# EVALUATION OF GROWTH PERFORMANCE, OXIDATIVE STRESS AND IMMUNE RESPONSE IN GILTHEAD SEABREAM FED WITH NOVEL FEED FORMULATIONS

A.M. Fernandes<sup>\*1,2</sup>, L.E.C. Conceição<sup>1</sup>, J.A. Calduch-Giner<sup>3</sup>, B. Silva<sup>1</sup>, G.V. Pereira<sup>1</sup>, B. Costas<sup>4</sup>, F. Naya-Català<sup>3</sup>, C. Piazzon<sup>3</sup>, J.M.O. Fernandes<sup>2</sup>, A. Sitjà-Bobadilla<sup>3</sup> and J. Pérez-Sánchez<sup>3</sup>

<sup>1</sup>Sparos Lda, Olhão, Portugal

<sup>2</sup>Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway

<sup>3</sup>Institute of Aquaculture Torre de la Sal (IATS-CSIC), Ribera de Cabanes, Castellón, Spain

<sup>4</sup>Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal E-mail: anafernandes@sparos.pt

### Introduction

As the aquaculture sector continues to expand while being more environmentally conscious, the development of sustainable aquafeeds is becoming increasingly important (FAO, 2020). Tolerance to the replacement of fishmeal and fish oil in feeds has been largely studied in gilthead seabream (Sparus aurata) (Gasco et al., 2018; Karapanagiotidis, Psofakis, Mente, Malandrakis, & Golomazou, 2019), and many products emerge now as potential alternatives to ingredients used in conventional formulations. A main goal of GAIN EU project is to evaluate emerging ingredients, already commercially available, using different formulation concepts that consider all fish nutritional requirements. GAIN diets are based on circularity principles, maximizing resource efficiency, while contributing towards zero waste in the agro-food value chain, feed cost-effectiveness, and having good social acceptance. The present study aims to understand the real impacts of these novel feed formulations on growth performance, nutritional condition, immunity, and oxidative status using biomarkers.

#### Methods

Quadruplicate groups of gilthead seabream (Sparus aurata) were fed ad libitum with four different diets. Three of them have been designed to facilitate aquaculture eco-intensification through increased circularity and resource utilization: NOPAP - formula without terrestrial animal by-products processed animal protein; PAP - formula with terrestrial animal by-products processed animal protein; PAP - formula with terrestrial animal by-products processed animal protein; PAP - formula with terrestrial animal by-products processed animal protein; and MIX - a mixture of NOPAP and PAP. The fourth feed followed a standard commercial formulation and was used as a control diet. After a 77-day feeding trial, plasma samples were collected to evaluate humoral parameters (protease, anti-protease, bactericidal activity and IgM). Liver and head kidney tissues were collected for the simultaneous profiling of a panel of 42 (liver) or 29 (head kidney) genes, as markers of growth performance, lipid and energy metabolism, and immune and antioxidant activities by qPCR. Liver samples were also used to analyse oxidative biomarker (Lipid peroxidation and catalase).

#### Results

Tested feed formulations did not affect growth performance or feed intake. However, fish fed PAP and MIX diets had a higher feed conversion ratio (FCR) and protein efficiency ratio than control and NOPAP groups. This impairment was accompanied by a decreased hepatic expression of *igf-i* and *ghr1*. NOPAP diet slightly increased innate immunity parameters, showing better results on bactericidal, IgM, and anti-protease activity, as well as a significant up-regulation of *il-8* in head kidney. Fish fed with PAP diet displayed an up-regulation of pro-inflammatory genes, namely *il-8* and other cytokines (*il-1* $\beta$ , *tnf-* $\alpha$ ), chemokines (*ck8*), and chemokine receptors (*ccr3*). The same pattern was found for the T-cell markers *cd3x*, *cd4*, and *cd8a*. The activity of the antioxidant enzyme catalase was significantly lower in fish fed with PAP and MIX diet, being a possible indication of decreased antioxidant defences. This is supported by the observed regulation of antioxidant genes (*mn-sod/sod2*, *gpr-170*, *gpr-94*, and *gpr-75*), although not statistically significant.

#### Discussion

The similar performance of novel formulations and the control diet indicates that they can be considered as viable options for seabream feeds. Differences in FCR suggest that NOPAP can promote a better bioavailability and/or increased absorption of key nutrients than PAP and MIX diets. Indeed, this impairment was also evidenced by their hepatic expression pattern of markers of growth performance. In general, PAP exhibited an opposite response to the NOPAP group. NOPAP was closer to the control diet, and MIX showed intermediate values between PAP and NOPAP in almost all parameters. The markedly pro-inflammatory head kidney expression profile in PAP fish may be also indicative of an impaired response at the mucosal level. In any case, the low proportion of differentially expressed genes between the experimental diets and control (18 out of 71) constitutes an additional and indirect confirmation of their suitability.

## Conclusions

Novel feed formulations for gilthead seabream seem to be viable options for a near future. In any case, all results are related to the formulation itself and cannot be attributed to a specific ingredient alteration. More studies are necessary to understand the cost-benefit of these new formulations and their market acceptability to optimize sustainability within the current/predictable European regulatory framework.

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