UNDERSTANDING THE FUNDAMENTAL CONTRIBUTION OF LIVE FOOD IN THE FEEDING REGIMES FOR MARINE FISH LARVAE. A CASE STUDY OF THE GILTHEAD SEABREAM *Sparus aurata* L.

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Introduction

Live food consumptions in the feeding regimes of marine fish larvae have been decreased considerably during the last decades, making the producers less reliant on the success of rotifer cultures and reducing the amounts of *Artemia* that are being used. A lot of progress has been made in diet formulations and production techniques, proving that early starter feeds are accepted well by marine larvae as soon as mouth opening occurs. In the meantime, quantities of live feed consumed during larval rearing have been reduced considerably. Despite these improvements, excluding completely the live preys from the menu does not result in successful larval production for any of the commercially most important marine species.

As nutrition has a large impact on fish gut mucosal health status, larval mucosal architecture and microstructure, larvae were examined through histology to evaluate diet-induced alterations. Additionally, transcriptome analysis by 3' end RNA-sequencing of pools of larvae was conducted to perform an in-depth analysis of the impact of live food deprivation on the fish larval health status. Fish growth and survival were evaluated as ultimate outcomes.

Materials and methods

Test set-up

Nearly hatched seabream larvae, originating from the same pool of eggs, were stocked at a density of 100 larvae per liter in 390L tanks. Seawater was provided through a semi-closed water renewal system at a temperature of 18±1°C. Dissolved oxygen levels were kept around 100% saturation. Larvae were reared under green water conditions. A standard live food regime (LFC) was compared to a treatment where no live food (No LF) was introduced, providing a special early start-feeding diet from the onset of exogenous feeding.

Weekly biometrics

20 Larvae per tank were sampled every week and controlled for Standard Length (SL).

Histology

6 Larvae per group were sampled at 20 and 35dph for histological analyses of the posterior intestine (PI). Whole larvae were fixed in Bouin's fixative and stained with May-Grünwald/Giemsa. PI folds height, width and enterocyte height were measured.

3' end RNA-sequencing

270 Larvae were sampled from the 2 experimental groups (LFC and No LF) at 20dph. Larvae were washed with PBS, proceeding with RNA extraction with Genezol. RNA concentration, purity and integrity were checked before proceeding with the RNA sequencing. A bioinformatic pipeline was applied to statistically analyze differentially expressed genes (DEGs) and assess enrichment of Gene Ontology (GO).

Results

The weekly length measurements showed very early significant differences between the No LF and LFC groups and the last survivor in the No LF group died at 36dph, indicating the poor biological performance of the latter.
Histomorphometric analyses of distal intestine from larvae at 20 and 35 dph revealed significantly shorter and narrower villi, and shorter enterocytes in the No LF compared to LFC treatment. Nevertheless, no coarse histological damages were visible in the gut mucosa in the No LF group.
RNA sequencing performed on pools of larvae at 20 dph highlighted 486 Differentially Expressed Genes (DEGs) between both groups: 260 and 226 were down- and up-regulated, respectively, in the No LF treatment compared to LFC. Based on statistical significance and extent of gene expression change, the absence of live food resulted in the disturbance of many Gene Ontology biological processes such as lipid transport, proteolysis, immune response, glycolysis and cartilage development.

Conclusions

This study indicates the types of biological processes that are highly influenced when the live food is removed from the standard larval feeding protocol. Live food remains fundamental, ensuring proper development and growth of gilthead seabream larvae and assesses its efficacy as a naturally derived functional feed.