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Research Article

The Effects of Seasons and Different Feeds on Fatty Acid Composition in Fillets of Cultured Gilthead Sea Bream (*Sparus aurata* L.) and European Sea Bass (*Dicentrarchus labrax* L.) in Turkey

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Abstract: Dietary and seasonal effects on the fatty acid composition in the fillets of sea bream *Sparus aurata* and sea bass *Dicentrarchus labrax* were studied. Samples of the fish and feed were taken at 4 fish farms on the Aegean coast of Turkey during the summer, winter and spring seasons of 2004. The 2 commercial pelleted feeds (A and C) and 2 commercial extruded feeds (B and D) used in the fish farms were analyzed. There were not significant differences among the seasonally fatty acid composition in feed and fish samples except for MUFA (monounsaturated fatty acid) in the fillets of sea bream. Sea bream fillets had significantly higher total MUFA concentration in winter samples (33.5 ± 1.40%) than summer (32.0 ± 0.37%) and spring (31.8 ± 0.87%) samples ($P < 0.05$). Predominant fatty acids of all feeds and fish fillets were 14:0, 16:0, 18:0, 16:1n-7, 18:1n-9, 18:2n-6, 20:5n-3 (eicosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA). The feeds generally had ArA (arachidonic acid) level ranging from 0.6% to 0.9% of the total fatty acids. Fatty acid composition in fish fillets generally reflected the fatty acid composition of the feeds. The changes in fatty acid composition of the fillets indicate that the MUFA were probably dispensable for sea bream and sea bass. EPA, DHA and ArA levels in the commercial feeds were adequate for both fish species. The results of this study indicated that both fish species were as a good source of n-3 HUFA in different seasons.

Key Words: Gilthead sea bream and European sea bass, different feeds, Seasonal effect, fatty acids in fillet.

Türkiye'de Yetiştirilen Çipura (*Sparus aurata* L.) ve Levrek (*Dicentrarchus labrax* L.) Balıklarının Filetolarındaki Yağ Asidi Kompozisyonuna Farklı Yemler ve Mevsimlerin Etkisi

Özet: Çipura (*Sparus aurata*) ve levrek (*Dicentrarchus labrax*) balıklarının filetolarındaki yağ asidi kompozisyonuna diyetlerin ve mevsimlerin etkisi incelenmiştir. Balık ve yem örnekleri 2004 yılı yaz, kış ve ilkbahar mevsimleri süresince Türkiye'nin Ege sahillerindeki dört balık işletmesinden alınmıştır. Bu işletmelerin kullandığı iki ticari pelet yem (A ve C yemi) ve iki ticari ekstrude yem (B ve D yemi) incelenmiştir. Çipura balığının filetosundaki tek doymamış yağ asitleri olan MUFA'lar dışında yem ve balık örneklerindeki mevsimsel yağ asidi kompozisyonları arasında önemli bir fark bulunmamıştır ($P > 0,05$). Çipura balığının filetosundaki toplam MUFA konsantrasyonu kış mevsiminde (% 33,5 ± 1,40), yaz (% 32,0 ± 0,37) ve ilkbahar (% 31,8 ± 0,87) mevsimlerine göre daha yüksek düzeyde bulunmuştur ($P < 0,05$). Yem ve balık örneklerindeki belirleyici yağ asitleri 14:0, 16:0, 18:0, 16:1n-7, 18:1n-9, 18:2n-6, 20:5n-3 (eikosapentaenoik asit, EPA) ve 22:6n-3 (dokosaheksaenoik asit, DHA)'lerdir. Yem örneklerindeki toplam yağ asitlerinin % 0,6 ile % 0,9'ü arasında değişen düzeylerde araşidonik asit (ArA) içerdiği görülmüştür. Genel olarak balık filetolarındaki yağ asitleri yemlerdeki yağ asidi kompozisyonunu yansıtmıştır. Fileto yağ asitleri kompozisyonundaki değişimler, MUFA'nın çipura ve levrek balıkları için esansiyel olmadığını göstermiştir. Ticari yemlerdeki EPA, DHA ve ArA düzeylerinin çipura ve levrek balıkları için yeterli olduğu görülmüştür. Bu araştırmanın sonuçları, her iki balık türünün farklı mevsimlerde n-3 HUFA bakımından iyi bir kaynak olduğunu göstermiştir.

Anahtar Sözcükler: Çipura ve levrek balıkları, farklı yemler, mevsimsel etki, filetodaki yağ asitleri.

Introduction

Gilthead sea bream *Sparus aurata* and European sea bass *Dicentrarchus labrax* are the most important marine finfish species cultured in the Mediterranean, and aquaculture production of both species is still expanding rapidly (1,2). Similarly, intensive aquaculture of these fish species in Turkey has greatly expanded in recent years (39,000 metric tons in 2003) (3). Gilthead sea bream

and European sea bass have dietary requirements for the polyunsaturated fatty acids (PUFA), arachidonic acid (ArA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3). These fatty acids are essential nutrients for marine fish. The essentiality of these fatty acids is based on the important roles they play as a structural unit of membrane phospholipids (4,5). Generally, marine fish have low or no capacity to synthesize highly unsaturated fatty acids (HUFA) from C18 fatty acids. Therefore, EPA, DHA, and ArA are considered EFA in the diets for normal growth and development of most marine fish (5-7). Lipids of marine fish species are characterized by low levels of linoleic (18:2n-6) acid and linolenic (18:3n-3) acid as well as high levels of long-chain n-3 polyunsaturated fatty acids. EPA and DHA are the predominant n-3 fatty acids in the lipids of marine fish species (8). There is evidence suggesting that long-chain n-3 polyunsaturated fatty acids also have beneficial effects on human health (4,8). Different dietary oils have an influence on the fatty acid profile of fish (9). Essential fatty acid (EFA) requirements of fish are influenced by the environmental factors such as temperature and salinity of water, and these differences are more complex in fish than in mammals (6). Therefore, the effects of water temperature and seasonal changes on the fatty acid composition of fish flesh have been studied for several species (10-13).

The aim of the present study was to determine the effects of fatty acid composition in different commercial feeds and seasonal variation on the fatty acid composition in fillets of gilthead sea bream and European sea bass. These fish were cultured at marine fish farms in the Aegean region of Turkey.

Materials and Methods

Materials

In this study, the growth trial for fish was not conducted in fish farms. All samples of fish (sea bream and sea bass) and commercial feeds were taken from 4 different fish farms in the Aegean Sea of Turkey during the summer, winter and spring of 2004. The different commercial feeds used in the different fish farms were classified with the letters A, B, C and D, respectively. The feeds A and C (6 mm) were pelleted feeds (produced in Turkey), and the feeds B and D (6 mm) were extruded feeds (import). In addition, the cultured fish species were classified with the same letters. Both fish species were cultured under the same culture conditions and fed the same feed by the same feeding techniques at the fish farms. Average water temperatures and the fish weight at the periods of sampling as well as feeding levels are given in Table 1. The proximate composition and fatty acid composition of the feed samples are shown in Tables 2 and 3, respectively. Fish samples (n = 9) were killed and packaged in a black nylon bags (packed into an insulated polystyrene box with dry ice) and then transported to the laboratory in a freezer. The samples were kept at –30 ºC until the fatty acid analyses.

Proximate analysis of feed samples

The methods of the Association of Official Analytical Chemists (14) were used to determine the moisture, crude protein, crude fat, crude fiber and ash content in feed samples. Crude protein was calculated as N X 6.25.

Table 1. Average water temperature, feeding levels and fish weight in different seasons.

Month	Temperature (ºC)*	Feeding level**		Weight (g)***	
		Pelleted	Extruded	Sea bream	Sea bass
July	27	1.8	1.2	433.7 ± 61.1	496.5 ± 61.6
January	15	0.8	0.5	341.2 ± 40.6	406.5 ± 49.9
April	18	1	0.7	327.2 ± 24.0	366.5 ± 45.8

* Average temperature at the aquaculture unit.
** Average daily feeding levels percentage of the wet fish weight.
*** Average weight of fish samples.

Table 2. Average proximate composition of the commercial feeds in different seasons.*

	Feed groups **			
	A (pelleted)	B (extruded)	C (pelleted)	D (extruded)
Proximate composition (%)				
Moisture	9.8 ± 0.67[a]	8.7 ± 0.34[ab]	8.7 ± 0.46[ab]	7.9 ± 0.40[b]
Crude protein	44.9 ± 0.71[a]	44.6 ± 0.26[a]	45.4 ± 0.18[a]	45.1 ± 0.31[a]
Crude fat	14.0 ± 0.36[b]	20.4 ± 0.41[a]	12.6 ± 0.26[c]	20.6 ± 0.24[a]
Ash	8.7 ± 0.17[b]	11.7 ± 0.33[a]	10.8 ± 0.45[a]	8.5 ± 0.32[b]
Crude fiber	3.2 ± 0.45[a]	2.7 ± 0.36[a]	2.6 ± 0.34[a]	2.5 ± 0.22[a]
Nitrogen free extract	19.3 ± 0.55[a]	11.8 ± 1.10[c]	19.4 ± 0.50[a]	15.5 ± 0.69[b]

*: Results represent means ± standard error, n = 6.
**: Feeds A, B, C and D were used by different fish farms, respectively. These feeds were produced by different commercial companies.
Results in each row with different superscript letters were significantly different (P < 0.05). Means were tested by ANOVA and ranked by Tukey's multiple range test.

Lipid extraction and fatty acid analysis

Total lipid was extracted from the fillets (n = 3) and feed samples by homogenization in chloroform/methanol (2/1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, according to Folch et al. (15). Fatty acid methyl esters were prepared from total lipid by acid-catalyzed transesterification using 2 ml of 1% H_2SO_4 in methanol plus 1 ml toluene as described by Christie (16), and the fatty acid analysis of the feed and fish samples were analyzed by gas-liquid chromatography (Perkin Elmer Auto System XL) using a 30 x 0.25 mm capillary column, FID detector (CP-2330 supelco). Helium was used as the carrier gas. Flame-ionization detection temperature 220 ºC, split rate 1/50, oven temperature programmed for rise from 120 ºC/2 min to 220ºC/15 min at a rate 5 ºC/min. Injector temperature was 240 ºC. Individual methyl esters were identified by reference to known standards (Sigma, 189-19).

Statistics

All the data are presented as means ± standard error. The statistical significance of differences in the fatty acid composition between groups was analyzed with one-way analysis of variance (ANOVA) and Tukey's multiple range test using a statistical software package (SPSS version 11.5); P < 0.05 was taken to indicate a statistically significant difference. The relation between the feeds and fish were also investigated using non-parametric correlation (Spearman rank correlations) analyses (17).

Results

Diet composition

According to the results of the analyses, there were not significant differences among the chemical composition in the same brand feed in 3 seasons. For this reason the data are presented as the mean amounts of every 3 seasons.

The crude protein was similar in all diets that contained 44.6% to 45.4% (P > 0.05). In contrast, the percentage of total crude fat was high in the extruded feeds B and D (20.4% and 20.6%, respectively), and low in the pelleted feeds A and C (14.0% and 12.6%, respectively) (P < 0.05) (Table 2). The main fatty acids in all feeds are 14:0, 16:0, 18:0, 16:1n-7, 18:1n-9, 18:2n-6, EPA and DHA. Total saturated fatty acids (SFA) level of feed D was significantly (P < 0.05) lower than that of the other 3 feeds. In contrast, total monounsaturated fatty acids (MUFA) level of feed D was significantly (P < 0.05) higher than that of the other feeds. Total n-6 PUFA levels of feeds C and D were high, compared with the other feed groups (P < 0.05). Total n-3 PUFA and n-3 HUFA levels of feeds C and D were significantly (P < 0.05) lower than those of feeds A and B. The DHA/EPA ratios of feeds A, B and C were similar and they were higher than that of feed D. Feeds A and B had a significantly (P < 0.05) lower ratio (0.6) of 18:1n-9/n-3 HUFA compared to the other feeds (Table 3).

Table 3. Average total lipid content (dry weight basis) and fatty acid composition (% of total fatty acids) in the different commercial feed samples in different seasons*.

Total lipid and Fatty acids	Feed groups **			
	A (pelleted)	B (extruded)	C (pelleted)	D (extruded)
Total lipid (%)	14.0 ± 0.5^{b}	20.4 ± 0.5^{a}	12.9 ± 0.3^{b}	20.6 ± 0.3^{a}
Fatty acids				
14:0	5.7 ± 0.14^{a}	6.3 ± 0.18^{a}	4.6 ± 0.11^{b}	4.7 ± 0.27^{b}
16:0	19.6 ± 0.13^{a}	19.4 ± 0.45^{a}	18.4 ± 0.07^{a}	15.6 ± 0.60^{b}
18:0	4.0 ± 0.09^{b}	3.9 ± 0.06^{ab}	5.1 ± 0.08^{a}	3.7 ± 0.03^{b}
16:1n-7	4.9 ± 0.15^{b}	6.0 ± 0.08^{a}	4.3 ± 0.14^{c}	4.8 ± 0.18^{bc}
18:1n-9	13.7 ± 0.33^{b}	14.0 ± 0.10^{b}	18.2 ± 0.81^{a}	15.5 ± 0.13^{b}
20:1n-9	1.2 ± 0.06^{b}	1.3 ± 0.02^{b}	0.9 ± 0.05^{b}	3.8 ± 0.38^{a}
22:1n-9	0.3 ± 0.05^{b}	0.5 ± 0.14^{b}	0.3 ± 0.10^{b}	2.7 ± 0.90^{a}
24:1n-9	0.6 ± 0.02^{a}	0.6 ± 0.02^{a}	0.4 ± 0.02^{b}	0.5 ± 0.02^{b}
18:2n-6	8.0 ± 0.73^{b}	3.6 ± 0.02^{c}	15.9 ± 0.63^{a}	15.1 ± 0.42^{a}
18:3n-6	0.2 ± 0.00^{a}	0.2 ± 0.00^{a}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}
20:4n-6	0.8 ± 0.03^{ab}	0.9 ± 0.01^{a}	0.6 ± 0.01^{c}	0.7 ± 0.04^{b}
18:3n-3	1.6 ± 0.09^{b}	1.3 ± 0.01^{c}	2.3 ± 0.04^{a}	2.3 ± 0.03^{a}
20:3n-3	0.1 ± 0.00^{a}	0.1 ± 0.00^{a}	0.1 ± 0.00^{a}	0.1 ± 0.01^{a}
20:5n-3	8.3 ± 0.37^{a}	8.6 ± 0.24^{a}	6.4 ± 0.28^{b}	6.5 ± 0.22^{b}
22:6n-3	13.9 ± 0.53^{a}	14.7 ± 0.40^{a}	9.8 ± 0.30^{b}	8.5 ± 0.29^{b}
∑ SFA	32.5 ± 0.81^{a}	33.0 ± 0.73^{a}	30.6 ± 0.11^{a}	26.2 ± 0.96^{b}
∑ MUFA	21.3 ± 0.46^{c}	23.1 ± 0.26^{bc}	24.6 ± 0.80^{b}	27.3 ± 1.10^{a}
∑ n-6 PUFA	8.7 ± 0.55^{b}	4.3 ± 0.21^{c}	16.4 ± 0.54^{a}	15.7 ± 0.56^{a}
∑ n-3 PUFA	23.7 ± 0.64^{a}	24.8 ± 0.64^{a}	18.5 ± 0.55^{b}	17.5 ± 0.49^{b}
∑ n-3 HUFA	22.2 ± 0.74^{a}	23.4 ± 0.62^{a}	16.2 ± 0.58^{b}	15.2 ± 0.51^{b}
18:1n-9/n-3 HUFA	0.6 ± 0.03^{b}	0.6 ± 0.01^{b}	1.1 ± 0.09^{a}	1.0 ± 0.03^{a}
DHA/EPA	1.7 ± 0.08^{a}	1.7 ± 0.02^{a}	1.5 ± 0.03^{a}	1.3 ± 0.02^{b}

*:Values are means ± SEM, n = 6.
**: Feeds A, B, C and D were used by different fish farms, respectively. These feeds were produced by different commercial companies.
Values in each row with different superscript letters are significantly different at $P < 0.05$. Means were tested by ANOVA and ranked by Tukey's multiple range test.
SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; HUFA, high unsaturated fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Fatty acid composition of fish

The fatty acid composition in the fillets of sea bream and sea bass are summarized in Tables 4 and 5. The fatty acid contents were similar within the same fish group except for the MUFA in the fillets of sea bream in the summer, winter and spring. For this reason, the fatty acid compositions of fish are presented as the average values of each 3 seasons. The fatty acid compositions of sea bream and sea bass reflected the fatty acid composition of the feeds. A strong positive correlation was found between the dietary n-3 HUFA levels and its levels in the fillets ($P < 0.01$) with correlation coefficients of 0.53 for sea bream and 0.82 for sea bass. The predominant fatty acids in the fillets of sea bream and sea bass were 14:0, 16:0, 18:0, 16:1n-7, 18:1n-9, 18:2n-6, EPA and DHA.

Total SFA in the fillets of sea bream fed feed A were highest (28.5%) and total SFA in the fillets of sea bream fed feed D were the lowest (23.0%) ($P < 0.05$). Total MUFA levels in the fillets of all sea bream groups were similar ($P > 0.05$). However, the MUFA content in the sea bream fillets was high in the winter (33.5 ± 1.40%) and low in the summer or spring (32.0 ± 0.37% and 31.8 ± 0.87%, respectively) ($P < 0.05$). Total n-6 PUFA level (13.0%) in the fillets of sea bream fed feed D were significantly ($P < 0.05$) higher than those in the other groups. Total n-3 PUFA (22.2%) and n-3 HUFA (21.1%) levels in the fillets of sea bream fed feed B were significantly ($P < 0.05$) higher than those in the other groups, but there were no significant ($P > 0.05$) differences in n-3 PUFA and n-3 HUFA levels among the fillets of the other 3 groups.

Table 4. Average fatty acid composition in the fillets of sea bream (% of total fatty acids) in different seasons*.

Total lipid and Fatty acids	Feed groups **			
	A	B	C	D
14:0	4.8 ± 0.28^{a}	4.7 ± 0.07^{a}	4.1 ± 0.06^{b}	3.8 ± 0.03^{b}
16:0	17.8 ± 0.26^{a}	15.2 ± 0.47^{bc}	16.4 ± 0.16^{b}	14.4 ± 0.26^{c}
18:0	3.4 ± 0.13^{b}	3.0 ± 0.10^{c}	3.8 ± 0.04^{a}	3.0 ± 0.06^{c}
16:1n-7	6.7 ± 0.18^{b}	7.5 ± 0.07^{a}	6.5 ± 0.08^{b}	6.0 ± 0.05^{c}
18:1n-9	22.4 ± 0.73^{ab}	22.0 ± 0.42^{b}	25.0 ± 0.56^{a}	22.0 ± 0.82^{b}
20:1n-9	1.2 ± 0.01^{b}	1.1 ± 0.00^{b}	1.0 ± 0.02^{c}	2.5 ± 0.03^{a}
22:1n-9	0.3 ± 0.06^{b}	0.3 ± 0.03^{b}	0.3 ± 0.02^{b}	1.0 ± 0.28^{a}
24:1n-9	0.5 ± 0.01^{a}	0.5 ± 0.01^{a}	0.4 ± 0.01^{b}	0.5 ± 0.01^{a}
18:2n-6	8.5 ± 0.72^{b}	5.9 ± 0.33^{c}	10.3 ± 0.52^{b}	12.4 ± 0.05^{a}
18:3n-6	0.1 ± 0.01^{a}	0.2 ± 0.00^{a}	0.2 ± 0.00^{a}	0.1 ± 0.00^{a}
20:4n-6	0.6 ± 0.01^{a}	0.8 ± 0.01^{a}	0.7 ± 0.07^{a}	0.7 ± 0.04^{a}
18:3n-3	1.3 ± 0.07^{b}	1.1 ± 0.01^{b}	1.4 ± 0.04^{b}	1.7 ± 0.11^{a}
20:3n-3	0.2 ± 0.02^{a}	0.1 ± 0.00^{b}	0.2 ± 0.02^{a}	0.2 ± 0.00^{a}
20:5n-3	4.4 ± 0.15^{b}	5.4 ± 0.04^{a}	4.2 ± 0.17^{b}	4.1 ± 0.09^{b}
22:6n-3	11.5 ± 0.45^{b}	15.8 ± 0.76^{a}	11.0 ± 0.26^{b}	10.7 ± 0.29^{b}
∑ SFA	28.5 ± 0.54^{a}	25.4 ± 0.54^{b}	26.6 ± 0.10^{b}	23.0 ± 0.38^{c}
∑ MUFA	30.9 ± 0.98^{a}	32.2 ± 0.55^{a}	33.8 ± 0.61^{a}	33.0 ± 0.95^{a}
∑ n-6 PUFA	9.1 ± 0.83^{b}	6.5 ± 0.35^{c}	10.9 ± 0.34^{b}	13.0 ± 0.20^{a}
∑ n-3 PUFA	17.5 ± 0.46^{b}	22.2 ± 0.73^{a}	16.6 ± 0.52^{b}	16.7 ± 0.38^{b}
∑ n-3 HUFA	16.2 ± 0.51^{b}	21.1 ± 0.74^{a}	15.6 ± 0.30^{b}	15.0 ± 0.39^{b}
18:1n-9/n-3 HUFA	1.4 ± 0.07^{a}	1.0 ± 0.02^{b}	1.6 ± 0.06^{a}	1.5 ± 0.10^{a}

*:Values are means ± SEM, n = 6.
**: Fish A, B, C and D were cultured by different fish farms, respectively. These fish were fed feeds A, B, C and D, respectively.
Values in each row with different superscript letters are significantly different at $P < 0.05$. Means were tested by ANOVA and ranked by Tukey's multiple range test.
SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; HUFA, high unsaturated fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Total SFA level in the fillets of sea bass fed the feeds A, B and C were found similar ($P > 0.05$). However, no significant differences ($P > 0.05$) were found among the B, C and D sea bass groups. Total MUFA level in the fillets from sea bass fed feeds A and C were similar ($P > 0.05$). Nevertheless, there were also no differences ($P > 0.05$) among B, C and D sea bass groups. Total n-6 PUFA level (6.5%) in the fillets of sea bass fed feed B was significantly ($P < 0.05$) lower than that of the other 3 sea bass groups. In contrast, the fillets of sea bass fed feed B had significantly ($P < 0.05$) higher levels of total n-3 PUFA (25.9%) and total n-3 HUFA (24.7%) compared to fish fed the other feeds. The fillets of sea bream and sea bass fed feed B had significantly ($P < 0.05$) lower ratios of 18:1n-9/n-3 HUFA compared to the other fish groups.

There was a high positive correlation between the fatty acids compositions in the fillets of sea bream and sea bass fed the same feed with correlation coefficients of 0.51 for total SFA ($P < 0.05$), 0.71 for total n-6 PUFA ($P < 0.01$), 0.75 for total n-3 PUFA ($P < 0.01$) and 0.77 for n-3 HUFA ($P < 0.01$).

Table 5. Average fatty acid composition in the fillets of sea bass (% of total fatty acids) in different seasons*.

	Fish groups **			
	A	B	C	D
14:0	3.9 ± 0.10^{b}	4.6 ± 0.17^{a}	3.5 ± 0.10^{b}	3.8 ± 0.17^{b}
16:0	18.2 ± 0.44^{a}	17.5 ± 0.44^{ab}	17.4 ± 0.36^{ab}	16.2 ± 0.29^{c}
18:0	3.4 ± 0.12^{a}	2.9 ± 0.03^{b}	3.4 ± 0.10^{a}	3.1 ± 0.03^{ab}
16:1n-7	5.3 ± 0.03^{b}	6.0 ± 0.10^{a}	4.9 ± 0.12^{c}	4.1 ± 0.07^{c}
18:1n-9	23.3 ± 0.62^{a}	18.7 ± 0.85^{b}	22.9 ± 1.09^{a}	18.7 ± 0.46^{b}
20:1n-9	1.5 ± 0.02^{b}	1.4 ± 0.04^{b}	1.2 ± 0.01^{c}	3.0 ± 0.07^{a}
22:1n-9	0.3 ± 0.06^{b}	0.3 ± 0.05^{b}	0.2 ± 0.03^{b}	2.2 ± 0.06^{a}
24:1n-9	0.4 ± 0.02^{a}	0.4 ± 0.02^{a}	0.3 ± 0.01^{b}	0.4 ± 0.00^{a}
18:2n-6	8.5 ± 0.55^{b}	5.8 ± 0.30^{c}	14.2 ± 0.17^{a}	13.2 ± 0.31^{a}
18:3n-6	0.2 ± 0.00^{a}	0.2 ± 0.00^{a}	0.2 ± 0.00^{a}	0.1 ± 0.00^{b}
20:4n-6	0.7 ± 0.03^{b}	0.8 ± 0.01^{a}	0.6 ± 0.00^{c}	0.6 ± 0.02^{c}
18:3n-3	1.4 ± 0.04^{c}	1.2 ± 0.01^{c}	1.9 ± 0.03^{b}	2.0 ± 0.03^{a}
20:3n-3	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}	0.11 ± 0.02^{a}	0.08 ± 0.00^{a}
20:5n-3	6.2 ± 0.16^{b}	7.1 ± 0.05^{a}	5.2 ± 0.15^{c}	5.4 ± 0.04^{c}
22:6n-3	13.6 ± 0.45^{b}	17.5 ± 0.78^{a}	11.5 ± 0.47^{bc}	11.2 ± 0.47^{c}
∑ SFA	27.8 ± 0.48^{a}	27.5 ± 0.58^{ab}	26.2 ± 0.53^{ab}	25.5 ± 0.48^{b}
∑ MUFA	31.6 ± 0.69^{a}	27.5 ± 0.49^{b}	30.0 ± 1.01^{ab}	29.4 ± 0.44^{ab}
∑ n-6 PUFA	9.1 ± 0.62^{b}	6.5 ± 0.43^{c}	14.8 ± 0.15^{a}	13.7 ± 0.24^{a}
∑ n-3 PUFA	21.2 ± 0.57^{b}	25.9 ± 0.86^{a}	18.7 ± 0.51^{c}	16.6 ± 0.41^{c}
∑ n-3 HUFA	19.8 ± 0.61^{b}	24.7 ± 0.84^{a}	16.8 ± 0.50^{c}	16.6 ± 0.44^{c}
18:1n-9/n-3 HUFA	1.2 ± 0.06^{a}	0.8 ± 0.05^{b}	1.4 ± 0.10^{a}	1.1 ± 0.01^{a}

*:Values are means ± SEM, n = 6.

**: Fish A, B, C and D were cultured by different fish farms, respectively. These fish were fed feeds A, B, C and D, respectively.

Values in each row with different superscript letters are significantly different at $P < 0.05$. Means were tested by ANOVA and ranked by Tukey's multiple range test.

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; HUFA, high unsaturated fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Discussion

Since the end of the 1970s, aquaculture diets have included 12%-14% crude fat for cultured sea bass. Then the extrusion technique was used in fish feed production and an improvement in performance was observed in salmonids fed diets characterized by high fat content. Similarly, sea bass and sea bream feeds include high fat and are currently used in commercial farms (18). In the present study, the lipid contents (average 20.5%) in the extruded feeds (imported; feeds B and D) were significantly higher than that in the pelleted (average 13.5%) feeds (produced in Turkey; feeds A and C). Peres and Oliva-Teles (19) reported that the increase of the dietary lipid level from 12% to 24% significantly improved lipid retention and energy utilization in sea bass. We did not find significant seasonal differences in the fatty acid compositions of the feed samples. These results showed that the formulation of feeds was stabilized.

It has been demonstrated that marine fish require n-3 HUFA, mainly EPA and DHA for normal development. The essentiality of these fatty acids is based on the

important structural role that they play as membrane phospholipid compenents, together with the inability of marine fish to synthesize EPA and DHA from linolenic acid (18:3n-3) (5). Feeding studies have shown that DHA is superior to EPA as an EFA for most marine fish (6,20). It has been suggested that ArA has an important physiological function in the membrane of fish, since it is known to be main precursor fatty acid of eicosanoids and is one of the main components of phosphatidylinositol (2,5). Increased attention has been paid during the recent years to ArA requirements of Mediterranean fish species (21). Therefore, juvenile marine fish generally require circa 0.5 - 1.0% of the dry weight of their diet as n-3 HUFA and ArA level ranging from 0.5% to 1.0% of the total fatty acids in their diet. Furthermore, sea bream and sea bass diet have a ratio of DHA/EPA of about 1:1.5 (5,21,22). The minimum level of dietary n-3 HUFA, including EPA and DHA required by gilthead seabream for optimum growth and development has been reported to be about 1.5% as a dry weight basis both larvae and broodstock and about 1% for both fingerlings and juveniles (23). Kalogeropoulos et al. (24) reported that the minimum requirement of gilthead bream for n-3 HUFA appears to be at least 7% of the dietary fatty acids or about 0.9% of the diet. Alexis (1) suggested that the EPA+DHA requirements of sea bass might be higher than that of sea bream with the requirement level being about 10% of dietary fatty acids or 1.3 of the diet. In the present study, n-3 HUFA (EPA+DHA) contents of the feeds A, B, C and D were about 3%, 5%, 2% and 3% of the dry diet or 22%, 23%, 16% and 15% of dietary fatty acids, respectively. ArA level ranged from 0.6% to 0.9% of total fatty acids in the diet, and the ratio of DHA:EPA ranged from 1.3% to 1.7%. These results of n-3 HUFA were higher, and ArA level and the ratio of DHA:EPA were similar compared to the findings reported by the researchers cited above.

In the present study, the main fatty acids in the fillets of both fish species and all feed groups were 14:0, 16:0, 18:0, 16:1n-7, 18:1n-9, 18:2n-6 and n-3 HUFA. Fatty acid composition of sea bream and sea bass fillets reflected the fatty acid composition of the feeds. There was a high correlation between fatty acid composition in the feed samples and fatty acid composition in the fish fillets. This positive correlation of dietary and fillets fatty acid composition had also been reported for sea bream (21,25,26) for sea bass (11,27-29) and for other fish species (6,30,31). The correlation between n-3 HUFA concentration in the feeds and in sea bream fillets was lower (correlation coefficient, 0.53) than that in sea bass fillets (correlation coefficient, 0.82). Both kinds of fish fillets had significantly higher concentration of total MUFA than the feeds ($P < 0.05$). Increasing MUFA in fish fillets indicates that n-3 PUFA, SFA (saturated fatty acid) and n-6 PUFA were preferred for catabolism while the MUFA were spared.

The fatty acid composition of fish flesh is influenced by temperature and seasonal changes, and this effect had been reported in cultured Japanese catfish *Silurus asotus* (Linnaeus, 1758) (12) and cultured crappie *Pomoxis* spp. (32). Alasalvar et al. (29) reported that wild sea bass had higher seasonal differences in their fatty acid compositions than cultured sea bass, and this could be due to a lack of uniform diet in the wild sea bass as compared with their cultured counterparts. In contrast, Cordier et al. (11) have observed that sea bass fed all year on the same industrial diet did not show a significant correlation between water temperature and DHA in the muscle. Similarly, we observed that different seasons (winter, summer and spring) did not cause any difference in the fatty acid profile of fish fillets except for total MUFA from the fillets of sea bream in the present study. This might indicate that feeding regimes being used for sea bream were not optimal in winter and possibly create problems in the metabolism at suboptimal temperatures. Nevertheless, an increase in the MUFA level in winter samples of the sea bream showed that the MUFA was accumulated due to the use of other fatty acids as an energy source instead of the MUFA. Similar results were also described for rainbow trout by Caballero et al. (33), for gilthead sea bream by Grigorakis et al. (10) and for Norwegian spring-spawning herring by Hamre et al. (13). Generally, the results of the fatty acid composition in the fillets of both fish species showed that the fatty acid profiles in the feeds or feeding regimes were optimal for sea bass in all 3 seasons and for sea bream in the summer or spring in our study.

It has been demonstrated that DHA, an abundant component of marine fish oil, can be used for the prevention and treatment of cardiovascular diseases, as an anticancer agent, and for the improvement of learning ability and visual function in human health (8,12). Alasalvar et al. (29) reported that sea bass was a good source of EPA and DHA. In the present study, similar

results were found in the fillets of sea bream and sea bass. The highest total n-3 HUFA and the lowest total n-6 PUFA concentrations were observed in the fillets of sea bream and sea bass fed feed B, extruded (import).

The ratio of 18:1n/n-3 HUFA, considered as an EFA index for gilthead bream (24), tended to decrease with increasing dietary n-3 HUFA level. In the same study, 18:1n/n-3 HUFA ratios of gilthead bream were less than 1 when dietary n-3 HUFA contents were satisfied. Lee et al. (6) found higher ratios in starry flounder fed diets containing sufficient n-3 HUFA. We were found lower (better) ratios in the fillets of sea bream and sea bass when they were fed feed B. These differences among fish groups were possibly due to variations in the fatty acid composition of dietary lipid sources used.

We found a high positive correlation among SFA, n-6 PUFA, n-3 PUFA and n-3 HUFA in the fillets of sea bream and sea bass. However, the highest correlation was found between n-3 HUFA in the fillets of sea bream and sea bass.

The results of this investigation have shown that there were not significant seasonal differences in the fatty acid compositions of the feed samples, and EPA, DHA and ArA concentrations in the feeds were in agreement with the literature (1,5,21,22,24). The dietary fatty acid composition influenced the fatty acid composition in the fillets of sea bream and sea bass. No significant differences were observed among the fatty acid compositions in the fillets of both fish species except for total MUFA from the fillets of sea bream. These results indicated that the cultured sea bream and sea bass in the Aegean Sea of Turkey were a good source of n-3 HUFA in different seasons.

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