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Comparison of muscle fatty acid and vitamin composition between wild and farmed common dentex (*Dentex dentex*)

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The present study was carried out to compare the tissue composition of wild and farmed common dentex (*Dentex dentex*) and the major nutrients in order to further explore the dietary requirements of this species. Wild (41 ± 14 g) and farmed (10 ± 2 g) fish were analyzed for proximate composition, fatty acid profile and vitamin C and E muscle and liver content. The fat content of the farmed population was almost six times higher than that measured in wild fish at the expense of water content. There were no significant differences in the protein and ash content between the two populations. The vitamin C content was insignificantly lower in wild fish liver while differences were significant in muscle compared to their farmed conspecifics. Vitamin E concentration was found to be significantly higher in both examined tissues of the farmed fish. The fatty acid profiles were different between the wild and farmed individuals. Wild fish livers were significantly richer in ω-6 fatty acids, poorer in total monounsaturates (MUFA), but no differences were observed for total polyunsaturates (PUFA) and saturated fatty acids (SFA). As regards muscle tissues, farmed fish were found to contain higher 22:6ω3 levels but significantly lower 16:1ω7 and 18:1ω9 levels than the wild ones. The information given by the present study should be evaluated for the further improvement of common dentex diets.

Key words: common dentex (*Dentex dentex*), nutrient composition, fatty acids, vitamin C, vitamin E.

INTRODUCTION

During the last decades, the Mediterranean aquaculture industry has developed an interest to farm new species in order to overcome the problem arising from the overproduction of the two main farmed species, namely gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) by product diversification (Theodorou, 2002; Cardia & Lovatelli, 2007). Common dentex (*Dentex dentex*) is a fast growing sparid which represents a possible candidate for the Mediterranean aquaculture (Tibaldi *et al.*, 1996). An extensive review on the biology and the high farming potentiality of common dentex has been given by Rueda & Martinez (2001). Significant mortalities mainly due to microbial infections and cannibalism behaviour have been associated with the first trials of common dentex farming (Rigos *et al*., 1997; 1999). Increased susceptibility of this species to stress and inadequate feeding management have been blamed for the high losses (Rigos *et al*., 1998). Koumoundouros *et al.* (2004) suggested the employment of an adapted semi-intensive version of mesocosm technology to confront the reduced survival of common dentex at least during the early stages of development.

Although there have been some attempts to formulate experimental diets best adopted for common dentex, especially in aspects of macronutrients needs (Skalli *et al*., 2004; Pérez-Jiménez *et al.*, 2009; Suárez *et al.*, 2009), and to determine this species fatty acid needs through starvation experiments (Giménez *et al*., 2008), there is lack of comparative knowledge between wild and farmed fish. This would give valuable information on the actual nutritional requirements of this species. Therefore, the aim of the present study

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was to investigate the tissue composition of wild and farmed common dentex and the important nutrients in order to further explore the requirements of this species and thus, to aid the development of feeds specific to common dentex.

MATERIALS AND METHODS

Fish and experimental set

Five wild common dentex weighting 41 ± 14 g were captured from Korinthiakos gulf during the spring period (water temperature 19-20°C) by long line and stored at –80°C until analysis. Five hundred farmed fish (10 ± 2 g) were transferred from Nireus Aquaculture SA (Nafpaktos, Sterea Ellada, Greece) and acclimated for a week in cylindrical (120 L) fiber-glass tanks prior to the beginning of the experiment. The tanks were supplied with mechanically filtered (5 μm) seawater (salinity 38‰). Water temperature was 22 ± 1°C (October-November) and dissolved oxygen concentration was 8 ± 1 mg l^{-1}. Fish were given commercial feed (Biomar, SA, Table 1) to satiation three times daily (09.00, 13.00 and 17.00) for 4 weeks.

Tissue preparation

Ten farmed fish, after being starved for a day, were randomly selected, weighed and measured after being killed by a blow to the head. Portions of liver and white muscle tissue (below the dorsal fin) devoid of skin and bone were taken and immediately frozen at –80°C until processed for vitamin E and C analysis. Three (total 10 fish) and two pools (total five fish) were, respectively, prepared from farmed and wild fish for the fatty acid profile determination. Portions of selected tissue samples were weighed and kept in 10 ml of chloroform:methanol solution 2:1 (v/v). For proximate composition determinations, the remaining fish tissues were minced and were further freeze-dried until analysis.

TABLE 1. Chemical composition (% dry matter) of the commercial diet (Biomar SA)

Component	
Total protein	47
Total lipids	22
Ash	11
Cellulose	1.6
Calcium	1.2
Vitamin C	250 mg kg^{-1}
Vitamin E	300 mg kg^{-1}

Chemical analyses

All proximate analysis was carried out according to AOAC (1984). The fatty acid analysis on muscle (1 g) and liver samples (0.5 g) was performed after chloroform:methanol (2:1 v/v) extraction. The extracted lipids were separated into neutral and polar lipid fractions by the solid phase extraction method of Kim & Salem (1990). Then, direct methyl-esterification of fatty acids was applied by treatment with 2% sulphuric acid-containing anhydrous methanol for 16 hrs at 50°C under nitrogen gas (Christie, 1989). Analysis of methylesters was conducted according to Kalogeropoulos *et al.* (1992) using gas chromatography (Varian 3300, Waters, MA, USA) equipped with a Flame Ionisation Detector (Waters, MA, USA). Peaks were extrapolated by external standards (Sigma Chemicals Co., St. Louis, USA) and by referenced fish oil.

Vitamin C (ascorbic acid) and E (alpha-tocopherol) contents were determined by High Performance Liquid Chromatography (HPLC) (600 Controler, 717 plus Autosampler, HPLC, Waters, MA USA), with a 3.9 × 300 mm C18 Bondapack column (Waters, Dublin, Ireland) and using an electrochemical detector (Waters 464 pulsed EC Detector, Waters, MA USA). In all cases, the used column was a C18 bondapack. The HPLC methodology of Kissinger & Pachla (1987) was used for ascorbic acid determination. Extraction of tissue ascorbate (vitamin C) with metha nol/aqueous metaphosphoric and sample preparation procedure was performed according to Wang & Seib (1990). The vitamin E (alpha-tocopherol) determination was carried out by a modification of the methodology of Bai & Gatlin (1993). Briefly, a quantity of 0.2 g of feed, or 0.2 g of liver tissue, or 0.4 g of white muscle was homogenized with 4 ml absolute ethanol containing 2% (v/v) pyrogalol, and heated at 70°C for 5 min. Subsequently 1 ml of 60% KOH solution was added and incubation at 70°C under nitrogen took place for 20 min, while periodical vigorous shaking of the samples occurred every 5 min. After cooling at room temperature 3 ml of the organic phase was received and completely dried by nitrogen. A quantity of 1 ml HPLC grade ethanol was added, solutions were vigorously shaken to solubilise the alpha-tocopherol and subsequent HPLC analysis took place.

Statistical analysis

Results from wild and farmed fish pools were checked for homogeneity of variance and comparisons among wild and farmed individuals for the studied

parameters were conducted with students *t-test*, by SPSS 13.0 statistic software. Levels of confidence were set at $p = 0.05$.

RESULTS AND DISCUSSION

The final body weight of the farmed fish at the end of the 4-week trial was 28.5 ± 2.1 g, while the standard growth rate (SGR) was 3.5%. The present growth rate is slightly higher than that mentioned for common dentex of similar sizes (Company *et al.*, 1999; Skalli *et al.*, 2004) and those of gilthead sea bream and European sea bass (Company *et al.*, 1999). Thus, the present results confirm findings indicating that common dentex is a fast growing species (Company *et al.*, 1999; Skalli *et al.*, 2004).

The whole body composition, vitamin C and E content and the fatty acid profile of the muscle and liver of wild and farmed common dentex are presented in Tables 2, 3 and 4, respectively. The ideal comparisons between wild and farmed fish would have been with individuals of similar size. In the present study, wild individuals had higher body weights, but this was due to the limited availability in wild fish sizes. Farmed common dentex had nearly six times more body fat content than their wild counterparts at

TABLE 2. Proximate composition of farmed and wild common dentex. Different letters (a, b) stand for statistically significant differences between wild and cultured counterparts for the same constitute

Composition (%)	Wild	Farmed
Water	75.6 ± 4.1 [b]	69.8 ± 2.2 [a]
Protein	18.7 ± 1.1	17.1 ± 0.9
Ash	4.00 ± 0.61	5.20 ± 0.83
Fat	1.32 ± 0.2 [a]	7.7 ± 1.3 [b]

TABLE 3. Vitamin C and E content in muscle and liver (μg g^{-1} tissue) of wild and farmed common dentex (average ± standard deviation). Different letters (a, b) stand for statistically significant differences between wild and cultured counterparts for the same tissue

	Liver		*Muscle*	
	Wild	Farmed	Wild	Farmed
Vitamin C	10.5 ± 3.12	12.8 ± 1.11	4.78 ± 1.12 [a]	7.0 ± 0.95 [b]
Vitamin E	28.6 ± 1.90 [a]	36.2 ± 1.34 [b]	3.53 ± 0.74 [a]	7.4 ± 0.43 [b]

TABLE 4. Main fatty acids and fatty acids groups in the liver and white muscle of wild and farmed common dentex. Values are expressed as percentage of the total (average ± standard deviation). Different letters (a, b) stand for statistically significant differences between wild and cultured counterparts for the same tissue

	Liver		*Muscle*	
	Wild	Farmed	Wild	Farmed
14:0	2.04 ± 0.74	3.4 ± 0.82	5.47 ± 2.35	6.16 ± 1.72
16:0	27.7 ± 3.32	25.2 ± 3.11	18.2 ± 4.57	21.3 ± 3.98
16:1ω-7	1.6 ± 0.93 [a]	4.28 ± 0.71 [b]	7.80 ± 1.56 [b]	6.07 ± 0.84 [a]
18:0	8.46 ± 1.32	8.92 ± 1.76	4.09 ± 0.76	4.94 ± 0.85
18:1ω-9	8.9 ± 1.27	10.0 ± 1.93	16.1 ± 3.11 [b]	9.97 ± 2.72 [a]
18:2ω-6	1.06 ± 0.83 [a]	4.28 ± 1.25 [b]	5.29 ± 1.19	4.98 ± 0.31
20:4ω-6	6.46 ± 1.07 [b]	2.08 ± 1.38 [a]	1.28 ± 0.31 [b]	0.71 ± 0.12 [a]
20:5ω-3	2.30 ± 0.97 [a]	8.32 ± 1.03 [b]	8.81 ± 1.71	9.34 ± 0.95
22:6ω-3	30.9 ± 4.12 [b]	23.3 ± 2.95 [a]	15.4 ± 4.37 [a]	21.5 ± 1.39 [b]
SFA	40.7 ± 5.92	37.5 ± 2.97	29.0 ± 4.45	32.4 ± 3.57
MUFA	15.5 ± 3.53 [a]	19.7 ± 2.11 [b]	32.6 ± 3.17 [b]	25.7 ± 2.56 [a]
PUFA	43.9 ± 4.11	42.8 ± 3.56	38.5 ± 3.11	41.9 ± 2.79
ω-3	34.5 ± 5.32	36.4 ± 3.15	30.8 ± 3.52 [a]	36.2 ± 2.11 [b]
ω-6	9.36 ± 2.15 [b]	6.36 ± 1.74 [a]	7.65 ± 1.01 [b]	5.69 ± 0.72 [a]
ω-9	11.8 ± 1.17	11.8 ± 0.99	19.9 ± 2.11 [b]	13.4 ± 1.25 [a]

the expense of water content. This can be attributed to the high fat content of the commercial diet and to increased energy input of the farmed fish (Shearer, 1994). Available data from literature also indicate higher fat levels in farmed species when compared to wild counterparts, including various Mediterranean aquaculture species such as red porgy (*Pagrus pagrus*) (Rueda *et al*., 1997), turbot (*Scophthalmus maximus*) (Serot *et al.*, 1998) and gilthead sea bream (Grigorakis *et al.*, 2002; Grigorakis, 2007).

There were no appreciable differences in the protein and ash content between the farmed and wild common dentex, which is in agreement with the comparable aforementioned studies. The present results on the whole body composition of farmed fish partly differ from those found for commercial-size farmed individuals (Özden & Erkan, 2008; Suárez *et al*., 2009), where higher muscle water and lower fat was reported, but are similar to other studies for adults from the same species (Chatzifotis *et al.*, 2004). This seems to be in contrast to the general rule that body fat levels and muscle fat in specific, increase with size (Grigorakis, 2007). However, no direct assumptions can be made on that, since fat deposition is a multiparameter regulated characteristic (Huss, 1988; Grigorakis, 1999, 2007).

The presently found vitamin C levels in farmed common dentex tissues were slightly lower than those observed for gilthead sea bream and European sea bass fingerlings fed diets with similar dietary levels (Alexis *et al.*, 1999). The vitamin C concentration in farmed fish has been demonstrated as a reflection of its dietary level (Dabrowski & Ciereszko, 1996; Alexis *et al.*, 1999) and also dependented on the dietary form of the incorporated ascorbic acid (Alexis *et al.*, 1999). Thus, liver ascorbic acid seemed to be higher in farmed fish fed with vitamin C-enriched diets (Murata *et al.*, 1996) than in wild fish. This is confirmed by the present findings where vitamin C content in liver and muscle of wild fish was lower in comparison with those in the farmed fish, with significant differences observed in the second tissue. Nettlenton & Exler (1992) reported slightly higher levels of vitamin C in fillet samples of farmed than wild coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Onchorynchus mykiss*).

Vitamin E analysis in muscle and liver of common dentex displayed a similar to vitamin C profile distribution. Both the examined tissues of the farmed fish displayed statistically higher levels of vitamin E, while comparable amounts of this vitamin were measured between wild and farmed European sea bass and gilthead sea bream (Orban *et al*., 2003). Thus, the distribution pattern of both these vitamins in the tissues of common dentex seemed to be directly affected by diet composition.

The fatty acid analysis of the commercial feed used in the present study was not available, but the diet was known as being enriched with ω-3 fatty acids. The fatty acid profiles were different between farmed and wild common dentex (Table 4). When comparing fatty acid groups, muscle tissue was found to be richer in monounsaturates (MUFA), while saturated fatty acids (SFA) were higher in liver. The total polyunsaturated fatty acids (PUFA) did not differ within either the two counterparts or their tissue distribution. However, wild fish liver and muscle were richer in ω-6 PUFA than the respective tissues in farmed counterparts. On the other hand, the farmed fish contained higher ω-3 fatty acids, although the difference was statistical significant ($p < 0.05$) only for muscle tissue. The fatty acid groups in muscle, but not in liver tissue, had the same pattern with that mentioned for various stages of common dentex larvae whole body (Giménez *et al*., 2008).

Among SFA, 16:0 was, by far, the most abundant one, in both tissues (liver and muscle) and for both wild and farmed fish. No individual differences were observed in this fatty acid for the counterparts, while higher contents were found in liver than in muscle tissue. Within the MUFA, 18:1ω-9 was the most common one, while 22:6ω-3 (DHA) dominated among ω-3 PUFA. The present fatty acid profiles generally agree with the literature findings for the same species, where 16:0 has been found to be the main SFA, 18:1ω-9 the MUFA and DHA the major ω-3 PUFA for both wild (İmre & Sağlik, 1998) and farmed (Özden & Erkan, 2008) common dentex. The levels of individual fatty acids in muscle were similar to those reported for the same species (İmre & Sağlik, 1998; Özden & Erkan, 2008), while no literature data are available for liver tissue. The present results also largely confirm the literature findings for other Mediterranean species, where 18:2ω-6 are more pronounced in farmed, while 20:4ω-6 in wild counterparts as a result of their presence in the feed-incorporated plant oils and in the marine food chain, respectively (Grigorakis, 2007).

For some fatty acids the differences between the two compared populations were not similar in liver and muscle samples. Thus, in the liver, 18:2ω-6 and 20:5ω-3 were more than 4 times higher in farmed fish

TABLE 5. Main fatty acids and fatty acids groups in the neutral (NL) and polar (PL) lipid fractions of liver and white muscle of wild and farmed common dentex. Values are expressed as percentage of the total SEM: standard error of the means

	Liver				*Muscle*				
	Wild		Farmed		Wild		Farmed		Pooled
	NL	PL	NL	PL	NL	PL	NL	PL	SEM
14:0	5.00	1.32	6.23	2.73	6.14	3.82	8.03	1.21	0.73
16:0	22.9	28.9	21.4	26.1	16.4	23.2	19.7	25.5	1.20
16:1ω-7	8.01	2.63	7.82	3.42	8.73	5.42	7.70	1.73	0.99
18:0	6.71	8.91	4.22	10.13	3.20	6.53	4.12	7.24	0.71
18:1ω-9	18.5	6.5	18.4	7.9	17.3	12.8	11.4	6.1	1.51
18:2ω-6	2.10	0.80	7.01	3.62	5.81	3.92	5.63	3.32	0.64
20:4ω-6	3.10	7.34	0.00	2.61	0.92	2.33	0.53	1.34	0.77
20:5ω-3	3.92	1.91	6.00	8.90	9.00	8.31	9.50	8.92	0.93
22:6ω-3	13.2	35.4	12.7	25.9	12.8	22.3	15.6	37.4	2.97
SFA	44.4	39.8	31.8	38.9	27.2	33.9	31.8	33.9	5.15
MUFA	30.2	11.8	36.4	15.5	36.5	22.2	30.4	13.1	9.52
PUFA	25.4	48.5	31.9	45.5	36.5	43.9	37.8	53.0	8.14
ω-3	18.6	38.5	24.9	39.3	28.9	36.1	31.7	48.4	8.27
ω-6	6.81	10.0	7.00	6.21	7.62	7.81	6.13	4.62	1.59
ω-9	18.5	10.1	22.10	9.20	22.0	14.4	15.2	8.50	5.03

than in wild fish, 16:1ω-7 almost 2.5 times higher, while 20:4ω-6 followed exactly the opposite pattern with wild fish containing 4 times higher levels of this fatty acid in their liver. Similar trends were not observed in muscle tissues. In total, wild fish livers were significantly richer in ω-6 fatty acids, poorer in total MUFA, but no differences were observed for total PUFA and SFA. On the contrary, higher values for PUFA were measured in wild compared to farmed European sea bass (Krajnovic-Ozretic *et al*., 1994).

As regards the muscle, farmed fish were found to contain higher DHA levels but significantly lower 16:1ω-7 and 18:1ω-9 than the wild ones. These findings were also reflected in the total ω-3, for which farmed fish muscle was found to be richer, and in the total muscle MUFA and ω-9 contents, for which wild fish were richer. Between the two lipid fractions, neutral lipids had higher MUFA than polar ones, while polar lipids showed higher total and ω3 PUFA, in both tissues (Table 5). These differences have been confirmed by published literature in various fish species including common dentex (Chatzifotis *et al.*, 2004).

In conclusion, this study demonstrated considerable differences between the composition of wild and farmed common dentex limited to the lipid content of the farmed fish which was higher at the expense of water content. The vitamin content analysis displayed a similar pattern between the two investigated vitamins, with both substances being higher in muscle and liver of the farmed fish. The comparison of the fatty acid profiles between the two populations demonstrated that they differentiate among wild and farmed counterparts. Since tissue fatty acids have been shown to mirror the dietary ones (Jobling, 2001), these information should be considered during formulation of commercial diets for common dentex. Due to the high differences in total body lipids between farmed and wild common dentex, it is suggested that a dietary lipid content of 22% leads to increased fat deposition. Although tissue concentration of both vitamins C and E was in most measurements significantly higher in farmed fish, higher dosages of both vitamins are recommended (>250 ppm) to enhance the immune system (Guerriero *et al.*, 2002) of fish against the battle of stress-related pathology (Rigos *et al*., 1998). A further investigation by seasonal analysis of wild fish and of wider variety of sizes should provide an additional knowledge of common dentex nutrient composition and possibly, of its feeding requirements in captivity.

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