Aquaculture 296 (2009) 87–95

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Assessment of the health and antioxidant trade-off in gilthead sea bream (*Sparus aurata* L.) fed alternative diets with low levels of contaminants

Alfonso Saera-Vila [a], Laura Benedito-Palos [a], Ariadna Sitjà-Bobadilla [b], Jaime Nácher-Mestre [c], Roque Serrano [c], Sadasivam Kaushik [d], Jaume Pérez-Sánchez [a,*]

[a] Nutrition and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal (CSIC), 12595 Ribera de Cabanes, Castellón, Spain
[b] Fish Pathology Group, Institute of Aquaculture Torre de la Sal (IATS, CSIC), 12595 Ribera de Cabanes, Castellón, Spain
[c] Research Institute for Pesticides and Water (IUPA). Avda. Sos Baynat, s/n. University Jaume I, 12071 Castellón, Spain
[d] UMR Nutrition, Aquaculture & Genomics, INRA, Unité-Mixte INRA-IFREMER-Univ. Bordeaux I, 64310 Saint-Pée-sur-Nivelle, France

ARTICLE INFO

Article history:
Received 17 April 2009
Received in revised form 20 July 2009
Accepted 25 July 2009

Keywords:
Fish oil
Vegetal oil
Plant proteins
Aryl hydrocarbon receptors
Cytochrome P450 1A1
Glucose regulated protein 75
Metallothionein
Glutathione
Alternative complement pathway
Lysozyme
Peroxidases
ROS production

ABSTRACT

The aim of the present work was to analyze the effect of partial and total replacement of fish oil (FO) by a blend of vegetable oils on the health and antioxidant status of gilthead sea bream (*Sparus aurata* L.) fed primarily plant protein-based diets. The study included measurements of feed-borne contaminants, gene expression analyses of detoxifying and antioxidant pathways and measures of antioxidant and innate immune descriptors. Polybrominated diphenyl ethers (PBDEs) were almost undetectable in all diets, and the loading-charges of polychlorinated biphenyls (PCBs), dioxin-like PCBs, organochlorine pesticides (OCs), and polycyclic aromatic hydrocarbons (PAHs) were at trace levels decreasing their concentrations according to the level of FO replacement with vegetable oils (0%, 33%, 66%, and 100%). Hepatic detoxifying pathways were down regulated by FO replacement, and the hepatic transcription of cytochrome P450 1A1 and aryl hydrocarbon receptor 1 was significantly reduced in fish fed the 100% vegetable oil diet. Dietary intervention did not alter the hepatic expression of the recycling glutathione reductase, whereas glutathione peroxidase-1 and phospholipid glutathione peroxidase were either down- or up-regulated by total FO replacement. This suggests that vegetable oils prime the *in situ* repair of peroxidized phospholipids rather than the increased turnover of membrane phospholipids from the undamaged pool of cytosolic free fatty acids. The hepatic expression of non-enzymatic antioxidants (metallothionein, glucose regulated protein 75) was down regulated in fish fed 66% and 100% vegetable oil diets. Hepatic glutathione levels and total plasma antioxidant capacity were also lowest in fish fed high levels of vegetable oils, but the concurrent increase in the GSH/GSSG ratio was interpreted as an index of reduced oxidative stress. This redox balance agrees with the enhanced respiratory burst of blood leucocytes after phorbol myristate acetate stimulation in fish feed the 100% vegetable oil. Total plasma peroxidases and plasma alternative complement pathway were not affected by dietary treatment, whereas plasma lysozyme was significantly decreased in fish fed the 66% vegetable oil diet. Taken together, the results suggest that the health and the antioxidant status of gilthead sea bream was not damaged by high levels of FO replacement in eco-friendly diets, but both the scavenging and production of reactive oxygen species were modulated in concert by complex and nutritionally mediated readjustments.

1. Introduction

The increasing demand for fish oil (FO) to meet the expanding aquaculture industry, together with the opposing trend of fisheries and the increasing use of FO in nutraceutical and agricultural industries, has lead to the search for alternative sources of dietary lipids in fish feeds (Miller et al., 2008). Different vegetable oils at different levels of inclusion have been tested with variable results in freshwater and marine fish (Webster et al., 2007; Bell and Waagbo, 2008). Indeed, vegetable oils are rich in C_{18} polyunsaturated fatty acids (PUFA), but they are lacking in n-3 long-chain polyunsaturated fatty acids (LC-PUFAs). This means that fish feeding on vegetable oils would have to desaturate and elongate C_{18} PUFAs to their LC-PUFA derivates. However, all marine fish so far studied, including gilthead sea bream, appear to have lost the ability to make such conversion (Mourente and Tocher, 1994; Seiliez et al., 2003; Zheng et al., 2004), and therefore they have absolute dietary requirements for C_{20} and C_{22} PUFAs. On the other hand, marine derived products are also the main source of environmental pollutants, even in human dietary supplements (Storelli et al., 2004). Furthermore, the high levels found in

* Corresponding author. Institute of Aquaculture Torre la Sal, 12595 Ribera de Cabanes, Castellón, Spain. Tel.: +34 964319500; fax: +34 964319509.
E-mail address: jperez@iats.csic.es (J. Pérez-Sánchez).

0044-8486/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2009.07.028

some farmed fish have led to reconsider the possible beneficial properties of fish consumption in some population groups (Foran et al., 2005; Hamilton et al., 2005). Thus, efforts to reduce this contaminant load have also been directed towards the use of alternative vegetable oils in fish feeds (Bethune et al., 2006) and even to engineering oil seeds to produce n-3 LC-PUFA (Damude and Kinney, 2008).

The anti-inflammatory effect of n-3 LC-PUFAs has been extensively documented, and supplementation of domestic foods with marine FO is becoming an accepted practice to improve the nutritional quality of most animal products (e.g., meat, milk, eggs). However, LC-PUFAs that are extremely vulnerable to oxidation and dietary antioxidants (e.g., vitamin C, vitamin E, polyphenols, carotenoids, biologically active peptides) help to counteract the negative effects of lipid peroxidation, having beneficial effects on growth, fertility, immunocompetence, ageing and pollutant susceptibility (Catoni et al., 2008; Erdmann et al., 2008; Fang et al., 2002). In fish, most studies dealing with antioxidant systems have focused on vitamin E, carotenoids and some minerals (Martínez-Alvarez et al., 2005; Mourente et al., 2007a), but now there is also evidence for the antioxidant properties of plant protein ingredients in practical diets for gilthead sea bream (Sitjà-Bobadilla et al., 2005), a highly valuated fish for the Mediterranean aquaculture. Also, we have earlier shown that both fish meal and FO can be replaced up to 65–75% without growth retardation and signs of histopathological damage (Benedito-Palos et al., 2007, 2008). The goal of the present study is to gain more understanding about the risk and benefits of these eco-friendly diets, in terms of the health and antioxidant status of the fish. For this issue, plant protein-based diets with a partial or total replacement of FO with vegetable oils were formulated, and the loading-charges of the most common persistent organic pollutants (POPs) were firstly monitored. The transcriptional and nutritionally mediated effects on detoxifying and antioxidant defence systems were assessed by hepatic mRNA measurements of aryl hydrocarbon receptors (AhR1 and AhR2), cytochrome P450 1A1 (CYP1A; EC 1.14.14.1), metallothionein (MT), glucose regulated protein 75 (GRP75), glutathione reductase (GR; EC 1.8.1.7), glutathione peroxidase (GPx-1; EC 1.11.1.9) and phospholipid glutathione peroxidase (PHGPx; EC 1.11.1.12). Hepatic glutathione levels and total plasma antioxidant capacity were monitored as antioxidant indexes. Lastly, immunological and pro-inflammatory status was assessed through the alternative complement pathway, leucocyte production of reactive oxygen species (ROS), and plasma measures of lysozyme and total peroxidase activities.

2. Materials and methods

2.1. Experimental setup

Animals and samples were the same as those described in a previous study (Benedito-Palos et al., 2008). Briefly, juvenile gilthead sea bream (*Sparus aurata* L.) of 16 g initial mean body weight were distributed into 12 fibreglass tanks (500 l) in groups of 60 fish per tank at the research experimental facilities of IATS (Castellón, Spain). Each triplicate group received from May 23rd to September 19th one of the four experimental diets nominally CTRL, 33VO, 66VO and VO (Table 1). Added oil was either Scandinavian FO (CTRL diet) or a blend of vegetable oils, replacing the 33% (33VO diet), 66% (66VO diet) and 100% (VO diet) of FO. All diets were manufactured using a twin-screw extruder at the INRA experimental research station of Donzaq (Landes, France), dried under hot air, sealed and kept in air-tight bags until use.

Fish were reared under natural day-length and water temperature following the natural changes at IATS latitude (40° 5′N; 0° 10′E). Water flow was 20 l/min and feed was offered by hand to apparent visual satiety twice a day (9.00 h–14.00 h). Each 3–4 weeks, fish were counted and group-weighed under moderate anaesthesia (3-aminobenzoic acid ethyl ester, MS 222; 100 μg/ml). There was no reduction in growth or feed efficiency (wet-weight gain/dry feed intake = 1.06–1.02) with the partial replacement of FO (183–186 g final mean body weight for fish fed CTRL 33VO, 66VO diets). A decrease in feed intake and weight gain of about 10% was found with the total FO replacement (VO diet).

Table 1
Ingredients and chemical composition of experimental diets.

Ingredient (g/kg)	CTRL	33VO	66VO	VO
Fish meal (CP 70%)[a]	150	150	150	150
CPSP 90[b]	50	50	50	50
Corn gluten	400	400	400	400
Soybean meal	143	143	143	143
Extruded wheat	40	40	40	40
Fish oil[c]	151.5	101.5	51.5	0
Rapeseed oil	0	8.5	17	25.8
Linseed oil	0	29	58	87.9
Palm oil	0	12.5	25	37.9
Soya lecithin	10	10	10	10
Binder	10	10	10	10
Mineral premix[d]	10	10	10	10
Vitamin premix[e]	10	10	10	10
$CaHPO_4 \cdot 2H_2O$ (18%P)	20	20	20	20
L-lys	5.5	5.5	5.5	5.5
Proximate composition				
Dry matter (DM, %)	93.42	94.16	94.79	95.38
Protein (% DM)	48.98	48.74	49.03	48.65
Fat (% DM)	22.19	22.26	22.11	22.31
Ash (% DM)	6.54	6.57	6.62	6.41

For details in amino acid and fatty acid composition see Benedito-Palos et al. (2007).
[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.

At the end of the feeding trial, randomly selected fish (4 fish per tank; 12 fish per treatment) were killed by a blow on the head prior to blood and tissue sampling. Blood was taken with heparinised syringes from caudal vessels, and kept on ice. One aliquot was immediately used to measure respiratory burst activity of circulating leucocytes. The remaining blood was centrifuged at 3000 *g* for 20 min at 4 °C, and plasma aliquots were stored at −80 °C until use. Liver was extracted and rapidly excised, frozen in liquid nitrogen, and stored at −80 °C until analyses.

2.2. Contaminant analyses

Organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), dioxin-like PCBs (DL-PCBs) and polybrominated diphenyl ethers (PBDEs) were analyzed in fish feeds as described elsewhere (Serrano et al., 2003a). Briefly, feed-borne contaminants were extracted by refluxing ca. 8 g during 4 h. Clean-up was performed by means of sulphuric acid digestion prior to normal phase liquid chromatography (NPLC). Identification and quantification of PCBs, DL-PCBs and selected OCPs were performed using a gas chromatograph (GC, Varian CP-3800) coupled to a Varian Saturn 4000 ion trap mass spectrometry detector (system operated in MS/MS mode). Instrumental determination of PBDEs was carried out by means of a GC system (Agilent 6890 N, Palo Alto, USA), equipped with an autosampler (Agilent 7683) coupled to a triple quadrupole (QqQ) mass spectrometer (Quattro Micro GC; Micromass, Boston, USA) operating in CI mode.

For polycyclic aromatic hydrocarbon (PAHs) analysis, a first saponification step was carried out. PAH analytes were then extracted twice with 8 ml of n-hexane and concentrated under gentle nitrogen