CAN THE GENETIC BACKGROUND OF GILTHEAD SEA BREAM CHANGE THE ACTION OF FEED ADDITIVES? ANSWERS FROM GUT MICROBIOME AND TRANSCRIPTOME INTERACTIONS

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Introduction

The sustainable growth of modern aquaculture must rely in the production of healthy and robust fish fed with diets overcoming the dependence on fish meal (FM) and fish oil (FO). Selective breeding and functional feeds should be keystones towards this development, though most of their synergies remains mostly unexplored. Certainly, recent studies in gilthead sea bream highlighted that selection for growth co-selects for a plastic microbiota capable of exerting a wide nutritionally-mediated metabolic response with less community changes (Piazzon et al., 2020). Otherwise, the use of feed additives as boosters of overall fish performance has expanded rapidly as an alternative to antibiotics and chemotherapeutics, with also the capacity to modify the gut microbiota and host transcriptional associations. However, we are far from establishing the ultimate mode of action of each feed additive for a given genetic background. To bridge this gap, we investigated the effect of a battery of feed additives upon gut microbiota and host transcriptomics in reference (REF) gilthead sea bream and genetically improved fish for growth (GS) within the PROGENSA® selection program.

Material and methods

Estimated breeding values ranged between -159.14 for REF fish and +223.18 for GS fish. The basal diet (CTRL; no feed additive) was formulated by Skretting to be a low FM diet (7.5%), completely devoid of FO. Feed additives (INVE Technologies) added to CTRL diet by oil-coating included a phytobiotic based on natural plant extracts (PHY), a mixture of organic acids (OA), and a *Bacillus*-based probiotic (PROB). After an acclimation period of two weeks with the CTRL diet, fish continued to be fed with either CTRL, PHY, OA or PROB diets until the end of the trial (97 days). At this end-point, tissue portions of anterior intestine (AI) were taken for transcriptional (RNA-seq) and AI scrapped mucus for adherent microbiota analyses, using the Illumina platform and RDP database. Additionally, AI and posterior intestine (PI) sections were used for histological survey.

Results

The GS fish presented higher growth rates and condition factors, lower feed conversion ratios, and an enhanced homogeneity in terms of microbiota composition, regardless of the additive. The PHY effects were especially remarkable in the intestinal transcriptome of higher growth GS-PHY fish, with a particular up-regulation of markers of epithelial integrity (*vil1*, *chmp2a-b*, *vps4b*), sphingolipid metabolism (*degs1*, *elovl1*, *sgpp1*, *plekha8*), high-density lipoproteins secretion to vascular tissues (*abcg8*, *abca1*, *nr1h3*), and bile salt-activated lipase and receptor (*cel*, *nr1h4*). Facing OA, the gut adherent microbiota of REF fish shifted towards a less pathogenic profile, with a reduction in *Staphylococcus*, *Streptococcus*, and *Neisseria* genera correlated with neutrophil degranulation genes. This profile showed inferred bacterial processes involving organic acids, such as ABE fermentation and TCA cycle, and was prone to exert vitamin K biosynthesis. The *Bacillus*-based PROB diet affected both microbiome and transcriptome

features. *Bacillus* genus was stablished in the fish gut regardless of the fish genotype, and anti-inflammatory histology patterns were increased in the AI and PI of PROB fish. GS-PROB showed an increased proportion of the nitrate reducer *Kocuria*, and a reduction of the pathogenic *Photobacterium damselae*, in parallel with a better feed efficiency of GS-PROB fish than the rest of the groups. This group also showed the up-regulation of markers of epithelial regeneration and integrity (*ezr*, *ncstn*, *plec*, and *neurog3*) in concurrence with the down-regulation of markers of protein synthesis, that correlated with *Chromohalobacter*, *Enhydrobacter*, *Vibrio*, and *Acinetobacter*.

Concluding remarks

As a general rule, it was confirmed that the gut microbiota variability among individuals was drastically reduced in GS fish (Naya-Català et al., 2022). The intensity and the specific effect of a given additive upon host transcriptomics and gut microbiota varied depending on the genetic background (Figure 1). Thus, PHY only shaped the transcriptome of GS fish. Conversely, OA shaped the gut microbiota of REF fish, whereas PROB triggered changes in both host transcriptome and gut microbiota of both GS and REF fish. Altogether, this work has generated a list of taxa and transcripts associated to a particular feed additive and fish genotype, which might help nutritionists, breeders and farmers to know which microbial and host elements are susceptible to be targeted in order to preserve and improve the gut function of PROGENSA® farmed fish.

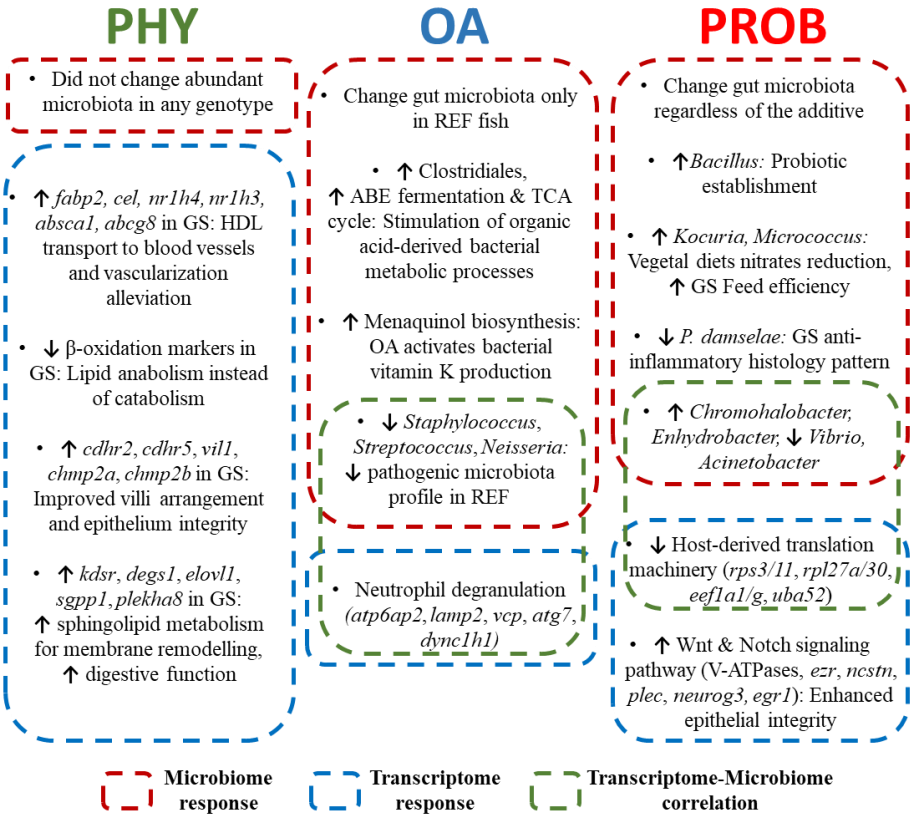

Figure 1: Proposed model for integrative associations of gut adherent microbiota, and intestinal transcriptome of gilthead sea bream fed with three additives.

References

Naya-Català F. et al. (2022); *Biology*, 11:1744.
Piazzon M.C. et al. (2020); *Microbiome*, 8:168.

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