

percent survival in relation to fish fed control diet. PHYTO diet reinforced fish capacity of stress response via protection of head kidney leucocytes from stress-related apoptotic processes. Additionally, dietary supplementation with GMOS and PHYTO compounds increased fish serum lysozyme and peroxidase activities.

Keywords: Functional feeds, prebiotics, phytonics, stress response, immune response

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Dietary and phytonics in low fish meal and fish oil diets for *Dicentrarchus labrax*: An effective tool to gut health and disease resistance

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Abstract

Fish intestinal mucosal surface supposes a potential route of entrance for pathogenic bacteria. An inflammatory gut reaction can be induced by a variety of factors, such as infection, stress or changes in feed composition. Particularly, for European sea bass (*Dicentrarchus labrax*) feeding low fishmeal (FM) and fish oil (FO) diets results in a gut inflammation-like status. The use of functional additives such as prebiotics and phytonics simultaneously with a low FM/FO based diet may help to buffer these associated gut health negative-side effects. Four low FM/FO (10%/6%) diets for European sea bass containing galactomannan oligosaccharides (GMOS), a mixture of garlic oil and labiate oils (PHYTO) were fed for 63 days before exposition to an intestinal *Vibrio anguillarum* infection in combination with a crowding stress. In order to evaluate functional diets efficacy in terms of gut mucosal health maintenance, structural, cellular and immune intestinal status was evaluated by optical and electron microscopy and gene expression analyses. A semi-automated software was adapted to determine variations on goblet cells area and mucosal mucus coverage along the challenge test. Functional diets fed did not affect growth performance, however PHYTO and GMOS dietary inclusion reduced European sea bass susceptibility to *V. anguillarum* after 7 days of challenge test. Rectum (post-ileorectal valve) presented longer ($p=0.001$) folds than posterior gut (pre-ileorectal valve), whereas posterior gut presented thicker submucosa ($p=0.001$) and higher mucus coverage as a result of an increased cell density compared to rectum. Functional diets did not affect mucosal folds length or the grade of granulocytes and lymphocytes infiltration in both intestinal segments. However, fish fed GMOS ($F=14.53$; $p=0.001$) and PHYTO ($F=5.52$; $p=0.019$) presented less posterior gut fold area covered by goblet cells. PHYTO ($F=3.95$; $p=0.049$) reduced posterior gut goblet cell size and increased rodlet cells density ($F=3.604$; $p=0.068$). Dietary GMOS reduced submucosa thickness ($F=51.31$; $p=0.001$) and increased rodlet cells density ($F=3.604$; $p=0.068$) in rectum. Structural TEM analyses revealed a normal intestinal morphological pattern. GMOS increased rectum microvilli length. PHYTO increased ($p\leq 0.10$) *Ocln*, *N-Cad* and *Cad-17* posterior gut gene expression. After bacterial intestinal inoculation posterior gut of fish fed PHYTO responded in a more controlled and belated way in terms of goblet cell size and mucus coverage in comparison to other treatments. Rectum pattern of response was similar for all dietary treatments

Keywords: European sea bass, functional additives, gut health, mucus production, disease resistance

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Health status of Senegalese sole (*Solea senegalensis*) post-larvae fed diets with microalgae inclusion

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Abstract

Senegalese sole (*Solea senegalensis*) is a highly valuable flatfish species targeted for aquaculture diversification in Southern-European countries and, as most farmed fish, are potentially subjected to stress and pathogens due to environmental factors (Reis et al., 2017; Pinto et al., 2018). Previous studies reported that algae can be used as antioxidant additive, high-quality dietary protein or source of bioactive compounds, thus promoting optimal growth and health in farmed fish (Becker, 2007; Teimouri et al., 2013). For these reasons, this study intended to evaluate the effects of dietary microalgae inclusion in both health status and growth performance of Senegalese sole post-larvae. Individuals with 41 days after hatching (DAH) were randomly distributed among 12 tanks with an initial density of 3000 post-larvae/m² and four experimental diets were randomly distributed by triplicate groups of tanks. Three experimental diets (CHLO-*Chlorella* sp., fermented; PHAEO-*Phaeodactylum* sp. and NANNO-*Nannochloropsis* sp.) were formulated to include 3% of each algal biomass to a basal diet, which served as CONTROL. The experimental diets were supplied through automatic feeders set up to supply 8 meals in a 24 h period. At 50 DAH, 20 post-larvae/tank were collected for analysis of immune and oxidative status. Also, at 61 DAH the total length, dry weight and survival were assessed. Homogenates had to be performed for the analyses of immune (i.e. lysozyme and protease activities) and oxidative stress (i.e. catalase activity) related parameters. Survival, relative growth rate and total length of individuals, at 61 DAH were not altered by the dietary treatments. However, post-larvae fed NANNO and CHLO dietary treatments increased dry weight at 61 DAH compared to those fed the CONTROL diet. Neither immune or oxidative stress status were altered by dietary treatments. According to these results, *Nannochloropsis* sp. and *Chlorella* sp. are potential candidates for inclusion in microdiets for Senegalese sole. Further analyses are being carried out to confirm the bioactive potential of these biomasses and optimal dietary inclusion levels.

Keywords: Early feeding, *Nannochloropsis*, *Chlorella*, immune status, catalase.

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