

Document downloaded from:

<http://hdl.handle.net/10251/140193>

This paper must be cited as:

Monge-Ortiz, R.; Tomas-Vidal, A.; Rodriguez-Barreto, D.; Martínez-Llorens, S.; Perez, J.; Jover Cerda, M.; Lorenzo, A. (2018). Replacement of fish oil with vegetable oil blends in feeds for greater amberjack (*Seriola dumerili*) juveniles: effect on growth performance, feed efficiency, tissue fatty acid composition and flesh nutritional value. *Aquaculture Nutrition*. 24(1):605-615. <https://doi.org/10.1111/anu.12595>



The final publication is available at

<https://doi.org/10.1111/anu.12595>

Copyright Blackwell Publishing

Additional Information

1 **Replacement of fish oil with vegetable oil blends in feeds for greater amberjack**  
2 **(*Seriola dumerili*) juveniles: effect on growth performance, feed efficiency, tissue**  
3 **fatty acid composition and flesh nutritional value.**

4

5 Raquel Monge-Ortiz<sup>1</sup>, Ana Tomás-Vidal<sup>1</sup>, Deiene Rodriguez-Barreto<sup>2</sup>, Silvia Martinez-  
6 Llorens<sup>1</sup>, Jose A. Pérez<sup>2</sup>, Miguel Jover-Cerdá<sup>1</sup>, Antonio Lorenzo<sup>2</sup>

7

8 <sup>1</sup>Research Group of Aquaculture and Biodiversity. Institute of Animal Science and  
9 Technology. Universitat Politècnica de València. Camino de Vera, 14. 46071 Valencia,  
10 Spain

11 <sup>2</sup>Departamento de Biología Animal, Edafología y Geología (U.D.I. Fisiología), Facultad  
12 de Ciencias, Sección Biología, Universidad de La Laguna. 38206 San Cristóbal de La  
13 Laguna, Tenerife, Spain

14

15

16

17

18 \* Corresponding author: R. Monge-Ortiz. Institute of Animal Science and Technology,  
19 Universitat Politècnica de València. Camino de Vera 14, 46071 Valencia. Tel: 34-  
20 963879752; Fax: 34-9638774. E-mail: ramonor@upv.es

21

22 **Abstract**

23 This study was undertaken to assess the effects of fish oil (FO) substitution by a mixture  
24 of alternative vegetable oils (VO) on *Seriola dumerili* culture performance. A 154-days  
25 feeding experiment was conducted using juveniles ( $39.2 \pm 1.6$  g average weight). Three  
26 isolipidic and isoenergetic meal-based diets were formulated varying their lipid  
27 component. The control diet contained 100% FO (FO100), whereas diets VO50 and  
28 VO100 included a 50% and 100% blend of palm oil (PO) and linseed oil (LO) as  
29 substitute for FO, respectively.

30 Dietary regime did not significantly affect growth performance, biometric indices, feed  
31 efficiency, plasma chemistry and liver and muscle lipid contents. Nonetheless, dietary  
32 VO inclusion impacted the fatty acid profile of target tissues, especially in the liver.  
33 Fatty acid profiles of the fillets reflected those of the dietary oils except that there was  
34 apparent selective utilization of palmitic acid (C16:0) and oleic acid (C18:1n-9) and  
35 apparent selective retention of long chain polyunsaturated fatty acids, especially  
36 eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3).

37 The nutritional value and the potential ability to prevent the development of coronary  
38 heart diseases of the flesh lipid fraction decreased with gradual FO substitution.

39

40 **Key words:** alternative oil sources, fatty acid composition, fish oil substitution, greater  
41 amberjack, palm oil, linseed oil.

## 42 **1. Introduction**

43 Marine fish oils (FO) have conventionally been used as the major dietary lipid  
44 component in aquaculture feeds, especially for fast-growing marine carnivorous fish  
45 which require the supply of long chain polyunsaturated fatty acids (LC-PUFA) such as  
46 eicosapentaenoic acid (20:5n-3, EPA), docosahexaenoic acid (22:6n-3, DHA), and  
47 arachidonic acid (20:4n-6, AA), considered essential fatty acids (EFA) for most marine  
48 finfish species. Supplying EFA-balanced diets is indispensable to sustain not only  
49 growth, survival and feed efficiency but also health and flesh nutritional quality in  
50 cultured specimens (Sargent *et al.* 2002; Tocher 2010).

51 Formulating suitable compound feeds is currently one of the main challenges for the  
52 aquaculture industry. The fast expansion of aquaculture production worldwide and the  
53 increasing demand of marine products along with the declining availability of fish meal  
54 (FM) and FO, make both economically and environmentally unsustainable to rely on  
55 finite marine natural resources (Kaushik *et al.* 2004; Tacon & Metian 2008).

56 Consequently, replacement of marine ingredients by terrestrial sources in aquafeeds is  
57 being a fairly widespread practice looking for suitable alternatives for the long-term  
58 sustainability of the aquaculture industry and vegetable oils (VO) have received an  
59 important attention as substitutes of the marine oil due to their comparative reduced  
60 cost, lower concentration of dioxins and other organic pollutants, and their suitable  
61 production levels (Sales & Glencross 2011). Numerous of these studies have covered a  
62 wide variety of fish species such as gilthead seabream (Benedito Palos *et al.* 2007,  
63 2008; Fountoulaki *et al.* 2009), European seabass (Izquierdo *et al.* 2003; Mourente &  
64 Bell 2006), red sea bream (Huang *et al.* 2007), turbot (Regost *et al.* 2003), cobia  
65 (Trushenski *et al.* 2011) and Atlantic salmon (Torstensen *et al.* 2000; Ruyter *et al.*  
66 2006). Little or no effect on fish performance has been observed in most of these

67 investigations as far as the minimum EFA requirements were covered. Nonetheless, fish  
68 fed VO have shown important modifications in their tissue fatty acid (FA) composition,  
69 including increased levels of C18 PUFA and reduced proportions of n-3 LC-PUFA,  
70 especially EPA and DHA, which may affect not only fish health (Bell *et al.* 2001; Bell  
71 & Sargent 2003; Alves-Martins *et al.* 2012) but also compromise the nutritional quality  
72 of flesh for human consumption, since n-3 LC-PUFA are human health-promoting  
73 compounds (Simopoulos 2008, 2011, 2016; Siriwardhana *et al.* 2012; Khankari *et al.*  
74 2015).

75 A blend of palm oil (PO) and linseed oil (LO) at a proportion of 4:1 was used in our  
76 present work to minimize potential changes derived from dietary substitution of FO. PO  
77 has high levels of C16 saturated fatty acids (SFA) and C18 monounsaturated fatty acids  
78 (MUFA), which are preferred substrates for energy production in fish species favoring  
79 diet-to-tissue transfer of LC-PUFA (Kiessling & Kiessling 1993; Henderson 1996)  
80 whereas LO is rich in PUFA, especially linolenic acid (C18:3n-3) which may result in  
81 tissues and organs of more favorable balanced FA. This combination of VOs should  
82 supply sufficient energy to maintain high growth, an n-6/n-3 PUFA ratio < 1 which is  
83 regarded as beneficial to human health and should not be detrimental for fish health  
84 (Bell *et al.* 2003b), and moderate levels of linoleic acid (C18:2n-6) trying to avoid an  
85 excessive deposition of this fatty acid which is reported as one of the most negative  
86 indicators to be taken into account when evaluating alternative lipid sources to FO for  
87 aquafeeds (Turchini *et al.* 2009).

88 The Carangidae family is a group of fish with exceptional consumer acceptance,  
89 considered of great potential for aquaculture diversification (updated by Sicuro &  
90 Luzzana 2016). Recently, several species within this family have been abundantly  
91 targeted for research, including the effects of replacing marine ingredients by terrestrial

92 sources in yellowtail kingfish (*Seriola lalandi*) (Bowyer *et al.* 2012a, b, 2013; Collins *et*  
93 *al.* 2014), Japanese yellowtail (*Seriola quinqueradiata*) (Seno-O *et al.* 2008; Sarker *et*  
94 *al.* 2012; Khaoian *et al.* 2014; Nguyen *et al.* 2015) and pompano (*Trachinotus spp.*)  
95 (Lech & Reig 2012; Lin *et al.* 2012; Rossi & Davis 2012). A further carangid species,  
96 the greater amberjack, *Seriola dumerili*, is a carnivorous pelagic fish with a broad  
97 geographical distribution, fast growth rate and large size which makes it suitable for  
98 product diversification and development of value-added products, excellent flesh quality  
99 and high market price (Nakada 2000). However, very scarce knowledge about EFA  
100 requirements or FO substitution in this species is available, being the studies published  
101 till date focused on the optimization of protein inclusion rates and the search of  
102 alternative plant protein sources to FM (Tomás *et al.* 2005; Takakuwa *et al.* 2006; Vidal  
103 *et al.* 2008; Uyan *et al.* 2009).

104 Therefore, the present study was conducted to determine whether partial (50%) or total  
105 dietary FO substitution by a blend of PO and LO (4:1) affects growth performance, feed  
106 efficiency, plasma chemistry and the degree of modification of the FA profile of liver  
107 and muscle of greater amberjack (*S. dumerili*) juveniles, including flesh lipid nutritional  
108 value. To the best of our knowledge, the present work may be considered as the first  
109 attempt to assess on the impact of FO replacement in this species.

110

## 111 **2. Materials and methods**

### 112 2.1 Fish and rearing conditions

113 A total of 185 *S. dumerili* juveniles were obtained from a fish farm (Futuna Blue S.A.  
114 Cádiz, Spain) and transported to the Fish Nutrition Laboratory of Universitat  
115 Politècnica de València (UPV, Spain). Prior to the feeding trial, fish were acclimatized  
116 to the experimental rearing conditions for four weeks by feeding a standard commercial

117 diet. After this period, groups of 20 fish (average weight  $39.2 \pm 1.6$  g) were randomly  
118 distributed into nine 1750-L cylindrical fibreglass tanks, three tanks per treatment.  
119 The culture was carried out under natural photoperiod conditions in a re-circulating  
120 seawater system of  $75 \text{ m}^3$  capacity equipped with a rotary mechanical filter and a  
121 gravity bio-filter ( $6 \text{ m}^3$ ). During the course of the trial, water temperature ( $21.5 \pm 2.4$   
122 °C), salinity ( $31.5 \pm 4.1 \text{ g L}^{-1}$ ), pH levels (7.5-8.0) and dissolved oxygen ( $6.6 \pm 1.3 \text{ mg}$   
123  $\text{L}^{-1}$ ) were monitored daily.

124

## 125 2.2. Experimental diets and feeding regime

126 Three iso-lipidic and iso-energetic practical feeds were formulated to contain 51% crude  
127 protein and 14% crude lipid in a dry weight basis. All ingredients were weighed  
128 individually before thoroughly mixed with water to form homogeneous dough and  
129 pelleted using a semi-industrial twin-screw extruder (CLEXTRAL BC-45, St. Etienne,  
130 France) at the Institute of Animal Science and Technology (UPV). All diets were stored  
131 at  $-20^\circ\text{C}$  for the duration of the trial. Fish were fed by hand to apparent satiation one of  
132 the three experimental diets for 154 days, twice a day (09:00 h and 17:00 h), 6 days a  
133 week. Any uneaten feed was collected daily to determine fish feed intake (FI).

134 The ingredients, proximate and FA composition of the experimental diets are shown in  
135 Table 1. Briefly, the diet containing FO as the sole lipid source was used as the  
136 reference diet (FO100) whereas a blend of VO consisting of PO and LO (4:1) replaced  
137 50% and 100% of the FO in the VO50 and VO100 diets, respectively. In all diets, 16:0  
138 accounted for the bulk of saturated fatty acids (SFA), 18:1n-9 for monounsaturated fatty  
139 acids (MUFA), 18:2n-6 for n-6 PUFA, and EPA and DHA for n-3 LC-PUFA.  
140 Moreover, gradual inclusion of the VO mixture increased dietary C16:0 and total SFA  
141 (30.2 to 36.1% of total FA), C18:1n-9 and total MUFA (22.4 to 29.3%), and C18:2n-6

142 and total n-6 PUFA (8.2 to 12.3%) while decreased EPA, DHA and total n-3 PUFA  
143 (32.4 to 19.1%) despite C18:3n-3 raised from 1.1 to 6.0% of total FA. DHA/EPA and  
144 EPA/ARA ratios remained unchanged among diets (Table 1).

145

### 146 2.3. Fish sampling and growth evaluation

147 Fish were anaesthetized with 10 mg L<sup>-1</sup> clove oil containing 87% eugenol (Guinama®,  
148 Valencia, Spain) for individual weight and fork length measurements at the beginning,  
149 end, and regularly at 30 days-intervals after the start of the feeding trial. In addition, at  
150 the end of the experiment eight fish from each treatment were collected for blood, liver  
151 and muscle sampling. Blood was drawn via the ventral aorta using 5-mL heparinized  
152 syringes, centrifuged at 3000 g for 5 min at 4 °C to separate the plasma which was  
153 stored at -30 °C until further analyses. Next, the fish were euthanized with an overdose  
154 of clove oil and portions of liver and dorsal muscle rapidly excised, frozen in liquid  
155 nitrogen and stored at -80 °C for subsequent biochemical determinations.

156 The effect of dietary treatments on culture performance was determined by evaluating  
157 growth, survival and nutrient utilization indices, including weight gain (WG), specific  
158 growth rate (SGR), feed intake (FI) and feed conversion ratio (FCR) at the end of the  
159 feeding trial (Table 2).

160 All procedures were carried out in accordance to the European Directive 2010/63/EU  
161 and Spanish national legislation (Spanish Royal Decree 53/2013), which regulate  
162 animal usage in experimentation and/or other scientific purposes.

163

### 164 2.4. Analytical procedures

165 Plasma glucose concentration (mg dL<sup>-1</sup>), activities of glutamate–oxalacetate  
166 transaminase GOT (AST) (EC 2.6.1.1) and glutamate–pyruvate transaminase GPT



167 (ALT) (EC 2.6.1.2) ( $\text{U L}^{-1}$  37 °C) were determined by enzymatic kits according to the  
168 manufacturer's instructions (Human, Wiesbaden, Germany). One unit (U) of  
169 aminotransferases activity was defined as 1  $\mu\text{mol}$  of NADH disappearance per minute.  
170 Concentrations of triglyceride ( $\text{mg dL}^{-1}$ ) and cortisol ( $\text{ng mL}^{-1}$ ) were measured with a  
171 diagnostic kit (Gernon, Barcelona, España) and an enzyme immunoassay kit (Arbor  
172 Assays, MI, USA), respectively. Lipase (E.C. 3.1.1) activity ( $\text{U L}^{-1}$  30 °C) was assayed  
173 by slight modifications of the method previously described by Gisbert et al. (2009)  
174 considering one unit of activity equivalent to 1  $\mu\text{mol}$  of p-nitrophenol myristate  
175 hydrolyzed per min.

176 Proximate composition of the experimental diets and whole body fish were determined  
177 according to the following procedures: moisture by oven thermal drying at 110 °C to  
178 constant weight, ash by combustion in a muffle at 550 °C overnight, and crude protein  
179 ( $\text{N} \times 6.25$ ) by sample digestion using the Kjeldhal method. Quantification of crude fat  
180 was performed by ether extraction with an Ankom XT10 Extraction System (NY, USA)  
181 (AOCS, 2005). Energy was calculated according to Brouwer (1965), from the C (g) and  
182 N (g) balance ( $\text{GE} = 51.8 \times \text{C} - 19.4 \times \text{N}$ ).

183 Liver and muscle total lipid (TL) was extracted by homogenization in  
184 chloroform/methanol (2:1, v/v) according to Folch *et al.* (1957). The organic solvent  
185 was evaporated under a stream of nitrogen, the lipid content gravimetrically determined  
186 (Christie 1982) and stored in chloroform/methanol (2:1) containing 0.01% butylated  
187 hydroxytoluene (BHT) at -20°C until further analysis. The lipid extract was subjected to  
188 acid-catalyzed transmethylation with 1% sulphuric acid (v/v) in methanol, and the  
189 resultant fatty acid methyl esters (FAME) purified by thin layer chromatography (TLC)  
190 (Christie 1982). During acid-catalyzed transmethylation, FAMES are formed  
191 simultaneously with dimethyl acetals (DMAs) which originate from the 1-alkenyl chain

192 of plasmalogens. FAME and DMA were separated and quantified on a TRACE-GC  
193 Ultra gas chromatograph (Thermo Scientific, Milan, Italy) equipped with an on-column  
194 injector, a flame ionization detector and a fused silica capillary column, Supelcowax  
195 TM 10 (30 m x 0.32 mm x 0.25  $\mu\text{m}$  film thickness) (Supelco Analytical, Bellefonte, PA,  
196 USA). Helium at a flow of 1.5 mL  $\text{min}^{-1}$  was used as the carrier gas. Individual FAME  
197 and DMA were identified by reference to authentic standards, and further confirmation  
198 of identity was carried out by mass spectrometry when necessary.

199

### 200 2.5 Indices of the nutritional quality of lipids

201 The influence of increasing levels of FO substitution on the nutritional quality of the  
202 fish fillet lipid fraction was monitored through indices based on the functional effects of  
203 its constituent FA. Equations [1-3] were used to determine the index of atherogenicity  
204 (IA) (Ulbricht and Southgate, 1991), the index of thrombogenicity (IT) (Ulbricht and  
205 Southgate, 1991), and the flesh lipid quality (FLQ) (Abrami *et al.* 1992), respectively.

$$206 \quad [1] \text{ IA} = [(C12:0 + (4 * C14:0) + C16:0)] \\ 207 \quad / [\sum \text{MUFA} + n6 \text{ PUFA} + n3 \text{ PUFA}]$$

$$208 \quad [2] \text{ IT} = (C14:0 + C16:0 + C18:0) \\ 209 \quad / [(0.5 * \sum \text{MUFA} + 0.5 * n6 \text{ PUFA} + 3 * n3 \text{ PUFA}) + (n3 \text{ PUFA} / n6 \text{ PUFA})]$$

$$210 \quad [3] \text{ FLQ} = (C20:5n-3 + C22:6n-3) / (\sum \text{total FA})$$

211 Briefly, the two first indices indicate that C12:0, C14:0 and C16:0 are atherogenic  
212 (favouring the adhesion of lipids to cells of the immunological and circulatory systems),  
213 and that C14:0, C16:0 and C18:0 are thrombogenic, facilitating the formation of clots in  
214 the blood vessels. The third equation, reveals the percentage relationship in which the  
215 main n-3 LC-PUFA (EPA and DHA) appear in muscle with respect to the totality of the  
216 lipids.

217

## 218 2.6. Statistical analysis

219 Prior to analysis, all data expressed as percentage were arcsine-transformed. Normal  
220 distribution was checked with the Kolmogorov–Smirnov test and homogeneity of  
221 variances by the Levene test. Comparisons among dietary groups (FO100, VO50 and  
222 VO100) were assessed by one-way ANOVA and significant differences identified by  
223 the Tukey post hoc test. When homoscedasticity and/or normality was not achieved,  
224 data were subjected to the non-parametric Kruskal–Wallis test followed by the Games–  
225 Howell test for post-hoc comparisons. Differences among means were accepted when  
226  $P < 0.05$ . Statistical analyses were carried out using the SPSS package version 20.0 for  
227 Windows (SPSS, Inc., Chicago, IL, USA).

228

## 229 **3. Results**

### 230 3.1. Growth performance and feed utilization

231 At the end of the feeding period, no negative effects were found with either the partial  
232 (50%) or complete FO substitution with the mixture of PO and LO (4:1) in growth and  
233 feed performance, although values were generally numerically inferior in fish fed the no  
234 FO-diet. Briefly, all dietary groups presented similar final body weight (390, 397 and  
235 375 g for FO100, VO50 and VO100, respectively) which resulted in steady weight  
236 gains of 894, 940 and 840%, respectively. All diets were readily accepted by the fish,  
237 with the mean daily FI being  $1.81 \text{ g } 100 \text{ g fish day}^{-1}$ , and the average FCR, 1.74. Final  
238 survival rate was 75% for all dietary groups (Table 2).

239

### 240 3.2. Biometric parameters and body proximate composition

241 None of the somatic parameters studied (condition factor, viscerosomatic,  
242 hepatosomatic and mesenteric fat indices, ingested fat retention and ingested energy  
243 retention) significantly varied with increasing FO replacement (Table 3). Similarly, no  
244 trend in protein, lipid or ash of fish whole-body was apparent in dietary groups. Only  
245 moisture content varied among treatments, being significantly lower in fish fed the  
246 control diet (FO100) than in those receiving VO50.

247

### 248 3.3. Plasma biochemical determinations

249 As it is shown in Table 4, glucose, triglycerides and cortisol concentrations remained  
250 fairly constant among treatments (186-223 mg dL<sup>-1</sup>, 89-98 mg dL<sup>-1</sup>, and 54-56 ng mL<sup>-1</sup>,  
251 respectively). Likewise, the activities of the enzymes GOT (10.9-20.6 U L<sup>-1</sup>), GPT (3.9-  
252 6.1 U L<sup>-1</sup>) and lipase (7.1-7.8 U L<sup>-1</sup>) were not affected by the diet.

253

### 254 3.4. Tissue biochemical composition

255 The TL contents of liver and muscle did not vary among treatments, neither when  
256 compared to the initial sample, although the liver presented significantly higher values  
257 than muscle ranging from 7.9 to 8.8% of fresh weight, and 0.7 to 0.9% of fresh weight,  
258 respectively (Figure 1). Both tissues followed similar patterns of FA profiles and  
259 variations with respect to the initial sample in response to increasing FO substitution  
260 (Tables 5 and 6, respectively). Briefly, despite the relative proportion of C16:0 was  
261 higher in fish fed the no FO diet (VO100), no significant variations among treatments  
262 existed in the total percentage of SFA. Total MUFA raised significantly with higher VO  
263 inclusion whereas total PUFA, n-6 and n-3 LC-PUFA showed the opposite trend.  
264 Individually, C18:1n-9 (which represented 50-80% of total MUFA), C18:2n-6 and  
265 C18:3n-3 were higher when complete FO substitution, whereas ARA, EPA, C22:5n-3

266 (DPA, docosapentaenoic acid) and DHA, reached higher values in fish fed the 100%  
267 FO-diet. Hepatic DHA/EPA ratio increased and EPA/ARA ratio decreased with reduced  
268 dietary FO (Table 5), which, conversely, remained unchanged in muscle (Table 6).  
269 Muscle and liver showed a tissue-specific fatty acid profile, with muscle containing  
270 lower proportions of MUFA, and higher PUFA, n-3 and n-6 LC-PUFA than liver. DMA  
271 were present exclusively in muscle (2.5 to 3.0% of total FA). Irrespective to diet, C18  
272 MUFA and C18:2n-6 proportions were 1.5 to 2-fold lower in muscle than in the liver  
273 (5.8, 9.4 and 14.0 vs 11.2, 19.8 and 26.8; 4.8, 7.2 and 10.7 vs 9.7, 12.4 and 15.3,  
274 respectively), whereas C22:6n-3 was 3 to 4-fold higher in the muscle (29.6, 26.7 and  
275 22.0 vs 9.9, 7.2 and 5.5% of total FA, respectively).

276

### 277 3.5 Indices of the nutritional quality of lipids

278 The indices used to assess the nutritional value of the flesh lipid fraction are shown in  
279 Table 6. Both PUFA/SFA and n-6/n-3 ratios were more favorable in terms of nutritional  
280 value in fish fed the diet with FO as the unique lipid source, decreasing with higher  
281 inclusion of the VO mixture. IA remained unchanged irrespective of dietary FO  
282 substitution (0.42-0.43) whereas complete FO replacement promoted a significant  
283 increase in IT ( $0.23 \pm 0.01$ ) compared to FO100 and VO50-fed fish ( $0.20 \pm 0.01$  and  
284  $0.21 \pm 0.01$ , respectively). Finally, FLQ decreased with gradual FO replacement ( $39.6 \pm$   
285  $2.0$ ,  $34.7 \pm 4.0$  and  $28.3 \pm 4.3$ , respectively).

286

## 287 **4. Discussion**

288 In the present study, the plant-based oil mixture consisting of PO and LO (4:1) used to  
289 partially (50%) or totally substitute FO did not significantly affect greater amberjack, *S.*  
290 *dumerili* growth performance and feed efficiency (Table 2). Both SGR and FCR of fish

291 receiving the VO-based blend are similar or even improve most values reported for fish  
292 of the same size class cultured in PVC tanks or floating cages fed fish scraps or FO-  
293 based diets in the western Mediterranean coast (reviewed by Mazzola *et al.* 2000).

294 A number of previous studies have reported that a large fraction (60-70%) of dietary FO  
295 may be replaced by VO blends without compromising fish production (Izquierdo *et al.*  
296 2003; Menoyo *et al.* 2004; Mourente & Bell 2006; Benedito-Palos *et al.* 2008; Peng *et*  
297 *al.* 2008; Fountoulaki *et al.* 2009). However, some species are negatively affected by  
298 total substitution of FO (Regost *et al.* 2003; Sales & Glencross, 2011; Nasopoulou &  
299 Zabetakis, 2012) while other reports show no effect (Glencross *et al.* 2016;  
300 Mozanzaded *et al.* 2016) so it became necessary to study carefully FO substitution  
301 effects for any particular fish species. Big pelagic marine carnivorous fish species such  
302 as *S. quinqueradiata* did not vary growth performance when receiving diets with  
303 increasing olive oil inclusion to completely replace FO (Seno-O *et al.* 2008) in a short-  
304 term feeding trial of 40 days. On the contrary, both cobia (*Rachycentron canadum*) and  
305 yellowtail kingfish (*S. lalandi*) juveniles production performance was compromised  
306 when FO was totally substituted by sunflower or canola oil, respectively (Trushenski *et*  
307 *al.* 2011; Bowyer *et al.* 2012a). Overall, successful fish performance may be achieved  
308 when FO sparing with alternative oils of terrestrial origin as long as their minimum  
309 EFA requirements are met. In our work, FO100, VO50 and VO100 diets provide 2.7,  
310 2.1 and 1.2% n-3 LC-PUFA of dry matter respectively, which is sufficient to cover the  
311 EFA requirements for most marine fish species (Glencross 2009; Tocher 2010).

312 Consequently, although *S. dumerili* nutritional requirements are still unknown and the  
313 EFA requirements vary qualitatively and quantitatively with both species and growth  
314 stage, it seems that formulation with 525 g kg<sup>-1</sup> of FM contributes to supply enough LC-  
315 PUFA to meet fish needs even in the absence of FO, since FM usually contains up to 8-

316 15% of crude lipid, with a 30-35% of n-3 LC-PUFA (Bimbo 2000). In fact, our present  
317 results seem to indicate that the EFA requirements of greater amberjack juveniles may  
318 be met by levels of n-3 LC-PUFA up to 1.2% of the dry weight of the diet. As far as we  
319 know, this is the first reference on the quantitative EFA requirements for this species.

320 Regardless of whether FO replacement affects fish growth and feed performance, its  
321 impact on tissue lipid deposition and fatty acid composition is controversial, varying  
322 depending on the species, dietary lipid content and substitute lipid source (Turchini *et*  
323 *al.* 2009). Previous research suggest that SFA and MUFA-rich lipid diets can make LC-  
324 PUFA utilization and/or diet-to-tissue transfer more efficient (Turchini *et al.* 2009;  
325 Pérez *et al.* 2014; Bowzer *et al.* 2016). The PO:LO (4:1) mixture used here seem to  
326 provide balanced proportions of SFA: MUFA: PUFA and n-6/n-3 ratio for maintaining  
327 or even improving DHA/EPA and EPA/ARA ratios in muscle (3.3 and 5.6 for VO50;  
328 3.5 and 5.7, for VO100, respectively) with respect to the initial fish (2.32 and 7.48) and  
329 fish receiving the 100% FO diet (3.0 and 5.8, respectively). The same tendency for both  
330 proportions was observed in the liver of VO-fed groups (0.76 and 9.10; 0.74 and 10.58;  
331 0.88 and 8.81; 1.20 and 7.68; for the initial, FO100, VO50 and VO100 fish,  
332 respectively). In addition, physiologically important DHA/EPA and EPA/ARA ratios  
333 obtained in our present work are similar to those previously reported for farmed greater  
334 amberjack adults and similar to wild counterparts (Rodriguez Barreto *et al.* 2012; Saito  
335 2012).

336 The liver is the major site of lipid storage in the majority of marine fish species being  
337 commonly used as indicator of unsuitable dietary fat ingestion. The diagnosis of healthy  
338 liver should allow optimized diets to be devised for a given species. It is well  
339 established that replacing dietary FO by terrestrial oils may produce the accumulation of  
340 fat in fish liver giving rise to a fatty liver syndrome (Sargent *et al.* 2002; Piedecausa *et*

341 *al.* 2007; Benedito-Palos *et al.* 2008; Díaz López *et al.* 2010), which may be associated  
342 with increased lipid peroxidation and impaired function such as inefficient nutrient  
343 utilization and necrosis (Tucker *et al.* 1997; Craig *et al.* 1999). In our study, both the  
344 liver fat content and the HSI of VO50 and VO100-fed fish were similar to the control  
345 and initial fish, suggesting no hepatic affection with increasing levels of PO:LO  
346 inclusion. These observations agree well with previous research on turbot (*Psetta*  
347 *maxima*) (Regost *et al.* 2003), European seabass (Richard *et al.* 2006) and gilthead sea  
348 bream (Bouraoi *et al.* 2011) where no impairment of lipogenic activity and lipid content  
349 in fish liver was detected when using PO and/or LO to replace FO. In line with this,  
350 Lemaire *et al.* (1991) found correlations between plasma biochemical parameters and  
351 hepatic histopathological condition. Thus, plasma parameters are often regarded as  
352 suitable monitoring tools of the physiological status of the fish (Coz-Rakovac *et al.*  
353 2008; Díaz López *et al.* 2009; Bowyer *et al.* 2012a; Kowalska *et al.* 2012) and could  
354 also be used as physiological indicators of lipogenesis affection with FO substitution  
355 (Richard *et al.* 2006). Under our experimental conditions, the inclusion of PO and LO  
356 did not affect plasma chemistry suggesting that fish were in acceptable nutritional status  
357 adding more evidences to the proper hepatic functioning even under FO absence.  
358 However, the higher relative content of C18:1n-9 and C18:2n-6, along with lower  
359 proportions of LC-PUFA, especially ARA, EPA and DHA, in the liver of VO-fed fish  
360 might have a long-term detrimental impact on lipid/lipoprotein metabolism, since they  
361 have been reported to modulate lipid metabolism at different levels (reviewed by  
362 Turchini *et al.* 2009). Thus, longer-term studies are needed to rule out possible hepatic  
363 damage caused by the PO:LO mixture not detected in the present 5 months-feeding  
364 trial.



365 Regardless of dietary inputs, muscle displayed higher relative content of n-3 LC-PUFA  
366 than the liver or diet. This indicates that LC-PUFA, particularly DHA, are selectively  
367 retained in greater amberjack fillets, as previously reported in salmon (Bell *et al.* 2001,  
368 2003a; Torstensen *et al.* 2004), and other marine fish species (Mourete & Bell 2006;  
369 Bowyer *et al.* 2012a; Pérez *et al.* 2014). The high supply of SFA, especially C14:0 and  
370 C16:0, and MUFA chiefly C18:1n-9, in VO50 and VO100 diets may have promoted  
371 their preferential use as metabolic energy for swimming (Mckenzie 2001; Bell *et al.*  
372 2003a; Torstensen *et al.* 2004; Stubhaug *et al.* 2007) enhancing muscle deposition of  
373 LC-PUFA.

374 There is currently increasing interest on the intake of marine-based feedstuff for its  
375 health-promoting benefits to humans. Several FA ratios and indices have been defined  
376 to assess the nutritional quality of food lipid for human consumption. According to  
377 nutritional recommendations, the PUFA/SFA ratio in human diets should be above 0.45  
378 (Wood *et al.* 2004) and, within the PUFA, a ratio of 1:1 to 2:1 n-6/n-3 should be the  
379 target ratio for health (Simopoulos 2011). Lower ratios of PUFA/SFA in the diet may  
380 increase the incidence of cardiovascular disease (WHO 2003). Further, fats with lower  
381 indices of atherogenicity (IA) and thrombogenicity (IT) can inhibit the aggregation of  
382 platelets and decrease the levels of esterified FA, cholesterol and phospholipids, thereby  
383 preventing the appearance of micro and macrocoronary diseases (Turan *et al.* 2007).  
384 The indices of lipid quality selected in the present work clearly indicate that flesh from  
385 greater amberjack juveniles is a nutritionally adequate food for human consumption  
386 although the gradual inclusion of the PO:LO mixture tended to partially reduce its  
387 value. In brief, and regardless of dietary treatment, both PUFA/SFA and n-6/n-3 are  
388 well within values recommended for healthy human. Although there is no  
389 recommended values for IA and IT, it is generally accepted that the lower the values the

390 healthier the ratios. So, the low values of both IA and IT indices together with high FLQ  
391 present in flesh suggest that its consumption may help to prevent the development of  
392 coronary heart diseases, being more favorable in terms of lipid quality for human  
393 consumption than gilthead seabream or European seabass (Grigorakis 2007; Pérez *et al.*  
394 2014).

395 In summary, the present work provides valuable information to the successful and  
396 economically viable culture of greater amberjack. The mixture of PO and LO (4:1) can  
397 effectively replace completely dietary FO in FM-based diets for *S. dumerili* juveniles  
398 without affecting growth performance, feed utilization and fish health. Based on these  
399 results, it appeared that a 1.2% of EFA in a dry weight basis may cover the EFA  
400 requirements for juveniles of this species. In terms of product quality, and regardless of  
401 dietary lipid, flesh of cultured specimens displayed good nutritional and healthy  
402 characteristics for human consumption, in line with current global guidelines for fat  
403 intake.

404

## 405 **5. Acknowledgments**

406 This study was supported by the grant from Ministerio de Ciencia e Innovación  
407 (MICINN) (Ref.: AGL2011-30547-C03-02). We would also like to thank LÍPIDOS  
408 SANTIGA, S.A. (Barcelona, Spain) for providing us the palm oil used in the present  
409 study.

410

## 411 **6. References**

412 Abrami, G., Natiello, F., Bronzi, P., McKenzie, D., Bolis, L. & Agradi, E. (1992) A  
413 comparison of highly unsaturated fatty acid levels in wild and farmed eels (*Anguilla*  
414 *anguilla*). *Comp. Biochem. Physiol. B*, **101**, 79–81.

415 Alves-Martins, D., Rocha, F., Martínez-Rodríguez, G., Bell, G., Morais, S.,  
416 Castanheira, F., Bandarra, N., Coutinho, J., Yúfera, M. & Conceição, L.E. (2012)  
417 Teleost fish larvae adapt to dietary arachidonic acid supply through modulation of  
418 the expression of lipid metabolism and stress response genes. *Br. J. Nutr.*, **108(5)**,  
419 864-74.

420 AOCS Official Procedure, Approved Procedure Am 5-04 (2005) Rapid determination of  
421 oil/fat utilizing high temperature solvent extraction. American Oil Chemists Society,  
422 Urbana, IL, USA.

423 Arts, M.T. & Kohler, C.C. (2009) Health and condition in fish: the influence of lipids  
424 on membrane competency and immune response. In: *Lipids in aquatic ecosystems*  
425 (Arts, M.T., Brett, M.T. & Kainz, M.J. Eds), pp. 237-256. Springer, New York,  
426 USA.

427 Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., Sargent, J.R. (2001)  
428 Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*)  
429 affects tissue lipid compositions and hepatocyte fatty acid metabolism. *J. Nutr.*,  
430 **131(5)**, 1535-1543.

431 Bell, J.G. & Sargent, J. R. (2003) Arachidonic acid in aquaculture feeds: current status  
432 and future opportunities. *Aquaculture*, **218(1)**, 491-499.

433 Bell, J.G., McGhee, F., Campbell, P.J. & Sargent, J.R. (2003a) Rapeseed oil as an  
434 alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*):  
435 changes in flesh fatty acid composition and effectiveness of subsequent fish oil  
436 “wash out”. *Aquaculture*, **218**, 515–528.

437 Bell, J.G., Tocher, D.R., Henderson, R.J., Dick, J.R. & Crampton, V.O. (2003b)  
438 Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing

439 linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing  
440 diet. *J. Nutr.*, **133**(9), 2793-2801.

441 Bell, J.G., Strachan, F., Good, J.E. & Tocher, D.R. (2006) Effect of dietary echium oil  
442 on growth, fatty acid composition and metabolism, gill prostaglandin production and  
443 macrophage activity in Atlantic cod (*Gadus morhua* L.). *Aquacult. Res.*, **37**, 606-617.

444 Benedito-Palos, L., Saera-Vila, A., Calduch-Giner, J.A., Kaushik, S. & Pérez-Sánchez,  
445 J. (2007) Combined replacement of fish meal and oil in practical diets for fast  
446 growing juveniles of gilthead sea bream (*Sparus aurata* L.): Networking of systemic  
447 and local components of GH/IGF axis. *Aquaculture*, **267**, 199–212.

448 Benedito-Palos, L., Navarro, J.C., Sitjà-Bobadilla, A., Bell, G.J., Kaushik, S. & Pérez-  
449 Sánchez, J. (2008) High levels of vegetable oils in plant protein-rich diets fed to  
450 gilthead sea bream (*Sparus aurata* L.): growth performance, muscle fatty acid  
451 profiles and histological alterations of target tissues. *Br. J. Nutr.*, **100**, 992-1003.

452 Bimbo, A.P. (2000) Fish Meal and Oil (Martin, R.E., Carter, E.P., Flick, G.J., Davis,  
453 L.M. Eds.), pp. 541–581. Technomic Publishing Co., Lancaster, UK.

454 Bouraoui, L., Sánchez-Gurmaches, J., Cruz-Garcia, L., Gutiérrez, J., Benedito-Palos, L.,  
455 Pérez-Sánchez, J. & Navarro, I. (2011) Effect of dietary fish meal and fish oil  
456 replacement on lipogenic and lipoprotein lipase activities and plasma insulin in  
457 gilthead sea bream (*Sparus aurata*). *Aquacult. Nutr.*, **17**(1), 54-63.

458 Bowyer, J.N., Qin, J.G., Smullen, R.P. & Stone, D.A.J. (2012a) Replacement of fish oil  
459 by poultry oil and canola oil in yellowtail kingfish (*Seriola lalandi*) at optimal and  
460 suboptimal temperatures. *Aquaculture*, **356**, 211-222.

461 Bowyer J.N., Rout-Pitt, N., Bain, P.A., Stone, D.A.J. & Schuller, K.A. (2012b) Dietary  
462 fish oil replacement with canola oil up-regulates glutathione peroxidase 1 gene

463 expression in yellowtail kingfish (*Seriola lalandi*). *Comp. Biochem. Physiol.*, **162**,  
464 100-106.

465 Bowyer J.N., Qin, J.G., Smullen, R.P., Adams, L.R., Thomson, M.J.S. & Stone, D.A.J.  
466 (2013) The use of a soy product in juvenile yellowtail kingfish (*Seriola lalandi*)  
467 feeds at different water temperatures: 1. Solvent extracted soybean meal.  
468 *Aquaculture*, **384-387**, 35-45.

469 Bowzer, J., Jackson, C. and Trushenski, J. (2016) Hybrid striped bass feeds based on  
470 fish oil, beef tallow, and eicosapentaenoic acid/docosahexaenoic acid supplements:  
471 Insight regarding fish oil sparing and demand for n-3 long-chain polyunsaturated  
472 fatty acids. *J. Anim. Sci.*, 2016.94. doi:10.2527/jas2015-9199.

473 Brouwer, E. (1965) Report of sub-committee on constants and factors. In: Blaxter, K.L.  
474 (Ed.), Proceedings of the Third EAAP Symposium on Energy Metabolism.  
475 Publication No. 11. Academic Press, London, pp. 441-443.

476 Collins, G.M., Ball, A.S., Qin, J.G., Bowyer, J.N. & Stone, D.A.J. (2014) Effect of  
477 alternative lipids and temperature on growth factor gene expression in yellowtail  
478 kingfish (*Seriola lalandi*). *Aquacult. Res.*, **45**, 1236-1245.

479 Coz-Rakovac, R., Smuc, T., Topic Popovic, N., Strunjak-Perovic, I., Hacmanjek, M. &  
480 Jadan, M. (2008) Novel methods for assessing fish blood biochemical data. *J. Appl.*  
481 *Ichthyol.*, **24**, 77-80.

482 Craig, S.R., Washburn, B.S. & Gatlin, D.M. (1999) Effects of dietary lipids on body  
483 composition and liver function in juvenile red drum, *Sciaenops ocellatus*. *Fish*  
484 *Physiol. Biochem.*, **21**, 249-255.

485 Christie, W.W. (1982) A simple procedure for rapid transmethylolation of glycerolipids  
486 and cholesteryl esters. *J. Lipid Res.*, **23(7)**, 1072-1075.

487 Díaz-López, M., Pérez, M.J., Acosta, N.G., Tocher, D.R., Jerez, S., Lorenzo, A. &  
488 Rodríguez, C. (2009) Effect of dietary substitution of fish oil by Echium oil on  
489 growth, plasma parameters and body lipid composition in gilthead seabream (*Sparus*  
490 *aurata* L.). *Aquacult. Nutr.*, **15(5)**, 500-512.

491 Díaz-López, M., Pérez, M.J., Acosta, N.G., Jerez, S., Dorta-Guerra, R., Tocher, D.R.,  
492 Lorenzo, A. & Rodríguez, C. (2010) Effects of dietary fish oil substitution by  
493 Echium oil on enterocyte and hepatocyte lipid metabolism of gilthead seabream  
494 (*Sparus aurata* L.). *Comp. Biochem. Physiol. B*, **155**, 371–379.

495 FAO (2014) The State of World Fisheries and Aquaculture. 223 pp. Rome, Italy.

496 FAO (2016) The State of World Fisheries and Aquaculture. Contributing to food  
497 security and nutrition for all. 200 pp. Rome, Italy.

498 Folch, J., Lees, M. & Sloane-Stanley, G.H. (1957) A simple method for the isolation  
499 and purification of total lipids from animal tissues. *J. Biol. Chem*, **226(1)**, 497-509.

500 Fountoulaki, E., Vasilaki, A., Hurtado, R., Grigorakis, K., Karacostas, I., Nengas, I.,  
501 Rigos, G., Kotzamanis, Y., Venou, B. & Alexis, M.N. (2009) Fish oil substitution by  
502 vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.); effects  
503 on growth performance, flesh quality and fillet fatty acid profile. Recovery of fatty  
504 acid profiles by a fish oil finishing diet under fluctuating water temperatures.  
505 *Aquaculture*, **289**, 317-326.

506 Gisbert, E., Giménez, G., Fernández, I., Kotzamanis, Y. & Estevez, A. (2009)  
507 Development of digestive enzymes in common dentex *Dentex dentex* during early  
508 ontogeny. *Aquaculture*, **287(3-4)**, 381-387.

509 Glencross, B.D. (2009) Exploring the nutritional demand for essential fatty acids by  
510 aquaculture species. *Rev. Aquacult.*, **1(2)**, 71-124.

511 Glencross, B., Blyth, D., Irvin, S., Bourne, N., Campet, M., Boisot, P. & Wade, N.M.  
512 (2016) An evaluation of the complete replacement of both fishmeal and fish oil in  
513 diets for juvenile Asian seabass, *Lates calcarifer*. *Aquaculture*, 298-309.

514 Grigorakis, K. (2007) Compositional and organoleptic quality of farmed and wild  
515 gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) and factors  
516 affecting it: A review. *Aquaculture*, **272**, 55–75.

517 Henderson, R.J. (1996) Fatty acid metabolism in freshwater fish with particular  
518 reference to polyunsaturated fatty acids. *Arch. Tierernahr.*, **49**, 5–22.

519 Huang, S.S.Y., Oo, A.N., Higgs, D.A., Brauner, C.J. & Satoh, S. (2007) Effect of  
520 dietary canola oil level on the growth performance and fatty acid composition of  
521 juvenile red sea bream, *Pagrus major*. *Aquaculture*, **271(1)**, 420-431.

522 Izquierdo, M.S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L. & Rosenlund,  
523 G. (2003) Dietary lipid sources for seabream and seabass: growth performance,  
524 tissue composition and flesh quality. *Aquacult. Nutr.*, **9(6)**, 397-407.

525 Kaushik, S.J., Coves, D., Dutto, G. & Blanc, D. (2004) Almost total replacement of fish  
526 meal by plant protein sources in the diet of a marine teleost, the European seabass,  
527 *Dicentrarchus labrax*. *Aquaculture*, **230**, 391-404.

528 Khankari, N.K.; Bradshaw, P.T., Steck, S.E., He, K., Olshan, A.F., Shen, J., Ahn, J.,  
529 Chen, Y., Ahsan, H., Terry, M.B., Teitelbaum, S.L., Neugut, A.I., Santella, R.M. &  
530 Gammon, M.D. (2015) Dietary intake of fish, polyunsaturated fatty acids, and  
531 survival after breast cancer: A population-based follow-up study on Long Island,  
532 New York. *Cancer*, **121**, 2244-2252.

533 Khaoian, P., Nguyen, H.P., Ogita, Y., Fukada, H., Masumoto, T. (2014) Taurine  
534 supplementation and palm oil substitution in low-fish meal diets for young yellowtail  
535 *Seriola quinqueradiata*. *Aquaculture*, **420-421**, 219-224.

536 Kiessling, K. -H. & Kiessling, A. (1993) Selective utilization of fatty acids in Rainbow  
537 trout (*Onchorhynchus mykiss* Walbaum) red muscle mitochondria. *Can. J. Zool.*, **71**,  
538 248–251.

539 Kowalska, A., Zakes, Z., Siwicki, A.K., Jankowska, B., Jarmolowicz, S. & Demska-  
540 Zakes, K. (2012) Impact of diets with different proportions of linseed and sunflower  
541 oils on the growth, liver histology, immunological and chemical blood parameters,  
542 and proximate composition of pikeperch *Sander lucioperca* (L.). *Fish Physiol.*  
543 *Biochem.*, **38**, 375–388.

544 Lech, G.P. & Reigh, R.C. (2012) Plant products affect growth and digestive efficiency  
545 of cultured Florida pompano (*Trachinotus carolinus*) fed compounded diets. *PLoS*  
546 *one*, **7(4)**, e34981, 11 pp.

547 Lemaire, P., Draï, P., Mathieu, A., Lemaire, S., Carrière, S., Giudicelli, J. & Lafaurie,  
548 M. (1991) Changes with different diets in plasma enzymes (GOT, GPT, LDH, ALP)  
549 and plasma lipids (cholesterol, triglycerides) of sea-bass (*Dicentrarchus labrax*).  
550 *Aquaculture*, **93(1)**, 63-75.

551 Lin, H., Chen, X., Chen, S., Zhuojia, L., Huang, Z., Niu, J. & Lu, X. (2012)  
552 Replacement of fish meal with fermented soybean meal in practical diets for  
553 pompano *Trachinotus ovatus*. *Aquacult. Res.*, **44(1)**, 151-156.

554 McKenzie, D.J. (2001) Effects of dietary fatty acids on the respiratory and  
555 cardiovascular physiology of fish. *Comp. Biochem. Physiol.*, **128**, 607–621.

556 Menoyo, D., Izquierdo, M.S., Robaina, L., Ginés, R., Lopez-Bote, C.J. & Bautista, J.M.  
557 (2004) Adaptation of lipid metabolism, tissue composition and flesh quality in  
558 gilthead sea bream (*Sparus aurata*) to the replacement of dietary fish oil by linseed  
559 and soyabean oils. *Br. J. Nutr.*, **92(1)**, 41-52.



560 Mourente, G. & Bell, J.G. (2006) Partial replacement of dietary fish oil with blends of  
561 vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass  
562 (*Dicentrarchus labrax* L.) over a long term growth study: Effects on muscle and liver  
563 fatty acid composition and effectiveness of a fish oil finishing diet. *Comp. Biochem.*  
564 *Physiol. B*, **145**, 389-399.

565 Mozanzadeh, M.T., Agh, N., Yavari, V., Marammazi, J.G., Mohammadian, T. &  
566 Gisbert, E. (2016) Partial or total replacement of dietary fish oil with alternative lipid  
567 sources in silvery-black porgy (*Sparidentex hasta*). *Aquaculture*, **451**, 232-240.

568 Nakada, M. (2000) Yellowtail and related species culture. In: *Encyclopedia of*  
569 *Aquaculture* (Stickney, R.R. Ed). pp. 1007-1036. John Wiley & Sons, Inc. New  
570 York, USA.

571 Nguyen, H.P., Khaoian, P., Fukada, H., Suzuki, N. & Masumoto, T. (2015) Feeding  
572 fermented soybean meal diet supplemented with taurine to yellowtail *Seriola*  
573 *quinqueradiata* affects growth performance and lipid digestion. *Aquacult. Res.*, **46**,  
574 1101-1110.

575 Nasopoulou, C. & Zabetakis, I. (2012) Benefits of fish oil replacement by plant  
576 originated oils in compounded fish feeds. A review. *Food Sci. Technol.* **47**, 217–244.

577 Peng, S., Chen, L., Quin, J.G., Hou, J., Yu, N., Long, K., Ye, J. & Sun, X. (2008) Effect  
578 of replacement of dietary fish oil by soybean oil on growth performance and liver  
579 biochemical composition in juvenile black seabream, *Acanthopagrus schlegeli*.  
580 *Aquaculture*, **276**, 154-161.

581 Pérez, J.A., Rodríguez, C., Bolaños, A., Cejas, J.R. & Lorenzo, A. (2014) Beef tallow as  
582 an alternative to fish oil in diets for gilthead seabream (*Sparus aurata*) juveniles:  
583 Effects on fish performance, tissue fatty acid composition, health and flesh  
584 nutritional value. *Eur. J. Lipid Sci. Technol.*, **116**, 571-583.

585 Piedecausa, M.A., Mazón, M.J., García-García B. & Hernández, M.D. (2007) Effects of  
586 total replacement of fish oil by vegetable oils in the diets of sharp snout seabream  
587 (*Diplodus puntazzo*). *Aquaculture*, **263**, 211-219.

588 Regost, C., Arzel, J., Robin, J., Rosenlund, G. & Kaushik, S.J. (2003) Total replacement  
589 of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta*  
590 *maxima*): 1. Growth performance, flesh fatty acid profile, and lipid metabolism.  
591 *Aquaculture*, **217**(1), 465-482.

592 Richard, N., Mourente, G., Kaushik, S. & Corraze, G. (2006) Replacement of a large  
593 portion of fish oil by vegetable oils does not affect lipogenesis, lipid transport and  
594 tissue lipid uptake in European seabass (*Dicentrarchus labrax* L.). *Aquaculture*,  
595 **261**(3), 1077-1087.

596 Rossi, W. & Davis, D.A. (2012) Replacement of fishmeal with poultry by-product meal  
597 in the diet of Florida pompano *Trachinotus carolinus* L. *Aquaculture*, **338**, 160-166.

598 Ruyter, B., Moya-Falcon, C., Rosenlund, G. & Vegusdal, A. (2006) Fat content and  
599 morphology of liver and intestine of Atlantic salmon (*Salmo salar*): Effects of  
600 temperature and dietary soybean oil. *Aquaculture*, **252**, 441-452.

601 Saito, H. (2012) Lipid characteristics of two subtropical *Seriola* fishes, *Seriola dumerili*  
602 and *Seriola rivoliana*, with differences between cultured and wild varieties. *Food*  
603 *Chem.* **135**(3), 1718-1729.

604 Sales, J. & Glencross, B. (2011) A meta-analysis of the effects of dietary marine oil  
605 replacement with vegetable oils on growth, feed conversion and muscle fatty acid  
606 composition of fish species. *Aquac. Nutr.* **17**, 271–287.

607 Sarker, M.S.A., Satoh, S., Kamata, K., Haga, Y. & Yamamoto, Y. (2012) Partial  
608 replacement of fish meal with plant protein sources using organic acids to practical  
609 diets for juvenile yellowtail, *Seriola quinqueradiata*. *Aquacult. Nutr.*, **18**(1), 81-89.

610 Sargent, J.R., Tocher, D.R. & Bell, J.G. (2002) The lipids. In: *Fish Nutrition, 3rd Edn.*  
611 (Halver, J.E. & Hardy, R.W. Eds), pp. 181–257. Academic Press, San Diego, CA,  
612 USA.

613 Seno-O, A., Takakuma, F., Hashiguchi, T., Morioka, K., Masumoto, T. & Fukada, H.  
614 (2008) Replacement of dietary fish oil with olive oil in young yellowtail *Seriola*  
615 *quinqueradiata*: effects on growth, muscular fatty acid composition and prevention  
616 of dark muscle discoloration during refrigerated storage. *Fish. Sci.*, **74**, 1297-1306.

617 Sicuro, B. & Luzzana, U. (2016) The state of *Seriola spp.* other than yellowtail (*S.*  
618 *quinqueradiata*). *Farm World, Rev. Fish. Sci. & Aquacult.*, **24(4)**, 314-325. DOI:  
619 10.1080/23308249.2016.1187583.

620 Simopoulos, A.P. (2008) The importance of the omega-6/omega-3 fatty acid ratio in  
621 cardiovascular disease and other chronic diseases. *Exp. Biol. Med.*, **233**, 674–688.

622 Simopoulos, A.P. (2011) The importance of the  $\omega$ -6/ $\omega$ -3 balance in health and disease:  
623 evolutionary aspects of diet. In: *Healthy Agriculture, Healthy Nutrition, Healthy*  
624 *People*, Vol. 102 (Simopoulos, A.P. Ed.), pp. 10–21. Karger, Basel, Switzerland.

625 Simopoulos A.P. (2016) An increase in the omega-6/omega-3 fatty acid ratio increases  
626 the risk of obesity. *Nutrients* **8**, 128.

627 Siriwardhana, N., Kalupahana, N.S. & Moustaid-Moussa, N. (2012) Health benefits of  
628 n-3 polyunsaturated fatty acids: Eicosapentaenoic acid and docosahexaenoic acid. In:  
629 *Advances in Food and Nutrition Research* (Kim, S.K. Eds), pp. 211–222. Academic  
630 Press, Amsterdam, The Netherlands.

631 Stubhaug, I., Lie, Ø. & Torstensen, B.E. (2007) Fatty acid productive value and  $\beta$ -  
632 oxidation capacity in Atlantic salmon (*Salmo salar* L.) fed on different lipid sources  
633 along the whole growth period. *Aquacult. Nutr.*, **13(2)**, 145-155.

634 Tacon, A.G.J. & Metian, M. (2008) Global overview on the use of fish meal and fish oil  
635 in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*,  
636 **285**, 146–158.

637 Takakuwa, F., Fukada, H., Hosokawa, H. & Masumoto, T. (2006) Optimum digestible  
638 protein and energy levels and ratio for greater amberjack *Seriola dumerili* (Risso)  
639 fingerling. *Aquacult. Res.*, **37(15)**, 1532-1539.

640 Tocher, D.R. (2010) Fatty acid requirements in ontogeny of marine and freshwater fish  
641 *Aquacult. Res.*, **41(5)**, 717-732.

642 Tomás, A., De La Gándara, F., García-Gomez, A., Pérez, L. & Jover, M. (2005)  
643 Utilization of soybean meal as an alternative protein source in the Mediterranean  
644 yellowtail, *Seriola dumerili*. *Aquacult. Nutr.*, **11(5)**, 333-340.

645 Torstensen, B.E., Li, Ø. & Frøyland, L. (2000) Lipid metabolism and tissue  
646 composition in Atlantic salmon (*Salmo salar* L.)-Effects of capelin oil, palm oil and  
647 oleic-acid enriched sunflower oil as dietary lipid sources. *Lipids*, **35**, 653-664.

648 Torstensen, B.E., Frøyland, L. & Lie, Ø. (2004) Replacing dietary fish oil with  
649 increasing levels of rapeseed oil and olive oil—effects on Atlantic salmon (*Salmo*  
650 *salar* L.) tissue and lipoprotein lipid composition and lipogenic enzyme  
651 activities. *Aquacult. Nutr.*, **10(3)**, 175-192.

652 Trushenski, J., Schwarz, M., Lewis, H., Laporte, J., Delbos, B., Takeuchi, R. &  
653 Sampaio, L.A. (2011) Effect of replacing dietary fish oil with soybean oil on  
654 production performance and fillet lipid and fatty acid composition of juvenile cobia  
655 *Rachycentron canadum*. *Aquacult. Nutr.*, **17(2)**, e437-e447.

656 Tucker, J.W., Lellis, W.A., Vermeer, G.V., Roberts, D.E. & Woodward, P.N. (1997)  
657 The effects of experimental starter diets with different levels of soybean or  
658 menhaden oil on red drum (*Sciaenops ocellatus*). *Aquaculture*, **149**, 323–339.

659 Turan, H., Sönmez, G. & Kaya, Y. (2007) Fatty acid profile and proximate composition  
660 of the thornback ray (*Raja clavata*, L. 1758) from the Sinop coast in the Black Sea. *J.*  
661 *Fish. Sci.*, **1(2)**, 97-103.

662 Turchini, G.M., Torstensen, B.E. & Ng, W.K. (2009) Fish oil replacement in finfish  
663 nutrition. *Rev. Aquacult.* **1**, 10-57.

664 Ulbricht, T.L.V. & Southgate, D.A.T. (1991) Coronary heart disease: Seven dietary  
665 factors. *The Lancet*, **338**, 985–992.

666 USDA, United States Department of Agriculture, Foreign Agricultural Service (July  
667 2016) Oilseeds: World Market and Trades, 37 pp.

668 Uyan, O., Koshio, S., Ishikawa, M., Yokoyama, S., Uyan, S., Ren, T. & Hernández,  
669 L.H.H. (2009) The influence of dietary phospholipid level on the performances of  
670 juvenile amberjack, *Seriola dumerili*, fed non-fishmeal diets. *Aquacult. Nutr.*, **15(5)**,  
671 550-557.

672 Vidal, A.T., De la Gándara García, F., Gómez, A.G. & Cerdá, M.J. (2008) Effect of the  
673 protein/energy ratio on the growth of Mediterranean yellowtail (*Seriola dumerili*).  
674 *Aquacult. Res.*, **39**, 1141–1148.

675 WHO, World Health Organization (2003) Diet, nutrition and the prevention of chronic  
676 diseases. WHO technical report series 916. Geneva, Switzerland. 148 pp.

677 Wood, J.D., Richardson, R.I., Nute, G.R., Fisher, A.V., Campo, M.M., Kasapidou, E.,  
678 Sheard, P.R. & Enser, M. (2004) Effects of fatty acids on meat quality: a review.  
679 *Meat Sci.*, **66(1)**, 21-32.

680

681 Table 1. Ingredients, proximate and main fatty acid composition of experimental diets.

	FO100	VO50	VO100
<i>Ingredients (g kg<sup>-1</sup>)</i>			
Fish meal	525	525	525
Wheat meal	235	235	235
Wheat gluten meal	130	130	130
Fish oil	90	45	0
Linseed oil	0	9	18
Palm oil	0	36	72
Vitamin and mineral premix <sup>a</sup>	20	20	20
<i>Proximate composition</i>			
Dry matter (DM, g kg <sup>-1</sup> wet weight)	886	894	899
Crude protein (g kg <sup>-1</sup> DM)	452	456	461
Crude lipid (g kg <sup>-1</sup> DM)	123	133	135
Ash (g kg <sup>-1</sup> DM)	91	87	87
Crude fibre (g kg <sup>-1</sup> DM)	237	245	240
<i>Fatty acids (% total fatty acids)</i>			
Total SFA <sup>1</sup>	30.17	33.28	36.13
14:0	5.65	4.24	2.33
16:0	19.96	24.26	28.56
18:0	3.67	3.77	4.17
Total MUFA <sup>1</sup>	22.42	26.09	29.29
16:1 <sup>2</sup>	7.74	5.70	3.12
18:1 <sup>2</sup>	13.24	18.73	24.94
20:1 <sup>2</sup>	0.95	0.79	0.49
22:1 <sup>2</sup>	0.50	0.42	0.37
Total PUFA <sup>1</sup>	44.71	38.51	32.87
18:2 n-6	7.04	9.31	11.77
18:3 n-3	1.08	3.14	5.99
18:4 n-3	2.11	1.46	0.83
Total n-6 LC-PUFA	1.13	0.85	0.51
20:4 n-6	0.78	0.56	0.30
22:5 n-6	0.35	0.28	0.21
Total n-3 LC-PUFA <sup>1</sup>	29.19	20.77	12.24
20:5 n-3	15.05	10.70	5.85
22:5 n-3	1.88	1.37	0.84
22:6 n-3	11.06	7.87	5.11
<i>Ratios</i>			
PUFA/SFA	1.48	1.16	0.91
n-6/n-3	0.25	0.40	0.64
DHA/EPA <sup>3</sup>	0.735	0.736	0.874
EPA/ARA <sup>3</sup>	19.178	19.038	19.228

682 <sup>a</sup> Contains: choline, 10 g; DL- $\alpha$ -tocopherol, 5 g; ascorbic acid, 5 g; Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, 5 g and a premix, 25 g.  
683 This premix contains per kg: retinol acetate, 1,000,000 IU; calciferol, 500 IU; DL- $\alpha$ -tocopherol, 10 g;  
684 menadione sodium bisulphite, 0.8 g; thiamine hydrochloride, 2.3 g; riboflavin, 2.3 g; pyridoxine  
685 hydrochloride, 15 g; cyanocobalamine, 25 mg; nicotinamide, 15 g; pantothenic acid, 6 g; folic acid, 650

686 mg; biotin, 70 mg; ascorbic acid, 75 g; inositol, 15 g; betaine, 100 g; polypeptides 12 g; Zn, 5 g; Se, 20  
687 mg; I, 500 mg; Fe, 200 mg; CuO, 15 g; Mg, 5.75 g; Co, 0.02 g; methionine, 1.2 g; cysteine, 0.8 g;  
688 lysine, 1.3 g; arginine, 0.6 g; phenylalanine, 0.4 g; tryptophan, 0.7 g.  
689 <sup>1</sup> Includes some minor components not shown.  
690 <sup>2</sup> Includes other isomers not shown. Mainly n-7 isomer for C16 and n-9 isomer for C18, C20 and C22.  
691 <sup>3</sup> DHA/EPA, 22:6 n-3/ 20:5 n-3; EPA/ARA, 20:5 n-3/ 20:4n-6.

692 Table 2. Growth performance and feed utilization of *Seriola dumerili* juveniles fed the  
 693 experimental diets for 154 days.

	FO100	VO50	VO100
Initial weight (g)	39.6 ± 3.7	37.9 ± 1.0	40.2 ± 2.9
Final weight (g)	390 ± 23.2	397 ± 24.4	375 ± 30.8
WG (%) <sup>1</sup>	894 ± 96	940 ± 75	840 ± 16
SGR (% day <sup>-1</sup> ) <sup>2</sup>	1.49 ± 0.07	1.50 ± 0.05	1.47 ± 0.02
FI (g 100 g fish day <sup>-1</sup> ) <sup>3</sup>	1.82 ± 0.26	1.81 ± 0.13	1.79 ± 0.02
FCR <sup>4</sup>	1.75 ± 0.27	1.75 ± 0.14	1.72 ± 0.03
Survival (%)	75 ± 6	74 ± 6	74 ± 7

694 Data are expressed as mean ± SD

695 <sup>1</sup>Weight gain = 100 x [(final weight – initial weight) / initial weight];

696 <sup>2</sup>Specific growth rate = 100 x (ln final weight – ln initial weight) / feeding days;

697 <sup>3</sup>Feed intake = 100 x feed consumption (g) / average biomass (g) x days;

698 <sup>4</sup>Feed conversion ratio = dry food fed (g) / wet weight gain (g).

699



700 Table 3. Biometric indices and proximate composition of *Seriola dumerili* juveniles fed  
 701 the experimental diets for 154 days.

	FO100	VO50	VO100
CF <sup>1</sup>	1.37 ± 0.17	1.30 ± 0.24	1.28 ± 0.10
VSI (%) <sup>2</sup>	4.32 ± 0.62	4.18 ± 1.10	4.33 ± 0.52
HSI (%) <sup>3</sup>	0.87 ± 0.20	0.78 ± 0.15	0.84 ± 0.10
MSI (%) <sup>4</sup>	0.18 ± 0.17	0.05 ± 0.15	0.10 ± 0.09
IFR (%) <sup>5</sup>	38.4 ± 4.17	34.1 ± 3.23	34.6 ± 4.60
IER (%) <sup>6</sup>	21.7 ± 0.91	20.6 ± 1.12	21.4 ± 0.83
<i>Whole body proximate composition g (kg w.w.)<sup>-1</sup></i>			
Moisture	696.5 ± 0.9 <sup>a</sup>	706.3 ± 0.9 <sup>b</sup>	702.5 ± 0.2 <sup>ab</sup>
Crude protein	192.2 ± 1.3	189.1 ± 1.5	188.7 ± 2.3
Total lipid	77.8 ± 2.9	72.9 ± 2.0	74.7 ± 1.6
Ash	28.1 ± 0.9	27.6 ± 0.3	26.9 ± 0.8

702 w.w., wet weight; Data are expressed as mean ± SD. Means with different superscripts letters are  
 703 significantly different (P < 0.05).

704 <sup>1</sup> Condition factor = 100 x (final weight / total length<sup>3</sup>);

705 <sup>2</sup> Viscerosomatic index = 100 x (viscera weight / final weight);

706 <sup>3</sup> Hepatosomatic index = 100 x (liver weight / final weight);

707 <sup>4</sup> Mesenteric fat index = 100 x (viscera fat / final weight);

708 <sup>5</sup> Ingested fat retention = 100 x (fish fat gain / crude fat intake);

709 <sup>6</sup> Ingested energy retention = 100 x (fish energy gain, kJ/ gross energy intake, kJ).

710

711 Table 4. Plasma parameters of greater amberjack juveniles fed the  
712 experimental diets for 154 days

	FO100	VO50	VO100
Glucose (mg dL <sup>-1</sup> )	223.4±48.4	190.9±35.5	185.9±55.5
GOT (U L <sup>-1</sup> )	20.6±11.5	12.6± 6.2	10.9± 0.8
GPT (U L <sup>-1</sup> )	6.1± 1.6	4.9± 1.2	3.9± 1.4
Triglycerides (mg dL <sup>-1</sup> )	94.4±18.6	89.0±25.8	97.8±28.8
Cortisol (ng mL <sup>-1</sup> )	54.2±28.3	56.1±16.3	55.2± 6.1
Lipase (U L <sup>-1</sup> )	7.8± 1.0	7.6± 0.7	7.1± 0.6

713 Data are expressed as mean ± SD (n=6).

714

715 Table 5. Total FA ( $\mu\text{g mg DM}^{-1}$ ) and main fatty acid composition (% total fatty acids)  
 716 of liver TL from cultured *Seriola dumerili* juveniles fed the experimental diets for 154  
 717 days.

	Initial sample	FO100	VO50	VO100
Total FA	211.74 $\pm$ 29.09	191.63 $\pm$ 24.92	222.07 $\pm$ 19.09	183.92 $\pm$ 28.56
Total SFA <sup>1</sup>	30.31 $\pm$ 1.00	30.25 $\pm$ 1.04	31.54 $\pm$ 1.28	31.46 $\pm$ 2.53
14:0	4.96 $\pm$ 0.18	4.62 $\pm$ 0.30 <sup>c</sup>	2.95 $\pm$ 0.25 <sup>b</sup>	1.67 $\pm$ 0.15 <sup>a</sup>
16:0	20.52 $\pm$ 0.87	20.33 $\pm$ 0.85 <sup>a</sup>	22.71 $\pm$ 1.21 <sup>ab</sup>	24.10 $\pm$ 2.12 <sup>b</sup>
18:0	3.63 $\pm$ 0.16	4.24 $\pm$ 0.51	5.04 $\pm$ 1.05	4.94 $\pm$ 0.99
Total MUFA <sup>1</sup>	28.39 $\pm$ 1.62	24.92 $\pm$ 1.75 <sup>a</sup>	30.52 $\pm$ 2.12 <sup>b</sup>	33.79 $\pm$ 2.18 <sup>b</sup>
16:1 <sup>2</sup>	9.27 $\pm$ 0.18	7.71 $\pm$ 0.08 <sup>c</sup>	5.39 $\pm$ 0.21 <sup>b</sup>	2.86 $\pm$ 1.10 <sup>a</sup>
18:1 <sup>2</sup>	18.08 $\pm$ 1.14	15.89 $\pm$ 1.62 <sup>a</sup>	23.92 $\pm$ 1.96 <sup>b</sup>	29.51 $\pm$ 1.93 <sup>c</sup>
20:1 <sup>2</sup>	0.61 $\pm$ 0.18	0.55 $\pm$ 0.07	0.52 $\pm$ 0.08	0.52 $\pm$ 0.13
Total PUFA <sup>1</sup>	38.16 $\pm$ 2.65	43.92 $\pm$ 1.12 <sup>b</sup>	37.37 $\pm$ 2.57 <sup>a</sup>	34.54 $\pm$ 2.26 <sup>a</sup>
18:2 n-6	8.80 $\pm$ 0.36	9.70 $\pm$ 0.39 <sup>a</sup>	12.40 $\pm$ 1.05 <sup>b</sup>	15.26 $\pm$ 1.38 <sup>c</sup>
18:3 n-3	0.93 $\pm$ 0.04	1.23 $\pm$ 0.15 <sup>a</sup>	3.25 $\pm$ 0.21 <sup>b</sup>	5.25 $\pm$ 0.71 <sup>c</sup>
18:4 n-3	1.29 $\pm$ 0.16	1.32 $\pm$ 0.11 <sup>c</sup>	0.73 $\pm$ 0.09 <sup>b</sup>	0.29 $\pm$ 0.07 <sup>a</sup>
Total n-6 LC-PUFA <sup>1</sup>	1.56 $\pm$ 0.06	1.96 $\pm$ 0.22 <sup>c</sup>	1.47 $\pm$ 0.16 <sup>b</sup>	1.12 $\pm$ 0.17 <sup>a</sup>
20:2 n-6	nd	0.26 $\pm$ 0.05	0.29 $\pm$ 0.07	0.30 $\pm$ 0.10
20:4 n-6	1.26 $\pm$ 0.05	1.28 $\pm$ 0.12 <sup>c</sup>	0.92 $\pm$ 0.10 <sup>b</sup>	0.62 $\pm$ 0.14 <sup>a</sup>
22:5 n-6	0.29 $\pm$ 0.02	0.29 $\pm$ 0.01	0.20 $\pm$ 0.08	0.18 $\pm$ 0.04
Total n-3 LC-PUFA <sup>1</sup>	23.77 $\pm$ 2.65	27.75 $\pm$ 1.00 <sup>c</sup>	18.50 $\pm$ 2.25 <sup>b</sup>	12.18 $\pm$ 1.84 <sup>a</sup>
20:4 n-3	0.76 $\pm$ 0.01	0.94 $\pm$ 0.14 <sup>c</sup>	0.67 $\pm$ 0.10 <sup>b</sup>	0.34 $\pm$ 0.05 <sup>a</sup>
20:5 n-3	11.50 $\pm$ 1.03	13.48 $\pm$ 0.33 <sup>c</sup>	8.07 $\pm$ 0.87 <sup>b</sup>	4.61 $\pm$ 0.55 <sup>a</sup>
21:5 n-3	0.39 $\pm$ 0.03	0.46 $\pm$ 0.01 <sup>c</sup>	0.31 $\pm$ 0.03 <sup>b</sup>	0.16 $\pm$ 0.02 <sup>a</sup>
22:5 n-3	2.37 $\pm$ 0.13	2.92 $\pm$ 0.44 <sup>c</sup>	2.28 $\pm$ 0.28 <sup>b</sup>	1.42 $\pm$ 0.21 <sup>a</sup>
22:6 n-3	8.75 $\pm$ 1.46	9.95 $\pm$ 1.32 <sup>b</sup>	7.18 $\pm$ 1.61 <sup>a</sup>	5.51 $\pm$ 1.46 <sup>a</sup>
<b>Ratios</b>				
PUFA/SFA	1.26 $\pm$ 0.13	1.45 $\pm$ 0.05 <sup>b</sup>	1.19 $\pm$ 0.12 <sup>a</sup>	1.11 $\pm$ 0.14 <sup>a</sup>
n-6/n-3	0.40 $\pm$ 0.05	0.39 $\pm$ 0.02 <sup>a</sup>	0.62 $\pm$ 0.09 <sup>b</sup>	0.92 $\pm$ 0.11 <sup>c</sup>
DHA/EPA <sup>3</sup>	0.76 $\pm$ 0.06	0.74 $\pm$ 0.09 <sup>a</sup>	0.88 $\pm$ 0.14 <sup>ab</sup>	1.20 $\pm$ 0.28 <sup>b</sup>
EPA/ARA <sup>3</sup>	9.10 $\pm$ 0.53	10.58 $\pm$ 0.78 <sup>b</sup>	8.81 $\pm$ 0.59 <sup>ab</sup>	7.68 $\pm$ 1.80 <sup>a</sup>

718 Results are expressed as means  $\pm$  SD (n=6-8) except for the initial sample where n=3. Means with different  
 719 superscript letters indicate significant differences (P < 0.05).

720 <sup>1</sup> Includes some minor components not shown.

721 <sup>2</sup> Includes other isomers not shown. Mainly n-7 isomer for C16 and n-9 isomer for C18 and C20.

722 <sup>3</sup> DHA/EPA, 22:6 n-3/ 20:5 n-3; EPA/ARA, 20:5 n-3/ 20:4 n-6.

723

724 Table 6. Total FA ( $\mu\text{g mg DM}^{-1}$ ) and main fatty acid composition (% total fatty acids)  
 725 of muscle TL, and indices of nutritional quality of lipids from cultured *Seriola dumerili*  
 726 juveniles fed the experimental diets for 154 days.

	Initial sample	FO100	VO50	VO100
Total FA	33.04 $\pm$ 6.45	16.89 $\pm$ 2.79	20.40 $\pm$ 5.70	25.63 $\pm$ 9.02
Total SFA <sup>1</sup>	28.44 $\pm$ 1.70	34.37 $\pm$ 0.56	33.62 $\pm$ 1.11	33.30 $\pm$ 0.93
14:0	2.47 $\pm$ 0.38	1.12 $\pm$ 0.29	0.97 $\pm$ 0.46	0.70 $\pm$ 0.26
16:0	17.79 $\pm$ 1.06	20.44 $\pm$ 0.38 <sup>a</sup>	20.44 $\pm$ 0.50 <sup>a</sup>	21.08 $\pm$ 0.44 <sup>b</sup>
18:0	7.27 $\pm$ 0.61	9.63 $\pm$ 0.50	9.36 $\pm$ 1.00	8.72 $\pm$ 0.90
Total MUFA <sup>1</sup>	20.22 $\pm$ 0.14	13.15 $\pm$ 1.70 <sup>a</sup>	16.31 $\pm$ 3.47 <sup>ab</sup>	19.52 $\pm$ 4.24 <sup>b</sup>
16:1 <sup>2</sup>	5.27 $\pm$ 0.50	2.72 $\pm$ 0.50 <sup>b</sup>	2.25 $\pm$ 0.71 <sup>ab</sup>	1.76 $\pm$ 0.46 <sup>a</sup>
18:1 <sup>2</sup>	13.31 $\pm$ 0.44	9.25 $\pm$ 1.13 <sup>a</sup>	12.84 $\pm$ 2.72 <sup>ab</sup>	16.72 $\pm$ 3.87 <sup>b</sup>
20:1 <sup>2</sup>	0.76 $\pm$ 0.14	0.35 $\pm$ 0.08	0.32 $\pm$ 0.06	0.31 $\pm$ 0.09
Total PUFA <sup>1</sup>	49.20 $\pm$ 2.13	51.84 $\pm$ 1.80 <sup>b</sup>	49.68 $\pm$ 2.73 <sup>ab</sup>	46.90 $\pm$ 3.40 <sup>a</sup>
18:2 n-6	5.08 $\pm$ 0.11	4.83 $\pm$ 0.50 <sup>a</sup>	7.17 $\pm$ 0.92 <sup>b</sup>	10.73 $\pm$ 0.56 <sup>c</sup>
18:3 n-3	0.65 $\pm$ 0.04	0.40 $\pm$ 0.04 <sup>a</sup>	1.40 $\pm$ 0.47 <sup>b</sup>	2.63 $\pm$ 0.64 <sup>c</sup>
18:4 n-3	0.91 $\pm$ 0.16	0.37 $\pm$ 0.09 <sup>b</sup>	0.31 $\pm$ 0.12 <sup>b</sup>	0.19 $\pm$ 0.07 <sup>a</sup>
Total n-6 LC-PUFA <sup>1</sup>	2.01 $\pm$ 0.06	2.69 $\pm$ 0.39 <sup>b</sup>	2.36 $\pm$ 0.37 <sup>ab</sup>	1.98 $\pm$ 0.40 <sup>a</sup>
20:2 n-6	nd	0.16 $\pm$ 0.01 <sup>a</sup>	0.18 $\pm$ 0.01 <sup>b</sup>	0.19 $\pm$ 0.01 <sup>b</sup>
20:4 n-6	1.43 $\pm$ 0.05	1.73 $\pm$ 0.26 <sup>b</sup>	1.44 $\pm$ 0.23 <sup>ab</sup>	1.16 $\pm$ 0.26 <sup>a</sup>
22:5 n-6	0.58 $\pm$ 0.03	0.76 $\pm$ 0.11	0.71 $\pm$ 0.12	0.63 $\pm$ 0.12
Total n-3 LC-PUFA <sup>1</sup>	39.29 $\pm$ 2.06	43.01 $\pm$ 1.77 <sup>c</sup>	38.06 $\pm$ 3.98 <sup>b</sup>	31.15 $\pm$ 4.24 <sup>a</sup>
20:4 n-3	0.52 $\pm$ 0.11	0.34 $\pm$ 0.03 <sup>b</sup>	0.30 $\pm$ 0.06 <sup>b</sup>	0.20 $\pm$ 0.04 <sup>a</sup>
20:5 n-3	10.73 $\pm$ 1.29	9.98 $\pm$ 1.22 <sup>c</sup>	8.00 $\pm$ 0.30 <sup>b</sup>	6.33 $\pm$ 0.59 <sup>a</sup>
21:5 n-3	0.35 $\pm$ 0.04	0.21 $\pm$ 0.04 <sup>b</sup>	0.21 $\pm$ 0.06 <sup>b</sup>	0.14 $\pm$ 0.04 <sup>a</sup>
22:5 n-3	3.05 $\pm$ 0.22	2.91 $\pm$ 0.35 <sup>b</sup>	2.88 $\pm$ 0.30 <sup>b</sup>	2.47 $\pm$ 0.15 <sup>a</sup>
22:6 n-3	24.64 $\pm$ 1.55	29.57 $\pm$ 2.11 <sup>b</sup>	26.66 $\pm$ 3.75 <sup>ab</sup>	21.98 $\pm$ 3.80 <sup>a</sup>
Total DMAs <sup>1</sup>	1.02 $\pm$ 0.11	2.97 $\pm$ 0.43	2.75 $\pm$ 0.60	2.51 $\pm$ 0.70
16:0 DMA	0.63 $\pm$ 0.03	1.60 $\pm$ 0.31	1.47 $\pm$ 0.32	1.41 $\pm$ 0.42
18:0 DMA	0.39 $\pm$ 0.11	0.82 $\pm$ 0.08 <sup>b</sup>	0.71 $\pm$ 0.10 <sup>ab</sup>	0.62 $\pm$ 0.12 <sup>a</sup>
Ratios				
PUFA/SFA	1.74 $\pm$ 0.17	1.51 $\pm$ 0.06 <sup>b</sup>	1.48 $\pm$ 0.06 <sup>ab</sup>	1.41 $\pm$ 0.07 <sup>a</sup>
n-6/n-3	0.17 $\pm$ 0.01	0.17 $\pm$ 0.02 <sup>a</sup>	0.24 $\pm$ 0.04 <sup>b</sup>	0.38 $\pm$ 0.04 <sup>c</sup>
DHA/EPA <sup>3</sup>	2.32 $\pm$ 0.33	3.01 $\pm$ 0.46	3.32 $\pm$ 0.37	3.45 $\pm$ 0.33
EPA/ARA <sup>3</sup>	7.48 $\pm$ 0.70	5.80 $\pm$ 0.29	5.68 $\pm$ 0.84	5.62 $\pm$ 0.78
IA		0.42 $\pm$ 0.02	0.42 $\pm$ 0.04	0.43 $\pm$ 0.02
IT		0.20 $\pm$ 0.01 <sup>a</sup>	0.21 $\pm$ 0.01 <sup>a</sup>	0.23 $\pm$ 0.01 <sup>b</sup>
FLQ		39.55 $\pm$ 2.03 <sup>b</sup>	34.66 $\pm$ 4.03 <sup>b</sup>	28.32 $\pm$ 4.34 <sup>a</sup>

727 Results are expressed as means  $\pm$  SD (n=8), except for the initial sample where n=3. Means with different  
 728 superscript letters indicate significant differences (P < 0.05).

729 <sup>1</sup> Includes some minor components not shown.

730 <sup>2</sup> Includes other isomers not shown. Mainly n-7 isomer for C16 and n-9 isomer for C18 and C20.

731 <sup>3</sup> DHA/EPA, 22:6 n-3/ 20:5 n-3; EPA/ARA, 20:5 n-3/ 20:4 n-6.

732 Figure 1. Total lipid content (% wet weight) of liver and muscle of *S. dumerili* juveniles  
733 fed the experimental diets for 154 days. The bars represent the mean of N replicates plus  
734 minus the SD.

735

736

