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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.

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 (Hernández et al., 2000; Hoekstra et al., 2003; Bocquené et al., 2005; Yang et al., 2007; Serrano et al., 2008a). 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 (Borga et al., 2001; Kidd et al., 2001; Hoekstra et al., 2003; Konwick et al., 2006; Serrano et al., 2008a,b; 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 and Gil, 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 (Hernández et al., 2002a,b,c; Pitarch et al., 2003; De Felip et al., 2004; Muñoz-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 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,b), 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, 2008; Drew et al., 2007). 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

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n-3) and docosahexaenoic acid (22:6 *n*-3) that become essential nutrients in marine fish aquafeeds (Sargent et al., 1999, 2002). However, among 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, 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.

Table 1
Ingredients and chemical composition of experimental diets. For more details in diet composition see Benedito-Palos et al. (2009).

Ingredient (%)	FO	33VO	66VO
Fish meal (CP 70%)[a]	15	15	15
CPSP 90[b]	5	5	5
Corn gluten	40	40	40
Soybean meal	14.3	14.3	14.3
Extruded wheat	4	4	4
Fish oil[c]	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[d]	1	1	1
Vitamin premix[e]	1	1	1
$CaHPO_4{\cdot}2H_2O$ (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

[a] Fish meal (Scandinavian LT).
[b] Fish soluble protein concentrate (Sopropêche, France).
[c] Fish oil (Sopropêche, France).
[d] Supplied 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.
[e] Supplied 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 B_{12} 0.025, ascorbic acid 250, inositol 500, biotin 1.25 and choline chloride 500.

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 nine fiberglass tanks (3000 L) 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 (Fig. 1).

At the beginning and at regular intervals through the finishing diet phase (0, 330, 360, 390 and 420 days) randomly selected fish (eight 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. (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 nos 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