

EFFECTS OF FEEDING YEASTS, *SACCHAROMYCES CEREVISIAE* AND *WICKERHAMOMYCES ANOMALUS*, ON GUT MICROBIOTA OF RAINBOW TROUT

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Introduction

Single cell proteins, such as yeasts, are an ideal feed alternative to plant and fish meals as they do not compete as food for humans. Previous studies have fed live yeast, such as *Saccharomyces cerevisiae*, in cold pelleted diets to farmed fish in order to increase beneficial microbiota in the gut (Gatesoupe, 2007). However, commercial salmonid diets are typically extruded at high temperatures, which can inactivate yeasts and reduce their effects on gut microbiota. In addition, previous studies have used culture-based methods to determine gut microbiota, while the development of next generation sequencing has improved identification of unculturable microbiota. The objective of this study was to determine the effect of feeding yeasts, *S. cerevisiae* and *Wickerhamomyces anomalus*, on microbiota in the distal intestine of rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

A diet of fish meal (FM) was used as a control against yeast diets that replaced 20, 40 and 60% of fish meal (digestible protein basis) with either *S. cerevisiae* (SC) or *W. anomalus*/*S. cerevisiae* mix (WA; Jästbolaget AB, Sweden). Diets were extruded at 120-130°C and later fed to triplicate tanks of 35 rainbow trout for 10 weeks. Afterwards, content and mucosa from the distal intestine of 3 fish per tank were collected. Ingredients, diets and gut samples were plated on yeast-peptone-D-glucose, then 26S rDNA were PCR-amplified and sequenced to determine live yeast counts and taxa. Diets and yeast ingredients were further examined microscopically to determine cell counts of yeast. For gut bacteria, 16S rDNA were PCR-amplified, barcoded and next generation sequenced using Illumina platform (SciLifeLab AB, Sweden). Significant differences ($p < 0.05$) between diets were determined using ANOVA and ANOSIM methods.

Results

The yeast ingredients, *S. cerevisiae* or *W. anomalus*/*S. cerevisiae* mix, contained between 9-10 log cfu g⁻¹ and SC and WA diets contained 7-8 log cfu g⁻¹ before extrusion. After extrusion, all diets had over 4-log reduction in cfu g⁻¹ (cultured yeast), however 7-8 log cells g⁻¹ of yeast were still viable. Diets of SC and WA contained between 96-100% *S. cerevisiae* and 0-20% *W. anomalus*. After 10 weeks of feeding, the distal intestine contained between 84-96 % *Debaryomyces hansenii* with few colonies of *S. cerevisiae* and no *W. anomalus*. For bacteria, the distal intestine contained between 37-62 % of *Leuconostocaceae*, *Lactobacillaceae* and *Photobacterium* and significant differences in taxa abundance and similarity were found between fish fed SC60, WA40 and WA60 compared with the FM diet (Fig. 1).

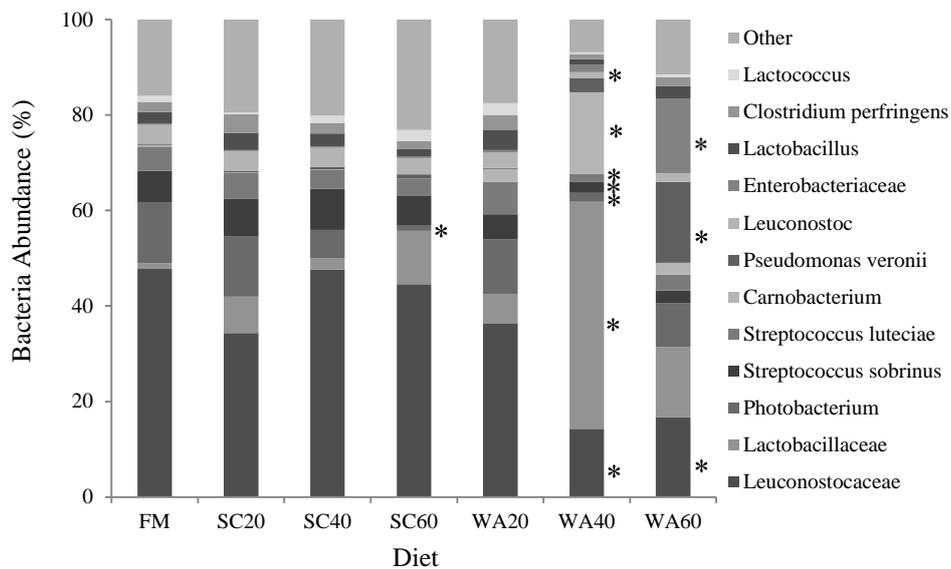


Fig. 1. Top 1% or higher abundant bacteria from the distal intestine of fish fed diets based on fish meal (FM) and 20, 40, 60% replacement with *Saccharomyces cerevisiae* (SC) or *Wickerhamomyces anomalus*/*S. cerevisiae* mix (WA).

Discussion and conclusion

This study is the first to analyse intestinal microbiota of rainbow trout fed yeast diets using next generation sequencing. In addition, few studies have investigated the viability and log reduction of yeast after feed extrusion. The low level of culturable yeast compared with the high level of intact yeast cells found after diet extrusion suggested that high temperature from extrusion inactivated yeasts, but did not disrupt the cells. Inactivated, non-disrupted yeast is not ideal because the yeast cannot cultivate the intestine or release nutrients for metabolic uptake. The lack of *S. cerevisiae* and *W. anomalus* in the distal intestine despite the high level of yeast ingestion, suggests that large amounts of yeast were metabolised. However, higher levels of unculturable yeast cells may have been present in the intestinal content and mucosa.

For gut bacteria, most were Lactic Acid Bacteria (Lactobacillales order), which includes *Carnobacteriaceae*, *Leuconstocaceae*, *Leuconstocaceae* and *Streptococcaceae* families. In comparison, Ingerslev et al. (2014) found similar levels of *Streptococcus*, *Leuconostoc*, Enterobacteriaceae and *Lactobacillus* in rainbow trout fed fish and plant-based diets. Significant differences in bacteria abundance for fish fed SC60, WA40 and WA60 diets indicated that *W. anomalus* had a higher effect than *S. cerevisiae* and that more than 20% replacement of fish meal with yeast affected gut microbiota in rainbow trout.

References

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