



# Evaluation of growth performance and intestinal barrier function in Arctic Charr (*Salvelinus alpinus*) fed yeast (*Saccharomyces cerevisiae*), fungi (*Rhizopus oryzae*) and blue mussel (*Mytilus edulis*)

A. VIDA KOVIC<sup>1</sup>, M. LANGELAND<sup>1</sup>, H. SUNDH<sup>2</sup>, K. SUNDELL<sup>2</sup>, M. OLSTORPE<sup>3</sup>,  
J. VIELMA<sup>4</sup>, A. KIESSLING<sup>1</sup> & T. LUNDH<sup>1</sup>

<sup>1</sup> Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden; <sup>2</sup> Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden; <sup>3</sup> Department of Microbiology, Uppsala BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden; <sup>4</sup> Natural Resources Institute Finland (Luke), Jyväskylä, Finland

## Abstract

Arctic charr (*Salvelinus alpinus*) were fed for 99 days on experimental diets with 40% of fish meal replaced, on a crude protein basis, with intact yeast (*Saccharomyces cerevisiae*) (ISC), extracted yeast (ESC), *Rhizopus oryzae* fungus (RHO) or de-shelled blue mussels (*Mytilus edulis*) (MYE). The fish were evaluated for growth performance, nutrient digestibility and fish intestinal function. Growth performance, retention of crude protein and sum of amino acids were not affected in fish fed diets ISC or MYE compared with those fed the reference (REF) diet. However, fish fed diet ISC displayed decreased digestibility of crude protein and indispensable amino acids and decreased intestinal barrier function compared with fish fed the REF diet. Fish fed diet ESC exhibited decreased growth performance and protein retention, but had comparable digestibility to fish fed the REF diet. Fish fed diets MYE and RHO showed similar performance in terms of growth, nutrient digestibility and intestinal barrier function. Overall, the results indicated that blue mussel and intact *S. cerevisiae* yeast are promising protein sources for Arctic charr.

**KEY WORDS:** aquaculture, arctic charr nutrition, intestinal barrier function, *Mytilus edulis*, *Rhizopus oryzae*, *Saccharomyces cerevisiae*

Received 13 February 2015; accepted 12 June 2015

Correspondence: A. Kiessling, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, P.O. Box 7024, SE-750 07 Uppsala, Sweden. E-mail: Anders.kiessling@slu.se

## Introduction

Arctic charr (*Salvelinus alpinus*) shares many traits with rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*), but has the distinct advantage for aquaculture in northern countries of maintaining higher growth at lower temperatures (Brännäs & Linnér 2000). This makes Arctic charr a potential complement to farming of salmonids in the northerly climate conditions typically found in large parts of Sweden.

Consumption of fish meal and fish oil by the aquaculture industry has doubled since 1998 (Naylor *et al.* 2009). Global supplies of fish meal and access to high-quality fish meal for the aquaculture industry are becoming increasingly limited due to declines in wild fish stocks (Tacon & Metian 2008). Consequently, a report by High Level Panel of Experts (HLPE, 2014) concluded that the expanding aquaculture industry needs to reduce its use of fish meal and fish oils and encourage the use of alternative feeds.

Plant materials are currently the main alternative protein source used to replace fish meal in fish feeds, but the presence of bioactive and antinutritional compounds in common plant-derived protein sources, such as oilseeds and legumes, limits their use in aquaculture feeds (Gatlin *et al.* 2007). Salmonids are especially affected by these compounds due to their carnivorous nature (Olli *et al.* 1994;

Krogdahl *et al.* 2003; Chikwati 2013). Furthermore, food demand from the growing global human population is limiting the availability of plant sources and is requiring the use of agricultural land for production of animal feed, including plant-derived aquaculture feedstuffs (Brown 2012).

The blue mussel, *Mytilus edulis*, is a filter-feeding mollusc with a high capacity for nitrogen (N) removal from aquatic environments. It represents a high-quality protein source (Lindahl *et al.* 2005), with an amino acid and fatty acid profile similar to fish meal (Berge & Austreng 1989). Previous studies on blue mussel diets for rainbow trout have shown that increased inclusion levels of whole, milled blue mussel cause a tendency for poor growth and lower protein digestibility, which has been attributed to high shell content (Berge & Austreng 1989). Pan (2013) and Langeland *et al.* (2014) showed that growth performance and digestibility in Arctic charr were unaffected when de-shelled blue mussels representing 520 g kg<sup>-1</sup> and 320 g kg<sup>-1</sup> of the diet (as is basis) were compared to fish meal-based diets.

Fungi, such as yeasts and filamentous fungi, may be suitable protein feed alternatives as they can utilize a variety of substrates and have a high reproductive rate (Kiessling 2009). The protein content in filamentous fungi and yeasts is high, and varies between 30 and 65% (Halasz & Lasztity 1991; Nasser *et al.* 2011). Most microbial protein sources contain high levels of nucleic acid (NA), which elevate plasma uric acid and generate toxicological effects on the metabolism of terrestrial animals (Rumsey *et al.* 1992). However, salmonid fishes are capable of metabolizing high NA levels due to higher activity of liver uricase (Kinsella *et al.* 1985; Rumsey *et al.* 1992; Andersen *et al.* 2006), suggesting high potential for use of fungi in fish feeds.

Baker's yeast, *Saccharomyces cerevisiae*, has previously been studied as a protein source for a number of fish species, such as lake trout (*Salvelinus namaycush*), European sea bass (*Dicentrarchus labrax*) and Atlantic salmon (Rumsey *et al.* 1990; Oliva-Teles & Gonçalves 2001; Øverland *et al.* 2013). Filamentous fungi, *Rhizopus oryzae*, possess a similar amino acid profile to fish meal and may be used as a suitable alternative (Mydland *et al.* 2007; Edebo 2008; Abro *et al.* 2014a). Apart from nutritional components such as protein and lipids, the cell wall of yeasts and other micro-organisms contains mannan oligosaccharides (MOS),  $\beta$ -glucans and chitin. These structural components have been shown to possess bioactive properties and to positively affect the intestinal health of fish, for example for sea bream (*Sparus aurata*) (Dimitroglou *et al.* 2010) and Atlantic salmon (Refstie *et al.* 2010).

The intestinal tract of fish serves several important biological functions, for example food digestion, nutrient uptake and osmoregulation (Sundell & Rønnestad 2011). In addition, the intestinal tract serves as an important defence mechanism for fish, referred to as the gastrointestinal barrier, which constitutes an extrinsic, intrinsic and immunological barrier (Sundell & Sundh 2012). The intrinsic barrier is made up of the epithelial cell monolayer and tight junction complexes that act as a primary physical barrier between the intestinal lumen and blood circulation. Increased leakage of ions and small-sized molecules, translocation of pathogens and intestinal inflammation are indicative of a disturbed barrier and may pose a threat to the health and welfare of fish (Segner *et al.* 2012).

The gastrointestinal barrier is of particular interest when developing new feed ingredients, as it is the first tissue to be exposed. Harmful feed ingredients can result in adverse effects on nutrient uptake, intestinal barrier integrity and local inflammation, which may lead to an increased risk of infection and disease susceptibility (Jutfelt *et al.* 2007; Knudsen *et al.* 2008; Chikwati 2013).

The aim of this study was to evaluate growth performance and nutrient utilization in Arctic charr fed intact and extracted *S. cerevisiae*, *R. oryzae* and *M. edulis* and to evaluate the possible effects on the intestinal barrier of these feed components. The study is part of a larger series of experiments using the same ingredients, at different dietary inclusion levels, comparing digestibility in Arctic charr and Eurasian perch (*Perca fluviatilis*) (Langeland *et al.* 2014) and investigating chitinolytic activity and gut health (Abro *et al.* 2014b), metabolomics (Abro *et al.* 2014a) and fatty acid deposition (Pan 2013).

## Materials and methods

### Facilities and fish

The experiment was carried out at Kälärne Research Station (Vattenbrukscentrum Norr AB, Kälärne, Sweden) using Arctic charr from the Swedish breeding programme designated 'Arctic superior' (Nilsson *et al.* 2010). The fish used in the experiment were hatched in February 2012. In total, 750 fish were netted, anesthetized with 100 mg L<sup>-1</sup> tricaine methane sulphonate (MS-222 Western Chemical Inc., Ferdale, WA, USA), weighed [47.8  $\pm$  8.6 g (mean  $\pm$  SD)] and randomly allocated in triplicate groups (50 fish per tank) to 15 square flowthrough fibreglass tanks. The tanks, each 700 L in volume, were supplemented with 10 L min<sup>-1</sup> flowthrough of water from Lake Ansjön with

**Table 1** Chemical composition (g kg<sup>-1</sup> DM) and energy content (MJ kg<sup>-1</sup> DM) of intact baker's yeast (*Saccharomyces cerevisiae*), extracted baker's yeast (*S. cerevisiae*), filamentous fungi (*Rhizopus oryzae*) and blue mussel (*Mytilus edulis*)

	Feed ingredient			
	<i>S. cerevisiae</i>			
	Intact	Extracted	<i>R. oryzae</i>	<i>M. edulis</i>
Crude protein	466	779	505	657
Sum of amino acids	428	498	274	472
Crude lipid	10	2	84	69
Ash	63	153	103	89
Gross energy	19.9	18.1	21.6	22.8
Indispensable amino acids				
Arginine	22.4	16.8	11.0	36.7
Histidine	10.4	10.4	9.8	10.2
Isoleucine	22.8	26.4	17.8	22.0
Leucine	32.1	36.7	23.3	33.7
Lysine	34.7	38.8	27.6	38.5
Methionine <sup>1</sup>	9.7	13.0	2.3	19.2
Phenylalanine	19.3	21.2	14.5	18.5
Threonine	22.9	20.6	8.6	21.8
Valine	28.1	32.8	21.8	24.9
Sum	202.3	216.8	136.6	225.5
Dispensable amino acids				
Alanine	24.4	37.8	20.3	25.7
Aspartic acid	45.4	53.4	25.2	49.7
Cysteine <sup>2,3</sup>	9.8	12.0	0.8	14.2
Glutamic acid	66.7	76.3	35.3	63.3
Glycine	22.1	26.2	19.7	31.1
Ornithine	1.0	0.0	8.6	0.3
Proline	15.4	33.8	3.8	18.5
Serine	18.3	21.1	15.9	21.3
Tyrosine <sup>3</sup>	22.9	19.6	8.2	21.8
Sum	225.9	281.3	137.8	246.0

<sup>1</sup> Amount present after oxidation of methionine to methionine sulphone.

<sup>2</sup> Amount present after oxidation of cysteine and cystine to cysteic acid.

<sup>3</sup> Conditionally indispensable (NRC, 2011).

an average temperature of 7.1 ± 1.8 °C. Duration of light exposure varied between 17 h 30 min and 19 h 30 min per day, following the natural day length in Northern Sweden.

The experiment was carried out in compliance with laws and regulations concerning experiments with live animals overseen by the Swedish Board of Agriculture and approved by the Ethical Committee for Animal Experiments in Umeå, Sweden.

### Diets and feeding

One reference (control) diet and four test diets were used in this experiment. The chemical composition of the test ingredients is given in Table 1, the diet formulation in Table 2 and diet composition in Table 3. The reference

**Table 2** Formulation (g kg<sup>-1</sup> as is) of the experimental diets

Ingredient	Experimental diet <sup>1</sup>				
	REF	MYE	ISC	ESC	RHO
Fish meal	467.9	280.4	280.9	281.7	279.0
Fish oil	89.4	89.3	91.7	97.0	81.6
Soya protein concentrate	36.4	36.4	28.1	31.3	36.2
Soya bean meal	114.3	103.9	83.2	114.7	113.7
Rapeseed oil	34.6	32.0	33.9	34.7	27.1
Wheat gluten	33.8	38.9	60.3	38.6	36.2
Wheat	124.8	124.6	102.0	130.1	100.2
Titanium oxide	5.2	5.2	5.2	5.2	5.2
Min-vit premix	15.6	15.6	15.6	15.6	15.5
Cellulose	78.0	53.5	10.0	77.9	44.7
<i>Mytilus edulis</i>	–	220.0	–	–	–
Intact <i>Saccharomyces cerevisiae</i>	–	–	289.0	–	–
Extracted <i>S.cerevisiae</i>	–	–	–	172.6	–
<i>R. oryzae</i>	–	–	–	–	260.1

<sup>1</sup> REF = reference diet, MYE = diet with blue mussel (*M. edulis*), ISC = diet with intact yeast (*S. cerevisiae*), ESC = diet with extracted yeast (ESC), RHO = diet with filamentous fungi *R. oryzae*.

(REF) diet was formulated similarly to a commercial diet for Arctic charr, with high-quality, low-temperature dried fish meal as the main protein source.

The experimental diets were formulated based on the reference diet, but with 40% of the fish meal replaced with test ingredients on a dry matter (DM) and crude protein (CP) basis. The test diets contained dried, de-shelled blue mussel *M. edulis* (diet MYE) (Royal Frysk Muscheln GmbH, Emmelsbüll-Horsbüll, Germany), intact yeast *S. cerevisiae* (diet ISC) (Jästbolaget AB, Rottebro, Sweden), extracted yeast *S. cerevisiae* (diet ESC) (Alltech, Senta, Serbia), or fungi *R. oryzae* (diet RHO) (Cewatech AB, Gothenburg, Sweden). Dietary recommendations for Arctic charr according to Jobling *et al.* (1993) were followed.

All diets were formulated as iso-nitrogenous (CP content = 45%) and iso-energetic (gross energy (GE) = 20 MJ kg<sup>-1</sup>). Titanium dioxide (TiO<sub>2</sub>) was used in all diets as an inert marker for digestibility determination. All diets were extruded at the Finnish Game and Fisheries Research Institute (Laukaa Research Station, Laukaa, Finland) on a twin-screw extruder (3 mm die, BC-45 model; Cletral, Creusot Loir, France). During the extrusion process, 20% of additional moisture was added to the feed mash, which was heated to 120–130 °C for 30 s, dried overnight by warm air and then sprayed with lipids using a vacuum coater (Pegasus PG-10VC; Dinnissen, Sevenum, the Netherlands).

**Table 3** Proximate chemical composition (g kg<sup>-1</sup> DM), energy content (MJ kg<sup>-1</sup> DM) and amino acid content (g kg<sup>-1</sup> DM) of the experimental diets

	Experimental diet <sup>1</sup>				
	REF	MYE	ISC	ESC	RHO
Dry matter (g kg <sup>-1</sup> )	912	917	913	929	908
Crude protein (N × 6.25)	493	498	492	494	480
Total amino acids	439	465	491	500	443
Crude lipid	201	201	190	174	186
Ash	76	74	67	75	73
Gross energy	24.1	24.4	23.9	23.2	23.9
Indispensable amino acids					
Arginine	28.1	30.6	28.4	27.5	25.3
Histidine	11.0	10.4	12.1	12.0	12.6
Isoleucine	21.4	19.5	23.4	23.4	22.8
Leucine	36.4	35.7	38.6	38.2	35.5
Lysine	31.6	33.0	34.0	34.3	32.5
Methionine <sup>2</sup>	18.4	14.2	13.4	15.7	14.1
Phenylalanine	20.1	20.3	22.5	22.0	20.7
Threonine	19.5	20.7	20.7	19.4	15.4
Valine	26.3	23.9	28.6	28.4	27.5
Sum	212.7	208.4	221.6	220.8	206.3
Dispensable amino acids					
Alanine	25.3	24.9	26.6	28.7	25.1
Aspartic acid	43.1	45.2	46.0	47.8	41.4
Cysteine <sup>3,4</sup>	8.1	8.7	9.0	10.5	9.4
Glutamic acid	79.3	81.4	92.8	90.3	76.8
Glycine	24.4	25.9	25.4	25.6	25.1
Proline	22.4	25.0	26.6	30.2	22.2
Serine	17.4	23.1	20.7	20.5	10.8
Tyrosine <sup>3</sup>	6.7	19.6	19.8	19.5	18.9
Ornithine	0.0	3.2	2.3	6.5	6.8
Sum	226.7	257.1	269.2	279.6	236.4

<sup>1</sup> REF = reference diet, MYE = diet with blue mussel (*Mytilus edulis*), ISC = diet with intact yeast (*Saccharomyces cerevisiae*), ESC = diet with extracted yeast (ESC), RHO = diet with filamentous fungi *Rhizopus oryzae*.

<sup>2</sup> Amount present after oxidation of methionine to methionine sulphone.

<sup>3</sup> Amount present after oxidation of cysteine and cystine to cysteic acid.

<sup>4</sup> Conditionally indispensable (NRC, 2011).

After the fish were placed in the experimental tanks, they were starved for 1 week in order to acclimate to the new tanks and avoid effects of handling stress on feed intake. Subsequently, the fish were fed the experimental diets distributed by automatic feeders (Arvo-Tec T 2000, Huutokoski, Finland). All fish were fed a restricted ration of 1% of the total fish biomass in each tank to avoid excess feed wastage and minimize the risk of compensatory feeding by increased feed intake. The feed ration was increased weekly based on a specific growth rate (SGR) of 0.7 at 6 °C (Linnér & Brännäs 2001) until experimental day 51, when all the fish were netted, anaesthetized with 50 mg L<sup>-1</sup> MS-222, measured and weighed. The feed allowance was then

corrected to 1% of total biomass based on the new values, and feeding was resumed 1 week after weighing to avoid the effect of handling stress on feed intake.

### Sampling of fish

The experiment lasted 99 days (March–June 2013). Prior to the start of the experiment, five fish were taken from the holding tanks, euthanized with an overdose of 300 mg L<sup>-1</sup> MS-222 and stored at -25 °C for reference whole-body analysis. At the end of the experiment, all fish were netted and anaesthetized with 100 mg L<sup>-1</sup> MS-222 solution. Body weight was recorded for each fish. For the purposes of whole-body analysis, additional five fish were randomly selected from each tank, euthanized and stored as previously described.

### Sampling for faeces and relative body indices

At the end of the trial, five fish from each tank were randomly selected, anesthetized with 200 mg L<sup>-1</sup> MS-222 and euthanized by cutting the branchial arches before dissection. The whole gastrointestinal tract, liver and visceral fat were removed and weighed for calculation of viscerosomatic index (VSI) and hepatosomatic index (HSI). Faeces samples were collected from the distal intestine of these fish and pooled as one sample per tank for digestibility analysis.

### Ussing chamber experiment

Possible effects of feed on the active transport functions of the intestinal epithelium were assessed in parallel with the intestinal barrier function using an Ussing chamber set-up according to Sundell *et al.* (2003) with modifications by Sundell & Sundh (2012). Intestines were sampled from the REF, MYE, ISC and RHO groups at experimental days 97–99. In brief, four fish from each tank replicate were randomly sampled in one quick dip netting and immediately anesthetized as previously described. The anesthetized fish were then killed with a sharp blow to the head, and the intestine was sampled as previously described (Abro *et al.* 2014b). After mounting, the intestinal segments were allowed 60 min of recovery for stabilization of the electrical parameters. Thereafter, the experiment was started by renewing the Ringer solution (4 mL in each half-chamber) on the serosal side and replacing the Ringer solution on the mucosal side with Ringer containing 4.6 µL mL<sup>-1</sup> of the hydrophilic marker molecule <sup>14</sup>C-mannitol

(0.1 mCi mL<sup>-1</sup> and 55.5 mCi mmol<sup>-1</sup>; Moravek Biochemicals, Brea, CA, USA) and 0.9 µL mL<sup>-1</sup> of <sup>3</sup>H-L-lysine (1 mCi mL<sup>-1</sup> and 91.6 Ci mmol<sup>-1</sup>; PerkinElmer, Boston, MA, USA) in combination with unlabelled lysine at a concentration of 0.5 mM in the final Ringer solution, resulting in a final specific <sup>3</sup>H-lysine activity of 1.9 mCi mmol<sup>-1</sup>. A 50 µL portion of the serosal Ringer was sampled at time points 0, 20, 30, 60, 80 and 90 min. Radioactivity was assessed in a liquid scintillation counter using a dual label (<sup>14</sup>C/<sup>3</sup>H) protocol (Wallac 1409 Liquid Scintillation Counter, Turku, Finland) after adding 5 mL Ultima Gold™ (PerkinElmer).

### Sample preparation and chemical analysis

Experimental feed and faeces were freeze-dried, ground with a coffee grinder (KG40; DeLonghi Appliances, Casula, NSW, Australia) and stored at -25 °C. Whole, non-processed fish were stored at -25 °C postsampling, thawed and homogenized with a mixer (B-400; Büchi Labortechnik AG, Flawil, Switzerland).

Ash was determined after incineration at 550 °C for a minimum of 3 h until the ash was white, and then cooled in a desiccator before weighing. The DM content was determined after heating the samples in an oven at 103 °C for 16 h and then cooling in a desiccator before weighing. Total N was determined according to the Kjeldahl method using a digester and analyser (2020 and 2400 Kjeltec; FOSS Analytical A/S, Hilleröd, Denmark). A factor of N × 6.25 was used to determine CP (Nordic committee on food analysis, 1976). Crude lipid (CL) was determined using a hydrolysis and extraction system (1047 Hydrolysing Unit and a Soxtec System HT 1043 Extraction Unit; FOSS Analytical A/S).

Determination of GE was performed with a bomb calorimeter (Parr 6300; Parr Instrument Company, Moline, IL, USA) and expressed as MJ kg<sup>-1</sup>. TiO<sub>2</sub> was analysed according to Short *et al.* (1996).

Amino acid levels in the feed, faeces and whole fish samples were determined as previously described by Langeland *et al.* (2014) using the AccQ-Tag™ method (Waters Corporation, Milford, MA, USA). The UPLC system was a Dionex Ultimate 3000 binary rapid separation LC system with a variable UV-detector (Thermo Fisher, Stockholm, Sweden). Empower 2 (Waters Corporation) software was used for system control and data acquisition. Reference feed samples were repeatedly analysed as an internal quality control. Within-laboratory relative variation was <10% for all amino acids analysed.

### Calculations

Weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) were calculated according to the equations:

$$\text{WG}(\%) = ((\text{FW} - \text{SW})/\text{SW}) \times 100$$

$$\text{SGR}(\% \text{ day}^{-1}) = 100 \times ((\ln \text{FW} - \ln \text{SW})/T)$$

$$\text{FCR} = \text{FI}/\text{WG}$$

where FW is the final weight (g) of the fish, SW is the initial weight of the fish (g), T is the duration of the experiment (days) and FI is the total feed intake (g). The relative weight of liver and viscera was expressed as hepatosomatic (HSI) and viscerosomatic (VSI) index, respectively, calculated according to the following equations:

$$\text{HSI}(\%) = (W_{\text{Liv}}/\text{FW}) \times 100$$

$$\text{VSI}(\%) = (W_{\text{Vis}}/\text{FW}) \times 100$$

where  $W_{\text{Liv}}$  is the weight of liver (g),  $W_{\text{Vis}}$  is the weight of viscera (g) and FW is the fish weight.

Nutrient retention was determined as:

$$(\text{Nutrient retained in the body}/\text{Nutrient ingested}) \times 100.$$

Apparent digestibility coefficient (ADC) was calculated as:

$$\text{ADC}_{\text{diet}} = [1 - (F/D \times D_i/F_i)] \times 100$$

where  $F$  = % nutrient (or kJ g<sup>-1</sup>GE) in faeces,  $D$  = % nutrient (or kJ g<sup>-1</sup>GE) in diet,  $D_i$  = % inert marker in diet and  $F_i$  = % inert marker in faeces.

Apparent permeability of mannitol,  $P_{\text{app}}$ , and uptake rate of L-lysine were calculated using the following equations:

$$P_{\text{app}} = dQ/dT \times 1/AC_0$$

$$\text{L-Lysine} = dQ/dT \times 1/A$$

where  $dQ/dT$  is the appearance rate of the molecule in the serosal compartment of the Ussing chamber,  $A$  is the area (cm<sup>2</sup>) of intestinal surface exposed in the chamber and  $C_0$  is the initial concentration (mol mL<sup>-1</sup>) on the mucosal side.

### Statistical analysis

Effects of the test diets on growth performance (SW, FW, WG and SGR) and relative organ weight (HSI and VSI)

were evaluated using the model PROC MIXED, including the fixed factor of test diet (REF, MYE, ISC, ESC and RHO) and the random factor of tank within test diet. Data analysis for growth parameters SW, FW, WG and SGR was run without outliers, which were identified as being outside the 97.5% confidence limits in a frequency distribution analysis. Effects of the test diets on FCR, ADC, nutrient and energy retention were evaluated using the statistical model PROC GLM, including the fixed factor of test diet. To adjust for multiple comparisons, Tukey's multiple comparisons test was used. Tank was the experimental unit, and significance level was set to  $P < 0.05$ . Data were analysed with Statistical Analysis System version 9.3 (SAS Institute Inc., Carry, NC, USA).

Ussing chamber data were analysed in a mixed linear model (MLM) with diet and tank nested within diet as a fixed factor and using type III sum of squares in cases of unbalanced data. Significant effects ( $P < 0.05$ ) of diet were subjected to subsequent post hoc analysis using Sidak-adjusted pairwise comparisons of the estimated marginal

means of each experimental diet to the control diet. Ussing chamber data were analysed using IBM SPSS Statistics software version 20 (IBM SPSS Statistics for Windows, Version 20.0.; IBM Corp., Armonk, NY, USA).

## Results

### Growth performance and feed utilization

There were no differences in start weight between fish in the different dietary treatments. Fish fed diets REF, MYE and ISC did not significantly differ in terms of FW, SGR or WG (Table 4). However, fish fed diets ESC and RHO had significantly lower FW, SGR and WG than fish fed the REF diet. There were no differences in FCR between diets, although FCR showed a tendency to be lower in fish fed diet RHO ( $P = 0.064$ ) than in fish fed diet REF. Dietary treatments did not influence HSI and VSI.

Nitrogen retention was significantly higher in fish fed diet REF than in fish fed diet ESC, while no differences

**Table 4** Growth performance, relative organ weight and nutrient retention in Arctic charr fed the experimental diets

	Experimental diet <sup>1</sup>					SE	P-value
	REF	MYE	ISC	ESC	RHO		
SW (g) <sup>2</sup>	48.6	47.5	46.6	47.8	48.3	0.81	0.652
FW (g) <sup>2</sup>	133.3 <sup>a</sup>	126.4 <sup>ab</sup>	125.6 <sup>ab</sup>	117.9 <sup>b</sup>	118.5 <sup>b</sup>	1.89	0.001
SGR (% day <sup>-1</sup> ) <sup>2</sup>	1.08 <sup>a</sup>	1.02 <sup>ab</sup>	1.04 <sup>ab</sup>	0.95 <sup>b</sup>	0.97 <sup>b</sup>	0.01	0.001
FCR (g g <sup>-1</sup> ) <sup>3</sup>	0.89	0.93	0.95	0.98	1.01	0.03	0.064
HSI (%) <sup>4</sup>	1.5	1.6	1.6	1.5	1.5	0.07	0.363
VSI (%) <sup>4</sup>	9.5	8.8	9.4	9.3	10.5	0.42	0.139
Nutrient and energy retention (%)							
Crude protein (N × 6.25) <sup>5</sup>	43.5 <sup>a</sup>	39.5 <sup>ab</sup>	40.2 <sup>ab</sup>	38.1 <sup>b</sup>	39.3 <sup>ab</sup>	1.05	0.041
Arginine	42.7	39.8	39.1	35.5	43.5	2.96	0.383
Histidine	42.9	50.0	38.7	35.6	36.8	3.47	0.083
Isoleucine	43.2	42.7	35.1	33.5	31.7	2.58	0.027
Leucine	41.8	43.0	36.4	34.5	37.9	2.07	0.071
Lysine	51.3	50.5	45.2	39.9	41.9	2.55	0.035
Methionine <sup>2</sup>	54.4 <sup>a</sup>	71.0 <sup>b</sup>	71.4 <sup>b</sup>	55.3 <sup>ab</sup>	65.6 <sup>ab</sup>	3.47	0.012
Phenylalanine	40.6	40.4	34.6	31.9	36.9	2.47	0.113
Threonine	42.4	44.2	37.7	38.2	53.3	3.22	0.061
Valine	43.7	44.4	36.3	34.6	34.6	5.26	0.015
Energy	43.6	42.3	40.7	39.8	38.2	1.24	0.075
Mortality (%)	0.67	0	0.67	0.67	0.67	–	–

SW, start weight; FW, final body weight; SGR, specific growth rate; FCR, feed conversion ratio; HSI, hepatosomatic index; VSI, viscerosomatic index; SE, pooled standard error.

Data presented are least square means.

Values within rows with different superscripts are significantly different ( $P < 0.05$ ).

<sup>1</sup> REF = reference diet, MYE = diet with blue mussel (*Mytilus edulis*), ISC = diet with intact yeast (*Saccharomyces cerevisiae*), ESC = diet with extracted yeast (ESC), RHO = diet with filamentous fungi *Rhizopus oryzae*.

<sup>2</sup> When analysing SW, FW and SGR,  $n = 145$  for REF;  $n = 145$  for MYE;  $n = 140$  for ISC;  $n = 141$  for ESC;  $n = 142$  for RHO.

<sup>3</sup> When analysing FCR,  $n = 3$ .

<sup>4</sup> When analysing HIS and VSI,  $n = 15$ .

<sup>5</sup> When analysing nutrient retention,  $n = 3$ .

were observed between diets REF, MYE, ISC and RHO. Similarly, fish fed diets REF, MYE, ISC and RHO did not differ in total amino acid retention, while fish fed ESC diet had lower total amino acid retention than those fed REF and MYE. There was a significant effect of diet on retention of isoleucine ( $P = 0.027$ ), lysine ( $P = 0.035$ ) and valine ( $P = 0.015$ ) when all diets were included in the same model. However, no significant effect was observed between specific diets when using adjustments for pairwise comparisons. There were no differences between dietary treatments in retention of the remaining IAA (Table 4). No effect of dietary treatment on energy retention was observed. There were minor mortalities in the study, but these were unaffected by diet (Table 4).

### Apparent digestibility of diets

Apparent digestibility coefficients (ADCs) for CP, energy and indispensable amino acids are shown in Table 5. Diet MYE had the highest ADC values for DM, although not significantly higher than diet ESC. Diet RHO had the lowest ADC for DM. The ADC for CP was higher for the REF, MYE and ESC diets than for diets ISC and RHO. Furthermore, the ADC of CP was higher for diet ISC than RHO. No differences in ADC of GE were observed between the diets.

**Table 5** Apparent digestibility coefficients (ADC; %) for dry matter (DM), crude protein (CP), gross energy (GE) and indispensable amino acids (IAA) of the experimental diets for Arctic charr,  $n = 15$

	Experimental diet <sup>1</sup>					SE	P-value
	REF	MYE	ISC	ESC	RHO		
ADC for DM	70.4 <sup>a</sup>	73.7 <sup>c</sup>	70.6 <sup>a</sup>	71.9 <sup>ac</sup>	62.2 <sup>b</sup>	0.51	<0.0001
ADC for CP	87.0 <sup>a</sup>	88.2 <sup>a</sup>	83.8 <sup>b</sup>	89.5 <sup>a</sup>	80.4 <sup>c</sup>	0.56	<0.0001
ADC for GE	78.8	87.1	85.3	78.7	71.5	4.41	0.178
ADC of IAA							
Arginine	93.4 <sup>ab</sup>	94.2 <sup>a</sup>	91.9 <sup>b</sup>	94.6 <sup>a</sup>	91.9 <sup>b</sup>	0.46	0.004
Histidine	89.9 <sup>ab</sup>	90.2 <sup>ab</sup>	88.7 <sup>a</sup>	91.7 <sup>b</sup>	89.7 <sup>ab</sup>	0.48	0.015
Isoleucine	91.7 <sup>ab</sup>	89.9 <sup>ab</sup>	89.0 <sup>a</sup>	93.6 <sup>b</sup>	90.0 <sup>ab</sup>	0.87	0.032
Leucine	92.4 <sup>abc</sup>	92.6 <sup>bc</sup>	89.9 <sup>a</sup>	94.0 <sup>b</sup>	90.3 <sup>ac</sup>	0.54	0.002
Lysine	92.2 <sup>ac</sup>	93.1 <sup>ac</sup>	89.3 <sup>b</sup>	94.0 <sup>c</sup>	91.2 <sup>ab</sup>	0.50	0.001
Methionine <sup>2</sup>	92.0 <sup>ac</sup>	90.7 <sup>ac</sup>	88.0 <sup>b</sup>	93.2 <sup>a</sup>	89.5 <sup>bc</sup>	0.58	0.001
Phenylalanine	91.7 <sup>ab</sup>	92.2 <sup>ab</sup>	89.8 <sup>a</sup>	93.8 <sup>b</sup>	90.0 <sup>a</sup>	0.52	0.002
Threonine	90.7 <sup>a</sup>	91.5 <sup>a</sup>	84.8 <sup>b</sup>	91.9 <sup>a</sup>	84.9 <sup>b</sup>	0.62	<0.0001
Valine	91.0 <sup>ab</sup>	90.3 <sup>ab</sup>	88.3 <sup>a</sup>	92.9 <sup>b</sup>	89.2 <sup>ab</sup>	0.85	0.029
Sum of IAA	91.9 <sup>ac</sup>	92.1 <sup>ac</sup>	89.1 <sup>b</sup>	93.5 <sup>c</sup>	89.9 <sup>ab</sup>	0.53	0.001

SE, pooled standard error.

Data presented are least square means.

Values within rows with different superscripts are significantly different ( $P < 0.05$ ).

<sup>1</sup> REF = reference diet, MYE = diet with blue mussel (*Mytilus edulis*), ISC = diet with intact yeast (*Saccharomyces cerevisiae*), ESC = diet with extracted yeast (ESC), RHO = diet with filamentous fungi *Rhizopus oryzae*.

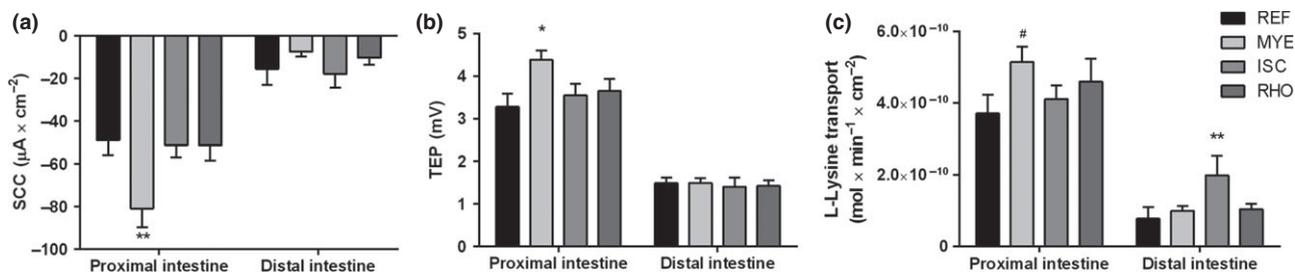
<sup>2</sup> Methionine = amount present after oxidation of methionine to methionine sulphone.

The ADC values for sum of indispensable amino acids (IAA) were relatively high for all diets, ranging from 89.1% (ISC diet) to 93.5% (MYE diet). The highest ADC of IAA was found for diets ESC, MYE and REF. Diet ISC showed lower digestibility of lysine, methionine and threonine compared with the REF and MYE diets. Moreover, diet ISC displayed lower ADC values for all amino acids compared with the ESC diet. There were no differences in ADC values for any of the IAA between ISC and RHO diets.

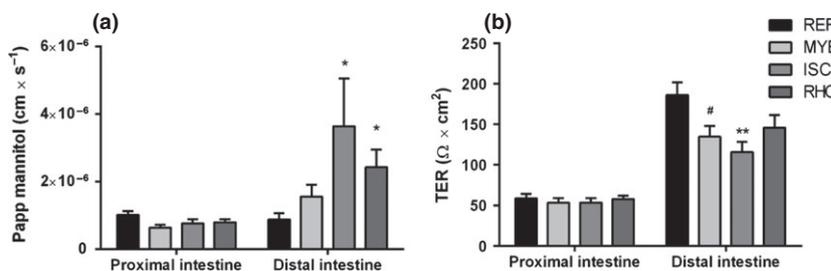
### Active intestinal transport

In the proximal intestine, diet had an overall effect on short-circuit current (SCC) and the subsequent post hoc test showed a more negative SCC with the MYE diet compared with the REF diet (Fig. 1a). Diet also affected the trans-epithelial potential (TEP) (Fig. 1b), with post hoc analysis revealing higher TEP in the MYE group compared with the REF group. No effects of diet on SCC or TEP were observed in the distal intestine.

Regarding uptake of the amino acid lysine, there was a tendency for higher transport in the proximal than in the distal intestine for diet MYE ( $P = 0.067$ ). In the distal intestine, diet had a significant effect on lysine transport, where the ISC diet showed higher uptake rate than the REF diet (Fig. 1c).



**Figure 1** Active intestinal transport in charr fed experimental diets as assessed by (a) short-circuit current (SCC), (b) transepithelial potential (TEP) and (c) lysine transport in the proximal and distal intestine,  $n = 12$ . Diet MYE increased active transport in the proximal intestine, as indicated by increased SCC, TEP and lysine transport ( $P = 0.067^{\#}$ ,  $P < 0.05^*$  and  $P < 0.01^{**}$ ). In the distal intestine, no effect of diet was seen for SCC or TEP, but diet MYE increased transport of lysine, possibly as a result of increased paracellular leakage.



**Figure 2** Intestinal barrier function of charr fed experimental diets as assessed by (a) the apparent permeability ( $P_{app}$ ) for mannitol and (b) transepithelial resistance (TER) in the proximal and distal intestine,  $n = 12$ . The diets did not affect the barrier function of the proximal intestine, but in the distal intestine diets, ISC and RHO impaired the intestinal barrier function, observed as increased diffusion rate of mannitol ( $P_{app}$ ) ( $P = 0.072^{\#}$ ,  $P < 0.05^*$  and  $P < 0.01^{**}$ ). TER tended to be lower in the MYE group and significantly lower in the ISC group, indicating reduced barrier function.

### Intestinal barrier function

The barrier function of the distal intestine, as assessed by  $P_{app}$ , was significantly affected by diet. Subsequent post hoc testing revealed higher  $P_{app}$  for the ISC and RHO diets compared with REF (Fig. 2a). No differences in paracellular permeability in the proximal intestine were observed between any diets (Fig. 2a).

A significant effect of diet was observed for transepithelial resistance (TER) in the distal intestine, and a subsequent post hoc test revealed a strong tendency for lower TER in the fish fed the MYE diet ( $P = 0.072$ ), as well as lower TER for the ISC dietary treatment compared with the REF diet (Fig. 2b). No significant differences in TER in the proximal intestine were observed between different diets (Fig. 2b).

### Chemical composition of feed ingredients and experimental diets

For ingredients, the highest CP content was found in extracted *S. cerevisiae*, followed by *M. edulis*, *R. oryzae* and intact *S. cerevisiae* (Table 1). Total amount and profile of amino acids were similar between test ingredients with

the exception of *R. oryzae*, which had lower total amino acid content. In general, all protein sources showed comparable levels of IAA with the exception of methionine, which was highest in *M. edulis*. Test ingredients varied in terms of crude lipid (CL) content from 2 to 84 g kg<sup>-1</sup> DM, with the lowest lipid content found in extracted *S. cerevisiae* and the highest in *R. oryzae*. Ash content varied from 63 to 153 g kg<sup>-1</sup> DM. Total GE content varied between 18.1 MJ kg<sup>-1</sup> DM (extracted *S. cerevisiae*) and 22.8 MJ kg<sup>-1</sup> DM (*M. edulis*).

For experimental diets, the chemical analyses showed that CP, CL, ash, GE and IAA were relatively uniform among the diets (Table 3). The CP content varied from 480 g kg<sup>-1</sup> DM (RHO) to 498 g kg<sup>-1</sup> DM (MYE). The GE varied from 23.2 MJ kg<sup>-1</sup> (ESC) to 24.4 MJ kg<sup>-1</sup> DM (MYE). The total amount of IAA was highest in diet ISC (221.6 g kg<sup>-1</sup> DM) and lowest in diet RHO (206.3 g kg<sup>-1</sup> DM).

### Discussion

The results of the present study show that de-shelled *M. edulis* and intact *S. cerevisiae* can replace 40% of fish

meal (on a CP basis) in the diet for Arctic charr without any significant effects on growth performance, based on FW, WG, SGR and FCR after 99 days of feeding. Conversely, charr fed diets supplemented with extracted *S. cerevisiae* and *R. oryzae* exhibited lower growth performance.

Only a limited number of published studies have evaluated the use of blue mussel meal as a protein source in fish diets. Berge & Austreng (1989) used whole, milled blue mussel for rainbow trout and showed that increasing the amount of mussel meal in the diet from 0 to 450 g kg<sup>-1</sup> reduced the digestibility of DM and caused an enlarged liver. These effects were attributed to the high shell fraction in the mussel meal. In the present study, there were no marked negative effects on growth or ADC values in Arctic charr fed mussel meal, which might be explained by the absence of the shell fraction. Stimulation of active transport mechanisms in the proximal intestine was observed for the MYE diet, as indicated by higher absolute values of SCC and TEP and supported by higher lysine transport. However, compared with the REF diet, the MYE diet may induce weakening of the intestinal physical barrier function, as there was a tendency for lower TER in the distal intestine with that diet.

The results of this study contradict earlier findings using intact *S. cerevisiae* in the diet of several other salmonid species. When 40% of fish meal (CP basis) was replaced with whole, intact *S. cerevisiae* in the diet for Atlantic salmon, nutrient retention, nutrient ADCs and growth performance were negatively affected (Øverland *et al.* 2013). In contrast, Rumsey *et al.* (1990) used disrupted baker's yeast to replace 50% of CP in the diet for lake trout and reported no negative effects on growth. Furthermore, Rumsey *et al.* (1990) concluded that the presence of intact cell walls in dried *S. cerevisiae* may lower digestibility and growth in fish. However, the diets used in the present study were produced by extrusion, which might have increased the availability and retention of CP and IAA for the ISC diet. Extrusion may have caused partial disruption of rigid yeast cell walls in the ISC diet, and consequently increased digestibility, whereas cold pelleting would not have had this effect. Positive effects of extrusion processing on digestibility and weight gain have been documented in gilthead sea bream, silver perch (*Bydianus bydianus*) and rainbow trout fed various protein sources (Booth *et al.* 2002; Venou *et al.* 2003, 2006; Barrows *et al.* 2007). Moreover, ADC values of IAAs were only slightly lower for ISC compared with the REF diet, whereas Øverland *et al.* (2013) reported 20–30% lower ADC values of IAA for *S. cerevisiae* compared with fish meal. Nonetheless, no firm conclusions on the

effect of extrusion can be drawn in the present study, and additional efforts exploring the effect of the extrusion process on disruption of cell walls in *S. cerevisiae* are ongoing. Furthermore, ADC of CP for the RHO diet in the previous study by Langeland *et al.* (2014) was higher than that of ISC and equal to that of other diets, whereas in the present study, ADC of CP was lowest for the RHO diet. An important finding was the difference in ADC values of RHO diet in the present study compared with that reported by Langeland *et al.* (2014), which used another batch of *R. oryzae* delivered by the same producer. This indicates that the quality fluctuates between microbial biomass batches produced and may have caused variations in ADC and growth. According to the *R. oryzae* biomass producer, spent sulphate liquor, used for production of *R. oryzae* biomass, may contain high levels of magnesium sulphate (MgSO<sub>4</sub>). Its concentration may differ between batches, mainly depending on how the *R. oryzae* biomass has been washed and dried. In earlier studies on rats and mice, MgSO<sub>4</sub> has been used for inducing diarrhoea and is known for its laxative effect (Izzo *et al.* 1994; Uddin *et al.* 2005). The consistency of the faeces samples acquired from fish fed RHO diet at the end of this trial was mainly liquid, which might indicate the onset of diarrhoea in Arctic charr fed RHO diet. This might partly explain unusually low ADC values of CP in charr fed RHO diet and the contradictory ADC values between this study and the earlier one by Langeland *et al.* (2014).

In case of nutrient retention, there is a general agreement between protein retention and retention of IAA between different dietary treatments. Higher methionine retention in fish fed diets ISC and MYE is not supported by the ADC values of methionine. Diets MYE and ISC had lower content of methionine when compared to other diets, which could explain higher methionine retention.

The present study and the previous study by Langeland *et al.* (2014), which used the same ingredients, both found lower ADC of CP for intact *S. cerevisiae* than for fish meal, *M. edulis* and extracted *S. cerevisiae*. Digestibility of REF, ISC and MYE diets in the present study agreed with that reported by Langeland *et al.* (2014) when compared as relative numbers, whereas the absolute values in the present study were lower. The differences between these studies in absolute ADC values for the REF, ISC and MYE diets could be the result of different faeces collection methods. In the present study, faeces were collected by dissection, while Langeland *et al.* (2014) used a settling column collection system. Settling column systems have been reported to overestimate ADC, whereas stripping can cause

underestimation (Hajen *et al.* 1993; Vandenberg & De La Noüe 2001).

The ISC and the RHO diets resulted in impaired intestinal barrier function (i.e. increased paracellular permeability), resulting in a leakier epithelium, which can lead to increased translocation of bacteria, viruses, antigens and other molecules. This in turn could increase disease susceptibility and the risk of developing intestinal inflammation (Segner *et al.* 2012). Consequences of a decreased physical barrier function may also be positive for the fish. For example, increased leakage of antigens across the epithelium could induce the immune system to counteract, and thereby decrease, the translocation rate of pathogenic bacteria (Niklasson *et al.* 2011; Torrecillas *et al.* 2011). Thus, a weakening of the physical intestinal barrier function may be an indication of a strengthened immunological barrier and better protection against disease, depending on whether an intestinal inflammation has developed or not. In the present study, it was not possible to assess the intestinal inflammatory status, and therefore, no conclusions can be drawn on the resulting effects of the increased paracellular permeability.

Yeast cell walls, which consist of mannoproteins,  $\beta$ -glucans and chitin, can account for 10–25% of total cell biomass (DM basis) of *S. cerevisiae* (Klis *et al.* 2006). In fish, these nutritional compounds have been shown to have immuno-modulating effects (Refstie *et al.* 2010; Navarrete & Tovar-Ramirez 2014), intestinal barrier and health-enhancing properties (Torrecillas *et al.* 2011) and positive effects on growth performance (Torrecillas *et al.* 2014). *Rhizopus oryzae* instead contains considerable amounts of chitin and chitosan, polysaccharides that have also been shown to generate positive immuno-modulatory effects in fish (Esteban *et al.* 2001; Harikrishnan *et al.* 2012). Diets ISC and RHO in the present study could be expected to contain high levels of polysaccharides, including a mixture of several of those listed above. However, in the present experiment, the RHO diet resulted in disturbed intestinal physical barrier function, lower CP digestibility and lower growth performance, indicating a negative rather than positive effect of the microbial cell wall compounds. In agreement with a previous dietary trial on Arctic charr, the *R. oryzae*-based diet also resulted in impaired physical intestinal barrier function (Abro *et al.* 2014b). As observed here, the ISC diet in that study resulted in disturbed intestinal physical barrier function and lower CP digestibility, but no negative impact was observed on growth performance, nutrient retention and ADC of DM and GE (Abro *et al.* 2014b). This indicates that a disturbed intestinal barrier

function did not negatively affect the growth performance in charr fed the ISC diet during the experimental period in this study. The mechanisms behind the dietary-induced decrease in intestinal physical barrier are currently unknown, but there are several possible pathways. In rainbow trout, high levels of chitosan in the diet have been suggested to be an irritating factor in the gastrointestinal canal that may result in direct mechanical damage to the epithelium (Mydland *et al.* 2009). Chitosan can also increase the paracellular permeability of the intestinal epithelia by affecting the tight junction (TJ) complexes (Thanou *et al.* 2001; Smith *et al.* 2004; Rosenthal *et al.* 2012). A reducing effect of chitosan on nitrogen digestibility in diets for rainbow trout has also been reported by Mydland *et al.* (2007).

In the present study, highest ADC values for DM, CP and IAA were observed for diets REF, MYE and ESC. In fact, the ADCs of CP and IAA were numerically highest for ESC diet. This is somewhat confusing, as these high values were not reflected in corresponding growth performance. A possible explanation is the chemical composition of extracted *S. cerevisiae*, which had a relatively high CP content but somewhat lower total amino acid content, indicating high non-protein nitrogen (NPN) content. Furthermore, the ESC diet had the lowest amount of CL, which might indicate that some of the protein and NPN were used for the energy needs of the fish. This would explain high digestibility values of CP and low growth performance. In addition, the method used for cell wall disruption during the production of extracted *S. cerevisiae* was autolysis. This might have led to partial hydrolysis of microbial protein resulting in high amount of short peptides and free amino acids, which could have caused an imbalance in amino acid absorption and possible use of amino acids for energy needs. This could partly explain both the lower retention of N, IAA and total amino acids in fish fed ESC diet compared with the REF and MYE diets and the lower overall growth performance while maintaining relatively high CP digestibility. Studies performed by Rumsey *et al.* (1990, 1991a) on lake trout and rainbow trout showed similar results when brewer's yeast cell walls were disrupted.

Few previous studies have focused on optimization of single cell protein (SCP) production with feed properties as the determining factor. In fact, in most dietary fish studies to date, such as those by Rumsey *et al.* (1990), Oliva-Teles & Gonçalves (2001), Abdel-Tawwab *et al.* (2008), Øverland *et al.* (2013) and Hauptman *et al.* (2014), non-feed microorganisms have been used. To improve the commercial

utilization of these sources as alternatives to fish meal and soya concentrate, more emphasis should be placed on optimization of dietary amino acid profiles. Levels of IAA such as methionine were consistently lower in all test diets, especially those containing *R. oryzae* and intact *S. cerevisiae*. However, inclusion of free crystalline methionine in diets supplemented with SCP has been tested in several studies over the years with no conclusive results (Murray & Marchant 1986; Rumsey *et al.* 1991b; Hauptman *et al.* 2014). This indicates that digestible protein, possibly in combination with appropriate extrusion configuration and methionine supplementation, should be considered in future studies with microbial protein sources. In addition, further studies exploring the effects of the dietary replacements tested in the present study on intestinal inflammatory status and immune system are needed to evaluate their feasibility as alternative feed ingredients in aquaculture.

## Acknowledgements

The authors would like to express their gratitude to a number of people who contributed to this manuscript: Kristina Andersson for help with statistical analysis and manuscript revisions, Hans Petersson and Anna-Greta Haglund for help in conducting UPLC analysis and the technical staff at Kälmarne Research Station (Vattenbrukscentrum Norr AB). The authors would also like to thank Hanna Carlberg, Liane Wagner and Pedro Gomez Requeni for their assistance during different stages of the experiment and David Huyben for valuable inputs during manuscript revisions. Finally, thanks to Cewatech<sup>®</sup>, Alltech<sup>®</sup> and Jästbolaget<sup>®</sup> for contributing the test ingredients. This work was supported by the Faculty of Veterinary Medicine and Animal Science at the Swedish University of Agricultural Sciences and by Formas (Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning).

## References

Abdel-Tawwab, M., Abdel-Rahman, A.M. & Ismael, N.E.M. (2008) Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for Fry Nile tilapia, *Oreochromis niloticus* (L.) challenged in situ with *Aeromonas hydrophila*. *Aquaculture*, **280**, 185–189.

Abro, R., Moazzami, A., Lindberg, J. & Lundh, T. (2014a) Metabolic insights in Arctic charr (*Salvelinus alpinus*) fed with zygomycetes and fish meal diets as assessed in liver using nuclear magnetic resonance (NMR) spectroscopy. *Int. Aquat. Res.*, **6**, 1–11.

Abro, R., Sundell, K., Sandblom, E., Sundh, H., Brännäs, E., Kiessling, A., Lindberg, J.E. & Lundh, T. (2014b) Evaluation of chitinolytic activities and membrane integrity in gut tissues of

Arctic char (*Salvelinus alpinus*) fed fish meal and zygomycete biomass. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.*, **175**, 1–8.

Andersen, Ø., Aas, T.S., Skugor, S., Takle, H., van Nes, S., Grisdale-Helland, B., Helland, S.J. & Terjesen, B.F. (2006) Purine-induced expression of urate oxidase and enzyme activity in Atlantic salmon (*Salmo salar*). *FEBS J.*, **273**, 2839–2850.

Barrows, F.T., Stone, D.A.J. & Hardy, R.W. (2007) The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **265**, 244–252.

Berge, G.M. & Austreng, E. (1989) Blue mussel in feed for rainbow trout. *Aquaculture*, **81**, 79–90.

Booth, M.A., Allan, G.L., Evans, A.J. & Gleeson, V.P. (2002) Effects of steam pelleting or extrusion on digestibility and performance of silver perch *Bidyanus bidyanus*. *Aquac. Res.*, **33**, 1163–1173.

Brännäs, E. & Linnér, J. (2000) Growth effects in Arctic charr reared in cold water: feed frequency, access to bottom feeding and stocking density. *Aquacult. Int.*, **8**, 381–389.

Brown, L.R. (2012) Full Planet, Empty Plates: The New Geopolitics of Food Scarcity. W. W. Norton, New York, USA.

Chikwati, E.M. (2013) Diet-induced physiological and pathophysiological responses in the intestine of Atlantic salmon (*Salmo salar* L.): effects of plant protein ingredients and antinutritional factors. Doctoral thesis, Norwegian School of Veterinary Science, DOI: 10.13140/rg.2.1.3574.7362

Dimitroglou, A., Merrifield, D.L., Spring, P., Sweetman, J., Moate, R. & Davies, S.J. (2010) Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead sea bream (*Sparus aurata*). *Aquaculture*, **300**, 182–188.

Edebo, L. (2008) Zygomycetes for fish feed. Google Patents.

Esteban, M.A., Cuesta, A., Ortuño, J. & Meseguer, J. (2001) Immunomodulatory effects of dietary intake of chitin on gilthead seabream (*Sparus aurata* L.) innate immune system. *Fish Shellfish Immunol.*, **11**, 303–315.

Gatlin, D.M., Barrows, F.T., Brown, P. *et al.* (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquac. Res.*, **38**, 551–579.

Hajen, W.E., Beames, R.M., Higgs, D.A. & Dosanjh, B.S. (1993) Digestibility of various feedstuffs by post-juvenile chinook salmon (*Oncorhynchus tshawytscha*) in sea water. 1. Validation of technique. *Aquaculture*, **112**, 321–332.

Halasz, A. & Lasztity, R. (1991) Use of Yeast Biomass in Food Production. CRC Press Inc, Boca Raton, FL, USA.

Harikrishnan, R., Kim, J.-S., Balasundaram, C. & Heo, M.-S. (2012) Immunomodulatory effects of chitin and chitosan enriched diets in *Epinephelus bruneus* against *Vibrio alginolyticus* infection. *Aquaculture*, **326–329**, 46–52.

Hauptman, B.S., Barrows, F.T., Block, S.S., Gibson Gaylord, T., Paterson, J.A., Rawles, S.D. & Sealey, W.M. (2014) Evaluation of grain distillers dried yeast as a fish meal substitute in practical-type diets of juvenile rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **432**, 7–14.

HLPE (2014) Sustainable fisheries and aquaculture for food security and nutrition. A report by the High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security, Rome, June 2014.

Izzo, A.A., Gagarella, T.S., Mascolo, N. & Capasso, F. (1994) Nitric oxide as a mediator of the laxative action of magnesium sulphate. *Br. J. Pharmacol.*, **113**, 228–232.

- Jobling, M., Jørgensen, E.H., Arnesen, A.M. & Ringø, E. (1993) Feeding, growth and environmental requirements of Arctic charr: a review of aquaculture potential. *Aquacult. Int.*, **1**, 20–46.
- Jutfelt, F., Olsen, R.E., Björnsson, B.T. & Sundell, K. (2007) Parr-smolt transformation and dietary vegetable lipids affect intestinal nutrient uptake, barrier function and plasma cortisol levels in Atlantic salmon. *Aquaculture*, **273**, 298–311.
- Kiessling, A. (2009) Feed-the key to sustainable fish farming. In: KSLA- Fish, Trade and Development (Ackefors, H., Cullberg, M., Wramner, P. & Sundberg, P. eds), pp. 303–322. Royal Swedish Academy of Agriculture and Forestry (KSLA), Halmstad Tryckeri, Sweden.
- Kinsella, J.E., German, B. & Shetty, J. (1985) Uricase from fish liver: isolation and some properties. *Comp. Biochem. Physiol. B*, **82**, 621–624.
- Klis, F.M., Boersma, A. & De Groot, P.W.J. (2006) Cell wall construction in *Saccharomyces cerevisiae*. *Yeast*, **23**, 185–202.
- Knudsen, D., Jutfelt, F., Sundh, H., Sundell, K., Koppe, W. & Frokiaer, H. (2008) Dietary soya saponins increase gut permeability and play a key role in the onset of soyabean-induced enteritis in Atlantic salmon (*Salmo salar* L.). *Br. J. Nutr.*, **100**, 120–129.
- Krogdahl, Å., Bakke-McKellep, A.M. & Baeverfjord, G. (2003) Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquac. Nutr.*, **9**, 361–371.
- Langeland, M., Vidakovic, A., Vielma, J., Lindberg, J.E., Kiessling, A. & Lundh, T. (2014) Digestibility of microbial and mussel meal for Arctic charr (*Salvelinus alpinus*) and Eurasian perch (*Perca fluviatilis*). *Aquac. Nutr.*, DOI: 10.1111/anu.12268 [Epub ahead of print].
- Lindahl, O., Hart, R., Hernroth, B., Kollberg, S., Loo, L.-O., Olrog, L., Rehnstam-Holm, A.-S., Svensson, J., Svensson, S. & Syversen, U. (2005) Improving marine water quality by mussel farming: a profitable solution for Swedish society. *Ambio*, **34**, 131–138.
- Linnér, J. & Brännäs, E. (2001) Growth in Arctic charr and rainbow trout fed temporally concentrated or spaced daily meals. *Aquacult. Int.*, **9**, 35–44.
- Murray, A.P. & Marchant, R. (1986) Nitrogen utilization in rainbow trout fingerlings (*Salmo gairdneri* Richardson) fed mixed microbial biomass. *Aquaculture*, **54**, 263–275.
- Mydland, L.T., Landsverk, T., Zimonja, T., Storebakken, T., Edebo, L. & Kiessling, A. (2007) Mycelium biomass from fungi (*Rhizopus oryzae*) grown on spent sulphite liquor from paper pulp as a protein source in diets for Rainbow trout (*Oncorhynchus mykiss*). In: *Aquaculture Europe 2007, Book of abstracts*, pp. 376–376, October 24–27, Istanbul, Turkey.
- Mydland, L.T., Skugor, S., Martinsen, T.S., Landsverk, S., Kiessling, A., Edebo, L. & Krasnov, A. (2009) Alterations in gene expression in gut tissue in Rainbow trout (*Oncorhynchus mykiss*) fed mycelium biomass from fungi (*Rhizopus oryzae*). In: *International Symposium on Genomics in Aquaculture*. Bodø, July 5–7, Norway.
- Nasseri, A.T., Rasoul-Amini, S., Morowvat, M.H. & Ghasemi, Y. (2011) Single cell protein: production and process. *Am. J. Food Technol.*, **6**, 103–116.
- Navarrete, P. & Tovar-Ramirez, D. (2014) Use of Yeasts as Probiotics in Fish Aquaculture. In: *Sustainable Aquaculture Techniques* (Hernandez-Vergara, M. ed.), pp. 135–172. InTech, Rijeka, Croatia.
- Naylor, R.L., Hardy, R.W., Bureau, D.P. et al. (2009) Feeding aquaculture in an era of finite resources. *Proc. Natl Acad. Sci. USA*, **106**, 15103–15110.
- Niklasson, L., Sundh, H., Fridell, F., Taranger, G.L. & Sundell, K. (2011) Disturbance of the intestinal mucosal immune system of farmed Atlantic salmon (*Salmo salar*), in response to long-term hypoxic conditions. *Fish Shellfish Immunol.*, **31**, 1072–1080.
- Nilsson, J., Brännäs, E. & Eriksson, L.O. (2010) The Swedish Arctic charr breeding programme. *Hydrobiologia*, **650**, 275–282.
- Nordic committee on food analysis (1976) Determination in Feeds and Faeces According to Kjeldahl, No6. NKML, Oslo, Norway.
- NRC (2011) Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC, USA.
- Oliva-Teles, A. & Gonçalves, P. (2001) Partial replacement of fishmeal by brewers yeast (*Saccharomyces cerevisiae*) in diets for sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture*, **202**, 269–278.
- Olli, J.J., Krogdahl, Å., van den Ingh, T.S.G.A.M. & Brattås, L.E. (1994) Nutritive value of four soybean products in diets for Atlantic Salmon (*Salmo salar*, L.). *Acta Agric. Scand. A Anim. Sci.*, **44**, 50–60.
- Øverland, M., Karlsson, A., Mydland, L.T., Romarheim, O.H. & Skrede, A. (2013) Evaluation of *Candida utilis*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* yeasts as protein sources in diets for Atlantic salmon (*Salmo salar*). *Aquaculture*, **402–403**, 1–7.
- Pan, J. (2013) Effects of non-fish based raw materials on the fish muscle quality of salmonids. Doctoral thesis No 2013:91, Department of Food Science, Swedish University of Agricultural Sciences, Uppsala.
- Refstie, S., Baeverfjord, G., Seim, R.R. & Elvebø, O. (2010) Effects of dietary yeast cell wall [beta]-glucans and MOS on performance, gut health, and salmon lice resistance in Atlantic salmon (*Salmo salar*) fed sunflower and soybean meal. *Aquaculture*, **305**, 109–116.
- Rosenthal, R., Gunzel, D., Finger, C., Krug, S.M., Richter, J.F., Schulzke, J.D., Fromm, M. & Amasheh, S. (2012) The effect of chitosan on transcellular and paracellular mechanisms in the intestinal epithelial barrier. *Biomaterials*, **33**, 2791–2800.
- Rumsey, G.L., Hughes, S.G. & Kinsella, J.L. (1990) Use of dietary yeast *Saccharomyces cerevisiae* Nitrogen by Lake Trout. *J. World Aquaculture Soc.*, **21**, 205–209.
- Rumsey, G.L., Hughes, S.G., Smith, R.R., Kinsella, J.E. & Shetty, K.J. (1991a) Digestibility and energy values of intact, disrupted and extracts from brewer's dried yeast fed to rainbow trout (*Oncorhynchus mykiss*). *Anim. Feed Sci. Technol.*, **33**, 185–193.
- Rumsey, G.L., Kinsella, J.E., Shetty, K.J. & Hughes, S.G. (1991b) Effect of high dietary concentrations of brewer's dried yeast on growth performance and liver uricase in rainbow trout (*Oncorhynchus mykiss*). *Anim. Feed Sci. Technol.*, **33**, 177–183.
- Rumsey, G.L., Winfree, R.A. & Hughes, S.G. (1992) Nutritional value of dietary nucleic acids and purine bases to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **108**, 97–110.
- Segner, H., Sundh, H., Buchmann, K., Douxfils, J., Sundell, K.S., Mathieu, C., Ruane, N., Jutfelt, F., Toften, H. & Vaughan, L. (2012) Health of farmed fish: its relation to fish welfare and its utility as welfare indicator. *Fish Physiol. Biochem.*, **38**, 85–105.
- Short, F.J., Gorton, P., Wiseman, J. & Boorman, K.N. (1996) Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Anim. Feed Sci. Technol.*, **59**, 215–221.
- Smith, J., Wood, E. & Dornish, M. (2004) Effect of chitosan on epithelial cell tight junctions. *Pharm. Res.*, **21**, 43–49.

- Sundell, K.S. & Rønnestad, I. (2011) Intestinal absorption. In: Encyclopedia of Fish Physiology (Editor-in-Chief: Anthony, P.F. ed.), pp. 1311–1321. Academic Press, San Diego, CA, USA.
- Sundell, K. & Sundh, H. (2012) Intestinal fluid absorption in anadromous salmonids: importance of tight junctions and aquaporins. *Front. Physiol.*, **3**, 388.
- Sundell, K., Jutfelt, F., Agustsson, T., Olsen, R.E., Sandblom, E., Hansen, T. & Björnsson, B.T. (2003) Intestinal transport mechanisms and plasma cortisol levels during normal and out-of-season parr-smolt transformation of Atlantic salmon, *Salmo salar*. *Aquaculture*, **222**, 265–285.
- Tacon, A.G.J. & Metian, M. (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture*, **285**, 146–158.
- Thanou, M., Verhoef, J.C. & Junginger, H.E. (2001) Chitosan and its derivatives as intestinal absorption enhancers. *Adv. Drug Deliv. Rev.*, **50**(Suppl 1), S91–S101.
- Torreillas, S., Makol, A., Benitez-Santana, T., Caballero, M.J., Montero, D., Sweetman, J. & Izquierdo, M. (2011) Reduced gut bacterial translocation in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides (MOS). *Fish Shellfish Immunol.*, **30**, 674–681.
- Torreillas, S., Montero, D. & Izquierdo, M. (2014) Improved health and growth of fish fed mannan oligosaccharides: potential mode of action. *Fish Shellfish Immunol.*, **36**, 525–544.
- Uddin, S.J., Shilpi, J.A., Alam, S.M.S., Alamgir, M., Rahman, M.T. & Sarker, S.D. (2005) Antidiarrhoeal activity of the methanol extract of the barks of *Xylocarpus moluccensis* in castor oil- and magnesium sulphate-induced diarrhoea models in mice. *J. Ethnopharmacol.*, **101**, 139–143.
- Vandenberg, G.W. & De La Noüe, J. (2001) Apparent digestibility comparison in rainbow trout (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility markers. *Aquac. Nutr.*, **7**, 237–245.
- Venou, B., Alexis, M.N., Fountoulaki, E., Nengas, I., Apostolopoulou, M. & Castritsi-Cathariou, I. (2003) Effect of extrusion of wheat and corn on gilthead sea bream (*Sparus aurata*) growth, nutrient utilization efficiency, rates of gastric evacuation and digestive enzyme activities. *Aquaculture*, **225**, 207–223.
- Venou, B., Alexis, M.N., Fountoulaki, E. & Haralabous, J. (2006) Effects of extrusion and inclusion level of soybean meal on diet digestibility, performance and nutrient utilization of gilthead sea bream (*Sparus aurata*). *Aquaculture*, **261**, 343–356.