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Fatty acids in muscles and liver of Tunisian wild and farmed gilthead sea bream, *Sparus aurata*

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Abstract

The total fat content and the fatty acids profile were analysed in the dorsal and ventral muscles and in the liver from wild and farmed gilthead sea bream (*Sparus aurata*). The amount of fish lipid was higher in farmed than in wild fish in all studied samples and the highest level of all was observed in liver. It was noted that, among all the samples studied for saturated fatty acids (SFA) and Monounsaturated fatty acids (MUFA), whether farmed or wild, palmitic (C16:0) and oleic (C18:1 $n-9$) acids were the principal saturated and monounsaturated fatty acids. The results showed that farmed fish contained a higher level of $n-3$ polyunsaturated fatty acids (PUFA), particularly docosahexaenoic acids (DHA) and eicosapentaenoic acids (EPA), whereas wild fish contained a higher level of $n-6$ PUFA. Arachidonic acid (C20:4 $n-6$) was the primary $n-6$ PUFA in wild fish whereas in farmed fish, linoleic acid (C18:2 $n-6$) was the major $n-6$ PUFA. Farmed fish were characterized by higher $n-3/n-6$ ratio for all samples studied, due to the abundance of $n-3$ PUFA, particularly DHA.

Keywords: Fish lipid; Fatty acids composition; *Sparus aurata*; Muscles; Liver; PUFA $n-3$

1. Introduction

It has been reported that, a high dietary consumption of marine $n-3$ fatty acids may prevent the development of atherosclerosis and thrombosis (Calder, 2004; Harris, 2004; Schmidt, Arnesen, Decaterina, Rasmussen, & Kristensen, 2005). Considering the high benefits of consumption of marine oils in human health, the Nutrition Committee of the American Heart Association, recommends eating fish of any type 2 or 3 times a week (Kris-Etherton, Harris, & appel, 2003). Indeed, long chain $n-3$ polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are found mostly in fish, but are contained in some other foods as well. However, fish oil is the major and the best source of these fatty acids. PUFA $n-3$ fatty acids are essential for neural development in the infant in pregnancy and during the first years after birth (Montano, Gavina, & Gavino, 2001). More importantly, $n-3$ PUFA have preventive effects in coronary heart diseases, inflammatory and autoimmune disorders, inflammation and arrhythmias (Belluzzi, 2001; Connor, 2001; Leaf, Xiao, Kang, & Billman, 2003).

Gilt head sea bream is considered to be a very important aquaculture fish species in Tunisia. The aquaculture production of this species has become intensive and there is much research with the aim of improving the quality of cultured gilthead sea bream. Lipid content and fatty acids profile of fish are known to vary between and within species

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(Haliloglu, 2001; Haliloglu, Aras, & Yetim, 2002). A number of factors, such as the temperature, the salinity, the season (Chaouch et al., 2003), the type and the availability of food, the habitat, the stage of maturity and the individual variability, are believed to be important factors contributing to these variations. The lipid composition of fish varies also with the part of the fish from which the sample is taken. The aim of this work was to compare the lipid content and fatty acid composition of dorsal, ventral muscles and liver in Tunisian farmed and wild gilthead sea bream (*Sparus aurata*).

2. Materials and methods

Cultured gilthead sea bream (*Sparus aurata*) obtained in the autumn of 2003, from the Station of Tunisian Aquaculture (water temperature: 22 °C; salinity: 40%; pH: 8.2) located in Hergla (Tunisia), with an average weight and total length of 53.49 ± 2.0 g and 14.29 ± 0.3 cm, were raised under typical farming conditions, on the same feed, and by the same feeding techniques. The commercial feed used contained 50% protein, 21% fat, 12% carbohydrates, 11.5% ashes and 1.5% raw fibre. The fatty acid profile of the feed used in this study is given in Table 1. Wild gilthead sea bream (average weight and total length of 42.01 ± 1.24 g and 14.15 ± 0.22 cm) were caught in the same season from the coastal waters (temperature: 25 °C; salinity: 38%; pH: 7.8) of Monastir (Central East of Tunisia).

Table 1
The fatty acid percentage profile (% of total fatty acids) of feed of cultured gilthead sea bream

Fatty acid	%
C14:0	6.01
C16:0	18.57
C17:0	2.30
C18:0	3.79
C20:0	2.10
C22:0	0.63
C24:0	1.46
SFA	34.88
C14:1 $n-5$	1.05
C16:1 $n-7$	6.70
C18:1 $n-7$	13.42
C18:1 $n-9$	3.17
C20:1 $n-9$	4.78
C22:1 $n-9$	0.70
C24:1 $n-9$	0.02
MUFA	29.87
C18:2 $n-6$	6.91
C20:2 $n-6$	0.03
C20:3 $n-6$	0.28
C20:4 $n-6$	0.94
C22:4 $n-6$	0.15
$n-6$ PUFA	8.34
C18:3 $n-3$	1.57
C20:5 $n-3$	10.68
C22:5 $n-3$	0.36
C22:6 $n-3$	14.20
$n-3$ PUFA	26.82
PUFA	35.16
$n-3/n-6$	3.21
EPA/DHA	0.75

Specimens were transported in ice to the laboratory where they were weighed and immediately processed. Dorsal and ventral muscle samples, and liver, were cut out and frozen at −80 °C, after being soaked in liquid nitrogen, until analyzed. Proteins were extracted from different samples according to Lowry, Rosebrough, Farr, and Randall (1951). Total lipids were extracted with chloroform/methanol (2:1) (Folch, Lees, & Sloane-Stanley, 1957, modified by Bligh & Dyer, 1959) and fatty acids were methylated with BF_3 in methanol. The fatty acid methyl esters were recovered with hexane (Metcalfe, Schmitz, & Pelka, 1966) and analyzed by capillary gas chromatography (column: 30 m × 0.25 mm HP-Innowax; flame ionization detect temperature at 280 °C; carrier gas N_2 at 1 ml/min; injector temperature 250 °C; oven temperature programmed from 180 to 250 °C) using a Hewlett-Packard HP 5890 capillary gas chromatograph linked to an HP Chemstation integrator.

The identification of fatty acid methyl esters was performed by external standards (all purchased from Sigma Chemical Co.) submitted to the same processes of manipulation as the biological samples analyzed. The values of fatty acids are presented as area percentage of total fatty acids.

Data are presented as mean ± SEM and subjected to student's *t*-test for determining significant differences between means.

3. Results and discussion

Results revealed that total fatty acids (mg/g protein) were higher in farmed fish in the three tissue samples studied (Table 2). Only in the ventral muscle is the difference statistically significant ($p < 0.05$). It was also observed in both farmed and wild gilthead sea bream, that the liver contained higher concentrations of lipids than do ventral and dorsal muscles.

The fatty acid profiles of dorsal and ventral muscles in farmed and wild *Sparus aurata* are listed in Table 3. Table 4 showed the gas chromatographic analyses of fatty acids extracted from the liver. The fatty acids analyzed were grouped as saturated (SFA), monounsaturated (MUFA) while di-, tri-, tetra-, penta- and hexa-noic fatty acids were grouped as polyunsaturated fatty acids (PUFA).

For dorsal and ventral muscle, and the liver, the percentage of total SFA was higher in wild fish compared with farmed gilthead sea bream. Total MUFA content, however, was lower in wild than in farmed in the dorsal and ventral muscles but the difference was significant only for ventral muscle. In the liver, the higher percentage of MUFA was observed in the wild fish than in the farmed fish. The major SFA and MUFA identified in all studied samples, whether wild or farmed, were (C16:0) (palmitic) and (C18:1 $n-7$) (oleic).

Table 2
Total fatty acids (mg/g protein) of dorsal, ventral muscles and liver of farmed and wild gilthead sea bream (*Sparus aurata*)

	Dorsal muscle		Ventral muscle		Liver	
	Farmed ($n = 7$)	Wild ($n = 15$)	Farmed ($n = 7$)	Wild ($n = 15$)	Farmed ($n = 8$)	Wild ($n = 9$)
Total lipid content (%)	0.149 ± 0.009	0.09 ± 0.008	0.234 ± 0.021	0.099 ± 0.008	0.666 ± 0.057	0.552 ± 0.019
Total fatty acids (mg/g protein)	0.037 ± 0.002	0.030 ± 0.004	0.058 ± 0.007	0.032 ± 0.003	0.115 ± 0.015	0.104 ± 0.007

For the three samples studied, the results revealed that cultured gilthead sea bream contained more $n-3$ PUFA than wild one, whereas its total $n-6$ PUFA content was lower and the difference was statistically significant. Similar results were observed for gilthead sea bream (Carpene, Martin, & Dalla Libera, 1998; Saglik et al., 2003) and for sea bass (Saglik et al., 2003). This difference was due to the variation of different fatty acids of each group.

DHA (C22:6 $n-3$) was the principal $n-3$ PUFA in both fish but its content was higher in the farmed fish than in the wild gilthead sea bream and the difference was statistically significant. The percentage of EPA (C20:5 $n-3$) was more important in the farmed fish but the difference was statistically significant only for liver.

For the group of $n-6$ PUFA, the primary fatty acid was arachidonic (C20:4 $n-6$) for wild fish and linoleic (C18:2 $n-6$) for farmed fish and the differences observed between both fishes were statistically significant. Linoleic acid (C18:2 $n-6$) has been observed in higher concentrations in cultured than in wild gilthead sea bream (Grigorakis, Alexis, Taylor, & Hole, 2002) and other species (Alasalvar, Taylor, Zubcov, Shahidi, & Alexis, 2002; Rueda, Lopez, Martinez, & Zamora, 1997; Saglik et al., 2003). Linoleic acid (C18:2 $n-6$) was found in high amounts in the feed used for cultured fish, so wild fish contained less amounts of C18:2 $n-6$, due to their reduced capacity for chain elongation and desaturation (Yamada, Kobayashi, & Yone, 1980). Wild sea bream,

Table 3
Comparison of the fatty acids compositions (% of total fatty acids[a]) in dorsal and ventral muscles of farmed and wild gilthead sea bream (*Sparus aurata*)

Fatty acid	Dorsal muscle		*F*	Ventral muscle		*F*
	Farmed	Wild		Farmed	Wild	
C14:0	4.50 ± 0.06	1.90 ± 0.14	*	4.11 ± 0.17	1.98 ± 0.19	**
C16:0	15.83 ± 0.40	19.39 ± 0.31	**	15.62 ± 0.26	19.41 ± 0.39	**
C17:0	0.76 ± 0.04	1.38 ± 0.07	**	0.69 ± 0.06	1.33 ± 0.10	**
C18:0	4.01 ± 0.19	7.56 ± 0.16	**	3.63 ± 0.25	7.42 ± 0.10	**
C20:0	1.28 ± 0.07	0.46 ± 0.04	**	1.43 ± 0.08	0.48 ± 0.06	**
C22:0	2.98 ± 0.52	0.60 ± 0.06	**	3.54 ± 0.60	1.24 ± 0.63	**
C24:0	2.23 ± 0.09	2.92 ± 0.13	**	2.25 ± 0.08	2.86 ± 0.12	**
SFA	31.59 ± 1.01	34.20 ± 0.53	*	31.26 ± 0.36	34.72 ± 0.74	*
C14:1 $n-5$	0.69 ± 0.14	0.99 ± 0.11	ns	0.67 ± 0.01	1.13 ± 0.14	**
C16:1 $n-7$	4.61 ± 0.74	4.86 ± 0.44	ns	5.89 ± 0.18	5.36 ± 0.32	ns
C18:1 $n-7$	15.33 ± 0.50	15.12 ± 0.74	ns	16.79 ± 0.26	14.12 ± 0.72	ns
C18:1 $n-9$	2.83 ± 0.14	$4.45 \pm 0,18$	**	2.69 ± 0.17	4.58 ± 0.19	**
C20:1 $n-9$	3.86 ± 0.66	0.88 ± 0.19	**	4.48 ± 0.72	0.82 ± 0.19	**
C22:1 $n-9$	0.24 ± 0.09	0.40 ± 0.08	ns	0.28 ± 0.06	0.63 ± 0.20	ns
C24:1 $n-9$	0.56 ± 0.32	0.48 ± 0.21	ns	0.37 ± 0.11	0.69 ± 0.16	ns
MUFA	28.11 ± 1.11	27.17 ± 1.05	ns	31.17 ± 0.83	27.32 ± 1.11	ns
C18:2 $n-6$	4.92 ± 0.09	3.00 ± 0.22	**	5.30 ± 0.09	3.09 ± 0.22	**
C20:2 $n-6$	0.42 ± 0.14	0.61 ± 0.07	ns	0.31 ± 0.04	0.68 ± 0.07	**
C20:3 $n-6$	0.48 ± 0.06	0.57 ± 0.05	ns	0.36 ± 0.06	0.61 ± 0.09	*
C20:4 $n-6$	1.63 ± 0.11	11.82 ± 0.82	**	1.29 ± 0.12	11.95 ± 0.79	**
C22:4 $n-6$	0.30 ± 0.06	3.19 ± 0.23	**	0.29 ± 0.03	2.85 ± 0.30	**
$n-6$ PUFA	7.75 ± 0.25	19.27 ± 0.94	**	7.54 ± 0.08	19.18 ± 0.91	**
C18:3 $n-3$	1.36 ± 0.09	0.96 ± 0.12	*	1.37 ± 0.05	0.99 ± 0.13	*
C20:5 $n-3$	7.68 ± 0.22	7.56 ± 0.30	ns	7.29 ± 0.16	7.01 ± 0.51	ns
C22:5 $n-3$	0.49 ± 0.13	1.69 ± 0.10	**	0.49 ± 0.10	1.68 ± 0.14	**
C22:6 $n-3$	23.01 ± 1.29	9.19 ± 0.99	**	20.86 ± 0.97	9.08 ± 0.89	**
$n-3$ PUFA	32.52 ± 1.39	19.40 ± 1.08	**	29.99 ± 1.10	18.75 ± 1.06	**
PUFA	40.28 ± 1.45	38.60 ± 1.44	ns	37.54 ± 1.13	37.93 ± 1.62	ns
$n-3/n-6$	4.21 ± 0.21	1.04 ± 0.08	**	3.97 ± 0.14	0.99 ± 0.06	**
EPA/DHA	0.34 ± 0.01	0.92 ± 0.07	**	0.35 ± 0.01	0.86 ± 0.09	**

ns = $p > 0.05$.
[a] Each fatty acid is presented as a percentage of the total.
* ($p < 0.05$).
** ($p < 0.01$).

Table 4
Fatty acids in liver of farmed and wild gilthead sea bream (*Sparus aurata*)

Fatty acid	Liver		*F*
	Farmed	Wild	
C14:0	3.62 ± 0.11	3.16 ± 0.19	ns
C16:0	14.07 ± 0.34	21.43 ± 0.81	**
C17:0	0.74 ± 0.04	1.60 ± 0.24	*
C18:0	4.73 ± 0.27	10.81 ± 0.69	**
C20:0	0.97 ± 0.06	0.28 ± 0.06	**
C22:0	1.96 ± 0.12	0.31 ± 0.05	**
C24:0	2.23 ± 0.09	2.18 ± 0.22	*
SFA	29.16 ± 0.44	39.77 ± 1.03	**
C14:1 $n-5$	0.62 ± 0.02	0.59 ± 0.16	ns
C16:1 $n-7$	5.80 ± 0.12	6.86 ± 0.68	ns
C18:1 $n-7$	14.95 ± 0.41	22.77 ± 2.48	**
C18:1 $n-9$	2.83 ± 0.06	3.82 ± 0.74	**
C20:1 $n-9$	3.11 ± 0.28	0.70 ± 0.06	**
C22:1 $n-9$	0.11 ± 0.03	0.24 ± 0.07	ns
C24:1 $n-9$	0.22 ± 0.09	$0{,}29 \pm 0{,}09$	ns
MUFA	27.64 ± 0.55	35.25 ± 1.47	**
C18:2 $n-6$	4.84 ± 0.11	3.04 ± 0.76	ns
C20:2 $n-6$	0.93 ± 0.32	1.03 ± 0.23	ns
C20:3 $n-6$	2.30 ± 0.19	0.69 ± 0.08	**
C20:4 $n-6$	1.51 ± 0.09	7.14 ± 1.13	**
C22:4 $n-6$	0.52 ± 0.09	2.10 ± 0.40	**
$n-6$ PUFA	10.09 ± 0.43	14.00 ± 1.23	*
C18:3 $n-3$	1.23 ± 0.05	0.65 ± 0.19	*
C20:5 $n-3$	7.62 ± 0.21	4.53 ± 0.14	**
C22:5 $n-3$	0.50 ± 0.01	0.71 ± 0.12	ns
C22:6 $n-3$	23.72 ± 0.54	5.08 ± 0.82	**
$n-3$ PUFA	33.07 ± 0.75	10.95 ± 1.18	**
PUFA	43.17 ± 0.65	24.96 ± 2.35	**
$n-3/n-6$	3.30 ± 0.17	0.78 ± 0.03	**
EPA/DHA	0.32 ± 0.00	1.00 ± 0.14	**

Each fatty acid is presented as a percentage of the total.
ns = $p > 0.05$.
* ($p < 0.05$).
** ($p < 0.01$).

on the other hand, had higher levels of arachidonic acid (C20:4). Similar results were found in previous studies (Grigorakis et al., 2002; Saglik et al., 2003). The high amount of C20:4 in wild fish was due probably to the diet of wild sea bream being rich in this fatty acid. However in cultured fish, levels of C20:4 $n-6$ are low because the feed used contained minimal amounts of this fatty acid (Sargent, Bell, Mcevoy, Tocher, & Estevez, 1999), as did the feed used in this study.

The ratio of $n-3$ to $n-6$ fatty acids was higher in cultured than in wild gilthead sea bream in contrast with the existing data from gilthead sea bream (Grigorakis et al., 2002; Saglik et al., 2003). This result was due to the abundance of $n-3$ PUFA in cultured fish because the feed used for farmed fish contain high proportions of lipids rich in $n-3$ PUFA.

The fatty acid profile of gilthead sea bream reflected the fatty acid composition of feed used in this study rich in PUFA particularly DHA, whereas the level of $n-6$ PUFA was low. It has been reported that there is a strong relationship between the fish lipid composition and the diets of fish (Grigorakis et al., 2002; Pirini, Gatta, Testi, Trigari, & Monetti, 2000).

The difference between wild and farmed gilthead sea bream was that the latter was being reared on an artificial diet containing a high level of $n-3$ PUFA, particularly DHA and EPA, blended with a small amount of vegetable oil higher C18:2 $n-6$. The low levels of $n-3$ PUFA in wild fish was probably due to the different diet that gilthead sea bream eat, mostly molluscs, which could contain variable amounts of C22:6 $n-3$. Moreover gilthead sea bream is considered as a marine fish with low levels of delta-5 desaturase (Mourente & Tocher, 1993). Considering the amounts of $n-3$ PUFA, the composition of the Tunisian gilthead sea bream, particularly farmed sea bream, will be an important contribution to the good health of the Tunisian people.

4. Conclusion

Differences in total lipid content and fatty acid proportions observed for dorsal muscle, ventral muscle and liver between wild and farmed gilthead sea bream may be attributed mainly to the dietary constituents of the fish. Cultured sea bream compared with wild fish provides the consumer with a high intake of desirable $n-3$ fatty acids, DHA and EPA. Liver and ventral muscle were as rich in $n-3$ PUFA as dorsal muscle and considering their high amounts of DHA and EPA they should be valued more highly.

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