Milián-Sorribes, MC.; Martínez-Llorens, S.; Cruz-Castellón, C.; Jover Cerda, M.; Tomas-Vidal, A. (2021). Effect of fish oil replacement and probiotic addition on growth, body composition and histological parameters of yellowtail (Seriola dumerili). Aquaculture Nutrition. 27(1):3-16. https://doi.org/10.1111/anu.13171

Effect of fish oil replacement and probiotic addition on growth, body composition and histological parameters of yellowtail (*Seriola dumerili)*

M. Consolación Milián-Sorribes[1], Silvia Martínez-Llorens[1], César Cruz-Castellón[2], Miguel Jover-Cerdá[1], Ana Tomás-Vidal[1].

[1]Research Group of Aquaculture and Biodiversity. Institute of Animal Science and Technology. Universitat Politècnica València. Camino de Vera, 14. 46022-Valencia, Spain

[2]Departamento Académico de Acuicultura e Industrias Pesqueras. Facultad de Pesquería. Universidad Nacional Agraria la Molina. Av. La Molina s/n La Molina, Lima, Peru.

Abstract

Fish (175 g of initial weight) were fed in triplicated groups with four diets formulated by 0% (FO 100), 75% (FO 25) and 100% (with and without probiotics, FO 0 and FO 0+) of fish oil replacement consisting of a mixture of linseed, sunflower and palm oils. After 109 days, growth and nutritional parameters were not affected by the treatment, however, fish fed with 0% of fish oil showed the lowest survival rate and without differences between the same diet with probiotics. As for biometric parameters, significant differences in the viscerosomatic index (VSI) were observed between fish fed the FO 0+ diet and the FO 100 and FO 25 diets. Results obtained from histological analysis did not detect inflammation in gut samples, while liver samples showed a remarkable steatosis in all four treatments. Total fish oil replacement produced a significant difference in the width of the lamina propria. The dietary inclusion of probiotics in the FO 0+ diet seems to favor a recovery of intestine histology. In addition, as fish oil substitution increased, the width of the lamina propria also increased.

In conclusion, it is possible to affirm that the four diets administrated to *Seriola dumerili* did not compromise the correct development of the animals.

Keywords: fish oil, *Seriola dumerili*, yellowtail, fatty acids, histology, probiotics

1. Introduction

The high demand and insecurity in the supply of fish oil (FO) is causing an increase in the price, which is leading aquaculture industries to search for vegetable oil substitutes.

This situation is relevant to aquaculture activity sustainability; because FO is the main lipidic source for carnivorous marine fish diets, due to its high content of essential fatty acids, in particular n-3 HUFA (Nasopoulou and Zabetakis, 2012). Several vegetable oils have been studied to substitute fish oil, such as soybean, rapeseed, linseed, palm, sunflower, canola oil, etc. A review of fish oil replacement in finfish has been published by Turchini *et al.* (2009) and a meta-analysis of the substitution of marine oil by plant oils has been reported by Sales and Glencross (2011).

Soybean and linseed oil are considered as a very good alternative lipid source for partial substitution in feed for freshwater fish and salmonids (Bell *et al.*, 2001; Rosenlund *et al.*, 2001, Acar *et al.*, 2018). Other sources and the combination of them have been assayed in salmon (Higgs *et al.*, 2006; Menoyo *et al.*, 2006; Nanton *et al.*, 2007; Pratoomyot *et al.*, 2008; Torstensen *et al.*, 2008; Bell *et al.*, 2002; Bogevik *et al.*, 2011), being possible a complete substitution of fish oil when a mixture of rapeseed, palm and cameline oils was used (Bell *et al.*, 2002). Complete replacement of fish oil has been reported by other works, but this is only possible when high/medium dietary levels of fish meal are present in the diets (Drew *et al.*, 2007, Acar and Türker, 2018). Also, several studies have been developed on sea bream

(Kalogeropoulos *et al.*, 1992; El-Kerdawy and Salama, 1997; Montero *et al.*, 2003; Izquierdo *et al.*, 2003, 2005; Caballero *et al.*, 2004; Martínez-Llorens *et al.*, 2007; Benedito-Palos *et al.*, 2008; Dias *et al.*, 2009; Fountoulaki *et al.*, 2009; Wassef *et al.*, 2009; Montero and Izquierdo, 2010) and a study on yellowtail (Monge-Ortiz *et al.*, 2018b). The supplementation with essential fatty acids (EFA) is not habitual, and only Ibeas *et al.* (1996, 1997, 2000) used HUFAs in diets with beef tallow.

Studies on the nutritional requirements of *S. dumerili* are scarce, because the studies have focused on alternative protein fish meal sources and nutrient levels (Jover *et al.*, 1999; Tomás-Vidal *et al.*, 2005, 2008; Takakuwa *et al.*, 2006; Uyan *et al.*, 2009; Monge-Ortiz *et al.*, 2018 a, b). Several species within the Seriola family have been researched both for nutritional requirements and replacement of fishmeal and fish oil in yellowtail kingfish (*S. lalandi*) (Bowyer *et al.*, 2012a, 2013; Collins *et al.*, 2014), and Japanese yellowtail (*S. quinqueradiata*) (Seno-O *et al.*, 2008; Sarker *et al.*, 2012; Khaoian *et al.*, 2014).

The main storage sites for lipids in fish are the liver, the perivisceral adipose tissue and the muscle, the only part that is really edible (Guillaume *et al.*, 2004). In numerous species, such as *Seriola lalandi*, rainbow trout, sea bream, *P. californicus*, and *Maccullochella peelii*, it has been demonstrated that that the fish fillet fatty acid profile is totally conditioned by the diet fatty acid profile (Caballero *et al.*, 2002; Izquierdo *et al.*, 2005; Badillo-Zapata *et al.*, 2010; Turchini *et al.*, 2011; Bowyer *et al.*, 2012b), similarly, this also happens in *S. dumerili*. The nature and content of a fats diet have a considerable influence on muscle composition (Guillaume *et al.*, 2004). Therefore, the composition of body FA reflects the diet FA. So, the substitution of the FO for an alternative lipid source, either plant or animal, not only affects the fish growth and the nutritional efficiency, but also affects (Turchini *et al.*, 2009) the

74 body fatty acids profile, as a consequence of the capacity of the species to
75 biosynthesize fatty acids.

76 In addition to the effects on growth and body composition as a result of the fish
77 oil replacement, intestinal changes can be produced, such as modifications in the
78 submucosa and muscular layers in the case of rainbow trout (Xu *et al.*, 2016). Also in
79 rainbow trout, Caballero *et al.* (2002) observed an increase in the accumulation of fat
80 in the liver in fish fed diets with high fish oil substitution. Sea bream fed high fish oil
81 substitution diets presented differences in the size of the hepatocytes by the
82 accumulation of fat and also the nuclei moved towards the periphery of the cell
83 (Wassef *et al.*, 2007), altering the cellular structure. Conversely, no intestinal and
84 hepatic changes were found in seabass, *Dicentrarchux labrax* (Figueiredo-Silva *et al.*,
85 2005) and sharpsnout seabream, *Diplodus puntazzo* (Nogales-Mérida *et al.*, 2017) fed
86 diets with soybean oil. In addition, previous studies reported that probiotics might
87 facilitate digestion (Najafabad *et al.*, 2016), due to it can modified the structure of the
88 intestine, such as villi elongation (Je *et al.* 2019), and intestinal fold height augment
89 (Yang *et al.*, 2019), and function of the gastrointestinal tract (Akter *et al.*, 2016)
90 optimising the nutrients absorption surface and therfore the nutrients digestibility
91 (John *et al.*, 2008). Nevertheless, in almost all of these studies address the interaction
92 beetween probiotics and dietary fish meal subtitution by alternative protein, but the
93 studies focus on the nexus between these additivites with FO susbtituion in fish diets
94 are scarce.

95 The use of probiotics and its effects on growth and survival has been studied in
96 aquaculture species such as the goldfish, *Carassius auratus* (Abraham *et al.*, 2008)
97 golden pompano, *Trachinotus ovatus* (Liu *et al.*, 2020) and the Nile tilapia,
98 *Oreochromis niloticus* (Aly *et al.*, 2008). The importance of the use of probiotics in

aquaculture is due to the fact that in fish; these would occupy and colonize sites in the digestive tract particularly in the epithelium of the gastrointestinal mucosa which, in turn, would displace pathogens and therefore improve the health status of organisms (Jöborn *et al.*, 1997; Macey and Coyne, 2006; Merrifield *et al.*, 2010; Lazado *et al.*, 2011; Korkea-Aho *et al.*, 2012). Likewise, the use of probiotics improves the activities of the innate immune system such as phagocytes (neutrophils and macrophages), respiratory status, lysozyme activity and the activity of peroxidase and antiprotease (Akhter *et al.*, 2015), modulates GI communities and improves the systemic immune responses (Merrifield and Ringø, 2014,). This is important since it helps the fish in the face of an adverse situation such as the appearance of pathogens and diseases in fish that have been fed with high replacement of fish meal and fish oil diets. It is also known that the use of probiotics in diets can modified in the microbiota associated with the gastrointestinal tract of the host and generate beneficial effects such as increased feed conversion and digestibility (De Schrijver and Ollevier, 2000; Ten Doeschate and Coyne, 2008; Diaz and Martinez-Silva, 2009, Je *et al.*, 2019). Stress and immunosuppression in fish fed non-fish meal and non-fish oil are subjected to increase the chances that the fish will be affected by pathogens specially in liver (Montero *et al.*, 2008; Martin and Król, 2017), since when marine ingredients replacement are high, mortality increases (Estruch *et al.*, 2018a, 2018b; Monge-Ortiz *et al.*, 2018a, 2018b). In order to improve the survival of fish fed with high vegetal ingredients dietary inclusion and its effect on immunosuppression, probiotics can be added with the objective to enhance the immune system of depressed fish (Dimitroglou *et al.*, 2011, Merrifield and Ringø, 2014, Ringø, 2020). The use of probiotics has been shown to significantly increase survival and fish performance (Acar *et al.*, 2018; Liu *et al.*, 2020).

The aim of this study was to evaluate the replacement of fish oil for a mixture of vegetable oils and the effect of the addition of a probiotic composed of *Lactobacillus brevis* and *L. buchneri*, to analyse the repercussion on the growth and nutritional parameters of *S. dumerili*, its fatty acid composition and the digestive histology effects.

2. Material and methods

2.1. Fish and experimental diets

The trial was carried out with juvenile fish (*S. dumerili*) obtained from the Futuna Blue S.A. company (Cádiz, Spain). The average weight of fish was 175 g. Before starting the growth trial, the animals had been allowed to acclimatize (one month) to the new conditions of the laboratory. During this time, fish were fed up to apparent satiation, twice daily (9:00 a.m.-16:00 p.m.) with a control diet, six days a week. Water parameters had been constantly monitored: temperature 21.5± 2.4°C, salinity 31 $g \cdot L^{-1}$ (31.5 ± 4.1 $g \cdot L^{-1}$), pH 7.5 to 8.0 and dissolved oxygen 8 $mg \cdot l^{-1}$. The photoperiod was natural and all tanks had similar light conditions. Following the acclimatization period, a total of 300 fish were randomly distributed in 12 tanks (25 fish/tank). The fish re-distribution into tanks was conducted in a way that limited stress. The animals were anaesthetized with clove oil at 30 $mg \cdot L^{-1}$, containing 87% of euglenol (Guinamas, Valencia, Spain). The period of the trial was of 109 days.

Four isolipidic diets (15% CF, crude fat) and isoproteic diets (52% CP, crude protein) were formulated. Diets were formulated with different levels of fish oil replacement with a mixture of vegetable oils. The diets composition is shown in Table 1. FO 0+ diet had the same formulation than FO 0 with the addition of probiotics

148 *Lactobacillus brevis* and *L. buchneri, which* were added daily to the pellet by spraying
149 it directly before eating (50 ml for 500 g).

150 2.2. Biometric parameters, growth indices and proximate composition

151 At the end of the growth trial, five fish were randomly sampled from each tank to
152 determine the biometric parameters and to carry out the proximate composition
153 analysis. The following indices were calculated:

154 Specific Growth Rate [% d^{-1}], SGR = {100 x ln [Final weight/Initial weight]}/d

155 Feed Intake ratio [g 100 g $fish^{-1}$ day^{-1}], FI = {100 x feed intake [g]}/{Average biomass
156 [g]. d}

157 Feed Conversion Ratio, FCR = Feed intake [g]/Weight gain [g]

158 Condition Factor [g·cm^{-3}], CF = 100 x Total fish weight [g]/Total $length^3$ [cm^3]

159 Viscerosomatic Index [%], VSI = 100 x Visceral weight [g]/Total fish weight [g]

160 Hepatosomatic Index [%], HSI = 100 x Liver weight [g]/Total fish weight [g]

161 Mesenteric Fat Index [%], MFI = 100 x Mesenteric fat weight [g]/Total fish weight
162 [g]

163 Dressout Percentage [%]: DP = 100 x (Total fish weight [g] - Visceral weight [g] -
164 Head weight [g])/Total weight

165 Muscle Index [%], MI: 100 x Muscle weight [g]/Total fish weight [g]

166 2.3. Chemical analysis

167 Chemical analyses of the dietary ingredients were performed prior to diet
168 formulation. The proximate composition of whole fish and fish diets were analysed
169 according to (AOAC, 1990) procedures: dry matter, official method 934.01 (105°C

to constant weight); ash, official method 942.05 (incinerated at 550°C for 5 h); crude protein, official method 990.03 (determined by direct combustion method DUMAS using LECO CN628) and crude lipid, official method 920.39 (extracted with methyl-ether using ANKOMXT10 Extractor). All analyses were performed in triplicate. The following indices were calculated:

Protein Productive Value [%], PPV = 100 x Protein fish gain [g]/Protein intake [g]

Fat Productive Value [%], FPV = 100 x Fat fish gain [g]/Fat intake [g]

Energy Productive Value [%], EPV = 100 x Energy fish gain [g]/Energy intake [g]

Fatty acids were determined by direct synthesis of methyl esters (FAME), and analysed by gas chromatography on a FINNIGAN FOCUS 6C chromatograph (AI 3000) (O'Fallon *et al.* 2007).

2.4. Histological analysis

Samples of proximal and distal intestine from three fish per tank were taken at the end of the experiment, fixed in formalin, dehydrated in a different ethanol concentration and fixed in paraffin. Sections (5µm) were stained with PAS-Alcian blue and observed through light microscopy.

The histological analysis performed was a quantitative analysis. Measurements used a combination of parameters proposed by different authors (Santigosa *et al.*, 2008, Adamidou *et al.*, 2009, Øverland *et al.*, 2009). Specifically, the histological analysis focused on the measurement of length and width of the intestinal villus. Six villi were measured for each of the three fish collected in each of the three tanks belonging to the same treatment. In addition, the thickness of the lamina propria, of the submucosa, muscular and serous layer were analysed

All the images of samples were taken with an optical microscope Nikon JAPON 0.90. The images were analysed using Photoshop software and a conversion into metric units.

2.5. Statistical analysis

Growth data, nutrient utilization, biometric parameters, body composition, fatty acids composition and histological analysis were treated using multifactor analysis of variance (ANOVA). The Newman–Keuls test was used to assess specific differences among diets at 0.05 significant levels (Statgraphics, Statistical Graphics System, Version Centurion XVI, Warrenton, Virginia, USA).

2.6. Ethical statement

The *Seriola dumerili* study complied with the European Union Council Directive 2010/63/UE, which sets out standards for the protection of animals and Spanish legislation (Spanish Royal Decree 53/2013) that protect animals used in experimentation. The experimental protocol was approved by the Ethics and Animal Welfare Committee of the Universitat Politècnica de València (UPV).

Fish in the tanks were checked twice daily. Additionally, every month fish were weighed individually and their health status was assessed by way of observation after sedation with clove oil dissolved in water (0.01 $mg \cdot L^{-1}$ of water). Animals were euthanized by an excess of clove oil (150 $mg \cdot L^{-1}$) and later dissected and analyzed.

3. Results

3.1. Composition of diets

Composition and concentration of the fatty acids diets reflect the concentrations of the types of oils used in each of them (Table 2). Thus, the diets containing fish oil lipid source had a higher quantity of highly unsaturated essential fatty acids of the n-

217 3 chain (HUFA n-3). On the other hand, diets where vegetable oils such as linseed,
218 sunflower and palm were used as a lipid source were characterized by a greater
219 quantity of linoleic and linolenic fatty acids respectively.

220 3.2. Growth, nutritional and biometric parameters

221 During the 109 days of the growth trial no negative effects were found with either
222 partial and complete fish oil substitution with vegetable oil or the inclusion of
223 probiotics on growth and feed performance of *S. dumerili* (Table 3). However, if
224 differences in survival were found, fish fed the FO 25 treatment showed the highest
225 value, while fish fed the FO 0+ diet the lowest. All diets were readily accepted by the
226 fish and no significant differences were reported in feed intake (1.1 g/100 fish per
227 day), food conversion ratio (1.6) or specific growth ratio (0.8 %/day).

228 In general, biometric indices were similar in all treatments analysed as is shown
229 in Table 4, except the viscerosomatic index (VSI), which was higher in yellowtail fed
230 with the FO0+diet.

231 3.3. Body composition

232 Body composition and productive values are shown in Table 5. No significant
233 differences were found in crude fat, dry matter and ashes in fish composition.
234 Conversely, crude protein results indicated significant differences between treatments
235 FO 25 and FO 0+. No significant differences were found in productive values.

236 The fatty acids profile (g 100 /g in wet weight) of the juveniles of yellowtail fed
237 during the experiment is shown in Table 6. Fish fed the FO 25, FO 0 and FO 0+ diets
238 (including high levels of vegetable oils) presented significantly higher concentrations
239 of α-linolenic acid and the EPA / DHA ratio, while fish fed the control diet (FO 100),
240 presented significantly greater proportions of 20:3n6 acid, arachidonic acid and

241 docosahexaenoic acid (DHA). The amount of eicosapentaenoic acid (EPA) did not
242 differ significantly between treatments that contained fish oil lipid source (FO 100
243 and FO 25) (Table 6).

244 Results of the fatty acids productive values are shown in Table 7. In comparison
245 with the control diet (58.9 g/100 g), the retention efficiency of fish fed diets with
246 100% vegetable mixtures oils (FO 0 (88.7 g/100 g) and FO 0+ (88.5 g/100 g)) showed
247 higher retention efficiencies of 17:0 acid, while only the group of fish fed the FO 0
248 diet indicated significant efficiencies under 18:3n-6, 22:4n-6 acid and 18:3n-3.In the
249 case of EPA and DHA, no significant differences were found between treatments.

250 3.4. Histological observations

251 The results of anterior and posterior intestine measurements are reported in Table
252 8 and 9, respectively.

253 In the anterior intestine differences only appear in the thickness of the submucosa
254 layer, thinner in the control diet. No statistically significant differences were
255 identified in the length and width of the villi and lamina propria. For all treatments,
256 the ratio between the width of the lamina propria and the width of the villus were very
257 similar.

258 In the posterior intestine the thickness of serosa (SL), muscularis (ML) and
259 submucosa (SML) of the four treatments showed no statistically significant
260 differences among fish fed the different diets. However, significant differences were
261 identified in the length of the villi and in the width of the lamina propria. Particularly,
262 FO 25 shows the lower length of villi value, which is statistically different from the
263 FO 100, FO 0 and FO 0+ diets.

264 Regarding liver histology, the cells count and their respective measurement
265 (diameter and core) were not possible because of the remarkable steatosis present in
266 samples of all treatments (Figure 1). As *Seriola dumerili* is a lean fish, accumulates
267 lipids in the liver. The hepatocytes, which should be of polygonal form with a central
268 nucleus, are deformed by the diffuse presence of lipid droplets to the point that their
269 shape is not perceptible.

270 **4. Discussion**

271 The present study indicates that it is possible to substitute practically all FO from
272 the feed for juveniles of yellowtail (*S. dumerili*) with a mixture of vegetable oils
273 (palm, linseed and sunflower), without causing adverse effects on growth, fish
274 performance and nutritional parameters. This is similar to what happens in other
275 species, such as the California halibut (*Paralichthys californicus*) (Badillo-Zapata *et*
276 *al.*, 2010), the gilthead seabream (*Sparus aurata*) in a work of Benedito-Palos *et al.*
277 (2007) or rainbow trout (*Oncorhynchus mykiss*) (Thanuthong *et al.*, 2011, Acar *et al.*,
278 2018, Parrino *et al.*, 2019).

279 The fatty acid composition of the diets formulated with 100 (FO 100) and 25%
280 (FO 25) fish oil as a lipid source, showed higher concentrations of essential fatty acids
281 (EFA), ARA, EPA and DHA, while, as was expected, feed formulated with 100%
282 mixtures of linseed, palm and sunflower oils without (FO 0) and with probiotic
283 supplementation (FO 0+), were characterized by high levels of 18: 1n-9, 18: 2n-6 and
284 18: 3n-3, respectively. The mixture of vegetable oils provides n-3 / n-6 and EPA /
285 DHA balanced ratios, similar to that obtained in our previous formulation in diets for
286 Mediterranean yellowtail (Monge-Ortiz *et al.*, 2018b). This did not suppose a
287 disadvantage, neither in the growth nor in the nutritious parameters of the M.
288 yellowtail, due to all the diets covered the requirements of EPA, DHA and of highly

289 unsaturated fatty acids of chain n-3 (n-3 HUFA), possibly because the amount of FO
290 present in FM (350g/kg) was sufficient to reach the minimum needs of EFA in
291 yellowtail 175 g initial weight. It is possible to make diets for *Seriola spp*. with levels
292 of EPA and DHA around 0.5% (Guillaume *et al.*, 2004), even in the case of EPA, it
293 could be with levels of 0.3%, since no differences in growth have been found

294 Although the substitution of fish oil for mixtures of vegetable oils did not affect
295 the growth and nutritional parameters in Mediterranean yellowtail, it has a negative
296 effect on survival, it could be explained by the deficiency of essential fatty acids in
297 diets without fish-oil, because this reduction may lead to immunosuppression in fish
298 (Montero *et al.*, 1998, 2003), as it has been reported in other fish species.

299 Substitutions above 75% of the FO by mixtures of VO significantly affect
300 yellowtail survival. The addition of the probiotic did not improve survival in this
301 experiment, similar to were reported in rabbitfish (*Siganus rivulatus*) (El-Dakar *et al.*,
302 2007) and in dentex (*Dentex dentex*) (Hidalgo *et al.*, 2006). Probiotics act on both
303 innate and adaptive gut immunity. The microorganisms they contain can stimulate the
304 production of certain components of the immune system, such as the secretion of
305 cytokines. In addition, certain probiotics can induce differentiation of mature B
306 lymphocytes and the production of antibodies, such as IgA. Despite the large number
307 of studies, it is currently unknown exactly how probiotics interact with lymphoid cells
308 in the intestine to achieve activation of the intestinal immune system but it is a fact
309 that so happens. The use of probiotics that had been studied in other species such as
310 poultry, pigs or cattle, has given good results, improving intestinal health and growth
311 in fish (Dimitroglou *et al.*, 2009). Probiotics (*Lactobacillus brevis* and *L. buchneri*)
312 were added to improve the digestibility of nutrients and to strengthen the immune
313 system of the host (Akhter *et al.*, 2015) in non-fish oil diet, but results were not as

314 expected. In other species, such as tilapia (*Oreochromis niloticus*) (Aly *et al.*, 2008),
315 sole (*Solea senegalensis*) (Sáenz De Rodrigáñez *et al.* 2009) and golden pompano
316 (*Trachinotus ovatus*) (Liu *et al.* 2020) the use of probiotics in diets has been shown
317 to improve nutrient digestibility and survival.

318 Nonetheless, the use of probiotics in aquaculture can pose difficulties, and it is
319 important to take into account two factors of great influence: the selection of the
320 strains and their stability that allows obtaining an effective density. It can be the cause
321 of the non-effectivity in present work, probiotics must be specifically selected from
322 the hosts in which they are to be used, since in this way the effects caused by the wide
323 differences between the environments in which organisms develop are minimized.
324 But similar results were obtained in Atlantic salmon, the use of probiotics increased
325 their mortality (Gildberg *et al.*, 1995) or in sea bream (*Sparus aurata*) where
326 probiotics addition caused intestinal damage (Cerezuela *et al.*, 2012, 2013).
327 Therefore, it is possible that the probiotics used could not have helped to strengthen
328 the immune system in the FO 0+ treatment because the species used are not the most
329 suitable for the yellowtail.

330 A close relationship between FA dietary level and fish survival was observed in
331 present trial. In fact, Izquierdo *et al.* (2003) reported that sea bream fed diets without
332 fish oil for 204 days demonstrated effects in both humoral and cellular immunology.
333 Montero *et al.*, (2008) reported that the inclusion of dietary vegetable oils modified
334 the fish content and ratio of arachidonic and eicosapentaenoic acids, altering in turn
335 the production of immunologically active eicosanoids derived from these fatty acids.
336 Specifically, in present work conditions, it is therefore possible to hypothesize that
337 the monthly handling of fish may have generated stressful situations for the animals.
338 Probably, fish fed with FO 0 and FO 0+ diets received enough *n*-3 HUFA, EPA and

DHA for their growth but insufficient to complete the immunologic system, whereas diets FO 0 and FO 0+ showed the lowest *n*-3 HUFA values. Specifically, *n*-3 HUFA requirements for marine species, such as red seabream (*Pagrus major*), yellowtail (*Seriola lalandi*) and turbot (*Psetta maxima*), range from 0.5 to 2.0% of dry diet (Kiron *et al.*, 1995; Montero *et al.*, 1998; Salze *et al.*, 2010) detected that the immune response of rainbow trout and sea bream was depressed when the diet was deficient in EPA and DHA. Furthermore, an EFA unbalanced contribution could change the membrane fatty acid composition of immune cells such as leukocytes (Montero *et al.*, 2008).

In fact, dietary fatty acids influence the lipid composition of membrane cells and their physical properties, exerting a profound effect on the activity of enzymes associated with membranes and receptors, and on immune response, as many of these responses are based on interactions on the cell membrane of leukocytes (e.g. cytokinin production). Although, dietary fatty acids may affect the production of eicosanoids derived from fatty acids of 20 carbon atoms (mainly EPA and arachidonic acid, AA). Eicosanoids include prostaglandins, leukotrienes and lipoxins, which are involved in various physiological processes such as osmoregulation and immune response (Uhing *et al.*, 1990; Rola-Pleszczynski and Stankova 1992).

While the effect of improving nutritional parameters in the diet is not reflected, due to fish fed with and without probiotics exhibited very similar results, some differences between both treatments were found in gut histology. The anterior intestine submucosa thickness of fish fed control and the FO 0+ diet was similar as the width of the lamina propria in the posterior, which can be explained by the assumption that probiotics have helped to reduce LP inflammation. In the other

histological parameters studied no differences were found between diet, as it has been reported in olive flounder (*Paralichthys olivaceus*) where the probiotic slightly modified the size of the villi without finding significant differences (Je *et al.* 2019). Despite this, in present work, the substitution of fish oil has not noticed very relevant effects on intestinal histology. The massive steatosis observed in all liver samples highlights a dysregulation of lipid metabolism. These results are in agreement with several previous works; Fountoulaki *et al.*, (2009) found that sea bream fed dietary lipids from vegetable origin seemed to increase lipid accumulation in liver at a different degree according to the fat origin, palm oil being superior to soybean oil, which was superior to rapeseed oil which was superior to fish oil. In particular, livers from fish fed with palm oil diet showed apparent steatosis, with intense lipid accumulation. The integrity of the hepatocytes was also affected; swelling and nuclei displacement were evident in all examined livers. Again, Benedito-Palos *et al.* (2008) studied the effect of feeding sea bream with diets rich in vegetable oil, and reported that fish showed fatty livers, in particular with signs of lipoid liver disease. Under present work conditions, all livers studied present steatosis, perhaps it is because the yellowtail needs diets with low fat level and the excess is accumulated in the liver, as was demonstrated by Tomás-Vidal *et al.* (2005) where yellowtail fed with 14% CF diets got better growth and nutritional results than fishes fed with 17% CF.

In particular, linoleic acid had perhaps been included in diets at too high concentrations for the requirements of yellowtail. As reported by Caballero *et al.*, (2004), this PUFA could be one of the main causes of lipid accumulation in the liver. The authors suggest that the type of non-essential fatty acid, characteristic of vegetable oils, induces the appearance of steatosis in the following order: linoleic acid > linolenic acid > oleic acid. Moreover, another possible explanation of the

remarkable steatosis presented could be related to the lack of the dietary phospholipids. Lu *et al.*, (2008) reported that the dietary inclusion of soybean phospholipid (PL) to *Pelteobagrus fulvidraco* larvae decreased the degree of lipid accumulation in the hepatocytes. Specifically, Ipatova *et al.*, (2004) reported that proven health benefits in the case of soybean phospholipids supplementation include lipid decrease, control of blood levels of cholesterol and triglycerides, stabilization of membrane functions and support of hepatic functions. In the case of *Seriola dumerili*, it is also possible to hypothesize that the rearing conditions have influenced the energy consumption of fish. It is possible to suppose that swimming limited to the area of the tanks compared to swimming in natural habitat may have contributed to decreasing energy consumption.

5. Acknowledgements

The financial support for this study was provided by the "Conselleria de Educació, Cultura i Esport" of Valencian government (Reference: AICO/2015/123). We also acknowledge David Harry Rhead for their English revision.

Data availability statement

No data to share.

6. References

Abraham, T.J., Mondal, S., and Surendra Babu, C. (2008). Effect of commercial aquaculture probiotic and fish gut antagonistic bacterial flora on the growth and disease resistance of ornamental fishes *Carassius auratus* and *Xiphophorus helleri*. *Journal of Fisheries & Aquatic Sciences*, 25 (1), 27–30.

Acar, Ü., Parrino, V., Kesbiç, O.S., Lo Paro, G., Saoca, C., Abbate, F., Yilmaz, S. and Fazio, F. (2018). Effects of different levels of pomegranate seed oil on some blood

parameters and disease resistance against *Yersinia ruckeri* in rainbow trout. *Frontiers in Physiology*, 9 (596), 1–7.

Acar, Ü. and Türker, A. (2018). Response of rainbow trout (*Oncorhynchus mykiss*) to unrefined peanut oil diets : Effect on growth performance, fish health and fillet fatty acid composition. *Aquaculture Nutrition*, 24, 292–299.

Adamidou, S., Nengas, I., Henry, M., Grigorakis, K., Rigos, G., Nikolopoulou, D., Kotzamanis, Y., Bell, G.J., and Jauncey, K. (2009). Growth, feed utilization, health and organoleptic characteristics of European seabass (*Dicentrarchus labrax*) fed extruded diets including low and high levels of three different legumes. *Aquaculture*, 293 (3–4), 263–271.

Akhter, N., Wu, B., Memon, A.M., and Mohsin, M. (2015). Probiotics and prebiotics associated with aquaculture: A review. *Fish and Shellfish Immunology*, 45 (2), 733–741.

Akter, M.N., Sutriana, A., Talpur, A.D., and Hashim, R. (2016). Dietary supplementation with mannan oligosaccharide influences growth, digestive enzymes, gut morphology, and microbiota in juvenile striped catfish, *Pangasianodon hypophthalmus*. *Aquaculture International*, 24, 127–144.

Aly, S.M., Abdel-Galil Ahmed, Y., Abdel-Aziz Ghareeb, A., and Mohamed, M.F. (2008). Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge infections. *Fish and Shellfish Immunology*, 25 (1–2), 128–136.

AOAC. (1990). *Official methods of analysis, 15th edn.* Association of Official Aalytical Chemists. Arlington, VA, USA.

437 Badillo-Zapata, D., Correa-Reyes, G., D'Abramo, L.R., Lazo, J.P., Toro-Vázquez. J.F.
438 and Viana, M.T. (2010). Effect of replacing dietary fish oil with vegetable oils on
439 the fatty acid composition of muscle tissue of juvenile California halibut
440 (*Paralichthys californicus*). *Ciencias Marinas*, 36 (2), 121–133.

441 Bell, J.G., Henderson, R.J., Tocher, D.R., Mcghee, F., Dick, J.R., Porter, A., Smullen,
442 R.P., and Sargent, J.R. (2010). Substituting fish oil with crude palm oil in the diet of
443 Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic
444 fatty acid metabolism. *American Society for Nutritional Sciences*, 222–230.

445 Bell, J.G., Mcevoy, J., Tocher, D.R., Mcghee, F., Campbell, P.J., and Sargent, J.R. (2001).
446 Replacement of fish oil with rapeseed oil in diets of atlantic salmon (*Salmo salar*)
447 affects tissue lipid compositions and hepatocyte fatty acid metabolism, 2 (22), 1535–
448 1543.

449 Benedito-Palos, L., Navarro, J.C., Sitjà-Bobadilla, A., Bell, J.G., Kaushik, S., and Pérez-
450 Sánchez, J. (2008). High levels of vegetable oils in plant protein-rich diets fed to
451 gilthead sea bream (*Sparus aurata* L.): Growth performance, muscle fatty acid
452 profiles and histological alterations of target tissues. *British Journal of Nutrition*,
453 100 (5), 992–1003.

454 Benedito-Palos, L., Saera-Vila, A., Calduch-Giner, J.A., Kaushik, S., and Pérez-Sánchez,
455 J. (2007). Combined replacement of fish meal and oil in practical diets for fast
456 growing juveniles of gilthead sea bream (*Sparus aurata* L.): Networking of systemic
457 and local components of GH/IGF axis. *Aquaculture*, 267 (1–4), 199–212.

458 Bogevik, A.S., Henderson, R.J., Mundheim, H., Olsen, R.E., and Tocher, D.R. (2011).
459 The effect of temperature and dietary fat level on tissue lipid composition in Atlantic
460 salmon (*Salmo salar*) fed wax ester-rich oil from *Calanus finmarchicus*.

461 *Aquaculture Nutrition*, 17 (3).

462 Bowyer, J.N., Qin, J.G., Adams, L.R., Thomson, M.J.S., and Stone, D.A.J. (2012a). The
463 response of digestive enzyme activities and gut histology in yellowtail kingfish
464 (*Seriola lalandi*) to dietary fish oil substitution at different temperatures.
465 *Aquaculture*, 368–369, 19–28.

466 Bowyer, J.N., Qin, J.G., Smullen, R.P., and Stone, D.A.J. (2012b). Replacement of fish
467 oil by poultry oil and canola oil in yellowtail kingfish (*Seriola lalandi*) at optimal
468 and suboptimal temperatures. *Aquaculture*, 356–357, 211–222.

469 Bowyer, J.N., Qin, J.G., Smullen, R.P., Adams, L.R., Thomson, M.J.S., and Stone, D.A.J.
470 (2013). The use of a soy product in juvenile yellowtail king fish (*Seriola lalandi*)
471 feeds at different water temperatures : 2. Soy protein concentrate. *Aquaculture*, 410–
472 411, 1–10.

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 Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Fernandez, A.J., and Rosenlund, G.
477 (2004). Histological alterations in the liver of sea bream, *Sparus auratus* L., caused
478 by short or long term feeding with vegetable oils as the sole lipid source. *Journal of*
479 *Fish Diseases*, 27, 531–541.

480 Caballero, M.J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M., and Izquierdo,
481 M.S. (2002). Impact of different dietary lipid sources on growth, lipid digestibility,
482 tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*.
483 *Aquaculture*, 214 (1–4), 253–271.

484 Cerezuela, R., Fumanal, M., Tapia-Paniagua, S.T., Meseguer, J., Morinigo, M.A. and
485 Esteban, M.A. (2012) Histological alterations and microbial ecology of the intestine
486 in gilthead seabream (*Sparus aurata* L.) fed dietary probiotics and microalgae. *Cell*
487 *and Tissue Research*, 350, 477–489.

488 Cerezuela, R., Fumanal, M., Tapia-Paniagua, S.T., Meseguer, J., Moriñigo, M.Á. and
489 Esteban, M.Á. (2013) Changes in intestinal morphology and microbiota caused by
490 dietary administration of inulin and *Bacillus subtilis* in gilthead sea bream (*Sparus*
491 *aurata* L.) specimens. *Fish and Shellfish Immunology*, 34, 1063–1070.

492 Collins, G.M., Ball, A.S., Qin, J.G., Bowyer, J.N., and Stone, D.A.J. (2014). Effect of
493 alternative lipids and temperature on growth factor gene expression in yellowtail
494 kingfish (*Seriola lalandi*). *Aquaculture research,* 45,1236–1245.

495 Dias, J., Conceição, L.E.C., Ribeiro, A.R., Borges, P., Valente, L.M.P., and Dinis, M.T.
496 (2009). Practical diet with low fish-derived protein is able to sustain growth
497 performance in gilthead seabream (*Sparus aurata*) during the grow-out phase.
498 *Aquaculture*, 293 (3–4), 255–262.

499 Díaz, L.V. and Martínez-Silva, M.A.(2009). Probióticos como herramienta
500 biotecnológica en el cultivo de camarón: reseña. *Boletin de Investigaciones Marinas*
501 *y Costeras*, 38 (2), 165–187.

502 Dimitroglou, A., Merrifield, D.L., Carnevali, O., Picchietti, S., Avella, M., Daniels, C.,
503 Güroy, D. and Davies, S.J. (2011) Microbial manipulations to improve fish health
504 and production: a Mediterranean perspective. *Fish and Shellfish Immunology*, 30,
505 1–16.

506 Dimitroglou, A., Merrifield, D.L., Moate, R., Davies, S.J., Spring, P., Sweetman, J., and
507 Bradley, G. (2009). Dietary mannan oligosaccharide supplementation modulates

508 intestinal microbial ecology and improves gut morphology of rainbow trout,
509 *Oncorhynchus mykiss* (Walbaum). *Journal of Animal Science,* 87 (10), 3226–3234.

510 Drew, M.D., Ogunkoya, A.E., Janz, D.M., and Van Kessel, A.G. (2007). Dietary
511 influence of replacing fish meal and oil with canola protein concentrate and
512 vegetable oils on growth performance, fatty acid composition and organochlorine
513 residues in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* 267(1-4), 260–268.

514 El-Dakar, A.Y., Shalaby, S.M., and Saoud, I.P. (2007). Assessing the use of a dietary
515 probiotic / prebiotic as an enhancer of spinefoot rabbitfish *Siganus rivulatus* survival
516 and growth. *Aquaculture Nutrition*, 13, 407–412.

517 El-Kerdawy, A. and Salama, A. (1997). Effect of dietary lipid sources on the growth and
518 fatty acid composition of gilthead seabream (*Sparus aurata*). *CIHEAM-Options*
519 *Mediterraneennes*, 241, 235–241.

520 Estruch, G., Collado, M.C., Monge-Ortiz, R., Tomás-Vidal, A., Jover-Cerdá, M.,
521 Peñaranda, D.S., Pérez Martínez, G., and Martínez-Llorens, S. (2018a). Long-term
522 feeding with high plant protein based diets in gilthead seabream (*Sparus aurata*, L.)
523 leads to changes in the inflammatory and immune related gene expression at
524 intestinal level. *BMC Veterinary Research*, 14 (1), 1–15.

525 Estruch, G., Tomás-Vidal, A., Nokrashy, A.M. El, Monge-Ortiz, R., Godoy-Olmos, S.,
526 Cerdá, M.J., and Martínez-Llorens, S. (2018b). Inclusion of alternative marine by-
527 products in aquafeeds with different levels of plant-based sources for on-growing
528 gilthead sea bream (*Sparus aurata*, L.): effects on digestibility, amino acid retention,
529 ammonia excretion and enzyme activity. *Archives of Animal Nutrition*, 72 (4), 321–
530 339.

531 Figueiredo-Silva, A., Rocha, E., Dias, J., Silva, P., Rema, P., Gomes, E., and Valente,

L.M.P. (2005). Partial replacement of fish oil by soybean oil on lipid distribution and liver histology in European sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) juveniles. *Aquaculture Nutrition*, 11 (2), 147–155.

Fountoulaki, E., Vasilaki, A., Hurtado, R., Grigorakis, K., Karacostas, I., Nengas, I., Rigos, G., Kotzamanis, Y., Venou, B., and Alexis, M.N. (2009). Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.); effects on growth performance, flesh quality and fillet fatty acid profile. Recovery of fatty acid profiles by a fish oil finishing diet under fluctuati. *Aquaculture*, 289 (3–4), 317–326.

Gildberg, A., Johansen, A., and Bøgwald, J. (1995). Growth and survival of Atlantic salmon (*Salmo salar*) fry given diets supplemented with fish protein hydrolysate and lactic acid bacteria during a challenge trial with *Aeromonas salmonicida*. *Aquaculture*, 138 (1–4), 23–34.

Guillaume, J., Kaushik, S., Bergot, P., and Metailler, R. (2004). *Nutrición y alimentación de peces y crustaceos*. Mundi-Prensa. Madrid.

Hidalgo, M.C., Skalli, A., Abella, E., Arizcun, M., and Cardenete, G. (2006). Dietary intake of probiotics and maslinic acid in juvenile dentex (*Dentex dentex* L.): effects on growth performance, survival and liver proteolytic activities. *Aquaculture Nutrition*, 12, 256–266.

Higgs, D.A., Balfry, S.K., Oakes, J.D., Rowshandeli, M., Skura, B.J., and Deacon, G. (2006). Efficacy of an equal blend of canola oil and poultry fat as an alternate dietary lipid source for Atlantic salmon (*Salmo salar* L .) in sea water. I : effects on growth performance, and whole body and fillet proximate and lipid composition. *Aquaculture Research*, 37, 180–191.

556 Ibeas, C., Cejas, J., Gómez, T., Jerez, S., and Lorenzo, A. (1996). Influence of dietary n-
557 3 highly unsaturated fatty acids levels on juvenile gilthead seabream (*Sparus aurata*)
558 growth and tissue fatty acid composition. *Aquaculture*, 142, 221–235.

559 Ibeas, C., Cejas, J.R., Fores, R., Badia, P., Gómez, T., and Lorenzo Hernández, A. (1997).
560 Influence of eicosapentaenoic to docosahexaenoic acid ratio (EPA/DHA) of dietary
561 lipids on growth and fatty acid composition of gilthead seabream (*Sparus aurata*)
562 juveniles. *Aquaculture*, 150, 91–102.

563 Ibeas, C., Rodrıguez, C., Badıa, P., Rosa, J., Santamarıa, F.J., and Lorenzo, A. (2000).
564 Efficacy of dietary methyl esters of n-3 HUFA vs. triacylglycerols of n-3 HUFA by
565 gilthead seabream (*Sparus aurata* L.) juveniles. *Aquaculture*, 190, 273-287.

566 Ipatova, O.M., Prozorovskaia, N.N., Torkhovskaia, T.I., Baranova, V.S., and Guseva,
567 D.A. (2004). Biological effects of the soybean phospholipids. *Biomeditsinskaia*
568 *khimiia*, 50 (5), 436—450.

569 Izquierdo, M.S., Montero, D., Robaina, L., Caballero, M.J., Rosenlund, G., and Ginés, R.
570 (2005). Alterations in fillet fatty acid profile and flesh quality in gilthead seabream
571 (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid
572 profiles by fish oil feeding. *Aquaculture*, 250 (1–2), 431–444.

573 Izquierdo, M.S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L., and Rosenlund,
574 G. (2003). Dietary lipid sources for seabream and seabass: Growth performance,
575 tissue composition and flesh quality. *Aquaculture Nutrition*, 9 (6), 397–407.

576 Je, W., Min, J., Hasan, T., Lee, B., Gu, S., and Kong, I., (2019). Effects of probiotic
577 supplementation of a plant-based protein diet on intestinal microbial diversity,
578 digestive enzyme activity, intestinal structure, and immunity in olive flounder
579 (*Paralichthys olivaceus*). *Fish and Shellfish Immunology*, 92, 719–727.

580 Jöborn, A., Olsson, J.C., Westerdahl, A., Conway, P.L., and Kjelleberg, S. (1997).
581 Colonization in the fish intestinal tract and production of inhibitory substances in
582 intestinal mucus and faecal extracts by *Carnobacterium* sp. strain K1. *Journal of*
583 *Fish Diseases*, 20 (5), 383–392.

584 John, S., Dimitroglou, A., Simon, D., and Silva, T., 2008. Nutrient Uptake. Gut
585 morphology a key to efficient nutrition. *International Aquafeed*, 11, 26–30.

586 Jover, M., García-Gómez, A., Tomás, A., De La Gándara, F., and Pérez, L. (1999).
587 Growth of mediterranean yellowtail (*Seriola dumerilii*) fed extruded diets containing
588 different levels of protein and lipid. *Aquaculture*, 179 (1–4), 25–33.

589 Kalogeropoulos, N., Alexis, M.N., and Henderson, R.J. (1992). Effects of dietary soybean
590 and cod-liver oil levels on growth and body composition of gilthead bream (*Sparus*
591 *aurata*). *Aquaculture*, 104 (3), 293–308.

592 Kesbiç, O.S., Acar, Ü., Yigit, M., Bulut, M., Gültepe, N., and Yilmaz, S. (2017).
593 Unrefined peanut oil as a lipid source in diets for juveniles of two-banded seabream
594 *Diplodus vulgaris*. *North American Journal of Aquaculture*, 78 (1), 64–71.

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

598 Kiron, V., Fukuda, H., Takeuchi, T., and Watanabe, T. (1995). Essential fatty acid
599 nutrition and defence mechanisms in rainbow trout *Oncorhynchus mykiss*.
600 *Comparative Biochemistry and Physiology Part A: Physiology*, 111 (3), 361–367.

601 Korkea-aho, T.L., Papadopoulou, A., Heikkinen, J., von Wright, A., Adams, A., Austin,
602 B., and Thompson, K.D. (2012). Pseudomonas M162 confers protection against

603 rainbow trout fry syndrome by stimulating immunity. *Journal of Applied*
604 *Microbiology*, 113 (1), 24–35.

605 Lazado, C.C., Caipang, C.M.A., Brinchmann, M.F., and Kiron, V. (2011). In vitro
606 adherence of two candidate probiotics from Atlantic cod and their interference with
607 the adhesion of two pathogenic bacteria. *Veterinary Microbiology*, 148 (2–4), 252–
608 259.

609 Liu, S., Wang, S., Cai, Y., Li, E., Ren, Z., and Wu, Y. (2020). Beneficial effects of a host
610 gut-derived probiotic, *Bacillus pumilus*, on the growth, non-specific immune
611 response and disease resistance of juvenile golden pompano, *Trachinotus ovatus*.
612 *Aquaculture*, 514 (July 2019), 734446.

613 Lu, S., Zhao, N., Zhao, A., and He, R. (2008). Effect of soybean phospholipid
614 supplementation in formulated microdiets and live food on foregut and liver
615 histological changes of *Pelteobagrus fulvidraco* larvae. *Aquaculture*, 278, 119–127.

616 Macey, B.M. and Coyne, V.E. (2006). Colonization of the gastrointestinal tract of the
617 farmed South African abalone Haliotis midae by the probionts *Vibrio midae* SY9,
618 *Cryptococcus* sp. SS1, and *Debaryomyces hansenii* AY1. *Marine Biotechnology*, 8
619 (3), 246–259.

620 Martin, S.A.M. and Król, E. (2017). Nutrigenomics and immune function in fish: new
621 insights from omics technologies. *Developmental and Comparative Immunology*,
622 75, 86–98.

623 Martínez-Llorens, S., Tomás-Vidal, A., Moñino, A.V., Pla-Torres, M., and Jover Cerdá,
624 M. (2007). Effects of dietary soybean oil concentration on growth, nutrient
625 utilization and muscle fatty acid composition of gilthead sea bream (*Sparus aurata*
626 L.). *Aquaculture Research*, 38 (1), 76–81.

627 Menoyo, D., Diez, A., Lopez-Bote, C.J., Casado, S., Obach, A., and Bautista, J.M. (2006).
628 Dietary fat type affects lipid metabolism in Atlantic salmon (*Salmo salar* L.) and
629 differentially regulates glucose transporter GLUT4 expression in muscle.
630 *Aquaculture*, 261 (1), 294–304.

631 Merrifield, D.L., Dimitroglou, A., Foey, A., Davies, S.J., Baker, R.T.M., Bøgwald, J.,
632 Castex, M., and Ringø, E. (2010). The current status and future focus of probiotic
633 and prebiotic applications for salmonids. *Aquaculture*, 302 (1–2), 1–18.

634 Merrifield, D.L. and Ringø, E. (eds) (2014). *Aquaculture Nutrition*. *Gut Health,*
635 *Probiotics and Prebiotics*. Wiley blackwell. DOI:10.1002/9781118897263.

636 Monge-Ortiz, R., Tomás-Vidal, A., Gallardo-Álvarez, F.J., Estruch, G., Godoy-Olmos,
637 S., Jover-Cerdá, M., and Martínez-Llorens, S. (2018a). Partial and total replacement
638 of fishmeal by a blend of animal and plant proteins in diets for *Seriola dumerili*:
639 Effects on performance and nutrient efficiency. *Aquaculture Nutrition*, 24 (4), 1163–
640 1174.

641 Monge-Ortiz, R., Tomás-Vidal, A., Rodriguez-Barreto, D., Martínez-Llorens, S., Pérez,
642 J.A., Jover-Cerdá, M., and Lorenzo, A. (2018b). Replacement of fish oil with
643 vegetable oil blends in feeds for greater amberjack (*Seriola dumerili*) juveniles:
644 Effect on growth performance, feed efficiency, tissue fatty acid composition and
645 flesh nutritional value. *Aquaculture Nutrition*, 24 (1), 605–615.

646 Montero, D., Grasso, V., Izquierdo, M.S., Ganga, R., Real, F., Tort, L., Caballero, M.J.,
647 and Acosta, F. (2008). Total substitution of fish oil by vegetable oils in gilthead sea
648 bream (*Sparus aurata*) diets: Effects on hepatic Mx expression and some immune
649 parameters. *Fish and Shellfish Immunology*, 24 (2), 147–155.

650 Montero, D. and Izquierdo, M. (2010). Welfare and health of fish fed vegetable oils as

651 alternative lipid sources to fish oil. In: *Fish oil replacement and alternative lipid*
652 *sources in aquaculture feeds*. 439–485.

653 Montero, D., Kalinowski, T., Obach, A., Robaina, L., Tort, L., Caballero, M.J., and
654 Izquierdo, M.S. (2003). Vegetable lipid sources for gilthead seabream (*Sparus*
655 *aurata*): Effects on fish health. *Aquaculture*, 225 (1–4), 353–370.

656 Montero, D., Tort, L., Izquierdo, M.S., Robaina, L., and Vergara, J.M. (1998). Depletion
657 of serum alternative complement pathway activity in gilthead seabream (*Sparus*
658 *aurata*) caused by α-tocopherol and n-3 HUFA dietary deficiencies. *Fish Physiology*
659 *and Biochemistry*, 18(4), 399-407.

660 Najafabad, K.M., Imanpoor, M.R., Taghizadeh, V., and Alishahi, A. (2016). Effect of
661 dietary chitosan on growth performance, hematological parameters, intestinal
662 histology and stress resistance of Caspian kutum (*Rutilus frisii kutum* Kamenskii,
663 1901) fingerlings. *Fish Physiology and Biochemistry*, 42 (4), 1063–1071.

664 Nanton, D.A., Vegusdal, A., Rørå, A.M.B., Ruyter, B., Baeverfjord, G., and Torstensen,
665 B.E. (2007). Muscle lipid storage pattern, composition, and adipocyte distribution in
666 different parts of Atlantic salmon (*Salmo salar*) fed fish oil and vegetable oil.
667 *Aquaculture*, 265 (1–4), 230–243.

668 Nasopoulou, C. and Zabetakis, I. (2012). Benefits of fish oil replacement by plant
669 originated oils in compounded fish feeds. A review. *LWT - Food Science and*
670 *Technology*, 47 (2), 217–224.

671 Nogales-Mérida, S., Martínez-Llorens, S., Moñino, A. V, Jover Cerdá, M., and Tomás-
672 Vidal, A. (2017). Fish oil substitution by soybean oil in Sharpsnout seabream
673 *Diplodus puntazzo*: Performance, fatty acid profile, and liver histology. *Journal of*
674 *Applied Aquaculture*, 29 (1), 46–61.

O'Fallon, J. V., Busboom, J.R., Nelson, M.L., and Gaskins, C.T. (2007). A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils, and feedstuffs. *Journal of Animal Science*, 85 (6), 1511–1521.

Øverland, M., Sørensen, M., Storebakken, T., Penn, M., Krogdahl, Å., and Skrede, A. (2009). Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (*Salmo salar*)-Effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. *Aquaculture*, 288 (3–4), 305–311.

Parrino, V., Kesbiç, O.S., Ümit, A., and Fazio, F. (2019). Hot pepper (*Capsicum* sp.) oil and its effects on growth performance and blood parameters in rainbow trout (*Oncorhynchus mykiss*). *Natural Product Research*, DOI: 10.1080/14786419.2018.1550769.

Pratoomyot, J., Bendiksen, E.Å., Bell, J.G., and Tocher, D.R. (2008). Comparison of effects of vegetable oils blended with southern hemisphere fish oil and decontaminated northern hemisphere fish oil on growth performance, composition and gene expression in Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 280 (1–4), 170–178.

Ringø, E. (2020). Probiotics in shellfish aquaculture. *Aquaculture and Fisheries*, 5 (1), 1–27.

Rola-pleszczynski, M. and Stankova, J. (1992). Leukotriene B4 enhances interleukin-6 (IL-6) production and IL-6 messenger RNA accumulation in human monocytes in vitro : Transcriptional and posttranscriptional mechanisms Leukotriene B4 enhances interleukin-6 (IL-6) production and IL-6 messenger RNA. *Blood*, 80, 1004–1011.

Rosenlund, G., Obach, A., Sandberg, M.G., Standal, H., and Tveit, K. (2001). Effect of

699 alternative lipid sources on long-term growth performance and quality of Atlantic
700 salmon (*Salmo salar* L.). *Aquaculture Research*, 32 (Suppl. 1), 323–328.

701 Sáenz De Rodrigáñez, M.A., Díaz-Rosales, P., Chabrillón, M., Smidt, H., Arijo, S., León-
702 Rubio, J.M., Alarcón, F.J., Balebona, M.C., Moriñigo, M.A., Cara, J.B., and
703 Moyano, F.J. (2009). Effect of dietary administration of probiotics on growth and
704 intestine functionality of juvenile Senegalese sole (*Solea senegalensis*, Kaup 1858).
705 *Aquaculture Nutrition*, 15 (2), 177–185.

706 Sales, J. and Glencross, B. (2011). A meta-analysis of the effects of dietary marine oil
707 replacement with vegetbles oils on growth, feed conversion and muscle fatty acid
708 composition of fish species. *Aquaculture Nutrition*, 17, 271–287.

709 Salze, G., McLean, E., Battle, P.R., Schwarz, M.H., and Craig, S.R. (2010). Use of soy
710 protein concentrate and novel ingredients in the total elimination of fish meal and
711 fish oil in diets for juvenile cobia, *Rachycentron canadum*. *Aquaculture*, 298 (3–4),
712 294–299.

713 Santigosa, E., Sánchez, J., Médale, F., Kaushik, S., Pérez-Sánchez, J., and Gallardo,
714 M.A.(2008). Modifications of digestive enzymes in trout (*Oncorhynchus mykiss*)
715 and sea bream (*Sparus aurata*) in response to dietary fish meal replacement by plant
716 protein sources. *Aquaculture*, 282 (1–4), 68–74.

717 Sarker, M.S.A., Satoh, S., Kamata, K., Haga, Y., and Yamamoto, Y. (2012). Partial
718 replacement of fish meal with plant protein sources using organic acids to practical
719 diets for juvenile yellowtail, *Seriola quinqueradiata*. *Aquaculture Nutrition*, 18 (1),
720 81–89.

721 De Schrijver, R. and Ollevier, F. (2000). Protein digestion in juvenile turbot
722 (*Scophthalmus maximus*) and effects of dietary administration of *Vibrio*

723 *proteotyticus*. *Aquaculture*, 186 (1–2), 107–116.

724 Seno-o, A., Takakuwa, F., Hashiguchi, T., and Morioka, K. (2008). Replacement of
725 dietary fish oil with olive oil in young yellowtail *Seriola quinqueradiata* : effects on
726 growth, muscular fatty acid composition and prevention of dark muscle
727 discoloration during refrigerated storage. *Fisheries science*, 74, 1297–1306.

728 Takakuwa, F., Fukada, H., Hosokawa, H., and Masumoto, T. (2006). Optimum digestible
729 protein and energy levels and ratio for greater amberjack *Seriola dumerili* (Risso)
730 fingerling. *Aquaculture Research*, 37 (15), 1532–1539.

731 Ten Doeschate, K.I. and Coyne, V.E. (2008). Improved growth rate in farmed *Haliotis*
732 *midae* through probiotic treatment. *Aquaculture*, 284 (1–4), 174–179.

733 Thanuthong, T., Francis, D.S., Senadheera, S.D., Jones, P.L., and Turchini, G.M. (2011).
734 Fish oil replacement in rainbow trout diets and total dietary PUFA content: I) Effects
735 on feed efficiency, fat deposition and the efficiency of a finishing strategy.
736 *Aquaculture*, 320 (1–2), 82–90.

737 Tomás-Vidal, A., De La Gándara, F., García-Gomez, A., Pérez, L., and Jover, M. (2005).
738 Utilization of soybean meal as an alternative protein source in the Mediterranean
739 yellowtail, *Seriola dumerili*. *Aquaculture Nutrition*, 11 (5), 333–340.

740 Tomás-Vidal, A., De La Gándara García, F., García-Gómez, A., and Jover-Cerdá, M.
741 (2008). Effect of the protein/energy ratio on the growth of Mediterranean yellowtail
742 (*Seriola dumerili*). *Aquaculture Research*, 39 (11), 1141–1148.

743 Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbø, R., Hemre, G.,
744 Fontanillas, R., Nordgarden, U., Hevrøy, E.M., Olsvik, P., and Berntssen, M.H.G.
745 (2008). Novel production of Atlantic salmon (*Salmo salar*) protein based on

combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. *Aquaculture*, 285 (1–4), 193–200.

Turchini, G.M., Francis, D.S., Senadheera, S.P.S.D., Thanuthong, T., and De Silva, S.S. (2011). Fish oil replacement with different vegetable oils in Murray cod: Evidence of an 'omega-3 sparing effect' by other dietary fatty acids. *Aquaculture*, 315 (3–4), 250–259.

Turchini, G.M., Torstensen, B.E., and Ng, W.K. (2009). Fish oil replacement in finfish nutrition. *Reviews in Aquaculture*, 1 (1), 10–57.

Uhing, R.J., Cowlen, M.S., and Adams, D.O. (1990). Chapter 14. Mechanisms Regulating the Production of Arachidonate Metabolites in Mononuclear Phagocytes. *Current Topics in Membranes and Transport*, 349–374.

Uyan, O., Koshio, S., Ishikawa, M., Yokoyama, S., Uyan, S., Ren, T., and Hernandez, L.H.H. (2009). The influence of dietary phospholipid level on the performances of juvenile amberjack, *Seriola dumerili*, fed non-fishmeal diets. *Aquaculture Nutrition*, 15 (5), 550–557.

Wassef, E.A., Saleh, N.E., and El-Abd El-Hady, H. (2009). Vegetable oil blend as alternative lipid resources in diets for gilthead seabream, *Sparus aurata*. *Aquaculture International*, 17, 421–435.

Wassef, E.A., Wahby, O.M., and Sakr, E.M. (2007). Effect of dietary vegetable oils on health and liver histology of gilthead seabream (*Sparus aurata*) growers. *Aquaculture Research*, 38 (8), 852–861.

Xu, H., Dong, X., Zuo, R., Mai, K., and Ai, Q. (2016). Response of juvenile Japanese seabass (*Lateolabrax japonicus*) to different dietary fatty acid profiles: Growth

769 performance, tissue lipid accumulation, liver histology and flesh texture.
770 *Aquaculture*, 461, 40–47.

771 Yang, G., Cao, H., Jiang, W., Hu, B., Jian, S., Wen, C., Kajbaf, K., Kumar, V., Tao, Z.,
772 and Peng, M. (2019). Dietary supplementation of *Bacillus cereus* as probiotics in
773 Pengze crucian carp (*Carassius auratus* var. Pengze): Effects on growth
774 performance, fillet quality, serum biochemical parameters and intestinal histology.
775 *Aquaculture Research*, 50, 2207–2217.

776

777 **Table 1.** Feed Ingredients and proximate composition of the experimental diets.

	FO 100	FO 25	FO 0
Ingredients (g/kg)[u]			
Fishmeal	350	350	350
Wheat meal	100	100	100
Wheat gluten	140	140	140
Soybean meal	185	185	185
Iberian pig meal	110	110	110
Fish oil	95	24	0
Linseed oil	-	28	38
Sunflower oil	-	21	28
Palm oil	-	22	29
[v]Multivitamin and minerals mix	20	20	20
Analysed composition (% dry weight)			
Dry matter (%DM)	89.3	89.7	89.4
Crude protein (%CP)	52.2	52.5	52.1
Crude lipid (%CL)	14.5	14.4	14.4
Ash (%)	7.3	9.1	7.4
Calculated values			
[µ]Energy (kJ/g)	21.4	21.3	21.2

778 [u] Fishmeal (crude protein, CP: 70.7%; crude lipids, CL: 8.9%; Carbohydrates, CHO: 6,0%; Ash:
779 15.1%); Wheat meal (CP: 14.0%; CL: 2.4%; CHO: 83.0%; Ash: 2.4); Wheat gluten (CP: 70.9%; CL:
780 1.3%; CHO: 34.1%; Ash: 1.5%), Soybean meal (CP: 34.3%; CL: 1.3%; CHO: 34.1%; Ash: 1.5%),
781 Iberian pig meal (CP: 66.4%; CL: 16.3%; Ash: 1.9%)

782 [v]Multivitamin and minerals mix (values are g/kg except those in parenthesis): Premix: 25; Choline, 10;
783 DL-a-tocopherol, 5; ascorbic acid, 5; $(PO_4)_2Ca_3$, 5. Premix composition: retinol acetate, 1000000 IU
784 kg−1; calcipherol, 500 IU/kg; DL-a-tocopherol, 10; menadione sodium bisulfite, 0.8; thiamine
785 hydrochloride, 2.3; riboflavin, 2.3; pyridoxine hydrochloride, 15; cyanocobalamin, 25; nicotinamide,
786 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07; ascorbic acid, 75; inositol, 15; betaine, 100;
787 polypeptides 12.

788 [µ]Energy (%) = (51.8 x (%C/100)) – (19.4 x (%N/100)). Calculated according to Brouwer (1965)

789

790 **Table 2.** Fatty profile (g 100/g, wet matter) of experimental diets.

	FO 100	FO 25	FO 0	FO 0+
14:0	0.319	0.249	0.185	0.160
15:0	0.002	0.003	0.002	0.002
16:0	1.839	2.045	2.122	1.894
17:0	0.052	0.025	0.018	0.016
18:0	0.494	0.510	0.528	0.482
Σ Saturated[a]	2.707	2.831	2.855	2.554
16:1	0.411	0.289	0.204	0.180
18:1n-9	2.643	3.096	3.663	3270
18:1n-7	0.384	0.307	0.273	0.245
22:1n-9	0.031	0.004	0.007	0.008
Σ MUFA[a]	3.470	3.695	4.147	3.703
18:2n-6	1.233	1.395	1.666	1.508

18:3n-6	0.010	0.009	0.010	0.008
20:3n-6	0.010	0.004	0.004	0.005
20:4n-6	0.099	0.061	0.039	0.036
22:4n-6	0.023	0.020	0.010	0.010
Σ n-6 PUFA[a]	1.375	1.488	1.730	1.567
18:3n-3	0.218	1.095	1.637	1.444
20:3n-3	0.015	0.008	0.006	0.005
20:5n-3 EPA	0.566	0.453	0.311	0.283
22:5n-3	0.126	0.076	0.047	0.046
22:6n-3 DHA	1.264	0.795	0.480	0.448
Σ n-3 PUFA[a]	2.189	2.427	2.481	2.227
Σ n-3 HUFA[a]	1.956	1.324	0.838	0.777
EPA/DHA[b]	0.448	0.569	0.648	0.632
DHA/EPA[b]	2.23	1.75	1.54	1.58
n-3/n-6	1.592	1.631	1.434	1.421

Σ Saturated: saturated fatty acids sum; Σ MUFA: monounsaturated fatty acids sum; Σ n-6 PUFA: n-6 polyunsaturated fatty acids sum; Σ n-3 PUFA: n-3 polyunsaturated fatty acids sum. Σ n-3 HUFA: n-3 highly unsaturated fatty acids sum.

[a]Including some minor components not shown.

[b]DHA/EPA, 22:6 n-3/20:5 n-3; EPA/ARA, 20:5 n-3/20:4n-6

Table 3. Effect of dietary fish oil replacement on growth performance, survival and nutrient utilization of *S. dumerili* after 109 days of feeding.

	Diets			
	FO 100	**FO 25**	**FO 0**	**FO 0+**
Initial weight (g)	175.2 ± 3.62	171.5 ± 3.62	175.3 ± 3.62	180.8 ± 3.62
Final weight (g)	422.7 ± 5.25	409.1 ± 5.25	419.3 ± 5.25	422.6 ± 5.25
WI[a] (g)	247.4 ± 7.03	241.0 ± 7.03	244.0 ± 7.03	241.8 ± 7.03
Survival (%)	89.67 ± 2.88[ab]	92.67 ± 2.88[a]	80.33 ± 2.88[b c]	77.33 ± 2.88[c]
SGR[b] (% day)	0.8 ± 0.013	0.8 ± 0.013	0.8 ± 0.013	0.8 ± 0.013
FI[c]	1.1 ± 0.034	1.1 ± 0.034	1.1 ± 0.034	1.1 ± 0.034
FCR[d]	1.5 ± 0.07	1.5 ± 0.07	1.6 ± 0.07	1.6 ± 0.07

The values represent the mean ± SD (n=3). Different superscripts letters indicate significant differences between treatments ($p<0.05$). Newman-Keuls Test..

[a] Weight Increase = final weight – initial weight

[b] Specific growth rate (%/day) SGR = 100 x ln (final weight/initial weight)/feeding days.

[c] Feed Intake (g 100 g /(fish day)). FI = 100 x feed intake (g)/average biomass (g) x days.

[d] Feed Conversion Ratio FCR = feed intake (g)/weight gain (g).

Table 4: Biometric indices of *S. dumerili* after 109 of feeding the experimental diets.

	FO 100	**FO 25**	**FO 0**	**FO 0+**
CF[a]	1.51 ± 0.02	1.50 ± 0.02	1.53 ± 0.02	1.58 ± 0.02
VSI[b] (%)	5.94 ± 0.15[b]	5.99 ± 0.15[b]	6.36 ± 0.15[ab]	6.55 ± 0.15[a]
HSI[c] (%)	1.69 ± 0.09	1.86 ± 0.09	1.94 ± 0.04	1.96 ± 0.09
MSI[d] (%)	0.36 ± 0.05	0.49 ± 0.05	0.45 ± 0.05	0.45 ± 0.05
DP[e] (%)	72.4 ± 0.32[a]	72.2 ± 0.32[ab]	71.2 ± 0.32[b]	71.9 ± 0.32[ab]

MI[f] (%)	48.5 ± 0.6	48.5 ± 0.6	47.5 ± 0.6	48.6 ± 0.6

The values represent the mean ± SD (n=3). Different superscripts letters indicate significant differences between treatments ($p<0.05$). Newman-Keuls Test.

[a]Condition factor. CF= = 100 x Total fish weight [g]/Total length^3 [cm^3]
[b]Viscerosomatic index(%). VSI = 100 x Visceral weight [g]/Total fish weight [g]
[c]Hepatosomatic index(%). HSI = 100 x Liver weight [g]/Total fish weight [g]
[d]Mesenteric fat index(%). MSI 100 x Mesenteric fat weight [g]/Total fish weight [g]
[e]Dressout percentage (%). DP = 100 x (Total fish weight [g] - Visceral weight [g] -Head weight [g])/Total weight
[f]Muscle index(%). MI = 100 x Muscle weight [g]/Total fish weight [g]

Table 5: Initial and final proximal composition (g 100/g, wet weight), crude energy content (MJ/kg) and productive values in *S. dumerili* fed with the different experimental diets, after 109 days of feeding the experimental diets.

	Initial	**FO 100**	**FO 25**	**FO 0**	**FO 0+**
Crude protein	17.94	18.65 ± 0.22^{ab}	19.5 ± 0.22^{a}	18.73 ± 0.22^{ab}	18.3 ± 0.22^{b}
Crude fat	6.07	8.69 ± 0.35	8.92 ± 0.35	8.58 ± 0.35	9.13 ± 0.35
Dry matter	26.4	30.45 ± 0.31	30.84 ± 0.31	29.64 ± 0.31	30.09 ± 0.31
Ash	2.65	2.87 ± 0.16	2.82 ± 0.16	2.79 ± 0.16	2.61 ± 0.16
Energy	6.02	7.18 ± 0.12	7.33 ± 0.12	7.11 ± 0.12	7.32 ± 0.12
PPV[a] (%)		25.7 ± 1.33	26.3 ± 1.33	24.1 ± 1.33	22.5 ± 1.33
FPV[b] (%)		52.6 ± 3.39	56.1 ± 3.39	46.7 ± 3.39	52.3 ± 3.39
EPV[c] (%)		26.1 ± 1.51	26.9 ± 1.51	24.0 ± 1.51	24.7 ± 1.51

The values represent the mean ± standard error (n=3). Different superscripts letters indicate significant differences between treatments ($p<0.05$). Newman-Keuls Test.
[a]Protein productive value. PPV= 100 x Protein fish gain [g]/Protein intake [g]
[b]Fat productive value. FPV = 100 x Fat fish gain [g]/Fat intake [g]
[c]Energy productive value. EPV = 100 x Energy fish gain [g]/Energy intake [g]

Table 6: Composition of fatty acids (g 100/g, wet weight) of the whole body of *S. dumerili* fed with experimental diets for 109 days.

	Initial	**FO 100**	**FO 25**	**FO 0**	**FO 0+**
14:0	0.120	0.178 ± 0.009^{a}	0.141 ± 0.009^{b}	0.119 ± 0.009^{b}	0.126 ± 0.009^{b}
15:0	0.007	0.006 ± 0.0003	0.007 ± 0.0003	0.007 ± 0.0003	0.007 ± 0.0003
16:0	0.762	1.20 ± 0.06	1.18 ± 0.06	1.14 ± 0.06	1.21 ± 0.06
17:0	0.017	0.031 ± 0.001^{a}	0.022 ± 0.001^{b}	0.019 ± 0.001^{b}	0.020 ± 0.001^{b}
18:0	0.365	0.46 ± 0.02	0.48 ± 0.02	0.47 ± 0.02	0.51 ± 0.02
Σ Saturated[a]	1.13	1.87 ± 0.10	1.82 ± 0.10	1.76 ± 0.10	1.88 ± 0.10
16:1	0.161	0.296 ± 0.013^{a}	0.225 ± 0.013^{b}	0.189 ± 0.013^{b}	0.204 ± 0.013^{b}
18:1n-9	1.290	2.286 ± 0.126^{b}	2.436 ± 0.126^{ab}	2.655 ± 0.126^{ab}	2.900 ± 0.126^{a}
18:1n-7	0.171	0.306 ± 0.012^{a}	0.261 ± 0.012^{ab}	0.231 ± 0.012^{b}	0.259 ± 0.012^{ab}
22:1n-9	0.013	0.023 ± 0.001^{a}	0.008 ± 0.001^{b}	0.004 ± 0.001^{b}	0.004 ± 0.001^{b}
Σ MUFA[a]	1.635	2.911 ± 0.149	2.929 ± 0.149	3.079 ± 0.149	3.368 ± 0.149
18:2n-6	0.967	1.144 ± 0.062^{b}	1.273 ± 0.062^{ab}	1.313 ± 0.062^{ab}	1.440 ± 0.062^{a}
18:3n-6	0.006	0.007 ± 0.0006	0.007 ± 0.0006	0.007 ± 0.0006	0.007 ± 0.0006

20:3n-6	0.005	0.010 ± 0.0007[a]	0.005 ± 0.0007[b]	0.003 ± 0.0007[b]	0.003 0.0007[b]
20:4n-6	0.409	0.066 ± 0.003[a]	0.049 ± 0.003[b]	0.036 ± 0.003[b]	0.039 ± 0.003[b]
22:4n-6	0.009	0.016 ± 0.0009[a]	0.013 ± 0.0009[ab]	0.009 ± 0.0009[b]	0.010 ± 0.0009[b]
Σ n-6 PUFA[a]	1.396	1.244 ± 0.067	1.347 ± 0.067	1.368 ± 0.067	1.500 ± 0.067
18:3n-3	0.256	0.279 ± 0.056[c]	0.672 ± 0.056[b]	0.830 ± 0.056[ab]	0.913 ± 0.056[a]
20:3n-3	0.007	0.016 ± 0.002[b]	0.020 ± 0.002[ab]	0.023 ± 0.002[ab]	0.028 ± 0.002[a]
20:5n-3 EPA	0.180	0.281 ± 0.020[a]	0.220 ± 0.020[ab]	0.163 ± 0.020[b]	0.173 ± 0.020[b]
22:5n-3	0.071	0.138 ± 0.009[a]	0.102 ± 0.009[b]	0.069 ± 0.009[b]	0.081 0.009[b]
22:6n-3 DHA	0.525	0.851 ± 0.051[a]	0.584 ± 0.051[b]	0.406 ± 0.051[b]	0.446 ± 0.051[b]
Σ n-3 PUFA[a]	1.039	1.565 ± 0.120	1.599 ± 0.120	1.490 ± 0.120	1.643 ± 0.120
n-3/n-6	0.744	1.258 ± 0.057	1.189 ± 0.057	1.085 ± 0.057	1.094 ± 0.057
EPA/DHA[b]	0.342	0.330 ± 0.011[b]	0.376 ± 0.011[a]	0.407 ± 0.011[a]	0.386 ± 0.011[a]

The values represent the mean ± standard error (n=3). Different superscripts letters indicate significant differences between treatments ($p<0.05$). Test de Newman-Keuls. Σ Saturated: saturated fatty acids sum; Σ MUFA: monounsaturated fatty acids sum; Σ n-6 PUFA: n-6 polyunsaturated fatty acids sum; Σ n-3 PUFA: n-3 polyunsaturated fatty acids sum.

[a]Including some minor components not shown.

[b]EPA/DHA calculated as 20:5 n-3/22:6 n-3

Table 7: Fatty acids productive values (FAPV) (% by wet weight) in *S. dumerili* fed with experimental diets for 109 days.

	FO 100	**FO 25**	**FO 0**	**FO 0+**
14:0	51.1 ± 3.47	47.9 ± 3.47	49.9 ± 3.47	51.3 ± 2.83
16:0	59.3 ± 3.07	54.3 ± 3.07	50.5 ± 3.07	52.6 ± 2.51
17:0	58.9 ± 6.67[b]	73.7 ± 5.44[ab]	88.7 ± 6.67[a]	88.5 ± 5.44[a]
18:0	68.6 ± 4.63	82.0 ± 4.63	79.6 ± 4.63	82.0 ± 3.78
Σ Saturated	69.1 ± 5.82	64.3 ± 5.82	61.6 ± 5.82	73.6 ± 5.82
16:1	70.6 ± 5.07	70.0 ± 5.07	78.3 ± 5.07	83.6 ± 4.14
18:1n-9	75.9 ± 5.14	71.9 ± 4.19	74.5 ± 5.47	81.0 ± 4.19
18:1n-7	76.77 ± 4.0	77.5 ± 4.0	75.9 ± 4.0	84.7 ± 3.26
Σ MUFA[a]	83.8 ± 5.72	79.3 ± 5.72	77.2 ± 5.71	88.6 ± 5.71
18:2n-6	66.2 ± 3.68	79.6 ± 3.68	69.9 ± 3.68	75.9 ± 3.0
18:3n-6	56.1 ± 2.88[a]	71.0 ± 3.52[a]	34.4 ± 3.52[b]	59.5 ± 3.52[a]
20:4n-6	55.1 ± 9.58	56.6 ± 9.58	63.5 ± 9.58	73.6 ± 9.58
22:4n-6	67.3 ± 3.53[ab]	56.1 ± 3.53[bc]	47.0 ± 3.53[c]	74.8 ± 3.53[a]
Σ n-6 PUFA[a]	90.4 ± 5.76	90.5 ± 5.76	81.3 ± 5.76	92.7 ± 5.76
18:3n-3	93.8 ± 3.24[a]	65.8 ± 3.24[b]	45.2 ± 3.24[c]	67.3 ± 3.24[b]
20:5n-3 EPA	39.5 ± 5.94	41.2 ± 5.94	38.9 ± 5.94	45.5 ± 5.94
22:6n-3 DHA	64.2 ± 8.91	54.1 ± 7.27	56.3 ± 8.91	63.0 ± 8.91
Σ n-3 PUFA[a]	71.5 ± 6.87	65.9 ± 6.87	60.1 ± 6.87	73.7 ± 6.87

The values represent the mean ± standard error (n=3). Different superscripts letters indicate significant differences between treatments ($p<0.05$). Newman-Keuls Test.

ΣSaturated: saturated fatty acids sum; Σ Ms: monounsaturated fatty acids sum; Σ n-6 PUFA: n-6 polyunsaturated fatty acids sum; Σ n-3 PUFA: n-3 polyunsaturated fatty acids sum.

[a]Including some minor components not shown.

Table 8: Histological measurements of the anterior intestine in yellowtail fed the experimental diets for 109 days.

	FO100	FO25	FO0	FO0+
SL (µm)	76.8 ± 10.1	102.8 ± 10.7	92.1 ± 9.6	94.4 ± 10.2
ML (µm)	258.4 ± 23.0	321.3 ± 25.0	329.7 ± 22.4	286.9 ± 24.0
SML (µm)	167.4 ± 16.3[a]	241.1 ± 19.3[b]	230.4 ± 171.1[b]	202.8 ± 17.0[ab]
VL (µm)	1623.8 ± 91.3	1692.0 ± 125.9	1555.6 ± 104.1	1796.7 ± 92.1
WVL (µm)	139.5 ± 6.0	119.2 ± 8.0	123.4 ± 6.7	139.5 ± 6.0
WLP (µm)	26.4 ± 2.0	26.4 ± 2.5	21.4 ± 2.1	26.8 ± 2.0
WLP/WVL	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.01

The values represent the mean ± standard error (n= 3). Different superscripts letters indicate significant differences between treatments ($p<0.05$). Newman-Keuls Test.

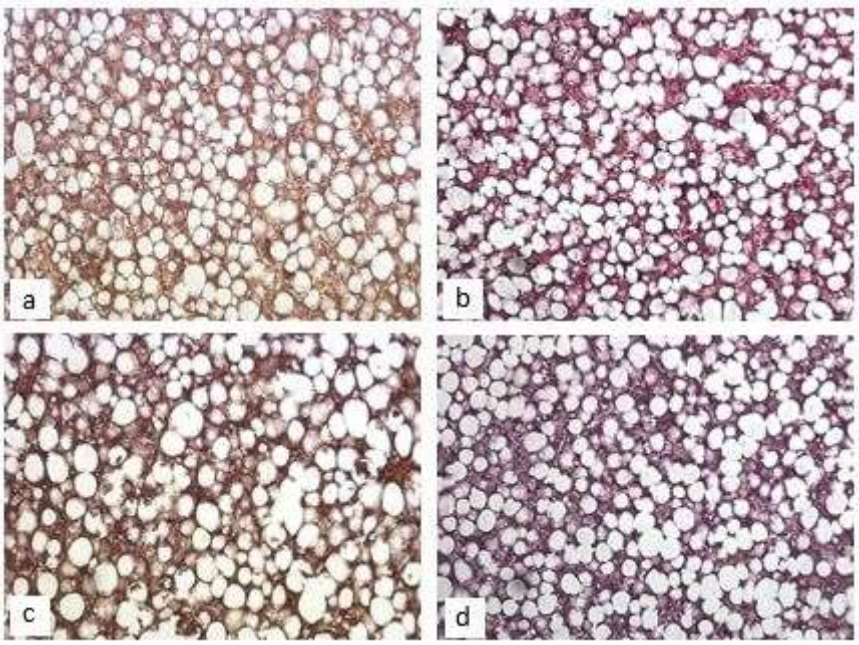
SL: Thickness of serosa layer, ML: Thickness of muscular layer, SML: Thickness of submucosa layer, VL: Villus length, WVL: Width villi, WLP: Width of *lamina propria*, WLP/WVL: ratio between Width of *lamina propria* and villi.

Table 9: Histological measurements of the posterior intestine in fish fed the experimental diets for 109 days.

	FO100	FO25	FO0	FO0+
SL(µm)	95.1 ± 8.4	67.0 ± 11.3	83.2 ± 8.1	96.9 ± 9.0
ML(µm)	200.8 ± 26.2	217.7 ± 33.0	278.2 ± 22.3	269.6 ± 24.9
SML(µm)	195.3 ± 15.3	207.3 ± 19.8	176.0 ± 14.7	199.7 ± 16.8
VL(µm)	1447.3[b] ± 68.4	993.7[a] ± 65.5	1448[b] ± 59.4	1539.2[b] ± 68.4
WVL(µm)	129.1 ± 6.6	136.8 ± 6.2	145.5 ± 5.7	131.9 ± 6.6
WLP(µm)	18.5[a] ± 1.6	22.0[ab] ± 1.5	25[b] ± 1.4	18.2[a] ± 1.6
WLP/WVL	0.1 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	0.1 ± 0.01

The values represent the mean ± standard error (n=3). Different superscripts letters indicate significant differences between treatments ($p<0.05$). Newman-Keuls Test.

SL: Thickness of serosa layer, ML: Thickness of muscular layer, SML: Thickness of submucosa layer, VL: Villus length, WVL: Width villi, WLP: Width of *lamina propria*, WLP/WVL: ratio between Width of *lamina propria* and villi.



862

863 **Figure 1**: Microphotographs of liver section (a) FO 100 (20x). b) FO 25 (20x). c) FO
864 0+ (20x). d) FO 0 (20x)) of yellowtail. Hematoxylin and eosine staining.

865