Aquaculture 498 (2019) 171–180

Contents lists available at ScienceDirect

Aquaculture

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

Interactive effects of dietary vegetable oil and carbohydrate incorporation on the innate immune response of European seabass (*Dicentrarchus labrax*) juveniles subjected to acute stress

Marina Machado[a,b,*], Carolina Castro[a,c], Aires Oliva-Teles[a,c], Benjamín Costas[a,b]

[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, Matosinhos, Portugal
[b] Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[c] Departamento de Biologia, Faculdade de Ciências da Universidade do Porto (FCUP), Rua do Campo Alegre 1021/1055, 4169-007 Porto, Portugal

ARTICLE INFO

Key-words:
Vegetable oils
Fish oil replacement
Carbohydrates
Immune responses
Acute stress
Cortisol

ABSTRACT

This study aims to understand the interactive effects of high dietary vegetable oils (VO) and carbohydrate (CH) content in European seabass cellular, humoral, and molecular innate immune responses to an acute stress. For that purpose, European sea bass juveniles (74.0 ± 1.5 g) were fed four diets differing in lipid source (fish oil (FO) or a blend of vegetable oils (VO)) and carbohydrate content (0% (CH–) or 20% (CH+) gelatinized starch). Nine fish per dietary treatment were sampled after 73 days of feeding and used as control whereas the remaining fish were subjected to an acute stress (netting, 1 min air exposure, and transfer to smaller tanks). Those fish were then sampled after 1 h and considered the stressed group. In the present study, dietary VO incorporation affected fish humoral immune parameters with a decrease of haematocrit, plasma peroxidase activity, and head-kidney *mc2r* and *gr1* mRNA expression levels. Regardless of dietary treatment, higher cortisol levels were accompanied by an increase of several haematological, cellular, and humoral parameters in response to an acute stress. Dietary VO incorporation led to lower neutrophil numbers, plasma antiproteases activity, and head-kidney *cox2* expression level in stressed fish. Fish fed CH+ diets showed a reduction of head-kidney *mc2r* expression levels in response to stress, while plasma cortisol remained unchanged, plasma NO decreases and antiproteases activity increased. In fish fed the VO diets, CH+ led to a decrease of plasma bactericidal activity compared to that of group CH-, while the opposite pattern was observed for plasma peroxidase. In conclusion, dietary substitution of FO by VO in CH– diet negatively affected the immune response in both undisturbed and stressed fish, while the effect of dietary CH incorporation in VO diet was not clear.

1. Introduction

Diets provided in aquaculture are generally species-specific and well-balanced, yet there is an increasing need to replace the use of some dietary ingredients, namely those of fisheries origin, leading to significant deviations from fish natural diets (Montero and Izquierdo, 2010). Together with the limited availability and increasing demand of fish oils (FO) and fish meal (FM), the higher availability and lower price of vegetable oils (VO) and plant proteins (PP) make these feedstuffs the most viable and practical alternatives to fisheries-based ingredients in aquafeeds (Montero et al., 2015; Hardy and Tacon, 2002). Despite being a suitable alternative to FO (Castro et al., 2015a, 2015b), VO are devoid of n-3 long-chain poly-unsaturated fatty acids (LC-PUFA), thus altering diet and fish fatty acid profiles (Bell et al., 2002), and decreasing LC-PUFA availability for important biological processes such as immune function. In addition, the use of plant feedstuffs (PF) in diet formulation increases dietary carbohydrate (CH) content, which in high quantities are not well utilized by carnivorous fish species (Dias et al., 2004), such as European seabass (*Dicentrarchus labrax*, Linnaeus, 1758). In this sense, recent studies have been focusing on the effects of dietary incorporation of PF and VO and the interaction between VO and CH on fish growth, body composition, and lipid metabolism (Castro et al., 2015a, 2015b). However, the concept of optimal nutrition is currently also associated with fish welfare, and well-balanced diets must provide nutrients that assure good health and allow fish to cope with stressful situations (Calder, 2006a, 2006b; Montero et al., 2010). In this sense,

* Corresponding author at: Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Novo Edifício do Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos S/N, 4450-208 Matosinhosdos, Portugal.
E-mail address: mcasimiro@ciimar.up.pt (M. Machado).

https://doi.org/10.1016/j.aquaculture.2018.08.050
Received 13 March 2018; Received in revised form 20 August 2018; Accepted 22 August 2018
Available online 27 August 2018
0044-8486/

dietary lipid source and the resultant n-3 to n-6 fatty acids profiles are key for adequate cell function and structure, tissue integrity, cell signaling, and eicosanoids production, but also for the whole immune response (Calder, 2006a, 2006b; Yaqoob and Calder, 2007). While being rich in C18 fatty acids as linoleic acid, α-linolenic acid, and oleic acid, VO lack LC-PUFA such arachidonic acid (20:4n-6, ARA), eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22: 3n, DHA) (Torstensen et al., 2005), which have key roles on fish health (Montero et al., 2010). Therefore, dietary VO incorporation may negatively affect fish health (Fracalossi et al., 1994).

It is widely known that CH are important energy sources, and a limited CH dietary content may be important for maintaining animal health (Wu et al., 2016). However, replacement of FM by PP may increase dietary CH content above recommended levels (Dias et al., 2004) and negatively affect innate immunity and ultimately increase disease susceptibility (Fletcher, 1997; Wu et al., 2016).

In the context of current fish feed formulations, it is also mandatory to understand potential interactions between dietary CH and lipid source to define a threshold for dietary FM and FO replacement while ensuring nutritional quality and fish health. Even though a few studies have already focused on mechanisms by which dietary lipids source or CH content may modulate fish immune response (Montero et al., 2003, Montero and Izquierdo 2010, Montero et al., 2010, Wu et al., 2016), there is a considerable gap of knowledge on the interaction between dietary VO inclusion and CH content upon immune activation by a stress stimulus. Having this in mind, the main goal of this study was to evaluate potential interactive effects of high dietary VO and CH content in European seabass innate immune system in response to a multifactorial acute stress.

2. Material and methods

2.1. Experimental diets

Four diets differing in lipid source (FO or VO blend) and carbohydrate supplementation (0% and 20% gelatinized starch, CH– and CH+, respectively) were formulated (Table 1). FM was added as a major dietary protein source to isolate the impacts of dietary VO and to avoid the interference of dietary plant protein on the evaluated parameters. The VO blend was composed of rapeseed (20%), linseed (50%), and palm (30%) oils, and replaced about 70% of dietary lipids, provided by cod liver oil and FM in the FO diet. All ingredients were finely ground, well mixed and dry pelleted in a laboratory pellet mill (California Pellet Mill, Crawfordsville, IN, USA), through a 3-mm die. The pellets were air dried for 24 h and stored in a refrigerator (4 °C) until use. Dietary carbohydrate content was increased at the expenses of dietary protein, which was kept above requirement for the species in all diets (Oliva-Teles, 2000). Details on diet preparation and analysis are given in Castro et al. (2016). Diets presented some differences in fatty acids (FA) composition due to the lipid sources (see (Castro et al., 2016)). Briefly, the proportion of total saturated fatty acids (SFA) was similar among diets, but monounsaturated fatty acids (MUFA) were higher in FO diets, and n-3 and n-6 polyunsaturated fatty acids (n-3, n-6 PUFA) were slightly higher in VO diets. Within MUFA, higher levels of oleic acid (18:1 n-9) were recorded in VO diets, while the opposite occurred for palmitoleic acid (16:1 n-7), eicosenoic acid (20:1 n-9), and erucic acid (22:1 n-9). FO diets had higher levels of EPA and DHA than VO diets. Total n-3 and n-6 PUFA were higher in VO diets, mainly due to linolenic acid (18:3 n-3) and linoleic acid (18:2 n-6) levels, respectively.

2.2. Animals, experimental design

This study 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 purpose. The trial was performed at the Marine Zoological Station, University of Porto, Portugal, with European seabass (*Dicentrarchus labrax*) juveniles (initial body weight: 74.0 ± 1.5 g). After a quarantine period, twenty fish were randomly distributed into each of 12 fiberglass of 300 L cylindrical tank, in a thermo-regulated recirculation water system with salinity averaging 35 ± 1.0 g l^{-1}; dissolved oxygen maintained at 90%, temperature regulated to 25.4 ± 0.5 °C, and photoperiod adjusted to 12 h light/12 h dark. After 2 weeks of adaptation to the experimental conditions, the experimental diets were randomly assigned to triplicate groups of fish, and the animals were hand-fed twice a day, 6 days a week, to apparent visual satiety for 73 days. Food intake and mortality were daily recorded. Growth performance of fish during the 73 days feeding period was not the aim of this study, and results were presented elsewhere (Castro et al., 2015a, 2015b). In brief, growth performance and feed efficiency were not affected by dietary treatments and final body mass averaged 221.7 ± 5.0 g. At the end of the feeding period, 3 fish were randomly collected from each tank after 18 h fasting and euthanized with a sharp blow to the head before sampling. These fish were used as the control, undisturbed group. Then, other three fish per tank ($n = 9$) was subjected to 1 min air exposure and then transferred to tanks of 100 l capacity. Fish were sampled after 1 h and considered the stressed group. Such a handling procedure (i.e. netting, air exposure, and transfer to smaller tanks) was considered a multifactorial acute stress. In both sampling groups, fish were weighed and sampled for blood and head-kidney collection.

Table 1
Ingredient and chemical composition of the experimental diets.

Diets	FO CH-	FO CH+	VO CH-	VO CH+
Ingredients (% dry weight)				
Fish meal[a]	86.5	64.5	86.5	64.5
Gelatinized maize starch[b]	0	20	0	20
Cod liver oil[c]	10	12	0	0
Vegetable oil blend[d]	0	0	10	12
Vitamins[e]	1.5	1.5	1.5	1.5
Minerals[f]	1.0	1.0	1.0	1.0
Binder[g]	1.0	1.0	1.0	1.0
Proximate Composition (% DM)				
Dry matter (DM)	89.5	90.4	91.3	91.0
Crude protein (CP)	62.4	46.6	62.4	47.1
Crude fat (CF)	18.4	18.4	18.2	18.3
Starch	0.8	18.7	0.8	17.5
Ash	17.6	14.0	17.6	13.8
Cholesterol	0.5	0.3	0.4	0.3

[a] Steam dried LT fish meal, Pesquera Diamante, Perú (CP:71.1% DM; GL: 8.8% DM).
[b] C-Gel Instant-12,018, Cerestar, Mechelen, Belgium.
[c] Labchem, Laborspirit Lda, Lisboa, Portugal.
[d] 30% palm oil (Colmi, Malasia), 50% linseed oil (Sociedade Portuense de Drogas, S.A.,Portugal) and 20% rapeseed oil (Huilerie Emile Noël S.A.S., France).
[e] Vitamins (mg kg^{-1} diet): retinol acetate, 18,000 (IU kg^{-1} diet); cholecalciferol, 2000 (IU kg^{-1} diet); alpha tocopherol acetate, 35; sodium menadione bisulphate, 10; thiamin-HCl, 15; riboflavin, 25; calcium pantothenate, 50; nicotinic acid, 200; pyridoxine HCl, 5; folic acid 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbic acid, 50; inositol, 400. Premix, Viana do Castelo, Portugal.
[f] 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; sodiumselenite, 0.66; zinc oxide, 37.5; dibasic calcium phosphate, 5.93 (g kg^{-1} diet); potassium chloride, 1.15 (g kg^{-1} diet); sodium chloride, 0.40 (g kg^{-1} diet). Premix, Viana do Castelo, Portugal.
[g] Aquacube (Guar gum, polymethyl carbamide, Manioc starch blend, hydrate calcium sulphate). Agil, England.

2.3. Haematological procedures

Blood was collected from the caudal vein using heparinized syringes. The haematological profile was assessed by the determination of