Nutrient digestibility and growth in Arctic charr fed microbial and mussel protein meal

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Description

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Abstract
The content of marine based feed sources must be decreased in feed for fish. New production has to be built on sustainable practices and technologies which include production of aquafeeds with other ingredients which are not suitable as food for humans. We have investigated the nutrient digestibility and performed a short term growth study on Artic charr fed microbial biomass and blue mussel meal instead of fish meal. Analysis of feed show different amino acid profiles with very low methionine content in especially *R. oryaze* (RHO) and extracted *Saccharomyces* (ESC), whereas intact *Saccharomyces* (ISC) showed similar amino acid profile as fish meal but with slightly lower methionine content.

The apparent digestibility coefficients of dry matter, crude protein, total amino acid and energy were highest for ESC, mussel meal (MYE) and RHO. No difference in weight gain was observed between fish fed the reference diet, diet ISC and diet MYE. Fish fed diet RHO and ESC showed 16 and 13% lower weight gain compared with fish fed diet REF.

The conclusion of the results is that intact *Saccharomyces* and mussel meal are good alternatives as a protein source instead of marine fish meal for feeding Arctic charr. The results suggest that ISC togheter with MYE is the best choice for developing locally produced feed resources and closing the nutrient loop in the Baltic Sea region. The results will be followed up with further evaluation in long-term feeding experiments with Arctic charr.

Keywords
Saccharomyses cerervisiae, Rhizopus oryzae, blue mussel, amino acid, apparent digestibility, weight gain

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1. Introduction

The aquafeed production has depended on marine based protein sources for decades. The increased debate about depletion of fish stocks and the increased demand for more sustainable aquaculture has moved the focus from marine to alternative protein sources of plant and microbial origin. New production has to be built on sustainable practices and technologies which include production of aquafeeds with other ingredients not suitable as food for humans.

Microbes are used in the industry to convert waste products from agro-and forestry – industries. Fungi of different species have been used for production of ethanol in the paper industry. Microbes grow very fast under the right conditions and microbe biomass contains high amounts of protein. Therefore, microbes are an attractive candidate as a protein source for feed production to farmed fish.

The Baltic blend concept is based on a sustainable aquafeed production of local sources not used for production of human food. The protein sources in Baltic blend feed origin from mussel and fish meal from the Baltic Sea region and of microbial biomass protein produced from non human food resources, one third of each. By using this type of locally produced protein sources to make feed for fish we are able to close the nutrient loop of circulating nutrients in the Baltic Sea region.

The aim of this study was to evaluate different microbial sources which should be used for making the Baltic blend feed for use in the long-term growth trial (Aquabest report, Performance of Arctic charr fed with Baltic Sea –sourced ingredients). The microorganisms used were chosen using the criteria that they should be safe for human handling and consumption and be able to be produced on agro- and forestry by-products. The nutrient content in feed, digestibility and growth of Artic charr were evaluated for three different microbial feed components, intact *Saccharomyses cervisae (ISC)*, extracted *Saccharomyces cerevisiae* (ESC) and *Rhizopus oryzae (*RHO*).* Mussel meal (MYE) was also included since mussel meal was one of the major ingredients in the Baltic blend feed concept.

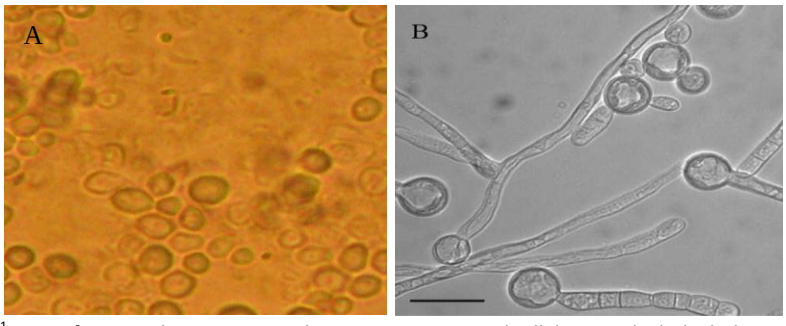

Figure 1. Feed ingredients for production of sustaniable feed production. A) Bakers yeast, *Saccharomysescerevisiae, B*[1]*)* Zygomycete fungi*, Rhizopus oryzae*

[1]Picture from Patrik Lennartssons Thesis, Zygomycetes and cellulose residuals: hydrolysis, cultivation and applications. Chalmers Univeristy of Technology, Gothenburg, Sweden.

2. Material and Methods

2.1. The feeds

The reference diet (REF) was formulated to resemble commercial diets with protein sources from commercial fish meal and soya. Test ingredients for replacement of fish meal in the fish diet were intact baker's yeast, *Saccharomyces cervisae (*Jästbolaget, Sweden*)* extracted yeast, *Saccharomyces*

*cervisae (*Alltech, Serbia, AD, Serbia), the filamentous micro fungi, *Rizhopus Oryzae* (Cewatech, Sweden) and blue mussel, *Mytilus Edulis*, (Royal Frysk Muscheln GmbH, Emmelsbüll-Hornsbül, Germany). The proximate compositon of test ingredients are presented in Table 1.

Table 1. Proximate chemical composition (g kg^{-1} DM), energy content (MJ kg^{-1} DM) and amino acid content (g kg^{-1} DM) test ingredients; of test ingredients used in experimental diet for the digestibilty study

	Intact S. *cervisae*	Extracted *S. cerivisae*	*R. oryzae*	*M. edulis*
Dry matter	935	938	947	868
Crude Protein	466	779	479	657
Sum of amino acids	428	498	220	472
Crude lipid	10	2	94	69
Ash	63	153	121	89
Gross energy	19.9	18.1	19.7	22.8

The experimental feeds for the digestibility and the growth study were produced by extrusion on a twin-screw Clextral (Creusot Loire) BC-45 extruder while the lipids were added to the extruded feed using a Dinnissen Pegasus PG-10VC vacuum coater. The diets were manufactured at Laukaa fish farn, Finland, of the Finish Game and Fisheries Research Institute and thereafter shipped to SLU, Uppsala, Sweden.

The feed formulation and proximal chemical composition of the diets used in the digestibility and growth trial is shown in Table 2 and 3.

Table 2. Feed ingredients and pproximate chemical composition (g kg^{-1} DM), energy content (MJ kg^{-1} DM) and amino acid content (g kg^{-1} DM) of the experimental diets in the digestibility trial.

	Experimental diet[1]				
	REF	ISC	ESC	RHO	MYE
Ingredients					
Fish meal	475	332	332	332	332
Soya bean protein concentrate	70	49	49	49	49
Soya bean meal	60	42	42	42	42
Wheat gluten	50	35	35	35	35
Wheat	170	119	119	119	119
Cellulose	20	14	14	14	14
Fish oil	120	84	84	84	84
Rapeseed oil	30	21	21	21	21
Titanium dioxide	0.5	0.35	0.35	0.35	0.35
Vitamin & mineral premix	5	3.5	3.5	3.5	3.5
Intact *Saccharomyses cervisae*		300			
Extracted *Saccharomyses cervisae*			300		
Rhizopus oryzae				300	
Blue mussel					300
Proximate composition					
Dry matter	953	921	917	927	954
Crude Protein	490	485	600	483	540
Sum of amino acids	417	369	436	344	470
Crude lipid	230	167	113	194	187
Neutral Detergent Fiber	61	37	43	75	46
Ash	84	74	110	84	84
Gross energy	23.8	22.7	21.4	22.8	23.2

[1]Ref= reference diet, ISC= intact saccharomyses, ESC= extracted saccharomyces and RHO= Rhizopus oryzae, MYE= blue mussel (*Mytilus edulis*)

Table 3. Feed formaulation and proximate chemical composition (g kg^{-1} DM), energy content (MJ kg^{-1} DM) and amino acid content of experimental diets in the growth trial.

	Diets				
	REF	ISC	ESC	RHO	MYE
Ingredients					
Fish meal	468	281	282	279	280
Fish oil	89	92	97	82	89
Soy protein concentrate	36	28	31	36	36
Soybean meal	114	83	115	114	104
Rapeseed oil	35	34	35	27	32
Wheat gluten	34	60	39	36	39
Wheat	125	102	130	100	125
Titanium oxide	5	5	5	5	5
Min-vit premix	16	16	16	16	16
Cellulose	78	10	78	45	54
Bakers yeast		289			
Yeast extract			173		
Zygomycetes				260	
Mussel meal					220
Proximate chemical composition					
Dry matter	912	913	929	908	917
Crude protein	493	492	494	480	498
Sum of amino acids	439	465	490	500	442
Crude lipid	201	190	174	186	201
Ash	76	66	75	73	74
Gross energy	24.1	23.9	23.2	23.9	24.4

[1]Ref= reference diet, ISC= intact saccharomyces, ESC= extracted saccharomyces and RHO= Rhizopus oryzae, MYE= blue mussel (*Mytilus edulis*)

2.2. Fish and rearing

2.2.1. Digestibility trial

Arctic charr, 200 fish in total (106.6 ± 4.9 g (mean ± s.d.) body weight (BW) in period 1 and 113.8 ± 7.9 g BW in period 2), from the Swedish breeding program (Nilsson et al., 2010) were obtained from Kälarne research station. Before starting the experiments the fish were kept in 1000 L flow-through tanks, separated by species, at the Swedish University of Agricultural Sciences, Uppsala, Sweden. Prior to the digestibility experiments the fish were fed commercial diets to apparent satiation twice daily.The fish were mildly anesthetized (triacine methanesuphonate, MS 222; Western Chemical Inc., Ferdale, WA, USA) before handling.

2.2.2. Growth trial

The fish were raised in 700L rectangular tanks at Kälarne research station. During this time fish families were kept separated and were fed the commercial diet. Two weeks prior to the experiment 750

fish from different families, average weight 48.05 g (±0.63g) were netted, anesthetized (100mg l^{-1} MS-222), individually tagged, weighed, measured and randomly allocated into fifteen rectangular flow through fiberglass tanks. Thus, 50 fish were placed in each tank. Average water temperature during the experimental period was 7.1 °C (±1.8°C) and water flow 10 l/min. The experimental period lasted for 99 days.

2.3. Experimental setup

2.3.1. Digestibility trial

The experimental unit was comprised of ten 90-L PVC tanks equipped with waste feed and faeces collectors (Cho *et al.*, 1982). The PVC tanks were connected to an indoor recirculating system with mechanical and biological filtration, and UV treatment. Average water temperatures were 10 C°.

The fish was fed by hand until provided feed were rejected. After feeding the feed waste were collected in 50 ml tube attached to the bottom of the collection column and stored in -25°c. The faeces were collected in the same way as the feed waste during the periods between feeding. The tube was removed just before the next feeding started, and centrifuged at 5000 x g for 10 minutes and the supernatant rejected. The pellets were stored in -25°C until analysis. Total collection time for the experiment was 2 weeks per period.

Figure 2. PVC tanks equipped with waste feed and faeces collectors. Foto. Aleksandar Vidakovic

2.3.2. Growth trial

Prior to the beginning of the experiment, five fish were taken from the holding tanks, for reference non-processed body analysis. After 99 days was the growth experiment finalized and all fish were netted and anesthetized with MS 222 (100 mg/L) solution. Body weight and length were recorded for each fish.

From each tank, five fish were randomly selected and 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

into common sample for each tank for digestibility analysis. For the purpose of whole body analysis additional randomly selected five fish from each tank, were euthanized as previously described and stored at -20°C.

Figure 3. Experimental tanks in Kälarne research station

3. Results

3.1. Amino acid profile

The amino acid profile differed between test ingredients. As expected the content of indispensable amino acid was highest in fishmeal except for phenylalanine, threonine and valine which was up to two times higher in intact *Saccharomyces*. In general *Rizhopus oryzae* contained less amino acid compared with the other components. Histidine and methione were exceptional low in *Rhizopus oryzae* and extracted *Saccharomyces* (Figure 4).

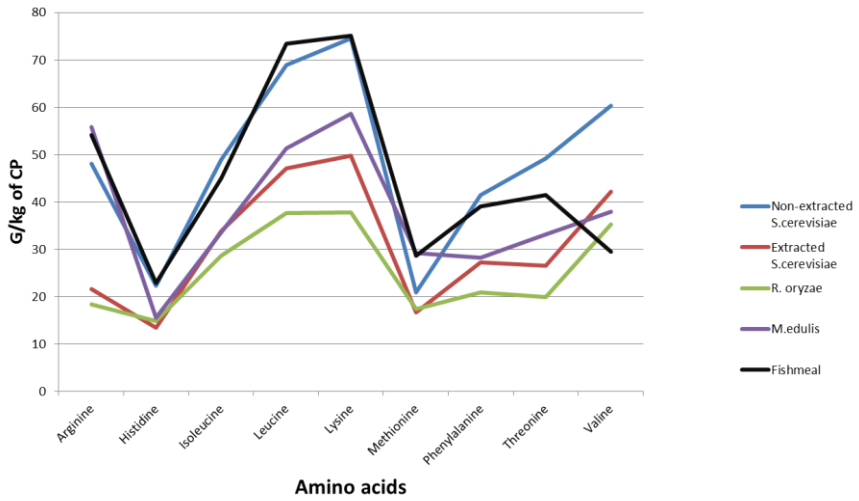

Figure 4. Amino acid content in test ingredients included in diets for digestibility and growth trials.

3.2. Digestibility

All experimental diets were well consumed by Arctic charr, with the exception for the RHO diet, which resulted in a lower feed intake. The weight gain ((final weight – initial weight)/initial weight × 100) ranged between 3.6% and 17.7%. The feed conversion ratio (FCR) (dry feed intake/wet weight gain) ranged between 0.75 and 2.87. The higher values in FCR for both species were recorded for fish fed the ESC diet, which had a poorer pellet quality than the other diets causing high losses when collecting feed wastes. In addition, the poor extrusion of this diet resulted in lower absorption of the added lipids.

For the test ingredients the apparent digestibility (ADC) of DM, SAA and energy of whole *S. cerevisiae* was significant lower than of extracted *S. cerevisiae, R. oryzae* and *M. edilus*, whereas ADC of CP did not differ between the test ingredients.

The ADCs of the reference and experimental diets for Arctic charr are presented in Figure 5. Diets based on extracted bakers yeast (ESC), the micro fungi *Rizhopus oryzae* (RHO) and mussel meal (MYE) had a higher ADC of DM than the reference diet (REF) and intact bakers yeast (ISC) ($P<0.001$). The ADC of CP in diet ESC (92.8%) was significantly higher than of diet ISC (87.5%), while the ADC of SAA was higher in both diets ESC and MYE than in diet ISC ($P<0.001$). Diet ESC had a higher ADC of energy than diets ISC and REF ($P<0.001$). Significant differences were found in the ADC of indispensable amino acids (IAA) between the experimental diets. Diets MYE and ESC had significantly higher ADC for most of the evaluated IAA than the other diets.

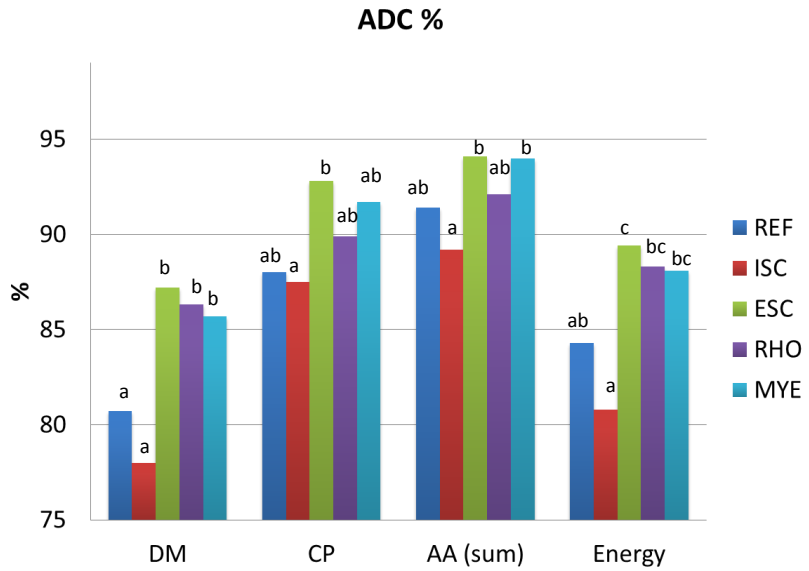

Figure 5. Apparent digestibility coefficients (%) of dry matter (DM), crude protein (CP), sum of amino acids (AA), and energy in reference and experimental diets for Arctic charr. Faeces samples from 10 fish per tank were collected by a Guelph system with four tanks per treatment. Data is presented as least square means. Reference diet (REF), Intact Saccharomyces (ISC), Extracted Saccharomyses (ESC), Rizhopus oryzae (RHO), Mussel meal (MYE)

3.3. Growth

There was no difference in final body weight (FW) among different treatments (Figure 6). Total weight gain (WG) was higher in fish fed REF than in fish fed RHO and ESC diets while there was no difference between fish fed diets REF, ISC and MYE ($P<0.05$). In addition, diet ISC and MYE had higher WG when compared to diet RHO.

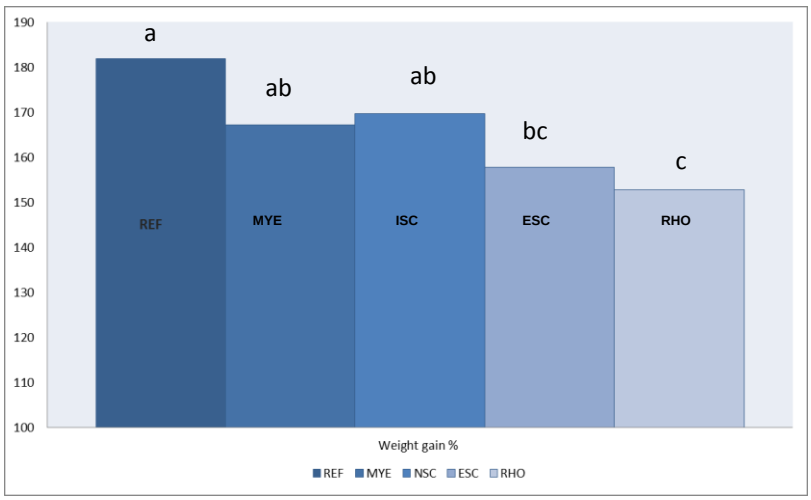

Figure 6. Weight gain (%) of Arctic charr fed different diets for 3 months. Reference diet (REF), Mussel meal (MYE), Intact Saccharomyces (ISC), Extracted Saccharomyces (ESC), Rizhopus oryzae (RHO).

The specific growth rate (SGR) varied from 0.97 to 1.08% and was higher for the REF than for RHO and ESC diets while no difference was found between REF, ISC and MYE diets. Furthermore, there was a tendency (p=0.0639) that the feed conversion ratio (FCR) was lower in fish fed diet REF (0.89) than diet RHO (1.01).

Body indices, hepato-somatic index (HIS) and viscero-somatic index (VSI) did not differ significantly between the treatments.

4. Discussion

The crude protein content in feed were analysed by the Kjeldahl method using the conversion factor of N x 6.25 for calculation. Based on these results we made an isonitrogenous diets in the growth trial. However, the amino acid analysis results show that the zygomycete fungi *R. oryzae*, contain much less true protein nitrogen compared with the other feed components The cell wall of R. *oryzae* contains high amounts of chitin and chitosan, glucose polymers with bound nitrogen (Lennartsson 2012), which explain the low amino acid content compared with crude protein values. Furthermore, we have found very high variation in amino acid concentration between batches which make it less usable for fish feed production until a stable production process of cultivating *Rhizopus oryzae* has been accomplished.

Our findings suggest that extracted *S. cerevisiae* and *M. edulis* have a high digestibility in Arctic charr, and that *R. oryzae* and in particular intact *S. cerevisiae* have lower digestibility. Further, we showed that the digestibility of *S. cerevisiae* might be improved by extraction.

The higher ADCs of DM, GE and SAA in extracted as compared to intact *S. cerevisiae* in Arctic charr could be an effect of the absence of cell wall material, ablated trough the extraction process. Similar results has been reported for lake trout (*Salvelinus namaycush*) and rainbow trout, fed brewer's yeast after cell wall disruption (Rumsey *et al.*, 1990; Rumsey *et al.*, 1991). The cell wall of yeast, consists of 85-90% polysaccharides (mixture of mannan, glucan and small amount of chitin) (Nguyen *et al.*, 1998; Klis *et al.*, 2006) The cell wall is relatively thick, tough and rigid and can constitute of 10-

25% of DM (Rumsey *et al.*, 1991). The rigid cell wall have been suggested to be the reason for the lower digestibility of the intact *S. cerevisiae* reported in several other studies (Rumsey *et al.*, 1991; Cheng *et al.*, 2004; Øverland *et al.*, 2013). However, the apparent digestibility of the intact *S. cerevisiae* presented in our study, are similar to values reported by Oliva-Teles & Gonçalves (2001).

No differences in growth were detected in fish fed with intact *Saccharomyces* and mussel meal. That fish fed extracted yeast show lower weight gain was surprising since the crude protein value was almost twice as high as for intact saccharomyces. The processes to make protein extracts of *Saccharomyces* seem to affect the protein quality. The results from amino acid analysis show clearly a lower content of indispenable amino acids in extracted *Saccharomyces*. Especially the methionine content was lower which indicates that the methionine content can be limitited for both extracted *Saccharomyses* and diets made on *R. oryzae.*

5. Conclusion

In conclusion, the result obtained in the present studies indicates that the digestibility of extracted *S. cerevisiae* and *M. edulis* is high in Arctic charr and that *R. oryzae* and particularly intact *S. cerevisiae* has lower digestibility. Further, the absence of intact cell walls and extrusion process seems to have a positive effect on digestibility of *S. cerevisiae* in Arctic charr. The best growth performances in Arctic charr were achived in fish fed intact Saccharomyses, mussel meal, extracted *Saccharomyses* and *R. oryzae* in descending order.

Thought lower digestibility of intact *Saccharomyces* compared with the other microbial protein sources, extracted *Saccharomyces* and *R. oryzae*, fish fed on intact *Saccharomyces* shown the best growth performance. Intact *Saccharomyces* and mussel meal are promising ingredients and have therefore been chosen for making diets for the long term feed trial of Arctic charr fed with Baltic Sea-sourced ingredients, see Reports of Aquabest project 10/2014 (Carlberg et al. 2014).

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