

Document downloaded from:

<http://hdl.handle.net/10251/111523>

This paper must be cited as:

Monge-Ortiz, R.; Tomas-Vidal, A.; Gallardo-Álvarez, F.; Estruch-Cucarella, G.; Godoy-Olmos, S.; Jover Cerda, M.; Martínez-Llorens, S. (2018). Partial and total replacement of fishmeal by a blend of animal and plant proteins in diets for *Seriola dumerili*: Effects on performance and nutrient efficiency. *Aquaculture Nutrition*. 24(4):1163-1174.
doi:10.1111/anu.12655



The final publication is available at

<https://doi.org/10.1111/anu.12655>

Copyright Blackwell Publishing

Additional Information

1 Running title: Fishmeal replacement in diets for *S. dumerili*

2 **Partial and total fishmeal replacement by a blend of animal and plant proteins in**
3 **diets for *Seriola dumerili* (Risso, 1810): Effects on performance and nutrient**
4 **efficiency.**

5 Raquel Monge-Ortiz, Ana Tomás-Vidal, Francisco J. Gallardo-Álvarez, Guillem Estruch,
6 Sergio Godoy-Olmos, Miguel Jover-Cerdá, Silvia Martínez-Llorens*.

7 Research Group of Aquaculture and Biodiversity. Institute of Animal Science and
8 Technology. Universitat Politècnica València. Camino de Vera, 14. 46071-Valencia,
9 Spain

10 ***Corresponding author:** Silvia Martínez-Llorens, Universitat Politècnica València.
11 Camino de Vera, 14. 46071-Valencia (Spain). Tel.: 34-96-3879752; Fax: 34-96-3877439.
12 E-mail: silmarll@dca.upv.es.

13

14

15

16

17

18

19

20

21 **Abstract:** A 154-day trial was carried out to assess the use of an alternative protein blend
22 (wheat gluten, corn gluten, krill and meat meal) as a substitute for fishmeal in diets for
23 juvenile yellowtail, using four isolipidic (140g kg⁻¹) and isoenergetic diets (24MJ kg⁻¹)
24 with the same digestible protein content (50%). The control diet was FM100, without
25 replacement and FM66, FM33 and FM0, fishmeal was replaced at 33, 66 and 100%,
26 respectively.

27 At the end of the experiment no differences in growth parameters were obtained. Fish fed
28 the FM0 diet exhibited the lowest survival (23%), maybe for a dietary amino acid
29 imbalance or some anti-nutrient factors contained in the alternative sources.

30 Feed intake, feed conversion ratio, digestible protein intake and protein efficiency ratio
31 were similar in all diets. However, the digestible energy intake and protein efficiency
32 retention were lowest in fish fed FM0 diet. Apparent digestibility coefficients for protein,
33 energy and amino acids diminished as fishmeal substitution increased. Significant
34 differences were observed in the ratio between diet and whole fish body profile (AAR)
35 for seven essential amino acids. In summary, total fishmeal replacement by the blend
36 assayed was not feasible for yellowtail. The FM66 diet obtained good growth, high
37 survival and good nutrient efficiency.

38 **Key words:** fishmeal replacement, survival, amino acids, digestibility, *Seriola dumerili*.

39

40 **1. Introduction**

41 Its fast growth, excellent meat quality, and its recent reproduction in captivity turns the
42 greater amberjack (*Seriola dumerili*, Risso 1810) into one of the most promising species
43 for Mediterranean aquaculture to enhance diversity of fish farms. However, to maintain

44 a profitable commercial culture of the greater amberjack, it is necessary to formulate
45 specific, healthy, sustainable and low cost diets. In this sense, to avoid the current
46 dependence on fishmeal for fish diets, it is necessary to include alternative ingredients for
47 economic and environmental benefits by reducing costs for fish feed, while lessening
48 fishing pressure on species harvested for fishmeal production, many of which also serve
49 as important resources in the marine food chain.

50 Tacon and Metian (2008) reported that 75% of the world fish stocks are currently
51 considered fully exploited or overexploited, including many small pelagic fish species
52 used to produce fishmeal for feed formulation worldwide. Since fishmeal production is
53 predicted to be unable to sustain the growth of the aquaculture sector, the quest for
54 alternative ingredients and protein sources and as well as the optimization of dietary
55 protein content are important goals. Studies carried out on the effects of alternative
56 protein inclusion in diets for the greater amberjack are scarce. Hitherto, there is only one
57 study on the subject which reported a range of 20% to 30% as the maximum level of
58 soybean dietary inclusion for the Mediterranean yellowtail, as the inclusion of a greater
59 amount led to poor growth and insufficient nutrient efficiency (Tomás *et al.*, 2005).

60 However, there are some studies available with other amberjack species. Several studies
61 on the nutritional requirements of the Japanese yellowtail (*Seriola quinqueradiata*) have
62 been published (Takeda *et al.*, 1975; Shimeno *et al.*, 1980; Shimeno, 1982, 1991;
63 Takeuchi *et al.*, 1992b; Ruchimat *et al.*, 1997a, b), including lipid (Takeuchi *et al.*,
64 1992c), and carbohydrate requirements (Furuichi *et al.*, 1986; Takeuchi *et al.*, 1992a).
65 Also, soybean meal as an alternative protein source has been considered as part of
66 Japanese yellowtail diets (Viyakarn *et al.*, 1992; Watanabe *et al.*, 1992), reporting
67 negative effects on growth with a fishmeal substitution above 30%. Blends of alternative
68 proteins have been used to achieve fishmeal substitution by up to 10-40% (depending on

69 the ingredients and their proportion in the blends) without negative effects on fish
70 performance. Soybean, gluten, rapeseed and meat meal (Shimeno *et al.*, 1993a, b),
71 soybean, corn gluten, meat meal and blood meal (Aoki *et al.*, 2000a), as well as poultry
72 and feather meal (Shimeno *et al.*, 2000) have been tested in fishmeal substitution trials.
73 However, these studies cannot be extrapolated to the Mediterranean yellowtail since it
74 has a lower growth than the Japanese yellowtail, which could have some influence on the
75 inclusion of optimum vegetable levels in diets, as its essential amino acid requirements
76 might be different.

77 Another amberjack studied is the yellowtail kingfish (*Seriola lalandi*), which is a highly
78 appreciated fish in many Asian countries such as Japan, China, Singapore and Korea.
79 Some alternative proteins have been assayed as a replacement of fishmeal in the
80 yellowtail kingfish. For example, the development of an experimental soy-based diet
81 (Jirsa *et al.*, 2011) has demonstrated the potential of the inclusion of soy protein in diets
82 for this species. On the other hand, recent studies on the yellowtail kingfish have observed
83 growth detriment and intestinal alterations with soybean meal and soy protein concentrate
84 use (Bowyer *et al.*, 2013a, b; Bansemer *et al.*, 2015) at 30% of dietary inclusion.

85 Amino acids (AAs) are responsible for the synthesis of most body tissues, enzymes,
86 hormones and other metabolic molecules, and it is clear that no fish can grow or reproduce
87 without a continuous supply of protein (Limin *et al.*, 2006). Since particular metabolic
88 functions require specific AAs, it is crucial that fish ingest, digest and bio-assimilate the
89 necessary AAs from protein sources. Therefore, protein quality is generally evaluated
90 according to its amino acid content. The ideal dietary AA profile could be simulated with
91 a protein mixture, and, thus a high fishmeal substitution could be successfully achieved
92 as reported in other carnivorous species (Kaushik *et al.*, 2004; Kissil & Lupatsch, 2004;
93 Espe *et al.*, 2006; Sánchez-Lozano *et al.*, 2009; 2011; Monge-Ortiz *et al.*, 2016.

94 Nevertheless, high or total fishmeal dietary replacement may produce non-desirable
95 effects on fish, mainly caused by anti-nutrients particularly contained in vegetable
96 proteins (Francis *et al.*, 2001). Protease enzyme activity decreases and therefore low
97 protein digestibility (Spinelli *et al.*, 1983), higher susceptibility to pathogenic infections
98 (Maita *et al.*, 1998), higher mortality (Estruch *et al.*, 2015) due to immunosuppression
99 (Sitjà-Bobadilla *et al.*, 2005), are some of the effects observed. The combination of animal
100 proteins (defatted krill and meat meal) in diets for fish could address some of these
101 deficiencies, reducing anti-nutrient compounds, the supplementation with synthetic AAs
102 (Lu *et al.*, 2015), lack of palatability and 'green liver' (Watanabe *et al.*, 1995).

103 So far, experimental cultures aimed at the development of the greater amberjack in the
104 Mediterranean have often failed due to parasite and/or pathogenic infections, particularly
105 in juvenile fish. Considering the above-listed negative effects of fishmeal replacement,
106 the design of specific diets suitable for the optimal rearing of the greater amberjack is
107 crucial.

108 Taking into account these facts and the scarcity of nutritional studies in the greater
109 amberjack on the effects of fishmeal replacement in diets, the aim of this work was to
110 assess fishmeal replacement by a blend of proteins in diets for this species not only with
111 regard to growth, but also evaluating nutritive and amino acid efficiency.

112

113 **2. Materials and methods**

114 *2.1 Production system*

115 The trial was carried out in 12 cylindrical fibreglass tanks (1750 L) inside a recirculated
116 seawater system (75 m³ capacity) with a rotary mechanical filter and a gravity bio-filter
117 (approximately 6 m³ capacity) at the Aquaculture Laboratory (Animal Science

118 Department at Polytechnic University of Valencia, Valencia, Spain). The marine water in
119 the system was changed once every three months.

120 The experimental period was 154 days (from January to June 2014). The water
121 temperature was maintained at $21.5 \pm 2.4^{\circ}\text{C}$ during the experimental period by a water
122 conditioning pump (TRANE CAN 490, 123.3 kW) installed in the system. All tanks were
123 equipped with aeration and the level of dissolved oxygen was $6.6 \pm 1.3 \text{ mg L}^{-1}$. Water
124 salinity was $31.5 \pm 4.1 \text{ g L}^{-1}$, pH 7.3 ± 0.4 , NO_3^- (25-150 mg L^{-1}), NO_2^- (0.05-0.5 mg L^{-1})
125 and the ammonium value was undetectable. The photoperiod was natural throughout
126 the experimental period (16L/8D in summer and 12L/12D in winter) and all tanks had
127 similar lighting conditions. All these parameters were measured on a daily basis from
128 Monday to Saturday.

129

130 *2.2 Fish and experimental design*

131 Greater amberjack (*Seriola dumerili*) juveniles were obtained from a fish farm (Futuna
132 Blue, Cádiz, Spain), transported live to the Aquaculture Laboratory of Polytechnic
133 University of Valencia and randomly distributed in experimental tanks.

134 Prior to the feeding trial, all fish were acclimatised to the indoor rearing conditions for 4
135 weeks and fed a standard diet (550 g/kg crude protein, CP; 140 g/kg crude lipid, CL; 110
136 g/kg ash; 22 g/kg crude fibre, CF; and 140 g/kg nitrogen free-extract, NFE). Groups of
137 19 fish (average weight $38.4 \pm 11.6 \text{ g}$) were housed in 12 cylindrical fibreglass tanks
138 (three per treatment).

139 All fish were weighed every 30 days. Previously, fish were anaesthetized with 30 mg L⁻¹
140 clove oil (Guinama®), containing 87% eugenol. The fish were not fed for one day before
141 weighing.

142 At the beginning of the experimental trial, 5 fish were sampled and stored at -30°C for
143 subsequent whole-body composition analyses. The fish were slaughtered by a thermo-
144 shock in a melting ice bath.

145 At the end of the growth trial, all fish per tank were sampled to determine biometric
146 parameters (viscerosomatic and hepatosomatic indices, condition factor and mesenteric
147 fat) and three specimens per tank were randomly sampled to determine the proximate and
148 amino acid body composition.

149

150 *2.3 Diets and feeding*

151 Four isolipidic (140 g kg⁻¹ of CL) and isoenergetic diets (24 MJ kg⁻¹ of gross energy, GE)
152 were formulated (Table 1) with the same digestible protein (50%, DP), and from 530 to
153 633 g kg⁻¹ of crude protein (CP). For diet formulation, individual ingredient digestibility
154 coefficients from a previous study (Tomás *et al.*, 2016) were taken to estimate protein
155 digestibility.

156 FM100 was used as the control diet without any fishmeal substitution. In the FM66, FM33
157 and FM0 diets, fishmeal was replaced by an alternative protein blend (wheat gluten meal,
158 corn gluten meal, krill meal and meat meal) at 33, 66 and 100%, respectively.

159 20 g kg⁻¹ of vitamins and minerals were added in all the diets, and the FM33 and FM0
160 diets were supplemented with synthetic L-Met and L-Lys in amounts of 3 to 5 g kg⁻¹,
161 respectively, to simulate the digestible amino-acid profile of the fishmeal diet. The

162 primary lipid source in all feeds was fish oil, with levels of about 90 g kg⁻¹ of dry matter.

163 The composition of the experimental diets and their proximate values are shown in Table

164 1.

165 Diets were prepared by cooking extrusion processing with a semi-industrial twin-screw

166 extruder (CLEXTRAL BC-45). The processing conditions were as follows: 0.63 g speed

167 screw, 110°C temperature, 40-50 atm pressure, producing pellets having a 3 to 5 mm

168 diameter. Experimental diets were assayed in triplicate. Fish were fed by hand twice a

169 day (9:00 and 17:00 h) from Monday to Saturday until apparent satiation. Pellets were

170 distributed slowly, allowing all fish to eat and the total amount of feed distributed was

171 recorded.

172

173 *2.4 Proximate composition and amino acid analysis*

174 Chemical analysis of the dietary ingredients was performed prior to diet formulation.

175 Dietary ingredients, diets (Table 1), as well as the whole fish, were analysed according to

176 AOAC (1995) procedures: dry matter (drying at 105 °C to constant weight), ash

177 (incinerated at 550 °C to constant weight), crude protein by the Kjeldahl procedure (N x

178 6.25) after acid digestion (2300 Kjeltac Analyzer Unit) and by the Dumas principle, crude

179 lipids were extracted with diethyl ether (ANKOM^{XT10}). Energy was calculated according

180 to Brouwer (1965), from the C (g) and N (g) balance ($GE = 51.8 \times C - 19.4 \times N$). Carbon

181 and nitrogen were analysed by the Dumas principle (TruSpec CN; Leco Corporation, St.

182 Joseph, MI, USA).

183 Following the method previously described by Bosch *et al.* (2006), amino acids of diets

184 (Table 2), fish carcasses and faeces were analysed in a Waters HPLC system (Waters

185 474) consisting of two pumps (Model 515; Waters), an auto sampler (Model 717; Waters),

186 a fluorescence detector (Model 474; Waters) and a temperature control module.
187 Aminobutyric acid was added as an internal standard after hydrolysis. Amino acids
188 were derivatised with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate).
189 Methionine and cysteine were determined separately as methionine sulphone and cysteine
190 acid after oxidation with performic acid. Amino acids were separated with a C-18 reverse-
191 phase column Waters AcQ. Tag (150 mm x 3.9 mm).

192 All analyses were performed in triplicate except faeces analysis, which was performed in
193 duplicate.

194 *2.5 Digestibility and retention estimations*

195 Simultaneously with the feeding trial, 16 fish were used in a trial designed to determine
196 the apparent digestibility of the experimental diets. The digestibility system was
197 constructed according to the Guelph System protocol (Cho, Slinger & Bayley, 1982),
198 using 4 digestibility tanks (4 fish/tank). The water temperature averaged $20.5 \pm 2.1^\circ\text{C}$.
199 The same four diets were used but chromium oxide (5 g kg^{-1}) was added as inert marker.
200 The fish groups were fed the experimental diets along a 30-35 day period, and wet faecal
201 content was collected and freeze-dried prior to analysis. Chromium oxide was determined
202 in the diets and in faeces using an atomic absorption spectrometer (Perkin Elmer 3300)
203 after acid digestion.

204 The apparent digestibility coefficients (ADCs) for protein, energy, dry matter and amino
205 acids for the diets tested were calculated with the following formula:

$$206 \text{ ADC (\%)} = 100 \times [1 - (F/D \times \text{DCr/FCr})],$$

207 where F is the percentage of nutrient or energy in faeces, D is the percentage of nutrient
208 or energy in the diet, DCr is the percentage of chromic oxide in the diet and FCr is the
209 percentage of chromic oxide in faeces (Cho & Kaushik, 1990).

210 Protein and AA retention efficiencies were calculated as follows:

- 211 • Ingested protein retention (IPR) or digested protein retention (DPR) :

212 $IPR (\%) = 100 \times \text{fish protein gain (g)} / \text{crude protein intake (g)}$

213 $DPR (\%) = 100 \times \text{fish protein gain (g)} / \text{digestible protein intake (g)}$

- 214 • Amino acid retention efficiency (AARE) or digestible amino acid retention
215 efficiency (DAARE):

216 $\text{Amino acid retention efficiency (AARE) (\%)} = 100 \times \text{fish amino acid gain}$
217 $(\text{g}) / \text{ingested amino acid (g)}$

218 $\text{Digestible amino acid retention efficiency (DAARE) (\%)} = 100 \times \text{fish amino acid}$
219 $\text{gain (g)} / \text{digested amino acid (g)}$

220 *2.6 Statistical analysis*

221 Results from growth data and nutritive parameters were treated using multifactor analysis
222 of variance (ANOVA), introducing the initial live weight as covariate (Snedecor &
223 Cochran, 1971) to assess the final weight and specific growth rate (SGR). Digestibility
224 and nutritive efficiency data were treated using a one-way ANOVA. The Newman–Keuls
225 test was used to assess specific differences among individual diets at 0.05 significant level
226 (Statgraphics, Statistical Graphics System, Version Plus 5.1).

227 *2.7 Ethical statement*

228 The *Seriola dumerili* study complied with European Union Council Directive 2010/63/
229 UE, which lays down minimum standards for the protection of animals, and was also in
230 accordance with Spanish national legislation (Spanish Royal Decree 53/2013) protecting
231 animals used in experimentation and for other scientific purposes. The experimental
232 protocol was approved by the Ethics Committee of Polytechnic University of Valencia
233 (UPV).

234 Fish in the tanks were checked on a daily basis. Also, fish were weighed individually
235 every four weeks and their health status was assessed through observation, after sedation
236 with clove oil dissolved in water (0.01 mg/l of water) to minimise animal suffering.
237 Animals were euthanised by an excess of clove oil (150 mg/l) and then dissected.

238

239 **3. Results**

240 Survival of fish at day 119 of the experiment was around 90%, but was negatively affected
241 at day 154 due to a non-specific fish disease causing high mortality (Figure 1). Fish fed
242 the FM0 diet exhibited the lowest survival (23%), while fish fed the FM100, FM66 and
243 FM0 diets presented similar survival rates (from 75 to 86%).

244 All experimental fish groups grew from the beginning of the trial, and at the end of the
245 experiment no differences in growth were obtained as shown in Table 3. Although no
246 significant differences were found, a clear tendency ($P < 0.05$) of diminishing fish growth
247 was observed when the protein mixture dietary levels increased.

248 With regard to nutritive parameters, no significant differences were obtained among the
249 diets. The digestible energy intake was lower in fish fed the FM0 diet (26.6 kJ 100 g fish⁻¹
250 day⁻¹) than in fish fed the FM100 and FM66 diets (33.0 and 30.2 kJ 100 g fish⁻¹ day⁻¹,
251 respectively). No differences were found among diets in the protein efficiency ratio

252 (PER), however, this index showed a significant diminishing tendency ($P < 0.05$) with the
253 dietary protein blend increase.

254 Concerning biometric parameters (Table 4), significant differences were observed in the
255 viscerosomatic index (VSI), showing the highest value in fish fed the FM0 diet (5.87%).
256 Neither the hepatosomatic index (HSI) nor the visceral fat index (MF) presented any
257 significant differences among diets.

258 In terms of whole-body composition, significant differences were found in protein
259 contents. Fish fed the control diet (FM100) showed the highest value (192.2 g kg^{-1}) and
260 fish fed the FM0 diet presented the lowest (177.7 g kg^{-1}). The moisture content, lipid and
261 ash were not affected by the level of fishmeal substitution.

262 Only protein efficiency retention showed significant differences (IPR, %). The value of
263 fish fed the FM0 diet (17.5%) was significantly lower than that related to the FM100 and
264 FM66 diets (23.7 and 21.9%, respectively), but no differences regarding the FM33 diet
265 were found. In general, the FM0 diet exhibited lower retention values than the other diets.
266 Regarding the dietary EAA level (Table 2), a slight decrease of Leu was observed in the
267 FM100 diet (42.9 g kg^{-1}). The level of other dietary EAAs was similar among
268 experimental diets. The FM66 and FM0 diets showed the lowest Lys content (29.2 and
269 28.0 g kg^{-1}); although the latter had been supplemented with synthetic L-Lys. However,
270 the dietary Met level did not present any differences. Moreover, the EAA/NEAA ratio
271 was lower in the FM0 diet than the FM100 diet (0.82 vs. 0.99) as a consequence of the
272 increased NEAA dietary levels as of dietary fishmeal substitution increased.

273 Regarding the ADC coefficients (Table 5), no differences were found in ADC for dry
274 matter. However, the ADC for protein diminished according to the fishmeal substitution,
275 so that the FM0 diet obtained a lower value (75.7 %) than the FM100 and FM66 diets

276 (93.7 and 86.8 %, respectively). Likewise, the energy ADC coefficient in the FM0 diet
277 was the lowest (68.5 %), followed by the FM33 diet (76.5 %).

278 The same tendency observed in the ADC for protein was shown in individual amino acids
279 (ADC^{AA}). In general, considering the EAA only, ADC^{AA} increased with the fishmeal
280 dietary content, and only the Met, Val and Thr did not show any significant differences.
281 Lys presented the lowest digestibility in the FM0 diet (69.4 %). For NEAA, the ADC^{AA}
282 was affected with significant differences in the case of Ala, Cys, Glu, Pro and Tyr.

283 Figure 2 shows the digestible EAA intake ratio (g AA Kg of fish⁻¹ day⁻¹, DAA). ADC^{AA}
284 had a high influence on this index, particularly on the Lys intake ratio, which resulted
285 significantly higher in fish fed the control diet (FM100) than those fed the FM0 diet.
286 Furthermore, no significant differences were detected between the FM66 and FM33 diets.

287 At the end of the trial, the experimental diets caused significant differences in the Arg,
288 and Gly levels of the entire fish-body (Table 6). Fish fed the FM100 and FM0 diets,
289 showed the highest and the lowest value, respectively, but no significant differences were
290 observed between fish fed the FM66 and FM33 diets. The EAA/NEAA ratio of whole
291 body was similar among all the experimental diets.

292 The amino acid retention efficiency (%) of ingested (A) and digested (B) EAA in fish fed
293 the experimental diets at the end of the experiment is shown in Figure 3A and Figure 3B,
294 respectively. Without considering the diet effect, His and Lys efficiency retention showed
295 the highest efficiency values (27.83 and 33.14 %), while Leu and Phe efficiency retention
296 presented the lowest values (18.88 and 18.12 %, respectively). Concerning the efficiency
297 retention of EAA ingested (%), fish fed the FM0 diet showed lower Met retention (19.79
298 %) than fish fed the FM100 and FM66 diets (26.16 and 30.25 %, respectively). In the
299 efficiency retention of EAA digested, fish fed the FM0 diet exhibited the highest Lys

300 efficiency retention (50.99 %) and fish fed the FM100 diet the lowest (30.94 %). The
301 efficiency retention of digested Met resulted higher in fish fed the FM66 diet (33.83 %),
302 than in fish fed the FM33 and FM0 diets (27.42 and 24.03 %, respectively).

303 The AA index, or ratio between diet and whole fish body EAA profile, is show in Figure
304 4. In the present experiment, significant differences were observed in the AA index, for
305 Arg, Ile, Leu, Lys, Met, Thr and Val EAA (Figure 4). Fish fed the FM0 diet exhibiting
306 an AA index below that of than 100, except for Leu and Phe, and fish fed the FM100 diet
307 presented values higher than 100 for Ile, Leu and Phe EAA.

308 In the AA index of Lys a tendency can also be appreciated, that decreased as the dietary
309 level of fishmeal substitution increased, particularly, showing a drastic decrease,
310 presenting the lowest values in fish fed the FM0 diet (45.43 %).

311

312 **4. Discussion**

313 The fish presented a satisfactory growth along the trial. The fact, the SGR average
314 obtained in the present experiment was higher than those obtained in previous studies
315 (Jover *et al.*, 1999; Tomás *et al.*, 2005; Takakuwa *et al.*, 2006b; Tomás *et al.*, 2008). The
316 lower protein crude dietary level (400-550 g kg⁻¹ of CP) formulated in all these
317 experiments, compared with those present experiment could be the main cause of a lower
318 growth. However, in a recent study (Dawood *et al.*, 2015), it has been demonstrated that
319 yellowtail fed with moist pellets exhibited a high SGR (2.9 % day⁻¹). In another study
320 (Takakuwa *et al.*, 2006a), good growth indices were also obtained, but only considering
321 the feeding period in juveniles.

322 Although, no significant differences were found in growth of fish fed experimental diets,
323 a significant negative tendency ($P>0.05$) was observed in growth when fishmeal
324 substitution was increased; this fact is in accordance with the results obtained in several
325 studies when soybean meal was used at the highest dietary levels as a partial substitute of
326 fishmeal (Tomás *et al.*, 2005; Dawood *et al.*, 2015) or poultry by-product meal
327 (Takakuwa *et al.*, 2006b). And it also agrees with *S. quinqueradiata* studies, in which the
328 fishmeal content in diets can be reduced to about 300 g kg⁻¹ diet by using alternative
329 protein sources (Watanabe *et al.*, 1994; Aoki *et al.*, 2000b), but further replacement of
330 fishmeal by alternative proteins results in inferior growth and feed utilization as well as
331 the development of abnormal physiological conditions, such as anaemia and a higher
332 incidence of green liver originated by biliverdin pigment (Maita *et al.*, 1997). Taurine
333 deficiency is the main reason for the outbreak of green liver in Japanese yellowtail
334 (Takagi *et al.*, 2006) and in red sea bream (Goto *et al.*, 2001). In our experiment, green
335 liver was not detected, because the diet contained high Tau concentration supplied in the
336 diets by defatted krill. Therefore, supplementary taurine into the substitute protein diets
337 to keep the hepatic taurine level higher will improve this abnormality by activating the
338 metabolism of the bile pigments.

339 The main problem of total fishmeal replacement by alternative protein sources in this
340 experiment was the high mortality observed during the last 30 days, causing the death of
341 75% of the fish fed that diet. The survival of fish fed the FM0 diet before this episode
342 was 95%, similar to the other diets fed. However, a direct causal agent causing this
343 mortality was not detected. From the biopsies carried out in dead fish, three species of
344 *Vibrio sp.* susceptible to quinolones and having an intermediate resistance to
345 tetracyclines, enhanced sulfonamides and penicillin were detected. However, the bacterial
346 infection was ruled out as the main cause of the mortality, but rather *Vibrio sp.* acted as

347 opportunistic agents in fish with a suppressed immune system, possibly due to the dietary
348 effect. Similar to the Japanese yellowtail, fish fed a non-fishmeal diet initially fed actively
349 and grew normally, but thereafter growth stagnated, and high mortality was found due to
350 a bacterial infection (Maita *et al.*, 1998). In addition, fish fed a non-fishmeal diet exhibited
351 anaemia and hypocholesterolemia (Maita *et al.*, 1997, 1998, 2006; Dawood *et al.*, 2015).
352 The susceptibility to opportunistic infections due to the weakening of the fish immune
353 system has also been seen in other species (Estruch *et al.*, 2015), when were feed without
354 dietary fishmeal.

355 This weakening on the immune system can be also explained in the nutrient availability
356 (there is a very clear decrease in the protein and energy digestibility of the FM0 diet)
357 caused by some anti-nutrient factors contained not only in vegetable meals but also in
358 krill by-product. In the case of krill, arthropods are usually poor in carbohydrates, but
359 they contain chitin, composed of an unbranched polymer of N-acetylglucosamine that
360 might reduce the access of chitinases or proteinases to their substrates and prevents
361 proteins and lipids absorption in the intestine producing a low nutrient efficiency and
362 decreasing growth (Tanaka *et al.*, 1997). In our experiment, lipid digestibility could not
363 be carried out, but the detriment of energy digestibility joined with the low content of
364 mesenteric fat according the dietary defatted krill meal increased would support this fact.
365 The reduction on nutrient digestion has previously been shown in mice and broiler
366 chickens (Han *et al.*, 1999; Razdan & Pettersson, 1994). However, the reduction of
367 nutrient digestibility in fish depends on the species; poor nutrient digestibility was
368 observed in rainbow trout fed 25% chitin and *Salmo salar* fed with 5% of dietary chitin
369 (Lindsay *et al.*, 1984; Karlsen *et al.*, 2015), nevertheless, *Gadus morhua* or Atlantic cod
370 a decrease in nutrient digestibility was not observed is able to digest and utilize the chitin.
371 Another problem associated with the high content of krill meal is the dietary content of

372 fluoride derived from Antarctic krill could also affect the digestibility, inhibit fish growth
373 (Yoshitomi & Nagano, 2012).

374 The relative low ADCs of energy obtained in fish fed the FM0 diet can be attributed to
375 several factors: the high content of chitin, non-digestible carbohydrates (Aslaksen *et al.*,
376 2007) and the high fibre content, that increase intestinal transit and reduce gut-retention
377 time of feed and time available for nutrient digestion (Fountoulaki *et al.*, 2005). Also, the
378 presence of chitin and its negative influence on lipid digestibility (Kroeckel *et al.*, 2012)
379 could affect the energy digested by fish fed a diet with a high content of krill meal as in
380 the FM0 diet. The detriment of digestible energy intake was as consequence of ADC
381 energy coefficients presented with this diet, and therefore, the efficiency retention showed
382 a clear tendency to increase when fishmeal substitution increased, although significant
383 differences were not observed, fish fed the FM0 diet showed an energetic deficiency.

384 Lys is one of the main limiting amino acid (Gatlin *et al.*, 2007). Its dietary imbalance
385 could be the main reason of fish mortality, also observed in midas (*Amphilophus*
386 *citrinellum*) by Dabrowski *et al.* (2007). A justification of the lower Lys digestibility
387 presented in the present trial can be related with the animal meal (meat meal) included in
388 high levels in the FM0 diet. The excessive heat applied during its production might
389 damage proteins, especially affecting Lys (Carpenter & Booth, 1973; Opstvedt *et al.*,
390 1984), which may contribute to lower protein digestibility. In addition, the protein source
391 (muscle, connective tissue, bones, etc.) also affects the digestibility. In this sense, Allan
392 *et al.* (2000), observed a lower Lys digestibility coefficient in meal obtained from bones
393 than in fishmeal.

394 Also, EAA digestibility diminishes with increasing dietary vegetable protein (Masumoto
395 *et al.*, 1996; Yamamoto *et al.*, 1998). Vegetable meals contain undigestible components,

396 but also protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins,
397 allergens (Francis *et al.*, 2001), that cause low protein availability, histological gut
398 alteration, an imbalanced microbiota (Estruch *et al.*, 2015), that may alter the immune
399 regulatory functions of the gut and contribute to the development of diseases (Pérez *et*
400 *al.*, 2010). Yellowtail kingfish (*Seriola lalandi*) fed with solvent-extracted soybean meal
401 and soy protein concentrate produced a development of subacute enteritis in the hindgut
402 that it may compromise fish health to long term feeding (Bansemer *et al.*, 2015).

403 Overall, the low EAA digestibility of FM0 diet caused the lowest intake of digested EAA
404 of fish fed with this diet. However, only digested Lys efficiency retention of fish fed FM0
405 diet exhibited the highest values. This indicates that Lys is the limiting amino acid for
406 protein synthesis in fish fed FM0 diet, on the contrary of Met.

407 The amino acid index is the result of the ratio between the EAA profile in experimental
408 diets and the whole body fish at the end of the trial. When this index is below 100, it
409 might indicate that the AA is deficient in the diet, as a consequence it would have a high
410 retention. Nevertheless, if the AA index is higher than 100, this AA could be in excess in
411 the diet, therefore the whole body efficiency retention would be low (Sánchez-Lozano *et*
412 *al.*, 2011). Moreover, the Lys amino acid index in the present experiment corroborates
413 that the percentage of digestible Lys in the FM0 diet did not cover the yellowtail Lys
414 requirements.

415 The results indicated that yellowtail did not decrease their feed intake (FI) with respect to
416 the fishmeal dietary substitution. On the contrary, Tomás *et al.* (2005), observed an
417 increase of FI when yellowtail was fed a high content of dietary soybean meal (40%) as
418 Watanabe *et al.* (1992) and Viyakarn *et al.* (1992) found in Japanese yellowtail. One
419 possible explanation could be attributed to an inadequate amino acid profile in diets with

420 high levels of fishmeal substitution, as an attempt of fish to compensate the deficiency of
421 some EAAs with a higher intake. In the present experiment, diets did not present negative
422 effects on palatability. Takakuwa *et al.* (2006b) observed that FI diminished when the
423 level of the dietary poultry by-product was increased, probably due to its lower
424 palatability.

425 In summary, from the results of this experiment it can be concluded that the total fishmeal
426 replacement by the alternative protein blend assayed was not feasible for yellowtail
427 feeding, causing a detriment of digestible EAA and energy and high mortality in long
428 term feeding. Fishmeal substitution at 66% dietary level obtained good growth and
429 nutrient efficiency and high survival.

430

431 **5. Acknowledgments**

432

433 Financial support of this study was provided by the “Ministerio de Ciencia e
434 Innovación” of Spain government (Project reference: AGL2011-30547-C03-02).

435 The authors have no financial or personal conflicts of interest to declare.

436

437 **6. References**

438 Allan, G.L., Parkinson, S., Booth, M.A., Stone D.A.J., Rowland, S.J., Frances, J. &
439 Warner-Smith, R. (2000) Replacement of fish meal in diets for Australian silver perch,
440 *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. *Aquaculture*, 186, 293–
441 310.

442 [AOAC] Association of Official Analytical Chemists (1995) Official methods of analysis.
443 Washington (DC): AOAC.

444 Aoki, H., Watanabe, T., Samada, Y., Yamagata, Y., Yamauchi, K. & Satoh, S. (2000a)
445 Use of alternative protein and lipid sources in practical feeds for yellowtail.
446 *Suisanzoshoku*, 48, 81–90.

447 Aoki H., Watanabe K., Satoh S., Yamagata Y. & Watanabe T. (2000b) Use of non-fish
448 meal diets for yellowtail: second trial. *Suisanzoshoku*, 48 (1), 73-79.

449 Aslaksen, M.A., Kraugerud, O.F., Penn, M., Svihus, B., Denstadli, V., Jørgensen, H.Y.,
450 Hillestad, M., Krogdahl, Å. & Storebakken, T. (2007) Screening of nutrient
451 digestibilities and intestinal pathologies in Atlantic salmon, *Salmo salar*, fed diets
452 with legumes, oilseeds, or cereals. *Aquaculture*, 272, 541–555.

453 Bansemer, M.S., Forder, R.E.A., Howarth, G.S., Sutor, G.M., Bowyer, J. & Stone, D.A.J.
454 (2015) The effect of dietary soybean meal and soy protein concentrate on the intestinal
455 mucus layer and development of subacute enteritis in Yellowtail Kingfish (*Seriola*
456 *lalandi*) at suboptimal water temperature. *Aquacult. Nutr.*, 21, 300–310.

457 Bosch, L., Alegria, A. & Farré, R. (2006) Application of the 6-aminoquinolyl-N-
458 hydroxysuccinimidyl carbamate (AQC), reagent to the RP-HPLC determination of
459 amino acids in infant foods. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*,
460 831, 176–183.

461 Bowyer, J.N., Qin, J.G., Smullen, R.P., Adams, L.R., Thomson, M.J.S. & Stone, D.A.J.
462 (2013a) The use of a soy product in juvenile yellowtail kingfish (*Seriola lalandi*) feeds
463 at different water temperatures: 1. Solvent extracted soybean meal. *Aquaculture*, 384–
464 387.

465 Bowyer, J.N., Qin, J.G., Smullen, R.P., Adams, L.R., Thomson, M.J.S. & Stone, D.A.J.
466 (2013b) The use of a soy product in juvenile yellowtail kingfish (*Seriola lalandi*) feeds
467 at different water temperatures: 2. Soy protein concentrate. *Aquaculture*, 410–411.

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

471 Carpenter, K.J. & Booth, V.H. (1973) Damage to lysine in food processing: its
472 measurement and its significance. *Nutr. Abstr. Rev.*, 43, 424–451. Cheryan, 1980.

473 Cho, C.Y., Slinger, S.J. & Bayley, H.S. (1982) Bioenergetics of salmonid fishes: Energy
474 intake, expenditure and productivity. *Comp. Biochem. Physiol. (B)*, 73, 25-41.

475 Cho, C.Y. & Kaushik, S.J. (1990) Nutritional energetics in fish: energy and protein
476 utilisation in rainbow trout (*Salmo gairdneri*). *World Rev. Nutr. Diet.*, 61, 132–172.

477 Dabrowski, K., Arslan, M., Terjesen, B.F. & Zhang, Y.F. (2007) The effect of dietary
478 indispensable amino acid imbalances on feed intake: is there a sensing of deficiency
479 and neural signaling present in fish? *Aquaculture*, 268, 136–142.

480 Dawood, M.A.O., Koshio, S., Ishikawa, M. & Yokoyama, S. (2015) Effects of partial
481 substitution of fish meal by soybean meal with or without heat-killed *Lactobacillus*
482 *plantarum* (LP20) on growth performance, digestibility, and immune response of
483 amberjack, *Seriola dumerili* juveniles. Hindawi Publishing Corporation. *BioMed*
484 *Research International*. Volume 2015, Article ID 514196, 11 pages.

485 Espe, M., Lemme, A., Petri, A. & El-Mowafi, A. (2006) Can Atlantic salmon (*Salmo*
486 *salar*) grow on diets devoid of fishmeal? *Aquaculture*, 255, 255-262.

487 Estruch, G., Collado, M. C., Peñaranda, D. S., Vidal, A. T., Cerdá, M. J., Martínez, G. P.,
488 & Martinez-Llorens, S. (2015). Impact of fishmeal replacement in diets for gilthead
489 sea bream (*Sparus aurata*) on the gastrointestinal microbiota determined by
490 pyrosequencing the 16S rRNA gene. PloS one, 10, e0136389.

491 Fountoulaki, E., Alexis, M.N., Nengas, I. & Venou, B. (2005) Effect of diet composition
492 on nutrient digestibility and digestive enzyme levels of gilthead sea bream (*Sparus*
493 *aurata*, L.). Aquac. Res., 36, 1243–1251.

494 Francis, G., Makkar, H.P.S. & Becker, K. (2001) Antinutritional factors present in plant-
495 derived alternate fish feed ingredients and their effects in fish. Aquaculture, 199, 197–
496 227.

497 Furuichi, M., Taira, H. & Yone, Y. (1986) Availability of carbohydrate in nutrition of
498 yellowtail. Bull. Jpn. Soc. Sci. Fish., 52, 99–102.

499 Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W.,
500 Herman, E., Hu, G., Kroghdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W.,
501 Skonberg, D., Souza, E.J., Stone, D., Wilson, R. & Wurtele, E. (2007) Expanding the
502 utilization of sustainable plant products in aquafeeds: a review. Aquac. Res., 38, 551–
503 579.

504 Goto, T., Takagi, S., Ichiki, T., Sakai, T., Endo, M., Yoshida, T., Ukawa, M. & Murata,
505 H. (2001) Studies on the green liver in cultured red sea bream fed low level and non-
506 fish meal diets: Relationship between hepatic taurine and biliverdin levels. Fisheries
507 Sci., 67, 58–63.

508 Han, L.K., Kimura, Y. & Okuda, H. (1999) Reduction in fat storage during chitin-chitosan
509 treatment in mice fed a high-fat diet. Int. J. obesity., 23 (2), 174-179.

510 Jirsa, D., Davis, A., Stuart, K. & Drawbridge, M. (2011) Development of a practical soy-
511 based diet for California yellowtail, *Seriola lalandi*. *Aquac. Nutr.*, 17, e869-e874.

512 Jover, M., García-Gómez, A., Tomás, A., De la Gándara, F. & Pérez, L. (1999) Growth
513 of the Mediterranean yellowtail (*Seriola dumerili*) fed extruded diets containing
514 different levels of protein and lipid. *Aquaculture*, 179, 25-33.

515 Karlsen, Ø., Amlund, H., Berg, A. & Olsen, R.E. (2015) The effect of dietary chitin on
516 growth and nutrient digestibility in farmed Atlantic cod, Atlantic salmon and Atlantic
517 halibut. *Aquac. Res.*, 1-11. doi:10.1111/are.12867.

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

521 Kissil, G.W. & Lupatsch, I. (2004) Successful replacement of fishmeal by plant proteins
522 in diets for the gilthead sea bream (*Sparus aurata L.*). *Isr. J. Aquacult-Bamid.*, 56,
523 188-199.

524 Kroeckel, S., Harjes, A.G.E., Roth, I., Katz, H., Wuertz, S., Susenbeth, A. & Schulz, C.
525 (2012) When a turbot catches a fly: evaluation of a pre-pupae meal of the Black
526 Soldier Fly (*Hermetia illucens*) as fish meal substitute – growth performance and
527 chitin degradation in juvenile turbot (*Psetta maxima*). *Aquaculture*, 364/365, 345–
528 352.

529 Limin, L., Feng, X. & Jing, H. (2006) Aminoacids composition difference and nutritive
530 evaluation of the muscle of five species of marine fish, *Pseudosciaena crocea* (large
531 yellow croaker), *Lateolabrax japonicus* (common sea perch), *Pagrosomus major* (red

532 seabream), *Seriola dumerili* (Dumeril's amberjack) and *Hapalogenys nitens* (black
533 grunt) from Xiamen Bay of China. *Aquac. Nutr.*, 12, 53-59.

534 Lindsay, G.J.H., Walton, M.J., Adron, J.W., Fletcher, T.C., Cho, C.Y. & Cowey, C.B.
535 (1984) The growth of rainbow trout (*Salmo gairdneri*) given diets containing chitin
536 and its relationship to chitinolytic enzymes and chitin digestibility. *Aquaculture*, 37
537 (1) 315-334.

538 Lu, F., Haga, Y. & Satoh S. (2015) Effects of replacing fish meal with rendered animal
539 protein and plant protein sources on growth response, biological indices, and amino
540 acid availability for rainbow trout *Oncorhynchus mykiss*. *Fisheries Sci.*, 81, 95-105.

541 Maita, M., Aoki, H., Yamagata, Y., Watanabe, K., Satoh, S. & Watanabe, T. (1997) Green
542 liver observed in yellowtail fed non-fish meal diet. *Nippon Suisan Gakk.*, 63, 400-
543 401.

544 Maita, M., Satoh, K., Fukuda, Y., Lee, H-K., Winton, J.R. & Okamoto, N. (1998) *Fish*
545 *Pathol.*, 33 (3), 129-133.

546 Maita, M., Maekawa, J., Satoh, K.I., Futami, K. & Satoh, S. (2006) Disease resistance
547 and hypocholesterolemia in yellowtail *Seriola quinqueradiata* fed a non-fishmeal
548 diet. *Fisheries Sci.*, 72, 513-519.

549 Masumoto, T., Ruchimata, T., Itob, Y., Hosokawa, H. & Shimeno, S. (1996) Amino acid
550 availability values for several protein sources for yellowtail (*Seriola quinqueradiata*).
551 *Aquaculture*, 146 (1-2), 109-119.

552 Monge-Ortiz, R., Martínez-Llorens, S., Márquez, L., Moyano, F.J., Jover-Cerdá, M. &
553 Tomás-Vidal, A. (2016) Potential use of high levels of vegetal proteins in diets for
554 marked-size gilthead sea bream (*Sparus aurata*). *Arch. Anim. Nutr.*, 70 (2), 155-172.

- 555 Opstvedt, J., Miller, R., Hardy, R. & Spinelli, J. (1984) Heat-induced changes in
556 sulfhydryl groups and disulfide bonds in fish protein and their effect on protein and
557 amino acid digestibility in rainbow trout (*Salmo gairdneri*). J. Agric. Food Chem., 32,
558 929–935.
- 559 Perez, T., Balcazar, J.L., Ruiz-Zarzuola, I., Halaihel, N., Vendrell, D., de Blas, I. &
560 Muzquiz, J.L. (2010) Host–microbiota interactions within the fish intestinal
561 ecosystem. Mucosal Immunol., 3, 355–360.
- 562 Razdan, A. & Pettersson, D. (1994) Effect of chitin and chitosan on nutrient digestibility
563 and plasma lipid concentrations in broiler chickens. Brit. J. Nutr., 72 (2), 277-288.
- 564 Ruchimat, T., Masumoto, T., Hosokawa, H. & Shimeno, S. (1997a) Quantitative
565 methionine requirements of yellowtail (*Seriola quinqueradiata*). Aquaculture, 150,
566 113–122.
- 567 Ruchimat, T., Masumoto, T., Hosokawa, H., Itoh, Y. & Shimeno, S. (1997b) Quantitative
568 lysine requirements of yellowtail (*Seriola quinqueradiata*). Aquaculture, 158, 331-
569 339.
- 570 Sánchez-Lozano, N.B., Martínez-Llorens, S., Tomas-Vidal, A. & Jover Cerdá, M. (2009)
571 Effect of high-level fish meal replacement by pea and rice concentrate protein on
572 growth, nutrient utilization and fillet quality in gilthead seabream (*Sparus aurata*, L.).
573 Aquaculture, 298, 83–89.
- 574 Sánchez-Lozano, N.B., Martínez-Llorens, S., Tomás-Vidal, A. & Jover Cerdá, M. (2011)
575 Amino acid retention of gilthead sea bream (*Sparus aurata*, L.) fed with pea protein
576 concentrate. Aquac. Nutr., 17, e604-e614.

577 Shimeno, S., Hosokawa, H., Takeda, M. & Kajiyama, H. (1980) Effect of calorie to
578 protein ratios in formulated diet on the growth, feed conversion and body composition
579 of young yellowtail. Bull. Jpn. Soc. Sci. Fish., 46, 1083–1087.

580 Shimeno, S. (1982) Effect of carbohydrates in feed on carbohydrate metabolism in
581 yellowtail. In: Balkema (Ed.), Studies on carbohydrate metabolism in fish, Rotterdam,
582 91–113.

583 Shimeno, S. (1991) Yellowtail, *Seriola quinqueradiata*. In: Wilson, R.P. (Ed.), Handbook
584 of nutrient requirements of finfish, CRC Press, Florida, 181–191.

585 Shimeno, S., Kumon, M., Ando, H. & Ukawa, M. (1993a) The growth performance and
586 body composition of young yellowtail fed with diets containing defatted soybean meal
587 for a long period. Nippon Suisan Gakk., 59 (5), 821–825.

588 Shimeno, S., Matsumoto, M., Hosokawa, H., Masumoto, T. & Ukawa, M. (2000)
589 Inclusion of poultry feather meal in diet for fingerling yellowtail. Nippon Suisan
590 Zoshuru, 48 (1), 99–104.

591 Sitjà-Bobadilla, A., Peña-Llopis, S., Gómez-Requeni, P., Médale, F., Kaushik, S. &
592 Pérez-Sánchez, J. (2005) Effect of fish meal replacement by plant protein sources on
593 non-specific defence mechanisms and oxidative stress in gilthead sea bream (*Sparus*
594 *aurata*). Aquaculture, 249, 387-400.

595 Snedecor, G. & Cochran, W. (1971) Métodos Estadísticos. Compañía Editorial
596 Continental, S.A. México.

597 Spinelli J., Houle C.R. & Wekell J.C. (1983) The effect of phytates on the growth of
598 rainbow trout (*Salmo gairdneri*) fed purified diets containing varying quantities of
599 calcium and magnesium. Aquaculture, 30, 71-83.

600 Tacon, A.G.J. & Metian, M. (2008) Global overview on the use of fish meal and fish oil
601 in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*,
602 285, 146–158.

603 Takakuwa, F., Fukada, H., Hosokawa, H. & Masumoto, T. (2006a) Optimum digestible
604 protein and energy levels and ratio for greater amberjack *Seriola dumerili* (Risso)
605 fingerling. *Aquac. Res.*, 37, 1532–1539.

606 Takakuwa, F., Fukada, H., Hosokawa, H. & Masumoto, T. (2006b) Availability of poultry
607 by-product meal as an alternative protein source for fish meal in diet for greater
608 amberjack (*Seriola dumerili*). *Aquac. Sci.*, 54 (4), 473-480.

609 Takagi, S., Murata, S., Goto, T., Hayashi, M., Hatate, H., Endo, M., Yamashita, H. &
610 Ukawa, M. (2006) Hemolytic suppression roles of taurine in yellowtail *Seriola*
611 *quinqueradiata* fed non-fishmeal diet based on soybean protein. *Fisheries Sci.*, 72
612 (3), 546-555.

613 Takeda, M., Shimeno, S., Hosokawa, H., Kajiyama, H. & Kaisyo, T. (1975) The effect of
614 dietary calorie-to-protein ratio on the growth, feed conversion and body composition
615 of young yellowtail. *Bull. Jpn. Soc. Sci. Fish.*, 41, 443–447.

616 Takeuchi, T., Arakawa, T., Shiina, Y., Satoh, S., Imaizumi, K., Sekiya, S. & Watanabe,
617 T. (1992a) Effect of dietary alpha and beta-starch of juvenile striped jack and
618 yellowtail. *Nippon Suisan Gakk.*, 58 (49), 701–705.

619 Takeuchi, T., Shiina, Y., Watanabe, T., Sekiya, S. & Imaizumi, K. (1992b) Suitable
620 protein and lipid levels in diet for fingerlings of yellowtail. *Nippon Suisan Gakk.*,
621 58 (7), 1333-1339.

622 Takeuchi, T., Shiina, Y., Watanabe, T., Sekiya, S. & Imaizumi, K. (1992c) Suitable levels
623 of n-3 highly unsaturated fatty acids in diet for fingerlings of yellowtail. Nippon
624 Suisan Gakk., 58 (7), 1341-1346.

625 Tomás, A., De La Gándara, F., García-Gómez, A., Pérez, L. & Jover, M. (2005)
626 Utilization of soybean meal as an alternative protein source in the Mediterranean
627 yellowtail, *Seriola dumerili*. Aquac. Nutr., 11, 333–340.

628 Tomás, A., De la Gándara, F., García-Gómez, A. & Cerdá, M. J. (2008) Effect of the
629 protein/energy ratio on the growth of Mediterranean yellowtail (*Seriola dumerili*).
630 Aquac. Res., 39, 1141–1148.

631 Tomás-Vidal, A., Monge, R., Jover-Cerdá, M. & Martínez-Llorens, S. (2016) Apparent
632 digestibility and protein quality evaluation of selected feed ingredients in *Seriola*
633 *dumerili*. In review (Aquac. Res.).

634 Viyakarn, V., Watanabe, T., Aoki, H., Tsuda, H., Sakamoto, H., Okamoto, N., Iso, N.,
635 Satoh, S. & Takeuchi, T. (1992) Use of soybean meal as a substitute for fish meal
636 in a newly developed soft-dry pellet for yellowtail. Nippon Suisan Gakk., 58, 1991–
637 2000.

638 Watanabe, T., Viyakarn, V., Kimura, H., Ogawa, K., Okamoto, N. & Iso, N. (1992)
639 Utilization of soybean meal as a protein source in a newly developed soft-dry pellet
640 for yellowtail. Nippon Suisan Gakk., 58, 1761–1773.

641 Watanabe, T., Viyakarn, V., Aoki, H., Tsuda, H., Sakamoto, H., Maita, M., Satoh, S. &
642 Takeuchi, T. (1994) Utilization of alternative protein sources as substitute for fish
643 meal in a newly developed soft-dry pellet for yellowtail. Suisanzoshoku, 42, 499-
644 506.

- 645 Watanabe, T., Watanabe, K., Takeuchi, T., Aoki H. & Yamagata Y. (1995) Abstr. Annu.
646 Meet. Jpn. Soc. Fish. Sci., 36 (in Japanese).
- 647 Yamamoto, T., Akimoto, A., Kishi, S., Unuma, T. & Akiyama, T. (1998) Apparent and
648 true availabilities of amino acids from several protein sources for fingerling rainbow
649 trout, common carp, and red sea bream. Fisheries Sci., 64, 448–458.
- 650 Yoshitomi, B. & Nagano, I. (2012) Effect of dietary fluoride derived from Antarctic krill
651 (*Euphausia superba*) meal on growth of yellowtail (*Seriola quinqueradiata*).
652 Chemosphere, 86, 891–897.