Fish & Shellfish Immunology 64 (2017) 437–445

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Supplementation of arachidonic acid rich oil in European sea bass juveniles (*Dicentrarchus labrax*) diets: Effects on leucocytes and plasma fatty acid profiles, selected immune parameters and circulating prostaglandins levels

S. Torrecillas [a, *], L. Román [a], F. Rivero-Ramírez [a], M.J. Caballero [a], C. Pascual [b], L. Robaina [a], M.S. Izquierdo [a], F. Acosta [a], D. Montero [a]

[a] Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Crta. Taliarte s/n, 35214 Telde, Las Palmas, Canary Islands, Spain
[b] Fisiología e Inmunología de Organismos Marinos. Universidad Nacional Autónoma de México, Unidad Académica Sisal. Puerto de Abrigo, S/N. 97356 Sisal, Hunucma, Yucatán, Mexico

ARTICLE INFO

Article history:
Received 11 January 2017
Received in revised form
23 February 2017
Accepted 26 March 2017
Available online 27 March 2017

Keywords:
European sea bass
Arachidonic acid
Immune system
Leucocytes fatty acid profiles
Plasma prostaglandins

ABSTRACT

The main objective of this study was to assess the effects of graded levels of dietary arachidonic acid (ARA), supplemented from alternative sources, on fatty acid composition of plasma and head kidney leucocytes of European sea bass (*Dicentrarchus labrax*). For that purpose, sea bass juveniles were fed four diets containing graded levels of ARA as follows: 0.5% (ARA0.5), 1% (ARA1), 2% (ARA2) and 4% (ARA4) during 60 days. At the end of the feeding trial fatty acid profiles of plasma and head kidney leucocytes were analyzed. Besides, plasma prostaglandins levels, head kidney leucocytes respiratory burst activity; peroxidase activity and phagocytic index were assayed. Reducing dietary ARA levels below 1% markedly reduced European sea bass growth performance. However, fish fed diet ARA0.5 tried to compensate this dietary ARA deficiency by a selective deposition of ARA on plasma and head kidney leucocytes, reaching similar levels to those fish fed diet ARA1 after 60 days of feeding. Nevertheless, head kidney phagocytic capacity was reduced as dietary ARA content in relation not only to variations on membrane composition but also to changes on fish basal prostaglandins levels. Results obtained demonstrated the importance to supply the necessary quantity n-6 LC-PUFA, and not only n-3 LC-PUFA levels, in European sea bass diets, in relation to not only growth performance but also immune system function.

1. Introduction

Long chain polyunsaturated fatty acids (LC-PUFA) have important roles in the regulation of fish growth performance, lipid metabolism, cell membrane structure and immune function [1–3]. During the past decades, most of the studies addressing LC-PUFA optimum marine fish dietary levels in relation to their biological and physiological role have been focused on n-3 LC-PUFA, particularly on docosahexaenoic acid (DHA, 22:6n−3) and eicosapentaenoic acid (EPA, 20:5n−3) [4–7]. However, studies focused on arachidonic acid (ARA, 20:4n-6) have been partially overlooked mainly due to its minor content as membrane component compared to DHA and EPA [8]. Indeed, it has been along the last years when more attention has been paid to ARA as essential fatty acid (EFA) in marine fish nutrition, predominantly due to its role as eicosanoid precursor [9,10]. ARA is recognized to be a main precursor of eicosanoids, the 2-series of prostaglandins (PGEs) and thromboxanes (TXs) and the 4-series of leucotrienes (LTs) and lipoxines thorough the action of cyclooxygenase (COX) and lipoxygenase enzymes [11]. Eicosanoids are involved in the regulation of several physiological processes, including reproduction, growth and development, immune system or stress response [12], thus playing a crucial role on the entire fish life cycle [13]. For instance, dietary ARA influences fish growth, survival and tissue fatty acid profiles [14–16], being preferentially retained in various fish

* Corresponding author. Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Crta. Taliarte s/n, 35214 Telde, Las Palmas, Canary Islands, Spain.
E-mail address: silvia.torrecillas@giaqua.org (S. Torrecillas).

http://dx.doi.org/10.1016/j.fsi.2017.03.041
1050-4648/

species during starvation [17]. Besides, ARA plays an important role in the regulation of reproduction [4,5,18–22], lipid metabolism [21,23], metamorphosis [24,25] pigmentation [26–29] and resistance to several stressors [30–34]. In terms of immune system function, the role of ARA is widely recognized, being preferentially retained in several fish species leucocytes [35–40]. Indeed, ARA deposition in membrane phospholipids influences cell membrane fluidity and stability by affecting several signaling pathways, ion transport, trafficking and vesicular transport and membrane-associated enzymes activities [3,41,42]. Besides, ARA alters the transcription of gene-encoding proteins involved in lipid metabolism that directly affect immune-related transcription factors (i.e. modulating the NF-kB) [43] and is involved in the assembly and activation of NADPH oxidase activity [40,44]. Eicosanoids derived from ARA, such as PGE_2 and LTB_4, increase vascular permeability and vasodilatation, induce leucocytes chemotaxis and promote generation of reactive oxygen species (ROS) [45]. Indeed, due to its role as precursor in eicosanoid synthesis, ARA has been also associated with regulation of cytokine release [3,45–48]. Several studies in fresh water species point to the effectiveness of an appropriate level of dietary ARA in modulation of immune system function and disease resistance [15,32,49]. For example in guppies (*Poecilia reticulate*) dietary enrichment with arachidonic acid (ARA)-rich triacylglycerols fraction improves disease resistance against *Tetrahymena* sp. [15]. Besides, dietary ARA affects leukocyte relative distribution following intraperitoneal injection with formalin-fixed *Staphylococcus aureus* in striped bass (*Morone saxatilis*) [49] and an optimum dietary ARA supplementation increases superoxide dismutase (SOD) and lysozyme activities in Japanese eel (*Anguilla japonica*) [50].

Likewise, dietary ARA levels (0.36–0.56% total FA) in juvenile Japanese sea bass (*Lateolabrax japonicus*) increase serum lysozyme, alternative complement pathway (ACP) and superoxide dismutase (SOD) activities, although do not affect respiratory burst activity of head kidney leucocytes and serum catalase (CAT) [8]. ARA levels in juvenile turbot (*Scophtalamus maximus*) regulate tissue PGE_2 and 6-ketoPGF$_1$ levels [51]. Feeding European sea bass with low ARA levels derived from dietary VO reduces plasma PGE_2 content [52], though PGEs production has been described to markedly differ among tissues of the same fish species [53]. Besides, fish immune system function is directly affected by dietary n-3/n-6 ratio [40,54], due to n-3 LC-PUFA and n-6 LC-PUFA interactions.

Despite the importance of European sea bass on European aquaculture production, the information available concerning its nutritional requirements is still incomplete compared to other cultured fish species such as salmonids or carps [55–57], and particularly those addressing n-6 LC-PUFA effects on European sea bass immune system. Thus, the aim of the present study is to assess the effects of graded levels of dietary ARA, supplemented from alternative sources, on European sea bass juvenile's fatty acid composition of plasma and head kidney leucocytes in relation to its immune potential.

2. Material and methods

All animal manipulation described in this paper, comply with the guidelines of the European Union Council (86/609/EU) and Spanish legislation (RD 1201/2005) for the use of laboratory animals and have been approved by the Bioethical Committee of the University of Las Palmas de Gran Canaria. The experimental tanks used within the present experiments are located at the aquaculture facilities belonging to the University of Las Palmas de Gran Canaria.

2.1. Experimental diets

Four isolipidic and isoproteic experimental dry pelleted diets based on a commercial formulation for European sea bass juvenile were prepared to contain 0.59%, 1%, 2%, and 4% of ARA, respectively. The desired ARA content was completed with commercially available ARA oil obtained from *Mortierella alpina* (Vevodar®, DSM Food Specialties, Netherlands). Supplementation of DHA and EPA was done using DHA50 and EPA50 (CRODA, East Yorkshire, UK). Diet ingredients, fatty acid profiles and proximate composition are detailed in Tables 1 and 2.

2.2. Experimental conditions

Six hundred and seventy two European sea bass juveniles were randomly stocked in 12 200 L fiber-glass tanks (56 individual per tank) with an average initial weight of 13.4 ± 0.29 g (mean ± SD) (3.7 kg m^{-3} initial stocking density). Diets were assayed by triplicate. Tanks were supplied in a flow-through system with sea water, at a temperature of 22.8–24.9 °C, and natural photoperiod (12 L:12D). Water dissolved oxygen ranged between 5 and 7 ppm. Fish were fed by hand until apparent satiation, three times a day, six days a week during 60 days.

At the end of the experimental period, whole fish population was sampled for determining survival and growth parameters. All animals were fasted for 24 h before sampling. Then, six animals per experimental tank (18 per diet) were euthanized by an anesthetic overdose (clove oil), and samples of blood were obtained by caudal sinus puncture using 1 ml heparinized syringes. Samples of plasma were obtained after immediate centrifugation of whole blood at

Table 1
Experimental diets main ingredients (g·kg^{-1}) and proximate composition (mean ± SD).

Ingredients	ARA0.5	ARA1	ARA2	ARA4
Fish Meal[a]	–	52.50	52.50	52.50
Fish oil[a]	–	14.50	12.60	11.40
Defatted Fish Meal[b]	46.50	–	–	–
Corn Meal[c]	7.00	6.00	6.00	6.00
Soy 44 Meal[c]	10.00	10.00	10.00	10.00
Wheat Meal[c]	5.50	5.50	5.50	5.50
Wheat Gluten[c]	7.00	7.00	7.00	7.00
Vegetable fats and oils[c]	14.50	0.00	0.00	0.00
Vitamins Mix[d]	2.00	2.00	2.00	2.00
Mineral Mix[e]	2.00	2.00	2.00	2.00
CMC[f]	0.50	0.50	0.50	0.50
ARA[g]	–	–	0.50	1.50
DHA and EPA[h]	5.00	–	1.40	1.60
Diets Proximate composition (g·kg^{-1}; d.w.)				
Crude Lipids	20.77	21.33	20.87	21.12
Crude Protein	43.71	43.32	44.93	44.61
Ash	9.75	10.51	10.47	10.39
Moisture	8.94	6.57	7.63	7.25

[a] Fish meal and oil, South American origin, (65% protein, 12% lipid).
[b] Defatted soymeal (GIA laboratory produced by 3 × chloroform extraction; 73% protein, 2% lipid).
[c] Vegetable ingredients locally found (SBM:46% protein, 3% lipid).
[d] Vitamin premix contains (mg kg−1 or IU/kg of dry diet): thiamine 40 mg, riboflavin 50 mg, pyridoxine 40 mg, calcium pantothenate 117 mg, nicotinic acid 200 mg, biotin 1 mg, folic acid 10 mg, cyanocobalamin, 0.5 mg, choline chloride 2700 mg, Myo-inositol 2000 mg, ascorbic acid 5000 mg, menadione 20 mg, cholecalciferol 2000 IU, ethoxyquin 100 mg, retinol acetate 5000 IU.
[e] Mineral premix contains (g/kg of dry diet): calcium orthophosphate 1.60 g, calcium carbonate 4 g, ferrous sulphate 1.5 g, magnesium sulphate 1.6 g, potassium phosphate 2.8 g, sodium phosphate 1 g, aluminium sulphate 0.02 g, zinc sulphate 0.24 g, copper sulphate 0.20 g, manganese sulphate 0.08 g, potassium iodate 0.02 g.
[f] Carboxymethyl cellulose (sodium salt, Sigma-Aldrich, Munich, Germany).
[g] Vevodar®, DSM Food Specialties, Netherlands.
[h] DHA50 and EPA50, CRODA, East Yorkshire, UK.