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

Abbreviated running title: Fatty acid descriptors in gilthead sea bream

Laura Benedito-Palos[1], Azucena Bermejo-Nogales[1], Alexandros I. Karampatos[1], Juan C. Navarro[1], Amalia Diez[2], José M. Bautista[2], J. Gordon Bell[3], Douglas R. Tocher[3], Alex Obach [4], Sadasivam Kaushik[5], Jaume Pérez-Sánchez[1*]

[1]Nutritional and Animal Health Research Unit, Institute of Aquaculture Torre la Sal, 12595 Castellón, Spain

[2]Department of Biochemistry and Molecular Biology, Facultad de Veterinaria, Universidad Complutense de Madrid, 28040 Madrid, Spain

[3]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK

[4] Research Center AS (ARC), Stavanger, Norway

[5]UMR Nutrition, Aquaculture and Genomics, INRA, Unité-Mixte INRA-IFREMER-Université Bordeaux I, 64310 Saint-Pée-sur-Nivelle, France

*Corresponding author contact information: Tel.: +34 964319500; Fax: +34 964319509; E-mail: jperez@iats.csic.es

ABSTRACT

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

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

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

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

2. Materials and methods

2.1. Diets

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

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

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

2. Animal care and experimental setup

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

The study included data from different feeding trials carried out at the IATS with juvenile fish purchased from different fish producers and fed different diets: i) Cupimar, Cádiz, Spain (A-D feeding trial, August-October 2003; original data, ii) Ferme Marine de Douhet (FMD), Bordeaux, France (E-H feeding trial, May-September 2005; published in Benedito-Palos et al., 2008) and iii) Valle Cà Zuliani, Cà Venier, Italy (I feeding trial, May-August 2009; original data). In all cases, juvenile fish were acclimatised to laboratory conditions for 20-30 days before the start of feeding trials. After this initial period, groups of 60 fish (16-34 g initial body weight) were placed into circular fiberglass tanks (500 l) in triplicate groups per dietary treatment. Water flow was 20 l/min and oxygen content of outlet water remained higher than 85% saturation. Day length and water temperature varied over the course of the study following natural changes at IATS latitude (40º5'N; 0º10'E). Feed was offered to satiety to maximize 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.
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 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,

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

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

2.5. Statistical analysis

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

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 ±

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

4. Discussion

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

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

In the present study, data for predictive equations were derived from fish with a body weight range of 60-140 g. But the model can be extrapolated to bigger fish (200-300 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

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

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

As mentioned earlier, a relation between dietary FA composition and flesh FA composition has been established in different species. But, whether the descriptors as 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

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

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Figure captions

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

Figure 2. Relationship between dietary and muscle fatty acid concentrations of 18:3n-3 (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 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.

	Prediction	*ad libitum* fish		CR fish	
FA profile	Values	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

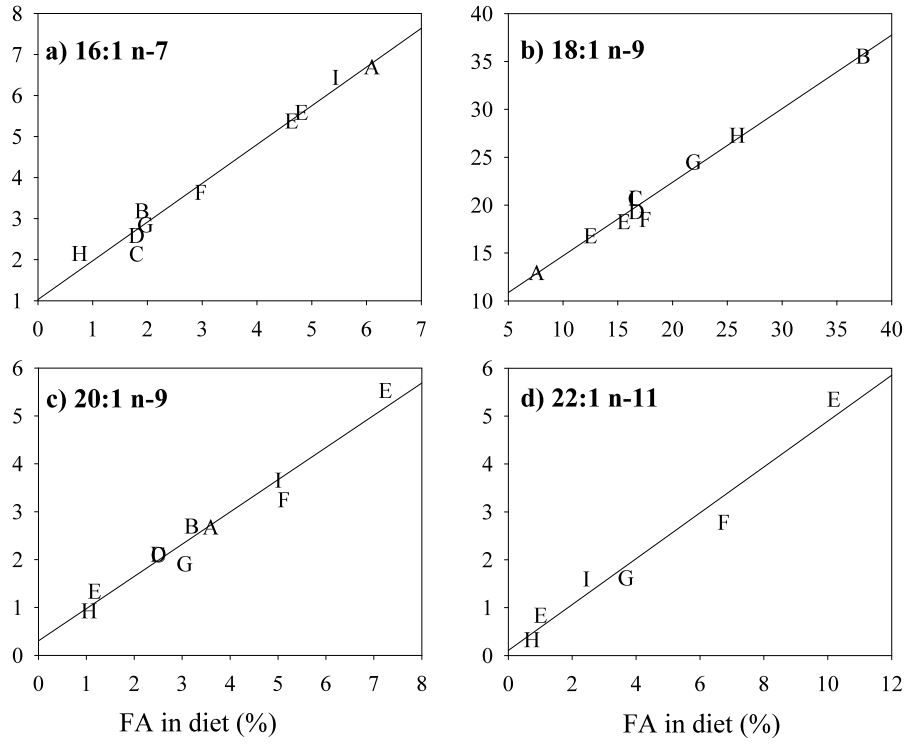

Figure 2