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Prediction of fillet fatty acid composition of market-size gilthead sea bream (*Sparus aurata*) using a regression modelling approach

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ABSTRACT

Gilthead sea bream (*Sparus aurata*) were fed in triplicate groups with a commercial standard diet from the juvenile stage to male–female sex reversal under natural day-length and temperature conditions. Every 3–4 months during the two-year production cycle, 9 fish were randomly selected and sampled for flesh composition analyses of total lipid levels and fatty acid (FA) composition. Curvilinear regressions fitting total lipid levels and % FAs in total lipids were made to underline the differential distribution of a given fillet FA within neutral and polar lipid fractions. This dataset along with published results on market-size fish were combined for multilinear regression approaches, with the aim of describing strong relationships ($P<0.0001$) between fillet FA composition and two independent variables: dietary FA composition and fillet lipid level. For saturated (14:0, 16:0, 18:0) and monounsaturated (16:1n-7, 18:1n-7, 18:1n-9, 20:1n-9) FAs, the overall variance in fillet FA composition is primarily explained by dietary FA composition and secondly by fillet lipid level. This second independent variable also contributes to explain the variations observed in arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3), but a statistically significant contribution is not found for linoleic acid (18:2n-6), linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3) and docosapentaenoic acid (22:5n-3). The consistency of these predictive equations in our particular rearing conditions was proved by means of a test validation trial, using fish fed an experimental diet based on plant proteins and fish oil.

1. Introduction

Dietary fatty acids (FA) in fish and terrestrial monogastrics are absorbed unchanged with highly predictable effects on meat FA composition (Chesworth et al., 1998; Kouba and Mourot, 2011). However, factors other than diet (e.g., genotype, gender, age and production system) have a significant influence on the fillet lipid level and thus on the FA composition of most animal products (Wood et al., 2008). In particular, the association between dietary and fillet FA composition is likely to be stronger in oily fish than in lean fish (Turchini et al., 2009). In addition, close associations between dietary and fillet FA composition are more likely to be produced with non-endogenously synthesised FAs. This is especially true for marine fish due to their limited ability to convert C18 FAs into long chain polyunsaturated FAs (LC-PUFAs) of n-6 and n-3 series (Sargent et al., 2002; Tocher, 2003).

Regarding gilthead sea bream (*Sparus aurata*), earlier studies have shown that the muscle tissue is especially sensitive to changes in dietary FA composition (Benedito-Palos et al., 2010). Thus, fillets of gilthead sea bream fed diets rich in plant oils show increased levels of linoleic acid (LA, 18:2n-6) and linolenic acid (LNA, 18:3n-3) with a concurrent decrease of eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acids (DHA, 22:6n-3), consistent with shifts in diet composition (Benedito-Palos et al., 2008; Izquierdo et al., 2005). The restoration of the fillet FA profile with a fish oil finishing diet follows a simple dilution process over the course of the summer growth spurt (Benedito-Palos et al., 2009). Also, linear regression equations derived from asynchronous studies closely relate dietary and fillet FA composition in one-year-old fish (Benedito-Palos et al., 2011). However, the extent to which such predictive equations are affected among other factors by season, fish size or reproductive status remains to be investigated in a protandric fish such as gilthead sea bream. Thus, the aim of the present study was to use multilinear regression approaches to check if dietary FA composition and fillet lipid levels effectively contribute to explain fillet FA composition from early juvenile stages to male–female sex reversal. If the model fits well, the regression equations might be extremely useful for modelling flesh FA composition, though they are specific to the particular conditions under which the data are obtained. Thus, in order to improve the predictive value of this empirical approach, regression equations were constructed with a complete dataset made with time-series data from a

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two-year production cycle along with our own published results on market-size fish (Benedito-Palos et al., 2009; De Francesco et al., 2007).

2. Material and methods

2.1. Experimental setup

Juvenile gilthead sea bream of Atlantic origin (Ferme Marine de Douhet, Ile d'Oléron, France) were acclimatised to laboratory conditions at the Institute of Aquaculture Torre de la Sal (IATS) for 20 days before the start of the growth study. Two hundred and ten fish of 17 g initial mean body weight were grown-out until 1 kg body weight in triplicate 500–3000 l fibreglass tanks at a maximum rearing density of 15 kg/m^3. Water flow (37‰ salinity) was 10–30 l/min, oxygen concentration remained higher than 85% saturation and unionised ammonia was below toxic levels (<0.02 mg/l). The growth trial was undertaken over 27 months from May 2008 to July 2010, and day-length and water temperature varied over the course of the study following the natural changes at IATS latitude (40º5′N; 0º10′E) with mortality less than 2%.

Fish were fed over the course of the study with extruded pellets (Excel, Skretting, Stavanger, Norway) of 3 consecutive sizes (2, 4, 6 mm), formulated to contain 47–48% protein and 20–21% lipids. Main ingredients were fish meal (35%), fish oil (7%), soybean meal (20%), corn gluten (11%), extruded peas (8%) and a blend of vegetable oils (60 soybean oil: 40 rapeseed oil) at the 7–8% inclusion level. The FA composition of diet is shown in Table 1 as the range of variation of the 3 feed batches corresponding to each pellet size.

Feed was offered by hand to visual satiety twice a day (9.00 and 14.00 h, 5–7 days per week) from May to September and once a day (12.00 h, 3–5 days per week) from October to May. Fish were counted and weighed every month under moderate anaesthesia (3-aminobenzoic acid ethyl ester, MS 222; 100 μg/ml). At regular intervals (3–4 months), 9 fish (3 per replicate) were randomly selected for fillet sampling. Fish were killed by a blow on the head and left side fillets without bones and skin were extracted, vacuum packed in plastic bags and stored at −80 °C until complete freeze drying (48 h at −55 °C) prior lipid analyses.

An additional feeding trial conducted at the IATS research experimental facilities from May 2008 to July 2009 was used for the test validation of predictive FA descriptors (multilinear regression equations). Triplicate groups of fish were fed with a practical diet based on plant proteins and fish oil (for details in diet composition see Benedito-Palos et al., 2007). The diet was manufactured by the Institut National de la Reserche Agronomique (INRA) at the experimental research station of Donzaq (Landes, France). At the end of trial, 12 fish (240–350 g) were randomly selected for fillet sampling and lipid composition analyses.

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.

2.2. 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 120 °C 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) 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) 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,

Table 1
Fillet lipid content (g/100 g fillet) and fatty acid composition (% fatty acid methyl esters) of gilthead sea bream grow-on a commercial diet. FA composition of diet is given as the range value of two technical replicates for each pellet size (2, 4 and 6 mm). Data on fillet FA composition are presented as mean and standard deviations of 8–9 individual fish samples. Statistically significant differences in fillet FA composition were found in all the analysed FAs in at least one sampling time (one-way ANOVA, P<0.001).

	Diet	July 08		November 08		March 09		July 09		November 09		March 10		July 10	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total lipids	20.0–20.4	6.5	1.15	7.0	1.88	6.2	1.19	8.8	1.60	10.0	1.24	7.5	1.62	10.7	2.24
Σ FAs (mg/g lipid)	631.0–760.1	645.2	62.39	685.6	47.49	616.4	73.23	690.6	70.93	669.2	18.00	672.4	91.65	715.0	67.05
FA (% FAME)															
14:0	3.8–4.6	3.3	0.09	3.5	0.11	3.6	0.62	3.2	0.23	3.1	0.11	2.9	0.18	3.1	0.09
16:0	14.0–16.7	16.0	0.29	15.9	0.42	15.5	1.53	14.3	1.37	14.9	0.46	13.2	0.68	14.4	0.31
18:0	3.3–3.8	4.4	0.23	3.7	0.14	3.8	0.42	3.2	0.32	3.4	0.10	3.3	0.20	3.2	0.16
SFA‡	22.0–26.3	24.5	0.47	23.8	0.57	23.6	2.56	21.4	1.87	22.0	0.52	20.0	0.85	21.3	0.36
16:1n-7	4.6–4.9	5.6	0.09	5.8	0.15	6.2	0.51	5.4	0.46	5.6	0.12	5.4	0.34	6.0	0.21
18:1n-7	2.7–3.2	2.6	0.07	2.9	0.06	2.8	0.16	3.2	0.29	3.0	0.05	2.9	0.06	3.2	0.13
18:1n-9	19.1–23.1	20.3	0.68	19.2	0.72	19.7	0.75	22.2	2.20	25.0	0.31	24.3	0.76	25.5	0.54
20:1n-9	0.7–1.2	1.3	0.02	0.8	0.04	0.7	0.01	0.8	0.07	0.7	0.03	0.7	0.04	0.7	0.03
22:1n-11	0.1–0.7	0.8	0.03	0.7	0.72	0.4	0.12	0.3	0.35	0.2	0.01	0.1	0.01	0.1	0.02
MUFA+	26.8–32.1	31.4	0.78	29.8	0.73	30.2	1.53	32.2	2.71	34.9	0.37	33.7	1.00	36.0	0.65
18:2n-6	20.0–22.7	18.7	0.20	21.5	0.37	22.4	0.42	22.4	4.98	18.6	0.37	20.2	0.81	18.3	0.50
20:2n-6	0.19–0.18	0.5	0.10	0.4	0.03	0.3	0.05	0.4	0.05	0.4	0.03	0.3	0.03	0.3	0.04
20:3n-6	0.08–0.13	0.4	0.06	0.2	0.03	0.3	0.03	0.3	0.03	0.2	0.02	0.3	0.03	0.2	0.03
20:4n-6	0.5–0.7	0.7	0.05	0.7	0.05	0.7	0.12	0.5	0.07	0.5	0.02	0.6	0.08	0.5	0.02
18:3n-3	2.6–3.9	2.1	0.02	2.2	0.04	2.1	0.15	2.8	0.26	3.0	0.05	2.8	0.11	3.0	0.10
18:4n-3	1.0–1.1	0.9	0.15	0.8	0.02	0.8	0.06	0.7	0.05	0.7	0.02	0.6	0.03	0.6	0.03
20:5n-3	8.8	6.5	0.34	7.0	0.17	6.4	0.96	5.8	0.28	5.8	0.19	5.9	0.27	5.7	0.29
22:5n-3	1.0–1.1	2.4	0.15	2.7	0.17	2.7	0.69	2.5	0.17	2.7	0.08	3.5	0.18	2.9	0.23
22:6n-3	4.3–4.7	7.3	0.56	6.6	0.61	6.3	1.86	4.9	0.41	5.1	0.23	6.8	0.68	5.3	0.34
PUFA♠	40.3–42.4	40.3	1.03	42.8	0.95	42.6	4.15	41.1	3.98	37.9	0.67	41.7	1.36	37.7	0.83

‡ Includes 15:0, 17:0, 20:0 and 22:0.
+ Includes 20:1n-7 and 22:1n-9.
♠ Includes 18:3n-6, 20:3n-3 and 20:4n-3.