Effects of fish oil replacement and re-feeding on the bioaccumulation of organochlorine compounds in gilthead sea bream (*Sparus aurata* L.) of market size

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Abstract

Organochlorine pesticide residues and polychlorinated biphenyls were determined in raw materials, fish feeds and fillets from fish exposed through the productive cycle (14 months) to experimental diets with different percentages of fish oil replacement with vegetable oils. Detectable amounts of organochlorine compounds were found in raw materials derived from fish sources with none being detected in vegetable ingredients. Fish feeds presented trace concentrations of contaminants at the ng/g level, which varied according to the contribution of the different resources used in their manufacture. Contaminants did not accumulate during the first 11 months of exposure, and low concentrations of organochlorine compounds were found both at the start and at the end of this feeding period. Fillets from fish fed the fish oil diet presented the highest concentrations of organochlorine compounds, with these decreasing in proportion to fish oil replacement. Three months of fish oil re-feeding during the finishing phase only produced significant bioaccumulation over the course of the first month. By optimizing fish meal and fish oil replacement with vegetable oils alternative feeds can contribute to significantly reduce the risk of organochlorine uptake by consumers.

Keywords: Organochlorine compounds, bioaccumulation, *Sparus aurata*, fish feed, marine aquaculture

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1. Introduction

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are non polar, highly lipophilic, and persistent ubiquitous environmental pollutants. Both are classified as Persistent Organic Pollutants (POP) and are present in the contamination pattern of marine environments worldwide. Despite the use of OCPs being strongly restricted and PCBs production being banned, these compounds are distributed across the marine environmental biota (et al., 2000; Hoekstra et al., et al., 2005; Yang et al., 2007; Serrano et al., 2008b). Special concern exists about dioxin-like PCB (DL-PCBs), which have been shown to cause toxic responses similar to those induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin, the most potent congener within polychlorinated dibenzo-p-dioxins (Van Den Berg et al., 1998). The characteristics of these compounds lead to high biomagnification in the food chain, and involve a wide range of trophic levels (Hoekstra et al., 2003; Borga et al., 2001; Kidd et al., 2001; Konwick et al., 2006; Serrano et al., 2008a; Serrano et al., 2008b, Sagratini et al., 2008). Some studies have shown that food is the major contributor for PCBs accumulation in farmed fish (Serrano et al., 2003a; Antunes et al., 2004). Thus, fish and in general seafoods have been considered the most important source of organochlorine compounds (OCs) in the human diet (Johansen et al., 1996; Bjerregaard et al., 2001; Tsukino et al., 2006), these compounds being frequently detected in human tissue lipids and fluids (H et al. et al. et al., 2002c; Pitarch et al., 2003; De Felip et al. -de-Toro et al., 2006; Tsukino et al., 2006; Lopez-Espinosa et al., 2008; Mueller et al., 2008).

Persistent organic pollutants are concentrating in lipid-rich feed grade fish used for production of fish oils, the major resource of these contaminants in aquaculture diets, and have been detected in both fish feed used in aquaculture and in farmed fish by several authors (Santerre et al., 2000; Easton et al., 2002; Hites et al., 2004; Navas et al., 2005; Bordajandi et

al., 2006; Maule et al., 2007; Ábalos et al., 2008; Serrano et al., 2008a; Serrano et al., 2008b), so dietary fish oil replacement with alternative oils can significantly reduce the load-charge of lipophilic contaminants in aquafeeds and thereby farmed fish (Bell et al., 2005; Berntssen et al., 2005; Bethune et al., 2006). Unfortunately, when fish oil is replaced with vegetable oils, the dietary supply of n-3 polyunsaturated fatty acids (PUFA) is also reduced (Bell et al., 2005; Benedito-Palos et al., 2007; Drew et al., 2007; Benedito-Palos et al., 2008). This is a major constraining factor for marine fish due to the inability of marine fish to convert C18 PUFA to long-chain PUFA, specially eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3) that become essential nutrients in marine fish aquafeeds (Sargent et al., 1999; Sargent et al., 2002). However, amongst others, recent studies in gilthead sea bream (*Sparus aurata* L.), indicate that fish oil can be replaced up to 66% with vegetable oils in plant protein-based diets without signs of growth retardation and histopathological tissue damage in a 8-month trial (Benedito-Palos et al., 2007; Benedito-Palos et al., 2008). Thus, a large amount of either fish meal or fish oil can be, and is practically replaced in the new sustainable diets for marine fish.

The feasibility of the fish oil replacement has been demonstrated through an entire production cycle, including a three month finishing fish oil diet phase (wash-out period), without negative effects on the growth performance of gilthead sea bream, a high valuable fish for the Mediterranean aquaculture. Also, a kinetic analysis of the fatty acids (FA) demonstrated that changes in the fillet FA profile arise because the existing stores become diluted as fish grow and deposit increasing amounts of dietary derived FAs (Benedito-Palos et al., 2009). The goal of the present study was to analyze, in the same fish, OCPs and PCBs bioaccumulation. Organochlorine compounds were determined in fish fillets, feeds and major raw materials of the diets, providing information on the end-product quality and safety from the consumer point of view.

2. Materials and Methods

2.1 Experimental Diets

Three isoproteic, isolipidic and isoenergetic plant protein-based diets were formulated with a low inclusion level (20%) of fish meal and fish soluble protein concentrates. Fish oil from the southern hemisphere was the only lipid source in the control diet (FO), which was also used as a finishing diet. The two remaining diets contained a blend of vegetable oils (2.5 rapeseed oil: 8.8 linseed oil: 3 palm oil), replacing 33% (33VO) and 66% (66VO) of the fish oil. All diets were manufactured using a twin-screw extruder (Clextral, BC 45) at the “Institut Scientifique de Recherche Agronomique” (INRA) experimental research station of Donzacq (Landes, France), dried under hot air, sealed and kept in air-tight bags until use. Ingredients and proximate composition are shown in Table 1.

Samples for pollutant analyses were collected and stored at -20°C until analysis. Determination of organochlorine residue and polychlorinated biphenyls in each diet was carried out by triplicate.

2.2 Bioaccumulation experiment

Gilthead sea bream of Atlantic origin (Ferme Marine de Douhet, Ile d’Oléron, France) were cultured at the Instituto de Acuicultura de Torre de la Sal (IATS) for 20 days before the start of the study. Fish of around 18 g initial mean body weight were allocated into 9 fiberglass tanks (3000 litres) in groups of 150 fish per tank. Water flow was 20 l/min and oxygen content of outlet water remained higher than 85% saturation. The growth study was undertaken over 14 months (July 11th, 2006 – September 2nd, 2007), and day-length and water temperature (10-26°C) varied over the course of the trial to match natural sea changes offshore from IATS (40° 5’N; 0° 10’E).

During the first 11 months of the trial, the three diets were randomly allocated to triplicate groups of fish, and feed was offered by hand to apparent visual satiety. During the

finishing diet phase (12 weeks, June 6th, 2007 – September 2nd, 2007), two tanks of 33VO and two of 66VO groups were fed with FO diet. The names of those groups became 33VO/FO and 66VO/FO respectively. Fish fed FO diet, and one tank of fish fed 33VO and 66VO diets were maintained on the initial diets until the end of the experiment. This meant that the bioaccumulation study consisted of five treatments: FO, 33VO, 66VO, 33VO/FO and 66VO/FO (Figure 1).

At the beginning and at regular intervals through the finishing diet phase (0, 330, 360, 390 and 420 days) randomly selected fish (8 fish from all tanks of the same treatment) were sacrificed by a blow on the head prior to tissue sampling. The left-side fillet (with skin and bone removed) was excised and stored at -20 °C until analysis. As reported by Benedito-Palos et al. (2009), body weight and fillet yield were not affected by the dietary treatment over the course of all feeding trial.

Sea water for fish culture was analyzed using the multi-residue method for pesticides described by Hernandez et al. (Hernandez et al., 1993) and OCs were not detected (limit of detection between 0.01 and 0.1 µg/l). Therefore, fish were cultured in sea water free of pesticides without any other known exposure to organochlorines except feed.

2.3. Analytical methodology.

Organochlorine compounds, including selected non polar pesticides and derivatives (DDTs -p,p'-DDT, p,p'-DDE, p,p'-DDD-, HCB, lindane, mirex, methoxychlor) and polychlorinated biphenyls IUPAC n° 28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 170, 180, as indicators of the presence of PCBs in the samples, were analyzed in raw materials, fish feeds and fish fillets following the method described by Serrano et al. (2003b). Eight fillets from each treatment (four from each replicate) were selected randomly to obtain three composite samples and were analyzed independently in triplicate. Fish diets and raw

materials were homogenized and analyzed in triplicate. In brief, extraction of fish feed, raw materials and muscle was carried out by refluxing ca. 8 g homogenized fresh sample in n-hexane for 4 h. Clean up of fish tissue was performed by means of normal phase liquid chromatography (NPLC) injecting 1 mL hexanic extract (4 g sample per mL) into a silicagel HPLC column. The liquid chromatography (LC) system used was comprised of a LC Pump Master 305 piston pump, Gilson (Middleton, USA), with a column 150 x 3.9 mm i.d. packed with 4 μ m silica Novapack (Waters, Milford, MA, USA); mobile phases were n-hexane and ethyl acetate for column regeneration after a clean up cycle; flow rate was 1 ml/min. For the fish feed and raw materials an additional clean-up step with concentrated sulphuric acid was necessary prior to normal phase HPLC clean up as a consequence of the high lipid content (from 20 to 100 % of fresh weight) (Serrano et al., 2003b).

Analysis of the fat-free LC fractions collected was performed by gas chromatography with tandem mass spectrometry (GC-MS/MS) using a gas chromatograph Varian CP-3800 coupled with an ion trap mass spectrometry detector (Saturn 4000, Varian) operating in the electron impact ionisation mode (EI) to identify and quantify PCBs and OCPs present in the diets and fish fillets. Separation of the analytes was carried out on a 30 m x 0.25 mm DB-5MS (0.25 μ m film thickness) Varian capillary column, using helium at 1 ml/min as the carrier gas. The temperature program was as follows: 90 °C for 1 min, increased to 180°C at a rate of 30 °C/min, increased to 260 °C at a rate of 4 °C/min, and finally increased to 300 °C at a rate of 20 °C/min, with a final isothermal stage of 4 min (total chromatographic analysis time was 30 min). Injection of 1 μ L of samples (injection port temperature 250 °C) in the splitless mode was carried out using a Varian 8400 autosampler equipped with a 10 μ L syringe. The gas chromatograph was directly interfaced with the Varian 4000 mass-spectrometer (ion trap) in the internal ionization mode with electron impact ionization energy of 70 eV in the positive

ion mode. Transfer line temperature was established at 260 °C and ion source and trap temperatures were adjusted to 200 °C (more details in Serrano et al., 2003b).

Quantification of samples was carried out by external calibration curve using the internal standard method. The limit of quantification has been calculated as three times the detection limit (i.e. nine times the background noise in the chromatograms), accepting coefficients of variation less than 30 %. For the quality control in analyses of samples, 50 ng of isotopically labeled standard (pp'-DDE-D₈) were added before extraction as surrogate. This allowed detection of possible analytical errors in every sample analysis. Statistical validation of the method included the study of linearity, accuracy, precision, selectivity and limits of quantification and detection. Moreover, the absence of matrix effect in the instrumental determination (GC-MS/MS) was proved by means of the Standard Additions Method (more details Serrano et al., 2003b).

The whole analytical process was carried out in the Good Laboratory Practices certified laboratories of the Research Institute for Pesticides and Water, University Jaume I, Spain.

2.4. Determination of fat

The total fat content in the sample extracts was determined gravimetrically, with evaporation at 95°C until constant weight.

2.5. Data analysis

PCBs and OCPs concentrations in raw materials and fish feed are expressed as ng/g fresh weight and in fillets also as lipid based concentrations. Biomagnification Factors (BMF) were calculated as the ratio between lipid based concentrations of organochlorine compounds in the fish fillets and in the fish feed at each sampling point. As two different fish feeds were used in 33VO/FO and 66VO/FO groups, calculations of BMF were made taking into account the time of exposure to each fish feed. Thus, the arithmetic mean of organochlorine

concentrations in the diet was calculated on both the basis of months of exposure to each fish feed and their concentration in each fish feed. The calculated data expressed as ng/fillet allowed to identify the amount of pollutant present in a whole fish fillet. The t-Student test ($P < 0.05$) was applied to compare data on diets and ingredients. Data for concentrations of PCBs and OCPs in fish fillets, BMF values and amounts of pollutants per fillet were compared by means of ANOVA I and “a posteriori” Scheffe’s test ($P < 0.05$). All data were transformed to Log10 before statistical analysis to achieve normality. Homoscedasticity of variances was tested by means of Bartlett’s test ($P < 0.05$). All the statistical tests were conducted using STATGRAPHICS plus for Windows version 4.1 (Statistical Graphics Corporation).

3. Results and Discussion

3.1. PCBs and OCPs content in experimental diets

The analytical method showed excellent sensitivity and selectivity as a consequence of the use of gas chromatography coupled tandem mass spectrometry (GC-MS/MS). It was validated by recovery experiments down to 5 ng/g, with lower spiked levels not being checked due to the presence of DDTs and PCBs in the blank samples (Serrano et al., 2003b). However, the powerful analytical characteristics of GC-MS/MS together with the efficiency of the HPLC clean up has allowed here analyte quantification at concentrations as low as 0.1 ng/g with acceptable precisions (below 30 %). Samples analysis were carried out in the Research Institute for Pesticides and Water facilities, whose laboratories are Good Laboratory Practices certified, which support the quality of the analytical data released in the present research.

Concentrations of PCBs and OCPs present in the raw materials used for the manufacture of experimental diets are shown in Table 2. As can be observed raw materials

derived from fish contain detectable amounts of organochlorine compounds, up to 76.4 ng/g of total organochlorine charge-load (34.8 ng/g total PCBs and 41.6 ng/g total OCPs). The predominant pesticide was pp'-DDT and its derivatives. For the PCBs investigated, the analytical methodology applied allowed the detection and quantification of 12 congeners from 15 considered as markers of total load. Congener 153 was the most abundant, as is usual in marine samples (Serrano et al., 2008a; Serrano et al., 2008b). This pattern is in agreement with the pollution pattern of different marine products (Zabik et al., 1996; Santerre et al., 2000; Johnson et al., 2007; Serrano et al., 2008a; Serrano et al., 2008b).

The presence of organochlorine contaminants in fish oil has been reported previously by other authors. Jacobs et al. (1997; 1998) reported concentrations up to 990 µg/l total PCBs and 119 µg/l total DDTs. Hilbert et al. (1998) reported concentrations up to 300 ng/g total PCBs and 60 ng/g total DDTs during the refining process. In all cases, levels reported are higher than those found in the raw materials used in this work. This fact could be related with the northern origin of the raw materials used in the cited papers.

Organochlorine compounds were not detected in any vegetable ingredient used to formulate the experimental diets, which allows the total load of contaminants in diets to be reduced with fish oil replacement. The non detectable levels of organochlorine contaminants in vegetable derivative resources and the low levels present in the marine derivatives used allow production of a new generation of fish feeds almost free of contaminants.

Table 2 also shows the concentrations of OCPs and PCBs present in the experimental diets used during the diet exposure experiment. The sum of OCPs only reached 7.6 ng/g fresh weight in the case of the FO diet, the diet with highest fish oil content. The sum of PCBs analyzed reached 4.4 ng/g fresh weight in the reference fish feed (FO).

The charge-load of contaminants present in the diets is in accordance with the contribution of the different resources used in their manufacture (t-Student, $P < 0.05$), which leads to very low levels of organochlorines. Thus fish oil replacement by vegetable oils without detectable levels of contaminants, decreases the charge of contaminants in correlation with the percentage of fish oil in the different diets ($r = 0.9978$, $P < 0.01$). Similar results have been previously obtained by other authors (Bell et al., 2005; Berntssen et al., 2005).

The concentrations of organochlorines in different fish feeds have been reported by several authors (Easton et al., 2002; Hites et al., 2004; Carubelli et al., 2007; Maule et al., 2007) with loads of contaminants in the different feeds higher or similar than levels found in the experimental diets used in this work. This highlights the quality of the new diets employed in this study in comparison to others. It is interesting to note that feeds for salmon farming present higher levels of contaminants than those used for Mediterranean aquaculture (Hites et al., 2004). This fact could be related to the use of fish oil from northern Atlantic origin and the higher percentage of fish oil used in salmonid diets (around 25-33 % versus 15-20 % in Mediterranean aquaculture feeds), or other factors such as the quality of raw materials used in manufacture.

3.2. Fish diet exposure

Tables 3 and 4 show the concentrations of PCBs and OCPs in fish fillets during the long term diet exposure experiments. Fish fed the FO diet presented the highest concentrations of organochlorine compounds at the end of the experiment (PCBs and OCPs) (Scheffe's test, $P < 0.05$) as would be expected due to the higher content of pollutants quantified in this diet. Following the expected trend, the fish fed 33VO presented lower organochlorine concentrations than those fed FO diet but were higher than 66VO group ($P < 0.05$). For the experiments with finishing diets, 33VO/FO and 66VO/FO, the supply of FO diet during the last three months of the experiment, provokes a tendency towards the increase

of the levels of organochlorine compounds in the fish fillets with respect to fish kept without finishing diets (33VO and 66VO), although not reaching the levels of PCBs and OCPs found in the control FO group.

Tables 3 and 4 also show the trend of PCBs and DDTs levels during the bioaccumulation experiment. During the first 11 months, contaminant concentrations increased very little compared with the last three months of the experiment. In fact contaminants did not accumulate during the first 11 months of exposure to the experimental diets, presenting similar concentrations (referred to both fresh and lipid weight) of organochlorine compounds at the start and finish of this feeding period (fish mean weights were 18 g at the start and 290 g after 11 months of exposure) (sampling times 0 and 1, July 06 and June 07, respectively) ($P>0.05$). The fact that levels of PCBs, dioxin like-PCBs and organochlorine pesticides in fish fillets did not increase in fresh weight and lipid based concentrations indicates the no bioaccumulation of these contaminants, suggesting the ability of fish to depurate the pollutants ingested in the period previous to the start of the experiment as well as in the first 11 months. Possibly dilution, excretion or detoxifying systems operate efficiently when toxicant load is low in diets with adequate nutritional composition.

From June 2007 to September 2007 fish ingested almost the same weight of feed as in the previous experimental period (from July 2006 to June 2007) (Figure 1). An increase in food intake during the finishing diet phase can cause major increases in the concentration of pollutants. Such increases can be seen after June 2007. As consequence of the different feeding behavior during the grow out and finishing periods, a kinetic model study was not considered. Treatments 33VO/FO and 66VO/FO showed a higher change compared to fish always fed FO, 33VO and 66VO diets. The ingestion of FO diet from June 2007 onwards as well as the higher feed intake resulted in a major increase in pollutants charge in the fish

fillets. In a short time fish accumulated more pollutants from the diet than could be eliminated by any detoxification route.

Figure 2 shows the Biomagnification Factors (BMF) at the four sampling points during the last three months of total experimental period. As can be observed, BMF values were close to one at sampling point 1 (July 07) and, fish fed on the diet with no fish oil replacement presented a steady, continuous increase over time. This same trend was observed but was softer in fish fed always 33VO and 66VO diets. Fish submitted to fish oil re-feeding showed stable BMF values (Scheffe's test, $P>0.05$) the three last sampling points. This observation supports the hypothesis discussed above on the high efficacy of detoxification routes, probably due to low oxidative stress because of the very low levels of contaminants and adequateness of the fish feeds composition supplied.

In September 2007 the total feeding trial finished as fish had reached commercial size. The concentration of the sum of PCBs and OCPs in fish fillets at this time revealed the low charge of contaminants and allowed the contaminant intake per fish by consumers to be checked (Figure 3). As shown, the charge of pollutants per fillet decreased with reduced percentages of fish oil in their diet. As well, experimental diets that included FO as finishing diets for the three last months did not increase the load of contaminants up to the load present in fish fed FO for the full 14 months (Scheffe's test, $P>0.05$). Besides, polybrominated biphenyl diethyl ethers (PBDEs) have been analyzed in fish feed and fish fillets studied in this work, showing very low levels of individual PBDEs around 0.2-0.3 ng/g fresh weight in fish feeds and below 0.1 ng/g fresh weight in fish fillets (data not shown).

Several authors have reported the presence of persistent organic contaminants in cultured fish. Santerre et al. (2000) noticed that farmed channel catfish, rainbow trout and red swamp crayfish from Southern USA sources presented absence or low levels of organochlorine contaminants, which were lower than the concentration levels reported in wild

fish. On the other hand, Easton et al. (2002) assessed the risk of the organochlorine contaminants in wild and farmed salmon, indicating higher levels of PCBs and OCPs in cultured fish as a consequence of the presence of these contaminants in fish feed. This fact has been confirmed in a recent study carried out by Hites et al. (2004), also suggesting the fish feed consumed is the cause of the high levels of contaminants in farmed salmon, especially in Northern Europe. In these samples, the levels of organic compounds were higher than those found in farmed fish from North and South America.

In regard to temperate marine aquaculture, Serrano et al. (2008a; 2008b) reported higher levels of contaminants in wild fish than those found in cultured fish from the Western Mediterranean region. This could be explained again by of the low contaminants load of fish feeds supplied to the farmed fish (see Table 2). From this point of view and in a food-safety framework, the results of the present work show that there is further margin for improvement in the elaboration of “cleaner” feeds and cultured fish.

Levels of pollutants in initial fish (T0) (Tables 3 and 4), can be attributed to the nutritional background of the previous diets that influenced the profile of pollutants. These were commercial diets with major levels of pollutants compared to the new generation of diets used in this work. Besides, it has to be taken into account that the maternal transfer of organochlorine compounds in fish has been reported as a possible source of accumulation (Miller et al., 1993; Russell et al., 1999; Monosson et al., 2003; Oka et al., 2006; Serrano et al., 2008c).

In this study fillets from fish fed FO diet presented trace level concentrations of organochlorine compounds with this decreasing in proportion to fish oil replacement. This fact has made it possible to produce a new generation of aquaculture fish feeds with optimal replacement of fish meal and fish oil with sustainable, alternative feed resources such as

vegetable oils and meals. In this way, feeds can be produced that contribute to ensure production of healthy, marketable fish, ensure also that the risk of organochlorine transfer to consumers is reduced, and improve sustainability of marine aquaculture by reducing the use of wild fish sources for feed production.

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Figure Captions:

Figure 1. Schematic representation of the long-term feeding trial over 14 months (for abbreviations see the text).

Figure 2. Biomagnification Factors for total OCs (ng/g lipid based) of fish fed different experimental diets with varying levels of fish oil substitution, at four sampling points (Fig. 1 for details).

Letters: comparison among BMF at different sampling points for each experimental diet, Scheffe's test, $P < 0.05$. bars: standard deviation

Figure 3. Concentration (ng/fillet fresh weight) of sum PCBs and OCPs in fillets of fish fed different experimental diets with varying levels of fish oil substitution at the end of the long-term feeding trial (September 2007). Fillet weight was not altered by dietary intervention and ranged between 113 and 126 g (Benedito-Palos et al., 2009).

Letters: comparison among diets for every category (Capitals: sum PCBs, Lower case: sum OCs). Scheffe's test, $P < 0.05$. bars: standard deviation

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520 **FIGURE 1**

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FIGURE 2

FIGURE 3

Table 1.

Ingredients and chemical composition of experimental diets. For more details in diet composition see Benedito-Palos et al., 2009.

Ingredient (%)	<i>FO</i>	<i>33VO</i>	<i>66VO</i>
Fish meal (CP 70%) ¹	15	15	15
CPSP 90 ²	5	5	5
Corn gluten	40	40	40
Soybean meal	14.3	14.3	14.3
Extruded wheat	4	4	4
Fish oil ³	15.15	10.15	5.15
Rapeseed oil	0	0.85	1.7
Linseed oil	0	2.9	5.8
Palm oil	0	1.25	2.5
Soya lecithin	1	1	1
Binder	1	1	1
Mineral premix ⁴	1	1	1
Vitamin premix ⁵	1	1	1
CaHPO ₄ ·2H ₂ O (18%P)	2	2	2
L-Lys	0.55	0.55	0.55
Proximate composition			
Dry matter (DM, %)	93.13	92.9	92.77
Protein (% DM)	53.2	52.81	52.62
Fat (% DM)	21.09	21	20.99
Ash (% DM)	6.52	6.69	6.57

¹Fish meal (Scandinavian LT)

²Fish soluble protein concentrate (Sopropêche, France)

³Fish oil (Sopropêche, France)

⁴Supplied the following (mg · kg /diet, except as noted): calcium carbonate (40% Ca) 2.15 g, magnesium hydroxide (60% Mg) 1.24 g, potassium chloride 0.9 g, ferric citrate 0.2 g, potassium iodine 4 mg, sodium chloride 0.4 g, calcium hydrogen phosphate 50 g, copper sulphate 0.3, zinc sulphate 40, cobalt sulphate 2, manganese sulphate 30, sodium selenite 0.3.

⁵Supplied the following (mg · kg /diet): retinyl acetate 2.58, DL-cholecalciferol 0.037, DL- α tocopheryl acetate 30, menadione sodium bisulphite 2.5, thiamin 7.5, riboflavin 15, pyridoxine 7.5, nicotinic acid 87.5, folic acid 2.5, calcium pantothenate 2.5, vitamin B₁₂ 0.025, ascorbic acid 250, inositol 500, biotin 1.25 and choline chloride 500.

591 **Table 2.**

592 Concentration (ng/g fresh weight) of organochlorine compounds in marine resources used in fish feed manufacture and experimental diets.

593 Pollutants in vegetable resources were no detected.

Compound	Fish oil		Fish meal		Fish soluble protein concentrate		Diet FO		Diet 33VO		Diet 66VO	
	Mean	CV	Mean	CV	Mean	CV	Mean	C.V.	Mean	C.V.	Mean	C.V.
PCB 28+31	1.6	4	<0.1	-	<0.1	-	0.2	9	0.1	9	<0.1	
PCB 52	1.2	14	<0.1		<0.1	-	0.2	6	0.2	8	0.1	
PCB 101	3.6	1	0.7	29	0.5	6	0.5	6	0.5	9	0.3	
PCB 118	4.7	9	<0.4	-	1.0	1	0.6	5	0.6	5	0.4	
PCB 153	6.5	19	2.1	4	3.5	14	1.6	3	1.5	6	0.9	
PCB 105	3.8	5	<0.2	-	0.3	22	<0.1	3	<0.1		<0.1	
PCB 138	4.9	8	1.4	20	1.9	3	0.8	14	0.7	7	0.5	
PCB 128	2.0	7	<0.2	-	0.3	27	<0.1		<0.1		<0.1	
PCB 156	1.9	4	<0.4	-	<0.4	-	<0.1		<0.1		<0.1	
PCB 180	4.8	14	0.4	14	0.9	12	0.4	8	0.4	37	0.3	
PCB 170	0.6	31	0.3	11	0.3	18	<0.2		<0.1		<0.1	
SUM PCBs	34.8	4	4.7	18	8.7	11	4.4	3	4.1	5	2.8	
HCB	<0.1	-	<0.1	-	<0.1	-	0.2	18	0.2	30	0.5	
p,p-DDE	16.8	3	5.5	7	3.6	7	2.5	12	2.0	10	1.7	
p,p-DDD	8.8	29	1.4	7	0.6	15	2.3	4	2.0	1	1.3	
p,p-DDT	15.9	6	1.5	12	1.0	11	2.5	10	2.1	2	1.4	
SUM OCPs	41.6	7	8.4	6	5.2	2	7.6	5	6.2	3	4.8	
Total Load	76.4	4	13.1	5	13.9	4	11.9	3	10.2	2	7.6	

620 CV: coefficient of variation. Compounds were quantified with CV<30.

621 **Table 3.**

622 Concentrations of total PCBs and total DL-PCBs^a in fillets (mean $\pm$ coefficient of variation, n=3) from fish exposure to the different
 623 experimental diets in long-term sea bream feeding trial (see Figure 1).

Compound	T0	FO		33VO		33VOFO	66VO		66VOFO
	July/06	June/07	Sept/07	June/07	Sept/07	Sept/07	June/07	Sept/07	Sept/07
<i>ng/g fresh weight</i>									
PCB 28+31	< 0.1	< 0.1	0.2(19)	< 0.1	0.1(26)	0.2(19)	< 0.1	0.2(4)	0.2(18)
PCB 52	< 0.1	0.1(27)	0.3(15)	< 0.1	0.2(35)	0.2(35)	< 0.1	0.2(9)	0.2(21)
PCB 101	< 0.1	0.2(30)	0.6(31)	0.1(22)	0.4(27)	0.4(31)	< 0.1	0.3(16)	0.5(15)
PCB 77	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
PCB 118	0.5(9)	0.5(13)	1(16)	0.2(23)	0.7(31)	0.8(16)	0.3(25)	0.6(12)	0.8(2)
PCB 153	2(26)	0.7(19)	1.8(26)	0.4(25)	1.4(29)	1.6(26)	0.5(28)	1.2(12)	1.8(14)
PCB 105	< 0.2	0.5(30)	0.6(25)	< 0.2	0.3(18)	0.4(25)	0.2(19)	0.3(21)	0.4(13)
PCB 138	1(11)	0.6(8)	1.1(22)	0.2(22)	0.8(29)	0.9(22)	0.4(23)	0.7(16)	1.0(5)
PCB 126	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
PCB 128	< 0.2	0.2(4)	0.3(19)	0.1(20)	0.2(14)	0.2(19)	0.1(24)	0.2(10)	0.2(24)
PCB 156	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4
PCB 180	0.5(26)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
PCB 169	< 0.1	0.5(33)	0.6(24)	0.2(30)	0.5(19)	0.5(24)	< 0.1	0.4(16)	0.6(23)
PCB 170	< 0.2	0.3(3)	0.2(23)	0.1(6)	0.2(23)	0.1(23)	0.1(4)	0.2(14)	0.1(20)
SUM PCBs	4.0 (3)	3.7 (11)	6.5 (5)	1.7 (10)	4.8 (11)	5.4 (11)	1.6 (14)	4.1 (5))	5.7 (6)
SUM DL-PCBs^a	0.4 (1)	1.2 (0.2)	1.3 (0.1)	0.5 (0.1)	1.0 (0.1)	1.0 (0.3)	0.5 (0.1)	0.8 (0.1)	1.1 (0.2)
<i>ng/g lipid weight</i>									
SUM PCBs	83.5 (4)	47.5 (2)	70.6 (6)	22.6 (2)	48.3 (5)	51.3 (5)	27.9 (4)	40.6 (2)	51.9 (3)
SUM DL-PCBs^a	9.8 (1)	15.4 (2)	14.6 (2)	7.1 (1)	10.2 (1)	9.7 (2)	6.9 (1)	8.5 (1)	10.1 (2)

^a see list of PCBs analyzed in Material and Methods section

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Table 4.

Concentrations of total OCPs in fillets (mean $\pm$ coefficient of variation, n=3) from fish exposure to the different experimental diets in long-term sea bream feeding trial (see Figure 1).

Compound	T0	FO		33VO		33VOFO	66VO		66VOFO
	July/06	June/07	Sept/07	June/07	Sept/07	Sept/07	June/07	Sept/07	Sept/07
<i>ng/g fresh weight</i>									
HCB	< 0.1	< 0.1	0.2(22)	< 0.1	< 0.1	0.2(34)	< 0.1	0.4(2)	< 0.1
p,p-DDE	2.2(5)	2(15)	3.6(19)	1.0(8)	2.5(20)	2.6(30)	0.7(18)	2.1(11)	2.0(5)
p,p-DDD	0.7(11)	1.4(19)	2.3(15)	0.4(29)	1.7(29)	2.1(28)	0.6(10)	1.3(8)	1.8(4)
p,p-DDT	< 0.4	1.2(24)	2.2(13)	0.3(29)	1.4(19)	1.6(28)	0.6(33)	1.0(19)	1.8(4)
SUM OCPs	2.9 (5)	4.6 (11)	8.3 (4)	1.7 (11)	5.8 (7)	6.6 (7)	1.8 (12)	4.8 (5)	5.9 (4)
<i>ng/g lipid weight</i>									
SUM OCPs	67.1 (1)	58.9 6	90.4 3	22.7 2	58.5 4	62.6 4	23.6 3	47.8 2	54.1 2