Research Article

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Fatty acid profiles of microdiets for marine fish in Turkey

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Abstract: Lipid contents and fatty acid profiles in the commercial microdiets for farming marine fish species were determined. Twenty microdiet samples were taken from marine fish farms on the Aegean region of Turkey throughout 2006-2007. Of the 20 microdiet samples, 5 were produced in Turkey and 15 were imported. The fatty acid analysis of the microdiets was made by gas-liquid chromatography using helium as the carrier gas. The lipid levels of the microdiets produced in Turkey by different companies ranged from 9.5 ± 0.05% to 11.6 ± 0.14% ($P < 0.05$). In contrast, the lipid levels of imported microdiets were between 10.6 ± 0.20% and 17.2 ± 0.14% ($P < 0.05$). n-3 polyunsaturated fatty acids (n-3 PUFA) levels in the microdiets ranged from 7.1 ± 0.03% to 24.8 ± 0.03% of total fatty acids ($P < 0.05$). Similarly, n-3 highly unsaturated fatty acid (n-3 HUFA) levels in the microdiets ranged from 6.1 ± 0.01% to 22.8 ± 0.02% of total fatty acids ($P < 0.05$). n-3 highly unsaturated fatty acids (n-3 HUFA) levels in the microdiets were found between 0.6 ± 0.00% and 3.6 ± 0.01% of dry weight of diets ($P < 0.05$). Docosahexaenoic acid (DHA) levels of these microdiets were between 0.3 ± 0.00% and 2.0 ± 0.03% of the dry weight of diets ($P < 0.05$). Eicosapentaenoic acid (EPA) levels in the dry weight of diets were between 0.3 ± 0.01% and 2.2 ± 0.01% ($P < 0.05$). Arachidonic acid (ArA) levels in the microdiets were also found between 0.3 ± 0.01% and 0.9 ± 0.01% of total fatty acids ($P < 0.05$). The value of DHA/EPA ratios in the microdiets ranged from 0.5 ± 0.01 to 2.0 ± 0.01 ($P > 0.05$).

The results showed that the total lipid or n-3 and n-6 HUFA percentages especially DHA, EPA, ArA levels, and DHA/EPA ratios in the microdiets covered essential fatty acid (EFA) needs for larvae or early juvenile sea bass and sea bream. However, microdiets 3 and 13 had a slightly lower ratio of n-3 HUFA levels required for larval or early juvenile of these species.

Key words: Marine fish, commercial microdiets, fatty acid profile, n-3 EFA

Türkiye'de deniz balıkları için kullanılan mikrodiyetlerin yağ asidi profilleri

Özet: Deniz balıkları yetiştiriciliğinde kullanılan ticari mikrodiyetlerdeki yağ oranları ve yağ asidi profilleri saptanmıştır. 2006-2007 yıllarında Türkiye'nin Ege Bölgesi'nde bulunan deniz balıkları işletmelerinden 20 adet mikrodiyet örneği alındı. Bu mikrodiyetlerin 5 tanesi Türkiye'de üretilmiş ve 15 tanesi ithal edilmişti. Mikrodiyetlerin yağ asidi analizleri helyum gazı kullanılarak gaz kromatografisi ile yapıldı. Türkiye'de farklı ticari firmalar tarafından üretilen mikrodiyetlerin yağ düzeyleri % 9,5 ± 0,05 ile % 11,6 ± 0,14 oranları arasında bulunmuştur ($P < 0,05$). Buna karşın, ithal edilen mikrodiyetlerin yağ düzeyleri % 10,6 ± 0,20 ile % 17,2 ± 0,14 oranları arasındaydı ($P < 0,05$). Mikrodiyetlerdeki n-3 çok doymamış yağ asidi (PUFA) düzeyleri toplam yağ asitlerinin % 7,1 ± 0,03 ile % 24,8 ± 0,03 oranları arasındaydı ($P < 0,05$). Benzer olarak,

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mikrodiyetlerdeki aşırı doymamış yağ asitlerinin (n-3 HUFA) düzeyleri kuru diyetin % 0,6 ± 0,00 ile % 3,6 ± 0,01 oranları arasında bulundu (P < 0,05). Bu mikrodiyetlerin dokosaheksaenoik asit (DHA) düzeyleri kuru diyetin % 0,3 ± 0,00 ile % 2,0 ± 0,03 oranlarındaydı (P < 0,05). Kuru mikrodiyetlerdeki eikosapentaenoik asit (EPA) düzeyleri % 0,3 ± 0,01 ile % 2,2 ± 0,01 oranlarındaydı (P < 0,05). Araşidonik asit (ArA) düzeyleri ise toplam yağ asitlerinin % 0,3 ± 0,01 ile % 0,9 ± 0,01 oranları arasında bulundu (P < 0,05). Mikrodiyetlerdeki DHA/EPA oranları % 0,5 ± 0,01 ile % 2,0 ± 0,01 arasında değişmiştir (P < 0,05).

Sonuçlar, mikrodiyetlerdeki toplam lipit ile n-3 ve n-6 HUFA'lardan özellikle DHA, EPA, ArA düzeyleri, ve DHA/EPA oranlarının çipura ve levrek balıklarının larval yada erken juvenil safhalarında gereksinim duydukları esansiyel yağ asitlerini karşıladığını göstermiştir. Ancak 3 ve 13 nolu mikrodiyetler bu türlerin larval yada erken juvenil safhalarında ihtiyaç duyduğu n-3 HUFA düzeylerinden biraz daha düşük değerlere sahiplerdi.

Anahtar sözcükler: Deniz balıkları, ticari mikrodiyetler, yağ asidi profili, n-3 EFA

Introduction

Sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) are the most important farmed marine fish species in Turkey. Total production of these species was 66.9 t in 2006 (1). The number of commercial hatcheries for marine aquaculture in Turkey was 14 and the total production of these hatcheries was about 190 million fry in 2004. Striped sea bream (*Lithognathus mormyrus*), white grouper (*Epinephelus aeneus*), common dentex (*Dentex dentex*), shi drum (*Umbrina cirrosa*), common pandora (*Pagellus erythrinus*), sharpsnout seabream (*Diplodus puntazzo*), and brown meagre (*Sciaena umbra*) are alternative fish species for marine aquaculture in Turkey (2). One of the important subjects in marine aquaculture is larval nutrition. Most of commercially-cultured marine fish species require live feeds until metamorphosis or 0.5-0.75 g weights of larvae. The use of microdiets for marine fish larvae has been studied for several decades (3,4). Nutrient values of microdiets considerably affected survival and growth performance of the fish larvae (4). Most of the commercial microdiets for marine aquaculture of Turkey are imported. The alternative fish species of marine aquaculture are also fed with commercial microdiets for sea bream or sea bass.

Dietary lipids and essential fatty acids (EFA) are generally recognized to play critical roles in optimum larval growth and development (5-7). The previous feeding investigations on the essentiality of polyunsaturated fatty acids (PUFA) in the fish diets (5,8,9) have showed that marine fish species require n-3 highly unsaturated fatty acids (n-3 HUFA) such as eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids. The essentiality of these fatty acids is based on the important structural role they play as structural components of membrane phospholipids. However, marine fish is not able to convert linolenic acid (18:3 n-3) to EPA or DHA. Some studies have indicated that DHA is superior to EPA for larvae of marine fish because of different physiological functions (5,7).

Several studies have reported that arachidonic acid (ArA, 20:4 n-6) was important for nutrition of marine fish species (8,10,11). However, marine fish species cannot convert linoleic acid (18:2n-6) to ArA. ArA and EPA are precursors of 2 groups of eicosanoids, prostaglandins, and leukotrienes (12). Therefore, marine fish diets should include EPA, DHA, and ArA. The HUFA requirements of marine fish species have been studied in larvae (6,13-15) and juveniles (9,11,16). A few studies have been performed on the fatty acid composition of commercial feeds for marine fish species in Turkey (17,18). However, there is no report in the literature on commercial microdiets currently used in marine fish hatcheries.

The aim of this study was to determine the total lipid and fatty acid profiles in commercial microdiets currently used by marine fish hatcheries in Turkey and to evaluate their efficacy to cover EFA needs for larvae and early juveniles of sea bream and sea bass.

Materials and methods

Feeds

Feed samples were obtained from different commercial marine fish hatcheries in the Aegean region of Turkey during 2006 and 2007. They were classified into 20 groups depending on brand and micron size (μm) to compare to total lipids or fatty acids in similar size microdiets produced by different companies. Twenty microdiets were analyzed; 4 were 50-200 µm (Table 1), 4 were 150-300 µm (1 of the feeds was granule 0) (Table 2), 4 were 200-400 µm (1 of the feeds was granule 1) (Table 3), 4 were 300-500 µm (Table 4), and 4 were 400-800 µm (Table 5). Details concerning the type of microdiets (extruded or granule), origin (locally or imported), and micron size (µm) are given in the respective tables.

Lipid extraction and fatty acid analysis

Total lipid content was gravimetrically measured after extraction with chloroform/methanol 2/1, v/v containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, according to the method of Folch et al. (19). 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 (20), and the fatty acid analysis of the microdiets was performed using gas-liquid chromatography (Perkin Elmer Auto System XL) with a 30 × 0.25 mm capillary column FID detector (CP-2330 Supelco). Helium was used as the carrier gas. Flame ionization detection temperature was 220 °C, split rate 1/50, and oven temperature was programmed for a rise from 120 °C/2 min to 220 °C/15 min at a rate of 5 °C/min. Injector temperature was 240 °C. Individual methyl esters were identified by reference to known standards (Sigma, 189-19).

Statistical analysis

All data were expressed as means ± SD (n = 2). For the total lipids and fatty acids, 2 samples per microdiets were analyzed and the average values used for each microdiets. Data were subjected to one way ANOVA, and subsequent comparison of means by Tukey's multiple range test was performed. All the above mentioned statistical analyses were performed using SPSS statistical software (Version 10 for Windows). Differences were considered statistically significant at $P < 0.05$ (21).

Results

The total lipid and main fatty acid profile in the commercial microdiets for larvae and early juvenile of marine fish are given in Tables 1, 2, 3, 4, and 5. Total lipid is expressed as a percentage of dry weight of the microdiets. Main fatty acid values are expressed as a percentage of total lipids in the microdiets. The fatty acids such as 16:0 and 18:1n-9 in all microdiets were most abundant within saturates or monoenes, respectively. Among the polyenes, the n-6 PUFA values were always less abundant than the n-3 PUFA except for the microdiets or granule 5, 6, 11, and 16. In n-3 PUFA, the EPA and DHA were the most abundant fatty acids. The n-6 PUFA were almost entirely contributed by 18:2n-6.

In Table 1, most values of total lipid contents in diets (50-200 µm) for larval fish were higher than 15% and only 1 diet contained less lipid (12.6%) ($P < 0.05$). Diets 3 and 4 had the highest levels of n-3 HUFA (2.9% and 3.0%, respectively) of dry diets ($P < 0.05$). Similarly, diet 4 had the highest EPA level (1.8%) and diet 3 had the highest DHA level (1.8%) of dry diet ($P < 0.05$). However, diet 4 had the lowest level of total n-6 PUFA and ratio of DHA/EPA ($P < 0.05$).

In Table 2, most values of total lipid contents in diets (150-300 µm) for larval fish were lower than 13.5% and only 1 imported diet contained higher lipid (16.3%) ($P < 0.05$). Diet 7 had the highest (2.9%) and diet 5 had the lowest (0.6%) levels of n-3 HUFA in dry diets ($P < 0.05$). Also, DHA and EPA levels in diet 5 were found significantly lower than those of other diets ($P < 0.05$). Total n-6 PUFA level in diet 6 and DHA/EPA ratio in diet 7 were significantly higher than those in other diets ($P < 0.05$).

In Table 3, most values of total lipid contents in diets (200-400 µm) for early juvenile were lower than 13.5% and only 1 imported diet contained higher lipid (17.2%) ($P < 0.05$). Diet 12 had the highest level (2.9%) of n-3 HUFA in dry diet ($P < 0.05$). However, DHA (1.8%) in diet 9 and EPA (2.2%) in diet 12 were significantly higher than those of other diets ($P < 0.05$). Total n-6 PUFA level of diet 6 was significantly lower than that of other diets ($P < 0.05$). In contrast, diet 9 had the highest DHA/EPA ratio.

In Table 4, most values of total lipid contents in diets (300-500 µm) for early juvenile were higher than 15% and only 1 diet produced in Turkey contained less lipid (10.2%) ($P < 0.05$). Diet 14 had the highest (3.0%) and diet 13 had the lowest (0.8%) levels of n-3 HUFA in dry diets ($P < 0.05$). Similarly, diet 14 had the highest DHA (2.0%) and diet 13 had the lowest

Table 1. Lipid and fatty acid profiles in microdiets of 50-200 μm for marine fish production[a].

Total lipid and fatty acids	Diet no.			
	1(I) Extruded 50-100 μm	2(I) Extruded 100-200 μm	3(I) Extruded 100-200 μm	4(I) Extruded 150-200 μm
Total lipid (%, DWB)	16.4 ± 0.28[a]	15.3 ± 0.22[b]	12.6 ± 0.13[c]	16.3 ± 0.21[a]
n-3 HUFA in diet (%, DWB)	2.2 ± 0.01[b]	2.0 ± 0.01[b]	2.9 ± 0.03[a]	3.0 ± 0.02[a]
DHA in diet (%, DWB)	1.3 ± 0.00[b]	1.2 ± 0.00[b]	1.8 ± 0.04[a]	1.2 ± 0.01[b]
EPA in diet (%, DWB)	0.9 ± 0.00[bc]	0.8 ± 0.00[c]	1.0 ± 0.00[b]	1.8 ± 0.00[a]
Fatty acids (% of total fatty acids)				
14:0	3.1 ± 0.01[c]	2.8 ± 0.04[c]	4.8 ± 0.04[b]	12.4 ± 0.11[a]
16:0	16.0 ± 0.05[b]	16.0 ± 0.22[b]	15.7 ± 0.07[b]	19.8 ± 0.12[a]
18:0	6.6 ± 0.01[a]	6.4 ± 0.01[a]	2.3 ± 0.00[b]	1.4 ± 0.01[c]
Total saturates[b]	27.6 ± 0.04[b]	27.4 ± 0.04[b]	25.4 ± 0.03[c]	38.2 ± 0.02[a]
16:1n-7	3.8 ± 0.01[c]	3.5 ± 0.05[d]	4.6 ± 0.02[b]	5.8 ± 0.03[a]
18:1n-9	11.2 ± 0.00[a]	11.4 ± 0.10[a]	10.9 ± 0.22[a]	10.8 ± 0.01[a]
20:1n-9	8.4 ± 0.01[a]	8.2 ± 0.03[a]	4.3 ± 0.01[b]	0.8 ± 0.00[c]
22:1n-9	9.0 ± 0.03[a]	8.8 ± 0.08[a]	4.0 ± 0.06[b]	0.4 ± 0.00[c]
24:1n-9	0.6 ± 0.00[a]	0.7 ± 0.01[a]	0.4 ± 0.03[b]	0.1 ± 0.00[c]
Total monoenes[c]	34.3 ± 0.02[a]	33.4 ± 0.07[a]	24.9 ± 0.03[b]	18.8 ± 0.02[c]
18:2n-6	11.4 ± 0.01[b]	12.8 ± 0.08[a]	10.6 ± 0.04[c]	2.0 ± 0.02[d]
18:3n-6	0.1 ± 0.01[b]	0.2 ± 0.02[a]	0.2 ± 0.01[a]	0.2 ± 0.00[a]
20:4n-6	0.9 ± 0.01[a]	0.4 ± 0.01[b]	0.8 ± 0.02[a]	0.4 ± 0.01[b]
18:3n-3	1.6 ± 0.00[b]	1.7 ± 0.01[b]	2.0 ± 0.00[a]	1.1 ± 0.00[c]
20:3n-3	ND	0.13 ± 0.04[a]	0.15 ± 0.01[a]	0.10 ± 0.01[a]
20:5n-3	5.6 ± 0.01[c]	5.3 ± 0.01[c]	7.9 ± 0.01[b]	10.9 ± 0.01[a]
22:6n-3	7.8 ± 0.01[b]	7.7 ± 0.08[b]	14.7 ± 0.27[a]	7.4 ± 0.03[b]
Total polyenes	27.4 ± 0.05[b]	28.2 ± 0.05[b]	36.4 ± 0.13[a]	22.1 ± 0.03[c]
Total n-6 PUFA	12.4 ± 0.04[b]	13.4 ± 0.03[a]	11.6 ± 0.03[b]	2.6 ± 0.05[c]
Total n-3 PUFA	15.0 ± 0.03[c]	14.8 ± 0.03[c]	24.8 ± 0.03[a]	19.5 ± 0.04[b]
Total n-3 HUFA	13.4 ± 0.02[c]	13.1 ± 0.02[c]	22.8 ± 0.02[a]	18.4 ± 0.02[b]
n-3/n-6	1.3 ± 0.00[c]	1.1 ± 0.00[c]	2.1 ± 0.03[b]	7.5 ± 0.02[a]
EPA/ArA	6.2 ± 0.02[d]	13.2 ± 0.04[b]	9.9 ± 0.03[c]	27.2 ± 0.06[a]
DHA/EPA	1.4 ± 0.02[b]	1.5 ± 0.00[b]	1.9 ± 0.03[a]	0.68 ± 0.00[c]

DWB: Dry weight basis. PUFA: Polyunsaturated fatty acids, HUFA: Highly unsaturated fatty acids, EPA: Eicosapentaenoic acid, ArA: Arachidonic acid, DHA: Docosahexaenoic acid.

[a] Values reported are means ± SD of 2 replicate measurements. Values in the same row with different letters are significantly different ($P< 0.05$). Means were tested by ANOVA and ranked by Tukey's multiple range test. I: Imported feed.

[b] Includes 15:0, 17:0, 20:0, 21:0, 22:0, 23:0 ,and 24:0. [c]Includes 14:1, 15:1, and 17:1.

Table 2. Lipid and fatty acid profiles in microdiets of 150-300 μm for marine fish production[a].

Total lipid and fatty acids	Diet no. 5(T) Extruded 150-300 μm	6(T) Granule 0 200-300 μm	7(I) Extruded 200-300 μm	8(I) Extruded 200-300 μm
Total lipid (%, DWB)	9.5 ± 0.05^{d}	11.2 ± 0.14^{c}	13.2 ± 0.21^{b}	16.3 ± 0.17^{a}
n-3 HUFA in diet (%, DWB)	0.6 ± 0.00^{d}	1.7 ± 0.01^{c}	2.9 ± 0.03^{a}	2.3 ± 0.01^{b}
DHA in diet (%, DWB)	0.3 ± 0.00^{d}	0.8 ± 0.00^{c}	1.9 ± 0.02^{a}	1.4 ± 0.01^{b}
EPA in diet (%, DWB)	0.3 ± 0.01^{c}	0.8 ± 0.01^{b}	1.0 ± 0.00^{a}	0.9 ± 0.00^{ab}
Fatty acids (% of total fatty acids)				
14:0	8.0 ± 0.02^{a}	5.1 ± 0.01^{b}	4.9 ± 0.02^{b}	2.9 ± 0.01^{c}
16:0	25.3 ± 0.06^{a}	17.2 ± 0.02^{b}	15.9 ± 0.04^{c}	15.9 ± 0.01^{c}
18:0	5.9 ± 0.01^{a}	4.4 ± 0.01^{c}	2.3 ± 0.01^{d}	5.2 ± 0.00^{b}
Total saturates[b]	41.6 ± 0.03^{a}	28.9 ± 0.01^{b}	25.3 ± 0.03^{c}	25.9 ± 0.02^{c}
16:1n-7	7.6 ± 0.01^{a}	5.1 ± 0.01^{b}	4.7 ± 0.01^{c}	3.6 ± 0.00^{d}
18:1n-9	17.7 ± 0.01^{a}	12.3 ± 0.01^{b}	11.3 ± 0.02^{c}	11.3 ± 0.01^{c}
20:1n-9	2.2 ± 0.01^{c}	1.9 ± 0.00^{d}	4.5 ± 0.02^{b}	8.6 ± 0.03^{a}
22:1n-9	1.5 ± 0.01^{d}	1.8 ± 0.01^{c}	4.1 ± 0.00^{b}	9.3 ± 0.06^{a}
24:1n-9	0.1 ± 0.01^{c}	0.1 ± 0.00^{c}	0.4 ± 0.01^{b}	0.7 ± 0.01^{a}
Total monoenes[c]	29.8 ± 0.01^{b}	22.3 ± 0.01^{d}	25.0 ± 0.02^{c}	34.3 ± 0.04^{a}
18:2n-6	7.4 ± 0.05^{d}	19.0 ± 0.04^{a}	10.4 ± 0.05^{c}	12.5 ± 0.04^{b}
18:3n-6	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.2 ± 0.01^{a}	0.1 ± 0.01^{b}
20:4n-6	0.5 ± 0.01^{b}	0.8 ± 0.01^{a}	0.4 ± 0.01^{c}	0.4 ± 0.01^{c}
18:3n-3	1.0 ± 0.00^{d}	2.8 ± 0.00^{a}	2.0 ± 0.02^{b}	1.6 ± 0.00^{c}
20:3n-3	0.1 ± 0.00^{a}	0.1 ± 0.01^{a}	0.1 ± 0.00^{a}	ND
20:5n-3	3.2 ± 0.01^{d}	7.3 ± 0.00^{b}	7.8 ± 0.02^{a}	5.5 ± 0.02^{c}
22:6n-3	2.8 ± 0.01^{d}	7.5 ± 0.06^{c}	14.2 ± 0.2^{a}	8.3 ± 0.03^{b}
Total polyenes	15.1 ± 0.01^{d}	37.6 ± 0.02^{a}	35.1 ± 0.04^{b}	28.4 ± 0.06^{c}
Total n-6 PUFA	8.0 ± 0.04^{d}	19.9 ± 0.03^{a}	11.0 ± 0.03^{c}	13.0 ± 0.04^{b}
Total n-3 PUFA	7.1 ± .03^{d}	17.7 ± 0.05^{b}	24.1 ± 0.02^{a}	15.4 ± 0.05^{c}
Total n-3 HUFA	6.1 ± 0.01^{d}	14.9 ± 0.06^{b}	22.1 ± 0.02^{a}	13.8 ± 0.01^{c}
n-3/n-6	0.9 ± 0.02^{c}	0.9 ± 0.03^{c}	2.2 ± 0.01^{a}	1.2 ± 0.02^{b}
EPA/ArA	6.4 ± 0.03^{d}	9.1 ± 0.01^{c}	19.5 ± 0.06^{a}	13.7 ± 0.03^{b}
DHA/EPA	1.0 ± 0.01^{c}	1.0 ± 0.03^{c}	1.8 ± 0.01^{a}	1.5 ± 0.01^{b}

DWB: Dry weight basis. PUFA: Polyunsaturated fatty acids, HUFA: Highly unsaturated fatty acids, EPA: Eicosapentaenoic acid, ArA: Arachidonic acid, DHA: Docosahexaenoic acid.

[a] Values reported are means ± SD of 2 replicate measurements. Values in the same row with different letters are significantly different ($P < 0.05$). Means were tested by ANOVA and ranked by Tukey's multiple range test. T: Feed produced in Turkey. I: Imported feed.

[b] Includes 15:0, 17:0, 20:0, 21:0, 22:0, 23:0 and 24:0. [c]Includes 14:1, 15:1 and 17:1.

Table 3. Lipid and fatty acid profiles in microdiets of 200-400 μm for marine fish production[a].

Total lipid and fatty acids	Diet no.			
	9(I) Extruded 200-400 μm	10(I) Extruded 200-400 μm	11(T) Granule 1 200-400 μm	12(I) Extruded 200-400 μm
Total lipid (%, DWB)	13.1 ± 0.19[b]	10.6 ± 0.20[c]	11.6 ± 0.14[c]	17.2 ± 0.14[a]
n-3 HUFA in diet (%, DWB)	2.8 ± 0.01[b]	1.7 ± 0.01[c]	1.7 ± 0.02[c]	3.4 ± 0.02[a]
DHA in diet (%, DWB)	1.8 ± 0.00[a]	0.6 ± 0.00[c]	0.9 ± 0.01[b]	1.1 ± 0.04[b]
EPA in diet (%, DWB)	1.0 ± 0.00[b]	1.1 ± 0.00[b]	0.8 ± 0.01[c]	2.2 ± 0.01[a]
Fatty acids (% of total fatty acids)				
14:0	4.5 ± 0.01[d]	14.2 ± 0.00[a]	5.2 ± 0.05[c]	5.8 ± 0.03[b]
16:0	15.9 ± 0.05[d]	21.5 ± 0.03[a]	17.4 ± 0.07[b]	16.6 ± 0.12[c]
18:0	2.7 ± 0.00[b]	1.6 ± 0.00[c]	4.4 ± 0.00[a]	4.3 ± 0.05[a]
Total saturates[b]	25.7 ± 0.04[c]	40.2 ± 0.06[a]	29.1 ± 0.04[b]	28.5 ± 0.01[b]
16:1n-7	4.8 ± 0.01[d]	8.9 ± 0.01[a]	5.2 ± 0.03[c]	6.1 ± 0.03[b]
18:1n-9	11.8 ± 0.03[c]	14.2 ± 0.14[a]	12.4 ± 0.01[b]	9.7 ± 0.07[d]
20:1n-9	4.6 ± 0.01[a]	1.1 ± 0.01[d]	1.9 ± 0.01[c]	2.3 ± 0.01[b]
22:1n-9	4.3 ± 0.02[a]	0.6 ± 0.01[d]	1.8 ± 0.01[c]	2.7 ± 0.01[b]
24:1n-9	0.5 ± 0.01[a]	0.1 ± 0.01[b]	0.1 ± 0.04[b]	0.4 ± 0.04[a]
Total monoenes[c]	26.7 ± 0.02[a]	26.4 ± 0.03[a]	22.5 ± 0.02[b]	22.7 ± 0.04[b]
18:2n-6	10.2 ± 0.03[b]	1.4 ± 0.02[c]	18.9 ± 0.00[a]	10.8 ± 0.03[b]
18:3n-6	0.2 ± 0.01[a]	0.2 ± 0.00[a]	0.1 ± 0.00[b]	0.1 ± 0.01[b]
20:4n-6	0.8 ± 0.00[a]	0.4 ± 0.02[c]	0.8 ± 0.01[a]	0.6 ± 0.08[b]
18:3n-3	1.8 ± 0.00[b]	0.7 ± 0.01[c]	2.8 ± 0.00[a]	1.6 ± 0.01[b]
20:3n-3	0.10 ± 0.01[b]	ND	0.10 ± 0.00[b]	0.14 ± 0.01[a]
20:5n-3	7.8 ± 0.00[c]	10.2 ± 0.01[b]	7.2 ± 0.00[d]	12.9 ± 0.06[a]
22:6n-3	13.8 ± 0.00[a]	5.6 ± 0.02[d]	7.2 ± 0.02[b]	6.6 ± 0.23[c]
Total polyenes	34.7 ± 0.02[b]	18.5 ± 0.07[d]	37.1 ± 0.01[a]	32.7 ± 0.06[c]
Total n-6 PUFA	11.2 ± 0.05[b]	2.0 ± 0.01[c]	19.8 ± 0.01[a]	11.5 ± 0.07[b]
Total n-3 PUFA	23.5 ± 0.06[a]	16.5 ± 0.02[c]	17.3 ± 0.01[c]	21.2 ± 0.03[b]
Total n-3 HUFA	21.7 ± 0.03[a]	15.8 ± 0.03[c]	14.5 ± 0.02[d]	19.6 ± 0.02[b]
n-3/n-6	2.1 ± 0.002[b]	8.3 ± 0.02[a]	0.9 ± 0.02[c]	1.8 ± 0.01[b]
EPA/ArA	9.7 ± 0.02[c]	25.5 ± 0.05[a]	9.0 ± 0.03[c]	21.5 ± 0.05[b]
DHA/EPA	1.8 ± 0.02[a]	0.5 ± 0.01[c]	1.1 ± 0.01[b]	0.5 ± 0.01[c]

DWB: Dry weight basis. PUFA: Polyunsaturated fatty acids, HUFA: Highly unsaturated fatty acids, EPA: Eicosapentaenoic acid, ArA: Arachidonic acid, DHA: Docosahexaenoic acid.

[a] Values reported are means ± SD of 2 replicate measurements. Values in the same row with different letters are significantly different ($P< 0.05$). Means were tested by ANOVA and ranked by Tukey's multiple range test. I: Imported feed. T: Feed produced in Turkey.

[b] Includes 15:0, 17:0, 20:0, 21:0, 22:0, 23:0 and 24:0. [c]Includes 14:1, 15:1 and 17:1.

Table 4. Lipid and fatty acid profiles in microdiets of 300-500 μm for marine fish production[a].

Total lipid and fatty acids	Diet no. 13(T) Extruded 300-500 μm	14(I) Extruded 300-500 μm	15(I) Extruded 300-500 μm	16(I) Extruded 300-500 μm
Total lipid (%, DWB)	10.2 ± 0.06[c]	15.3 ± 0.42[b]	16.7 ± 0.24[a]	15.1 ± 0.28[b]
n-3 HUFA in diet (%, DWB)	0.8 ± 0.01[d]	3.0 ± 0.03[a]	2.1 ± 0.03[b]	1.3 ± 0.03[c]
DHA in diet (%, DWB)	0.4 ± 0.01[d]	2.0 ± 0.03[a]	1.2 ± 0.00[b]	0.8 ± 0.02[c]
EPA in diet (%, DWB)	0.4 ± 0.00[c]	1.0 ± 0.01[a]	0.9 ± 0.00[a]	0.6 ± 0.01[b]
Fatty acids (% of total fatty acids)				
14:0	7.7 ± 0.00[a]	4.8 ± 0.04[b]	3.0 ± 0.01[c]	2.7 ± 0.01[d]
16:0	24.0 ± 0.02[a]	17.6 ± 0.10[b]	16.2 ± 0.03[c]	17.0 ± 0.01[b]
18:0	5.7 ± 0.03[c]	3.8 ± 0.00[d]	6.8 ± 0.00[b]	9.9 ± 0.00[a]
Total saturates[b]	40.0 ± 0.02[a]	29.2 ± 0.03[c]	28.0 ± 0.04[c]	31.1 ± 0.01[b]
16:1n-7	7.3 ± 0.01[a]	4.6 ± 0.03[b]	3.7 ± 0.01[c]	3.2 ± 0.00[c]
18:1n-9	17.0 ± 0.01[a]	12.9 ± 0.02[b]	11.1 ± 0.01[c]	10.2 ± 0.05[d]
20:1n-9	2.2 ± 0.00[d]	4.8 ± 0.06[c]	8.2 ± 0.01[a]	7.5 ± 0.04[b]
22:1n-9	1.5 ± 0.00[d]	5.5 ± 0.12[c]	8.8 ± 0.00[b]	9.9 ± 0.09[a]
24:1n-9	0.1 ± 0.01[c]	0.6 ± 0.01[b]	0.6 ± 0.00[b]	0.7 ± 0.01[a]
Total monoenes[c]	28.8 ± 0.01[c]	30.9 ± 0.04[b]	33.2 ± 0.03[a]	32.3 ± 0.04[ab]
18:2n-6	7.8 ± 0.01[c]	7.2 ± 0.06[d]	11.6 ± 0.02[b]	16.1 ± 0.01[a]
18:3n-6	0.1 ± 0.00[a]	0.1 ± 0.01[a]	0.1 ± 0.01[a]	0.1 ± 0.00[a]
20:4n-6	0.6 ± 0.01[a]	0.6 ± 0.01[a]	0.4 ± 0.00[b]	0.4 ± 0.00[b]
18:3n-3	1.1 ± 0.00[d]	1.4 ± 0.00[c]	1.7 ± 0.02[b]	2.2 ± 0.01[a]
20:3n-3	0.1 ± 0.00[a]	0.10 ± 0.01[a]	0.10 ± 0.01[a]	0.1 ± 0.00[a]
20:5n-3	4.0 ± 0.02[c]	6.6 ± 0.06[a]	5.4 ± 0.01[b]	3.8 ± 0.00[c]
22:6n-3	4.0 ± 0.03[d]	13.2 ± 0.18[a]	7.4 ± 0.01[b]	5.1 ± 0.05[c]
Total polyenes	17.7 ± 0.01[c]	29.2 ± 0.06[a]	26.7 ± 0.03[b]	27.8 ± 0.01[ab]
Total n-6 PUFA	8.5 ± 0.02[c]	7.9 ± 0.03[d]	12.1 ± 0.02[b]	16.6 ± 0.04[a]
Total n-3 PUFA	9.2 ± 0.01[d]	21.3 ± 0.05[a]	14.6 ± 0.03[b]	11.2 ± 0.04[c]
Total n-3 HUFA	8.1 ± 0.01[d]	19.9 ± 0.04[a]	12.9 ± 0.03[b]	9.0 ± 0.05[c]
n-3/n-6	1.1 ± 0.02[b]	2.7 ± 0.02[a]	1.2 ± 0.01[b]	0.7 ± 0.02[c]
EPA/ArA	6.7 ± 0.03[c]	11.0 ± 0.04[b]	13.5 ± 0.02[a]	9.5 ± 0.05[b]
DHA/EPA	1.0 ± 0.02[c]	2.0 ± 0.01[a]	1.4 ± 0.02[b]	1.3 ± 0.02[b]

DWB: Dry weight basis. PUFA: Polyunsaturated fatty acids, HUFA: Highly unsaturated fatty acids, EPA: Eicosapentaenoic acid, ArA: Arachidonic acid, DHA: Docosahexaenoic acid.

[a] Values reported are means ± SD of 2 replicate measurements. Values in the same row with different letters are significantly different (P< 0.05). Means were tested by ANOVA and ranked by Tukey's multiple range test. T: Feed produced in Turkey. I: Imported feed.

[b] Includes 15:0, 17:0, 20:0, 21:0, 22:0, 23:0 and 24:0. [c]Includes 14:1, 15:1 and 17:1.

Table 5. Lipid and fatty acid profiles in microdiets of 400-800 µm for marine fish production[a].

Total lipid and fatty acids	Diet no. 17(I) Extruded 400-600 µm	18(I) Extruded 500-800 µm	19(I) Extruded 500-800 µm	20(T) Extruded 500-800 µm
Total lipid (%, DWB)	11.5 ± 0.14[b]	17.0 ± 0.12[a]	17.0 ± 0.28[a]	10.1 ± 0.05[c]
n-3 HUFA in diet (%, DWB)	1.9 ± 0.02[b]	3.6 ± 0.01[a]	3.4 ± 0.03[a]	1.1 ± 0.01[c]
DHA in diet (%, DWB)	0.7 ± 0.00[b]	1.5 ± 0.01[a]	1.6 ± 0.02[a]	0.6 ± 0.01[b]
EPA in diet (%, DWB)	1.1 ± 0.00[b]	2.0 ± 0.02[a]	1.8 ± 0.01[a]	0.5 ± 0.00[c]
Fatty acids (% of total fatty acids)				
14:0	13.3 ± 0.04[a]	6.3 ± 0.07[b]	6.2 ± 0.04[b]	6.7 ± 0.01[b]
16:0	21.3 ± 0.02[a]	17.8 ± 0.13[b]	17.1 ± 0.02[b]	21.4 ± 0.03[a]
18:0	1.6 ± 0.00[c]	4.0 ± 0.01[b]	3.6 ± 0.00[b]	5.3 ± 0.01[a]
Total saturates[b]	40.0 ± 0.08[a]	30.2 ± 0.01[c]	28.8 ± 0.02[d]	35.7 ± 0.01[b]
16:1n-7	8.5 ± 0.01[a]	6.1 ± 0.05[c]	6.0 ± 0.02[c]	6.8 ± 0.01[b]
18:1n-9	13.8 ± 0.01[b]	11.7 ± 0.03[c]	10.1 ± 0.01[d]	15.7 ± 0.01[a]
20:1n-9	1.1 ± 0.00[c]	2.3 ± 0.03[b]	4.1 ± 0.01[a]	2.1 ± 0.00[b]
22:1n-9	0.6 ± 0.01[d]	3.2 ± 0.25[b]	5.4 ± 0.01[a]	1.4 ± 0.01[c]
24:1n-9	0.1 ± 0.00[b]	0.5 ± 0.06[a]	ND	0.1 ± 0.01[b]
Total monoenes[c]	24.8 ± 0.04[b]	24.5 ± 0.02[b]	27.0 ± 0.01[a]	27.5 ± 0.01[a]
18:2n-6	1.8 ± 0.07[d]	8.0 ± 0.04[b]	7.5 ± 0.01[c]	8.9 ± 0.02[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	0.3 ± 0.01[c]	0.6 ± 0.07[b]	0.7 ± 0.01[a]	0.8 ± 0.00[a]
18:3n-3	0.7 ± 0.01[c]	1.2 ± 0.01[b]	1.4 ± 0.00[a]	1.2 ± 0.01[b]
20:3n-3	0.10 ± 0.01[b]	0.13 ± 0.01[a]	0.10 ± 0.00[b]	0.11 ± 0.00[b]
20:5n-3	9.9 ± 0.03[c]	11.9 ± 0.11[a]	10.4 ± 0.01[b]	5.7 ± 0.01[d]
22:6n-3	6.2 ± 0.03[c]	9.0 ± 0.07[a]	9.3 ± 0.05[b]	5.6 ± 0.00[d]
Total polyenes	19.2 ± 0.04[c]	31.0 ± 0.03[a]	29.6 ± 0.01[a]	22.4 ± 0.01[b]
Total n-6 PUFA	2.3 ± 0.03[c]	8.8 ± 0.04[b]	8.4 ± 0.02[b]	9.8 ± 0.02[a]
Total n-3 PUFA	16.9 ± 0.02[b]	22.2 ± 0.02[a]	21.2 ± 0.03[a]	12.6 ± 0.01[c]
Total n-3 HUFA	16.2 ± 0.03[b]	21.0 ± 0.05[a]	19.8 ± 0.03[a]	11.4 ± 0.04[c]
n-3/n-6	7.3 ± 0.02[a]	1.8 ± 0.04[c]	2.5 ± 0.02[b]	1.3 ± 0.02[d]
EPA/ArA	33.0 ± 0.06[a]	19.8 ± 0.04[b]	14.9 ± 0.02[c]	7.1 ± 0.05[d]
DHA/EPA	0.6 ± 0.00[c]	0.8 ± 0.02[b]	0.9 ± 0.02[b]	1.2 ± 0.02[a]

DWB: Dry weight basis. PUFA: Polyunsaturated fatty acids, HUFA: Highly unsaturated fatty acids, EPA: Eicosapentaenoic acid, ArA: Arachidonic acid, DHA: Docosahexaenoic acid.

[a] Values reported are means ± SD of 2 replicate measurements. Values in the same row with different letters are significantly different (P< 0.05). Means were tested by ANOVA and ranked by Tukey's multiple range test. I: Imported feed. T: Feed produced in Turkey.

[b] Includes 15:0, 17:0, 20:0, 21:0, 22:0, 23:0 and 24:0. [c] Includes 14:1, 15:1 and 17:1.

EPA (0.4%) levels ($P < 0.05$). Total n-6 PUFA level of diet 16 and DHA/EPA ratio of diet 14 were significantly higher than those of other diets ($P < 0.05$).

In Table 5, total lipid contents in diets (400-800 µm) for early juvenile ranged from 10.1% to 17.0% ($P < 0.05$). DHA levels in diets ranged from 0.6% to 1.6% and EPA levels ranged from 0.5% to 2.0%. Diet 14 had the highest (3.0%) and diet 13 had the lowest (0.8%) levels of n-3 HUFA in dry diets ($P < 0.05$). Similarly, diet 14 had the highest DHA and diet 13 had the lowest EPA levels ($P < 0.05$). Total n-6 PUFA level of diet 17 was significantly lower than that of other diets ($P < 0.05$). However, diet 20 had the highest DHA/EPA ratio.

Discussion

The dietary fats are the source of the energy, essential fatty acids (EFA), and fat-soluble vitamins (A, D, E and K). Lipids are the main energy source in developing larvae (7), while carnivorous fish species, such as sea bream and sea bass, use less carbohydrate compared to omnivorous or herbivorous fish species (22). Many studies have been conducted to determine the optimal lipid level in diets formulated for marine fish larvae. The best growth was obtained in larval diets for sea bream (18% lipid), compared to diets containing 13%, 23%, or 27% lipid (23). Commercial high fat diets used presently in gilthead bream nutrition provide n-3 HUFA in excess of requirements (7). Growth and survival of sea bass larvae were directly related to the lipid content of the diet. Best results were obtained with the diet containing 30% lipid, supplied as cod liver oil and soybean lecithin (24). However, Barnabe (25) stated that the optimum lipid level in the diets of juvenile sea bass was around 9%-15%. Similarly, Peres and Oliva-Teles (26) reported that the increase of dietary lipid level from 12% to 24% did not improve the growth performance of sea bass juveniles. The results of studies cited above indicated that optimum lipid level of diets for larvae of marine fish, such as sea bass or sea bream, was between 9% and 18%. In the present study, lipid levels in the microdiets were found adequate for larvae and early juveniles of both of the fish species.

Several studies confirmed the importance of a sufficient supply of n-3 HUFA particularly in different life stages, such as larval and juvenile. It has been demonstrated that high percentages of n-3 HUFA in the diet can improve growth and survival of fish larvae in a number of marine fish species (6,8). Many authors (9,13-15) have also indicated that certain levels of n-3 HUFA are required to obtain optimal growth and survival for larvae of marine fish species. It is known that dietary phospholipids requirement of fish larvae is higher than that of juveniles (6,7). The n-3 HUFA requirements in the dry diet for larvae or early juveniles of some marine fish species, such as red sea bream (*Pagrus major*), yellowtail (*Seriola quinqueradiata*), and gilthead sea bream (*Sparus aurata*), were 2.1% (with 1.0% DHA), 3.9% (DHA/EPA = 0.5), 5.5% (DHA/EPA = 0.3) or 1.5% (DHA/EPA = 2), respectively (7). Sea bass larvae require dietary DHA/EPA ratio of 2 and EPA/AA ratio of 1 for optimum performance (6).

The optimum level of n-3 HUFA required by fingerlings and juveniles of gilthead sea bream is close to 1% (16). However, reduction in growth of gilthead sea bream juveniles has also been found when fed a diet containing 5% n-3 HUFA in the form of triglycerides (9). Parpoura and Alexis (27) reported that juvenile sea bass has a minimum requirement of 1.35% EPA+DHA for optimum performance. Nevertheless, Skalli and Robin (28) stated that the requirement for growth of n-3 HUFA of juvenile sea bass was at least 0.7% of the dry diet. Levels of n-3 HUFA, DHA, or EPA in microdiets (Tables 1-5) of our study agree with the above cited literature except for microdiets 5 and 13. However, EPA/ArA ratio in all microdiets was found in considerably higher levels. These results indicated that high levels of EPA content lipid sources could be supplemented in the analyzed microdiets.

Koven et al. (14) reported that dietary ArA improved survival and alleviated the acute and chronic stressors during rearing. Nevertheless, the ArA requirement of juvenile marine fish has not been determined quantitatively. The increase in the ArA level from 0.1% to 1.0% of total fatty acids in experimental microdiets significantly improved the growth of gilthead sea bream larvae after 3 weeks of feeding (8). However, in juveniles of the same species, Fountoulaki et al. (10) did not observe any effect of ArA on growth (minimal level 0.2% of dietary fatty acid). There are no data on the ArA requirement for

European sea bass juveniles. Dietary arachidonic acid is supplied at low level by marine products, but is lacking in available vegetable sources (28). In the present study, the levels (as percent of dietary fatty acids) of dietary ArA were found ranging from 0.4% to 0.9% in the larval or early juvenile microdiets (Tables 1-5). These ArA levels appeared to cover requirements of larval and early juvenile marine fish species according to the results reported by the researchers cited above. Furthermore, ArA levels from 0.5% to 1.0% of total fatty acids in diets for larvae of marine fish were accepted as optimum (29).

In conclusion, the results of our study showed that total lipid or the n-3 and n-6 HUFA percentages especially DHA, EPA, ArA levels, and DHA/EPA ratio in the microdiets covered essential fatty acids (EFA) requirements for larvae or early juvenile sea bass and sea bream. However, microdiets 3 and 13 had lower n-3 HUFA levels than levels required for larval or early juvenile of these species. More research is required concerning the optimum levels of dietary ArA for optimal growth and development in larval or juvenile stages of sea bass. Besides, researchers have to determine the dietary EFA levels required for a good growth performance of alternative marine fish species in the Mediterranean.

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