# PERFORMANCE AND INTESTINAL HEALTH OF SALMONIDS FED SINGLE-CELL PROTEINS BASED DIETS.

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## Introduction

To find alternatives to fish meal (FM) in aquaculture feeds is one of the main challenges in the establishment and expansion of sustainable aquaculture. Plant protein sources, such as soy bean meal and other legumes, are still the major alternative to FM in most commercial fish diets, despite issues relating to anti-nutritional compounds and negative fish welfare (Gatlin et al., 2007). Another sustainable alternative is proteins from single-cell organisms, e.g. yeasts, bacteria and microalgae, as some single-cell proteins (SCP) can be produced using industrial by-products as substrates, allowing long-term sustainable use of resources (Nasseri et al., 2011). However, evaluation of new alternative feed ingredients must include maintained health and welfare of the fish, in general and of the intestine in particular. Even though research has been performed on inclusion of different SCP in fish diets, studies on high inclusion of whole yeast in diets for salmonids are scarce and SCP is still not included to any larger degree in commercial fish diets (Vidakovic et al., 2015). .

The aim of the present study was to investigate effects on overall performance as well as intestinal health of two salmonids, rainbow trout (*Oncorhynchus mykiss*) and arctic charr (*Salvelinus alpinus*) when replacing FM in the feed with the two species of yeast, *S. cerevisiae* and *W. anomalus* alone or in combination.

## Material and methods

Rainbow trout: Four weeks before the experiment, 840 fish, with average body weight 93.7 ± 3.8 g (mean ± s.d.) were netted and anesthetised with 100 mg $L^{-1}$ tricaine methane sulphonate. Weight and length were recorded and 35 fish were randomly allocated to each of 24 cubical, fibreglass tanks, each 340 L in volume. The tanks were supplied with 10 L $min^{-1}$ flow through fresh water (FW; 12.9 ± 1.2°C, derived from Lake Ansjön after passage through a rotating drum filter). The control diet used in the experiment was similar to a commercial diet for rainbow trout and contained 30% FM. Six test diets were based on this control diet with the FM replaced with different test ingredients on a digestible protein basis. The test ingredients were intact yeast *S. cerevisiae* replacing 20% (diet S20), 40% (diet S40) and 60% (diet S60) of the FM in the control feed, or a mix of *W. anomalus* and *S. cerevisiae* (70:30) replacing 20% (diet WS20), 40% (diet WS40) and 60% (diet WS60) of FM. All diets were formulated on a iso-nitrogenous basis, accounting for a 10% lower crude protein (CP) digestibility of yeast than FM. The diets were supplemented with crystalline L-methionine up to a total methionine content of 9 g $kg^{-1}$ diet. The fish were fed the experimental diets for 10 weeks (72 days) and no mortality was recorded during the trial. Weight gain, specific growth rate and feed conversation ratio were monitored. At the end of the dietary trial the intestines were sampled and fixed in 4% buffered formalin for 24h at 4°C and embedded in paraffin wax for later histological assessment of intestinal morphology.

Arctic charr: 750 fish (47.8±8.6 g) were randomly placed in 15 square fiber glass tanks (50 fish per tank). The tanks, each 700 L in volume, were supplemented with 10 L $min^{-1}$ flow through FW from Lake Ansjön with an average temperature of 7.1 ± 1.8 °C. The

control diet used in this experiment was formulated on a iso-nitrogenous basis, accounting for a 10% lower CP digestibility of yeast than FM. The control diet had FM as the main protein source (47%) following recommendations for Arctic charr diet according to Jobling et al. (1993). The experimental diet was formulated based on this control diet, but with 40% of the FM replaced with intact yeast *S. cerevisiae* (S40-charr) thus resembling the S40 diet used in for rainbow trout. The fish were fed experimental diets for 99 days during which weight gain, specific growth rate and feed conversion efficiency were recorded. At the end of the dietary trial the intestines were sampled and effects of S40-charr on intestinal active transport functions and intestinal barrier function were assessed, in parallel, using an Ussing chamber set-up.

**Results**

Rainbow trout: In general the S60 and W60 groups tended to have lower final weight. Fish fed diet W60, showed significantly lower SGR while fish fed diet S60 did not differ from the reference diet. Two standard morphometric analyses, intestinal villi height and width, were performed on 7 µm sections from the anterior intestine of 8-12 fish. In addition the number of goblet cells/mm of epithelia was counted. There were no apparent effects of diet on the height of the villi. All groups, including the control, displayed some degree of oedema at the villi tips, but the oedema was enhanced in fish fed diets containing *S. cerevisiae*, with significantly wider villi tips in fish fed the S60 diet. The more pronounced oedema observed in fish fed *S. cerevisiae* diet was not apparently infiltrated by any immune cells and could therefore not be classified as enteritis, but could be an indication of an early stage of inflammation. The number of goblet cells/mm epithelium showed a trend towards decreased number with increasing amount of *S. cerevisiae* in the diet. Fish fed the mixture of *S. cerevisiae* and *W. anomalus* displayed decreased number of goblet cells compared to control fish irrespective of inclusion level. The decrease in number of goblet cells may indicate an increased rate of mucus secretion, which can be due to induction, mechanically or immunologically, from components in the yeasts.

**Arctic charr**: No significant effect could be observed on growth performance by the S40-charr diet. However, the intestinal barrier function was impaired in the distal intestine. This was observed as decreased transepithelial electrical resistance (TER) and increased apparent permeability of the paracellular marker molecule, mannitol (Papp). Further, the S40-charr showed an increased L-lysine transfer in the distal intestine. No effects were observed on barrier function or active transport parameters in the proximal intestine.

In conclusion, small but significant effects were apparent in intestinal morphology after SCP inclusion in trout feed. In charr, intestinal barrier function was reduced by SCP based feeds also in the absence of effects on growth. The results will be discussed in relation to alternative feed development and health and performance indicators.

**References**

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