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PROOF

Effects of Fish Oil Substitution with Two Different Vegetable Oil Classes on Fatty Acid Digestibility in Juvenile European Sea Bass, *Dicentrarchus labrax*

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Abstract

This study evaluated dietary polyunsaturated fatty acid (PUFA)/highly unsaturated fatty acid (HUFA) ratios in European sea bass subjected to feeds containing fish oil (FO) or two different vegetable oils (the monounsaturated fatty acid: MUFA-rich rapeseed oil, RO; and the n-6 PUFA-rich cottonseed oil, CSO). Triplicate groups of twenty fish (35-g ± 0.2) were fed three fish-based diets in which the added lipid was 100% fish oil (FO), 100% refined low-erucic-acid-rapeseed oil (RO), 100% cottonseed oil (CSO) for a period of 130 days. As a result of this study, final weight, weight gain and specific growth rate were significantly higher in fish fed FO and RO diet compared to fish fed CSO. Final weight in fish fed the RO diet were significantly higher (87.3 ± 3.1 g) in comparison to fish fed the FO (84.3 ± 1.2 g) and CSO diets (80.4±2.3, $p<0.05$). The concentration of eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in flesh was significantly reduced with increasing levels of MUFA and n-6 PUFA in the diet. Accordingly, DHA value in fish fed the CSO diet was 9.41% whereas fish fed the RO diet had lower (7.93%) DHA content in the flesh. As expected, the absorption of individual dietary fatty acids decreased with chain length and increased with the degree of unsaturation. The apparent total lipid digestibility decreased with increasing the PUFA/HUFA ratio in the diet.

Keywords: β-oxidation, chain length, degreee of unsaturation, digestibility

Balık Yağına İkame Eden İki Farklı Bitkisel Yağ Sınıfının Juvenil Avrupa Deniz Levreğinde *(Dicentrarchus Labrax)* Besinsel Sindirime Etkileri

Özet

Bu çalışmada, balık yağı (BY) ve iki farklı bitkisel yağ (tekli doymamış yağ asitlerince zengin kolza yağı, KY ve n-6 çoklu doymamış yağ asitlerince zengin pamuk tohumu yağı; PTY) içeren yemlere tabi tutulan Avrupa deniz levreğindeki çoklu doymamış yağ asidi (PUFA)/ yüksek doymamış yağ asidi (HUFA) oranı değerlendirilmiştir. 20 adet Avrupa deniz levreği, (35-g) 130 gün boyunca %100 balık yağı (BY), %100 rafine edilmiş kolza yağı (KY) ve %100 rafine edilmiş pamuk tohumu yağı ilave edilmiş 3 yemle beslenmiştir. Final ağırlığı, ağırlık kazancı ve spesifik büyüme oranı karşılaştırıldığında BY ve KY yemi ile beslenen balıklar PTY yemi ile beslenenlere göre önemli düzeyde daha yüksek bulunmuştur. KY ile beslenen balıklarda final ağırlığı (87.3 ± 3.1 g), BY(84.3 ± 1.2 g) ve PTY (80.4±2.3, $p<0.05$) ile beslenenlere kıyasla önemli ölçüde daha yüksek bulunmuştur. Filetodaki eikosapentaenoik asit, (EPA) ve dokosahekzaenoik Asit (DHA) yoğunluğu, yemlerdeki MUFA ve n-6 PUFA seviyelerinin artmasıyla azalmıştır. Buna göre, PTY ile beslenen balıkların DHA miktarı %9.41 iken KY (%7.93) ile beslenen balıkların kaslarındaki DHA miktarı daha düşüktür. Tahmin edildiği üzere, diyetlerdeki bireysel yağ asitlerinin emilimi zincir uzunluğu ile azalmakta ve doymamışlık derecesiyle artmaktadır. Diyetlerdeki PUFA/HUFA oranının artmasıyla görünen total yağ sindirilebilirliği azalmaktadır.

Anahtar Kelimeler: β-oksidasyon, doymamışlık derecesi, sindirilebilirlik, zincir uzunluğu

Introduction

Aquaculture has been successful in converting low value fish meal and oil, derived from industrial fisheries, into high value food for the human consumer (Tidwell and Allan, 2002). However, as aquaculture production continues to grow, particularly for carnivorous species, it is clear that global fish oil supplies are inadequate to support the rising demand (NRC, 2011). Therefore, over the last decade or so, considerable research attention has been given to the identification of alternative vegetable oil sources

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(corn, sunflower, linseed etc.) that are easy to produce, economically viable and rich in essential fatty acids (Dernekbaşı and Karayücel, 2010; NRC 2011). However, in marine fish feeds the use of vegetable oils as the sole lipid source is limited by the low ability of these species to convert α-linoleic (LA, 18:2n-6) and linolenic (LNA, 18:3n-3), abundant in many plant oils, into arachidonic (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Thus, the partial replacement of fish oil by vegetable oils would be only possible when these fatty acids are present in the diets in sufficient levels to meet essential fatty acid requirements, which can generally be achieved when fishmeal is included in the diet in the range of 30 – 50% (Izquierdo *et al.,* 2005; Benedito-Palos *et al.,* 2008; Ng and Gibon 2010; Eroldoğan *et al.,* 2013).

Compared with other temperate and warm water marine species, European sea bass (*Dicentrarchus labrax*) is a strict carnivore with a low ability to convert fatty acid acids to higher homologues, although products of each of the key enzymes (i.e. elongase, Δ-6 desaturase and Δ-5 desaturase) involved in the desaturation and elongation of C_{18} fatty acids have been reported (Mourente *et al.,* 2005a; Turchini *et al.,* 2009). However, previous studies have demonstrated that the dietary fish oil component of the diet can be substituted to levels of 50-60% (Montero *et al.,* 2005; Mourente *et al.,* 2005 b; Richard *et al.,* 2006) and 100% (Eroldoğan *et al.,* 2012; Eroldoğan *et al.,* 2013). However, one of the principal effects of fish oil substitution is the unavoidable and detrimental impact on the final fillet concentrations of long chain omega-3 fatty acids (n-3 LC PUFA) and the modification of specific fatty acid digestibility. Thus, the potential for dietary alternative vegetable lipids to be used in fish diets is limited by their degree of digestibility.

In general, fatty acid digestibility is dictated by chain length and degree of unsaturation. Higher fatty acid digestibility is therefore observed for LC PUFA, followed by C_{18} PUFA, MUFA, and SFA (Francis *et al.* 2007). In this sense, there are large variations between the fatty acid compositions of the major vegetable oils used by the aqua feed industry. Of the oils available for utilization in aquafeed formulations, several studies have investigated the potential inclusion of those rich in MUFA or other n-6 PUFA (Torstensen *et al.,* 2004; Stubhaug *et al.,* 2007; Turchini and Francis, 2009) However, specific and direct comparisons of these two types of oils are scarce in relation to individual fatty acid digestibility. A recent study with sea bass demonstrated that the apparent *in vivo* β-oxidation of dietary fatty acids was significantly affected by the dietary lipid source (Eroldoğan *et al.,* 2013). However, the fatty acid or lipid digestibility in terms of dietary PUFA/HUFA ratio has always been reported as none or minimal (Eroldoğan *et al*., 2013; Francis *et al.,* 2007). Therefore, the present study was undertaken to evaluate the efficacy of using refined RO and CSO substitute for the supplemental fish oil in a practical diet for European sea bass (35-g) taking into account the lipid digestibility and dietary PUFA/HUFA ratio.

Materials and Methods

Three diets (3 mm) of equivalent crude protein (~460 g crude protein kg^{-1} dry weight), energy (~ 21.9 KJ/kg) and lipid (~ 200 g lipid kg^{-1} dry weight) concentration were formulated. The diets were formulated to satisfy the nutritional requirement of marine fish (NRC, 2011). The diets had identical ingredient compositions, with the exception of the lipid source which consisted of either 100% fish oil (FO), 100% refined low-erucic-acid-rapeseed oil (RO) and 100% cotton seed oil (CSO) (100 g/kg diet) with the remainder originating from anchovy oil (*Engraulis encrasicolus*) (Table 1). All diets contained 510 g/kg of fish meal as the main protein sources and were cold-pelleted with a laboratory pellet mill.

European sea bass (*Dicentrarchus labrax*) juveniles used in this study were obtained from a commercial hatchery (Akuvatur, Ltd., Adana, Turkey) and transported to an indoor system where they were acclimated in fiberglass tanks for a four week period. Following this, 180 fish (initial weight 35.4 g ± 0.2) were anaesthetized (2-phenoxyethanol at 0.5 ml/L), individually weighed and randomly distributed amongst 9 fiberglass tanks of 400-l with 20 fish per tanks -1. Tanks were continuously provided aerated seawater (38 ppt) at a flow rate of approximately 2 L min -1. Water parameters such as pH and dissolved oxygen were continuously monitored with an YSI model salinometer (Yellow Springs Instrument Yellow springs, OH, USA), an oxygen meter and pH meter (pH 315i Set, WTW Measurement Systems, Germany). The water temperature was maintained at 26.0±0.7ºC and fish were subjected to a 12-h light/12-h dark photo-period regime over the course of the study. Triplicate groups of fish were each fed one of the three aforementioned diets by hand to apparent satiation three times daily (0900 h, 1300 h and 1800 h) for 130 days. Utmost care was taken to assure that all feed was consumed. Feed intake was determined daily and all fish were individually weighed every 10 days.

At the beginning of the experiment, an initial sample of 15 fish from a common pool of fish was culled and stored at -20°C for subsequent determination of their initial proximate and lipid compositions (the analyses were conducted on composite samples of fish each; n=3). At the end of the experiment, all fish were individually weighed each tanks and three fish were sampled randomly from each replicate group (tank) per diet treatment for subsequent determination of whole body and flesh proximate and flesh fatty acid composition. Whole

Table 1. Formulation and proximate compositions (%) of experimental diets fed to European sea bass for 130 days

	Diets		
	FO	RO	CSO
Dietary Ingredients (g kg^{-1})			
Fish meal [a]	510	510	510
Corn gluten [b]	225	225	225
Dextrin [b]	70	70	70
Fish oil [a]	100	-	-
Refined low-erucic-acid-rapeseed oil [c]	-	100	-
Cottonseed oil [c]	-	-	100
Carboxymethyl cellulose [d]	37	37	37
Cr_2O_3 [e]	10	10	10
Di Calcium Phosphate [d]	23	23	23
Mineral premix [f]	15	15	15
Vitamin premix [f]	10	10	10
Proximate composition (%)			
Dry matter	86.0 ± 0.16	86.1 ± 0.26	86.0 ± 0.69
Protein	45.9 ± 0.64	45.9 ± 0.66	46.9 ± 1.05
Lipid	20.4 ± 0.73	20.0 ± 0.96	19.5 ± 0.36
Ash	12.7 ± 0.21	12.3 ± 0.07	12.3 ± 0.25
Gross energy (kJ/g) [g]	21.0 ± 0.29	19.9 ± 0.33	20.0 ± 0.18

[a] Supplied by Sibal Black Sea Feed, İzmir, Turkey (Anchocy meal-crude protein 70%).
[b] Supplied by Sunar Mısır, Adana, Turkey.
[c] Çukurova Corporative Enterprise, Adana, Turkey.
[d] Interlab Laboratory Supplies, İstanbul, Turkey.
[e] Sigma-Aldrich, Inc., St Louis, MO, USA.
[f] Vitamin and mineral added minimum to NRC recommendations. Supplied by Sibal Black Sea Feed, İzmir, Turkey.
[g] Calculated based on the standard physiological fuel values: 19 kJ g^{-1} for protein, 36 kJ g^{-1} for lipid and 15 kJ g^{-1} for carbohydrate.

body and flesh samples from each replicate tank were pooled and ground to a homogeneous consistency using a centrifugal mill fitted with a 0.25 mm and stored at -20°C for subsequent chemical analysis. All fish handling procedures complied with Turkish guidelines for animal care (No. 28141) set by the Ministry of Food, Agriculture and Livestock.

Faecal samples were collected during the last 15 days of the feeding trial. Prior to collection, a specific faecal collection column was fitted to each of the experimental tanks using the Guelph system (Cho *et al*. 1982), which was modified as described by Tibbetts *et al.* (2006). Faecal samples were collected on a daily basis. After the final meal (1800 h), tanks were siphoned to remove the uneaten feed and the feacal collection columns set on each tank were covered with ice in a plastic bag to minimize possible bacterial growth. The following morning, the faeces deposited between the pm and am feed were collected and stored at -20°C until analysis. The diets and freeze-dried faecal samples (around 35 g wet faeces for each tank) were analyzed for chromic oxide (Cr_2O_3) content according to Furukawa and Tsukahara (1966). The apparent digestibility coefficients of fatty acids were calculated from standard formula (Maynard *et al* .,1979).

Determinations of moisture, ash and protein in diets and fish samples were conducted as described below. Percent moisture samples were dried to constant weight at 103 ºC. Ash content was determined by burning the samples at 450 ºC for 5 h (AOAC, 1995). Protein (N x 6.25) content was determined using an automated Kjeldahl Kjeltec 2200 (Foss Tecator, Högans, Sweden). Gross energy content of the test diets was calculated on the basis of 19, 36 and 15 kJ g^{-1} for protein, lipid and carbohydrate, respectively. Lipids were extracted according to the procedure of Folch *et al.* (1957). Following the lipid extraction, fatty acid methyl esters (FAME) were prepared according to Metcalfe & Schmitz (1961) and analyzed as described previously (Czesny and Dabrowski, 1998) with some modifications. Briefly, the FAME obtained were separated by gas chromatography (Agilent 6890 N), equipped with a flame ionization detector and fitted with a DB 23 capillary column (60 m, 0.25 mm i.d. and 0.25 µm) injector and detector temperature program was 190 ºC for 35 min then increasing at 30 ºC per min up to 220 ºC where it was maintained for 5 min. Carrier gas was hydrogen (2 ml min^{-1} and split ratio was 30:1). The individual fatty acids were identified by comparing their retention times to that of a standard mix of fatty acids (Supelco 37 component FAME mix).

The effect of the dietary treatment on the growth performance of the fish was assessed using the following formulas:

(1) Wet weight gain (WG) (g) = (final mean wet weight (FW) (g) −initial mean wet weight (IW) (g))

(2) Specific growth rate (SGR) (% body weight/day) = [(ln FW (g) −ln IW (g))/time (days)] × 100, (Company *et al*., 1999),

(3) Dry feed intake (DFI) (g/fish) = total

daily dry feed intake/fish over 130 days

(4) Feed conversion ratio (FCR) = dry feed fed (g) / weight gain (g), (Santinha *et al*., 1999),

All data were reported as means ± standard error (n=3; N= 9) throughout the text. Data were analyzed by one-way analysis of variance (ANOVA) at a significance level of 0.05% following confirmation of normality and homogeneity of variance. Two-way ANOVA was used to separate the effects of dietary treatment, fatty acid structure and their interaction. Where significant differences were detected, data were subjected to Duncan's post hoc test for identifying homogeneous subset (SPSS16.0, SPSS Inc., Chicago, IL, USA).

Results

The proximate composition of the three fish meal-based diets was uniform in all respects (protein, lipid, and gross energy), differing only in the source of the dietary lipid (Table 1). Lipid and protein content ranged from 19.5% to 20.4% g and 45.9% to 46.9%, respectively. The fatty composition of the three experimental diets clearly reflected that of the oil source used in the formulation (Table 2). The FO diet was characterized by a high content of total n-3 LC-PUFA (15.87%), including EPA (5.28%) and DHA (7.0 %). The RO diet containing low-erucic-rapeseed oil was dominated by the highest levels of oleic acid, 18:1n-9 (47.65%), while the CSO diet was dominated by LA (45.70%). The PUFA / HUFA ratio of the diets were 2.1, 11.4 and 29.3 for FO, RO and CSO, respectively. All the experimental diets were well accepted by the treatment groups and subsequently no significantly differences in feed intake were observed (P>0.05).

During the experimental period, mortality was low and unaffected by the dietary treatment. However, the dietary treatments did exert an influence on growth performance. European sea bass fed the RO diet were significantly larger (87.3 ± 3.1 g) in comparison to fish fed the FO (84.3 ± 1.2 g) and CSO diets (80.4±2.3, P<0.05).

Accordingly, weight gain percentage was significantly higher in fish fed the RO diet and FO diet in comparison to fish fed the CSO diet. No

Table 2. Total fatty acid composition (% total lipid) of the experimental diets

	Diets		
Fatty acids	FO	RO (CO)	CSO (CSO)
14:0	3.93	1.61	1.92
16:0	15.23	11.16	22.40
18:0	3.39	3.05	3.12
20:0	0.46	0.73	0.45
22:0	3.35	1.08	0.36
SFA*	26.98	18.2	28.70
14:1	0.19	0.09	0.08
16:1n-7	4.55	1.68	1.77
18:1n-9	25.96	47.65	18.72
18:1n-7	2.87	3.10	1.37
20:1n-11	0.49	0.06	0.04
20:1n-9	3.69	1.64	0.41
22:1n-11	0.05	0.09	0.01
MUFA*	39.24	55.12	23.00
18:2n-6	12.47	18.55	45.70
18:3n-6	0.10	0.07	0.19
20:2n-6	0.54	0.14	0.07
20:4n-6	0.42	0.08	0.06
22:4n-6	0.36	0.07	0.01
n-6 PUFA*	14.34	18.94	46.06
18:3n-3	2.62	5.36	0.49
18:4n-3	0.94	0.10	0.06
20:3n-3	0.23	0.05	0.02
20:4n-3	0.95	0.08	**
20:5n-3	5.28	0.92	0.57
22:5n-3	2.06	0.27	0.36
22:6n-3	7.35	0.97	0.69
n-3 PUFA	19.43	7.75	2.19
PUFA	33.79	26.68	48.25
LC-PUFA	16.23	2.61	1.81
n-6 LC- PUFA	0.36	0.32	0.17
n-3 LC-PUFA	15.87	2.29	1.64
PUFA/HUFA	2.1	11.4	29.3

* Includes: 15:0 for Saturated, 15:1, 17:1, 22:1n-9 and 24:1n-9 for Monoenes and 20:3-6 and 22:2n-6 for n-6 PUFAs.
**Not detected.

significant differences in feed conversion ratio or daily feed intake were noted among the groups over the 130-day experimental period (Table 3). However, somatometric parameters differed significantly among the dietary treatments. The hepatosomatic index (HSI) of fish receiving the FO and CSO diets were lower than fish receiving the RO diet, while the highest viscerosomatic index (VSI) was observed in fish fed the CSO diet (Table 3).

The chemical composition of whole body and fillet samples was significantly affected by the diets. While there were no significant differences in the fillet protein composition across all groups ($P>0.05$), whole body protein content in fish fed the CSO diet was significantly higher than all other groups ($P<0.05$). Lipid levels of whole body samples were lower in fish fed the RO diet (7.7%) than fish fed the CSO diet and the FO diet (8.7% and 8.3%) (Table 3). On the other hand, lipid levels in fish fillets were significantly higher in fish receiving RO diet in comparison to other groups.

As expected, the fatty acid content of fish fillets mirrored that of the experimental diets. However, the concentrations of EPA and DHA were the exception here, and were somewhat conserved irrespective of the alternative oil treatment received. (Table 4). As such, fish receiving the RO and CSO diets contained high concentrations of 16:0, oleic acid (18:1n-9, OA) and LA, while fish fed the FO diet were characterized by high concentrations of LC-PUFA. Total saturated fatty acids (SFA) (primarily 16:0) were highest in fish fed the FO diet and lowest in fish fed CSO, however, no significant differences were noted amongst the dietary treatment for this class. Total monounsaturated fatty acid concentrations were close to identical across the treatments, but the major monoenes, OA, was significantly higher in the fillets of fish fed the RO and CSO diets due to a higher dietary inclusion. On the contrary, total PUFA content of the fish fed the RO diet was lower than the fish fed the CSO and FO diets.

As shown in Table 5, DHA and SFA (namely 18:0 and 20:0), 20:1n-11, 20:4n-6 and 20:3n-3 were present in higher concentrations in fillet lipids than in the dietary lipid, suggesting that these fatty acids were selectively deposited in fillet lipids. Similar results were also evident for LC-PUFA, n-3 LC PUFA and n-6 LC PUFA across all dietary groups. However, LA was present in lowest concentration in fish fed the RO and FO dies. ALA and OA were present in lower concentration in fillet lipids than in the dietary lipid in fish fed the CSO diet and FO diet respectively. EPA was highly present in flesh lipid than in dietary lipid by fish-fed RO and CSO, whereas the lowest values were recorded in fish fed the FO diet. Clearly, DHA was preferentially deposited in flesh lipid relative to its level in dietary lipid among the experimental groups.

Generally regardless of dietary treatment, each specific fatty acid was digested efficiently (Table 6). The absorption of individual dietary saturated fatty acids decreased with chain length (Table 7). Moreover, the apparent digestibility of unsaturated fatty acids decreased as the position of the first double bond moved from the methyl end of the carbon chain, with n-3 fatty acids absorbed at a higher rate compared to n-6 and n-9 fatty acids (Table 6). Similarly, the inverse was apparent with regard to apparent digestibility and the degree of unsaturation. Individual fatty acids all recorded relatively high

Table 3. Growth performance, proximate composition whole body and fillet (% as is, mean ± SE, n=3) of European sea bass fed the experimental diets for 130 days.

	Diets		
Growth parameters	FO	RO	CSO
Initial Weight (g)	35.2 ± 0.2	35.7 ± 0.2	35.8 ± 0.2
Final Weight (g)	84.3 ± 1.2^{ab}	87.3 ± 3.1^{a}	80.4 ± 2.3^{b}
Weight Gain (g/fish)	49.1 ± 1.7^{ab}	51.6 ± 2.2^{a}	44.6 ± 0.6^{b}
SGR (% g day^{-1})	0.67 ± 0.1^{a}	0.69 ± 0.1^{a}	0.62 ± 0.1^{b}
DFI (g/day)	17.3 ± 0.6	18.2 ± 0.6	17.1 ± 0.8
FCR	2.4 ± 0.1	2.4 ± 0.1	2.6 ± 0.1
HSI	1.2 ± 0.2^{b}	1.7 ± 0.3^{a}	1.3 ± 0.1^{b}
VSI	1.2 ± 0.5^{c}	2.2 ± 0.7^{b}	2.6 ± 0.7^{a}
Proximate composition			
Whole Body			
Protein	18.6 ± 0.2^{a}	18.6 ± 1.6^{a}	17.6 ± 0.7^{b}
Lipid	8.3 ± 0.2^{ab}	7.7 ± 0.2^{b}	8.7 ± 0.2^{a}
Dry Matter	35.5 ± 0.4^{a}	34.5 ± 0.4^{b}	31.4 ± 0.3^{c}
Ash	6.6 ± 0.2^{a}	5.3 ± 0.4^{b}	5.1 ± 0.1^{b}
Fillet			
Protein	19.6± 0.1	19.3 ± 0.3	19.8 ± 0.5
Lipid	3.34 ± 0.1^{b}	3.6 ± 0.1^{ab}	2.7 ± 0.2^{c}
Dry Matter	25.4 ± 0.4^{a}	24.7 ± 0.4^{ab}	23.9 ± 0.3^{c}
Ash	1.7 ± 0.1^{a}	1.6 ± 0.1^{ab}	1.5 ± 0.1^{bc}

Table 4. Flesh fatty acid composition of sea bass fed different diets over a 130 day period.

	Diets		
Fatty acids	FO	RO	CSO
14:0	3.05 ±0.2[a]	2.46 ± 0.1[b]	2.40 ± 0.2[b]
16:0	16.67 ± 0.8	16.17 ± 0.0	16.01± 0.0
18:0	3.72 ± 0.4[b]	3.86 ± 0.1[b]	4.77 ± 0.0[a]
20:0	3.65 ± 0.1[b]	4.32± 0.1[a]	2.64 ± 0.0[c]
22:0	1.77 ± 0.1[a]	1.79 ± 0.0[a]	1.34 ± 0.0[b]
SFA	29.23 ± 0.6	28.92 ± 0.5	27.40 ± 0.2
14:1	0.07 ± 0.1[b]	0.08 ± 0.0[a]	0.06 ± 0.0[b]
16:1n-7	0.05 ± 0.0	0.05 ± 0.0	0.05 ± 0.0
18:1n-9	25.32 ± 0.4[c]	31.93 ± 0.5[a]	28.84 ± 0.6[b]
18:1n-7	2.50 ± 0.1[a]	2.36 ± 0.0[b]	2.35 ± 0.1[b]
20:1n-11	0.52 ± 0.1	0.52 ± 0.0	0.46 ± 0.0
20:1n-9	0.07 ± 0.1[a]	0.04 ± 0.0[b]	0.01 ± 0.0[c]
22:1n-11	0.17 ± 0.1[a]	0.14 ± 0.0[b]	0.11 ± 0.0[c]
MUFA	29.66 ± 0.6[c]	36.10± 0.6[a]	32.71 ± 0.6[b]
18:2n-6	17.62 ± 0.5[b]	17.92± 0.0[b]	22.92 ± 0.2[a]
18:3n-6	0.08 ± 0.1[a]	0.06± 0.0[b]	0.08 ± 0.0[a]
20:2n-6	0.01± 0.0	0.01± 0.0	0.01 ± 0.0
20:4n-6	0.63 ± 0.1[a]	0.53± 0.0[b]	0.65 ± 0.0[a]
22:4n-6	0.37 ± 0.1	0.01± 0.0	0.01 ± 0.0
n-6 PUFA	18.78 ± 0.5[b]	18.58 ± 0.0[b]	23.67 ± 0.1[a]
18:3n-3	0.66 ± 0.0[a]	0.49 ± 0.0[c]	0.57 ± 0.0[b]
18:4n-3	0.70 ± 0.0[a]	0.71 ± 0.0[a]	0.62 ± 0.1[b]
20:3n-3	0.54 ± 0.0[a]	0.46 ± 0.0[b]	0.44 ± 0.0[b]
20:4n-3	0.33 ± 0.0	0.01± 0.0	0.01 ± 0.0
20:5n-3	4.88 ± 0.0[a]	4.73 ± 0.2[a]	3.57 ± 0.2[b]
22:5n-3	2.50 ± 0.0[a]	1.87 ± 0.1[b]	1.09 ± 0.2[c]
22:6n-3	12.73 ± 0.0[a]	7.93 ± 0.1[c]	9.41 ± 0.1[b]
n-3 PUFA	22.34 ± 0.0[a]	16.20 ± 0.3[b]	15.70 ± 0.1[c]
PUFA	41.12 ± 0.3[a]	34.78 ± 0.3[c]	39.36± 0.0[b]
LC-PUFA	22.05 ± 0.5[a]	15.60 ± 0.0[b]	15.18 ± 0.0[bc]
n-6 LC- PUFA	1.08 ± 0.7[a]	0.60 ± 0.3[c]	0.67 ± 0.2[b]
n-3 LC-PUFA	20.98 ± 0.6[a]	15.00 ± 0.0[b]	14.51± 0.0[c]

*** Values in the same row with different superscripts are significantly different (P<0.05)

digestibility values. The apparent digestibility of saturated fatty acids recorded a significant negative trend in relation to chain length. Individual dietary fatty acid digestibility increased with the degree of unsaturation. This trend was particularly apparent for SFA classes which increased (P<0.05) from 95.7 ± 0.0% to 96.1± 0.1%, as was also the case for PUFA (Figure 1). The PUFA/HUFA ratio increased considerably as the fish oil component of the diet was substituted with RO and CSO.

Discussion

The present study indicates that the fish oil component of practical diet for European sea bass can be replaced by MUFA-rich rapeseed oil with minimal adverse effects on growth, survival and proximate composition. In contrast, the 100% substitution of fish oil with cottonseed oil did result in reduced growth performance of sea bass. In a similar study on sea bass, Izquierdo *et al.* (2003) reported that the replacement of 60% of fish oil with linseed oil (n-3 PUFA-rich), soybean oil (n-6 PUFA-rich), rapeseed oil (MUFA-rich) and a mixed-blend did not negatively impact on fish performance, when high lipid content (25%) diets were evaluated. As in many instances, this result is similar to a number of the other earlier studies with European sea bass and salmonids where the complete replacement of dietary fish oil with alternative vegetable oils has resulted in no affect on fish growth performance (Guillou *et al.*, 1995; Tocher *et al.*, 2000; Torstensen *et al.*, 2000; Bell *et al.*, 2001, 2002, Mourente *et al.*, 2005b; Turchini *et al.*, 2011b; Eroldoğan *et al.*, 2012; Eroldoğan *et al.*, 2013).

In the present study, the final weight, weight gain and specific growth rate of the fish reared on the RO and FO diets were significantly higher than those fish fed the CSO diet. It is important to underline that diets used in the present study contained a relatively large amount of fish meal (510 g/kg), and thus contained some n-3 LC-PUFA (varying from 0.28 to 0.38 % of dry diet in CSO and RO, respectively). Even though all diets contained the same quantity of the fish meal, the CSO diet exhibited slightly lower growth performance than the other diets and can therefore be deemed unsuitable for complete fish oil substitution for sea bass. It is well known that MUFA-rich diets are more efficiently transformed into energy via the process of β-oxidation than n-6 PUFA-rich

Table 5. Fatty acid concentration in flesh including the difference (Δ) between diet and fatty acid values for FO, RO and CSO

Fatty Acid	ΔFO	ΔRO	ΔCSO
14:0	-0.88	0.85	0.48
16:0	1.44	5.01	-6.39
18:0	0.33	0.81	1.65
20:0	3.19	3.59	2.19
22:0	-1.58	0.71	0.98
SFA	2.25	10.80	-1.30
14:1	-0.12	-0.01	-0.02
16:1n-7	-4.50	-1.63	-1.72
18:1n-9	-0.64	-15.72	10.12
18:1n-7	-0.37	-0.74	0.98
20:1n-11	0.03	0.46	0.42
20:1n-9	-3.62	-1.60	-0.40
22:1n-11	0.12	0.05	0.05
MUFA	-12.58	-19.02	9.72
18:2n-6	5.15	-0.63	-22.78
18:3n-6	-0.02	-0.01	-0.11
20:2n-6	-0.53	-0.13	-0.07
20:4n-6	0.21	0.45	0.59
22:4n-6	0.01	-0.06	0.00
n-6 PUFA	4.43	-0.36	-22.33
18:3n-3	-1.96	-4.87	0.08
18:4n-3	-0.24	0.61	0.56
20:3n-3	0.31	0.41	0.42
20:4n-3	-0.62	-0.07	0.00
20:5n-3	-0.40	3.81	3.00
22:5n-3	0.44	1.60	0.27
22:6n-3	5.38	6.96	8.72
n-3 PUFA	2.90	8.46	13.5
PUFA	7.33	8.10	-8.84
LC-PUFA	5.77	12.99	13.37
n-6 LC- PUFA	0.66	0.28	0.50
n-3 LC-PUFA	5.11	12.71	12.87

Negative values Δ indicates lower values in flesh compared with diet whereas positive values indicate accumulation in flesh relative to diet.

oils as MUFA-rich oils are more digestible than n-6 PUFA-rich oils (Turchini *et al.,* 2011a; Eroldoğan *et al.,* 2013). Thus, this might in part explain the higher growth performance in fish fed the RO diet in the present study.

In the present study, by replacing fish oil with rapeseed oil, slight increases in HSI was observed which is in accordance with some other studies (Caballero *et al.,* 2004, Wassef *et al.,* 2007, Benedito-Palos *et al., 2*007, Piedecausa *et al.,* 2007, Leaver *et al.,* 2008). In many instances, when n-6 PUFA rich alternative oils were used to replace fish oil, the modification of the hepatic lipid content was recorded (Caballero *et al.,* 2004; Wassef *et al.,* 2007; Benedito Palos*et al.,* 2007; Piedecausa *et al.,* 2007; Leaver *et al.,* 2008; Eroldoğan *et al.,* 2012). Increased hepatic lipid deposition is commonly associated with the morphological alteration known as steatosis, which is due to increased synthesis and deposition of triacylglycerols in hepatocyte vacuoles (Montero and Izquierdo, 2010).

The fatty acid compositions of tissue lipids of cold/temperate species are known to be highly influenced by dietary fatty acids (Torstensen *et al.,* 2000; Rosenlund *et al.,* 2001; Eroldoğan *et al.,* 2012) and linear correlations exist between individual fatty acids in fillet total lipid and their concentrations in dietary lipids (Bell, *et al.,* 2001; Bell *et al.,* 2002). Previous studies have shown that although dietary fatty acids correlated to fatty acids deposited in flesh, specific fatty acids were selectively utilized or retained (Bell *et al.,* 2001; Bell *et al.,* 2002; Torstensen *et al.,* 2004). This was amply confirmed in the present study. It appeared that while dietary fatty acids influence fillet fatty acids, DHA was selectively retained in the muscle compared to other fatty acids. Higher levels of DHA in fish fillets in comparison to the concentration present in the diet was also observed in Atlantic salmon (Bell *et al.,* 2001, 2002), rainbow trout, *Oncorhynchus mykiss* (Caballero *et al.,* 2002) and turbot, *Psetta maxima* (Bell *et al.,* 1994; Regost *et al.,* 2003). The possible mechanisms underlying this selective deposition include the high specificity of fatty acyl transferase for DHA. Within this context, the results of the present study, in line with previous published studies, clearly suggest that some dietary fatty acids particularly MUFA can be preferentially β-oxidized when provided in dietary

Table 6. Mean percentage (± SE) apparent fatty acids digestibility of the vegetable oil blend diets.

	Diets		
	FO	RO	CSO
14:0	99.5±0.1[a]	93.4±0.4[c]	95.0±0.3[b]
16:0	95.7±0.1[ab]	94.2±0.5[b]	96.1±0.1[a]
18:0	94.0±0.1	93.8±0.8	92.8±0.2
20:0	94.1±0.0	93.1±1.6	92.9±0.3
22:0	99.4±0.0	98.8±0.5	98.7±1.0
SFA	96.1±0.1	96.0±0.6	95.7±0.0
14:1	96.7±0.2	89.5±9.1	92.0±1.2
16:1n-7	99.8±0.0[a]	95.2±0.4[b]	95.2±0.5[b]
18:1n-7	97.0±0.1[b]	99.2±0.1[a]	98.4±0.5[a]
18:1n-9	97.5±0.0[b]	98.6±0.1[a]	97.1±0.1[c]
20:1n-9	98.7±0.1[a]	98.4±0.3[a]	94.6±0.4[b]
22:1n-11	98.3±1.2[a]	78.2±11.4[b]	66.9±7.2[b]
MUFA	99.4±0.0[a]	99.2±0.3[a]	97.3±0.1[b]
18:2n-6	97.7±0.1[c]	98.2±0.2[b]	99.5±0.1[a]
18:3n-6	99.8±1.1[a]	75.8±1.4[b]	99.7±0.4[a]
20:2n-6	99.6±0.1[a]	82.5±6.5[b]	84.9±1.5[b]
20:3n-6	99.6±0.9[a]	87.4±0.0[b]	77.0±19.4[c]
20:4n-6	98.8±0.1[a]	73.7±8.1[b]	98.4±0.8[a]
22:4n-6	99.1±0.2[a]	69.0±0.0[b]	81.2±1.8[ab]
n-6 PUFA	98.8±0.1[a]	97.7±0.1[b]	97.8±0.2[b]
18:3n-3	99.8±0.1[a]	99.1±0.1[a]	96.5±0.2[b]
18:4n-3	99.6±0.1[a]	85.1±4.3[b]	95.4±02.7[b]
20:3n-3	99.8±0.7[a]	68.2±9.4[b]	98.3±4.8[a]
20:4n-3	99.7±0.5[a]	85.4±9.6[b]	99.0±0.0[a]
20:5n-3	98.9±0.0[a]	88.5±3.2[c]	94.8±0.4[b]
22:5n-3	99.6±0.6[a]	92.7±8.6[b]	96.1±1.6[ab]
22:6n-3	99.6±0.0[a]	94.3±1.4[b]	94.5±0.7[b]
n-3 PUFA	99.2±0.1[a]	96.0±1.0[b]	96.6±0.8[b]
PUFA	98.8±0.1[a]	97.1±0.0[b]	96.8±0.2[b]
n-6 LC-PUFA	98.2±0.1[a]	84.5±8.3[b]	90.5±10.0[a]
n-3 LC-PUFA	98.8±0.1[a]	94.0±3.2[b]	95.2±11.9[b]

Table 7. The *F* and *P* value of two-way ANOVA for dietary treatment and acid structure (and their interaction) on the apparent digestibility in Sea bass.

	Dietary treatment	FA structure	Interaction
Chain length (14:0>16:0>18:0> 20:0>22:0)	7.969***	37.512***	3.925***
Degreee of unsaturation (18:0>18:1>18:2>18:3>18:4)	9.787***	35.563***	12.725***
Position of first double bond (n-3>n-6>n-9)	7.000***	5.438**	12.971***
Fatty acid class (SFA>MUFA>PUFA>HUFA)	13.374***	19.817***	8.765***

P: *** <0.001; **<0.01

surplus (Turchini *et al.*, 2011c). Therefore, it is worth at this point to underline that DHA can be spared from catabolism and thereby significantly improve its deposition into the flesh, as was also observed in other experiments (Turchini *et al.*, 2011b; Eroldoğan *et al.*, 2012; Eroldoğan *et al.*, 2013).

Obviously, the fatty acid composition of a vegetable oil alternative diet differs dramatically from the fatty acid composition of the FO and hence, dietary inclusion of RO and CSO at the expense of FO will affect the flesh fatty acid composition of the fish. LA, LNA and oleic acid were preferentially utilized in flesh, when present at high concentrations in the diet, as reported previously (Bell *et al.*, 2002; Ng *et al.*, 2003). These data suggest that the monoene, oleic acid as well as LA and LNA, are readily oxidized when present in high concentrations. This difference between fillet oleic acid concentrations compared to dietary oleic concentration suggests an active biosynthesis (liponeogenesis) of this fatty acid. This observation is entirely consistent with the results of a previous study on the same species (Montero *et*

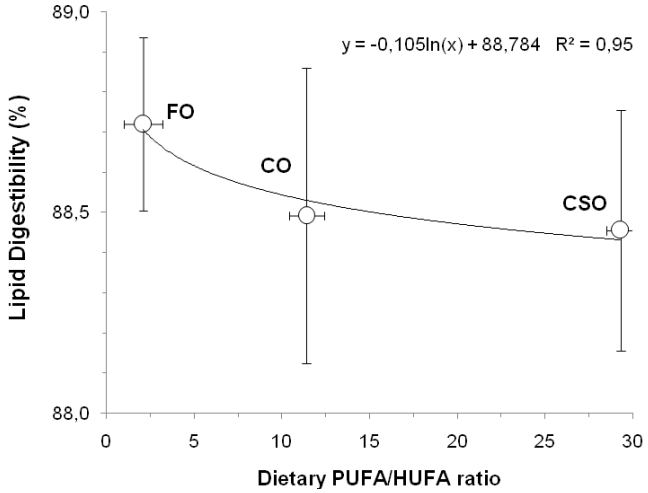

Figure 1. The apparent lipid digestibility in relation to the dietary PUFA/HUFA ratio. Dietary PUFA/HUFA ration for the FO, CO and CSO were 2.3, 12.8, 29.9 respectively.

al., 2005). As mentioned above, dietary fatty acids generally influence flesh fatty acids, but some specific fatty acids have been suggested to be selectively retained or deposited (Turchini and Francis 2009). This has also been observed in marine carnivorous species such as red sea bream, *Pagrus major* (Huang *et al.,* 2007); turbot (Bell *et al.,* 1994; Regost *et al.,* 2003) and European sea bass (Mourente *et al.,* 2005a). CSO is rich in saturated fatty acids and n-6 PUFA, largely 16:0 and 18:2n-6, respectively, and RO is rich in MUFA while it lacks n-3 HUFA, resulting in a substantially increased PUFA/ HUFA ratio. It is clearly evident in our study that a higher dietary content of 18:2n-6, and therefore higher PUFA/ HUFA ratio, resulted in lower β-oxidation and higher deposition of HUFA (mainly DHA).

Lipid digestibility can be affected by many factors, with the digestibility of individual fatty acids varying greatly (Olsen and Ringo, 1997). In fish it is well known that fatty acids digestibility decrease with chain length (Sigurgisladottir *et al.,* 1992; Turchini *et al.,* 2010). Early nutritional evidence suggested that fatty acid composition, particularly the degree of unsaturation and the chain length, determines the lipid melting point (Olsen and Ringo 1997). In the present study, regardless of the substitution level, fatty acids present in the highest concentrations in the test diets were 16:0, 18:1 n-9, 18:2 n-6 and 18:3 n-3, which have individual melting points of 61.0, 13.0, -5.0 and -11.0 °C, respectively. Thus the low melting point of MUFA rich oil is a good indication of the fact that they are potentially a viable source of easily digestible energy. Eroldoğan *et al.,* (2013) noted that the high melting point of 18:1n-9 compared to 18:2n-6 can explain the decreased overall apparent fatty acid absorption of LC-PUFAs in sea bass fed the canola diet compare to cottonseed oil and mixture of these two vegetable oil (w:w).

According to this information, the FO and vegetable oil sources were close to identical in their content of SFA, MUFA, n-3 and n-6 classes and that the principal difference was relative to a substantially increased PUFA/ HUFA ratio. Ultimately, the combination of chain length, degree of unsaturation and the melting point of individual fatty acids resulted in the apparent digestibility of SFA> MUFA> PUFA>HUFA and short chain>longer chain fatty acids, as reported extensively for several species (Turchini *et al.,* 2010; Francis *et al.,* 2007). This was the case in the present study, where the digestibility of saturated fatty acids for each of the dietary treatments was 14:0>16:0>18:0.>20.0>22:0. Fatty acid digestibility increased relative to the degree of unsaturation and PUFA/HUFA ration, as reported for various finfish species (Austreng *et al.,* 1980; Schwarz *et al.,* 1988; Olsen *et al.*, 1998; Røsjø *et al.,* 2000; Morais *et al.,* 2005; Turchini *et al.,* 2010; Francis *et al.*, 2007) and in this study.

In general, sn-1 to sn-3 positions of L-glycerol affects the lipid digestibility, i.e. SFA and MUFA are preferentially located in the sn-1 and sn-3 position of glycerol, whereas PUFA are preferentially located in the sn-2 position of glycerol (Sargent *et al.,* 2002). Therefore, highest lipid digestibility in fish fed with FO containing abundance of n-3 PUFA (especially EPA and DHA) can be explained by the dietary PUFA/ HUFA ratio which affects fatty acid hydrolysis and subsequent absorption. These results are in line with previous studies in mammals (Mu and Høy 2004), in rat (Brink *et al.,* 1995) and in fish (Koven *et al.,* 1994). In line with the previous studies, the lower lipid digestibility detected in fish fed with CSO was possibly associated with increased faecal lipid load (data not reported) and poor digestibility of saturates through poor lipid hydrolysis. The digestibility of individual fatty acids could have also been affected by other factors relative to emulsification, enzymatic hydrolysis, lipase specificities and micellar incorporation (Francis *et al.,* 2007).

In conclusion, the results of the present study, aiming to investigate the effects of PUFA/ HUFA

ratio in diets of European sea bass, showed no negative results in growth, feed utilization and proximate composition of the fish. This suggests that RO and CSO can be successfully used as a sustainable alternative to FO in European sea bass nutrition. However, the dietary RO and CSO inclusion resulted in significant changes in the FA compositions of the fish tissues. These changes, including reductions in n-3 LC-PUFA may have a serious impact on the quality of the final product, in terms of its nutritional value to the human consumer, although, it should be noted that the reduction in EPA and especially in DHA were only modest. Thus, it is important to carefully consider the inclusion levels of CSO to optimize sea bass diets due to its lower digestibility.

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