Fish and Shellfish Immunology 79 (2018) 209–217

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

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

Full length article

Humoral and mucosal immune responses in meagre (*Argyrosomus regius*) juveniles fed diets with varying inclusion levels of carob seed germ meal

Francisco Antonio Guardiola[a,1], Carolina Barroso[a,b,1], Paula Enes[a,1], Ana Couto[a], Patricia Díaz-Rosales[a], António Afonso[a,c], Erika Kanashiro[b], Helena Peres[a], Elisabete Matos[d], Aires Oliva-Teles[a,b], Pedro Pousão-Ferreira[e], Benjamín Costas[a,c,*]

[a] *Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208, Porto, Portugal*

[b] *Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, Ed. FC4, 4169-007, Porto, Portugal*

[c] *Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313, Porto, Portugal*

[d] *Sorgal, Sociedade de Óleos e Rações S. A., EN 109, Lugar da Pardala, 3880-728, S. João Ovar, Portugal*

[e] *Instituto Português do Mar e da Atmosfera (IPMA), Estação Piloto de Piscicultura de Olhão, Av. 5 de Outubro s/n, 8700-305, Olhão, Portugal*

ARTICLE INFO

Keywords:
Carob seed germ meal
Fish meal replacement
Alternative protein sources
Cell-mediated immunity
Humoral and mucosal immunity
Meagre (*Argyrosomus regius*)

ABSTRACT

Many studies have assessed the effects of incorporation of plant feedstuffs in fish diets on growth performance, whereas few studies have addressed the effects of fish meal replacement by plant protein sources on fish immune parameters. Thus, the aim of this study was to evaluate the effects on immune response of different inclusion levels of carob seed germ meal (CSGM) as partial replacement for fish meal in diets for meagre (*Argyrosomus regius*) juveniles. Fish were fed four experimental diets with increased CSGM inclusion levels [0% (control), 7.5% (CSGM7.5), 15% (CSGM15) and 22.5% (CSGM22.5)]. After 1, 2, and 8 weeks of feeding fish were sampled to determine haematological profile and several humoral parameters in plasma and intestine. Results showed that dietary inclusion of CSGM did not negatively affect the immune parameters of meagre. In addition, total numbers of red and white blood cells, as well as thrombocytes, lymphocytes, monocytes, and neutrophils counts were not affected by dietary treatments. All parameters evaluated in plasma were unaffected by dietary CSGM inclusion after 1 and 2 weeks of feeding, with only the haemolytic complement activity showing an increase in fish fed diets with CSGM after 1 week and in fish fed CSGM22.5 diet after 2 weeks. Regarding the innate immune parameters analysed in the intestine, it could be highlighted the increase in alkaline phosphatase and anti-protease activities in fish fed the diet with the higher inclusion of CSGM at 8 weeks. Overall, results suggest that high dietary CSGM inclusion do not compromise immune status or induce an inflammatory response in meagre juveniles.

1. Introduction

The aquaculture sector has rapidly expanded worldwide to meet the increasing demand for seafood; consequently, increasing amounts of raw feed materials are needed by this industry to sustain this rapid growth [1]. In the last years, replacement of fish meal (FM) by sustainable, low-cost, and eco-friendly plant protein (PP) sources is a major trend in aquafeeds [2]. To date, FM has been the most important protein source in aquafeeds, especially for carnivorous fish species, because it is an excellent source of high quality protein, essential nutrients, attractants, and potentially unidentified growth factors [3]. Nonetheless, it is not expected that FM production will increase to supply the growing demand for aquafeed protein with the intense expansion of aquaculture. Therefore the search for alternative protein sources needs to be intensified [4,5].

Fish nutritional status can be considered a major factor that modulates immune mechanisms, acting as immunostimulator or immunosupressor [6–8]. The potential to modulate the activity of the immune system by supplying additional resources to the animal and therefore providing improved protection has been reported in the last years. However, the incorporation of PP sources in carnivorous fish diets can lead to nutritional imbalances, inflammation process, and

* Corresponding author. Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208, Porto, Portugal.
E-mail address: bcostas@ciimar.up.pt (B. Costas).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.fsi.2018.05.027
Received 16 January 2018; Received in revised form 13 May 2018; Accepted 14 May 2018
1050-4648/

physiological disorders, among others (reviewed by Ref. [9]), due to the presence of antinutritional factors (ANFs) and deficiency in certain indispensable amino acids [5,10]. Consequently, immune function may be affected and this represents a major obstacle in the maintenance of health and disease resistance [7].

Many studies have currently assessed the incorporation of plant feedstuffs in fish diets on growth performance of different fish species. However, few studies have addressed the effects of FM replacement by PP sources in fish immune parameters [11–16]. For instance, Bransden et al. [11] did not observe changes in immune parameters of Atlantic salmon (*Salmo salar*) fed diets with 40% of lupin (*Lupinus angustifolius*) meal replacement. Sahlmann et al. [16] tested the early responses of Atlantic salmon fed a 20% soybean meal (SBM) diet and verified an up-regulation of several immune related genes in the first 3–5 days following first feeding. More recently, Azeredo et al. [15] observed changes in immune parameters of European seabass (*Dicentrarchus labrax*) fed diet with inclusion of different PP at a ratio of 30 FM:70 PP.

Carob seed germ meal (CSGM) has the potential to compete with SBM due to its reduced cost, similar percentage of protein (45–50%), a relatively balanced amino acid profile, and high production in Mediterranean countries [17–19], thus contributing to the sustainability of fish-farming in the Mediterranean region. Despite this, few studies assessed CSGM inclusion in fish diets [19–23]. Significant alterations were observed in growth, haematological parameters, and intestine morphology when dietary CSGM increased over 45% in rainbow trout (*Oncorhynchus mykiss*) [20] and 52% in gilthead seabream (*Sparus aurata*) [22]. In these studies, a dose-dependent effect was verified, the worst results being observed with the highest dietary CSGM inclusion levels. In meagre, a previous study at our laboratory showed that inclusion of CSGM replacing dietary FM as protein source, was suitable up to 22.5% [23].

In contrast, a decrease of digestive enzymes activity was observed, together with decreased protein retention in fish fed the higher levels of CSGM. At the histomorphology level, lipid deposition was not affected in the liver, while distal intestine showed hyaline droplets of varying sizes with the increase of CSGM levels in the diets. However, no structural signs of inflammation were recorded in the intestine [23]. This is interesting, since the inclusion of ANFs, such as soybean saponins, into fish diets has been associated with morphological changes and inflammation of the intestinal epithelium in several species, such as Atlantic salmon (*Salmo salar*, L.), common carp (*Cyprinus carpio* L.) and Japanese flounder (*Paralichthys olivaceus* L.) [24–26].

Thus, the aim of this study was to evaluate the effects of different dietary inclusion levels of CSGM on the immune status of meagre (*Argyrosomus regius*), an emerging species for Mediterranean aquaculture diversification. Due to the limited knowledge of CSGM effects on fish immune status, cell-mediated, humoral and mucosal intestine immune responses were assessed in meagre juveniles fed diets including CSGM, in order to gather health-related information and to confirm the suitability of the newly developed plant-based diets in farmed fish.

2. Material and methods

2.1. Fish and experimental diets

Meagre (*Argyrosomus regius*) juveniles were obtained from IPMA-EPPO facilities (Olhão, Portugal) and transported to the Marine Zoology Station facilities (Porto University, Porto, Portugal). Fish were submitted to a quarantine period of 30 days after transportation. During this period, fish were fed with a commercial diet (56% crude protein and 22% crude lipids).

Four experimental diets were formulated to be isonitrogenous (53% crude protein) and isolipidic (18% crude lipids) and to meet the nutritional requirements for the species. A fish meal (FM) based diet was used as control whereas CSGM was incorporated in the other three diets at increasing levels, 7.5% (CSGM7.5), 15% (CSGM15), and 22.5% (CSGM22.5), at expenses of FM (Table 1). Before use, CSGM was autoclaved (110 °C; 10 min; 0.4 bar) and then dried in an oven (60 °C) for 24 h. All dietary ingredients were finely ground, well mixed and dry pelleted in a laboratory pellet mill (California Pellet Mill, CPM Crawfordsville, IN, USA) through a 3 mm die. The pellets were then dried in an oven (40 °C) for 24 h and stored at −20 °C until used. Chemical analysis of the experimental diets was done as described in Couto et al. [23]. Amino acid composition of FM and CSGM, and of the experimental diets are presented in Couto et al. [23].

Table 1
Ingredient composition and proximate analysis of the experimental diets (control, CSGM7.5, CSGM15 and CSGM22.5) [23].

	Diets			
	Control	CSGM7.5	CSGM15	CSGM22.5
Ingredients (% dry weight basis)				
Fish meal[a]	66.4	61.8	57.1	52.4
Wheat meal[b]	18.6	15.7	12.8	9.9
Fish oil	11.5	11.6	11.7	11.8
Carob seed germ meal[c]	–	7.5	15.0	22.5
Vitamin premix[d]	1.0	1.0	1.0	1.0
Mineral premix[e]	1.0	1.0	1.0	1.0
Choline chloride	0.5	0.5	0.5	0.5
Binder[f]	1.0	1.0	1.0	1.0
Proximate analysis (% dry weight basis)				
Dry matter	92.4	94.7	92.2	93.5
Crude protein	52.5	52.5	53.2	52.5
Crude fat	17.8	17.1	17.9	18.0
Ash	14.5	13.7	13.9	13.3
Starch	10.9	8.3	8.2	6.2
Gross Energy (KJ g^{-1} DM)	22.7	22.4	22.7	22.9

DM, dry matter; CP, crude protein; GL, gross lipid.
[a] Pesquera Diamante, Steam Dried LT. Austral Group, S.A. Peru (CP: 71.4% DM; GL: 9.3% DM).
[b] Sorgal, S.A. Ovar, Portugal (CP: 13.9% DM; GL: 1.8% DM).
[c] Sorgal, S.A. Ovar, Portugal (CP: 50.0% DM; GL: 5.2% DM).
[d] Vitamins (mg kg^{-1} diet): retinol, 18 000 (IU kg^{-1} diet); cholecalciferol, 2000 (IU kg^{-1} diet); alpha tocopherol, 35; menadion sodium bis., 10; thiamin, 15; riboflavin, 25; Ca pantothenate, 50; nicotinic acid, 200; pyridoxine, 5; folic acid, 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbyl monophosphate, 50; inositol, 400.
[e] Minerals (mg kg^{-1} diet): cobalt sulphate, 1.91; copper sulphate, 19.6; iron sulphate, 200; sodium fluoride, 2.21; potassium iodide, 0.78; magnesium oxide, 830; manganese oxide, 26; sodium selenite, 0.66; zinc oxide, 37.5; potassium chloride, 1.15 (g kg^{-1} diet); sodium chloride, 0.44 (g kg^{-1} diet).
[f] Aquacube. Agil, England (guar gum, polymethyl carbamide, manioc starch blend, hydrate calcium phosphate).

2.2. Experimental conditions

The trial was conducted in a thermoregulated recirculating water system, equipped with a battery of fiberglass tanks (100 L capacity) supplied with a continuous flow of filtered seawater (6.0 L min^{-1}). Twelve groups of 9 fish with an initial mean body weight of 35.00 ± 0.01 g were randomly established in each tank and each diet was randomly assigned to triplicate groups. Fish were fed by hand until apparent visual satiation, 6 days a week, during 8 weeks. Utmost care was taken to assure that all feed supplied was consumed. During the trial, water temperature averaged 23.6 ± 0.9 °C; salinity 37.5 ± 0.8 g L^{-1}; dissolved oxygen kept near saturation (> 7.0 mg L^{-1}); and fish were subject to a 12 h light/12 h dark photoperiod regime.

This experiment was directed by trained scientists (following FELASA category C recommendations) and conducted according to the European Union Directive 2010/63/EU on the protection of animals for scientific purposes.