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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.

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Abstract

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

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

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

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

1. Introduction

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

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

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

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

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

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

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

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

2. Materials and methods

2.1 Fish and rearing conditions

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

diet. After this period, groups of 20 fish (average weight 39.2 ± 1.6 g) were randomly distributed into nine 1750-L cylindrical fibreglass tanks, three tanks per treatment.

The culture was carried out under natural photoperiod conditions in a re-circulating seawater system of 75 m^3 capacity equipped with a rotary mechanical filter and a gravity bio-filter (6 m^3). During the course of the trial, water temperature (21.5 ± 2.4 °C), salinity (31.5 ± 4.1 g L^{-1}), pH levels (7.5-8.0) and dissolved oxygen (6.6 ± 1.3 mg L^{-1}) were monitored daily.

2.2. Experimental diets and feeding regime

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

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

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

2.3. *Fish sampling and growth evaluation*

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

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

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

2.4. Analytical procedures

Plasma glucose concentration (mg dL^{-1}), activities of glutamate–oxalacetate transaminase GOT (AST) (EC 2.6.1.1) and glutamate–pyruvate transaminase GPT

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

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

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

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

2.5 Indices of the nutritional quality of lipids

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

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

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

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

Briefly, the two first indices indicate that C12:0, C14:0 and C16:0 are atherogenic (favouring the adhesion of lipids to cells of the immunological and circulatory systems), and that C14:0, C16:0 and C18:0 are thrombogenic, facilitating the formation of clots in the blood vessels. The third equation, reveals the percentage relationship in which the main n-3 LC-PUFA (EPA and DHA) appear in muscle with respect to the totality of the 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–Smirnoff 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 g 100 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^{-1}, 89-98 mg dL^{-1}, and 54-56 ng mL^{-1},
251 respectively). Likewise, the activities of the enzymes GOT (10.9-20.6 U L^{-1}), GPT (3.9-
252 6.1 U L^{-1}) and lipase (7.1-7.8 U L^{-1}) 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

(DPA, docosapentaenoic acid) and DHA, reached higher values in fish fed the 100% FO-diet. Hepatic DHA/EPA ratio increased and EPA/ARA ratio decreased with reduced dietary FO (Table 5), which, conversely, remained unchanged in muscle (Table 6).

Muscle and liver showed a tissue-specific fatty acid profile, with muscle containing lower proportions of MUFA, and higher PUFA, n-3 and n-6 LC-PUFA than liver. DMA were present exclusively in muscle (2.5 to 3.0% of total FA). Irrespective to diet, C18 MUFA and C18:2n-6 proportions were 1.5 to 2-fold lower in muscle than in the liver (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, respectively), whereas C22:6n-3 was 3 to 4-fold higher in the muscle (29.6, 26.7 and 22.0 vs 9.9, 7.2 and 5.5% of total FA, respectively).

3.5 Indices of the nutritional quality of lipids

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

4. Discussion

In the present study, the plant-based oil mixture consisting of PO and LO (4:1) used to partially (50%) or totally substitute FO did not significantly affect greater amberjack, *S. 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 not effect (Glencross *et al.* 2016;
300 Mozanzaded *et al.* 2016) so it becames 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^{-1} 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*

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

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

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

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

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

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680

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

	FO100	VO50	VO100
Ingredients (g kg^{-1})			
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[a]	20	20	20
Proximate composition			
Dry matter (DM, g kg^{-1} wet weight)	886	894	899
Crude protein (g kg^{-1} DM)	452	456	461
Crude lipid (g kg^{-1} DM)	123	133	135
Ash (g kg^{-1} DM)	91	87	87
Crude fibre (g kg^{-1} DM)	237	245	240
Fatty acids (% total fatty acids)			
Total SFA[1]	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[1]	22.42	26.09	29.29
16:1[2]	7.74	5.70	3.12
18:1[2]	13.24	18.73	24.94
20:1[2]	0.95	0.79	0.49
22:1[2]	0.50	0.42	0.37
Total PUFA[1]	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[1]	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
Ratios			
PUFA/SFA	1.48	1.16	0.91
n-6/n-3	0.25	0.40	0.64
DHA/EPA[3]	0.735	0.736	0.874
EPA/ARA[3]	19.178	19.038	19.228

682 [a] Contains: choline, 10 g; DL-α-tocopherol, 5 g; ascorbic acid, 5 g; $Ca_3(PO_4)_2$, 5 g and a premix, 25 g.
683 This premix contains per kg: retinol acetate, 1,000,000 IU; calcipherol, 500 IU; DL-α-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 [1] Includes some minor components not shown.
690 [2] Includes other isomers not shown. Mainly n-7 isomer for C16 and n-9 isomer for C18, C20 and C22.
691 [3] 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 (%)[1]	894 ± 96	940 ± 75	840 ± 16
SGR (% day^{-1})[2]	1.49 ± 0.07	1.50 ± 0.05	1.47 ± 0.02
FI (g 100 g fish day^{-1})[3]	1.82 ± 0.26	1.81 ± 0.13	1.79 ± 0.02
FCR[4]	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 [1]Weight gain = 100 x [(final weight – initial weight) / initial weight];
696 [2]Specific growth rate = 100 x (ln final weight – ln initial weight) / feeding days;
697 [3]Feed intake = 100 x feed consumption (g) / average biomass (g) x days;
698 [4]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[1]	1.37 ± 0.17	1.30 ± 0.24	1.28 ± 0.10
VSI (%)[2]	4.32 ± 0.62	4.18 ± 1.10	4.33 ± 0.52
HSI (%)[3]	0.87 ± 0.20	0.78 ± 0.15	0.84 ± 0.10
MSI (%)[4]	0.18 ± 0.17	0.05 ± 0.15	0.10 ± 0.09
IFR (%)[5]	38.4 ± 4.17	34.1 ± 3.23	34.6 ± 4.60
IER (%)[6]	21.7 ± 0.91	20.6 ± 1.12	21.4 ± 0.83
Whole body proximate composition g (kg w.w.)$^{-1}$			
Moisture	696.5 ± 0.9 ^{a}	706.3 ±0.9 ^{b}	702.5 ± 0.2 ^{ab}
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 [1] Condition factor = 100 x (final weight / total length^3);
705 [2] Viscerosomatic index = 100 x (viscera weight / final weight);
706 [3] Hepatosomatic index = 100 x (liver weight / final weight);
707 [4] Mesenteric fat index = 100 x (viscera fat / final weight);
708 [5] Ingested fat retention = 100 x (fish fat gain / crude fat intake);
709 [6] 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^{-1})	223.4 ± 48.4	190.9 ± 35.5	185.9 ± 55.5
GOT (U L^{-1})	20.6 ± 11.5	12.6 ± 6.2	10.9 ± 0.8
GPT (U L^{-1})	6.1 ± 1.6	4.9 ± 1.2	3.9 ± 1.4
Triglycerides (mg dL^{-1})	94.4 ± 18.6	89.0 ± 25.8	97.8 ± 28.8
Cortisol (ng mL^{-1})	54.2 ± 28.3	56.1 ± 16.3	55.2 ± 6.1
Lipase (U L^{-1})	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 (µg 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 ±29.09	191.63 ± 24.92	222.07 ± 19.09	183.92 ± 28.56
Total SFA[1]	30.31 ± 1.00	30.25 ± 1.04	31.54 ± 1.28	31.46 ± 2.53
14:0	4.96 ± 0.18	4.62 ± 0.30 [c]	2.95 ± 0.25 [b]	1.67 ± 0.15 [a]
16:0	20.52 ± 0.87	20.33 ± 0.85 [a]	22.71 ± 1.21 [ab]	24.10 ± 2.12 [b]
18:0	3.63 ± 0.16	4.24 ± 0.51	5.04 ± 1.05	4.94 ± 0.99
Total MUFA[1]	28.39 ± 1.62	24.92 ± 1.75 [a]	30.52 ± 2.12 [b]	33.79 ± 2.18 [b]
16:1[2]	9.27 ± 0.18	7.71 ± 0.08 [c]	5.39 ± 0.21 [b]	2.86 ± 1.10 [a]
18:1[2]	18.08 ± 1.14	15.89 ± 1.62 [a]	23.92 ± 1.96 [b]	29.51 ± 1.93 [c]
20:1[2]	0.61 ± 0.18	0.55 ± 0.07	0.52 ± 0.08	0.52 ± 0.13
Total PUFA[1]	38.16 ± 2.65	43.92 ± 1.12 [b]	37.37 ± 2.57 [a]	34.54 ± 2.26 [a]
18:2 n-6	8.80 ± 0.36	9.70 ± 0.39 [a]	12.40 ± 1.05 [b]	15.26 ± 1.38 [c]
18:3 n-3	0.93 ± 0.04	1.23 ± 0.15 [a]	3.25 ± 0.21 [b]	5.25 ± 0.71 [c]
18:4 n-3	1.29 ± 0.16	1.32 ± 0.11 [c]	0.73 ± 0.09 [b]	0.29 ± 0.07 [a]
Total n-6 LC-PUFA[1]	1.56 ± 0.06	1.96 ± 0.22 [c]	1.47 ± 0.16 [b]	1.12 ± 0.17 [a]
20:2 n-6	nd	0.26 ± 0.05	0.29 ± 0.07	0.30 ± 0.10
20:4 n-6	1.26 ± 0.05	1.28 ± 0.12 [c]	0.92 ± 0.10 [b]	0.62 ± 0.14 [a]
22:5 n-6	0.29 ± 0.02	0.29 ± 0.01	0.20 ± 0.08	0.18 ± 0.04
Total n-3 LC-PUFA[1]	23.77 ± 2.65	27.75 ± 1.00 [c]	18.50 ± 2.25 [b]	12.18 ± 1.84 [a]
20:4 n-3	0.76 ± 0.01	0.94 ± 0.14 [c]	0.67 ± 0.10 [b]	0.34 ± 0.05 [a]
20:5 n-3	11.50 ± 1.03	13.48 ± 0.33 [c]	8.07 ± 0.87 [b]	4.61 ± 0.55 [a]
21:5 n-3	0.39 ± 0.03	0.46 ± 0.01 [c]	0.31 ± 0.03 [b]	0.16 ± 0.02 [a]
22:5 n-3	2.37 ± 0.13	2.92 ± 0.44 [c]	2.28 ± 0.28 [b]	1.42 ± 0.21 [a]
22:6 n-3	8.75 ± 1.46	9.95 ± 1.32 [b]	7.18 ± 1.61 [a]	5.51 ± 1.46 [a]
Ratios				
PUFA/SFA	1.26 ± 0.13	1.45 ± 0.05 [b]	1.19 ± 0.12 [a]	1.11 ± 0.14 [a]
n-6/n-3	0.40 ± 0.05	0.39 ± 0.02 [a]	0.62 ± 0.09 [b]	0.92 ± 0.11 [c]
DHA/EPA[3]	0.76 ± 0.06	0.74 ± 0.09 [a]	0.88 ± 0.14 [ab]	1.20 ± 0.28 [b]
EPA/ARA[3]	9.10 ± 0.53	10.58 ± 0.78 [b]	8.81 ± 0.59 [ab]	7.68 ± 1.80 [a]

718 Results are expressed as means ± 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 [1] Includes some minor components not shown.
721 [2] Includes other isomers not shown. Mainly n-7 isomer for C16 and n-9 isomer for C18 and C20.
722 [3] DHA/EPA, 22:6 n-3/ 20:5 n-3; EPA/ARA, 20:5 n-3/ 20:4 n-6.
723

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

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

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

[1] Includes some minor components not shown.

[2] Includes other isomers not shown. Mainly n-7 isomer for C16 and n-9 isomer for C18 and C20.

[3] 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.

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