FISH MEAL-FREE DIETS SUPPLEMENTED WITH HEALTH PROMOTERS SUPPORT OPTIMAL GROWTH IN GILTHEAD SEA BREAM, WITH BENEFITIAL CHANGES IN GENE EXPRESSION, INTESTINAL MICROBIOTA AND IMPROVED INTESTINAL DISEASE RECOVERY

M.C. Piazzon[1], F. Naya-Català[2], G.V. Pereira[3], I. Estensoro[1], R. Del Pozo[1], J.A. Calduch-Giner[2], W.G. Nuez-Ortín[4], O. Palenzuela[1], A. Sitjà-Bobadilla[1], J. Dias[3], L.E.C. Conceição[3], J. Pérez-Sánchez*[2]

[1]Fish Pathology Group and [2]Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal, CSIC, Spain. [3]Sparos Lda, Olhão, Portugal. [4]Adisseo, Dendermonde, Belgium
E-mail: jaime.perez.sanchez@csic.es

Introduction

The exponential growth of the aquaculture sector requires the development of sustainable aquafeeds with less dependence on marine products. Tolerance to fish meal (FM) and fish oil replacement in the economically important gilthead sea bream (*Sparus aurata*) is being extensively studied with many products emerging as alternative feed ingredients. It has been demonstrated that alternative diets influence the composition of intestinal adherent microbial populations, which have a key role on host metabolism, health and disease resistance. In addition, low fish meal diets showed an increased susceptibility to enteric parasites (Piazzon et al., 2017). Clearly, differences in diet have an impact on the overall health and metabolism of the fish and many parameters have to be taken into account when studying alternative diets for their use in aquaculture. In this study we evaluated the effect of a novel feed formulation (NoPAP SANA) with total replacement of FM by insect meal and bacterial fermentation biomass, and supplemented with the health-promoter additive SANACORE®GM (Palenzuela et al., 2020), on growth performance, gene expression, intestinal microbiota and disease resistance in gilthead sea bream.

Methods

Tagged gilthead sea bream of mean weight 21.3 g were distributed in two open-flow tanks (160 fish/tank) and fed *ad libitum* during 34 days with control or NoPAP SANA diets. Twelve fish/diet were sacrificed and head kidney (HK), liver (L) and posterior intestine (PI) were taken for RNA extraction. From the same fish, the adherent bacteria of PI were collected and immediately used for DNA extraction. RNA from HK, L and PI was used to run three customized PCR-arrays including genes of interest for each tissue, with markers of performance and metabolism (L), immune system (HK and PI), epithelial integrity, nutrient transport and mucins (PI). Using the bacterial DNA, the V3-V4 region of the 16S rRNA of each individual sample was amplified and sequenced by Illumina MiSeq. After quality filtering, taxonomic assignment was performed with a custom-made pipeline using the RDP database. Alpha diversity was calculated using Phyloseq and beta diversity using PERMANOVA and PLS-DA models. Metagenome prediction and pathway analysis were performed using Piphillin. Differential gene expression and OTU presence and abundance correlations were studied using the corrplot R package. From the remaining fish, 70 fish/group were challenged with the intestinal parasite *Enteromyxum leei* by effluent exposure and the remaining fish were used as controls. The challenge lasted 78 days, including a non-lethal diagnosis sampling at day 40. At the end of the challenge all fish were sampled for histological and molecular diagnosis. Biometric values from all fish were taken in all sampling points.

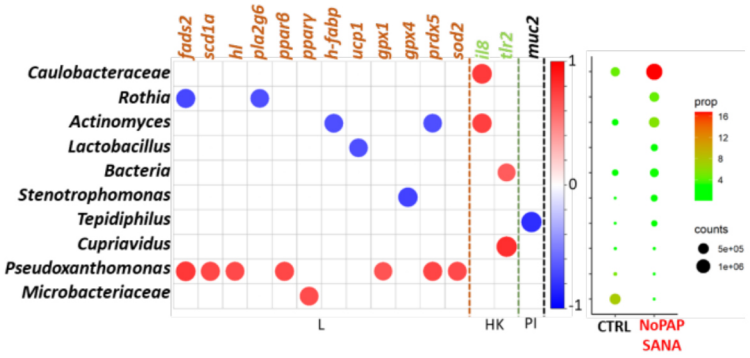

Figure 1: Correlation plot showing significant correlations between OTUs and liver (L), head kidney (HK) and posterior intestine (PI) differentially expressed genes. Positive and negative correlations are represented in red and blue, respectively. On the right, dotplot depicting the relative abundance of the respective OTUs in proportion (colour scale) and normalized counts (dot size).

(Continued on next page)

Results

A slight decrease in condition factor and specific growth rate was detected in the NoPAP SANA group. However, all fish grew efficiently considering gilthead sea bream standards. NoPAP SANA group showed differential expression of 17 out of 44 genes in L, two out of 29 in HK, and 4 out of 44 in PI. The bacterial composition at the PI showed no major differences in diversity or at the phylum level. However, 29 abundant (>1%) OTUs significantly changed with the diet. From these, 10 OTUs were significantly correlated with differential expression of genes in the different tissues, highlighting *Pseudoxanthomonas* which was positively correlated with the expression of seven L genes, or *Actinomyces*, significantly correlated with the expression of L and HK genes (Fig. 1). Inferred metagenome analyses revealed that the altered microbiota with NoPAP SANA diet could account for changes in 15 metabolic pathways. The intensity and prevalence of infection after the parasite challenge was not significantly different between diets. In fact, infected fish from both groups showed similar recovery rates.

Conclusions

NoPAP SANA promoted good growth parameters and efficient conversions arising as a good alternative for a FM-based diet in gilthead sea bream diets. This diet modulated the expression of several genes in L showing the capacity to reduce lipogenesis, mitochondrial activity and the risk of oxidative stress and, at the same time, promoting an anti-inflammatory gene expression profile in HK and PI. Changes were also detected in the adherent bacterial populations of PI, with significant changes of OTUs that could potentially account for significant metabolic alterations. The correlations between presence and abundance of intestinal bacteria with changes in gene expression of different tissues, together with the pathway analysis results, show that microbiota changes can have an impact on host metabolism at a systemic level, and *vice versa*. Clearly, the changes induced by this novel FM-free diet supported an accelerated growth with an overall feed conversion ratio close to 1 and no increased susceptibility against this intestinal parasite, as often observed in studies when replacing a FM-based diet.

References

Palenzuela O., Del Pozo R., Piazzon M.C., Isern-Subich M.M., Ceulemans S., Coutteau P., Sitjà-Bobadilla A. 2020. Effect of a functional feed additive on mitigation of experimentally induced gilthead sea bream *Sparus aurata* enteromyxosis. *Diseases of Aquatic Organisms*, 138: 111-120.

Piazzon M.C., Calduch-Giner J.A., Fouz B., Estensoro I., Simó-Mirabet P., Puyalto M., Karalazos V., Palenzuela O., Sitjà-Bobadilla A., Pérez-Sánchez J. 2017. Under control: how a dietary additive can restore the gut microbiome and proteomic profile, and improve disease resilience in a marine teleostean fish fed vegetable diets. *Microbiome*, 5:164.

Acknowledgements: GAIN (EU-H2020 #773330); RYC2018-024049-I/AEI/10.13039/501100011033.