroform/methanol/water in a ratio 2:2:1. After phase separation, the chloroform extracts were evaporated until dryness. The TL content was determined gravimetrically for each fish species in triplicate. The results were expressed as g per 100g raw tissue (g 100g⁻¹r.t.).

Fatty acids analysis

Fatty acid compositions of TL at edible fish tissue were determined by GC of the corresponding methyl esters. The residual lipid fraction was methylated by base-catalyzed transmethylation using 2M methanolic potassium hydroxide and n-hexane according to BDS EN 5509:2000 (1). After 10 minutes centrifugation (3500 rps), the hexane layer was taken for GC analyses. Gas chromatography was performed by a model FOCUS Gas Chromatograph with autosampler A 3000, equipped with Polaris Q MS detector (Thermo Scientific, USA). The capillary column used was a TR-5 MS (Thermo Scientific, USA) universal column 30m length and 0.25mm i.d, with a wide range of applications from food analysis. Helium was used as a carrier gas at flow rate 1 ml/min. Chromatographic separation was achieved by temperature range: initial temperature – 40°C for 4 min followed by 10°C per minute until 235°C and final temperature reach was 280°C for 5 min. The

sample volume was 1µl. The three parallel analyses were made from each methanolysed sample. The injector was a split/splitless injector operated in the split mode. Peaks were identified according to two parameters: Retention Time (RT) based on available FAME mix standard (SUPELCO 37 F.A.M.E. Mix C4-C24) and mass spectra (ratio m/z) – compared to internal Data Base (Thermo Sciences Mass Library, USA). FAMES were identified and quantified by comparison with the RT and peak areas of SUPELCO standards. Three replicate GC analyses were performed. The values of FA were expressed as g of 100 g edible tissues as mean value and \pm standard deviation (SD) (2). All of the chemicals used in the experiments were analytical grade and GC grade (Sharlau, Sharlab Sourcing Group, Spain).

Statistical analysis

The results of analysis are presented as mean values \pm standard deviation (SD). Student's t-test was used to evaluate the seasonal differences between Fatty Acid groups. $P < 0.05$ value was considered as significant. Statistical analysis was done using Grap Pad Prizm 5 software.

RESULTS AND DISCUSION

Total lipids

Fish are usually classified into groups according to their overall lipid content including lean (<2%), low-fat (2–4%), medium-fat (4–8%) and high-fat (>8%) (10). Significant seasonal differences in the TL contents were found in both species (Table 1). Lowest TL content was observed for spring red mullet (5.38 g per 100g edible tissue), whereas shad showed relatively constant high lipid levels.

The present data showed that spring red mullet is classified as medium-fat, whereas shad (in both seasons) and red mullet showed in autumn higher to-

tal lipid contents and can be named as to highly fat fish species. The observed results are similar to other relevant publications describing seasonal changes in TL of pelagic and demersal species from Mediterranean and Black Sea (15, 19).

Fatty Acid content

A lipid analysis enabled the classification and quantitative determination of FA as well as the sum of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). FA contents in both species followed a relative pattern with MUFA>SFA>PUFA in spring season. In contrast to the red mullet, shad (autumn) contained a higher amount of SFA than that of MUFA (e.g. SFA>PUFA>MUFA) whereas the red mullet presented a different pattern of FA composition as evidenced by the relatively greater SFA compared to PUFA and MUFA (e.g. SFA>MUFA>PUFA). On figure 1 are shown seasonal differences obtained for FA groups in shad (1A) and red mullet (1B).

Contents of these FA groups presented significant differences in their values during the seasons ($p < 0.001$). Those visible FA variations observed in the red mullet in both seasons are in accordance with the data presented from Polat et al. (19) for the Mediterranean red mullet. Kocatepe and Turan (15) presented similar results for FA profile of spring shad and red mullet from the Sinop Region of the Black Sea.

The major FA identified as n-3 VLCPUFA is DHA. Other important FA is EPA which found in significant amounts too. The obtained results (see Table 2) for EPA, DHA, n-3 and n-6 content and n-6/n-3 ratios for both species are varied significant according to seasons and species.

The maximum value of DHA was defined in shad (74.50% of total PUFA) and the minimum- in red

Table 1. Biometric characteristics and total lipids content (mean \pm SD)

| | Shad | | Red mullet | |
|--|-------------------|-------------------|------------------|------------------|
| | Spring | Autumn | Spring | Autumn |
| Total lipids (g 100g ⁻¹ e.t.) | 13.15 \pm 0.70 | 12.70 \pm 0.80 | 5.38 \pm 0.40 | 14.69 \pm 0.55 |
| Mean weight (g) | 325.00 \pm 5.00 | 315.00 \pm 5.20 | 35.00 \pm 5.00 | 47.50 \pm 4.00 |
| Mean length (cm) | 25.50 \pm 2.50 | 28.50 \pm 3.50 | 15.00 \pm 1.50 | 16.00 \pm 2.00 |

SD - standard deviation

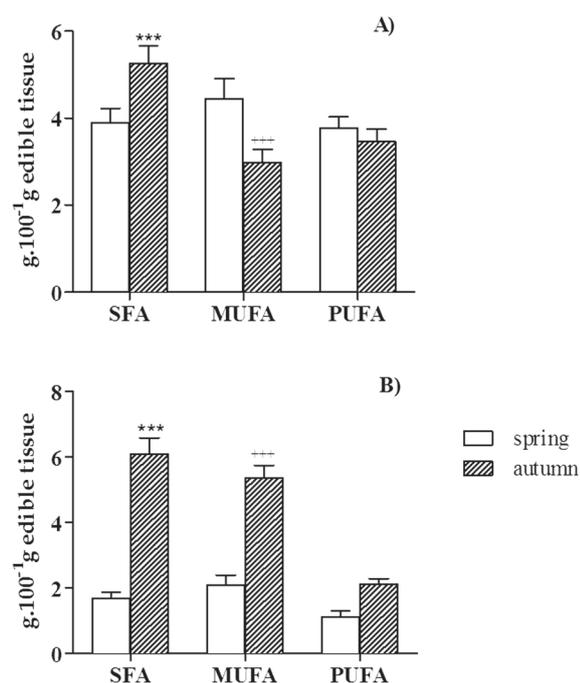


Fig.1. Seasonal changes of total SFA, MUFA and PUFA content (g.100⁻¹g edible tissue) in shad and red mullet
 *** *p* < 0.001 SFA spring vs SFA autumn; *** *p* < 0.001 MUFA spring vs MUFA autumn

mullet (48.80% of total PUFA). In this investigation for all species EPA levels were lower than those of DHA, in both seasons. When presenting the VLCPUFA content in absolute amounts (g.100g⁻¹ edible tissue) is possible to provide more useful and accurate information for observed seasonal changes. The obtained concentration of EPA in both species ranged from 0.020 to 0.152 g.100⁻¹g edible tissue, while that of DHA ranged from 0.302 to 2.44g.100¹g edible

tissue, which are significant higher than presented for the same species from Turkish part of Black Sea (15). In this study was found that 100 g of fish fillets from spring shad contained 2.46g of EPA+DHA which significantly decreased (*p*<0.001) in autumn (by 49%) to 1.25g. Our results for EPA+DHA contents in both seasons for analyzed species are significant higher than those presented for the same fishes (0.25g.100g⁻¹ fish for shad and 0.15g.100 g⁻¹ fish for red mullet) from South part of the Black Sea (15).

Stancheva et al (23) were found significant low EPA+DHA amounts: from 13.15 to 55.00 mg .100⁻¹ g e.t. for Black Sea sprat and goby. Saglik (20) presented n-3PUFA content in some Mediterranean fish species from Turkish water and reported similar results for EPA+DHA amounts for Mediterranean high fat bluefish and significant lower for bonito and horse mackerel. Soltan and Gibson (22) were investigated the levels of n-3VLCPUFAs in twenty-six wild Australian fish species. Authors reported similar results only for three seawater species as salmon, swordfish and gem fish which presented omega 3FA in ranges from 1.36 to 2.52 g.100g fish and assumed that many of most popular eating fish species are poor sources of analyzed VLCPUFAs.

As mentioned in introduction the several health agencies and professional organizations as the British Nutrition Foundation (BNF) recommended that people who have a balanced and healthy diet consume minimum 0.25 g of EPA and DHA daily (5). The American Heart Association (AHA), the European Society for Cardiology, Food Based Dietary Guidelines for Adults in Bulgaria recommended that

Table 2. Seasonal changes of EPA, DHA, n-3and n-6content (g.100⁻¹g edible tissue) and omega 6/omega 3 ratios (mean ± SD) in edible fish tissue

| | Shad | | Red mullet | |
|-----------------|------------|---------------|------------|---------------|
| | Spring | Autumn | Spring | Autumn |
| EPA | 0.020±0.02 | 0.152±0.06*** | 0.050±0.03 | 0.090±0.05** |
| DHA | 2.440±0.38 | 1.100±0.09*** | 0.302±0.14 | 0.600±0.12*** |
| Omega-3 | 2.740±0.17 | 1.710±0.11*** | 0.460±0.05 | 0.784±0.09** |
| Omega-6 | 0.654±0.09 | 1.210±0.12*** | 0.530±0.06 | 1.036±0.12*** |
| Omega-6/omega-3 | 0.24±0.03 | 0.71±0.08*** | 1.15±0.07 | 1.32±0.10* |

*** *p* < 0.001, ** *p* < 0.01, * *p* < 0.05

all adults should include fish in their diet at least twice a week, especially oily fish. For patients with documented coronary heart disease, the AHA recommended about 1g of EPA+DHA (combined) per day. As shown in Table 2, the highest EPA+DHA content was obtained from spring shad, whereas the lowest amount was found for spring red mullet. Following the EPA and DHA recommendations for healthy adults according to BNF, AHA and EFSA, 100g portion of shad and red mullet filets in both seasons is enough to cover the daily recommended intake of these FAs.

It was observed in this study that shad presented higher content of n-3 than n-6 in both seasons, whereas red mullet showed opposite trend. The n-6/n-3 PUFA ratio is known to be of dietetic importance, since it is a key factor for balanced synthesis of eicosanoids in the organism (17, 22). A previous study revealed that this ratio in marine fishes is between 0.7 and 14.4 (4, 10). The obtained results for both species in two seasons are in the range of 0.25 to 1.32 (table 2). The observed differences in VLCPUFA contents between seasons results in a slightly increase of n-6/n-3 ratio (13%, $p < 0.05$) in red mullet edible tissue in autumn, while in shad was found significant decrease - with 65% ($p < 0.001$) in autumn. According to the current WHO recommendations, n-6/n-3 PUFA should not be higher than 4.0 (7, 21). In analysed fish species (in both seasons) this ratio remains significantly below the cut-off value of 4.0.

The results presented in this study represent important information for consumers who may believe that they are ingesting adequate amounts of n-3 VLCPUFA from a single serve of Black Sea shad and red mullet. In fact, they would need to consume 1 times per week 100 g shad fillets and 2 times per week 100 g red mullets fillets, to ingest 1 g EPA+DHA. There is need to provide adequate and useful data to inform consumers about n-3 content of these commercially important and preferred Bulgarian Black Sea fish species during the all year.

CONCLUSION

This study was carried out to determine the seasonal changes of n-3 content in shad and red mullet from Bulgarian Black Sea waters. A 100 g portion of fillet of both analyzed species delivers between 350 and 2690 mg sum of EPA+DHA, depend-

ing on the season. This is over than 100% of daily recommended amounts of these PUFAs. In modern nutritional studies namely the sum of EPA and DHA is used as one of the key biochemical characteristics of products consumed by human population. This information would be especially important for a population with low levels of fish consumption, because higher intakes of EPA+DHA and fish may protect against fatal CHD. Intake of only a small amount of fish may be beneficial to cardiac health. The lipids of analyzed Bulgarian Black Sea fish species shad and red mullet are rich in EPA and DHA VLCPUFA during the analyzed yearly seasons. Taking into an account that n-3 PUFA deficiency involves mainly VLCPUFA content, analyzed species are an excellent source of these PUFA and would be recommended as part of a healthy or therapeutic diet for adults in Bulgaria.

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