Veterinary Parasitology 175 (2011) 141–150

Contents lists available at ScienceDirect

Veterinary Parasitology

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

# The nutritional background of the host alters the disease course in a fish–myxosporean system

Itziar Estensoro[a,1], Laura Benedito-Palos[b,1], Oswaldo Palenzuela[a], Sadasivam Kaushik[c], Ariadna Sitjà-Bobadilla[a,*], Jaume Pérez-Sánchez[b]

[a] Fish Pathology Group, Department of Marine Species Biology, Culture and Pathology, Instituto de Acuicultura de Torre de la Sal (CSIC), 12595 Ribera de Cabanes, Castellón, Spain
[b] Fish Nutrition and Growth Endocrinology Group, Department of Marine Species Biology, Culture and Pathology, Instituto de Acuicultura de Torre de la Sal (CSIC), 12595 Ribera de Cabanes, Castellón, Spain
[c] UMR 1067, Nutrition, Aquaculture & Genomics, INRA, Unité-Mixte INRA-IFREMER-Univ. Bordeaux I, 64310 Saint-Pée-sur-Nivelle, France

ARTICLE INFO

*Article history:*
Received 2 April 2010
Received in revised form 7 September 2010
Accepted 15 September 2010

*Key words:*
Myxozoa
*Enteromyxum*
Parasites
Immune response
*Sparus aurata*
Anorexia
Vegetable oils

ABSTRACT

The aim of the present work was to determine if a practical plant protein-based diet containing vegetable oils (VO) as the major lipid source could alter the disease course when challenged with the myxosporean *Enteromyxum leei*, a wide-spread parasite in the Mediterranean basin causing heavy economic losses. Gilthead sea bream (*Sparus aurata*) fed for 9 months either a fish oil (FO) diet or a blend of VOs at 66% of replacement (66VO diet) were challenged by exposure to parasite-contaminated water effluent. All fish were periodically and non-lethally sampled to obtain biometrical data and to know their infection status. After 102 days of exposure, fish were euthanized and haematological, biometrical, histological, immunological, glutathione and anti-oxidant data were obtained from tissue, blood and serum samples. Anorexia appeared in both exposed groups, but feed intake reduction was higher in 66VO fish. The signs of disease (lower growth, condition factor, specific growth rate, haematocrit) as well as the disease course were worse in fish from 66VO group, with a higher prevalence and intensity of infection, a higher percentage of fish harbouring the parasite in the entire intestinal tract, and a faster establishment of the parasite. Parasite intensity of infection was negatively correlated with growth parameters and haematocrit in both groups, and with complement, lysozyme and hepatic total glutathione in 66VO fish.



## 1. Introduction

Parasites are a major constraint on animal production through the world, and recent cases of massive losses in salmon culture due to sea lice are an outstanding example (Costello, 2009). Economic losses are due not only to mortality but also to poor growth performance, low reproduction efficacy, emaciation or other external signs that turn fish into unmarketable products (Sitjà-Bobadilla, 2004, 2009; Guo and Woo, 2009). Gilthead sea bream (*Sparus aurata*) is the main cultured fish species in the Mediterranean area, with a production of more than 150,000 tonnes in 2008 (APROMAR, 2009). Diseases and feed costs are the main limitations for enhancing the productivity. *Enteromyxum leei* is a widely spread myxosporean responsible for one of the most threatening parasitic diseases in Mediterranean fish cultures (Palenzuela, 2006). This parasite invades the intestine of gilthead

* Corresponding author at: Instituto de Acuicultura de Torre de la Sal, Consejo Superior de Investigaciones Científicas, Torre de la Sal s/n, 12595 Ribera de Cabanes, Castellón, Spain. Tel.: +34 964319500; fax: +34 964319509.
*E-mail address:* ariadna@iats.csic.es (A. Sitjà-Bobadilla).
[1] These authors contributed equally to this article.

0304-4017/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2010.09.015

sea bream producing a slow-progressing disease, which induces anorexia, cachexia and eventually the death of fish. Its impact is further enhanced due to its direct fish-to-fish transmission either by cohabitation with infected fish, by contact with contaminated effluent and *per os* (reviewed in Sitjà-Bobadilla et al., 2007). Thus far, there are neither preventive nor curative treatments for enteromyxosis. Therefore, there is an urgent need in advancing our knowledge of the parasite itself and the host-parasite interaction.

Substitution of fish meal (FM) and fish oil (FO) by optimised levels of vegetable ingredients stands as one of the current strategies for reducing the cost of fish feeds and the dependency on fisheries to produce aquafeeds (Tacon and Metian, 2008). While using such alternative ingredients, not only the possible effects on growth performance, but also animal health and welfare should be analyzed in an integrative manner. In this context, studies on the effect of FO substitution by vegetable oils (VO) on fish health and the possible nutritional modulation of resistance to infectious diseases are of major importance. In previous works in gilthead sea bream, it has been demonstrated that FO can be replaced in plant-protein based diets up to 66% without detrimental effects on growth, redox balance, immunocompetence or on the intestinal and hepatic architecture (Benedito-Palos et al., 2007, 2008, 2009; Saera-Vila et al., 2009). Thus, a further step was to test whether plant proteins and VO optimised diets could alter the disease outcome when confronted with a pathogen. For this purpose, chronic exposure to *E. leei* by contact with parasite-containing water was chosen to mimic the natural infections. The present work aimed to determine the effects of dietary fat sources on infection levels, growth performance, host immune response and protection against oxidative stress.

## 2. Material and methods

### 2.1. Experimental design and sampling procedure

Naïve gilthead sea bream were obtained from a commercial fish farm and checked for the absence of the parasite (see below). Fish were divided into two experimental groups, which were fed two different diets (Supplementary Table S1). After 9 months of feeding with the corresponding two diets, 60 fish from each diet were individually tagged with passive integrated transponders (PIT-tags) (Trovan, Spain), transferred to the Pathology unit of the Instituto de Acuicultura de Torre de la Sal (IATS) and acclimated for 2 weeks before the parasite challenge. Each diet group was divided again into two groups, control (C, $n = 30$) and recipient (R, $n = 30$) in 500-L fibre-glass tanks. The average initial weight before the challenge was 223.7 g. The two R-tanks (one for each dietary treatment) were exposed to *E. leei*-contaminated effluent as previously described (Sitjà-Bobadilla et al., 2007). Briefly, R tanks were set to receive exclusively the effluent water from another tank containing 30 infected fish (donors; average weight = 240.2 g; prevalence of infection = 100%; fed a standard commercial diet). Control fish were kept under the same conditions, but without receiving *E. leei*-contaminated water.

Day length followed natural changes at IATS latitude (40°5′N, 0°10′E) and water temperature was kept always above 18 °C (average = 21.3 °C, range = 18–26 °C, sea water (37.5‰ salinity) was pumped from ashore (open system), 5 μm-filtered and UV irradiated. Each diet group was fed *ad libitum* with the same experimental diet it was receiving before the challenge, and daily food intake in each tank was registered. Disease signs and daily mortality were recorded.

The progression of the infection and the evaluation of growth performance were monitored by sampling both C and R groups at 32, 53 and 88 days post exposure (p.e.). At each sampling, all fish were sized and weighed and non-lethally (NL) sampled for parasite diagnosis with a PCR test (see below). An additional last sampling was performed at 102 days p.e. Then, all R remaining fish and 15 fish from each C group were killed by over-exposure to benzocaine (3-aminobenzoic acid ethyl ester, 100 mg $l^{-1}$, Sigma, St. Louis, MO, USA) and blood and tissue samples were taken for histological, immunological, and anti-oxidant analyses. Length and body weight, liver and spleen weights were measured and the condition factor (CF = [weight (g) × length $(cm)^{-3}$] × 100), and the hepatosomatic (HSI) and splenosomatic (SSI) indexes were calculated as the ratio between the organ weight and body weight. Specific growth rates (SGR) of all fish were calculated for the period ranging from one week previous to the challenge up to the end of the experiment (109 days) as follows: $SGR\ (\%) = 100 \times (\ln W_t - \ln W_0)/t$, where $W_0$ represents weight at the beginning of the period, $W_t$ the weight at the end of the trial and $t$ the number of growth days.

One heparinised blood aliquot was immediately used to measure the respiratory burst activity and another aliquot was drawn into heparinised capillary tubes, centrifuged at $1500 \times g$ for 30 min, and the haematocrit measured. The remaining non-heparinised blood was allowed to clot overnight at 4 °C, centrifuged at $3000 \times g$ for 20 min at 4 °C, and serum aliquots were stored at −80 °C until used in immunological, anti-oxidant and glutathione assays.

One replicate tank per group was established to minimize the number of experimental fish (a mandatory requirement of the ethical committee) and to guarantee an infective effluent to the R tanks compatible with a good water quality. According to our previous experience, no tank effect has been found when two tanks are infected from the same D tank under these experimental conditions (Sitjà-Bobadilla et al., 2007, 2008). As all fish were individually monitored along the entire experimental period, each fish was considered the experimental unit (Fisher, 2000).

### 2.2. Parasite diagnosis

Parasite diagnosis was performed by histology (at 102 days p.e.) or by NL-PCR (at 32, 55 and 88 days p.e.). For histological examination, pieces of anterior, medium and posterior intestine were fixed in 10% buffered formalin, embedded in paraffin, 3 μm-sectioned and stained with haematoxylin and eosin. NL samples were obtained by probing the rectum with a cotton swab and PCR diagnosis was carried out as described in Palenzuela and