

## MODULATION OF GILTHEAD SEA BREAM GUT MICROBIOTA BY A BIOACTIVE EGG WHITE HYDROLYSATE

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### Introduction

A bioactive egg white hydrolysate (EWH) treated with pepsin has demonstrated potent *in vitro* and *in vivo* antioxidant and anti-inflammatory properties, improving oxidative stress and inflammation biomarkers on genetically and diet induced obese rats (Requena et al., 2017). However, the effects of protein hydrolysates and bioactive food-derived peptides on gut microbiome remain relatively poorly studied in mammals and fish in particular. Thus, the aim of this study was to unravel the main effects on fish performance, histopathological scoring and mucosal adherent gut microbiota of EWH supplementation in a fish fed a formulation with a high replacement of marine feedstuffs by alternative plant ingredients, using gilthead sea bream as a farmed fish model.

### Methods

The feeding trial lasted 8 weeks (May-July) under natural photoperiod and temperature conditions. Juvenile fish (20-24 g initial body weight, 4.8-4.9 kg/m<sup>3</sup>) were fed near to visual satiety with control (CTRL) or low fish meal (FM)/fish oil (FO) diets with/without egg white hydrolysate (EWH) supplementation (7.5%). DNA from the adherent bacteria of the anterior intestine was collected and the V3-V4 region of the 16S rRNA of each sample was amplified and sequenced by Illumina MiSeq. 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 partial least-squares discriminant analysis (PLS-DA) models. Metagenome prediction and pathway analysis were performed using Piphillin.

### Results

Daily specific growth rates (SGR) varied significantly from 2.16 in CTRL fish to 1.88 in EWH fish as a result of a reduced feed intake. A slight impairment of feed conversion ratio, from 1.03 to 1.10, was also observed. Intermediate values on growth performance parameters were reported with the low FM/FO diet without EWH supplementation. No changes in total plasma antioxidant capacity, and faecal concentrations of lactic acid and short-chain fatty acids were found among dietary groups. The dietary replacement of FM/FO triggered a hyperplastic inflammation of the anterior intestine submucosa that was not alleviated by EWH supplementation. Conversely, alterations on the staining pattern and amount of goblet cells at the level of anterior intestine were reversed in EWH fish, together with a decreased accumulation of lipid vacuoles in the epithelium of posterior intestine, a high abundance of hepatic melanomacrophage centers, and depletion of hepatocyte lipid depots until the restoration of CTRL fish values. Illumina sequencing reads were assigned to 2,117 OTUs and a significantly lower richness was found in the EWH group. Indeed, at the phylum level, *Proteobacteria* reached the highest proportion in CTRL and EWH fish, whereas *Firmicutes* were decreased and *Actinobacteria* increased with the replacement of FM/FO. The proportion of *Actinobacteria* was restored to CTRL values with the dietary EWH supplementation. Additionally, EWH triggered the highest amount of *Bacteroidetes* and *Spirochaetes* phyla. Detailed differences in microbiota composition were analysed with a statistically validated PLS-DA which clearly separated CTRL fish from fish fed low FM/FO diets along x-axis (component 1, 37.4%), whereas component 2 (43.2%) separated the low FM/FO diets with/without EWH along y-axis (Fig. 1). This analysis disclosed 165 OTUs discriminating among diets (VIP ≥ 1), with 46 OTUs representing at least the 1% in one of the groups. For these abundant bacteria, a first type of response was mediated by 17 OTUs that were increasing with the FM/FO replacement and decreasing again in EWH fish. In this group, *Neisseriaceae* family and species of *Ralstonia*, *Lactobacillus*, *Streptococcus*, *Corynebacterium* and *Nocardioides* genera were included. A group of 14 OTUs were present in high proportion in the CTRL group, but decreased in fish fed the two low FM/FO diets. In this case, the dietary plant ingredients drove the decrease of the *Comamonadaceae* family and *Mesorizhobium*, *Brochotrix*, *Bacillus*, *Clostridium sensu stricto* and *Exiguobacterium* genera. The remaining 15 OTUs increased their proportion in fish fed the EWH diet, being in a very low proportion in the other two dietary groups. This response triggered the presence

of *Bacteroidetes* phylum, *Rhodospirillales* order and *Granuicatella*, *Bradyrhizobium*, *Propionibacterium* and *Streptophyta* genera. Inferred metagenome results showed two pathways corresponding to primary bile acid biosynthesis and steroid degradation consistently underrepresented in the microbiota of EWH fish when compared to the other two groups.

### Conclusions

These results reinforce the central role of gut microbiota in the regulation of host metabolism and lipid metabolism in particular (Hegyí et al., 2018), supporting a main role of the EWH as an anti-obesity and satiety factor in fish as suggested in rat models of obesity. The potential use of this functional food ingredient in finishing diets, and the role of gut microbiota in tuning fillet fatty acid composition of marketable fish merits further research.

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### References

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