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RESEARCH PAPER

Fatty Acid Composition of Gilthead Sea Bream (*Sparus aurata*) Fillets as Affected by Current Changes in Aquafeed Formulation

Mauro Vasconi[1,*], Fabio Caprino[1], Federica Bellagamba[1], Vittorio Maria Moretti[1]

[1] The University of Milan, Department of Health, Animal Science and Food Safety Via Trentacoste 2, 20134 Milano, Italy.

* Corresponding Author: Tel.:+39.025 0315759 ; Fax: +39.025 0315746;
E-mail: mauro.vasconi@unimi.it

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Abstract

The aquafeed industry is facing the lack of availability of fish meal and oil. The need of an economic and environmental sustainability has increased the use of plant-derived oils. In order to provide an overview of which ingredients are the most frequently used in GSB diet and as they affect the lipid composition of aquafeeds, we analyzed 26 feed samples collected from farmers. At the same time, 44 farmed GSB were analyzed to compare the fatty acid (FA) composition with 21 wild GSB and 46 GSB farmed in 2005. Twenty-four different ingredients were declared in the analyzed feeds. Fish meal and oil were present in all samples, even if they were not the principal protein and lipid source in the analyzed feeds. If we consider the FA n-3/n-6 ratio, feeds presented a range from the lowest value of 0.35, to the maximum of 2.26. Concerning fish FA, 2014-farmed GSB presented an increased difference with wild GSB, if compared with those reared in 2005, due to the higher presence of oleic and linoleic acid. Our study confirms the increase of use of plant ingredients in aquafeeds and the decrease of the fish meal and oil inclusion in feeds.

Keywords: Fish meal and oil replacement, fish feed formulation, aquafeed ingredients.

Introduction

The steady decline in catch of wild fish used in the fish meal (FM) and fish oil (FO) industry and the rise of aquaculture, which is the larger user of FM and FO, are creating a lack of availability of these two raw materials and the gradual increase of their price. In 2012, 16.3 million tons of wild fish were used in the production of FM and FO (FAO, 2014); this amount remains static and its increase is not expected. In the same year, aquaculture consumed about 68% of global production of FM and 74% of FO (Tacon & Metian, 2015). The global aquafeed production shows a continuously increasing trend; in 2025, aquaculture will consume 87.1 million tons of feed (Tacon & Metian, 2015) and naturally, the FM and FO content of feed are going to decrease.

FM and FO are the basic component of fish feed, in particular for carnivorous species like salmonids and marine fish. The raw materials cost covers the 75% of total cost of aquafeeds for the salmon feed production (Rana, Siriwardena & Hasan, 2009), and in general the cost of aquafeed accounts for more than 50% of the expenses for a fish farmer. FM price reached its maximum value of 2,388$/ton in December 2014 and this continuous rise leads the use of cheaper feed ingredients, such as plant products, which would allow the aquafeed industry to maintain the feed price low and stable. Further, sustainability of aquaculture is becoming a demanded priority for consumers and public opinion, and the overexploitation of ocean resources is one of the critical point of the thorny debate (Byelashov & Griffin, 2014).

The aquafeed industry is making efforts to reduce the dietary inclusion of marine derivate ingredients, searching alternative dietary proteins and lipid sources and formulating more efficient eco-friendly feeds. Widely cultivated terrestrial plant, like soya, corn, wheat and sunflower, provide protein meals and oils that have been largely introduced in fish diet formulation (Glencross, Booth & Allan, 2007; Sales, 2009; Turchini & Torstensen, 2009). Processed animal protein (PAP), obtained from the rendering industry, are another notable alternative protein sources, and their recent re-admission in aquafeeds (REG CE 56/2013), after the bans caused by the transmissible spongiform encephalopathies crisis, could represent an important resource for the European aquafeed industry. Several other protein and oil sources have been studied and proposed by several authors for replacing FM and FO, like algae

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(Hemaiswarya, Raja, Ravi Kumar, Ganesan, & Anbazhagan, 2011; Chauton, Reitana, Norsker, Tveterås & Kleivdal, 2015) or insects' meal (Hanry, Gasco, Piccolo & Fountoulaki, 2015). Nevertheless, the high price and the scarce availability of some of these new ingredients have limited their use in commercial feeds.

Gilthead sea bream (*Sparus aurata*) (GSB) is one of the most important farmed species in the Mediterranean area. Its production has a positive trend, and reached a volume of 173000 t in 2013. It is mainly reared in the Mediterranean, with Greece being the largest producer, followed by Turkey and Spain (FAO FishStat, 2015). As a consequence of the progressive increase of GSB production, farmers had to face a decline in sale price and a diminution in profitability. The only way that GSB farmers have to maintain a profit margin is to reduce the cost of feeding, with diets that include cheaper ingredients (Martinez-Llorens, Tomas-Vidal & Jover Cerda, 2012). Plant oils are rich in 18-carbon (18:2n-6 and 18:3n-3) polyunsaturated fatty acid (PUFA) but are deficient in n-3 long chain PUFA (LC-PUFA) (Sargent, Tocher & Bell, 2002). The substitution of FO with plant oils, causes alteration of muscle fatty acid profile of GSB, reducing the amount of n-3 LC-PUFA and consequently their nutritional value, since fish is the principal source of these fatty acids, recognized as fundamental for human health (Grigorakis, 2007). In addition, long periods of feeding soybean oil may result into immunosuppression in seabream (Montero *et al*., 2003). Italian GSB farmers report an increase of disease incidents in their fish and diet rich in vegetable oils is considered a possible cause of these phenomena.

The aim of present study was to determine the extent and type of ingredients that are used at present in commercial feeds for GSB and their fatty acid composition. Furthermore, two fatty acid datasets of a total of 90 fillet samples from farmed market-sized GSB during the last decade were used to discuss the consequences of the substitution of FM and FO with alternative ingredients on the lipid and FA composition of GSB fillets.

Material and Methods

Standards and Reagents

Analytical grade reagents and chemicals were purchased from Sigma-Aldrich (Milan, Italy). Demineralized water was obtained from an Elga purification system (Veolia Water Solutions and Technologies, Italy). Standards of individual fatty acids were purchased from Sigma-Aldrich (Milan, Italy). Stock solutions of standard compounds were prepared in hexane at a concentration of 10 mg ml^{-1} and stored at -20°C. Further standard dilutions were prepared in hexane individually or in mixtures to reduce the concentration to 0.2-1 mg ml^{-1} before GC analysis. A 37 Fatty acids methyl esters (FAME) mixture in dicloromethane and standard Menhaden fish oil were obtained from Supelco (Supelco, Bellafonte, PA, USA) and were used as reference standard.

Feed and Fish Sampling

Two sampling campaigns of GSB were organized on Italian market, through distributors, retailers and fish markets during the winter season. The first sampling was done during 2005, in which 46 farmed fish of commercial size (400-600 g) and 19 wild fish were sampled. Farmed fish were obtained from Greece (18), Italy (15), Croatia (10) and Turkey (3), according to their labels.

The second campaign was organized in 2014. Forty-four samples of farmed GSB of the same size were purchased: Fish were farmed in Italy (18), Greece (14), Turkey (6), Croatia (3) and Malta (3). In addition, other 2 wild GSB ware collected.

During the same period (2014) twenty-six aquafeed samples were collected directly from GSB farmers on Italian territory. Our sampling strategy was focused on grow-out diets used at that moment by farmer, as these are the diets used for the greatest proportion of time under culture.

Lipid Extraction and Fatty Acids Analysis

The extraction and determination of total lipids was performed according to the Folch (1957) method with chloroform:methanol (2:1), using 500 mg of feed and 1.5 g of GSB muscle collected from dorsal, ventral and caudal regions of fillets. The preparation of fatty acid methyl esters was performed according to Christie (2003). Briefly, the lipid sample (20 mg) was dissolved 10% methanolic hydrogen chloride (2 ml). A 1 ml solution of tricosanoic acid (1 mg ml^{-1}) in toluene was added as internal standard. The sample was sealed and heated at 50°C overnight; then, 2 ml of a 1 M potassium carbonate solution and 5 ml of 5% NaCl were added to each sample. The FAMEs were extracted with 2×2 ml of hexane and the mixture was evaporated under nitrogen. The sample was dissolved in 1 ml hexane and 1 µl sample was injected into the gas-chromatograph, in split mode (split ratio 1:100). Fatty acid analysis was carried out on an Agilent gas-chromatograph (Model 6890 Series GC) fitted with an automatic sampler (Model 7683) and FID detector. The carrier gas was helium with a flow rate of 1.0 ml min^{-1} and an inlet pressure of 16.9 psi. A HP-Innowax fused silica capillary column (30 m×0.25 mm I.D., 0.25 µm film thickness; Agilent Technologies) was used to separate fatty acid methyl esters. The oven temperature program for separation was from 100 to 180°C at 3°C min^{-1}, then from 180 to 250°C at 2.5°C min^{-1} and held for 10 min. Carrier gas was helium at 1.0 ml min^{-1}, inlet pressure 16.9 psi. Fatty acids were identified relative to known external standards and were expressed as percentage of total fatty acids.

Statistical Analysis

Normal distribution and homogeneity of variance was confirmed and comparison between means was performed by analysis of variance. The Student Newman Keuls was used as post hoc test for comparison of the means among different GSB origins. Significance was accepted at probabilities of 0.05 or less.

Principal component analysis was performed using fatty acid profiles in order to compare different samples and to detect the most important fatty acids affecting the distribution of GSB samples. All the statistical analysis were performed by SPSS version 22.0 (SPSS Inc. Chicago, Illinois) and The Unscrambler version 10.0 (Camo, Norway).

Results

Feed samples collected in 2014 originated from four different manufacturers, among the major companies in the aquafeed market of the Mediterranean basin. Feed extrude diameter were between 3-6 mm. The proximate composition (Table 1) of the 26 feeds, as declared in label, showed a common pattern with minor variations. Feed used in the grow-out phase of farming was rich in protein (43%) and fat (20%). These values match to the actual grow out GSB nutritional requirement (Koven, 2002). Feed ingredients used in feed preparation are reported in Table 2. According to the Reg CE 767/2009 feed's ingredients were listed in label in descending order by weight calculated on the moisture contents of the feed compound. Twenty-five different ingredients were found in GSB feeds. Some of them were obtained from the same raw material; for instance, soybean was present as protein concentrate, expeller, meal and oil. Fish meal was used in all the feeds, but it was not the main protein source of all GSB diets, being the main protein source in only 4 out of the 26 feeds. Krill meal, the other marine meal found in feed samples, was reported in 5 feeds. Corn gluten, soybean and sunflower protein meal or expeller are the most common plant protein source found in GSB feed, while rapeseed, pea, bean, guar and rice were present

Table 1. Proximate composition (g /100 g) of the 26 gilthead sea bream feed as reported in label (mean ± St Dev)

Protein	42.9	±2.49
Lipid	19.7	±1.75
Fiber	2.8	±0.66
Ash	6.2	±1.03

Table 2. Raw material used in gilthead sea breams feed a their relative frequency

Ingredients	Frequency (%)
Fish meal	100
Fish oil	100
Whole weat	85
Corn gluten	85
Sunflower meal	73
Soy protein concentrate	67
Rapeseed oil	42
Soybean oil	42
Rapeseed expeller	42
Wheat meal	33
Wheat gluten	27
Soybean expeller	19
Krill meal	19
Wheat bran	19
Guar protein	19
Sunflower oil	13
Linseed oil	13
Sunflower expeller	13
Hemoglobean meal	8
Rice protein	4
Pea protein	4
Whole peas	4
Bean protein concentrate	4
Rapenseed meal	4
Soybean meal	4

in only few samples. Fish oil was the first pure lipid ingredient in most of the samples and it was present in all the feed samples. We have no direct evidence about the fish species utilized as raw materials to produce the fish oil used in our study. Rapeseed and soybean were the plant oils present in higher proportions, followed by linseed and sunflower oils. Other ingredients, like expellers, can possible also contribute with considerable amount of lipid in feed. The dietary starch was provided mainly as wheat, which was present, in different form, in all feeds.

The fatty acid composition of feeds is shown in Table 3. Saturated fatty acid (SFA) were the fatty acids present in smaller amounts, with the exception of one feed where they were in balance with monounsaturated fatty acid (MUFA). Palmitic acid (PA 16:0) was the most representative SFA in all samples. Among MUFAs, which were dominant in half of the feeds, oleic acid (OA 18:1 n-9) was the fatty acid with the highest relative concentration in 18 of the 26 samples; remaining feeds presented linoleic acid (LA 18:2 n-6) as dominant fatty acid. Long chain monounsaturated fatty acids, like gadoleic (GA 20:1 n-11) and cetoleic (CA 22:1 n-11) acids, were more abundant in five feeds. The α linolenic acid (ALA 18:3 n-3) was more abundant in three feeds, that were the ones which contain linseed oil in their formulation. PUFA were dominant in half of feeds. Among PUFA, eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) were particularly present in four feeds, characterized also by having the highest n3/n6 ratio, reaching the maximum value of 2.26.

Table 4 shows the results of lipid content and fatty acid analysis of GSB fillets. Farmed GSB were fatter than wild fish, whilst no significant difference was found between 2005 and 2014 farmed fish. Wild fish did not show any statistical difference between 2005 and 2014 and they were merged in a one group. They were characterized by a higher content of PUFA, followed by SFA and MUFA. DHA was the dominant fatty acid, followed by PA and OA. LA was present by only in limited amounts; 20 - 22 C MUFAs were present only in traces. The n-3/n-6 FA ratio resulted 3.91, due to the high amount of n-3 and the poor presence of n-6. The most representative n-6 FA was arachidonic acid (ARA, 20:4 n-6).

Fillets FA composition of GSB farmed in 2014 differed statistically from that of 2005 fish. There was an increase of MUFA, mainly due to the raise of OA (from 17.6 % in 2005 to 27.5% in 2014). The increase of MUFA was counterbalanced by the decrease of SFA. Among MUFA, 20:1 and 22:1 FA showed a decrease from 2005 to 2014. Total PUFA were similar between the two groups, but their composition varied among years. In fact, in 2005 there was a predominance of LC-PUFA such as DHA, EPA and ARA, while in 2014 the main PUFA were LA and ALA. n-3/n-6 ratio decreased from the value of 2.04 in 2005 to the value 1.24 of GSB farmed in 2014. This difference was due mainly to the decrease of EPA and DHA, only partially compensated by the increase of ALA, and to the simultaneous increase of LA from 12.5% to 16.7%.

Table 3. Fatty acid composition (percentage of total fatty acid) of 26 gilthead sea bream feeds (mean ± St Dev)

	Mean± Std dev	Min	Max
14:0	2.9±1.56	1.2	8.7
16:0	12.6±3.21	7.4	22.0
16:1n-7	3.2±1.32	1.1	7.2
18:0	3.2±0.74	1.7	4.9
18:1n-9	28.1±8.96	13.8	44.9
18:1n-7	2.5±0.83	0.2	3.7
18:2n-6	22.27.65	10.0	37.2
18:3n-3	4.8±2.75	1.1	11.3
18:4n-3	1.3±0.77	0.3	3.6
Σ20:1*	3.0±2.36	0.3	10.6
20:3n-6	0.7±1.53	0.0	5.8
20:4n-6	0.5±0.21	0.2	0.9
20:4n-3	0.5±0.14	0.2	0.9
20:5n-3	5.9±2.86	1.9	14.4
22:1n-11	4.4±4.12	0.3	15.9
22:1n-9	0.3±0.29	0.0	1.0
22:5n-3	1.2±0.38	0.4	2.0
22:6n-3	6.8±2.43	2.7	13.1
SFA	18.8±4.92	11.2	34.5
MUFA	41.9±9.09	23.6	61.4
PUFA	43.3±7.41	29.6	59.3
n-3	19.9±5.40	11.0	35.0
n-6	22.9±7.52	10.6	37.6
n3/n6	1.0±0.52	0.3	2.3

* Sum of 20:1 n-7, 20:1 n-9 and 20:1

The FA profile of wild, farmed in 2005 and 2014 GSB has been submitted to principal component analysis (PCA) which is presented in Figure 1 and 2. PCA was performed using 24 fatty acid variables. PCA explained the 66% of the variance with PC1 accounting for 42% and PC2 for 24% of the total variance. GSB appeared well separated according their diet/year. Wild GSB, sampled in 2005 and 2014, were grouped in the upper-left part of graph (Figure 1), being characterized mainly by ARA, PUFA, and n-3 PUFA. GSB farmed during 2014 were also well differentiated and grouped in a small part of graph, the area where MUFA, OA, LA and ALA are abundant. GSB farmed in 2005 occupy an

Table 4. Lipid content (g/100g) and fatty acid composition (percentage of total fatty acid) of gilthead sea bream muscle. Data are expressed as mean ± standard deviation.

	2005 (n= 46)	2014 (n=44)	Wild (n=21)
Lipid	2.6 ±0.14^{b}	2.8 ±1.31^{b}	0.7 ±0.13^{a}
14:00	3.28 ± 1.169^{c}	1.84 ± 0.413^{b}	1.33 ± 0.611^{a}
16:00	21.36 ± 3.275^{b}	14.67 ± 1.931^{a}	21.21 ± 1.298^{b}
16:1n-7	4.89 ± 1.181^{b}	3.33 ± 0.649^{a}	4.37 ± 1.476^{b}
18:00	5.20 ± 0.807^{b}	4.57 ± 0.890^{a}	6.97 ± 0.606^{c}
18:1n-9	17.57 ± 1.749^{b}	27.49 ± 4.070^{c}	12.50 ± 2.986^{a}
18:1n-7	2.52 ± 0.246^{a}	2.66 ± 0.195^{a}	2.97 ± 0.633^{b}
18:2n-6	12.47 ± 4.097^{b}	16.70 ± 4.072^{c}	1.29 ± 1.060^{a}
18:3n-6	0.03 ± 0.044	0.11 ± 0.122	0.11 ± 0.243
18:3n-3	1.55 ± 0.509^{b}	3.61 ± 2.012^{c}	0.30 ± 0.193^{a}
18:4n-3	0.87 ± 0.265^{c}	0.43 ± 0.135^{b}	0.28 ± 0.149^{a}
*20:1	3.09 ± 1.638^{c}	2.21 ± 0.503^{b}	0.63 ± 0.359^{a}
20:2n-6	0.36 ± 0.150^{a}	0.85 ± 0.210^{b}	0.36 ± 0.109^{a}
20:4n-6	0.80 ± 0.328^{a}	1.40 ± 2.246^{a}	7.39 ± 3.433^{b}
20:5n-3	5.57 ± 1.215^{b}	3.87 ± 2.055^{a}	10.02 ± 2.524^{c}
**22:1	2.04 ± 1.096^{c}	1.28 ± 0.506^{b}	0.30 ± 0.337^{a}
22:2n-6	0.25 ± 0.223^{a}	0.17 ± 0.161^{a}	1.81 ± 0.937^{b}
22:5n-3	2.57 ± 0.442^{a}	2.86 ± 1.439^{a}	5.55 ± 1.522^{b}
22:6n-3	15.57 ± 4.930^{b}	11.95 ± 2.532^{a}	22.64 ± 7.302^{c}
SFA	29.84 ± 4.725^{b}	21.09 ± 2.526^{a}	29.51 ± 1.588^{b}
MUFA	30.12 ± 4.283^{b}	36.97 ± 5.159^{c}	20.76 ± 4.021^{a}
PUFA	40.04 ± 7.291^{a}	41.94 ± 3.446^{a}	49.73 ± 5.091^{b}
n-3	26.12 ± 5.605^{b}	22.71 ± 4.446^{a}	38.79 ± 5.555^{c}
n-6	13.91 ± 4.092^{b}	19.23 ± 2.860^{c}	10.95 ± 3.813^{a}
n3/n6	2.04 ± 0.722^{b}	1.24 ± 0.482^{a}	3.91 ± 1.315^{c}

Value within the same raw not sharing a common letter are significantly different (P<0.05)
* Sum of 20:1 n-7, 20:1 n-9 and 20:1 n-11
** Sum of 22:1 n-9 and 22:1 n-11

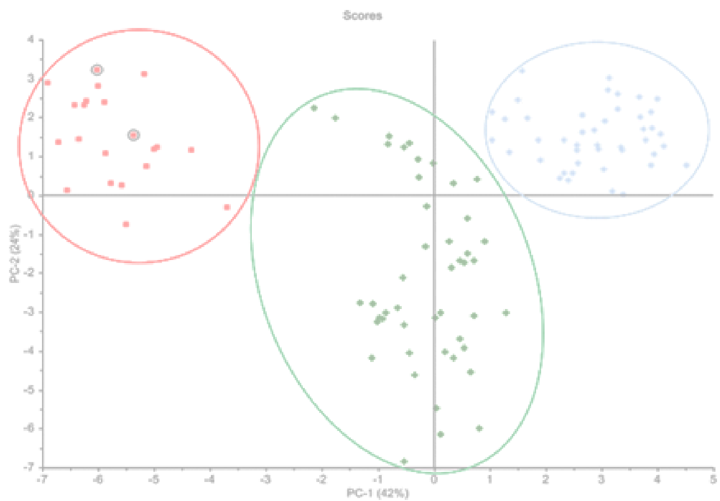

Figure 1. Principal component analysis. Red square wild gilthead sea bream 2005 (circled 2014); green diamond farmed gilthead sea bream 2005; blue star farmed gilthead sea bream 2014.

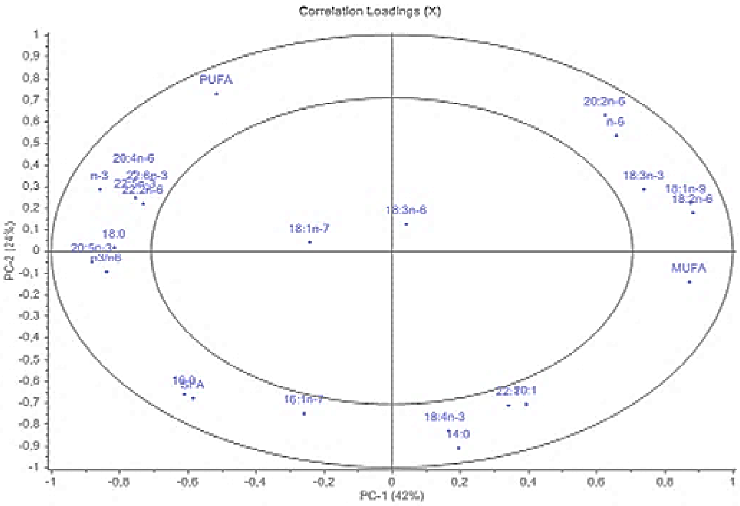

Figure 2. Principal component analysis: correlation loadings plot of the fatty acids in the bidimensional space of the first two PC. The outer ellipse indicates 100% of the explained variance. The inner ellipse indicates 50% of the explained variance.

intermediate position between wild GSB and farmed GSB sampled in 2014; they show the higher spatial spread; only some of them were characterized by 20:1 and 22:1 FA.

Discussion

The composition of analyzed feed was found constituted by a limited range of ingredients if we consider the total of potential ingredients suggested by the research community for the nutrition of GSB.

Several authors proposed alternative protein (Robaina, Moyano, Izquierdo, Socorro, Vergara, & Montero, 1995; Robaina *et al*., 1997; Kissil, Lupatsch, Higgs & Hardy, 2000; Pereira & Oliva-Teles, 2003; Pereira & Oliva-Teles, 2004; Gomez-Requeni *et al*., 2004; De Francesco *et al*., 2007; Sanchez-Lozano *et al*., 2007; Emre, Sevgili, & Sanli, 2008; Martinez-Llorens *et al*., 2008; Piccinno *et al*., 2013) and oil sources (Montero *et al*., 2003; Montero *et al*., 2008; Benedito-Palos *et al*., 2008; Fountoulaki *et al*., 2009; Pérez, Rodríguez, Bolaños, Cejas & Lorenzo, 2014; Castro *et al*., 2015) for the feeding of GSB. Even if the research provided a wide range of raw materials and ingredients for feed formulation and manufacture, only few economic and largely available plant ingredients like soybean, sunflower and rapeseed are commonly used to replace FM and FO. Even after the processed animal proteins (PAPs) were re-allowed by the European regulations from the June 2013 (Reg CE 56/2013) we didn't found any PAP in our samples. Hemoglobin meal has never been banned from use in feed for aquaculture but it was present only in two samples of 26. Martinez –Llorens *et al*. (2012) proposed a new tool for determining the optimum fish meal and vegetable meal inclusion in GSB in order to maximize the profitably. Their study consider the price of raw material and the feed conversion rate of fish fed with the use of the same ingredients as substitute of FM, proposing two index to evaluate the economic profitability of substitution. According to their study, a pea-rice mixture was the most profitable alternative vegetable source in GSB nutrition. In our survey only two diets contained rice or pea, and none of them their mixture.

The analysis of feeds fatty acid composition is an important tool to understand the source of the raw materials used and it could give an idea of the proportion of the ingredients used in feed.

Monoenoic long chain fatty acids, like CA and GA, could considered as a marker of the use of Northern hemisphere fish oils in feeds, since they are present in fish, like herrings and capelin, originating from North Atlantic, where they eat with copepods rich in these fatty acids (Pascal & Ackman, 1976). Otherwise, these MUFAs are not common in the Mediterranean Sea food chain (Özogul, Özogul, Çiçek, Polat, & Kuley, 2009). This is also confirmed by the analysis of the present study wild GSB, that were caught in the Mediterranean and didn't present considerable amounts of these fatty acids. The presence of CA and GA was apparent in only five feeds. The presence of these fatty acids in GSB muscle decrease from 2005 to 2014, suggesting a reduction of the use of fish oil coming from North Atlantic. The aquafeed industry is now preferably using fish oil from different origins or sources, as for example the South American fish oil or the oil obtained from the processing of farmed fish.

Some feeds have a relevant content of ALA. These feed were formulated using linseed oil, which is the principal source of α linoleic acid used in fish nutrition (Castro *et al*., 2015). EPA and DHA are considered essential fatty acids for marine fish species so they must be included their fish diets. Marine fish evolved in an environment naturally rich in EPA and

DHA, produced by phytoplankton, and they lost the ability of conversion of ALA to EPA and DHA (Tocher, 2003). Fish oil is the principal source of EPA and DHA for fish nutrition and even if its inclusion is strongly decreased during last 20 years, it remains an irreplaceable ingredient for commercial aquatic feeds (Shepherd & Jackson, 2013).

The FA composition of lipids extract from GSB mirrored the presence of FA of the commercial feeds used in the Mediterranean area. OA and LA were the dominant fatty acids in both fish and feeds sampled in 2014; the main sources of these two FAs are soybean and rapeseed (Tacon, Metian & Hasan, 2009), that were included in almost all the feeds we analyzed. Even though we did not have data about the feed' composition and the FA profile used in 2005, we can assume that their formulation affected the 2005 farmed GSB FA composition. The use of fish oil, especially the one made from Northern Atlantic fish, was higher in 2005 than 2014. Though all the 2014-feeds contained fish oil, it seems that its amount was limited just to the quantity needed to satisfy the EPA and DHA requirement of GSB, (EPA + DHA: 0.7 to 0.9% of DM, according to Benedito-Palos, Navarro, Kaushik & Pérez-Sánchez, (2010).

The PCA analysis of fatty acid profile suggests that the difference in fatty acid profiles of wild and farmed GSB during the last 10 years has increased. The 2014-GSB group presented higher difference to the wild counterparts than 2005-GSB. Analyzing the spatial spread of samples displayed in Figure 1, PCA showed a decrease of intragroup variability in farmed GSB fatty acid profile among years. 2014-fish were very close to each other, indicating a small variation among group while 2005- fish presented a higher variability. The FA profile of fish is strongly related to the one of the diet (Kirsh, Iverson, Bowen, Kerr, & Ackman, 1998; Budge, Penney & Lall, 2012). The FA signature of GSB farmed in 2014 suggested that all fish were fed with feeds with a similar fatty acid composition. This similitude increased between the two sampling, since 2005-farmed GSB showed a higher spatial spread in the PCA plot, compared to 2014 fish.

We found higher variability in feed fatty acids profile than we expected observing the results of 2014-fillet FA profile. This phenomenon could be explained with the attitude of fish farmers that are used to utilize more than one feed during the production cycle of fish, switching between feed commercial lines but also between aquafeed company.

Conclusions

The data presented confirm that the use of marine-derivate ingredients in the formulation of GSB feeds has been reduced in the last decade. Although the research has proposed several alternative ingredients to substitute FM and FO in aquafeed, only soybean, cereal gluten, sunflower and rapeseed meals and oils are commonly used. The 2014-GSB present a more similitude between samples, indicating a standardization trend, common to all the countries where GSB are farmed, if they are compared with 2005-fish. Farmed GSB present a modified FA profile, with the prevalence of OA and LA and a decrease of EPA and DHA, compared with wild GSB and the gap has increased over the period 2005-2014.

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