Domestic feed sources to farmed Arctic Charr (Salvelinus alpinus). An investigation of nutritional implications and impact on the ecological footprint.

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Summary

This study presents an overview of recapturing nutrients from Baltic Sea thru mussel farming and producing mussel meal, detoxification of polluted fish into purified high quality fish meal and oil and introducing microorganisms as waste consumers as well as good protein sources for fish diets. Work on this study is focused on comparing growth performance of test diet, which has been made strictly of domestic (Baltic Sea) protein sources to commercial diet. Also, the study backs up the idea that use/reuse of protein sources and nutrients, i.e. making diets of sources from Baltic Sea and use them in aquaculture makes positive impact on ecological footprint, restores balance in aquatic ecosystem and flow of nutrients that can compete with present commercial diets in growth performance and price. Study is based on practical experiment, literature investigation and personal communication with people involved in this issue. Experiment is based on comparing growth performance of fish fed commercial (control) feed and test (experimental) feed.

The thesis intends to show that recapture of nutrients is a way to establish a nutrition positive and environmental sustainable aquaculture in the Baltic Sea.

Acknowledgements

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1. Introduction

1.1. Background

Aquaculture is the most rapidly expanding sector of animal production worldwide and requires increased production of fish feeds. Increased production of fish feed will require increasing of feed ingredients, mainly protein sources. Most high-value species of fish raised by aquaculture are carnivore fish requiring feeds containing 400 g per kg, or more protein, generally supplied by fish meal (Hardy 1996).

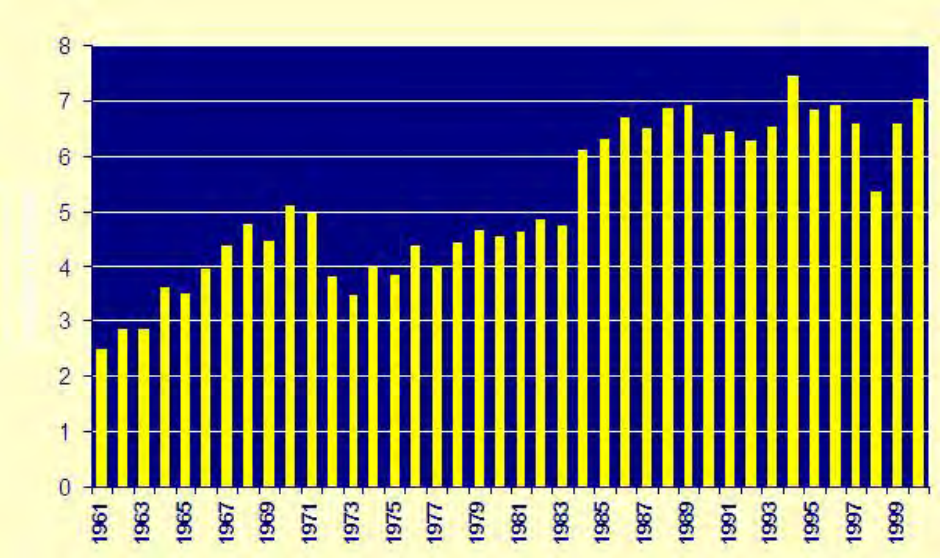


Figure 1. Fishmeal Production (1961-2000), Source: www.iffo.net

Historically, fish meal has been the source of protein for salmonid feeds (Rumsey 1993).
World fish meal production increased to 6-7 million mt (metric tons) between the 1960s and the 1980s and has remained relatively constant since then (Hardy 1996). This requires an annual catch of 25 - 30 million tons of feed-grade fish and fish processing waste; in other words 4 - 5 kilos of wet fish yield 1 kilo of dry fishmeal (www.iffo.net). In addition, world fish meal production is not expected to increase beyond current levels (Hardy 1996).

Seafood production from fish catch is not expected to increase more than current levels and Increased demand for seafood must be supplied by expansion of aquaculture production.

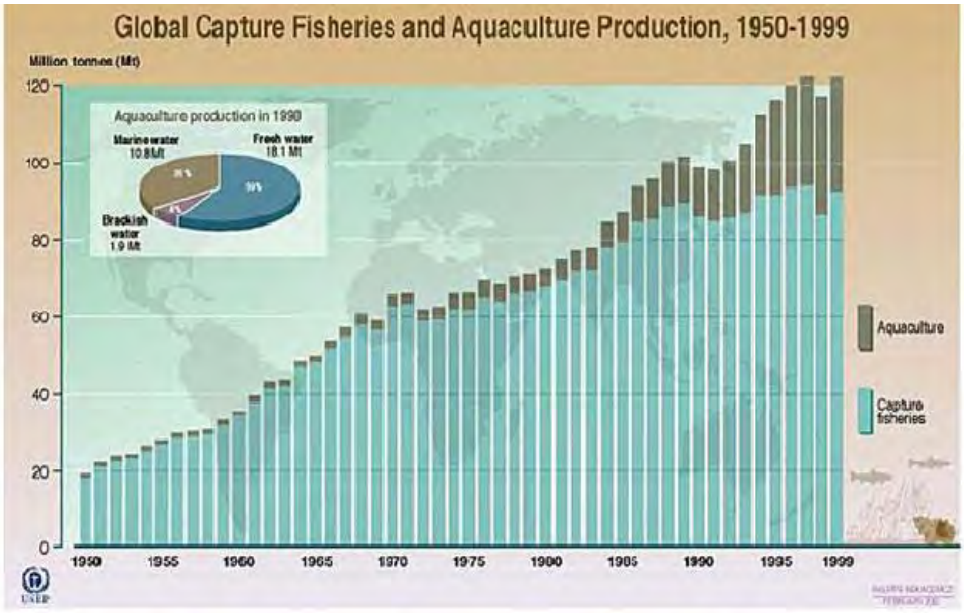


Figure 2. Global Capture Fisheries and Aquaculture Production, 1950-1999 (UNEP/GRID 2002)

Table 1. World fisheries and aquaculture production and utilization (FAO 2006)

	2000	2001	2002	2003	2004	2005[1]
			(million tonnes)			
PRODUCTION						
INLAND						
Capture	6.6	6.7	6.5	6.6	6.8	7.0
Aquaculture	6.0	6.5	7.0	7.6	8.3	8.8
Total inland	**12.6**	**13.3**	**13.5**	**14.2**	**15.1**	**15.8**
MARINE						
Capture	72.0	69.8	70.2	67.2	71.3	69.7
Aquaculture	4.9	5.3	5.6	6.1	6.6	6.6
Total marine	**76.9**	**75.2**	**75.8**	**73.3**	**77.9**	**76.3**
TOTAL CAPTURE	78.6	76.6	76.7	73.8	78.1	76.7
TOTAL AQUACULTURE	10.9	11.9	12.6	13.8	14.9	15.4
TOTAL FISHERIES	**89.5**	**88.4**	**89.3**	**87.5**	**93.0**	**92.1**
UTILIZATION						
Human consumption	63.9	65.7	65.7	67.5	68.9	69.0
Non-food uses	25.7	22.7	23.7	20.1	24.0	23.1
Population *(billions)*	4.8	4.9	5.0	5.0	5.1	5.1
Per capita food fish supply *(kg)*	13.3	13.4	13.3	13.4	13.5	13.4

Capture fisheries and aquaculture supplied the world with about 142 million tons of food fish in 2005 (preliminary estimate in 2004). Of this total, aquaculture participated 43 percent (FAO 2006). At present the production of aquaculture has increased, constituting nearly 50%

of all aquatic products for human consumption (FAO 2009). Global fisheries catch is declining in most fishing areas. Nowadays, most fishing areas are producing lower yields than in the past, and it is unlikely that sustainable increase will ever again be possible (FAO, 2006). The production of farmed salmonid has increased remarkably during the last decade (FAO 2006, FISHSTAT). In the Nordic countries salmonids, a highly carnivorous fish with diets consisting of up to 40 % protein, dominates the production.

At present plant sources dominates as alternatives to fish meal as protein source in intensive fish farming. However, the most sustainable way for alternative protein sources is a scenario in which the farmed animal becomes a net contributor, i.e. transforms "non-human" food resources into human ones in an ecologically sound way (Kiessling, 2009). Plant sources and soy, besides being excellent human food contain substances that are produced by plant as protection from grazing or as hormones. We call these substances "antinutrients" because they have physiological effects on animal, often reducing feed utilization. Salmonids are sensitive to these substances in whole soy, causing everything from reduced protein and mineral digestion to severe inflammation of the hindgut (Baeverfjord and Krogdahl, 1996). Also modern intensive and large scale plant production may not be characterised as sustainable (Kiessling, 2009). Another reason for unsustainability of plant sources from ecological point of view is linear flow of nutrients.

1.2. Nutrient leakage and alternative feed sources

All factors mentioned above showed that overfishing and imbalance in aquatic food chain is taking place. This resulted in research on alternatives to fish meal (as main protein source in fish diets) to supply protein in fish feeds, such as animal, plant protein source (with attention to soybean meals (Ozgul et al. 2006), fish processing by-product meals, oilseed proteins and concentrates, protein concentrates produced from grains (Hardy, 1996), marine zooplankton and krill (Moren et al., 2006), detoxified, high quality fish meal, mussel meal, evertebrate meal (especially worms but also insects) and micro-organisms.

Fish living in water has an easy way of disposing nitrogen, the by-product of protein catabolism, and phosphorus. From the environmental point of view this is undesirable, since it is nearly impossible to collect these eutrophicathing substances, once they dissolve in larger water volume. There are also other sources of polluting and organic leaks to the water, like human waste, agriculture fertilization leaks, etc.

Leak of nutrients in to water leads to "eutrophication"of the water. Eutrophication is defined, by Khan and Ansari in 2005 as the "sum of the effects of the excessive growth of phytoplankton leading to imbalanced primary and secondary productivity and a faster rate of succession from existence to higher serial stage, as caused by nutrient enrichment through runoffs that carry down overused fertilizers from agro ecosystems and/or discharged human waste from settlements" (Khan and Ansari, 2005). This means that oversupply of fertilizers,

discharged human waste, fish farm waste, poorly digested feed from fish, and other organic runoffs, leads to excessive growth of phytoplankton and creates imbalance in aquatic system.

Eutrophication can occur in a several scenarios, e.g. an effluence of nutrients being above the carrying capacity of the local ecosystem and/or in combination with fishing pressure that is too high on the large predatory fish, resulting in an increase of smaller prey fish. Hereon we have chain effect where smaller prey fish is feeding on too much zooplankton and lack of zooplankton is providing phytoplankton in a nutrient rich environment to bloom (algae bloom) resulting in a too large biomass of the system with oxygen deficiency followed by dead bottoms. And this is a major environmental problem (Kiessling, 2009).

Damages caused from eutrophication have been reported for several seas and lakes globally during several decades, such as the Black Sea, Mississippi delta, Cheasepeake Bay, Mediterranean Sea and Baltic Sea as a classic example. (see e.g. Turner et al., 1999; Bodungen and Turner).

Picture 1.Example of the lake eutrophication (algae bloom) (http://www.mistra.org)

In all considering facts given above it is crucial that we either reduce leakage of nutrient from fish farming or recapture nutrients from the environment balancing the leakage from production. A first solution would be to reduce use of fertilizers in agriculture fields, to reduce waste on fish farms, to produce more balanced and more digestible feeds. In the latter alternative, it is a prerequisite that it is possible to connect the leakage and the recapture in a causative way and that the local levels do not exceed the carrying capacity of the local water system.

Several models for combating eutrophication through recapture of nutrients, and include them back in to the food chain, using “catch crops” to capture them and make them available again, as new alternative feed components has been suggested. In the aquatic environment these models include mussel farming, being significant consumers of organic matters from water,

detoxification of fish meal from polluted fish to make high quality fish meal and use of microorganisms produced on e.g. pulp mill waste water as consumers of nutrients in the Baltic Sea water shed.

The major aim of this study is to evaluate a specific case of producing high value human food from “non-human” product though farmed fish. A fish feed was made of detoxified fish meal, mussel meal and meal from the mycelium of the microorganism Zygomycet as protein and fat sources and tested to farmed Arctic charr.

1.3. Models for combating eutrophication

1.3.1. Blue Mussel farming

Blue mussel is a filter feeder and feeds on phytoplankton and organic material. From a human nutrition point of view they have excellent protein and fat (EPA and DHA) composition. Under favourable conditions one mussel can filter 2-3 litres of water per hour (Lindhal et al., 2005).

In this model, mussel can be considered as an aquatic “catch crop“. Mussel farming is an easy, flexible and realistic measure and can be a cost-effective method to decrease the negative effects of eutrophication in marine and brackish waters, and serves as bioremediation measure (Gren et al. 2009). At the same time, healthy marine food is produced from a low level of the food chain, and nutrients are recycled from sea to land. Farming mussels in eutrophic water positive effect is achieved as nutrients are taken out at harvest. However, in normal production there will always be discards, either due to algae toxins, to small mussels or scale defects. These mussels are today discarded as waste and in best case scenario used as fertilizers. These mussels have the potential to be used as protein meal in animal/fish feed. Mussels farmed in brackish water will not reach market size for human consumption. However, such small mussels are excellent to use for mussel protein meal as the scale meat ratio is higher than in larger mussels.

Picture 2. Blue mussels attached to long line rope. (www.awi.de)

Harvest of mussels will decrease the nutrient level in the coastal zone. One kilogram of live mussels can remove 8.5–12 g of nitrogen, 0.6– 0.8 g of phosphorous and about 40–50 g of carbon. Good example of trading nutrient emissions and combating eutrophication is the "Lysekil case"at Swedish west coast. Here, the volume of farmed mussels was raised significantly and nitrogen was emitted from the sewage plant, supposing that the same amount of nitrogen will be ''harvested'' and brought ashore by 3,900 tons of farmed blue mussels. The cost of 150,000 € to expand the mussel farm for the Lysekil community was far below the price for nitrogen removal in the sewage plant.

Funds went to a mussel farming enterprise, which has been contracted for the removal of 39 tons of nitrogen. This was estimated to correspond to 100% nitrogen treatment of the emission from the sewage treatment plant. Phosphorus (3.6 tons) was also removed from the water through the mussel harvest, which also could be traded (Lindahl et al., 2005).

Mussels also contribute to clean the water where oversupply of nitrogen and phosphorus has leaked from surrounding agricultural industry (Jönsson, 2009). Blue mussels farmed in waters like Baltic Sea high in xenobiotics do not accumulate high levels of lipid soluble substances like dioxins and PCB. Therefore they might be used in recycling nitrogen and phosphorus back into the human food system in contaminated waters (Kiessling, 2009). As mentioned above, mussels from some basins of the Baltic Sea are too small to be used as seafood. Options for the use of these small Baltic mussels are as organic feedstuff replacing fish meal in feed for e.g. laying hens and chicken poultry (Jönsson, 2006), replacing fish meal in fish diets (Berge, Austreng, 1986), or as organic fertilizations (Lindahl et al., 2005).

Lindhal and Kollberg, 2009 suggested that the EU agro-environmental aid program could be extended into the coastal zone in order to combat eutrophication. In practice it meant that this should involve financial support to mussel farming enterprises through their harvest of

mussels (and thus their harvest of nutrients) in the same way as support is paid to agricultural farmers for operations that reduce nutrient leakage from their farmland. They made calculation and comparison of prices based on calculation of The Swedish Commission for the Environment of the Seas that catch crops and spring cultivation together decrease nitrogen release by 2,000 tons. This gave a price of 11 € per kg of retained nitrogen. If the mussels were to be compensated according to the same price for retained nitrogen, it meant an environmental subsidy of about 0.11 € per kg of live mussels. This is roughly 25% of what a mussel farm enterprise needs as gross income for harvested mussels.

Modern mussel farming has been established in Sweden since early 80's. Off-bottom cultivation with long line systems where mussels are attached to the ropes, hanging from the back-bone rope supported by large floats are most commonly used in Swedish mussel farms. The production capacity of an average long line unit is about 140-180 tons of mussels during farming cycle of 18 months.

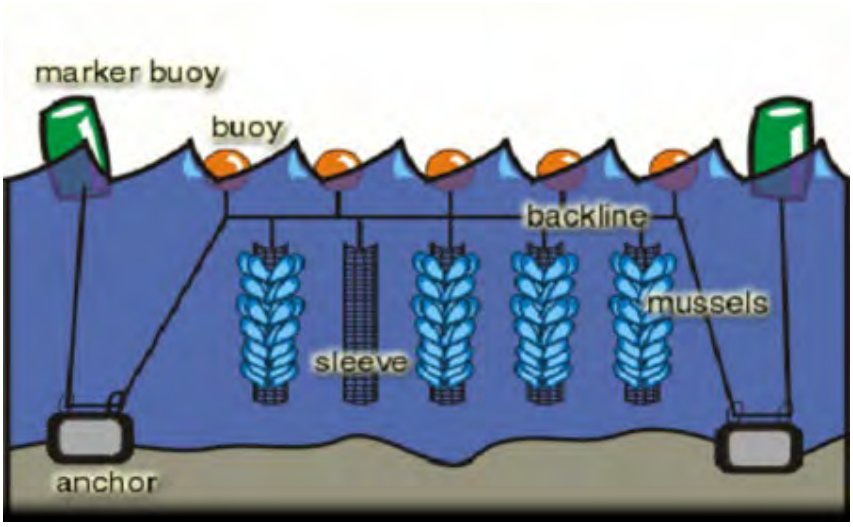


Figure 3. Schematic drawing of long-line mussel farm

Each unit occupies water surface of around 2000 m^2 (Lindhal et al., 2005). As quoted by Jönsson 2009, Lutz said that approximately 1% and 0.1% of the wet weight of mussels are nitrogen (N) and phosphorus (P), respectively. That means that one farm brings back around 1.5 tons of nitrogen and 150 kg of phosphorus from the sea to land in 18 months period.

1.3.2. Microorganisms (mycelium from fungi *Rhizopus oryzae*) as contributors to recapture nutrients

Micro-organisms are the most effective producers of organic material in nature, often exceeding 50% of dry weight in protein content being similar to animal origin meals. In order to have such bio-production, high levels of nucleotides are required (DNA and RNA >12% of

DW). If these organisms are eaten directly by man in large volumes it will result in diseases as kidney stones and gout, but the metabolic ability of the fish is capable to utilise high levels of microorganisms in diet. They can excrete the waste products, nitrogen from purin catabolism, as urea while in humans the final product is uric acid (not soluble in water and will therefore form crystals in joints and in the urine system (Kiessling 2009).

Mydland et al. 2009 conducted a study in which they showed that a 30% fish meal replacement by R. oryzae biomass did not affect feed intake and growth of rainbow trout. Histological examinations revealed to some minor impact on the immune system, but repeating the experiment in juvenile salmon, a species and live stage known to be even more sensitive than rainbow, revealed absolutely no immune stimulating effect (Brännäs, Kiessling, Pickova, and personal communication). Furthermore, using micro array including 2500 genes, specifically aimed at stress and immune response, from several tissues of rainbow trout revealed no significant up regulation of any genes (Mydland et al, 2009).

Rhizopus oryzae is a chitin/chitosan rich, filamentous fungus (Zygomycetes), which is able to assimilate the hexoses glucose, mannose and galactose, the pentoses xylose and arabinose, as well as acetic acid, all which are present in SSL.

Spent sulphite liquor (SSL) is a by-product from the paper pulp industry with a high organic content that contains approximately 50% of the dry weight of wood in a dissolved form (50–65% lignosulfonate and 15–22% sugars such as mannose, xylose, galactose, glucose and arabinose) (Taherzadeh et al., 2003). Taherzadeh et al. investigated in 2003, cultivation conditions for Rhizopus oryzae grown in synthetic medium and paper pulp spent sulphite liquor (SSL) to achieve high biomass. The fungus assimilated the hexoses; glucose, mannose and galactose, and the pentoses; xylose and arabinose as well as acetic acid which are present in SSL. They report highest biomass yields of 0.18 and 0.43 g biomass/g sugar.

Conversion of carbohydrate by-products to value added products is of great importance for production from renewable resources in a sustainable aquaculture industry (Kiessling 2009). If we take for example one medium size pulp mill that produces 40.000 tons of SSL, it means that we can produce 20.000 tons of dry weight of Zygomycetes, with price estimated to be comparable to fish meal (Edebo, L. and Kiessling, A. personal communication).

1.3.3. High quality detoxified fish meal

Detoxified fish is good possibility to gain high quality fish meal. Fish in many contaminated waters is unfit for direct human consumption due to high loads of lipid soluble environmental contaminants, especially dioxin and PCB like dioxins. Dioxins, generates for example, during the production of paper, volcanic eruptions and man-made incineration processes. Modern cleaning procedures can be used to decontaminate this polluted biomass. The contaminated

fish will thereby be transformed into high-quality food via fish farming. Production of high quality fish meal with low bone content resulted in remarkable reduction of both phosphorus and nitrogen leaks per kg produced fish compared to the use of low quality products (Kiessling, 2009). A general method to detoxify polluted fish is to press out the oil, which contains the majority of the lipid soluble components. The fish meal may then in most cases be used directly in a diet containing 25-30% fish meal. The oil is then purified by the active carbon method. If further detoxification is needed of the meal, lipid solvents are needed in order to produce a defatted meal, a much more expensive technique. Also heavy metals etc may be removed by different purification techniques. Below is schematic drawing of extraction process.

Figure 4. Fish meal extraction scheme. (www.999.dk)

In the scheme above, step 3 is very important as it is refers to process of extraction fish oil from fish meal by adding Isohexane. Isohexane is solvent for removing oil from meal. It is in fact in fish oil that toxins are found. Mixture of fish oil and Isohexane is then transported to evaporator (step 5) where Isohexane is separated from fish oil in vapour form. The vapour is then transformed in to liquid form and returned to the extractor. Oil containing dioxins is transported to oil tank store and it's ready to be purified in filtration plant. Fish meal is purified in a toaster (step 4) which has eight sections through which defatted fish meal is falling adding steam to it. This heating cause Isohexane to vaporise. From the bottom the toaster purified fish meal is transported to dryer/cooler from where it goes to storage tanks.

First factory to producing detoxified fish meal and oil started in Denmark in 2005. This is the world's very first plant that is able to remove dioxin and other POP's from fish meal with the

aid of a so-called extraction process. Persistent Organic Pollutants (POPs) are chemical substances that persist in the environment, bio accumulate through the food chain, and pose a risk of causing adverse effects to human health and the environment. These include: Aldrin, chlordane, dieldrin, dioxins, DDT, endrin, furans, heptachlor, hexachlorbenzene, mirex, PCB´s and toxaphene (www.chem.unep.ch).

EU regulation on dioxin and industry capability to meet those demands

EU has set limits for how much dioxin is permissible in fish meal and fish oil, which is also used in feedstuffs for farmed fish and mammals that are used as human food. Maximum limit for dioxin in fish oil is set by EU to 6 ng/kg, and limits for fish meal is set to 1,25 ng/kg.

Industry is capable to respond to these strict regulations especially in fish oil where they produce fish oil purified to have only 2 ng/kg, or less. As far as fish meal is concerned, concentration of dioxin will vary depending on raw material (e.g. Sprat fish from Baltic is high in dioxin) and time of the year (in spring, when the fat deposits of the fish are small, the concentration of dioxin are therefore large). (www.999.dk)

2. Material and Methods

The performance of Arctic charr fed the experimental diet was compared with reference groups fed a standard commercial diet.

Experimental animals and rearing conditions

Arctic charr (*Salvelinus alpinus*) used in experiment originated from a multiple families breeding program, including 150 families "Arctic superior" of 7th generation at Kälarne research station. Each diet was given to five replicate tanks, in all 10 tanks, with 100 fish in each tank. The fish had an average start weight of 30.5 gr. Experiment was performed in Kälarne research station (Sweden) using 1m^3 sized fibre glass tanks equipped with external standpipes. Water was pumped from two depths in Lake Kälarne (form 2 m depth and 7 m depth). Water temperature during the experiment is given in Fig 1.

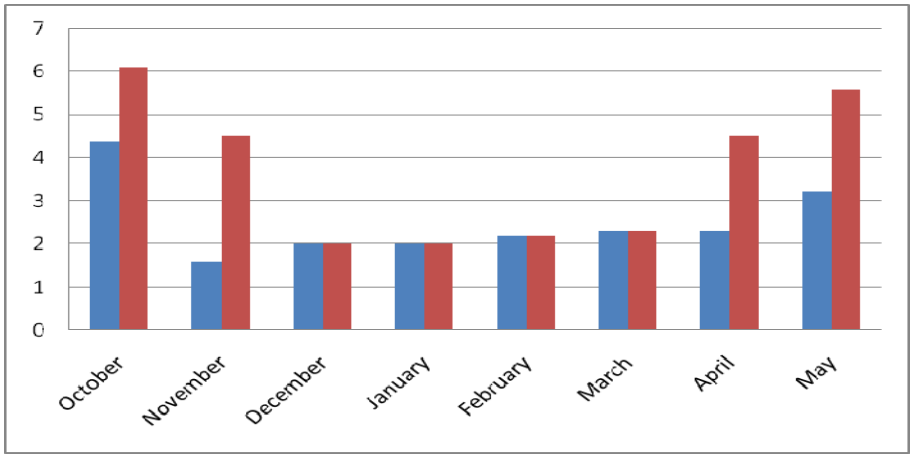


Figure 5. Water temperature during the experiment (minimum and maximum monthly temperatures) in °C.

Before the start of the feeding experiment, on 27th Oct. 2009, 200 fish was tagged and measured by weight and length and distributed in to 10 tanks (20 fish to each tank, 1m^3 of volume), then 800 fish was group measured, weighed and 80 fish was added in to each tank summing up to a total of 100 fish. The fish tanks were cleaned and disinfected before the start of the experiment.

Picture 3. Research facility in Kälarne (Sweden)

Tagging of fish

Fish was first collected and sedated with Tricane methanesulfonate (MS-222) solution. The solution was made by mixing 25 g of MS-222 in 1 litre water and 20 ml of this solution was used per 10 litres of water. Fish was then tagged with passive integrated transponder (PIT) tag. Pit-tagging was done by cutting 1-2 mm incision in abdominal area with thin scalpel and inserting the PIT tag in abdominal cavity. Fish was then measured, weighed, PIT tag reading was performed and data recorded on to PC- hard disk.

Diets used in experiment

Experimental diet *("Baltic blend")* was handmade at SLU-Umeå laboratory, at the department of Wildlife, Fish and Environmental studies. Formulations of experimental diets are given in Table 3, 4, 5 and 6. As the protein part of diet 1 (equal protein source mixture, in further text diet 1), fish meal (FM), Mussel meal (MM) and freeze dried zygomycete (*Rhizopus oryzae*) were used in 24%, 20% and 23% ratio, respectively as part of the total diet composition. The lipid source consisted of fish oil (FO), rapeseed oil (RO) and sesamesead oil (SO) in 13%, 7% and 1% respectively of total diet composition. Gelatine and sodium alginate were used as binders, 6,5% and 2% respectively. As carbohydrate and fibre source extruded wheat is used as 2% of total diet composition. 1% of Mineral- vitamin premix supplied diet with minerals and vitamins and as digestibility marker, 0.5% of titanumdioxid is used.
Diets 2, 3 and 4 were made as single protein source diets with maximum of each protein source (Fish meal, Mussel meal and Mycellium biomass, in further text diet 2, diet 3, and diet 4, respectively). For formulation of all diets see table 3, 4, 5 and 6.

Feed was mixed in ordinary home mixer/grinder with multiple functions. The mixing process was done in several steps:

First, the protein components were mixed together in bowl for mixing. Wheat was mixed with vitamin-mineral premix and marker (to get marker well spread into other ingredients) and added to protein component, and everything was mixed together. Oil components were carefully measured and mixed into each other while sodium alginate was added to it (to get sodium alginate dissolve in oil). Oil was then added to the mixture and well mixed. Gelatine was added to hot water and heated in a microwave oven until it was dissolved. The gelatine solution is finally added to the rest of the mixture and mixed into a good texture (for better texture, more water is eventually added to the whole diet mixture).

Second, after achieving desirable texture, while still hot, diet is shaped to several smaller pieces to fit grinding machine with the sizing knife removed. Then the feed was placed into a grinder and pressed through to get spaghetti shape feed which is placed on to trays.

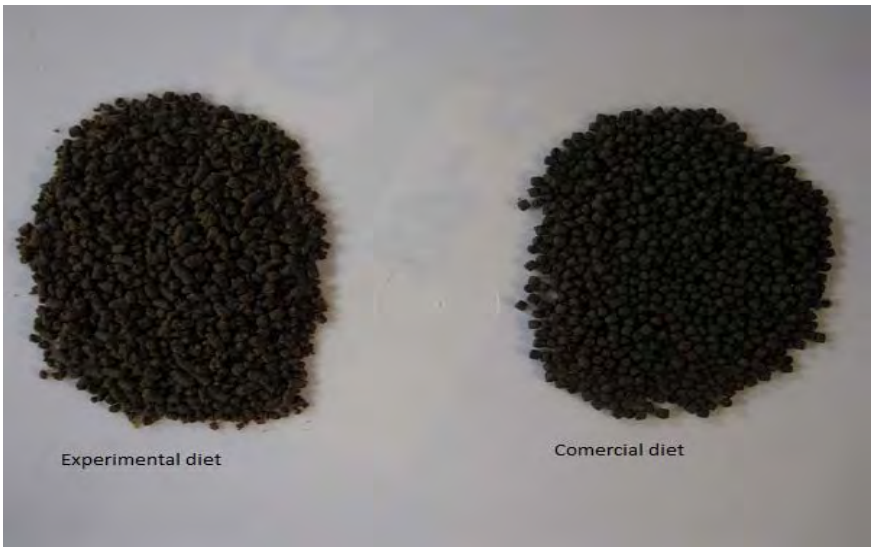
Third, feed was then left in refrigerator (6°C), for one hour to cool down.

Fourth, feed is taken out from refrigerator and chopped into small pellets ≈2mm.

Fifth, feed it is taken to heat dryer (≈45°C) for at least 24 hours, to dry.

After the feed was dried, it was packed to plastic bags, marked and shipped to Kälarne research station.

As *control diet*, an commercial feed "Skretting Nutra Parr 2 mm" was used.



Picture 4. Comparison between experimental and control diet (Skretting Nutra Parr 2mm)

Digestibility experiment

To perform a digestibility test of experimental diets, 3 "pure protein diets" were made (diet 2, diet 3 and diet 4).

In these diets, as much of a single protein source has been used, i.e. fish meal or mussel meal or zygomycete (mycelium biomass from Rhizopus orizae fungi). Diet composition of digestibility diets are given in Table 2 – 4.

Each diet was given to three parallel tanks with 10 fish each (identical to tanks described above) using the same fish stock as in growth experiment. Each diet was fed for four weeks before the faeces was sampled by carefully scraping/pushing faeces out of the dissected

intestine to avoid including any faeces caudal to the distal intestine. Samples of faeces was pooled per tank and analysed by standard procedure (HUV, SLU, analytic lab.).

Unfortunately the analysis of the faeces samples was not performed in time to be included in to the written part of the thesis. The thesis has a final date for delivery. These data will therefore be shown and discussed during the oral presentation of the thesis.

Chemical composition of experimental diet and it's components

Table 2. Chemical composition of "diet 1" and its protein components

				% av förtorkat prov				g / kg torrsubstans			
Prov	**För-ts %**	**Ts 2 %**	**Ts %**	**Aska**	**Råprot**	**EG-fett**	**NPN**	**Aska**	**Råprot**	**EG-fett**	**NPN**
Fiskskit											
Test diet	15,4	91,2	14,0	16,7	35,6	15,5		183	390	169	
Control	16,4		0,0								
Zyg	15,6	90,2	14,1	18,3	26,9	9,9		203	298	110	
Musslor	15,7	90,8	14,2	19,3	32,8	8,6		212	361	94	
Fiskmjöl	17,5	90,7	15,9	27,1	24,9	4,4		299	274	48	

Feeding experiment description

Experiment started on 4th November 4, 2009 (in further text labelled as "w1" for the first weight measurement of the on this date and "l 1" for measuring the length of fish) , with 100 fish (20 tagged) in each tank (10 tanks), with approximately same biomass in each tank. Fish was left in tanks without feeding for seven days (starved). " Band feeders" were mounted on tanks and filled with experimental and control diets, in a way that every second tank was given different diet (e.g. tank 37 experimental diet, tank 38 control diet, etc..). At the beginning the amount of feed given to fish was 2% of tank's biomass, per day, both experimental and control diet. Experimental diet was given to 5 tanks, i.e. to 500 fish, and other 5 tanks were fed with control diet. No mortality occurred during the experimental period. Until February 18th amount of feed given to fish in each tank was 15 g/day. From that date to 1st April 30 g/day was given. Until May 20th, 45 g/day of feed per tank was given.
Test diet ran out in 3rd week of April, after that all fish were fed with control diet.

The second measuring and weighing was conducted on 3rd December 2009 (in further text labelled as "w2" for weight measurement of fish on this date and "l 2" for measuring length of fish). Same procedure was followed as used in initial measuring and weighing (no fish was tagged this time). Gathered data was merged with data from previous measuring and stored. Same procedure was conducted on 18th February 2010 (in further text labelled as "w3" for weighing of fish on this date and „l 3" for measuring length of fish) and 20th May (last measurement) (in further text labelled as weighing "w4" for weight measurement of fish on this date and "l 4" for measuring length of fish).

Statistical data analysis

After performing all measurements, all data is collected and imported to Microsoft Excel program for simple analysing. Means of body weight (BW) and body length (BL) of tagged and all fish from tanks are calculated.

Unfortunately and out of our control, not all groups were sampled equally by only 3 test groups (diet 1) and 1 control group. These were given the same amount of feed as the complete group why these hade to be compared separately.

Average and standard deviation was calculated using excel (Microsoft Inc.). Statistical comparisons were made by F-test using the Proc-GLM procedure of SAS (Statistical analytic System, ver. 8.2). Multiple comparison including all three samplings or by samplings were evaluated using the same procedure.

Table 3. Diet 1 (experimental diet with approximately equal ratio of protein sources)

Feed components	Quantity	Protein		Fat		NFE*		Fibre		Ash		n3 HUFA	
Fish meal	**0,24000**	70,0	0,17	10,0	0,02	0,1	0,00	0	0,00	5	0,01	5	0,01
Fish oil	**0,13000**	0,0	0,00	99,0	0,13	0,0	0,00	0	0,00	1	0,00	10	0,01
Mussel meal	**0,20000**	60,0	0,12	3,0	0,01	0,1	0,00	0	0,00	20	0,04	1,5	0,00
Zygomycete	**0,23000**	50,0	0,12	5,0	0,01	20,0	0,05	5	0,01	5	0,01	0	0,00
Rapesead oil	**0,07000**	0,0	0,00	95,0	0,07	0,0	0,00	0	0,00	0	0,00	0	0,00
Sesame oil	**0,01000**	0,0	0,00	99,0	0,01	0,0	0,00	0	0,00	0	0,00	0	0,00
Wheat	0,02000	11,0	0,00	1,5	0,00	82,5	0,02	1	0,00	0	0,00	0	0,00
Alginat	0,02000	0,0	0,00	0,0	0,00	10,0	0,00	10	0,00	2	0,00	0	0,00
Gelatin	0,06500	65,0	0,04	0,0	0,00	25,0	0,02	0	0,00	0	0,00	0	0,00
Mineral/Vitamin mix	0,01000	0,0	0,00	0,0	0,00	0,0	0,00	0	0,00	0	0,00	0	0,00
Titaniumdioxid	0,00500	0,0	0,00	0,0	0,00	0,0	0,00	0	0,00	0	0,00	0	0,00
	1,00		0,45		0,25		0,08		0,01		0,0652		0,0280

Table 4. Diet 2 (experimental diet with fish meal as only source of protein)

	Quantity	Protein		Fat		NFE*		Fibre		Ash		n3 HUFA	
Fish meal	**0,58000**	70,0	0,41	10,0	0,06	0,1	0,00	0	0,00	5	0,03	5	0,03
Fish oil	**0,13000**	0,0	0,00	99,0	0,13	0,0	0,00	0	0,00	1	0,00	10	0,01
Mussel meal	**0,00000**	60,0	0,00	3,0	0,00	0,1	0,00	0	0,00	20	0,00	1,5	0,00
Zygomycete	**0,00000**	50,0	0,00	5,0	0,00	20,0	0,00	5	0,00	5	0,00	0	0,00
Rapesead oil	**0,07000**	0,0	0,00	95,0	0,07	0,0	0,00	0	0,00	0	0,00	0	0,00
Sesame oil	**0,01000**	0,0	0,00	99,0	0,01	0,0	0,00	0	0,00	0	0,00	0	0,00
Wheat	0,08000	11,0	0,01	1,5	0,00	82,5	0,07	1	0,00	0	0,00	0	0,00
Alginat	0,05000	0,0	0,00	0,0	0,00	10,0	0,01	10	0,01	2	0,00	0	0,00
Gelatin	0,06500	65,0	0,04	0,0	0,00	25,0	0,02	0	0,00	0	0,00	0	0,00
Mineral/Vitamin mix	0,01000	0,0	0,00	0,0	0,00	0,0	0,00	0	0,00	0	0,00	0	0,00
Titaniumdioxid	0,00500	0,0	0,00	0,0	0,00	0,0	0,00	0	0,00	0	0,00	0	0,00
	1,00		0,46		0,26		0,09		0,0058		0,0313		0,0420

Table 5. Diet 3 (experimental diet with mussel meal as main source of protein)

	Quantity	Protein		Fat		NFE*		Fibre		Ash		n3 HUFA	
Fish meal	**0,15000**	70,0	0,11	10,0	0,02	0,1	0,00	0	0,00	5	0,01	5	0,01
Fish oil	**0,15000**	0,0	0,00	99,0	0,15	0,0	0,00	0	0,00	1	0,00	10	0,02
Mussel meal	**0,52000**	60,0	0,31	3,0	0,02	0,1	0,00	0	0,00	20	0,10	1,5	0,01
Zygomycete	**0,00000**	50,0	0,00	5,0	0,00	20,0	0,00	5	0,00	5	0,00	0	0,00
Rapesead oil	**0,07000**	0,0	0,00	95,0	0,07	0,0	0,00	0	0,00	0	0,00	0	0,00
Sesame oil	**0,01000**	0,0	0,00	99,0	0,01	0,0	0,00	0	0,00	0	0,00	0	0,00
Wheat	0,00000	11,0	0,00	1,5	0,00	82,5	0,00	1	0,00	0	0,00	0	0,00
Alginat	0,02000	0,0	0,00	0,0	0,00	10,0	0,00	10	0,00	2	0,00	0	0,00
Gelatin	0,06500	65,0	0,04	0,0	0,00	25,0	0,02	0	0,00	0	0,00	0	0,00
Mineral/Vitamin mix	0,01000	0,0	0,00	0,0	0,00	0,0	0,00	0	0,00	0	0,00	0	0,00
Titaniumdioxid	0,00500	0,0	0,00	0,0	0,00	0,0	0,00	0	0,00	0	0,00	0	0,00
	1,00		0,46		0,26		0,02		0,0020		0,1134		0,0303

Table 6. Diet 4 (experimental diet with Zygomycete as main source of protein)

		Protein		Fat		NFE*		Fibre		Ash		n3 HUFA	
Fish meal	**0,23000**	70,0	0,16	10,0	0,02	0,1	0,00	0	0,00	5	0,01	5	0,01
Fish oil	**0,13000**	0,0	0,00	99,0	0,13	0,0	0,00	0	0,00	1	0,00	10	0,01
Mussel meal	**0,00000**	60,0	0,00	3,0	0,00	0,1	0,00	0	0,00	20	0,00	1,5	0,00
Zygomycete	**0,48000**	50,0	0,24	5,0	0,02	20,0	0,10	5	0,02	5	0,02	0	0,00
Rapesead oil	**0,07000**	0,0	0,00	95,0	0,07	0,0	0,00	0	0,00	0	0,00	0	0,00
Sesame oil	**0,01000**	0,0	0,00	99,0	0,01	0,0	0,00	0	0,00	0	0,00	0	0,00
Wheat	0,00000	11,0	0,00	1,5	0,00	82,5	0,00	1	0,00	0	0,00	0	0,00
Alginat	0,00000	0,0	0,00	0,0	0,00	10,0	0,00	10	0,00	2	0,00	0	0,00
Gelatin	0,06500	65,0	0,04	0,0	0,00	25,0	0,02	0	0,00	0	0,00	0	0,00
Mineral/Vitamin mix	0,01000	0,0	0,00	0,0	0,00	0,0	0,00	0	0,00	0	0,00	0	0,00
Titaniumdioxid	0,00500	0,0	0,00	0,0	0,00	0,0	0,00	0	0,00	0	0,00	0	0,00
	1,00		0,44		0,25		0,11		0,0240		0,0368		0,0245

3. Results

Four experimental diets were tested (diet 1, diet 2, diet 3 and diet 4). Diet 1 was tested for growth performance (see table 7). diets 2,3 and 4 were tested for digestibility tests (Data pending, the thesis is restricted in time and due to late analysis these data could not be included in this version and will therefore be published under separate cover and presented in the oral presentation of the thesis, see above). Diet 1 and its protein components (Fish meal, Mussel meal and Zygomycete) was chemically analysed (table 2) for raw protein, fat, ash percentage in dry matter and in g/ kg in dry matter.

Table 7. Weight and legth data of 5 groups fed „diet 1" and five groups fed commercial diet, per tank and measurement period. E – Experimental „diet 1", C – control diet.* Only 80 fish but the same amount of feed as the groups with 100 individuals. The total average is separated in to 80 and 100 fish.

tank/feed	37 E	38 C	39 E	40 C	41 E	42 C	43 E	44 C	45 E	46 C	total average E	total average C
mean tagged l1	139,52	132,11	134,70	136,25	137,35	136,70	137,45	135,38	135,30	135,76	136,08	135,67
mean tagged w1	288,10	315,05	298,00	311,90	309,40	305,15	315,45	297,43	298,45	306,14	304,33	306,33
mean tagged l2	143,75	144,47	141,80	142,35	143,10	141,90	143,10	134,19	141,94	142,19	141,85	141,67
mean tagged w2	328,15	326,05	307,35	326,75	322,15	326,15	320,60	295,10	324,89	330,38	319,69	319,94
mean tagged l3	158,70	155,00	154,65	157,47	154,95	157,42	154,37	153,19	153,47	157,00	155,47	155,28
mean tagged w3	488,70	454,26	445,70	491,21	449,58	478,74	422,79	423,10	396,68	488,48	450,08	450,06
mean tagged l4	186,00	186,22	183,53	181,84	184,60	182,94	185,63	184,19	181,13	187,69	184,01	184,20
mean tagged w4	694,60	838,33	756,32	778,42	802,00	802,22	818,75	835,71	675,56	898,75	777,99	800,67
mean all in tank l2	143,47	143,52	144,48	142,49	142,82	142,10	141,32	141,46	142,39	144,55	142,67	142,79
mean all in tank w2	331,40	336,51	335,86	331,29	330,87	328,41	315,55	327,74	329,45	346,35	329,68	331,34
mean all in tank l3	156,22	161,15	156,50	155,05	155,46	155,26	153,40	155,99	159,66	159,73	156,52	156,91
mean all in tank w3	471,04	477,05	473,03	472,26	464,95	464,97	424,36	460,46	438,89	510,81	460,78	465,20
mean all in tank l4	184,21	186,82	183,96	185,17	184,43	186,69	181,61	186,76	182,81	189,45	184,72	185,30
mean all in tank w4	775,19	858,99	790,61	852,40	822,21	851,46	841,76	854,21	782,41	936,15	782,90	854,26
					*		*		*	*	822,9*	936,2*

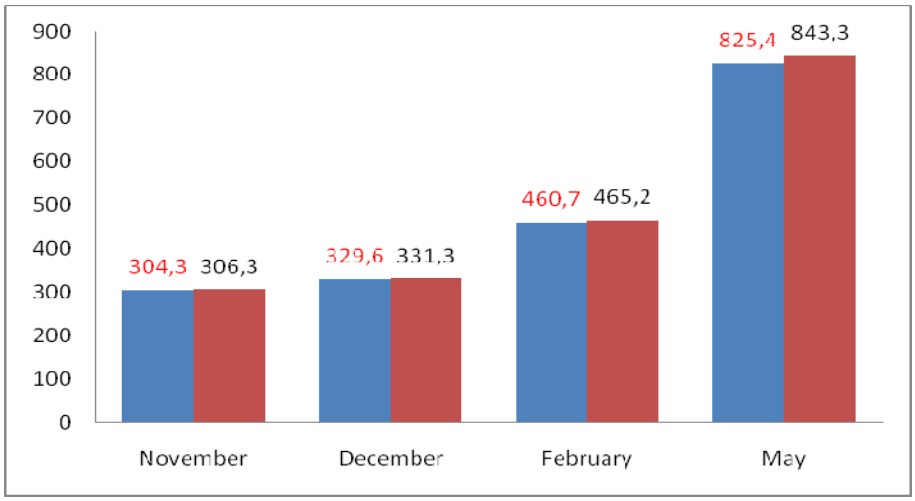


Figure 6. Body weight of fish fed with „diet 1" and control diet, thru entire experiment. Blue columns equal fish fed Baltic Blend (experimental diet) and red columns equal fish fed control (commercial) diet. May, only tanks with 100 fish.

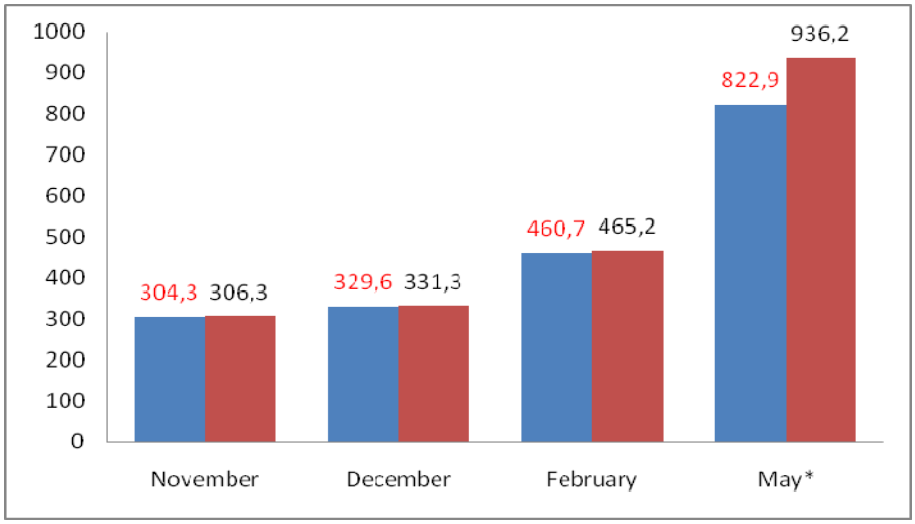


Figure 7. Body weight of fish fed with „diet 1" and control diet, thru entire experiment. Blue columns equal fish fed Baltic Blend (experimental diet) and red columns equal fish fed control (commercial) diet. May*, only tanks with 80 fish.

Table 8. Growth performance, compared between different measurement periods.* Only 80 fish but the same amount of feed as the groups with 100 individuals. The total average is separated in to 80 and 100 fish.

Pittaged (W4-W3)	37 E	38 C	39 E	40 C	41 E*	42 C	43 E*	44 C	45 E*	46 C*	total average E	total average C
Bw diff	205,90	384,07	310,62	287,21	352,42	323,49	395,96	412,62	278,87	410,27	258	352
Percent Bw diff	42%	85%	70%	58%	78%	68%	94%	98%	70%	84%	56%	77%
SGR	0,46	0,80	0,69	0,60	0,75	0,67	0,86	0,88	0,69	0,79	0,57	0,74
											total average E*	total average C*
Pittaged (W3-W2)	37 E	38 C	39 E	40 C	41 E	42 C	43 E	44 C	45 E	46 C	342,42	410,27
											81%	84%
											0,77	0,79
Bw diff	160,55	128,21	138,35	164,46	127,43	152,59	102,19	128,00	71,80	158,10	120	146
Percent Bw diff	49%	39%	45%	50%	40%	47%	32%	43%	22%	48%	37%	46%
SGR	0,52	0,43	0,48	0,53	0,43	0,50	0,36	0,47	0,26	0,51	0,41	0,49
Pittaged (W2-W1)	37 E	38 C	39 E	40 C	41 E	42 C	43 E	44 C	45 E	46 C		
Bw diff	40,05	11,00	9,35	14,85	12,75	21,00	5,15	-2,33	26,44	24,24	18,75	13,75
Percent Bw diff	14%	3%	3%	5%	4%	7%	2%	-1%	9%	8%	6%	4%
SGR	0,17	0,04	0,04	0,06	0,05	0,09	0,02	-0,01	0,11	0,10	0,08	0,06
All in tank (W4-W3)	37 E	38 C	39 E	40 C	41 E	42 C	43 E	44 C	45 E	46 C		
Bw diff	304,15	381,94	317,58	380,14	357,26	386,49	417,40	393,75	343,51	425,34	347,98	393,53
Percent Bw diff	65%	80%	67%	80%	77%	83%	98%	86%	78%	83%	77%	82%
SGR	0,65	0,76	0,67	0,77	0,74	0,79	0,89	0,80	0,75	0,79	0,74	0,78
All in tank (W3-W2)	37 E	38 C	39 E	40 C	41 E	42 C	43 E	44 C	45 E	46 C		
Bw diff	139,64	140,54	137,17	140,98	134,08	136,56	108,81	132,71	109,45	164,46	125,83	143,05
Percent Bw diff	42%	42%	41%	43%	41%	42%	34%	40%	33%	47%	38%	43%
SGR	0,46	0,45	0,44	0,46	0,44	0,45	0,38	0,44	0,37	0,50	0,42	0,46
All in tank (W4-W2)	37 E	38 C	39 E	40 C	41 E	42 C	43 E	44 C	45 E	46 C		
Bw diff	443,79	522,48	454,75	521,11	491,34	523,05	526,22	526,47	452,96	589,80	473,81	536,58
Percent Bw diff	134%	155%	135%	157%	148%	159%	167%	161%	137%	170%	144%	161%
SGR	1,10	1,22	1,11	1,23	1,18	1,24	1,27	1,24	1,12	1,29	1,16	1,24
All in tank (W4-W1)	37 E	38 C	39 E	40 C	41 E	42 C	43 E	44 C	45 E	46 C		
Bw diff	487,10	543,94	492,61	540,50	512,81	546,31	526,31	556,78	483,96	630,01	500,56	563,51
Percent Bw diff	169%	173%	165%	173%	166%	179%	167%	187%	162%	206%	166%	184%
SGR	1,29	1,30	1,27	1,31	1,27	1,33	1,27	1,37	1,25	1,45	1,27	1,35

Table 9. Average body weight and standard deviation of weigh per tank, and of the test (E) and control (C) diet.

tank	37 E	38 C	39 E	40 C	41 E	42 C	43 E	44 C	45 E	46 C	st deviation E	st deviation C
average (w1, w4)	466,43	496,90	474,37	491,96	481,86	487,50	474,28	484,96	462,30	524,86	6,83	14,40
st. deviation per tank	190,65	218,11	193,84	217,11	205,34	218,82	216,77	221,83	192,04	249,53		

4. Discussion

As fish meal is reaching its maximum production from wild catches, and its production expected to decrease in future (at least for certain period), research for alternatives to fish meal are taking place. First alternative to begin with was plant protein sources. This turn out not to be sustainable as replacement to whole amount of fish meal in fish diets (see above under introduction). Furthermore a need for sustainable solution of fish meal replacement together with environmental issues of eutrophicating and polluting waters emerged with a possible solution in reuse of nutrients. This “cause-consequence” principle seems in theory to be working. The aim of the present project was therefore to test this in practise.

Over fishing, catching wild fish and transforming it into fish meal to feed farmed carnivorous fish, constant expansion of aquaculture and therefore increased demand for fish meal nutrient, pollutant and fertilizer leaks to water are all factors that have serious impact on Aquatic ecosystem. This thesis presents fish diets that is made of ingredients which use (reuse) can significantly decrease mentioned impacts to one aquatic ecosystem, in this case Baltic Sea's and its tributaries’ ecosystem. However, it shows that the use of these diets does not negatively affect growth or health of Arctic charr fed this diet.

Several studies showed that using mycelium biomass, blue mussel and purified fish meal can successfully be use as alternative protein source in fish diets, instead of fish meal (Berge and Austreng 1989, Nestor et al. ms). More specifically, fish fed with 20% feed enriched of zygomicete showed comparable growth performance with fish fed with feed based only on fish meal (Kaszowska personal communication) and fish feed 100% of protein from de-shelled blue mussel meal showed identical growth to fish feed the same diet but with mussel meal replaced by fish meal. Similar results were found in early measurements of feed experiment investigated in this thesis.

Farmed fish have a much higher capability to utilize high level of micro organism in diets compared to vertebrates why this non-human protein source is a very promising alternative ingredient in the future fish feed (Skrede et al., 1998). However, micro organisms contain cell walls of varying digestibility, but there are species and strains that have already been found to be suitable in feed to carnivorous fish (Kiessling, 2009). The experiments by Mydland (unpublished), and the ongoing studies (Brännäs et al, unpublished) indicate that mycelium

from Zygomycetes may be included in diets to salmonids with at least 30% replacement level of fish meal. Also student studies using Torula yeast in diets to Arctic charr (Kiessling pers. Com.) indicate that even higher inclusion levels are possible, as they used a 50% replacement of fish meal with no negative effect on growth. Microbial exponential growth provides quick increase in biomass. Exponential growth is a physiological state of division cycles, such that the population doubles in number every generation time. A bacterial generation time is also known as it's doubling time. Note that during exponential growth there is no change in average cell mass, though individuals cells are constantly changing in mass as they increase in mass, then divide thus rapidly decreasing in mass (while increasing in number) and then each cell regain mass before dividing again. For example, if there are 10 cells present at time 0, and 100 cells present at time 200, then at time 400 there will be 10,000 (100*100) cells present. Furthermore, our society is producing lots of biological (organic) side products that need to be broken down by the use of micro organisms. Properties mentioned above makes micro organisms very interesting for use in future composition of fish diets, as pulp mill and other wood industry waste removal media, and most certainly for further investigation from both, nutritional and ecological point of view.

The study of Berge and Austreng (1989) that showed comparable growth potential in mussel meal also showed low digestibility of mussel meal. The most likely cause of the low digestibility was that blue mussels were not de-shelled. That also led to unavailability of the concept, back in time. Unavailability of the concept was primarily due to high ash content (especially Calcium 7 - 8%). As quoted by Berge and Austreng (1989). Cowey et al. (1977) stated that renal calcinosis is known to occur when fish are fed a diet high in calcium and low in magnesium, but additional magnesium reduces this effect. This will not happen if the mussel meal is produced from de-shelled mussels (mussel meat meal). However, meat from de-shelled mussels could replace fish meal without reducing growth in Arctic charr (Nestor et al. ms).

Data from another unpublished test from Nestor et al. (see table 9) is comparing growth performance of Arctic charr fed with two different diets. First diet with 100% fish meal (FM) as protein source, and second 100% de-shelled mussel meal (MM). Results from this study show that de-shelled mussel meal is absolutely comparable and can in total replace fish meal, from fish nutritional point of view.

Table 10. Comparison between different groups of fish fed with 100% Fish meal and 100% mussel meal as protein source in fish diet. (Unpublished data from Nestor et al. ms)

diet	Group (replicas)	Mean	Group (replicas)	Mean	
1	a	44.91	a	92.68	FM (1)
1	b	45.80	b	91.97	
1	c	45.51	c	93.27	
2	d	42.12	d	88.83	MM (2)
2	e	41.15	e	89.71	
2	f	44.21	f	90.03	

In addition, dried mussel meal contains 10 % of high valuable fatty acids (Pickova pers.com). This is one of solid arguments for mussel meal to be used as substitution to fish meal.

Phosphorus is an important mineral for fish as it is a major component of bone, scale and teeth. The majority of phosphorus in plant sources is bound by phytic acid making it more or less unavailable to the fish. The use of plant sources may therefore led to the very odd situation with a high dietary content of phosphorus, low availability and thereby high level of discard to the environment. In blue mussel the majority of phosphorus bounds are in the shell (Jönsson, 2009) and should be available to the fish. It may therefore be of interest to evaluate the optimal level of shell in "de-shelled" mussel meal in order to provide optimal growth levels of phosphorus and calcium (high levels in shell, and they are of importance for normal growth) with minimum discard to surrounding water.

Comparing mussels and microbial diet from human nutrition point of view, we will see that mussels are usable in direct human consumption (opposite effect of microorganisms). Therefore one can argue that mussels are suppose to be use as human food rather than used as mussel meal in fish diets, poultry diets ect. Considering mussels from Baltic Sea, as too small and of low interest for human consumption, they are all used as fertilizer which is horrible waste of useful nutrients.

Therefore we argue for Baltic Sea mussel to be used as component of fish diets. Mussels being used in that sense are than indirectly used as human food thru fish as medium. The positive side of blue mussels, from human nutrition point of view is that they have a similar fat composition as fish meal considered to have a beneficial effect for human's health. This as fatty fish will have a fatty acid profile of its flesh reflecting that of diet (Kiessling 2009). Since mussels are at the second step of the marine food-chain, the use of mussels instead of fish for meal production also has a large ecological advantage.

As quoted by Kiessling, Duinker et al. did a study (2005) on harvesting "waste" of small mussels as alternative to fish meal for ecological poultry production. The study showed that cost for producing de-shelled mussel meal was not economically viable with fish meal price of 20 NOK/kg. What is important to mention here is that this study was done in Norway not considering the possible environmental service performed by mussel farming. So far the price is too high compared to fish meat and the techniques for producing de-shelled dry mussel meat need to be improved. At present a price of 25 SEK / kg mussel meal from feed mussel production is estimated to be possible if the farmer receive environmental support of 2 SEK per kg raw mussel. This would translate to an increase in consumer price of 15 SEK per kg and an increase in 5 SEK in production costs if mussel meal replaces 30% of fish meal at a cost of 12 SEK per kg (Odd Lindahl, personal communication). Problems reported by Berge and Austreng (1985) with low digestion of mussel meal can now be efficiently overcome with use of new techniques in de-shelling the mussels (Lindahl O. personal communication). Combining environmental service with use of de-shelled mussel meal as protein and fat

source in fish diets should offer an economical viable and high quality alternative to fish meal in diets to salmonids pending either that the consumer is prepared to pay an 10% increase in retail price, cost of producing mussel meal is decreased with increased production volumes, present price of fish meal of 13 SEK will increase due to higher demand for fish meal as protein source and constant level of fishing, or a combination of the above.

Prediction for fish meal to become more expensive due to expansion stagnation of its production, will definitively lead the price of fish meal to meet price of mussel meal, which is expected to drop with increase of production, introducing new technologies and gaining subsidy for farming mussels.

In case of detoxified fish (fish meal and fish oil), we have similar scenario as mussel meal. That similarity reflects in unavailability to use these two in direct human consumption. When talking about detoxified fish meal and oil we find first obstacle in difficulty to sell anything rather than whole fish, for human consumption. Another obstacle is that fish meal will still have low level of xenobiotics after detoxification. If used directly as human food, the risk of low dose accumulation effects are high as humans would have consume it for long period of time, e.g. 40-80 years, in opposite to fish which would only eat it for 1-2 years and therefore avoid risk of accumulating xenobiotics over a long period of time. In this case fish serves as dilution filter and would increase the safety margin for the human consumer of detoxified fish (fish meal and oil).

Another advantage of using detoxified fish meal is that we will remove xenobiotics from the Baltic in the purification stage. M. Pandelova et al., 2008 reports 40 nanogram of dioxin per kg fresh weight (fw) pending on age and location in Baltic Sea. We can now make an estimate how much of detoxified fish meal/oil would be used in Sweden in a future estimates of a production of 70.000 tons of fish (SOU 2009:26). With a feed conversion of 1.4 this is equivalent to 100.000 ton feed. 25% of that amount would be detoxified fish meal/oil, i.e. 25.000 ton fish meal. To produce 25.000 tons of fish meal we need 5 times of that amount in fish i.e. 125.000 ton fish. This number multiplied with a 40 nanogram allows us to estimate amount of Dioxin (0,5 kg) removed from Baltic Sea every year.

Example above as well as other examples and studies presented in discussion, we found as strong reasons to introduce new, domestic feed sources to carnivorous fish diets. Results from experiment at Kälarne (table 7, figure 6) indicate very equal growth performance of tested “diet 1“ in average, compared to control commercial diet especially during the period of first three measurements (October – March). Latest measuring conducted in May showed that fish fed with control diet started to perform better in growth than fish fed with test “diet 1“. The reason behind this may be in the different process of producing these two diets. Control diet was industrially produced, i.e. extruded and fat coated, pellet size was more equal, taste enhancers used in diet and other factors which couldn't be mimicked in the handmade test diet. Furthermore, the handmade diet was pelleted yielding a harder texture and more crumbling possible also contributing to a larger feed waste compared to the extruded commercial diet. No feed waste collectors, beyond ocular inspection of the tank after feeding,

were applied in the experimental tanks. This might be the reasons for fish to have an apparent better appetite and to perform better with control diet rather than test diet in spite being fed the same percent of body mass independent of diet.

If observed per tank, highest score in biomass gained tank nr. 46, fed with control diet. Biomass in this tank has tripled, scoring 206 % of body weight difference since initial weight in November. Score of 165% of body weight since initial weight measurement was in tank nr. 39 where fish was fed with test „diet 1“. This was the lowest score in percentage body weight difference. Other tanks performed similar. Standard deviation calculated among tanks with fish fed „diet 1“was 6,83 in May. Tanks with fish fed control diet had standard deviation of 14,40 indicating a larger variation in feed intake among fish fed control diet. This may in fact indicate a general preference for our “Baltic Blend”.

Furthermore, latest measuring of fish showed that average body weight of all fish in tank including pit-tagged fish (regardless of which tank and diet), compared to pit-tagged fish from the same tank, was higher from 0,3 – 4,0 gr. The cause for this we found in lesser population of tagged fish and in initial sedation and tagging stress, also average body weight of all fish from tank is expected to be bigger than average body weight of pit-tagged fish due to larger dispersion of all fish. Tested diet showed very good growth performance compared to commercial control diet in spite that the chemical analysis of tested diet showed a content of 36,5% of protein and 15,5% of f. In comparison with the commercial diet composition of 51% of protein and 21% of oil component (Lysfjord 2004). This lower protein content of test diet is the result of large variations in composition between batches of feed ingredients. This is explained by the fact that all these products so far being produced in small batches under non commercial conditions. All diets were composed based on analysis from earlier batches and the target composition was naturally the same as the commercial diet. However, due to large inter batch variation in composition the test diet had a much lower protein composition than intended. As production of mussel meal and micro meal is improved, more standardised products are expected. Even so it is not wordy that the fish given the same ratio of test diet managed to keep up in growth with that of fish given a higher protein content of the commercial diet. The only plausible explanation is a much higher digestibility or biological accessibility of the protein in the test diet. The commercial diet has besides fish meal also different plant protein sources, including soy. Soy is well known to cause intestinal problems in salmonids. I.e. the Baltic blend, besides being environmental friendly may in fact also be “animal” friendly and support a higher protein growth per feed ingested and thereby also support a better “Fish in – Fish out” ratio than today’s commercial diets. However, this are to some degree speculative before the data including digestibility and protein retention are available. This will therefore be further discussed in the oral presentation of the thesis as the result from the faeces is expected to be available at that time.

Growth of fish fed with both commercial and test diet was pending on water temperature also, as water temperature rose in April, fish fed test diet (tanks with 100 fish) increased its body weight by 56% in period February – May, and fish fed test diet (tanks with 80 fish) had 81% of body weight increase, in same period. The overall better growth in last period of measuring

the fish is certainly due to water temperature increase of almost 4°C (Bottengård L. and Jørgensen E. H., 2008). However, in this period tank 43 (test diet) almost doubled its biomass, and had increase in body weight of 94%. Only tank can match this score was tank 44 (commercial diet), which was expected to have high increase in biomass due to feeding its fish with commercial diet. Fish fed with commercial diet (tanks with 100 fish) had an average increase in body weight of 77%, and fish fed commercial diet (tanks with 80 fish) had an average increase in body weight of 84%, but none of the tanks had managed to get even close to performance of tank 43. Discussing this reason might lead to fact that it was 20 fish that was removed from tank 34, but also from tanks 41, 45 and 46 fish has been removed and still they didn't managed to cope with tank 43. Again underlining a possible biological potential of the ingredients used in the "Baltic Blend" yielding the concept additional interest beyond that of pure environmental service.

5. Conclusion

1. Alternative protein sources from Baltic Sea in fish diets are definitely able to replace fish meal to certain percent, especially de shelled mussel meal which can substitute fish meal by 100%.
2. Using alternative proteins in fish feed will make farmed fish a "link" in human diet to access other feed sources otherwise non usable by humans.
3. Technology of fish meal and oil purification has contributed in accessing feed sources that were unusable.
4. Governmental support is important in developing mussel farming and microbial biomass, as important contributors, in battle against eutrophication in Baltic Sea and as good and sustainable source of feed.
5. Tested diet was able to compete with commercial diet, especially if taken in consideration hand made feed versus industrial feed.
6. The data indicate a possible "biological potential" of the "Baltic Blend" ingredients not merely explained by protein level but also source of the protein that needs to be further investigated.
7. It is significant to use domestic protein sources from ecological point of view, as it makes flow of nutrients circular, rather than linear.

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