Effect of Dietary Lipid Levels on Fatty Acids Composition of Cultured Sparusaurata

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Abstract: The aim of this study was to determine the progressive evolution of fish muscle fatty acids composition feeding by different lipid levels of farmed Mediterranean gilthead sea bream (Sparusaurata). During twenty one weeks, Sparusaurata were hand-feed two diets with protein and lipid ratios 47/20 (Diet A) and 45/22 (Diet B) respectively. At the end of the feeding period, lipid contents of muscle, as well as fatty acids profiles of different portions of the muscles were studied. Results indicated the effects of fatty acids profiles of diets throughout the liver and white muscle fillet (dorsal, middle and ventral). These were more noticeable when Sparusaurata was fed on the diet A that decreased the content of monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in all the muscles during the period of feeding while the saturated fatty acids (SFA) content tended to increase and it was lower than ones in the muscles of Sparusaurata fed on diet B. The highest amount of PUFA and docosahexaenoic acid, (22: 6n-3 DHA) were found among the different portions of ventral muscles. In addition, the progressive evolution of different parts of muscle’s fatty acid profiles showed significant differences in contents of SFA, PUFA and especially the n-3 PUFA. However, the content of MUFA seems to be alike in different muscles and tended to decrease in dorsal and ventral muscle from D₀ to D₇ and inversely when the fish were fed on diet A.

Key words: Fatty acids, Liver, Muscles, cultured fish, Sparusaurata.

1. Introduction

In recent years, many studies reported the high benefits of consumption of marine oils in human health according to Simopoulos 2003 [1]; Schmidt et al., 2005 [2] and Kremer 2000 [3]. Coinciding with this and the overexploitation of fishing grounds in World bank. 2013 [4], aquaculture activities have stirred growing interest. In the Mediterranean area, notable success has been achieved in the production of diverse species, such as sea bream (Sparusaurata) and sea bass (Dicentrarchuslabrax). Therefore, rearing these species has been redirected with the improvement of the final eating quality of the product.

The sea bream (Sparusaurata) is considered to be a very important aquaculture fish species. On the other hand, such as marine fish, Sparusaurata is considered as a source of highly unsaturated fatty acids family (HUFA) [5]. However, the types and amounts of fatty acids in fish tissues vary with the geographic location, size, age, aquaculture feeds, reproductive status and seasons [6-9]. The short or long period of feeding (period of rearing to have a marketable commercial size) may, also, influence the fatty acids profile of the fillet quality as is shown in many reared fish species [10, 11].

The objective of this study was to evaluate the effects of two lipids levels of extruded diets on fatty acid composition of different edible muscles of reared Sparusaurata at different dates during the period of breeding.

2. Materials and Methods

2.1 Diets and Feeding

The study was based on two dietary experiments. The experience was performed at National Institute of Marine Sciences and Technologies of Monastir, Tunisia;
from February 2009 to July 2009 (total 21 weeks).

Two commercial diets contained two different lipids levels (20% and 22%) were used for feeding the juvenile *Sparus aurata*.

At the start of the feeding trial, two groups of 180 fish of 133.11 ± 3.48 g mean body weight were randomly weighed and stocked into two round tanks. The fish were fed to satiation by hand, twice a day. Each diet was randomly allocated to duplicate groups.

### 2.2 Sampling Procedure

Samplings were performed regularly after 21 days, so fish from each tank were weighed at first day 0 (D₀), then after 11 weeks (D₁) and after 21 weeks (D₂). From each diet, five fish were sampled and approximately 0.5 g of muscle from different edible parts of fish (dorsal muscle: DM, middle muscle: MM and ventral muscle: VM) and the liver were dissected out and stored at -80 °C for subsequent lipids and fatty acids analyses.

### 2.3 Lipids and Fatty Acids Determination

Lipids for fatty acid analysis were extracted from diets and different muscle samples with chloroform and methanol according to the procedure of Folch et al., (1957) [12], then methylated and transesterified with boron trifluoride in methanol [13]. The internal standard (19: 0) was added at 1% of the total lipid weight for quantitative measurement of individual fatty acids present in the diets and muscles. Fatty acid methyl esters were resolved and analyzed by Hewlett-Packard HP 5890 capillary gas chromatograph linked to an HP Chemstation integrator. The capillary column was of HP-Innowax silica (30 m long, 0.25 mm internal diameter with 0.25 µm thickness of film of the stationary phase).

The quantitative analysis of the fatty acids was made by comparing the retention times of the fatty acids in the chromatograms corresponding to the reaction products, with those of a standard mixture, from Sigma Chemical Co.

### 2.4 Statistical Analysis

Data obtained were all subjected to one-way analysis of variance (ANOVA) to test the effects of different dietary lipid source and the breeding period on fatty acid composition of different muscles. Differences between means were determined by Duncan’s Multiple Range test and were considered to be significant when $P < 0.05$. All analysis was performed using the PASW statistics 18 for Windows.

### 3. Results and Analysis

#### 3.1 Fatty Acid Composition of the Diets

The analytic and fatty acid composition of the commercial diets were shown in Tables 1 and 2. According to the results of analyses, there were significant differences among the levels of different fatty acids.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Ingredients and analytical composition of the two commercial diets.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diets</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Ingredients</td>
<td></td>
</tr>
<tr>
<td>Analytical composition</td>
<td></td>
</tr>
</tbody>
</table>
Table 2  Fatty acid profile (% of total fatty acids) of the commercial diets (wet weight).

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Diet A</th>
<th>Diet B</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>1.76±0.00 a</td>
<td>1.47±0.27 a</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.24±0.08 a</td>
<td>0.11±0.06 a</td>
</tr>
<tr>
<td>C16:0</td>
<td>43.14±0.16 b</td>
<td>45.01±0.99 b</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.34±0.11 a</td>
<td>0.77±0.05 b</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.22±0.24 a</td>
<td>2.01±0.03 a</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.38±0.06 a</td>
<td>0.58±0.02 b</td>
</tr>
<tr>
<td>C22:0</td>
<td>1.90±0.03 b</td>
<td>3.24±0.16 a</td>
</tr>
<tr>
<td>C24:0</td>
<td>1.21±0.09 a</td>
<td>1.63±0.05 b</td>
</tr>
<tr>
<td>ΣSFA</td>
<td>51.21±0.45 b</td>
<td>54.84±0.47 b</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.49±0.09 a</td>
<td>0.69±0.09 b</td>
</tr>
<tr>
<td>C15:1</td>
<td>0.36±0.34 a</td>
<td>0.08±0.04 a</td>
</tr>
<tr>
<td>C16:1</td>
<td>1.92±0.05 a</td>
<td>2.04±0.04 a</td>
</tr>
<tr>
<td>C17:1</td>
<td>0.30±0.00 b</td>
<td>0.67±0.03 b</td>
</tr>
<tr>
<td>C18:1n-9</td>
<td>13.22±0.28 b</td>
<td>9.27±0.02 a</td>
</tr>
<tr>
<td>C18:1n-7</td>
<td>1.38±0.06 a</td>
<td>1.29±0.06 a</td>
</tr>
<tr>
<td>C20:1n-9</td>
<td>0.46±0.00 a</td>
<td>0.63±0.04 b</td>
</tr>
<tr>
<td>C22:1</td>
<td>1.55±0.07 b</td>
<td>2.11±0.11 b</td>
</tr>
<tr>
<td>C24:1</td>
<td>0.16±0.04 b</td>
<td>0.18±0.02 b</td>
</tr>
<tr>
<td>ΣMUFA</td>
<td>19.85±0.52 b</td>
<td>16.96±0.23 a</td>
</tr>
<tr>
<td>C18:2n-6</td>
<td>14.46±0.48 b</td>
<td>10.77±0.41 a</td>
</tr>
<tr>
<td>C20:2n-6</td>
<td>1.36±0.00 b</td>
<td>1.84±0.06 b</td>
</tr>
<tr>
<td>C20:3n-6</td>
<td>0.66±0.00 a</td>
<td>0.72±0.02 b</td>
</tr>
<tr>
<td>C20:4n-6</td>
<td>0.55±0.10 a</td>
<td>1.01±0.11 b</td>
</tr>
<tr>
<td>C22:4n-6</td>
<td>0.76±0.06 b</td>
<td>1.18±0.18 b</td>
</tr>
<tr>
<td>Σn-6PUFA</td>
<td>17.79±0.64 b</td>
<td>15.52±0.66 a</td>
</tr>
<tr>
<td>C18:3n-3</td>
<td>2.37±0.13 b</td>
<td>2.26±0.34 b</td>
</tr>
<tr>
<td>C20:5n-3 (EPA)</td>
<td>2.93±0.27 a</td>
<td>3.06±0.06 a</td>
</tr>
<tr>
<td>C22:5n-3</td>
<td>0.61±0.09 a</td>
<td>0.81±0.02 b</td>
</tr>
<tr>
<td>C22:6n-3 (DHA)</td>
<td>5.06±0.08 b</td>
<td>6.30±0.10 b</td>
</tr>
<tr>
<td>Σn-3PUFA</td>
<td>10.98±0.40 b</td>
<td>12.43±0.16 b</td>
</tr>
<tr>
<td>ΣPUFA</td>
<td>28.77±0.24 b</td>
<td>27.95±0.50 b</td>
</tr>
</tbody>
</table>

Values are the averages of three replicates ±SEM. Numbers in rows having different letters indicate that treatment are significantly different.

The two diets had a similar average of SFA (≈ 50%) and PUFA (≈ 28%) with an important level of n-6 PUFA (≥ 15 %) respectively (Table 2). Comparing between two diets, the diet B contained higher proportions of SFA and n-3 PUFA. Whereas the diet A had a higher proportion of shorter 18C chain-length unsaturated fatty acids and n-6 PUFA.

In the two diets, palmitic and stearic acids were the primary saturated fatty acid (SFA), contributing approximately 90% to the total SFA content of the lipids. Oleic acid was identified as the primary monoenoic fatty acid and was significantly ($P < 0.05$) higher in diet A than in B.

Among n-6 series of the fatty acids, linoleic acid (18: 2n-6) had a higher level. This fatty acid is present in plant oils used in the feed ingredient of cultured fish [14-16].

Among the n-3 series, both diets were good sources of EPA and DHA. The percentages of EPA and DHA were (2.93% in diet A vs 3.06% in diet B) and (5.06% in diet A vs 6.30% in diet B) respectively.

3.2 Fatty Acids Composition of the Liver

The fatty acid profiles of liver of the two groups of...
cultured *Sparus aurata* are presented in figures 1 (a and b). The percentage of total saturated acids was higher in the group of fish fed on diet B than on diet A, whereas its total monoenoic and polyenoic content was lower.

The major polyunsaturated fatty acids identified in both group of fish were 20: 5n-3 (eicosapentaenoic acid, EPA) and 22: 6n-3 (docosahexaenoic acid, DHA). Liver of cultured sea bream contained significantly (*P* < 0.05) higher proportions of n-3 PUFA and specially a higher proportion of DHA than n-6 PUFA.

At the same time, the figure 1 showed the evolution of fatty acids values at the Di and Df. The values of different groups of fatty acids were, on average, similar regardless the date of sampling.

Fig. 1  Fatty acids composition (% of total fatty acids) and distribution in the liver of each group of *Sparus aurata* fed on diet A and diet B. Values are the average of three replicates ±sem. Numbers in histograms having different letters indicate that treatment are significantly different *P* < 0.05.
But statistical analyses revealed that during the first experimental days (D1), differences were not very important in SFA profiles in liver compared between the initial profile (D0) and the final one at Df. But, MUFA and PUFA tended to decrease (from 33.91% to 28.91% for the MUFA and from 35.16% to 31.58% for the PUFA) in the liver of the fish fed on diet B and inversely in the fish fed on diet A.

3.3. Fatty Acids Composition of Different Edible Muscles.

The fatty acid compositions of different body parts (the dorsal, middle and ventral muscles) of Sparus aurata are presented in figures 2 (a, b and c) respectively.

Comparing fatty acids class’s level in each group of gilthead seabream, values showed that all muscles had significantly higher levels of SFA and PUFA than the MUFA levels. Moreover, among the PUFA group, the n-6 PUFA levels were significantly lower than n-3 PUFA which been expressed essentially by the DHA acid.

Muscle fatty acid composition of total lipids showed the effect of the diets from the intermediate sampling (D1) to the end of feeding diets (Df) when fish reached the commercial size.

Saturated fatty acids (SFA) increased from the beginning sampling until the end of feeding experience (Df).

However, MUFA decreased in the muscle of the fish fed on diet A and B in the intermediate sampling but did not further increase in the final sampling (Figs. 2 a, b and c).

Moreover, PUFA decreased along the first period in the diets A and B proportionally to their level in the diets but up to the level of ≈ 40% of total fatty acids, total PUFA decrease at the end of experiment.

In comparison between fish fed on diet A and B, n-3 PUFA muscle contents in the first sampling reduced by 3.5% in the average in all muscles. Then, the levels of these fatty acids were further reduced until the end of the experimental period reaching 13% and 20% reductions in fish fed 20% and 22%. Reduction of fatty acids along the diet B was more pronounced for DHA than EPA in the intermediate and in the final sampling (Fig. 2).

(a) Dorsal muscle (DM) of Sparus aurata fed on diet A (20% of lipid) and diet B (22% of lipid).
Effect of Dietary Lipid Levels on Fatty Acids Composition of Cultured *Sparus aurata*.

(b) **Middle muscle (MM) of Sparus aurata** fed on diet A (20% of lipid) and diet B (22% of lipid).

(c) **Ventral muscle (VM) of Sparus aurata** fed on diet A (20% of lipid) and diet B (22% of lipid).

Fig. 2 Fatty acids composition (% of total fatty acids) and distribution in the Dorsal muscle (MD)-a-, Middle muscle (MM)-b- and Ventral muscle (MV)-c- of each group of *Sparus aurata* fed on diet A (with 20% of lipids) and diet B (with 22% of lipid) from initial to final period of experiment. Values are the average of three replicates ±sem. Different letters in histograms indicate that treatment are significantly different $P < 0.05$. 
4. Discussion

In the present study, fish were fed on two diets (20% and 22% lipid levels) during 21 weeks to marketable size fish. As such, the deposition of fatty acids in liver and different analysed muscles of *Sparus aurata* was influenced by the fatty acid composition of the experimental diets. In general, fatty acids found in high concentrations in the diet were also reflected to a certain extent in the fatty acid composition of muscle tissue. These results are in agreement with other studies reporting that the fatty acid pattern of fish tissue reflects that of dietary lipids [17, 18].

In this study, there are marked differences in fatty acid deposition in different muscles of cultured *Sparus aurata*. Generally, the dorsal and middle parts (DM and MM) had a higher concentration of SFA and PUFA, especially in fish fed on 22% of lipid level. In comparison between the two groups, the muscle of the fish fed on diet A, contained a significantly higher level of PUFA, n-6 PUFA and MUFA. Similar to these, high proportions of DHA were also observed in the dorso-ventral muscle.

However, DHA content was double that of EPA and was not increased by the feeding with the two diets for 21 weeks. Moreover, feeding with diet A (20% of lipid level) for 11 weeks effectively increased DHA contents in muscle of fish, improving the nutritional value of seabream fillets for human [19]. But EPA’s fillet contents were not fully recovered even after 21 weeks, this fatty acid being also very important for human health, as a potent hypotriglyceremic factor [20] and more effective than DHA in inhibiting platelet aggregation [21].

So, as it shown, these results are similar to the results found on gilthead seabream fed on vegetable oils for a long term period [22], a significantly higher level of SFA, MUFA and PUFA were stored in the flesh compared to the beginning of diet experiment due to the introduction of certain fatty acids of vegetal origin such as α-linolenic acid in formulation of fish diets.

In comparison with the evolution of DHA muscle contents, this higher reduction of DHA and lower incorporation of EPA after feeding with diets A and B may be related with several factors. On one hand, it could be due to a preferential oxidation of EPA over DHA in agreement with the higher reduction of EPA in the neutral lipids, since white muscle seem to play a key role in the overall fatty acid oxidation capacity in fish, mitochondrial beta-oxidation dominating over peroxisomal oxidation in this tissue [23], and EPA is mainly oxidized by mitochondria, whereas DHA seems to be oxidized by the peroxisomes and to a lower extent than EPA [24]. On the other hand, there is a high affinity of phosphatidylcholine (PC) and, especially, phosphatidylethanolamine (PE) synthetases for DHA, particularly in the 2n position, and in gilthead sea bream larvae elevation of dietary DHA inhibits EPA incorporation into PE, whereas elevation of dietary EPA levels enhances DHA incorporation into PC and PE [25]. In agreement with those authors, in the present experiment the elevation of DHA enhanced the incorporation of DHA rather than that of EPA.

From start-feeding to marketable size of fish, the higher proportion of SFA and PUFA appeared to be the retention and storage fatty acids, such as the case DHA. However, MUFA and n-6 PUFA were being the actively synthesized because they were limited in muscle tissue but abundant in oil diets.

Thus, there maining fatty acids found in both species (about 70%) were saturated and polyunsaturated fatty acids (SFA+ PUFA). These values are higher than the reported values (15.7%) in silver carp (*Hypophthalmichthys molitrix*) [26].

In general, fish are relatively low in saturated fatty acid (< 30%), except for certain species [27]. Similar results for wild zander [28] and other freshwater fish have also been reported in the literature [26].
The saturated fatty acid content didn’t remain constant and increased amongst the different fillet portions and/or the different fish sizes. This is in agreement with studies of Turchini et al. (2003) [29] and Francis et al. (2006) [30] in that SFA are not used efficiently by gilthead sea bream (Sparus aurata) as an energy source and are therefore accumulated at an optimal level compared to other fatty acid classes. On the contrary to SFA, there was a decrease in the PUFA content and an increase in that of MUFA in the muscle for the first days.

Overall, in highly fat-rich fillet portions n-6 fatty acids were less abundant, whereas n-3 were predominant, suggesting that n-3 are preferentially deposited as “stored lipid”, while n-6 fatty acid are important part of the “functional lipid”. The eicosapentaenoic acid content remained fairly uniform in the different fillet portions, suggesting that its percentage content is similar in both stored and functional lipids.

In fish, generally, accumulation of certain fatty acids in muscle tissue is dependent on their dietary concentration. However, in this study, the n-3 PUFA values were higher in muscle than in the diet. The same trend has been observed in salmonids released by Turchini et al., 2003 [29], suggesting that Sparus aurata tend to accumulate and store n-3 while using n-6 as an energy source.

5. Conclusions

In summarising, it may be concluded that the period of breeding of Sparus aurata fed on a medium differences of lipid’s diet levels (20% in the diet A) and (22% in the diet B), did not produce diverge in terms of contents of EPA and DHA acids, the most valuable to a consumer, or in the values of such indicators of the nutritional quality of lipids as n-3/n-6 and EPA/DHA ratios.

Nevertheless, there was an increased fat deposition as the fish size increased, suggesting that Sparus aurata utilises lipid at a faster rate during early growth stages, and then starts accumulating fat as growth decreases. This is in accordance with that described by Bell et al. (2003) [31] and Palmeri et al. (2007) [32] for Atlantic salmon and Murray cod.

Despite these changes in muscle fatty acid composition, fish fillets remained being highly nutritious for human health.

References

seasonal variations.” *International Journal of Food Science and Technology* 37: 477-84.


