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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.

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

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1. Introduction

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

There is also now increased interest for ensuring the nutritional value of seafood products. For instance, many marine fish species are known to be excellent dietary sources of n-3 long chain polyunsaturated fatty acids (LC-PUFA), especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). However, given the variations in fat content of flesh from fatty, medium or lean fish, the total EPA or DHA levels can vary in a large extent (www.nutraqua.com). With regard to farmed fish, it is also known that dietary fish meal and fish oil (FO) levels modify the muscle FA profiles, but continuous efforts have been directed towards the reduction of wild-fishery derived raw materials in the feeds of farmed fish. Hence, the inclusion 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.

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100   **2. Materials and methods**

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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 Researche 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.

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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 (40º5'N; 0º10'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 ºC 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.

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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 ºC

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 ºC at 40 ºC/min and then to 220

168 ºC at 3 ºC/min. Peaks were recorded in a personal computer using software package

169 (version 4.0.2.0. Azur, Datalys, St Martin d'Heres, 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).

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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   **3. Results**

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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 ±

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.

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224   **4. Discussion**

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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 derivates 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

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324 predictions would strengthen the potential for tailoring flesh FA composition and to

325 ensure the nutritional value of farmed seafood.

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333   **References**

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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
Proximate composition										
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
Fatty acid profile										
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	Cupimar, Spain				Ferme Marine de Douhet, France				Valle Cà Zuliani, Italy
Diet (see Table 1)	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

[a]Mean body weight values and standard deviation of fish from triplicate tanks are presented.
[b]Mean 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, a*d libitum fish;* and 30% calorie-restricted diet, CR fish) upon predictable values on muscle fatty acid composition.

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

[a]*P* values result from statistical t-test to determine if observed values are statistically distinguishable from predicted values.
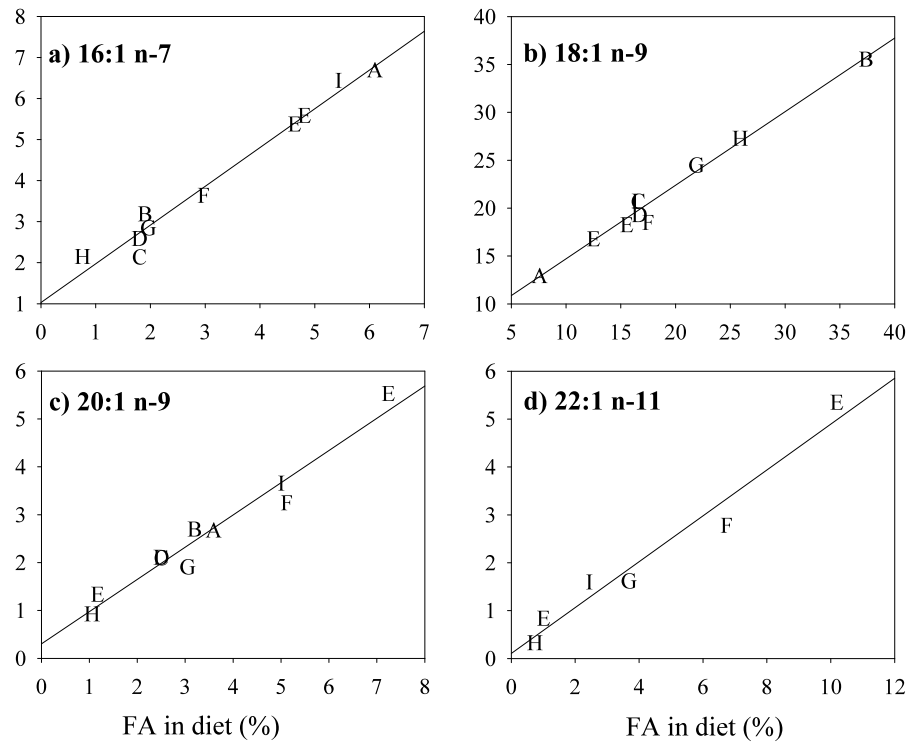
Figure 1

Figure 2