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1 **Modelling the predictable effects of dietary lipid sources on the fillet fatty acid**
2 **composition of one-year-old gilthead sea bream (*Sparus aurata* L.)**

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4 **Abbreviated running title:** Fatty acid descriptors in gilthead sea bream

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22 **ABSTRACT**

23 The present study aimed to ascertain the different fatty acid (FA) descriptors linking
24 dietary and muscle FA composition in one-year-old gilthead sea bream. For that
25 purpose, our own published data along with additional data from the present study were
26 compiled and analysed. High linear correlations ($r^2 = 0.90$, $P < 0.001$) between dietary
27 and muscle fatty acid composition were reported for monoenes, C18 polyunsaturated
28 FA (PUFA) and long-chain PUFA. Prediction deviations due to changes in muscle
29 fatness were analyzed in an independent trial with two different feeding levels (full
30 ration size, 30% restriction ration). Regardless of feeding regimen, predicted values for
31 muscle FA at low concentrations deviated ($P < 0.001$) from observed values, but good
32 predictions with less than 6% deviations were found for abundant fatty acids (16:1n-7,
33 18:1n-9, 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3, 22:6n-3). All this highlights the predictable
34 effects of dietary oils in the muscle FA composition of gilthead sea bream, although
35 further research is needed to cover all the range of commercial fish size and for the up-
36 scaling of laboratory results to different fish farming conditions.

37

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39 **Keywords:** Fatty acid descriptors, fish oil, vegetable oil, muscle, ration size.

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41 **1. Introduction**

42

43 The nature of lipid digestion has a substantial effect on the transfer of fatty acids
44 (FA) from the diet into the animal product (Woods & Fearon, 2009). In ruminants,
45 dietary FA are rapidly hydrogenated by rumen microorganisms into more highly
46 saturated end products (Demeyer & Doreau, 1999). This partial hydrogenation also
47 produces many other minor FA including branched and odd-numbered FA, as well
48 intermediate products such as conjugate linoleic acids (CLA), among which C18:2c-9,
49 t-11 is the most important isomer (Bhattacharya, Banu, Rahman, Causey & Fernandes,
50 2006). By contrast, in terrestrial monogastrics such as pig and poultry, FA are absorbed
51 unchanged and have more predictable effects on tissue FA composition (Chesworth,
52 Stuchbury & Scaife, 1998), although a wide range of factors including age, gender,
53 genotype and fatness influence the FA composition of edible matter in non-ruminant
54 animal products (Daza, Lopez-Bote, Olivares, Menoyo & Ruiz, 2007; Wood et al.,
55 2008; Ntawubizi, Raes, Buys & De Smet, 2009).

56 There is also now increased interest for ensuring the nutritional value of seafood
57 products. For instance, many marine fish species are known to be excellent dietary
58 sources of n-3 long chain polyunsaturated fatty acids (LC-PUFA), especially
59 eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3).
60 However, given the variations in fat content of flesh from fatty, medium or lean fish, the
61 total EPA or DHA levels can vary in a large extent (www.nutraqua.com). With regard
62 to farmed fish, it is also known that dietary fish meal and fish oil (FO) levels modify the
63 muscle FA profiles, but continuous efforts have been directed towards the reduction of
64 wild-fishery derived raw materials in the feeds of farmed fish. Hence, the inclusion
65 level of such marine feedstuffs have been steadily declining for the last ten-years not

66 only due to increasing costs, but also to ensure the sustainability of fish farming (Tacon
67 & Metian, 2008).

68 Of note, gilthead sea bream is a major finfish species farmed in the
69 Mediterranean area and there is ample evidence that practical diets with less than 25%
70 of fish meal plus fish oil can support optimal growth when the theoretical needs of
71 essential amino acids and FA are supplied (Benedito-Palos, Saera-Vila, Calduch-Giner,
72 Kaushik & Pérez-Sánchez, 2007; Benedito-Palos, Navarro, Sitjà-Bobadilla, Bell,
73 Kaushik & Pérez-Sánchez, 2008; Benedito-Palos, Navarro, Kaushik & Pérez-Sánchez,
74 2010). By examining the kinetics of muscle FA as affected by dietary FA profiles, it
75 was shown that the muscle FA composition of gilthead sea bream fed vegetable oils
76 follows a simple dilution model with possibilities of tailoring the FA profile with
77 adequate dietary and feeding regimes (Benedito-Palos, Navarro, Bermejo-Nogales,
78 Saera-Vila, Kaushik & Pérez-Sánchez, 2009). According to this a finishing period with
79 a FO-based diet can restore the FA profile and the efficacy of that has been
80 demonstrated in a number of species, including rainbow trout, turbot, Atlantic salmon,
81 European sea bass, red sea bream and warm fresh water species such as Murray cod
82 (revised in Turchini, Torstensen & Ng, 2009). However, predictive equations examining
83 the association between dietary FA intake and FA composition of edible matter are
84 practically reduced to Atlantic salmon (Bell, McEvoy, Tocher, McGhee, Campbell &
85 Sargent, 2001; Bell et al., 2002; Bell, Tocher, Henderson, Dick & Crampton, 2003) and
86 Atlantic cod (Karalazos et al., 2007). Furthermore, results accumulated so far remain
87 insufficient or still equivocal and do not allow to develop a proper strategy for
88 increasing the beneficial FA in farmed fish.

89 Regarding gilthead sea bream, we have shown earlier that season and fish size
90 have negligible effects on the muscle FA composition of juvenile fish fed different

91 dietary oil sources (Benedito-Palos et al., 2008). This is indicative that FA composition
92 remains mostly constant in one-year-old farmed fish, and the aim of the present study
93 was to underline the descriptors linking dietary and muscle FA composition. For that
94 purpose, our own published data along with additional data derived from the present
95 study were compiled and analysed. Prediction deviations due to changes in fatness
96 were subsequently analysed in an independent trial under restricted and un-restricted
97 feeding conditions.

98

99

100 **2. Materials and methods**

101

102 **2.1. Diets**

103 Data on composition of the different diets used in the different studies is
104 summarized in Table 1. A short description of the diets is given below. Extruded pellets
105 were manufactured by the Skretting Company (Stavanger, Norway) or the Institut
106 National de la Recherche Agronomique (INRA) at the experimental research station of
107 Donzaq (Landes, France). Diets A-D (manufactured by Skretting) were fish meal-based
108 diets containing 449 g of crude protein/kg and were supplemented with South
109 American FO (A), rapeseed oil (B), linseed oil (C) or soybean oil (D). Diet J was a
110 commercial Skretting diet (D-2 Excel 1P) based on fish meal (350 g/kg) and FO (70
111 g/kg),supplemented with a blend of vegetable oils (60 soybean oil: 40 rapeseed oil).
112 Diets E to H (manufactured by INRA, France) were practical diets based on plant
113 proteins (150 g/kg) and Scandinavian FO (E), partially (F-G) or totally (H) replaced
114 by a blend of vegetable oils (17 rapeseed oil: 58 linseed oil: 25 palm oil) (for details see
115 Benedito-Palos et al., 2007; Benedito-Palos et al., 2008). Diet I (manufactured by

116 INRA) was a plant protein-based diet with Scandinavian FO (150 g/kg) as the only
117 dietary lipid source.

118 All diets (A-J) contained similar crude protein levels around 47-48% of dry
119 matter, whereas the total lipid content varied from 19% to 24% of dry matter. The
120 inclusion of plant ingredients at the expense of fish meal and FO had a direct effect on
121 the FA composition of the diets. In particular, EPA and DHA largely decreased,
122 whereas an opposite trend was found for C18 PUFA.

123

124 ***2. Animal care and experimental setup***

125 Fish rearing was according to the guidelines set out by the Spanish Council of
126 Animal Care under a protocol approved by the Review Board of the Institute of
127 Aquaculture Torre de la Sal (IATS, Castellón, Spain).

128 The study included data from different feeding trials carried out at the IATS with
129 juvenile fish purchased from different fish producers and fed different diets: i) Cupimar,
130 Cádiz, Spain (A-D feeding trial, August-October 2003; original data, ii) Ferme Marine
131 de Douhet (FMD), Bordeaux, France (E-H feeding trial, May-September 2005;
132 published in Benedito-Palos et al., 2008) and iii) Valle Cà Zuliani, Cà Venier, Italy
133 (I feeding trial, May-August 2009; original data). In all cases, juvenile fish were
134 acclimatised to laboratory conditions for 20-30 days before the start of feeding trials.
135 After this initial period, groups of 60 fish (16-34 g initial body weight) were placed into
136 circular fiberglass tanks (500 l) in triplicate groups per dietary treatment. Water flow
137 was 20 l/min and oxygen content of outlet water remained higher than 85% saturation.
138 Day length and water temperature varied over the course of the study following natural
139 changes at IATS latitude (40°5'N; 0°10'E). Feed was offered to satiety to maximize
140 growth two times per day, six days per week over the course of 12-17 weeks. Overall,

141 body weight at slaughter was increased 3-6 fold times. Randomly selected fish (three
142 fish per replicated tank; nine fish per treatment) were killed by a blow on the head
143 before tissue sampling. Fillets (devoid of bone and skin) were rapidly excised and
144 stored at -80 °C until analyses of chemical and FA composition.

145 In an additional feeding trial (May-August 2009; original data), juvenile fish of
146 17 g initial body weight (FMD origin) were fed with a commercial diet (diet J)
147 distributed at two different ration levels: i) full ration (*ad-libitum* group) and ii) 30%
148 restricted ration (R group). Each experimental group was arranged in triplicate 500 l
149 tanks and reared over the course of 11 weeks. Fish rearing and tissue sampling was
150 carried out as indicated above for the other feeding trials.

151

152 **2.4. Chemical composition and fatty acid analyses**

153 The composition of diets and fish samples was analysed by standard procedures
154 as described elsewhere (Benedito-Palos et al., 2009). Total lipids for FA analyses were
155 extracted by the method of Folch, Less & Sloane-Stanley (1957), using
156 chloroform:methanol (2:1) containing 0.01% butylated hydroxytoluene (BHT) as
157 antioxidant. After the addition of nonadecanoic FA (19:0) as internal standard, total
158 lipids (TL) were subjected to acid-catalysed transmethylation for 16 hours at 50 °C
159 using 1 ml toluene and 2 ml of 1% (v/v) sulphuric acid in methanol (Christie, 1982).
160 The FA methyl esters (FAME) were extracted with hexane:diethyl ether (1:1, v/v), and
161 purified by thin layer chromatography (Silica gel G 60, 20 x 20 cm glass plates; Merck,
162 Darmstadt, Germany) using hexane:diethyl-ether:acetic acid (85:15:1.5, v/v) as a
163 solvent system. The FAME were then analyzed with a gas chromatograph (GC 8000
164 Series, Fisons Instruments, Rodano, Italy), equipped with a fused silica 30 m x 0.25 mm
165 open tubular column (Tracer, TR-WAX; film thickness: 0.25 µm; Teknokroma,

166 Barcelona, Spain) and a cold on-column injection system. Helium was used as a carrier
167 gas, and temperature programming was from 50 to 180 °C at 40 °C/min and then to 220
168 °C at 3 °C/min. Peaks were recorded in a personal computer using software package
169 (version 4.0.2.0. Azur, Datalys, St Martin d'Herès, France). Individual FAME were
170 identified by reference to well characterized FO standards, and the relative amount of
171 each FA was expressed as a percentage of the total amount of FA in the analysed
172 sample.

173 BHT and internal standard (19:0) were obtained from Sigma-Aldrich (Madrid,
174 Spain). All solvents in lipid extraction and FA analyses were HPLC grade and were
175 obtained from Merck (Darmstadt, Germany).

176

177 ***2.5. Statistical analysis***

178 Linear regression equations between dietary and tissue FA were calculated with
179 the following model, $Y = aX + b$, where Y = muscle tissue fatty acid (% of total FAME)
180 and X = dietary fatty acid (% of total FAME). Prediction deviations of the model were
181 analyzed using a statistical t-test to determine if the predicted FA value (result from the
182 regression equation) was statistically distinguishable from the observed value at a
183 significance level of 5%. All analyses were made using the SPSS package version 15.0
184 (SPSS Inc., Chicago, IL, USA).

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187

187 3. Results

188

189 In all the analysed studies, gilthead sea bream exhibited good specific growth
190 rates (SGR, 1.6-1.8) and low feed:gain ratios (FGR, 0.9-1). Body weight at slaughter
191 varied between 60 and 140 g without significant differences in whole body (12-14% fat,
192 wet matter basis) and muscle fat stores (6-8 %, wet matter basis) independently of fish
193 origin and diet composition. Regarding the effects of diets on muscle FA composition
194 (Table 2), fish fed diets with a higher proportion of FO contained higher n-3 LC-PUFA
195 in combination with reduced amounts of 18:1n-9, 18:2n-6 and 18:3n-3, as compared to
196 fish fed diets with a higher proportion of vegetable oils. These values in muscle ranged
197 between 31% and 6% for n-3 LC-PUFA in the two extreme groups, and between 17%
198 and 63% in the case of the sum of C18 PUFA.

199 The linear regressions of muscle FA composition against FA composition of
200 diets A-I are shown in Table 3. Slopes, Y-axis intercepts, correlation coefficients (r^2)
201 and P values were considered for 15 FA at detectable levels in all the analyzed fish
202 samples. A significant correlation ($P < 0.05$) was established for all FA including
203 saturated FA (14:0, 16:0, 18:0). However, strong and positive correlations were
204 especially evident ($P < 0.001$) for C18 PUFA and LC-PUFA. Data on the relation
205 between dietary FA and flesh FA composition for monoene FA (16:1n-7, 18:1n-9,
206 20:1n-9 and 22:1n-11) are presented in Figure 1 and those for 18:2n-6, 18:3n-3, 20:4n-
207 6, 20:5n-3 and 22:6n-3 in Figure 2.

208 When considering the effects of ration size, the final body weight of fish fed the
209 full ration was greater ($P < 0.001$) than that of C fish (*ad-libitum* fish: 72.55 ± 0.07 ; R
210 fish: 59.99 ± 0.52). Both groups of fish grew efficiently, although a slight improvement
211 in FCR was found in R fish (0.99 ± 0.005) in comparison to *ad libitum* fed fish ($0.92 \pm$

212 0.07). Dietary intervention decreased ($P = 0.002$) muscle body fat stores from 6.5% in
213 wet matter basis in *ad-libitum* fish to 4.5% in R fish. Only minor changes were found in
214 FA composition between groups and regarding the predicted values, a significant ($P =$
215 0.01) deviation (less than 6%) was found for 18:1n-9 and 18:2n-6 in fish from the R
216 group but not in *ad libitum* fed fish. Only minor changes were found in FA composition,
217 and regarding the predicted values a slight but significant ($P = 0.01$) deviation (less than
218 6%) was found for 18:1n-9 and 18:2n-6 in R fish but not in *ad libitum* fed fish. The
219 predicted values for FA at low concentrations (20:1n-9 and 22:1n-11) deviated ($P <$
220 0.001) from the observed values regardless of feeding regimen. In both experimental
221 groups, predicted values mainly agreed ($P > 0.05$) with observations for 16:1n-7, 18:3n-
222 3, 20:4n-6, 20:5n-3 and 22:6n-3.

223

224

224 4. Discussion

225

226 The FA descriptors for one-year-old gilthead sea bream give close associations
227 between dietary and muscle FA composition. Data on muscle FA composition were
228 derived from feeding trials conducted at different times with fish originating from three
229 major European producers, which assured a representative fish population of farmed
230 gilthead sea bream. Partial and total replacement of either fish meal or FO was also
231 considered in the experimental setup, and the replacement strategy of marine raw
232 materials with plant ingredients covered a wide range of changes in the FA composition
233 of diets containing 20-24 % crude lipid, which represents the normal range for dietary
234 lipids in most commercial feeds used for gilthead sea bream farming..

235 The slopes and Y-intercepts of the relations between muscle FA and dietary FA
236 composition were specific for each FA, and correlation coefficients were particularly
237 high for monoenes, C18 PUFA and LC-PUFA. Saturated FA, especially 16:0 and 18:0,
238 gave low correlation coefficients ($r^2 = 0.5$). These results are explained by the fact that
239 the C14, C16 and C18 saturated FA are mainly the products of endogenous lipogenesis
240 and interconversions between them limiting the impact of dietary supply levels. By
241 contrast, marine fish species including gilthead sea bream have a very limited capacity
242 to elongate and desaturate C18 vegetable oils into long chain C20 and C22 PUFA
243 (Sargent, Tocher & Bell, 2002), and most of these FA in the flesh are entirely derived
244 from the diet, which enables the mathematical modelling of FA composition with a high
245 level of confidence.

246 In the present study, data for predictive equations were derived from fish with a
247 body weight range of 60-140 g. But the model can be extrapolated to bigger fish (200-
248 300 g) because season and fish size components have a negligible effect on the muscle

249 FA composition of juvenile fish grown out for 8-month productive cycle under natural
250 light and temperature conditions (Benedito-Palos et al., 2008). Attempts in salmonids
251 for the nutritional modelling of FA composition remain yet uncertain, and collectively,
252 data from the literature (Bell et al., 2001; 2002; 2003) suggest that selective retention or
253 metabolism of individual FA is influenced to a large extent by the blend of dietary oils,
254 fish size, age and fat level in the fish. Even in lean fish such as the Atlantic cod, the FA
255 composition of muscle is highly influenced by diet, but with relatively high levels of
256 18:1n-9 and DHA in polar lipids which remain fairly constant, irrespective of whether
257 the fish were fed a diet with FO or vegetable oil (Karalazos et al., 2007). This is
258 indicative that perhaps a meta-analysis approach (e.g. warm vs. cold fish and lean vs.
259 muscle fat fish) is needed for precise guidelines in managing beneficial fish FA for
260 human health.

261 The muscle FA composition in pigs, sheep and cattle is also dependent upon the
262 amount of fat in the carcass and in the muscle (Wood et al. 2008). Thus, as fat content
263 of the animal and meat increases between early life and the time of slaughter, the
264 proportion of FA changes. This has been ascribed to an increased contribution of *de*
265 *novo* synthesis of saturated and monounsaturated FA and a relative decline for the direct
266 incorporation of C18 and derivatives from the diet. Thus, in young lean animals,
267 genetically lean animals or animals fed low energy diets, the FA composition of
268 phospholipids (PL) has a major influence on total muscle FA composition. But as body
269 fat increases, neutral lipid (NL) predominates in overall FA composition (Kiessling,
270 Pickova, Johansson, Asgard, Storebakken & Kiessling, 2001). In the present study,
271 body fat stores in the muscle samples used for the predictive modelling remained fairly
272 constant (6-8% in wet matter basis) and FA proportions increased linearly in the muscle
273 as the corresponding FA level in the diet increased. However, the FA composition of PL

274 and NL is different because a selective incorporation of FA tend to dominate in PL,
275 whereas FA composition of NL, as fat storage form, is more dependent on diet
276 regardless of tissue function in both gilthead sea bream (Benedito-Palos et al., 2010)
277 and other fish species (Sargent et al., 2002; Tocher, 2003). Thereby, it appears likely
278 that 2-3 fold increases in body fat stores would lead to changes in muscle FA
279 proportions (total lipids) when comparisons are made between one year (< 300 g body
280 mass) and 2-3 year-old gilthead sea bream (0.5-1 kg body mass). This, however, needs
281 to be confirmed and long-term studies analyzing the age and fatness effects on FA
282 composition are underway to cover the full range of commercial size (300g- 1 kg).

283 The effects of fat gain on FA composition were analyzed by comparing fish fed
284 to satiety against those fed at a reduced ration level. Decreases in body weight and body
285 fat stores paralleled dietary restriction, but even then, a 30% reduction in muscle fat
286 stores did not have a noteworthy effect on the FA profile. Similarly, Kiessling, Pickova,
287 Eales, Dosanjh & Higgs (2005) found slight variations in Chinook salmon given a 25%
288 reduced ration. Under practical farming conditions, it is common practice to resort to
289 slightly restricted rationing, in order to avoid feed wastages as well as to increase
290 efficiency. Therefore, for the given size-class studied, it appears likely that the proposed
291 equations can be up-scaled to most farm conditions. Thus overall deviations from
292 predicted values are less than 6% for C18 PUFA and LC-PUFA, whereas FA
293 descriptors for less abundant FA (< 1.5 %) such as 20:1n-9 and 22:1n-11, are
294 substantially less accurate since probably low concentrations are by themselves a source
295 of error.

296 As mentioned earlier, a relation between dietary FA composition and flesh FA
297 composition has been established in different species. But, whether the descriptors as
298 established here for gilthead sea bream are applicable to other species needs to be

299 verified. For instance, the intrinsic potential for bioconversion of 18:2n-6 and 18:3n-3
300 fatty acids to n-6 and n-3 long chain PUFA is reported to be higher in freshwater fish
301 than in marine teleosts (Henderson & Tocher, 1987). Besides the ecological niche
302 occupied by the species, FA profiles are reportedly affected by water temperature
303 (Jobling & Bendiksen, 2003; Skalli, Robin, Le Bayon, Le Delliou & Person-Le Ruyet,
304 2006) and salinity (Haliloglu, BayIr, Sirkecioglu, Mevlüt Aras & Atamanalp, 2004),
305 linked to the cell membrane fluidity and permeability. Additionally, the amount of
306 muscle fat stores can differ between species, and the concordance with the model will
307 be greater in species with high fat deposition, which contain more NL than PL. These
308 factors need to be taken into account for tailoring flesh FA composition of fish,
309 especially when increasing levels of alternatives to FO are used in the feeds of farmed
310 fish. Furthermore, a better understanding of the mechanisms leading to tissue FA uptake
311 and turnover are needed from intra- and inter-species comparative perspective to draw
312 guidelines on the means to tailor flesh FA profile and to supply the recommended
313 dietary allowance in EPA and DHA for human consumers.

314 In summary, with the given regression formulas, the muscle FA profile of
315 gilthead sea bream can be predicted for a given class of fish size as based on the FA
316 composition of the diet. The data collected here correspond to fish which had undergone
317 similar experimental rearing conditions under the same standards of handling and
318 maintenance. This unavoidably leads to a decrease in the experimental statistical error
319 that ultimately translates into the increase of the quality of the regression results,
320 leading to high predictability to show the relation between dietary and muscle FA
321 levels. Further work is in progress to complete the construction of a FA database for
322 bigger fish in order to evaluate the specificity of predictive equations within and
323 between different marine fish species and farming conditions. The application of such

324 predictions would strengthen the potential for tailoring flesh FA composition and to
325 ensure the nutritional value of farmed seafood.

326

327

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333 **References**

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336 Bell, J. G., Henderson, R. J., Tocher, D. R., McGhee, F., Dick, J. R., Porter, A.,
337 Smullen, R. P., & Sargent, J. R. (2002). Substituting fish oil with crude palm oil in the
338 diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic
339 fatty acid metabolism. *Journal of Nutrition*, 132, 222-230.

340 Bell, J. G., McEvoy, J., Tocher, D. R., McGhee, F., Campbell, P. J., & Sargent,
341 J. R. (2001). Replacement of fish oil with rapeseed oil in diets of Atlantic salmon
342 (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism.
343 *Journal of Nutrition*, 131, 1535-1543.

344 Bell, J. G., Tocher, D. R., Henderson, R. J., Dick, J. R., & Crampton, V. O.
345 (2003). Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets
346 containing linseed and rapeseed oils can be partially restored by a subsequent fish oil
347 finishing diet. *Journal of Nutrition*, 133, 2793-2801.

348 Benedito-Palos, L., Navarro, J. C., Kaushik, S., & Pérez-Sánchez, J. (2010).
349 Tissue-specific robustness of fatty acid signatures in cultured gilthead sea bream
350 (*Sparus aurata* L.) fed practical diets with a combined high replacement of fish meal and
351 fish oil. *Journal of Animal Science*, In press.

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

356 Benedito-Palos, L., Navarro, J. C., Bermejo-Nogales, A., Saera-Vila, A.,
357 Kaushik, S., & Pérez-Sánchez, J. (2009). The time course of fish oil wash-out follows a
358 simple dilution model in gilthead sea bream (*Sparus aurata* L.) fed graded levels of
359 vegetable oils. *Aquaculture*, 288, 98-105.

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

364 Bhattacharya, A., Banu, J., Rahman, M., Causey, J., & Fernandes, G. (2006).
365 Biological effects of conjugated linoleic acids in health and disease. *Journal of*
366 *Nutritional Biochemistry*, 17, 789-810.

367 Chesworth, J.M., Stuchbury, T., Scaife, J.R., 1998. An introduction to agricultural
368 biochemistry. Chapman and Hall, London, UK.

369 Christie, W.W., 1982. Lipid Analysis. Isolation, Separation, Identification and
370 Structural Analysis of Lipids, 2nd ed. Pergamon Press, Oxford, UK.

371 Cruz-Garcia, L., Saera-Vila, A., Navarro, I., Calduch-Giner, J., & Pérez-
372 Sánchez, J. (2009). Targets for TNF alpha-induced lipolysis in gilthead sea bream
373 (*Sparus aurata* L.) adipocytes isolated from lean and fat juvenile fish. *Journal of*
374 *Experimental Biology*, 212, 2254-2260.

375 Daza, A., Lopez-Bote, C. J., Olivares, A., Menoyo, D., & Ruiz, J. (2007). Age at
376 the beginning of the fattening period of Iberian pigs under free-range conditions affects
377 growth, carcass characteristics and the fatty acid profile of lipids. *Animal Feed Science*
378 *and Technology*, 139, 81-91.

379 Demeyer, D., & Doreau, M. (1999). Targets and procedures for altering
380 ruminant meat and milk lipids. *Proceedings of the Nutrition Society*, 58, 593-607.

381 Folch, J., Less, N., & Sloane-Stanley, G. H. (1957). A simple method for
382 insolation and purification of total lipids from animal tissues. *Journal of Biological*
383 *Chemistry*, 226, 497-509.

384 Haliloglu, H. I., BayIr, A., Sirkecioglu, A. N., Mevlüt Aras, N., & Atamanalp,
385 M. (2004). Comparison of fatty acid composition in some tissues of rainbow trout
386 (*Oncorhynchus mykiss*) living in seawater and freshwater. *Food Chemistry*, 86, 55-59.

387 Henderson, J. R., & Tocher, D. R. (1987). The lipid composition and
388 biochemistry of freshwater fish. *Progress in Lipid Research*, 26, 281-347.

389 Jobling, M., & Bendiksen, E. A. (2003). Dietary lipids and temperature interact
390 to influence tissue fatty acid compositions of Atlantic salmon, *Salmo salar* L., parr.
391 *Aquaculture Research*, 34, 1423-1441.

392 Karalazos, V., Treasurer, J., Cutts, C. J., Alderson, R., Galloway, T. F.,
393 Albrektsen, S., Arnason, J., MacDonald, N., Pike, I., & Bell, J. G. (2007). Effects of fish
394 meal replacement with full-fat soy meal on growth and tissue fatty acid composition in
395 Atlantic cod (*Gadus morhua*). *Journal of agricultural and food chemistry*, 55, 5788-
396 5795.

397 Kiessling, A., Pickova, J., Johansson, L., Asgard, T., Storebakken, T., &
398 Kiessling, K. H. (2001). Changes in fatty acid composition in muscle and adipose tissue
399 of farmed rainbow trout (*Oncorhynchus mykiss*) in relation to ration and age. *Food*
400 *Chemistry*, 73, 271-284.

401 Kiessling, A., Pickova, J., Eales, J. G., Dosanjh, B., & Higgs, D. (2005). Age,
402 ration level, and exercise affect the fatty acid profile of chinook salmon (*Oncorhynchus*
403 *tshawytscha*) muscle differently. *Aquaculture*, 243, 345-356.

404 Ntawubizi, M., Raes, K., Buys, N., & De Smet, S. (2009). Effect of sire and sex
405 on the intramuscular fatty acid profile and indices for enzyme activities in pigs.
406 *Livestock Science*, 122, 264-270.

407 Saera-Vila, A., Calduch-Giner, J. A., Navarro, I., & Pérez-Sánchez, J. (2007).
408 Tumour necrosis factor (TNF) α as a regulator of fat tissue mass in the Mediterranean
409 gilthead sea bream (*Sparus aurata* L.). *Comparative Biochemistry and Physiology Part*
410 *B: Biochemistry and Molecular Biology*, 146, 338-345.

411 Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids. In: Halver, J.E.,
412 Hardy, R.W. (Eds.), *Fish Nutrition*. Academic Press, San Diego, CA, pp. 181-257.

413 Skalli, A., Robin, J. H., Le Bayon, N., Le Delliou, H., & Person-Le Ruyet, J.
414 (2006). Impact of essential fatty acid deficiency and temperature on tissues' fatty acid
415 composition of European sea bass (*Dicentrarchus labrax*). *Aquaculture*, 255, 223-232.

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

419 Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in
420 teleost fish. *Rev. Fish. Sci.*, 11, 107-184.

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

423 Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R.
424 I., Hughes, S. I., & Whittington, F. M. (2008). Fat deposition, fatty acid composition
425 and meat quality: A review. *Meat Science*, 78, 343-358.

426 Woods, V. B., & Fearon, A. M. (2009). Dietary sources of unsaturated fatty
427 acids for animals and their transfer into meat, milk and eggs: A review. *Livestock*
428 *Science*, 126, 1-20.

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431 **Figure captions**

432

433 Figure 1. Relationship between dietary and muscle fatty acid concentrations of 16:1n-7
434 (a), 18:1n-9 (b), 20:1n-9 (c), and 22:1n-11 (d) in gilthead sea bream fed A-I diets in
435 asynchronous trials.

436

437 Figure 2. Relationship between dietary and muscle fatty acid concentrations of 18:3n-3
438 (a), 18:2n-6 (b), 20:5n-3 (c), 20:4n-6 (d), and 22:6n-3 (e) in gilthead sea bream fed A-I
439 diets in asynchronous trials.

Table 1. Chemical composition and fatty acid profile (% of total fatty acid methyl esters) of diets.

| | A | B | C | D | E | F | G | H | I | J |
|------------------------------|------|------|------|------|------|------|------|------|------|------|
| <i>Proximate composition</i> | | | | | | | | | | |
| Dry matter (DM, %) | 94.9 | 95.0 | 96.3 | 96.8 | 93.4 | 94.1 | 94.7 | 95.3 | 95.1 | 89.1 |
| Protein (% DM) | 47.7 | 47.3 | 46.9 | 47.5 | 48.9 | 48.7 | 49.0 | 48.6 | 47.8 | 48.2 |
| Fat (% DM) | 23.6 | 23.6 | 24.6 | 24.3 | 22.1 | 22.2 | 22.1 | 22.3 | 19.6 | 19.9 |
| <i>Fatty acid profile</i> | | | | | | | | | | |
| 14:0 | 6.6 | 2.0 | 1.9 | 1.9 | 5.0 | 3.7 | 1.8 | 0.5 | 6.8 | 5.0 |
| 16:0 | 17.0 | 8.7 | 9.0 | 12.6 | 16.7 | 16.9 | 16.9 | 16.7 | 20.6 | 17.5 |
| 16:1n-7 | 6.1 | 1.9 | 1.8 | 1.8 | 4.6 | 2.9 | 1.9 | 0.7 | 5.4 | 4.8 |
| 18:0 | 3.5 | 2.1 | 3.2 | 3.0 | 2.5 | 2.9 | 3.4 | 3.7 | 4.1 | 4.1 |
| 18:1 n-9 | 7.6 | 37.4 | 16.6 | 16.7 | 12.5 | 17.5 | 21.9 | 25.9 | 16.3 | 15.5 |
| 18:1 n-7 | 2.3 | 2.6 | 1.2 | 1.6 | 1.9 | 1.6 | 1.4 | 1.2 | 2.6 | 2.9 |
| 18:2 n-6 | 4.4 | 15.8 | 13.0 | 37.2 | 12.1 | 15.7 | 19.2 | 21.3 | 7.9 | 21.4 |
| 18:3 n-3 | 1.2 | 6.5 | 32.1 | 4.8 | 1.5 | 8.9 | 16.3 | 23.2 | 0.8 | 2.3 |
| 18:4 n-3 | 3.1 | 1.3 | 1.3 | 1.2 | 2.1 | 1.4 | 0.8 | 0.2 | 1.0 | 0.8 |
| 20:1 n-9 | 3.6 | 3.2 | 2.5 | 2.5 | 7.2 | 5.1 | 3.0 | 1.0 | 5.0 | 1.1 |
| 20:4 n-6 | 0.7 | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 | 0.1 | - | 0.5 | 0.6 |
| 20:4 n-3 | 0.7 | 0.2 | 0.2 | 0.2 | 0.4 | 0.2 | 0.1 | - | 0.6 | 0.3 |
| 20:5 n-3 | 12.5 | 4.1 | 4.0 | 3.7 | 6.8 | 4.6 | 2.7 | 0.9 | 6.6 | 7.5 |
| 22:1 n-11 | 3.5 | 3.0 | 2.7 | 2.8 | 10.1 | 6.7 | 3.6 | 0.7 | 2.4 | 1.0 |
| 22:5 n-3 | 1.4 | 0.4 | 0.4 | 0.3 | 0.6 | 0.4 | 0.1 | - | 1.3 | 0.9 |
| 22:6 n-3 | 13.5 | 4.5 | 4.5 | 4.2 | 8.3 | 5.6 | 3.3 | 1.0 | 7.2 | 4.5 |

Table 2. Initial body weight, final body weight and muscle fatty acid profile at slaughter (% of fatty acid methyl esters) in fish fed experimental diets.

| Origin of fish Diet (see Table 1) | Cupimar, Spain | | | | Ferme Marine de Douhet, France | | | | Valle Cà Zuliani, Italy |
|--------------------------------------|----------------|-----------|-----------|-----------|--------------------------------|----------|----------|----------|----------------------------|
| | A | B | C | D | E | F | G | H | I |
| Initial body weight (g) ^a | 34.0±0.1 | 34.0±0.1 | 34.2±0.1 | 34.3±0.1 | 16.1±0.1 | 16.3±0.1 | 16.3±0.1 | 16.1±0.1 | 17.1±0.1 |
| Final body weight (g) ^a | 144.0±1.3 | 138.5±4.3 | 136.5±2.5 | 137.7±2.5 | 91.7±0.6 | 91.3±1.5 | 91.1±2.0 | 80.9±0.5 | 62.7±1.0 |
| Fatty acids (%) ^b | | | | | | | | | |
| 14:0 | 4.9±0.2 | 2.1±0.1 | 1.9±0.1 | 0.9±0.2 | 4.5±0.3 | 2.6±0.4 | 1.7±0.3 | 1.1±0.4 | 4.1±0.2 |
| 16:0 | 19.8±0.4 | 13.4±0.5 | 14.1±0.8 | 14.1±0.6 | 18.3±0.9 | 19.0±0.4 | 17.2±0.6 | 16.1±0.5 | 18.0±0.4 |
| 16:1n-7 | 6.7±0.1 | 3.2±0.1 | 2.1±1.3 | 2.5±0.2 | 5.4±0.5 | 3.6±0.5 | 2.8±0.3 | 2.1±0.5 | 6.4±0.1 |
| 18:0 | 4.6±0.3 | 3.1±0.2 | 4.4±0.2 | 4.1±0.1 | 3.0±0.2 | 4.1±0.5 | 3.9±0.6 | 4.4±0.6 | 4.1±0.2 |
| 18:1 n-9 | 12.9±0.4 | 35.5±1.0 | 20.7±1.8 | 19.3±0.7 | 16.0±0.9 | 18.5±2.8 | 24.5±2.2 | 27.3±3.1 | 20.7±1.1 |
| 18:2 n-6 | 4.4±0.2 | 13.7±0.3 | 8.7±5.8 | 30.8±1.3 | 11.8±0.2 | 14.9±1.5 | 17.4±0.1 | 20.5±1.6 | 7.9±0.3 |
| 18:3 n-3 | 1.1±0.1 | 4.8±0.2 | 23.9±1.8 | 3.6±0.1 | 1.1±0.1 | 5.8±0.6 | 12.1±1.5 | 15.8±1.7 | 0.8±0.1 |
| 18:4 n-3 | 2.1±0.1 | 0.8±0.1 | 0.8±0.4 | 0.8±0.1 | 1.3±0.1 | 0.8±0.1 | 0.7±0.1 | 0.5±0.1 | 0.9±0.1 |
| 20:1 n-9 | 2.6±0.1 | 2.7±0.1 | 2.1±0.1 | 2.1±0.1 | 5.5±0.2 | 3.2±0.1 | 1.9±0.2 | 0.9±0.5 | 3.6±0.1 |
| 20:4 n-6 | 0.8±0.1 | 0.3±0.1 | 0.5±0.3 | 0.4±0.1 | 0.3±0.1 | 0.4±0.1 | 0.2±0.1 | 0.1±0.1 | 0.6±0.1 |
| 20:4 n-3 | 1.0±0.1 | 0.5±0.1 | 0.7±0.1 | 0.5±0.1 | 0.6±0.1 | 0.5±0.1 | 0.4±0.1 | 0.3±0.1 | 0.7±0.1 |
| 20:5 n-3 | 10.1±0.1 | 3.5±0.3 | 3.8±0.5 | 3.7±0.3 | 5.0±0.3 | 4.3±0.7 | 2.5±0.7 | 1.5±0.7 | 5.9±0.2 |
| 22:1 n-11 | 2.4±0.1 | 2.1±0.1 | 1.9±0.1 | 1.9±0.1 | 5.3±0.5 | 2.7±0.4 | 1.6±0.4 | 0.3±0.1 | 1.6±0.1 |
| 22:5 n-3 | 3.1±0.1 | 1.3±0.2 | 1.1±0.8 | 1.4±0.1 | 1.5±0.1 | 1.2±0.1 | 0.6±2.5 | 0.4±0.1 | 2.4±0.1 |
| 22:6 n-3 | 17.1±1.2 | 7.5±1.8 | 8.2±3.2 | 8.5±0.5 | 10.6±2.0 | 8.8±2.6 | 6.0±2.5 | 3.5±1.8 | 10.5±0.7 |

^aMean body weight values and standard deviation of fish from triplicate tanks are presented.

^bMean fatty acid values and standard deviation of individual fish are presented (n = 9).

Table 3. Correlation coefficients (r^2), slopes, Y-axis intercepts and P values for the regression analysis of dietary fatty acid concentrations versus muscle fatty acid concentrations. Data were derived from fish fed A-I diets in asynchronous gilthead sea bream trials.

| Fatty acid | r^2 | slope | Y-axis intercept | P |
|------------|-------|-------|------------------|--------|
| 14:0 | 0.87 | 0.60 | 0.60 | <0.001 |
| 16:0 | 0.53 | 0.51 | 9.64 | 0.016 |
| 16:1n-7 | 0.97 | 0.94 | 1.02 | <0.001 |
| 18:0 | 0.55 | 0.91 | 1.99 | 0.014 |
| 18:1 n-9 | 0.97 | 0.76 | 7.02 | <0.001 |
| 18:2 n-6 | 0.96 | 0.82 | 1.05 | <0.001 |
| 18:3 n-3 | 0.99 | 0.72 | 0.05 | <0.001 |
| 18:4 n-3 | 0.89 | 0.55 | 0.24 | <0.001 |
| 20:1 n-9 | 0.95 | 0.67 | 0.30 | <0.001 |
| 20:4 n-6 | 0.91 | 0.89 | 0.20 | <0.001 |
| 20:4 n-3 | 0.86 | 0.88 | 0.28 | <0.001 |
| 20:5 n-3 | 0.98 | 0.74 | 0.72 | <0.001 |
| 22:1 n-11 | 0.95 | 0.47 | 0.10 | <0.001 |
| 22:5 n-3 | 0.91 | 1.70 | 0.48 | <0.001 |
| 22:6 n-3 | 0.96 | 1.02 | 2.93 | <0.001 |

Table 4. Effect of ration size (full ration, *ad libitum* fish; and 30% calorie-restricted diet, CR fish) upon predictable values on muscle fatty acid composition.

| FA profile | Prediction | <i>ad libitum</i> fish | | CR fish | |
|------------|------------|------------------------|------------------------------|------------------|------------------------------|
| | Values | Mean \pm SD | <i>P</i> -value ^a | Mean \pm SD | <i>P</i> -value ^a |
| 16:1n-7 | 5.53 | 5.59 \pm 0.03 | 0.17 | 5.37 \pm 0.10 | 0.17 |
| 18:1 n-9 | 18.80 | 18.27 \pm 0.22 | 0.13 | 17.41 \pm 0.30 | 0.01* |
| 18:2 n-6 | 18.60 | 18.75 \pm 0.06 | 0.13 | 17.48 \pm 0.30 | 0.01* |
| 18:3 n-3 | 1.71 | 1.88 \pm 0.45 | 0.71 | 1.78 \pm 0.03 | 0.09 |
| 20:1 n-9 | 1.04 | 1.34 \pm 0.01 | <0.001* | 1.34 \pm 0.03 | <0.001* |
| 20:4 n-6 | 0.73 | 0.65 \pm 0.05 | 0.17 | 0.66 \pm 0.05 | 0.22 |
| 20:5 n-3 | 6.27 | 6.48 \pm 0.11 | 0.20 | 6.07 \pm 0.09 | 0.06 |
| 22:1 n-11 | 0.57 | 0.84 \pm 0.01 | <0.001* | 0.87 \pm 0.04 | <0.001* |
| 22:6 n-3 | 7.52 | 7.33 \pm 0.18 | 0.46 | 7.25 \pm 0.27 | 0.36 |

^a*P* values result from statistical t-test to determine if observed values are statistically distinguishable from predicted values.

Figure 1

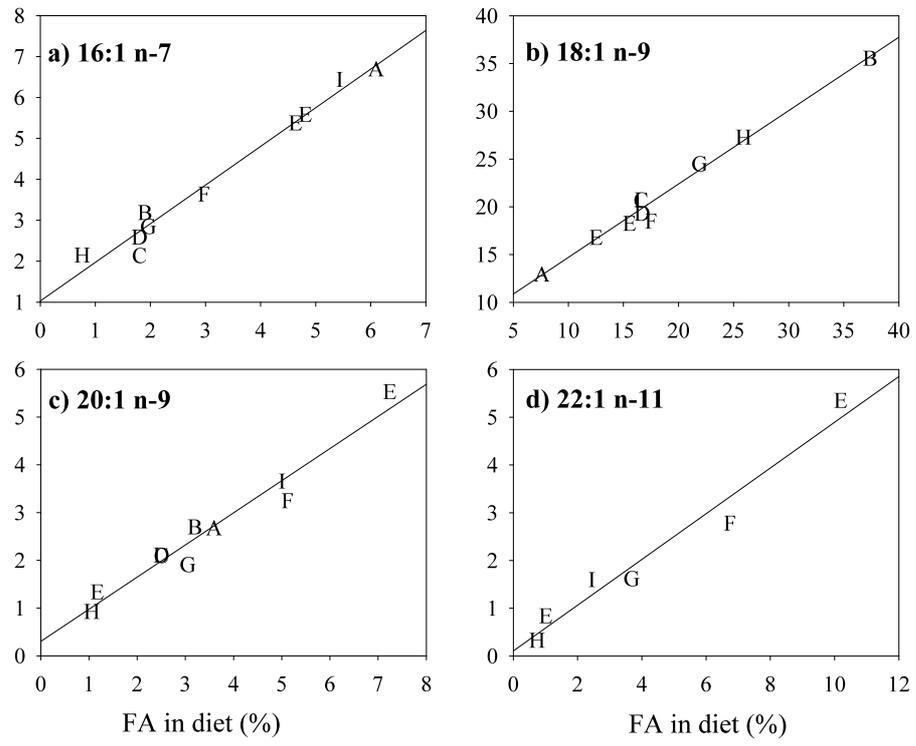


Figure 2

