Dummy regression analysis for modelling the nutritionally tailored fillet fatty acid composition of turbot and sole using gilthead sea bream as a reference subgroup category

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Abstract

Farmed turbot and sole were sampled at different stages of the production cycle for analysis of fillet lipid content and fatty acid (FA) composition. The entire dataset along with our own published data on gilthead sea bream were fitted to dummy regression equations with turbot and sole as dummy variables, gilthead sea bream as a reference subgroup category, and diet FA composition and fillet lipid content as independent variables. The relative contribution of each independent variable to the total variance was found to vary within and among FAs and fish species, but strong correlation coefficients ($0.76 < r^2 > 0.99$) were found for almost all of the FA equations, including saturated FAs, monoenes and long-chain polyunsaturated fatty acids (PUFA) of n-3 and n-6 series. Given the differences in lipogenic activities of the fish species, major interaction effects between fillet lipid content and dummy variables were found for monoenes and saturated FAs. The proposed equations (hosted at www.nutrigroup-iats.org/aquafat) were able to fit different proportions of EPA, DPA and DHA underlying the fish species differences in FA desaturation/elongation pathways. The robustness of the model was proven with extra data from the three fish species, allowing a close linear association near to equality for the scatterplot of observed and predicted values.

Keywords: fish quality, fillet, fatty acids, EPA, DPA, DHA

Introduction

Global fisheries are in decline, whereas the aquaculture industry is still growing; therefore, several efforts have been directed towards the reduction of wild-fishery-derived raw materials in feeds of farmed fish (Tacon *et al*. 2010). Much attention has been focused on plant ingredients and there is now accumulating interest for a large and combined replacement of fish meal and fish oil in fish feeds for salmonids and marine fish (Torstensen *et al*. 2008; Dias *et al*. 2009). For instance, fish oil can be totally replaced by a blend of vegetable oils in practical gilthead sea bream (*Sparus aurata*) diets with a 35% inclusion level of fish meal (Bouraoui *et al*. 2011). Alternatively, up to 65-70% of fish oil can be replaced in diets with a 15-20% of fish meal inclusion level with no detrimental effects on growth performance (Benedito-Palos *et al*. 2007) and fatty acid (FA) composition of phospholipids (Benedito-Palos *et al*. 2010), since the theoretical requirements for essential FAs are met by the diet.

Another important issue is the effect of alternative dietary oils on the nutritional and quality characteristics of edible fish matter. Certainly, high levels of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) are important quality factors in human foods. For example, it is not well known if salmonids and freshwater fish have specific requirements in n-3 LC-PUFA, but any substitution of fish oils with vegetable oils should be carried out in such a way as to ensure the qualities that make cultured fish a healthy food option (Bell *et al*. 2003; Thanuthong *et al*. 2011). This reinforces the interest in reliable FA descriptors linking dietary and muscle FA composition. In particular, powerful linear regression equations for monoenes, C18 FAs and LC-PUFAs have been reported in gilthead sea bream for a given class of fish size (Benedito-Palos *et al*. 2011). This is not surprising because marine fish have a very limited capacity to elongate and desaturate C18 FAs into long chain C20 and C22 PUFAs, which enables the predictive modelling of FA composition year-round and throughout the life-cycle

when the lipid fillet content was used as a second independent variable in multi-linear regression models (Ballester-Lozano *et al*. 2011). Nevertheless, the nutritional tailoring of fish FA composition is still in its infancy and the aim of the present study is to develop and validate an integrative tool based on dummy regression analysis for predictive modelling of the fillet FA composition of turbot (*Scophthalmus maximus* L.) and sole (*Solea solea* L.), using gilthead sea bream as the reference subgroup category. Since turbot and sole are in the list of "new fish species" for European and Spanish aquaculture, the practical implications of the study are obvious, helping to face the nutritional human recommendations in LC-PUFA and the concomitant policies advising the sustainable utilisation of marine resources as fish feed ingredients.

Materials and methods

Fish rearing and sampling

Juveniles of turbot and sole were grown-out from fingerlings to harvest size in two fish farms of the Stolt Sea Farm in the Northwest of Spain (Galicia). Over the entire production cycle (turbot, 24 months; sole, 20 months), fish were fed with commercial diets from two different feed producers (Skreeting, Stavanger, Norway; Biomar, Aarhus, Denmark). Lipid content (20-22%) and FA composition of commercial diets was checked regularly and were maintained at almost constant levels with a high content of n-3 LC-PUFAs (26-28% FAMEs) regardless of the origin and pellet size (Table 1).

Table 1

For a given fish farm and fish species subgroup, 8-10 individuals were randomly selected and sampled for fillet lipid content and FA composition analyses at different stages of the production cycle of turbot (300 g, 500 g, 1 kg, 2.5 kg) and sole (100 g, 300 g, 500 g). As a general rule, fish were weighed under intensive anaesthesia (3-aminobenzoic acid ethyl ester, MS 222; 100 μg mL^{-1}), killed by a cut on the head and deboned fillets from dorsal-eyed side were vacuum packed in plastic bags and stored at −80°C until analysis.

All procedures were carried out according to national and institutional regulations (Consejo Superior de Investigaciones Científicas, IATS Review Board) and the current European Union legislation on handling experimental animals.

Lipid composition analyses

Lipid content in freeze-dried fillet samples (0.5 g) was determined gravimetrically using the Soxhlet 4001046 Auto extraction apparatus (Selecta, Barcelona, Spain) with 50 mL diethyl ether at 120ºC as extracting solvent.

Total lipids (TL) for analyses of fillet FA composition were extracted in freeze-dried samples by the method of Folch *et al*. (1957), using chloroform-methanol (2:1, v v^{-1}) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant. After the addition of nonadecanoic FA (19:0) as internal standard, TL were subjected to acid-catalysed transmethylation for 16 h at 50°C using 1 mL toluene and 2 mL of 1% (v v^{-1}) sulphuric acid in methanol (Christie 1982). FA methyl esters (FAME) were extracted with hexane:diethyl ether (1:1) and purified by thin layer chromatography (Silica gel G 60, 20 × 20 cm glass plates, Merck, Darmstadt, Germany), using hexane:diethyl-ether:acetic acid (85:15:1.5) as a solvent system. FAMEs were then analysed with a Fisons Instruments GC 8000 Series (Rodano, Italy) gas chromatograph as described elsewhere (Ballester-Lozano *et al*., 2011). Individual FAMEs were identified by comparison with well characterised sardine oil (Marinol, Fishing Industry Research Institute, Rosebank, South Africa) and the FAME 37 mix from Supelco (Bellefonte, PA, USA). BHT and internal standards (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.

Regression equations

Our own published data (De Francesco *et al*. 2007; Benedito-Palos *et al*. 2009; Ballester-Lozano *et al*. 2011), along with the data derived from the present study, were compiled in an FA repository database hosted at www.nutrigroup-iats.org/aquafat. Gilthead sea bream was used as the reference subgroup category with more than 100 independent entries derived from the large replacement of dietary fish meal and fish oil with plant ingredients. Data from turbot and sole were restricted to currently commercial formulations, leading a complete fish dataset (gilthead sea bream, turbot, sole) with more than 150 entries that were fitted to multiple regression equations:

$$Y'_i = \beta_0 + \beta_1 X_{i1} + \beta_2 X^{-1}_{i2} + [\beta_3 X_{i3} + \beta_4 X_{i4}] + [\beta_5 X_{i1} X_{i3} + \beta_6 X_{i1} X_{i4}] + [\beta_7 X^{-1}_{i2} X_{i3} + \beta_8 X^{-1}_{i2} X_{i4}]$$

Where Y'_i = forecasted fillet FA in mg g^{-1} lipid; β_0 = Y axis intercept; $\beta_{1...8}$ = regression coefficients; X_{i1} = dietary FA composition (mg g^{-1} lipid); X^{-1}_{i2} = inverse of fillet lipid content (% wet matter); X_{i3} = 1 if turbot, 0 otherwise; X_{i4} = 1 if sole, 0 otherwise; $X_{i1}X_{i3}$ = interaction effect between X_{i1} and X_{i3}; $X_{i1}X_{i4}$ = interaction effect between X_{i1} and X_{i4}; $X^{-1}_{i2}X_{i3}$ = interaction effect between X^{-1}_{i2} and X_{i3}; $X^{-1}_{i2}X_{i4}$ = interaction effect between X^{-1}_{i2} and X_{i4}. The $\beta_3 X_{i3}$ and $\beta_4 X_{i4}$ terms are written inside brackets in order to stress that they correspond to dummy variables; the same applies to the interaction terms of dummy variables and independent variables ($[\beta_5 X_{i1} X_{i3} + \beta_6 X_{i1} X_{i4}]$, $[\beta_7 X^{-1}_{i2} X_{i3} + \beta_8 X^{-1}_{i2} X_{i4}]$), which were analysed as blocks with a given statistical significance for each of them.

Statistical analysis

Data on fillet lipid content and FA composition were checked for normal distribution and homogeneity of variances, and arcsin transformation was performed when necessary. Means were then compared by one-way ANOVA followed by Student-Newman-Keuls (SNK) test at a significance level of 5%. Parameters of regression equations were estimated by the least squares method. The absence of inter-correlation between independent variables was checked. Residuals from regression equations were checked for normal distribution by the Shapiro-Wilk test, and its dispersion was visualised by plotting them against the corresponding predictions of fillet lipid content. Additional sets of samples (8 fish from each fish species) were used to validate the multi-specific FA descriptors, and deviations from the model were analysed using a statistical t-test to determine if the predicted FA values (results from the regression equations) were statistically distinguishable from the observed values at the significance level of 5%. All analyses and graphs were conducted using the SPSS (version 20) and SigmaPlot (version 12.0) software packages.

Results

As a general rule, the fillet lipid content of turbot increased with the increase in body weight, ranging from 3-4% (wet weight basis) in 300 g fish to 7-9% in 2.5 kg fish (Table 2). Likewise, fillet lipid content increased in sole from 4-6% to 6.5-7.5% in fish of 100 g and 500 g average body weight, respectively (Table 3). However, the latter

trend was not statistically significant ($P > 0.05$) due to the relatively high individual variability of sole under farming conditions.

Table 2 and Table 3

Regarding the fillet FA composition of turbot, saturated FAs remained unchanged with the increase in fish size and fillet lipid content. In contrast, the parallel rise in fish size and fillet lipid content was highly associated with the increase of monoenes, mostly represented by oleic acid (OA, 18:1n-9), whereas an opposite trend was found for n-3 LC-PUFAs, calculated as the sum of eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Table 2). In sole, the fillet FA composition remained mostly unchanged in the three classes of fish size, although saturated FAs (16:0, 18:0) decreased significantly in larger animals, showing monoenes (20:1n-9, 22:1n-11) and linolenic acid (LNA, 18:3n-3) an opposite trend (Table 3). Moreover, when comparing sole and turbot, it is noteworthy that the two species shared a different EPA/DHA ratio that was markedly higher ($P < 0.001$) in turbot (1.12) in comparison to sole (0.67), irrespective of fish size and fillet lipid content.

Table 4

As shown in Table 4, FA descriptors for the most relevant FAs (> 0.2% FAME) considered in the dummy regression analysis fit well to empirical equations with statistically significant coefficients of correlation ($P < 0.0001$), which varied between 0.76 for 18:1n-7 and 0.99 for LNA. Since almost all β_1 and β_2 partial regression coefficients were significant at the 5% level, the two independent variables highly contributed to explain the total variance, although it was apparently more consistent for the diet FA composition variable. Dummy variables illustrated the FA differences from the reference species in absolute values. These variables also contributed significantly to the explanation of the variance of all FAs considered in the model in at least one of the two flat fish species.

Regarding interaction effects, there were no statistically significant interactions between diet composition and dummy variables detected for any FA descriptor. This implies that the relationship between diet and fillet FA composition is of the same order for all fish species subgroups; therefore, the corresponding blocks of interactions were not included in the regression equations. Nevertheless, significant interactions between fillet lipid content and fish species subgroup were found for saturated FAs (14:0, 16:0, 18:0) and monoenes (OA, 16:1n-7, 18:1n-7, 20:1n-9); this block of interaction is included in the regression equation. This was not the case for the n-6 (18:2n-6, 20:4n-6) and n-3 (LNA, EPA, DPA, DHA) series, and again the interaction blocks were not included in the regression equations due to their poor contribution to the total variance.

Figure 1 and Figure 2

The scatter plots of residuals (observed–predicted values, Y-axis) against fillet lipid content (X-axis) are shown in Fig.1 and Fig. 2 for saturated, monoenes and PUFAs. The scale of residuals represents 100% of model deviations for a given FA descriptor with a homogenous distribution for plotted values regardless of the fish species subgroup and FA descriptor; this strengthens the robustness of categorical regression equations. When extra data from the three fish species were compiled to validate the proposed dummy regression equations, the predicted values for fillet FA composition did not differ significantly from the observed values. Thus, a close linear association near to equality can be seen in the scatter plot of observed (X-axis) and predicted (Y-axis) values of fillet FA composition, regardless of the fish species subgroup (Fig. 3).

Figure 3

Based on the predictive FA descriptors, the amount of n-3 LC-PUFAs per 150 g ration portion is theoretically of the same order of magnitude in turbot (2.5 kg), sole (500 g) and gilthead sea bream (500 g) marketable fish. The calculated amounts of monoenes and saturated FAs are higher in gilthead sea bream, which also showed a

lower saturated/monoenes ratio (Fig. 4). The calculations were made with a theoretical diet formulation that mimics the FA composition of the current commercial gilthead sea bream diets, which contains 4.1% saturated FAs, 3.8% monoenes and 1.8% n-3 LC-PUFA on a dry matter basis.

Figure 4

Discussion

Dummy regression analyses have been widely used in econometrics and social sciences (Kutner *et al*. 2004), and are now being seen as highly informative tools for the predictive modelling of productive and physiological traits in biological sciences. For instance, dummy variables have been used to underline the complex effects of dietary FAs on the kinetics of gamma-glutamyltranspeptidase in the epididymis tissue of mice (Basso *et al*. 2006). This approach has also been used to characterise the spatial and inter-annual trends of mercury accumulation in sport fish (Melwani *et al*. 2009). Likewise, we used herein a categorical regression approach to predict the fillet FA composition of two flat fish species of a high value for European and Spanish aquaculture in particular, using our own published data in gilthead sea bream as a reference subgroup category.

As suspected, the relative contribution of diet FA composition and fillet lipid content to the total variance of fillet FA composition varied within and among FAs and fish species. Otherwise, the regression model assumes that the contribution of independent variables to the total variance is best estimated by pooling all data from the different species subgroups when statistically significant interactions between independent and dummy variables are not found. This explains, at least in part, why the

coefficients of correlation for FA were higher overall in the present study than in the previously published work of Ballester-Lozano *et al*. (2011), who only considered data from gilthead sea bream in a bivariate regression model, with diet composition and fillet lipid content as independent variables. This is true for a wide range of FAs, including stearic acid (0.79 *vs*. 0.43), OA (0.97 *vs*. 0.89), arachidonic acid (0.97 *vs*. 0.68), LNA (0.99 *vs*.0.88), DPA (0.98 *vs*. 0.67) and DHA (0.98 *vs*.0.86), which, in turn, substantiates a higher power of prediction with each dataset update.

Unlike dietary FA composition, the contribution of the variable fillet lipid content to the total variance is highly dependent on fish species, and interactions on blocks were statistically significant for almost all of the descriptors of monoenes and saturated FAs, but not for those of LC-PUFAs. The physiological significance of this finding can be more complex than initially envisaged, although it is probably prone to fish species' differences in lipogenic enzymes, such as stearoyl-CoA desaturases, which are conserved in tetrapods and fish as five different paralogous genes (SCD1–SCD5) (Castro *et al*. 2011). The precise regulation of the SCD genes has been poorly studied in fish, but experimental evidence supports the role of two SCD1 isoforms as strong lipogenic markers in the skeletal muscle of gilthead sea bream (Benedito-Palos *et al*. in press). These SCD1 isoforms are probably conserved in flat fish species and, these genes or closely related paralogous genes, might contribute to explain the already identified differences in muscle lipid deposition rates between gilthead sea bream, and turbot and sole, which were initially considered semi-fat and lean fish species, respectively.

As a general statement, marine fish have a limited capability for LC-PUFA biosynthesis due to deficiencies in the PUFA desaturation/elongation pathway. For instance, Δ6 desaturase activity has been found in a wide range of marine fish species

(Zheng *et al*. 2004), but the lack of a Δ5 desaturase activity contributes to explain the inability of gilthead bream (Tocher & Ghioni 1999) and some flat fish species (Ghioni *et al*. 1999) to produce DHA from C18 FAs of n-3 and n-6 series. This metabolic feature means that the predictive modelling of fillet FA composition from the diet becomes theoretically easier in marine fish rather than in salmonids and typically freshwater fish. Regardless, it is noteworthy that the proposed FA descriptors were able to predict different EPA, DPA and DHA ratios for edible matter of the three fish species considered in the study. The precise mechanisms remain to be determined, but it is noticeable that a nutritionally-regulated FA acyl desaturase with Δ4 activity has been reported in sole (Morais *et al*. 2012). This enzyme activity has only been found in another vertebrate, the marine fish *Siganus canaliculatus* (Li *et al*. 2010), which suggests that the synthesis of DHA from EPA in some fish can be conducted through the direct Δ4 desaturation of DPA as an alternative to the "Sprecher pathway", which involves FA elongation, Δ6 desaturation and peroxisomal β-oxidation steps (Sprecher 2000).

The robustness of the regression equations was validated with data from other production cycles, which closely confirmed the predictions made for a wide range of FA descriptors. This agrees with the scatter plot analysis of residuals where a continuous (homogenous) distribution of plotted values along the X-axis was found regardless of the species subgroup. However, scaling up the predictive model to other fish species and/or experimental conditions is difficult, since precise information on fillet lipid content and FA composition is not always available, as highlighted by the recent literature overview of more than 390 scientific publications (Karakatsouli 2012). This is not the case for the data used in the present study and it can be predicted that the fillet content in n-3 LC-PUFA is of the same order of magnitude at a marketable size of 2.5

306 kg for turbot and 500 g for sole and gilthead sea bream. In addition, on the basis of the
307 proposed FA equations, the weekly consumption of two fillet ration portions (150 g
308 each) of either gilthead sea bream or turbot and sole fed currently gilthead sea bream
309 diets fulfils the human nutritional recommendations for n-3 LC-PUFA (EFSA 2010) (3
310 g $week^{-1}$). However, more emphasis is needed in other FAs when the nutritional value
311 of fish products is considered as a whole (Larsen *et al*. 2011). Interestingly, the
312 proposed FA descriptors are highly informative to assess the fish species differences in
313 monoenes and saturated FAs levels and their concomitant ratios.
314 In summary, dummy regression analysis is becoming a powerful multi-species
315 tool for predictive modelling of the nutritionally-tailored fillet FA composition of
316 marine farmed fish when the subgroup category covers a wide range of experimental
317 variance. This skill becomes especially evident in the absence of statistically significant
318 interactions between dietary FA composition and fish species subgroups, which
319 reinforces the possibility of producing more tailored and healthy fish products for
320 human consumers. For the practical use of a wide audience (fish farmers, fish feed
321 producers and public in general), FA algorithms for the predictive modelling of turbot,
322 sole and gilthead sea bream fillet FA composition are hosted in an interactive database
323 www.nutrigroup-iats.org/aquafat. Further updates with data from these or other fish
324 species will offer the possibility of interrogating the composition of new seafood
325 products, as well as improving the strength of the predictions made for the species
326 already considered in the model.
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Table 1. Fatty acid composition of commercial diets (% total fatty acid methyl esters). Values are the mean and standard deviation of pellets of graded size.

[1] Skretting diet (turbot, Couso fish farm).

	Diet A[1]	Diet B[2]	Diet C[3]
Moisture (%)	6.81 ± 1.23	6.94 ± 1.17	6.64 ± 1.28
Total lipids (%)	21.9 ± 3.69	20.6 ± 1.90	20.2 ± 0.66
∑ FA (mg g^{-1} lipid)	645.7 ± 41.74	655.3 ± 65.06	648.4 ± 50.89
FA (% FAME)			
14:0	6.5 ± 0.44	6.6 ± 0.28	6.6 ± 0.27
16:0	18.5 ± 0.72	18.6 ± 0.57	18.5 ± 0.47
18:0	3.6 ± 0.13	3.8 ± 0.11	3.7 ± 0.13
SFA[4]	29.7 ± 1.08	30.0 ± 0.81	29.7 ± 0.80
16:1n-7	7.7 ± 0.51	7.7 ± 0.39	7.7 ± 0.23
18:1n-7	3.0 ± 0.13	3.0 ± 0.15	3.0 ± 0.13
18:1n-9	10.1 ± 1.52	11.0 ± 0.59	11.2 ± 1.28
20:1n-9	1.0 ± 0.28	0.7 ± 0.13	0.9 ± 0.13
22:1n-11	0.4 ± 0.18	0.3 ± 0.13	0.5 ± 0.16
MUFA[5]	22.7 ± 1.18	23.1 ± 0.42	23.8 ± 1.48
18:2n-6	5.2 ± 1.32	4.9 ± 0.81	5.4 ± 1.44
20:2n-6	0.2 ± 0.03	0.1 ± 0.01	0.2 ± 0.06
20:3n-6	0.1 ± 0.05	0.1 ± 0.04	0.1 ± 0.05
20:4n-6	1.0 ± 0.08	0.9 ± 0.04	0.9 ± 0.03
18:3n-3	0.9 ± 0.22	1.0 ± 0.16	1.0 ± 0.24
18:4n-3	2.2 ± 0.21	2.1 ± 0.16	2.0 ± 0.22
20:5n-3	15.5 ± 1.69	15.5 ± 0.99	15.1 ± 0.83
22:5n-3	1.9 ± 0.09	1.9 ± 0.16	1.9 ± 0.13
22:6n-3	10.6 ± 0.96	9.9 ± 0.88	9.5 ± 1.06
n-3 LC-PUFA	28.0 ± 1.21	27.3 ± 0.95	26.4 ± 1.52

Pellet size (mm): 2, 3, 5, 7, 10, 13, 15, 17, 22.

[2] Biomar diet (turbot, Quilmas fish farm).

Pellet size (mm): 1.9, 3, 4.5, 6.5, 9.

[3] Biomar diet (sole, Quilmas fish farm).

Pellet size (mm): 1.5, 1.9, 3, 4.5.

[4] Includes 15:0, 17:0, 20:0 and 22:0.

[5] Includes 20:1n-7 and 22:1n-9.

Table 2. Body weight, fillet lipid content (wet weight basis) and fillet fatty acid profile (% total fatty acid methyl esters) of turbot grow-out in two different fish farms. Mean values and standard deviation of individual fish are presented ($n = 8$). Mean values within each fish farm with unlike superscript letters are significantly different ($P < 0.05$).

		Diet A			Diet B
Body weight (g)	282.6 ± 21.15^{a}	953.3 ± 98.64^{b}	2592.5 ± 145.48^{c}	298.5 ± 15.15^{a}	494.2 ± 20.71^{b}
Fillet lipids (%)	3.6 ± 0.93^{a}	5.3 ± 0.84^{b}	7.9 ± 1.47^{c}	4.6 ± 1.07	4.8 ± 1.00
∑ FA (mg g^{-1} lipid)	694.2 ± 10.16^{a}	725.3 ± 27.09^{b}	672.8 ± 24.65^{a}	715.89 ± 21.84	709.7 ± 17.16
FA (% FAME)					
14:0	5.4 ± 0.33	5.6 ± 0.41	5.6 ± 0.17	5.4 ± 0.54	5.5 ± 0.29
16:0	14.9 ± 0.55	14.9 ± 0.68	15.1 ± 0.15	14.1 ± 1.26	14.3 ± 0.45
18:0	2.6 ± 0.19	2.4 ± 0.38	2.5 ± 0.05	2.4 ± 0.56	2.4 ± 0.21
SFA[1]	23.6 ± 0.86	23.6 ± 1.00	23.8 ± 0.18	22.5 ± 1.31	22.8 ± 0.35
16:1n-7	7.5 ± 0.35^{a}	8.0 ± 0.48^{b}	7.8 ± 0.09^{ab}	7.9 ± 0.93	8.1 ± 0.27
18:1n-7	3.3 ± 0.06^{a}	3.4 ± 0.06^{b}	3.4 ± 0.04^{b}	3.3 ± 0.09^{a}	3.4 ± 0.10^{b}
18:1n-9	11.1 ± 0.69^{a}	11.6 ± 0.52^{a}	12.4 ± 0.33^{b}	11.6 ± 0.52	11.5 ± 0.36
20:1n-9	0.9 ± 0.13	0.9 ± 0.07	0.9 ± 0.05	0.9 ± 0.13	0.9 ± 0.08
22:1n-11	0.2 ± 0.09	0.2 ± 0.07	0.2 ± 0.06	0.4 ± 0.11	0.4 ± 0.11
MUFA[2]	23.4 ± 0.96^{a}	24.4 ± 1.00^{a}	25.2 ± 0.44^{b}	24.5 ± 1.63	24.7 ± 0.54
18:2n-6	7.4 ± 0.88^{a}	6.5 ± 0.33^{b}	6.7 ± 0.30^{ab}	4.7 ± 0.53	4.9 ± 0.47
20:2n-6	0.4 ± 0.05^{a}	0.4 ± 0.05^{a}	0.5 ± 0.04^{b}	0.2 ± 0.03	0.2 ± 0.02
20:3n-6	0.2 ± 0.03	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.01
20:4n-6	1.2 ± 0.14^{a}	1.1 ± 0.25^{a}	1.0 ± 0.04^{b}	1.3 ± 0.30	1.1 ± 0.07
18:3n-3	0.8 ± 0.07^{a}	0.9 ± 0.10^{a}	1.0 ± 0.04^{b}	0.9 ± 0.12	0.9 ± 0.06
18:4n-3	1.8 ± 0.22	2.0 ± 0.24	1.8 ± 0.09	2.2 ± 0.33	2.2 ± 0.13
20:5n-3	12.9 ± 0.35	13.8 ± 0.70	13.7 ± 0.47	15.4 ± 1.21	15.5 ± 0.37
22:5n-3	4.2 ± 0.29^{a}	4.2 ± 0.28^{a}	4.5 ± 0.17^{b}	3.9 ± 0.39	3.9 ± 0.25
22:6n-3	14.8 ± 1.04^{a}	12.8 ± 1.51^{ab}	11.7 ± 0.34^{b}	13.6 ± 2.63	12.8 ± 0.72
n-3 LC-PUFA	31.5 ± 1.21^{a}	30.9 ± 0.70^{a}	30.0 ± 0.57^{b}	32.9 ± 1.95	32.2 ± 0.53

[1] Includes 15:0, 17:0, 20:0 and 22:0
[2] Includes 20:1n-7 and 22:1n-9

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Table 3. Body weight, fillet lipid content (wet weight basis) and fillet fatty acid profile (% total fatty acid methyl esters) of sole. Mean values and standard deviation of individual fish are presented ($n = 8$). Mean values with unlike superscript letters are significantly different ($P < 0.05$).

	Diet C		
Body weight (g)	100.5 ± 12.72^{a}	325.6 ± 21.54^{b}	512.0 ± 31.11^{c}
Fillet lipids (%)	5.7 ± 1.90	6.6 ± 1.21	7.3 ± 1.64
∑ FA (mg g^{-1} lipid)	726.0 ± 32.49	740.8 ± 20.75	752.4 ± 28.07
FA (% FAME)			
14:0	5.8 ± 0.28	5.7 ± 0.15	5.7 ± 0.31
16:0	17.9 ± 0.36^{a}	17.2 ± 0.51^{b}	16.7 ± 0.61^{c}
18:0	3.2 ± 0.29^{a}	3.0 ± 0.29^{ab}	2.8 ± 0.09^{b}
SFA[1]	27.7 ± 0.53^{a}	26.7 ± 0.64^{b}	25.8 ± 0.88^{c}
16:1n-7	8.4 ± 0.54	8.6 ± 0.32	8.8 ± 0.18
18:1n-7	3.4 ± 0.13	3.4 ± 0.20	3.5 ± 0.22
18:1n-9	12.4 ± 0.85	12.7 ± 0.71	12.9 ± 0.66
20:1n-9	0.7 ± 0.08^{a}	0.8 ± 0.04^{b}	0.8 ± 0.05^{ab}
22:1n-11	0.3 ± 0.08^{a}	0.4 ± 0.05^{b}	0.4 ± 0.05^{ab}
MUFA[2]	25.5 ± 1.63	26.3 ± 1.16	26.8 ± 1.10
18:2n-6	4.3 ± 0.24^{a}	4.5 ± 0.20^{ab}	4.6 ± 0.23^{b}
20:2n-6	0.1 ± 0.01^{a}	0.1 ± 0.01^{b}	0.1 ± 0.01^{b}
20:3n-6	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01
20:4n-6	0.9 ± 0.10	0.9 ± 0.03	0.9 ± 0.05
18:3n-3	0.8 ± 0.04^{a}	0.9 ± 0.04^{b}	0.9 ± 0.04^{b}
18:4n-3	1.8 ± 0.07	1.8 ± 0.16	1.8 ± 0.11
20:5n-3	8.4 ± 0.63	8.0 ± 0.92	7.8 ± 0.75
22:5n-3	8.6 ± 0.38	8.7 ± 0.55	9.0 ± 0.51
22:6n-3	12.1 ± 1.02	12.3 ± 1.02	12.2 ± 0.90
n-3 LC-PUFA	29.0 ± 1.80	29.1 ± 1.36	29.1 ± 1.15

[1] Includes 15:0, 17:0, 20:0 and 22:0

[2] Includes 20:1n-7 and 22:1n-9

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Table 4. Correlation (r^2) and regression (β_1- β_6) coefficients of dummy regression equations with dietary fatty acid composition (mg g^{-1} lipid) and inverse of fillet lipid content (%) as independent variables.

FA	r^2	β_0	P	β_1	P	β_2	P	β_3	P	β_4	P	β_5	P	β_6	P
14:0	0.931	9.44	<0.001	0.70	<0.001	-45.44	<0.001	3.12	0.081	12.60	<0.001	28.22	0.011	-11.63	0.423
16:0	0.777	57.35	<0.001	0.63	<0.001	-143.65	<0.001	-28.26	<0.001	9.05	0.168	131.64	<0.001	60.00	0.166
18:0	0.794	11.24	<0.001	0.64	<0.001	-15.26	0.018	-10.31	<0.001	-7.15	<0.001	19.54	0.019	32.95	0.003
SFA	0.789	77.13	<0.001	0.65	<0.001	-202.64	<0.001	-34.65	<0.001	15.32	0.930	177.71	<0.001	79.61	0.185
16:1n-7	0.939	15.12	<0.001	1.04	<0.001	-66.12	<0.001	-6.95	0.002	13.53	<0.001	44.46	0.001	-36.60	0.032
18:1n-7	0.765	18.98	<0.001	0.30	<0.001	-32.67	<0.001	-0.23	0.848	4.53	0.003	20.08	<0.001	7.52	0.780
18:1n-9	0.969	93.78	<0.001	0.72	<0.001	-290.16	<0.001	-48.40	<0.001	-29.62	<0.001	229.01	<0.001	150.88	0.001
20:1n-9	0.954	1.45	<0.001	0.94	<0.001	-5.48	0.046	-0.67	0.290	0.195	0.807	6.10	0.124	-6.94	0.185
MUFA	0.887	184.59	<0.001	0.50	<0.001	-472.18	<0.001	-71.11	<0.001	-24.87	0.018	386.86	<0.001	192.62	0.004
18:2n-6	0.979	8.53	0.004	0.89	<0.001	-30.51	0.017	10.25	0.001	-0.10	0.973				
20:4n-6	0.972	0.25	0.129	0.83	<0.001	4.05	0.004	1.21	<0.001	0.48	0.198				
n-6 PUFA	0.977	8.68	0.004	0.89	<0.001	-25.12	0.049	11.40	<0.001	0.14	0.959				
18:3n-3	0.999	1.43	<0.001	0.78	<0.001	-8.60	<0.001	2.00	<0.001	1.60	<0.001				
20:5n-3	0.940	3.26	0.306	0.68	<0.001	-23.20	0.077	33.01	<0.001	-7.18	0.004				
22:5n-3	0.980	7.04	<0.001	2.06	<0.001	-14.85	0.005	-0.13	0.899	34.85	<0.001				
22:6n-3	0.978	-4.90	0.003	1.11	<0.001	93.67	<0.001	1.88	0.374	10.34	<0.001				
n-3 LC-PUFA	0.983	3.52	0.374	0.87	<0.001	90.71	<0.001	39.98	<0.001	42.88	<0.001				

Statistically significant contribution in analysis on blocks is not detected ($P < 0.05$) for n-3 and n-6 PUFAs, and the corresponding interactions coefficients are not reported.

Figure legends

Fig. 1. Scatter plots of residuals against fillet lipid content values (wet weight basis) for monoenes and saturated fatty acids (mg g^{-1} lipid). Sole (black circles), turbot (grey circles), gilthead sea bream (white circles).

Fig. 2. Scatter plots of residuals against fillet lipid content values (wet weight basis) for representative polyunsaturated fatty acids (mg g^{-1} lipid). Sole (black circles), turbot (grey circles), gilthead sea bream (white circles).

Fig. 3. Plot prediction of the fillet FA profile (mg g^{-1} lipid) for extra data not included in the construction of the model. Data values are the mean and standard deviation of 8 fish per each fish species. Sole (black circles), turbot (grey circles), gilthead sea bream (white circles). The identity of each fatty acid is not reported to simplify the figure plot.

Fig. 4. Predicted fillet fatty acid composition of marketable sole, turbot and gilthead sea bream according to dummy regression analysis. The calculations for the three species are made on the basis of a relative low fish oil that mimics the currently composition of gilthead sea bream diets. Body weight (BW), saturated fatty acids (SAT), monoenes (MUFA), long chain polyunsaturated fatty acids (LC-PUFA).

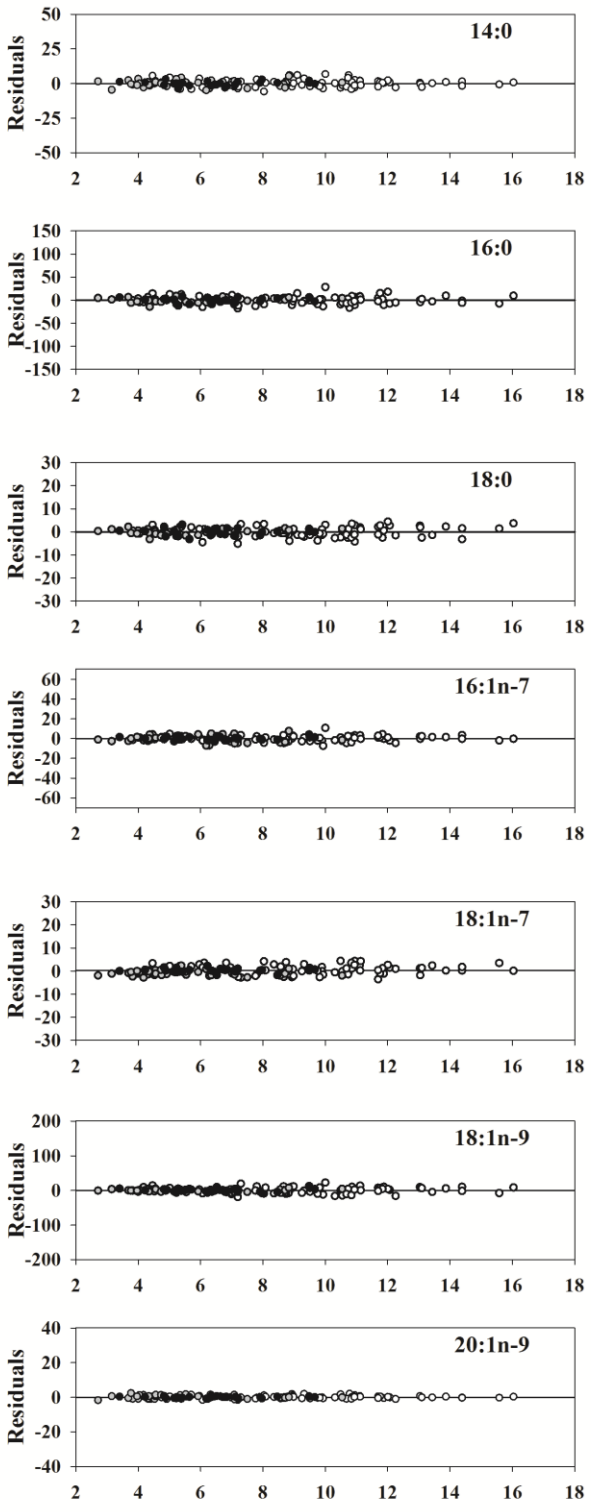

Fig. 1.
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Dummy regression analysis for modelling the nutritionally tailored fillet fatty acid composition of turbot and sole using gilthead sea bream as a reference subgroup category.

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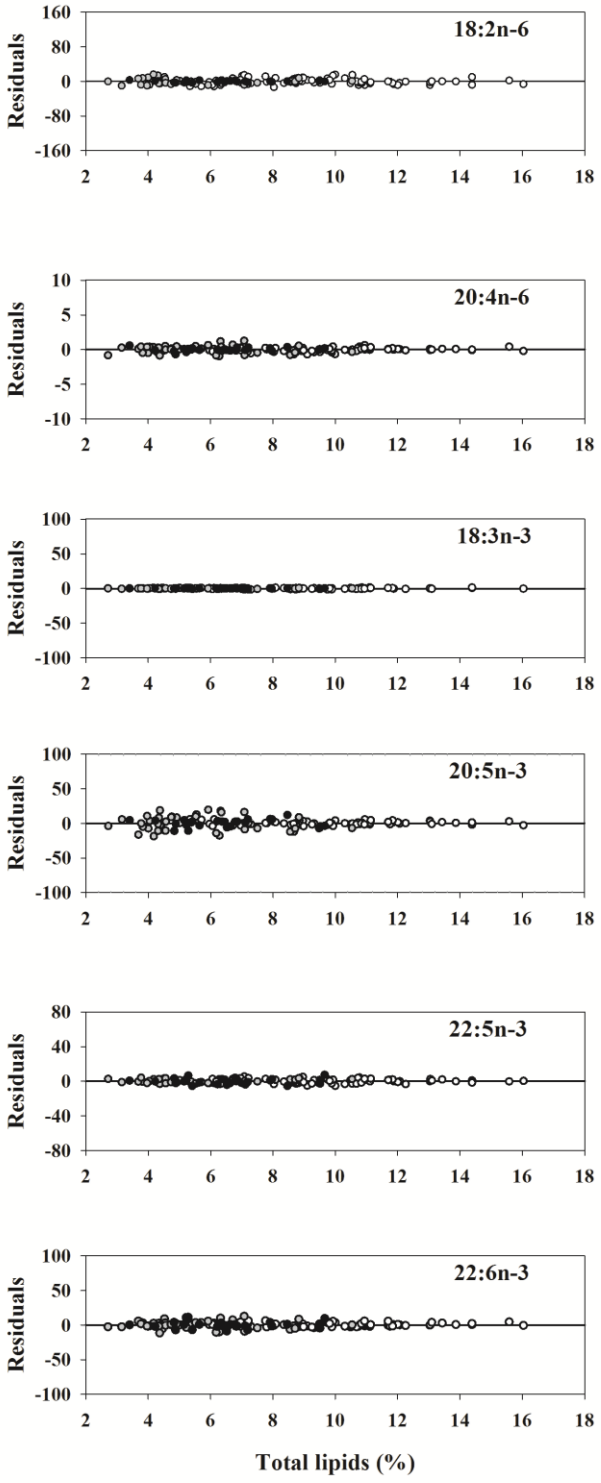

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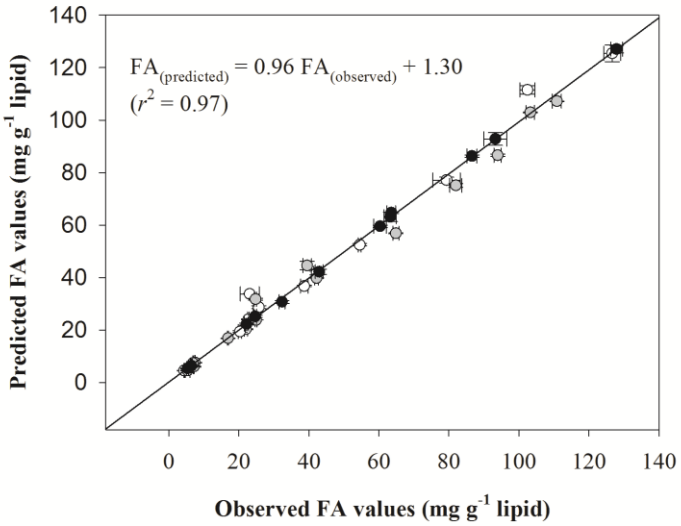

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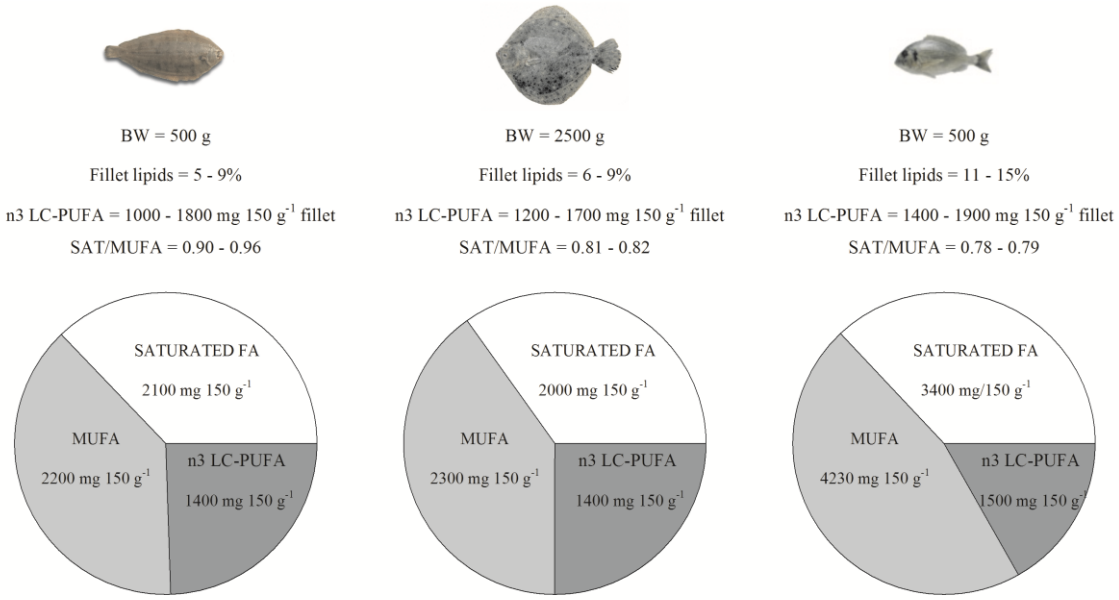

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