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Full Length Research Paper

Impact of a blood-sucking parasite on the chemical composition of fatty acids in the white muscle of garfish (*Belone belone*, Belonidae) from Tunisian coasts (Central Mediterranean)

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The objective of this study was to compare the composition of fatty acids in the garfish (*Belone belone*) white muscle removed from parasitized and non parasitized specimens. The chemical analysis results revealed low contents of total fatty acids for both parasitized and non-parasitized specimens. Their values, less than 2 g/100 g of fresh muscle, allowed the classification of the garfish as a lean fish. High ratios of saturated fatty acids were found in the garfish muscle reaching 58.4% of total fatty acids. These fatty acids were represented mainly by lauric, miristic and palmitic at a level of 50.3%. As a lean fish, garfish contains 16% polyunsaturated fatty acids (n-3). Two major fatty acids are docosahexaenoic and eicosapentaenoic with respective percentages of 9 and 1.17% of total fatty acids. The parasitized garfish showed increase in their fatty acids, mainly in pentadecanoic, pentadecenoic, docosahexaenoic and arachidonic acids and decreases in saturated acids especially lauric, miristic and palmitic. This drop is correlated with a very significant increase in PUFA from 16 to 26% of total fatty acids. In order to obtain 0.5 g/day of EPA + DHA, the amount of garfish required is 641 g of non-parasitized and 436 g parasitized fish.

Key words: Garfish, blood-sucking, parasite, parasitized fish, fatty acid analysis, Tunisia.

INTRODUCTION

Fish are known for their high content in protein and lipids. These, rich in polyunsaturated fatty acids (PUFA) n-3 such as eicosapentaenoic acid (EPA, 20: 5 n-3) and docosahexaenoic acid (DHA, 22: 6 n-3), are stored in various organs of the fish; liver, muscle, gonad, skin and perivisceral adipose tissue (Corraze and Kaushik, 1999). Specifically, it has been shown that the biochemical composition of muscle and PUFA content present significant differences according to its nature and its location in the various regions of the body; anterior, middle or caudal (Ben Smida et al., 2009). The quantitative variability of PUFA in a given organ is closely related to the life cycle of the fish. As such, Henderson and Tocher (1987) showed increased levels of muscle lipids in freshwater fishes according to the age and the size. Later, Mörköre and Rørvik (2001) confirmed these results in salmon. Jaloustre et al. (2012) found very large fluctuations in levels of triglycerides (TAG) in relation to

Abbreviations: PUFA, Polyunsaturated fatty acids; **EPA,** eicosapentaenoic acid; **DHA,** docosahexaenoic acid; **TAG,** triglycerides; **GC,** gas chromatography; **TFA,** total fatty acids; **MUFA,** monounsaturated fatty acids; SFA, saturated fatty acid.

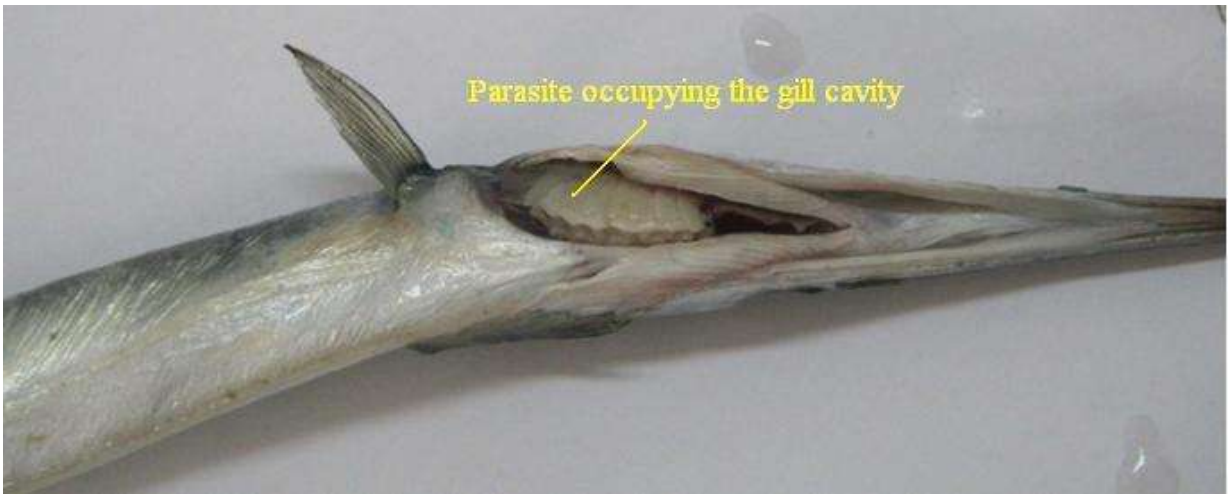

Figure 1. Photo showing a blood-sucking inside the garfish gill cavity (by Bedoui-Fehri R).

total lipids of muscle and liver of sardine and anchovy in relation to their sexual cycle; much more variable in the sardine (30-40% during the period of sexual rest against 5% in the period of the intense sexual activity). It is also important to mention that the fishes move in an open environment and live in constant dynamic interaction with their environment as adoption of diverse and complex behavioral strategies to insure their position in the food chain.

However, fish are never protected against the environmental stresses such as pollution and parasitism (Amilhat, 2007). Parasitism involves a wide diversity of species among the endo and ectoparasites of fish. However, the impact of ectoparasites on fish, including bloodsucking crustacean isopods remains little known and the available information is limited to the effect of the parasite on the outer aspect of the fish (Tombi et al., 2011). It is even reported that ectoparasites are not generally damaging to fishes and do not affect the quality of meat (Natural Resources Quebec, 2006-2013).

To our knowledge, no studies on the lipids biochemical composition of parasitized fish have been performed. It is in this context and in order to assess the real impact of the blood-sucking parasite on garfish from Tunisian coast, we undertake a comparative study of the fatty acid composition between parasitized and non parasitized fish.

MATERIALS AND METHODS

Garfish samples examined were collected from fishermen in July 2011. This month corresponds to the spawning period of the fish (Bedoui et al., 2002). The total length and the weight of the non-infected specimens (n = 6) varied between 27 and 28.5 cm and 66.6 and 80.7 g, respectively. Those of infected specimens (n = 6) varied between 23 and 29.5 cm (length) and between 42.9 and 88.9 g (weight). The infection rate was one parasite by gill cavity (Figure 1).

In total, six parasites were collected. The parasite was identified as being an isopod belonging to *Mothocya belonae sp.* (Bruce, 1985). Approximately, a one-gram sample of white muscle was taken from the left anterior side of each specimen. The total lipid extraction was performed according to the method of Folch et al. (1957). The total lipids obtained were stored in chloroform - methanol- butylated hydroxytoluene (BHT) at -28°C. Before being analyzed by gas chromatography (GC), fatty acids were made volatile by esterification using sodium methylate (Cecchi et al., 1985). An internal standard, the nonadecanoate methyl $C_{19:0}$ (Sigma) were added in order to quantify the fatty acids. Methyl esters of total fatty acids were separated, identified, determined and titrated by a gas chromatograph type HP 6890 with a split/splitless injector with electronic pressure control and a flame ionisation detector. The apparatus was equipped with a capillary column HP Innowax 30 m long, with an internal diameter of 250 µm and whose film thickness was 0.25 µm. Polar stationary phase used in this column was polyethylene glycol. The oven temperature was programmed as follows: from 50 to 180°C at a rate of 4°C/min; from 180 to 220°C to 1.33°C/min; 220°C for 7 min. To obtain an accurate identification of the various fatty acids of the lipids of the garfish, we compared the retention times of the fatty acids in our samples with those of a mixture of methyl esters of reference (Supelco-3 PUFA). The results represent the average of six replicates (n = 6). For data analysis, each fish sample was subjected to a one-way analysis of variance, with a confidence level of 5%, using the Duncan test. Statistical analyzes were performed using the Statistical Package for the Social Sciences (SPSS) 13 software.

RESULTS AND DISCUSSION

The content of muscle tissue (without the skin) of total fatty acids (TFA) of parasitized and non-parasitized garfish collected in July is shown in Table 1. These contents are less than 2 g/100 g (fresh muscle). According to the classification of Ackman (1994), garfish can be considered as a lean fish. This value (0.78 g) is close to that of garfish captured in May in the Baltic Sea, that was 2.44 g/100g (fresh muscle) (Kolakowska et al., 2000) and is low compared to that captured from October to March, in the Black Sea, 5 g/100 g (fresh muscle)

Table 1. Contents of TFA in the garfish white muscle (g/100g fresh muscle).

Parameter	Garfish		F	P
	Non parasitized	Parasitized		
Total fatty acids) (g/100g fresh muscle)	0.78 ± 0.13	0.68 ± 0.09	0.04	*

Mean ± ES; n=6; F: Fischer test; *Significant *P ≤ 0.05*.

(Boran and Karaçam, 2011). It seems that TFA from the garfish vary greatly depending on the season. This variation is related to seasonal environmental changes such as water temperature, the current patterns, the availability of prey resources whose impact on fish behavior are not negligible. We mention, as an example, periods of starvation or intense feeding activity, feeding or reproduction migration to other more suitable areas. Given that the garfish spawns intermittently, Kompowski (1965a, b) reported that this fish maintains its rate of feeding during the different stages of the sexual cycle. The long period of egg maturation reported by Zorica et al. (2010) could explain the low fat content in the non-parasitized fish (Table 1).

The TFA value increased from 0.78 g / 100 g (fresh muscle) in the non-parasitized fish to 0.68 g/100 g (fresh muscle) in parasitized fish (Table 1). On this subject, Macnab and Barber (2012) reported that infected fish behave differently; probably parasites affect their behavior, forcing them to move and look for warmer temperatures. This decrease in the amount of total fatty acids was confirmed by Van den Broek (1978) who found that the parasite *Lernaeocera branchialis*, caused, in its host, *Merlangius merlangus*, a significant decrease in body weight associated with a decrease in the lipid content of the liver and an increase in the rates of cholesterol. Thus, we suggest that the effect of parasitism would result in a decrease in the amount of total fatty acids in parasitized fish. The majority of fatty acids identified in the non-parasitized fish are lauric, the pentadécénoïque the palmitic and DHA with percentages of 25.6, 17.3, 14.8 and 9% of TFA. Saturated fatty acids are present with a percentage of 58.3% TFA. They are represented by the $C_{12:\ 0}$, $C_{14:\ 0}$ and $C_{16:\ 0}$ that have a cumulative percentage of 50.3%. The biochemical composition of fish is strongly affected by the composition of their food (Orban et al., 2007). Hughes et al. (1996) have shown that stearic acid ($C_{18:\ 0}$), palmitic ($C_{16:\ 0}$) and myristic ($C_{14:\ 0}$) or lauric acid ($C_{12:\ 0}$), have different metabolisms and should be considered separately.

In all living organisms, monounsaturated fatty acids (MUFA) are provided from endogenous synthesis and from their alimentation (Legrand, 2007). According to Table 3, the pentadecenoic fatty acid ($C_{15:\ 1}$) was identified as a major monounsaturated fatty acid. Oleic acid ($C_{18\ :1n-9}$), a characteristic of fish tissues (Steffens, 1997), was present at the percentages of 3.3 and 2.5% in the parasitized garfish muscle and non-parasitized garfish muscle, respectively. Kołakowska et al. (2000) observed in reared garfish percentages of 0.81% for $C_{15:\ 1}$ and 24.2% for the C_{18} complex: $_{1n-9}$, $C_{18:\ 1n-7}$. We suggest that the $C_{15:\ 1}$ is a product that does not come from endogenous synthesis, but rather comes from the garfish alimentation. The muscles of the garfish non-parasitized are poor in PUFA 16% TFA. This result is confirmed by the value of PUFA / saturated fatty acid (SFA) ratio which is 0.29 whereas the ratio recommended by the HMSO (1994) is 0.45. Among the fatty n-3 and n-6 acids, fatty acids DHA and arachidonic dominate with percentages of 9 and 1.9% TFA, respectively. Probably, the lack of fatty acid n-6 and n-7 might be attributed to environmental conditions and / or fish food. The ratio n-3/n-6 is a conclusive criterium to compare the nutritional value of fish oils (Piggot and Tucker, 1990).

Fish or fishery produce rich in fatty acids type n-3 fatty and poor in fatty acids type n-6 are beneficial to human health (Sargent, 1997). In the garfish, the ratio n-3/n-6 ratio is 5.9. The effect of the blood-sucking parasite on the muscle of the garfish results in a significant variation ($p \leq 0.01$) PUFA / SFA ratio, going from 0.29 to 0.63. This increase is explained, on the one hand, by a significant increase in the percentage of PUFA (16 to 26.4%) and, on the other hand, by a highly significant decrease in the percentage of the group, $C_{12:\ 0} + C_{14:\ 0} + C_{16:\ 0}$, going from 50.3 to 9.7% TFA. The fatty acid profile of the parasitized garfish depends on the enzymatic activity of lipid metabolism vis-à-vis the SFA and MUFA. The parasitized muscles of garfish accumulate PUFAs with a transformation of SFA that is more important than that of MUFA. These results are corroborated by the ratio PUFA / MUFA and PUFA / SFA (Table 2) with respective values of 0.7 to 0.8 ($p > 0.05$) and 0.29 to 0.63 ($p \leq 0.01$).

At the level of PUFA, we note a significant increase in fatty acids of the type n-3 (13.1 to 20.9% TFA) and a very significant increase ($p \leq 0.001$) in the percentage of fatty acids type n-6 (from 2.2 to 5.1% TFA). The n-3 fatty acids are represented by the DHA with a percentage of 14.9% TFA, while those of type n-6 are represented by arachidonic with a percentage of 4.8% TFA acid. The value of the n-3/n-6 ratio is 4.2. Thus, the impact of the blood-sucking on the garfish shows a low value of this ratio. According to the study of Hossain (2011), a lower value of n-3/n-6 ratio means that the enzymes that convert fats into the forms in which they are active in the body are rather directed towards the synthesis of n-6 fatty acids. According to the study of Ackman (1980), fish are

Table 2. Composition on fatty acids in the garfish (*Belone belone*) from Tunisian coasts.

Fatty acids	Garfish specimen		*P*
	Non Parasitized	Parasitized	
C12 : 0	25.64±5.79[a]	7.58±2.63[b]	*
C14 : 0	9.81±3.34[a]	1.29±0.32[b]	*
C15 : 0	2.09±0.28[b]	23.90±2.66[a]	* **
C16 : 0	14.86±2.36[a]	0.81±0.11[b]	**
C17 : 0	1.03±0.10[a]	1.30±0.10[a]	ns
C18 : 0	5.10±0.49[a]	7.04±0.67[a]	ns
C15 : 1	17.37±6.04[a]	23.60±2.56[a]	ns
C16 : 1n-9	2.21±0.31[a]	1.27±0.11[b]	*
C16 : 1n-7	1.10±0.16[a]	0.63±0.05[b]	*
C18 : 1n-9	2.45±0.37[b]	3.27±0.43[a]	*
C18 : 1n-7	1.44±0.17[a]	1.96±0.17[a]	ns
C20 : 1	0.35±0.12[a]	0.24±0.07[a]	ns
C22 : 1	0.46±0.06[a]	0.79±0.28[a]	ns
C16 : 2n-4	0.61±0.14[a]	0.17±0.03[b]	*
C18 : 2n-6	0.19±0.07[a]	0.29±0.13[a]	ns
C20 : 2n-6	0.14±0.00[a]	0.07±0.01[b]	**
C18 : 3n-3	0.04±0.01[b]	0.11±0.03[a]	**
C18 : 4n-3	1.20±0.64[a]	0.27±0.18[a]	ns
C20 : 4n-6	1.90±0.20[b]	4.79±0.37[a]	***
C20 : 4n-3	0.25±0.04[a]	0.44±0.11[a]	ns
C20 : 5n-3	1.17±0.05[b]	2.26±0.20[a]	*
C22 : 5n-3	1.50±0.17[b]	2.92±0.24[a]	*
C22 : 6n-3	9.00±0.88[b]	14.89±1.65[a]	*
C12:0+C14 :0+C16 : 0	50.32±6.79[a]	9.69±3.06[b]	**
SFA	58.36±6.68[a]	41.74±2.16[a]	ns
MUFA	25.41±5.80[a]	31.80±2.21[a]	ns
PUFA	16.02±1.08[b]	26.24±1.22[a]	**
UFA	41.44±6.68[a]	58.05±2.16[a]	ns
n-3	13.15±1.00[b]	20.90±1.13[a]	**
n-6	2.25±0.25[b]	5.16±0.40[a]	***
n-7	2.55±0.75[a]	2.60±0.15[a]	ns
n-9	4.56±0.49[a]	4.55±0.43[a]	ns
EPA+DHA	10.17±0.84[b]	17.16±1.47[a]	**
n-3/n-6	5.95±0.49[a]	4.21±0.38[b]	**
UFA/SFA	0.78±0.22[b]	1.40±0.12[a]	*
PUFA/MUFA	0.70±0.11[a]	0.83±0.70[a]	ns
PUFA/SFA	0.29±0.05[b]	0.63±0.04[a]	**

SFA, $C_{12:0}+C_{14:0}+C_{15:0}+C_{16:0}+C_{17:0}+C_{18:0}$; MUFA, $C_{15:1}+C_{16:1n-9}+C_{16:1n-7}+C_{18:1n-9}+C_{18:1n-7}+C_{20:1}+C_{22:1}$; PUFA, $C_{16:2n-4}+C_{18:2n-6}+C_{20:2n-6}+C_{18:3n-3}+C_{18:4n-3}+C_{20:4n-6}+C_{20:4n-3}+C_{20:5n-3}+C_{22:5n-3}+C_{22:6n-3}$; UFA, MUFA+ PUFA; *, ** and ***, significant at P≤0.05, P≤0.01 and P≤0.001, respectively; ns, not significant (P>0.05). Mean ± ES; n=6; P<0.05.

inclined to adjust their lipid composition to the demands of the environment and to their own physiological requirements. Their behaviors and food preferences support this goal.

A number of countries such as Canada, United Kingdom, World Health Organization (WHO) and North Atlantic Treaty Organization have advocated dietary recom-mendations for PUFA (n-3). These recommendations are 0.3 to 0.5 g/jour EPA + DHA (Kris-Etherton et al., 2002). According to Table 3, these recommendations can easily be fulfilled through the consumption of garfish with a daily intake of 641 g if it is non-parasitized and 436 g if it is parasitized.

Conclusion

It appears from this study that *M. belonae,* parasite of the

Table 3. Quantities of EPA+DHA in the garfish white muscle and proposed daily intake of fish flesh.

Specimen	EPA+DHA (mg/100 g)	EPA+DHA (mg/150 g)	Quantity of consumed fish (g) to provide 0.5 g EPA+DHA/day
Non-parasitized	78	117	641
Parasitized	115	172	436

garfish, modifies the fatty acid composition of its white muscle. This change improves the nutritional quality of this pelagic fish. Thus, it results in a significant decrease in the percentage of group $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$, which rose from 50.3 to 9.7%. This effect is also confirmed by the increase in the UFA / SFA ratio by about 100% (0.78 to 1.40). The positive effect of this parasite allows a significant increase in PUFA, changing from 16 to 26.2% TFA. Because of both the low fat content of garfish and the need to guarantee the intake of 0.5 g EPA + DHA/day, the recommended intake of this fish is a portion of more than 150 g.

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