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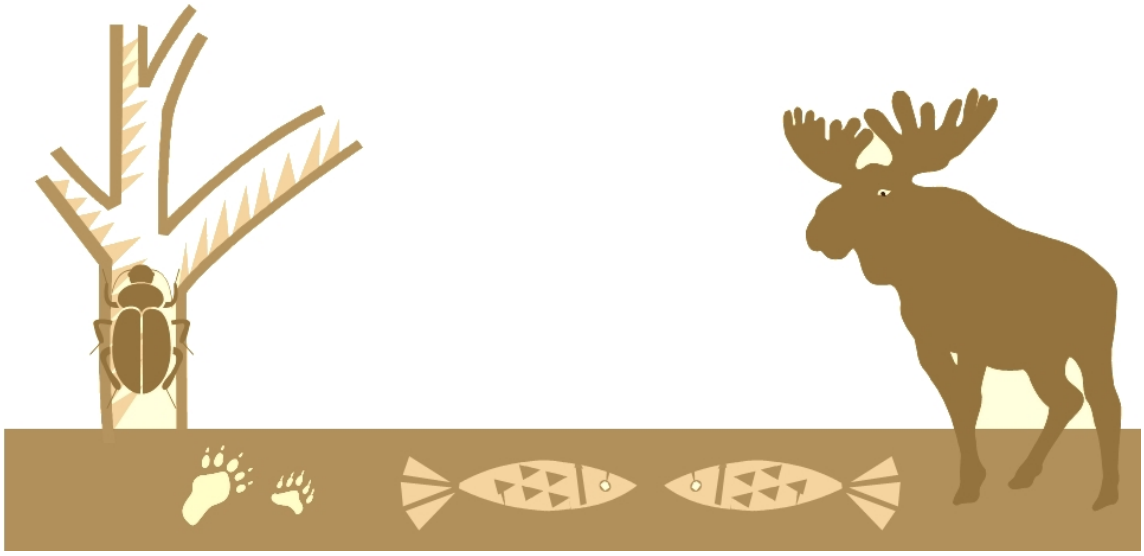

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AQUABEST

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Description

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Title
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Abstract

Performance of Arctic charr fed a feed containing Baltic Sea-sourced ingredients has been evaluated. The feed ingredients blue mussels, detoxified Baltic Sea fish, microbes and by-products from regional crops initiate regional nutrient movements from the eutrophicated Baltic Sea. Arctic charr farming is conducted in nutrient depleted utraoligotrophic water bodies affected by hydropower regulations in northern Sweden. These systems can receive Baltic Sea nutrients through farming and close the loop of nutrients that will improve the farming industry's sustainability.

The novel Baltic Sea-sourced feed, Baltic Blend, was tested in triplicate groups and compared to groups fed a fishmeal based control feed mirroring a commercial feed. The trial lasted a full production cycle and fish grew from a mean weight of 50 to 670 g. The groups fed the Baltic Blend had a 12 % lower final weight than fish fed the control diet. Fat and astaxanthin content in the flesh did not differ between treatments. In order to evaluate if the Baltic Blend had a genetic effect on growth in Arctic charr, the test groups consisted of identifiable individuals from 15 sibling groups. All sibling groups showed similar differences in final weight with respect to diet, thus demonstrating no genetic effect on how the new diet affects growth. A sensory evaluation on the final product was made and the panel found fish fed Baltic Blend and the control feed similarly tasty.

A feed preference experiment with individual Arctic charr in aquarium was conducted and shows a preference by the fish for the new Baltic Blend feed. A pilot study to evaluate the decontaminated Baltic Sea fishmeal and fish oil and to learn more about its suitability for inclusion in fish feed was also conducted.

The results are promising. The Baltic Sea sourced ingredients that are of little or no value for direct human consumption can be harvested to produce Arctic charr, a high quality exclusive product on the table market. In addition, the loop of nutrients potentially improves the ecological status of both the receiving and the donating ecosystem, resulting in for example increased production of wild fish. This nutrient loop could support and increase sustainability in the limited farming industry for Arctic charr in Sweden and thereby the industry can gain better acceptance by the general public and other stakeholders.

Keywords
Mussel meal; Yeast Saccharomyces cerevisiae; Arctic charr farming

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Cover page picture taken by Oddmund Gröttan

AQUABEST

Contents

1. Introduction

Aquaculture has great potential to supply our growing human population with fish as the wild stocks are declining. However, new production has to be based on sustainable practices and technologies. Aquabest creates a strong basis for new environmental regulation policies. The project strives to demonstrate that the Baltic Sea Region aquaculture has the potential to become a sustainable and responsible food production system, accepted by all stakeholders.

The aim of this project, is to recycle Baltic Sea Region nutrients from both the aquatic and terrestrial environment back in to the food chain. This in the form of fish feed composed of detoxified Baltic Sea fish, farmed mussels, microbes and by-products from regional crops. This part of the project follows the flow; feed ingredients - fish feed - pilot farming. In addition, when recycling nutrients back into the food chain, food safety is a prime objective.

Arctic charr (*Salvelinus alpinus*) is the most northern distributed salmonid in the world (Johnson 1980). It is well adapted to cold temperatures and, in comparison to other salmonids; Arctic charr has the best ability to grow at low temperatures, making it optimal for farming in cold waters (Eriksson, Alanärä et al. 2010; Siikavuopio, Knudsen et al. 2010). Arctic charr is considered a delicacy and its market value is steadily increasing (SOU 2009:26). This species has shown to be a suitable target-species for feed replacements of fishmeal and fish oil with alternative feed ingredients (see e.g. (Tocher, Bell et al. 2001; Pettersson, Pickova et al. 2009). Replacements of fishmeal and fish oil increase sustainability of the farming industry.

Arctic charr is the second most farmed species in Sweden with a modest production of 1849 metric tonnes in 2012 (SCB 2013). Through a national selective breeding program initiated in the mid 1980´s by the Swedish University of Agricultural Sciences (SLU) the Swedish industry has access to a fast growing strain, the Arctic Superior. This has strongly contributed to the successful development of the Arctic charr farming industry in Sweden (Eriksson, Alanärä et al. 2010; Nilsson, Brannas et al. 2010). Within a near future the industry is expected to further increase its production considerably.

In Sweden, the species is mainly farmed in net-pens situated in large oligotrophic water bodies in the northwestern parts of the country (Eriksson, Alanärä et al. 2010). These water bodies are, as a result of damming, nutrient depleted (Stockner, Rydin et al. 2000). Arctic charr fish farms located in oligotrophic water bodies have been conceptually targeted for the Aquabest project. The nutrient added from fish farming to the surrounding water and neighbouring ecosystems create a positive loop of nutrients. A fish feed with ingredients from the Baltic Sea and its catchment area would move nutrients from the europhic southern parts of the Baltic Sea to the nutrient-depleted water bodies in the northwest where the farming is conducted. Taking nutrients from the rich and giving to the poor is a conceptual model called the Robin Hood model (Figure 1) (Eriksson, Alanärä et al. 2010). The model makes up for the basis of this project.

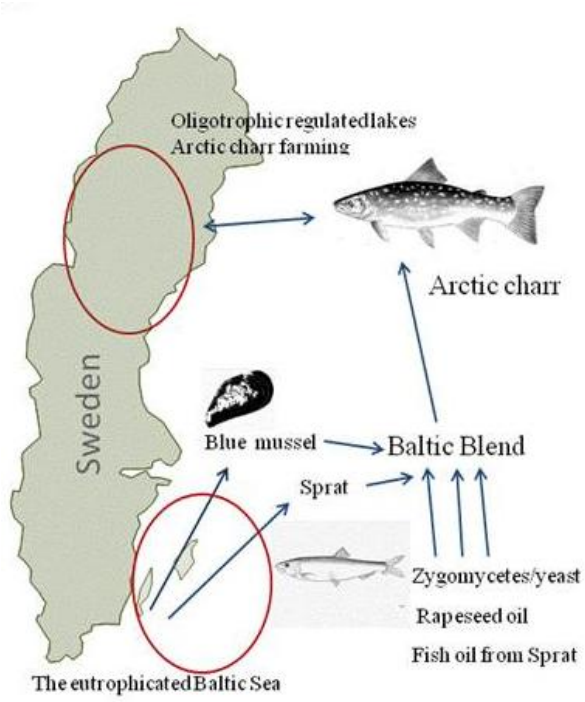

Figure 1. The Robin Hood Model. Nutrients (nitrogen and phosphorous) is transported from the nutrient rich, eutrophic Baltic Sea to the nutrient poor, oligotrophic water bodies affected by hydropower regulations in Northern Sweden where Arctic charr is farmed. Through producing a fish feed, the Baltic Blend, from Baltic Sea mussels, de-toxified fatty fish such as sprat, microbe meal and a fraction of rapeseed oil a loop of nutrients can be created.

It is a broad and long process to thoroughly evaluate a new fish feed and its components. Fish welfare is the core but also reaching production goals, the consumer's opinions as well as food safety are important in the process. The product must not only be suitable for the fish but also give rise to a tasty product. It is important that the composition of the diet fulfils the nutritional needs of the species. An animal given its total nutrition as a pelleted one-choice feed can only regulate intake of a specific nutrient by eating less or more (Simpson and Raubenheimer 2001). If the feed is sub-optimally formulated relative to the nutritional needs of the fish, this inevitably leads to either under- or over-consumption of another nutrient. Resulting in possible deleterious consequences regarding growth, survivorship and feed utilization (and, hence, effluent composition) as well as affecting the welfare of the fish.

Replacing fishmeal is a challenge. Using ingredients that are not suitable for human consumption is one possible way to reach increased sustainability. Sprat (*Sprattus sprattus*) and herring (*Clupea harengus*) are today, presumably as an effect of intensive cod fisheries and ecosystem imbalances very abundant in the Baltic Sea. Unfortunately, high levels of PCBs and dioxins limit direct human consumption. Removing dioxins and other harmful anthropogenic substances through an industrial method developed by the Danish fish meat and oil companies produce a detoxified fishmeal product suitable for inclusion in fish feed (Kiessling 2009).

Blue mussels (*Mytilus edulis*) are very efficient plankton assimilators. Farming of bivalves contributes to a net removal of nutrients from the water column. By farming mussels in eutrophic waters nutrients is taken out of the water column at harvest, leading to a recycling of excess nutrients in the water that has been added through anthropogenic activities (Lindahl and Kollberg 2009). At present, the majority of all bivalve farming is conducted in a marine environment. Neither freshwater mussels

nor blue mussels that grow in low salinity will reach a size suitable for the human food market. However, considering the environmental gain of nutrients removal from the water column, low salinity or freshwater-farmed mussels produced for inclusion in animal feeds may very well be profitable. In particular if the mussel meat is used to produce high-value organic certified fish and poultry products (Lindahl and Kollberg 2008; Lindahl and Kollberg 2009; Goedkoop, Naddafi et al. 2011). Complete substitution of fish protein with cultured mussels in feed for rainbow trout *(Oncorhynchus mykiss*) and Arctic charr show no reductions in growth (Brännäs et al. In prep).

Single-cell organisms, microorganisms, are also promising protein sources for sustainable replacements. They are fast-growing organisms that potentially also can grow on human waste-materials. Microorganisms as such are usually not suitable for direct human consumption and can theoretically be custom-made to suit the needs of a farmed fish. Research to develop microorganisms suitable for inclusions in fish feed is ongoing, feed trials with rainbow trout show promising results (Mydland, Landsverk et al. 2007). Baker's yeast, (*Saccharomyces cerevisiae*) is a good candidate for this type of inclusion.

As fish are fed with a certain feed during a full production cycle possible shortcomings are easier to detect. Evaluation of genetic variations of growth, feed efficiency and effects of diets on selection in breeding programs has been emphasized as important (Silverstein, Hostuttler et al. 2005). In order to evaluate the new Baltic Sea-sourced feed, several experiments have been conducted. Our experiments evaluate the growth performance, genetic effects on growth, taste, and preference as well as several physiological factors on Arctic charr.

To understand the importance of detoxification of the fish oil from the Baltic Sea pelagic fisheries of mainly herring and sprat a feeding trail with Arctic charr was designed. The levels of persistent organic pollutants (POPs) in the Baltic Sea fatty fish has been evaluated as too high, therefore distillation of the fish oil has been developed and is now practiced. The EU and WHO regulations on tolerable weekly intake (TWI) of dioxins and dl-PCBs are used as guidelines for negative health effects of these substances in children and women in childbearing years (TWI, 14 pg, TEQ/kg body weight/week) (SLV 2014). These levels are decreased by 95 % when fish oils are distilled with active carbon. In addition, methods for reduction of the remaining toxicants are also available.

Sesamin as bioactive compounds added in fish diet has been studied a lot in recent years. Sesamin has an effect on lipid metabolism with increasing the proportions of DHA in rainbow trout white muscle (Trattner, Kamal-Eldin et al. 2008a; Trattner, Kamal-Eldin et al. 2008b). Meanwhile, Wagner et al. (2013; 2014) have shown that sesamin resulted in a metabolic disturbance in Atlantic salmon (*Salmo salar*) liver and muscle, and an elevated level of EROD (ethoxyresorufin-O-deethylase) activity in the liver of Atlantic salmon and common carp (*Cyprinus carpio*). Thus, sesamin could be considered as a xenobiotic compound, in action possibly in similar way as POPs.

The aim of the Baltic Sea fishmeal and fish oil evaluation study was to evaluate the decontaminated fish meal and fish oil from the Baltic Sea used in feed for Arctic charr to learn more about its suitability for inclusion in fish feed. For this purpose, lipid and fatty acid (FA) composition and the expression of genes involved in lipid metabolism were measured. Additionally, metabolomics of liver and muscle by means of NMR-based metabolomic approach, and the hepatic activity of EROD as a biomarker of organic pollutants were analyzed.

This report presents results from three different feeding trails with Baltic Sea-sourced feed fed to Arctic charr. A long-term trial over a full production cycle with Arctic charr fed a Baltic Sea-sourced feed compared with fish fed a diet mirroring a commercial recipe. A preference test was conducted

where the fish could choose what feed they preferred. Furthermore the decontaminated Baltic Sea fishmeal and oil was also evaluated.

2. Material and Methods

2.1. The feeds

Two feeds were used for the long-term and preference experiments. A novel Baltic Sea-sourced feed, hereafter called the Baltic Blend, BB, and as control feed a fishmeal based feed, corresponding to a commercial feed in its formula. See Table 1 for recipes and Table 2 for composition and total energy content. Feeds were manufactured in Jyvaskyla, Finland by the Finish game and Fisheries Research Institute during 2013 and thereafter shipped to Aquaculture Centre North, ACN, in Kälarne where the long-term experiment was conducted.

Table 1. Feed recipe for the Baltic Blend and the fishmeal based control feed used in the long-term and preference experiments. 40 mg/kg astaxanthin was added to each diet.

Feed composition (g kg^{-1})	**Baltic Blend**	**Control**
Regular fish meal		0.332
Baltic Sea fish meal	0.216	
Fish oil, "regular"	0.071	0.073
Mussel meal, not from Baltic Sea	0.212	
Yeast	0.253	
Rapeseed oil	0.047	0.049
Wheat gluten	0.050	
Wheat meal	0.131	0.259
Soy protein concentrate		0.267
Mineral/Vitamin mix	0.015	0.015
Titanium oxide	0.005	0.005
Total	1.000	1.000

All diets additionally contain 40 mg astaxanthin/kg

Table 2. Proportion (%) of wet feed sample of protein, fat and ash as well as total energy content (MJ/kg) of the two experimental diets used in the long-term and preference experiments.

	Baltic Blend	Control
% of wet sample		
Crude protein	44.4	44.4
Fat	19.9	17.7
Ash	7.6	6.5
Total energy (MJ/kg)	21.9	21.9

2.2. Long-term trial

2.2.1. Fish and rearing

Arctic charr from the selected strain Arctic superior reared at ACN were used in the long-term trial. Fish from 15 sibling groups, each group with a distinct pedigree, were anaesthetized with MS-222 (40 mg L^{-1}), and individually tagged with PIT-tags (Passive integrated Transponders, Biomark HPT12) in October 2012. Initial length and weight were recorded. The fish were divided into six different tanks. All six tanks contained 33 fish from each sibling group, in total 495 fish per tank. The fish had an average initial weight of 32.7 g (S.D ± 10.1) with no significant difference between tanks (ANOVA, p=0.65).

During the experiment the fish were kept in a flow-through system (65 l min^{-1}) in circular concrete tanks, 2 m in diameter with a water volume of 5 m^3. From October 2012 to February 2013 the fish were fed commercial feeds (Skretting Nutra MP 1.9 mm and Skretting Optime 1P 2.5 mm) by belt-feeders to grow large enough to accept 3 mm pellets, the smallest pellet size that could be produced for this trial. Feeding was made *ad libitum* by experienced staff that assessed feeding behavior and waste- pellets daily, to ensure that correct amounts of feed were given. Feed was delivered from 7 a.m. until 4 p.m. The fish were kept in a light/dark-cycle mirroring natural conditions but never with a shorter day-length than 7 a.m.-4 p.m. during winter. The temperature from October to February ranged between 8.5° C to 0.8° C (± 0.1°C).

2.2.2. Experimental set-up

The study was conducted with three replicates (Figure 2) Starting in February 2013 the commercial feed was mixed 1:1 with experimental feeds to allow the fish to acclimatize to the new feeds and minimize the risk of a sudden appetite loss. After one month, experimental feeds were given in full rations. Pellets were 3 mm until July 1$^{st;}$ thereafter 4 mm pellets were used. Temperature from February to December 2013 ranged from 1.2 – 13.8 °C (± 0.1°C) and was measured daily. Fish weight, length and general condition was recorded monthly between February to May 2013 (See Figure 3 for a description of the experimental protocol).

Figure 2. The six experimental tanks

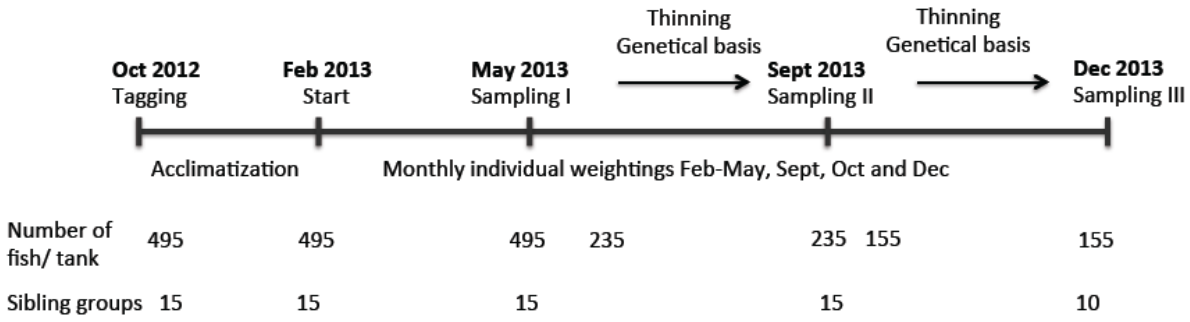

Figure 3. Schematic description of the experimental protocol

In May a thinning of the fish was conducted to ensure suitable biomass for the tank and good fish welfare. The 13 smallest individuals from each of the 15 sibling groups from the April measurement were removed from the experiment using a modified version of Biomark Tag Manager v1.0 for sorting. In May, fillet, liver and faces samples from 8 fish per tank were also taken for further analysis.

Another sampling was done in September, fish were weighed, measured and had their general condition assessed. A thinning was conducted to ensure suitable biomass in the tank and good fish welfare. All fish from five sibling groups were excluded and further also the smallest individual from the remaining sibling groups. The sibling groups that were excluded were one of the fastest growing, one of the poorest growing and three sibling groups with intermediate growth. Fillet, liver and feces were taken from eight fish per tank. From another five fish per tank, feces were collected for microbial activity-analysis.

In October, a sample of the remaining fish from each tank were weighed and measured to securely adjust the feed rations.

The long-term trial ended on December 2nd 2013, final weights and lengths were measured and general condition assessed. Fish (n=216) were selected for sampling; six fish from six sibling groups in all six tanks. The six sibling groups were selected based on growth differences between treatments; small, intermediate, and large growth differences. From each sibling group two small, two intermediate and two large fish were selected. Muscle, heart, liver, blood plasma, feces and whole fish were taken for analysis. The tissues will be used for evaluation of fatty acid profiles, metabolomics, digestibility, microbial activity, heart size, toxicity-tests, total fat content, and fillet colour (some of these data are presented in separate reports). In addition, 20 fish from each treatment were not sedated but removed

to another tank and saved for slaughter after 10 days of starvation. Slaughter of the 20+20 fish was performed through giving the fish a blow to the head and thereafter bleeding. Intestine were removed, fish were rinsed and stored on ice until they were used for a sensory evaluation.

2.2.3. Sensory evaluation

The sensory evaluation test was conducted in cooperation with Umeå University School of Restaurants and Culinary Arts. Fish were filleted and each fillet cut into three parts. The fish were cooked for 4 min 58 sec to 52-54° C in a steam oven (Jonsson, Marklinder et al. 2007). Thereafter fish were served to a panel of 26 participants for sensory evaluation. The panel contained of staff and students at Umeå University School of Restaurants and Culinary Arts. Participants were all very familiar with fish as food and had an interest for food quality and taste, some with many years of experience from the restaurant business. The tasting was a blind-test. Participants were asked to fill out a form to answer questions about different aspects of the fish regarding taste, smell, texture and appearance. The participants were also asked questions of more general character regarding how they perceived the fish from a consumer point of view.

2.2.4. Flesh colour

The levels of axtanxanthin in the fillets were analysed using a simplified method without separation of carotenoids. Astaxanthin in a piece of muscle was extracted in acetone, followed by evaporation and dilution with isopropanol. The amount of astaxanthin was measured through spectrophotometry at 477nm.

2.2.5. Fat content of fillet

Fillet samples from ten fish from each treatment were used to analyse the muscle fat content. Approximately 3 g from the left fillet was homogenised in a food processor together with an alkaline detergent (LOSsolver Fish, MIRIS AB, Uppsala, Sweden) at 45 °C. The solution was analysed using a Mid-Infrared-Transmission (MIT) spectroscopy method (MIRIS AB, Uppsala, Sweden) according to the manufacturer's instructions. To compare the lipid content, the average MIT value of two to three subsamples of each homogenised fillet was used.

2.3. Feed preference test

2.3.1. Fish and rearing

Arctic charr from the strain Arctic superior aged 0.5 years with an average weight of 40 g were transported from ACN to Umeå Marine Science Centre in Norrbyn in October 2012. Fish were held in 0.5 m^3 tanks, supplied with flow through brackish water (3 ‰) and fed a commercial feed (Skretting Nutra MP T 1.9 mm and Skretting Optime 1P 2.5 mm) using an automatic feeder. The light/dark cycle was kept to 12:12. The water temperature followed the natural variation, starting with 10° C in October and dropping down to 0.3°C at the lowest during the winter months. Three weeks before the trail water temperature was slowly increased from 1°C to 10°C by mixing the natural tempered water with heated 10° C water.

2.3.2. Experimental set-up

Sixteen Arctic charr were anaesthetized with MS-222 (40 mg L^{-1}) and initial weight and length was recorded. The initial weights ranged between 72.2 - 149.2 g (average 118.6 g, S.D ± 18.1). The fish were placed in one aquarium each. The aquariums had a total volume of 70 l and were divided into two compartments separated by a gray plastic wall with a hole in the middle, allowing the fish to freely swim between the two compartments. Each compartment was equipped with a water inlet; an oxygen stone and an artificial see grass shelter (Figure 4). The aquariums were supplied with flow through water with a temperature of 10° C (± 0.5°C) and fish were kept in a light/dark cycle of 12:12. Fish were acclimatized to their aquarium for 10 days and during that time fed with a commercial feed (Skretting Optime 1P 2.5 mm) with battery driven feeders (Fish mate T14) located above each of the two compartments.

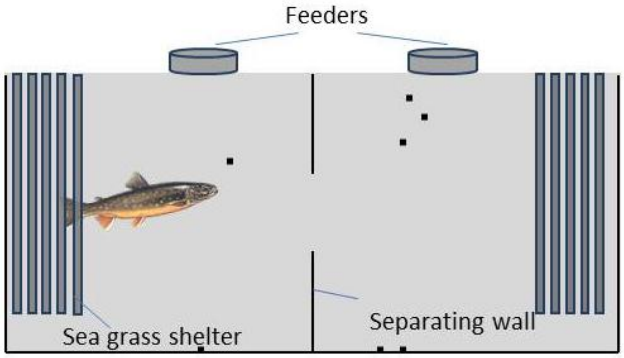

Figure 4. The experimental set-up for the fish preference test.

Fish preference of the two different feeds was tested; the Baltic Blend and the Fishmeal-based commercial type feed as control, both 3 mm. The two feeds were tested for durability in water to ensure that uneaten pellets were not destroyed when left for 5 h in the aquarium.

The initial weights of the fish were used to determine the feed ration that was set to 1.5 % of Body weight (BW) day-1 for each of the two feeds. This resulted in a total feed ration of 3 % BW day-1 to ensure that feed was given in excess. During the 6th day of the trial the feed ration was adjusted for seven fish that had a particularly good appetite. The feeding-levels for these fish were at day six 1.66 - 2.05 % of BW, in comparison to the remaining eight fish where levels ranged between 1.24 - 1.42 % of BW.

During the trial, feed was delivered two times per day, at 8 and 11 am, each feeding lasted approximately 1h and 40 min. Baltic Blend was fed in one compartment of the aquarium and the control feed fed in the other. Uneaten pellets were collected from each tank with a small net daily and thereafter counted. After nine days the fish showed a preference for one the feeds and the position of the feeds was changed on day ten in all aquaria. The trial continued for another seven days, giving a total trail time of sixteen days.

2.4. Baltic Sea fishmeal and fish oil evaluation

2.4.1. Fish and rearing

Arctic charr from the selected strain Arctic superior reared at ACN, Kälarne, Sweden, were used in the feeding trial. The water temperature during the trial was approximately 6 ^{o}C.

2.4.2. Experimental set-up

Ten fish per tank were kept in totally six tanks for nine weeks. The feeds were composed by two fish oil and two fishmeal qualities. A control (standard commercial diet, Skretting Arctic charr feed) 3 mm was included and one negative control diet was composed with addition of the sesame oil lignan sesamin (Table 3 and 4). At termination, muscle and liver was sampled from 8-10 Arctic charr per treatment. Fatty acid and metabolomic profile of muscle and liver as well as gene expression were analyzed. The following genes were chosen to be relevant for the study design and to reveal if pollutants have effects on the metabolism and expression of genes related to the lipids PPAR-α, PPAR-β, PPAR-γ, GHR-I, IGF-I, IGF-II.

2.4.3. The feeds

Table 3. Feed formulations in the five experimental diets (content in %). CFM crude fish meal; DFM defatted fish meal; CFO crude fish oil; PFO purified fish oil; S sesamin.

Components	DFM+PFO	DFM+CFO	CFM+PFO	CFM+CFO	CFM+PFO+S
Casein	10	10	12	12	12
Fish meal defatted	62	62	-	-	-
Fish meal	-	-	62	62	62
Fish oil purified	22	-	20	-	20
Fish oil crude	-	22	-	20	-
Vitamin + Mineral premix	2	2	2	2	2
Gelatin	3	3	3	3	3
Carboxyl methyl-cellulose	1	1	1	1	1
Sesamin	-	-	-	-	0.58

Table 4. Lipid content and relative fatty acid composition (%) in the diets. CFM crude fish meal; *DFM* defatted fish meal; *CFO* crude fish oil; *PFO* purified fish oil; *S* sesamin.

	Control	DFM+PFO	DFM+CFO	CFM+PFO	CFM+CFO	CFM+PFO+S
Lipid content	19.6	28.0	27.2	27.4	26.6	26.8
16:0	15.2	17.7	18.9	17.9	18.7	18.0
18:1n-9	32.9	19.4	18.6	18.2	17.3	18.0
18:2n-6	16.2	2.7	2.6	2.6	2.5	2.7
18:3n-3	4.3	2.2	2.1	2.1	2.1	2.2
20:4n-3	1.5	7.7	8.2	8.6	8.8	7.4
20:5n-3	5.7	7.9	7.6	7.9	7.9	8.3
22:6n-3	4.3	10.6	10.2	10.2	10.1	10.7
SAFA	23.5	27.1	28.5	27.5	28.6	27.9
MUFA	41.8	37.2	36.3	36.5	35.5	35.9
PUFA	34.7	35.7	35.2	35.9	35.9	36.1
n3	17.8	31.9	31.5	32.4	32.4	32.4
n6	16.9	3.8	3.7	3.6	3.5	3.7
n3/n6	1.1	8.3	8.5	9.0	9.2	8.7

SAFA saturated fatty acids (14:0, 15:0, 16:0, 17:0, 18:0, 20:0); *MUFA* monounsaturated fatty acids (14:1, 16:1n-7, 17:1, 18:1n-11, 18:1n-9, 18:1n-7, 18:1n-5, 20:1n-11, 20:1n-9, 22:1n-9, 24:1); *PUFA* polyunsaturated fatty acids (18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-6, 20:4n-6, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-6, 22:5n-3, 22:6n-3); n3 (18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3); n6 (18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6,20:4n-6, 22:5n-6).

Table 5. Contaminants in the fish raw materials used in the experimental diets (analysed by accredited laboratory).

	Fish oil L10		Fish meal	
uk/kg	Non-purified	Purified	Non-defatted	Defatted
2,4,4´ – trichlorobiphenyl	3.70	3.28	0.11	0.24
2,2´,5,5´– tetrachlorobiphenyl	5.62	5.33	0.14	0.39
2,2´,4,5,5´– pentachlorobiphenyl	15.86	15.79	0.45	1.36
3,3´,4,4´- tetrachlorobiphenyl	1.26	1.09	0.06	0.11
2,3´,4,4´,5´– pentachlorobiphenyl	13.86	13.41	0.40	1.06
2,2´,4,4´,5,5´– hexachlorobiphenyl	36.52	37.08	0.95	2.84
2,3,3´,4,4´– pentachlorobiphenyl	4.70	4.58	0.11	0.31
2,2´,3,4,4´,5´– hexachlorobiphenyl	23.83	24.06	0.67	1.86
2,3´,4,4´,5,5´– hexachlorobiphenyl	1.18	1.21	<0.05	<0.05
2,3,3´,4,4´,5´– hexachlorobiphenyl	1.89	1.87	<0.05	0.15
2,2´,3,4,4´,5,5´– heptachlorobiphenyl	8.84	8.83	0.17	0.65
Benzo[c]fluorene	15.56	2.87	<0.05	0.31
Benz[a]anthracene	26.13	0.76	0.10	0.22
Cyklopenta[c,d]pyrene	48.97	2.32	1.61	0.79
Chrysene	28.91	<0.25	2.95	0.52
Benzo[b]fluoranthene	9.00	<0.25	0.07	0.07
Benzo[k]fluoranthene	4.10	<0.25	<0.05	0.05
Benzo[j]fluoranthene	5.99	<0.25	<0.05	0.06
Benzo[a]pyrene	12.04	<0.25	0.05	0.21
Dibenz[a,h]anthracene	0.62	<0.25	<0.05	0.07
Indeno[1,2,3-cd]pyrene	3.36	<0.25	<0.05	0.12
Benzo[g,h,i]perylene	3.92	<0.25	0.09	0.24
Dibenzo[a,e]pyrene	0.52	<0.25	<0.05	<0.05
2,2´,4,5´ tetrabromodiphenyl ether	1.42	1.56	0.09	0.18
2,2´,4,4´ tetrabromodiphenyl ether	3.59	3.49	0.22	0.48
2,2´,4,4´,6 pentabromodiphenyl ether	1.15	1.24	0.09	0.11

2.5. Data management

2.5.1. Long-term trial

Data from all measurements was paired with Microsoft Office Access 2007 (Windows 8).

The weights of the fish in each tank were compared with repeated measurement ANOVA using mean weight per sibling group (n=10 at termination) at each sampling occasion as dependent variable and feed (Baltic Blend and control) as factor. Differences in weight (tank means) on single sampling occasions were analyzed with One-Way ANOVA. The data was analyzed in Statistica 12 or SPSS 19. Level of significance was set to $p<0.05$ for all analyses in this report.

The effect of sibling group on the relationship between diet and final weight was analyzed with Univariate-ANOVA with sibling group as random factor.

Differences in fat content and astanxhantin-levels were analyzed with Student t-test using Minitab 16 Statistical software.

2.5.2. Feed preference test

In the feed preference test, 200 pellets from each diet were weighed and the average weight pellet was calculated. Feed intake was calculated through subtracting the left-over pellets weight from the total weight of feed given per day. To compensate for variation in the fish initial weights, preference results are presented in feed intake % BW day^{-1}. A linear estimated weight increase between start and final weight was assumed for calculating the feed intake as % BW day^{-1}.

Data from the preference test was analyzed with repeated measurement ANOVA using sampling occasion as dependent variable and feed (Baltic Blend and control) as factor. Feed intake between each collecting occasion and day one to day sixteen as dependent variables and feed as factor. The statistics were calculated in Statistica 12 or SPSS 19.

2.5.3. Baltic Sea fishmeal and fish oil evaluation

Analyses of the results were performed by one way ANOVA, statistical methods are given together with the data found in the appendix.

3. Results

3.1. Long-term trail

3.1.1. Growth

Fish grew satisfactory and reached an acceptable size for the market. The fish almost two-folded their weights from February until May. During this time-period there was no significant difference between the two treatments comparing average weight of the fish in each tank (n=3+3) (Figure 5). From May until September the fish increased their weight more than three times in all tanks. The groups fed with the control feed had a significantly higher mean weight increase than the fish fed the Baltic Blend (repeated measurement ANOVA $p<0.0001$ for the effect of feed and fed*time). This difference remained until the termination in December when fish reached market-size. On average, the fish fed Baltic Blend were almost 80 g smaller than the fish fed control fed (Figure 5 and Table 6).

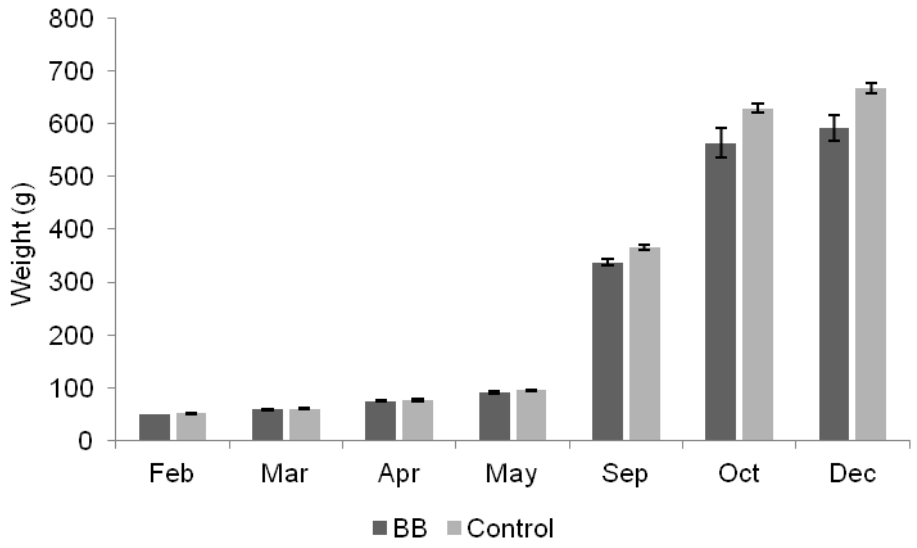

Figure 5. Mean weight (g) of the three replicate groups fed with Baltic Blend (dark grey) and the control feed (gray), error bars show ± S.D.

Table 6. Mean weights (g) from all individuals and ± S.D.

	Control		Baltic Blend	
	Weight (g)	± S.D	Weight (g)	± S.D
February	50.8	13.6	49.4	12.7
March	59.8	16.2	58.3	15.2
April	77.1	20.8	74.4	19.3
May	94.6	25.4	90.8	23.8
September	366.0	69.0	337.3	62.4
October	628.7	119.6	562.7	99.9
December	667.9	122.3	590.5	109.7

3.1.2. Genetic effects

There was a significant feed and sibling group (genetic) effect on final weight but no interaction between feed and sibling groups (Univariate-ANOVA, $F1=67.4$, $p<0.001$ for feed, $F9=9.1$, $p<0.001$ for sibling group and $F9=1.0$, $p=0.409$ for sibling group*feed). In other words, there was no sibling group (genetic) effect of the diets on the final weight (Figure 6).

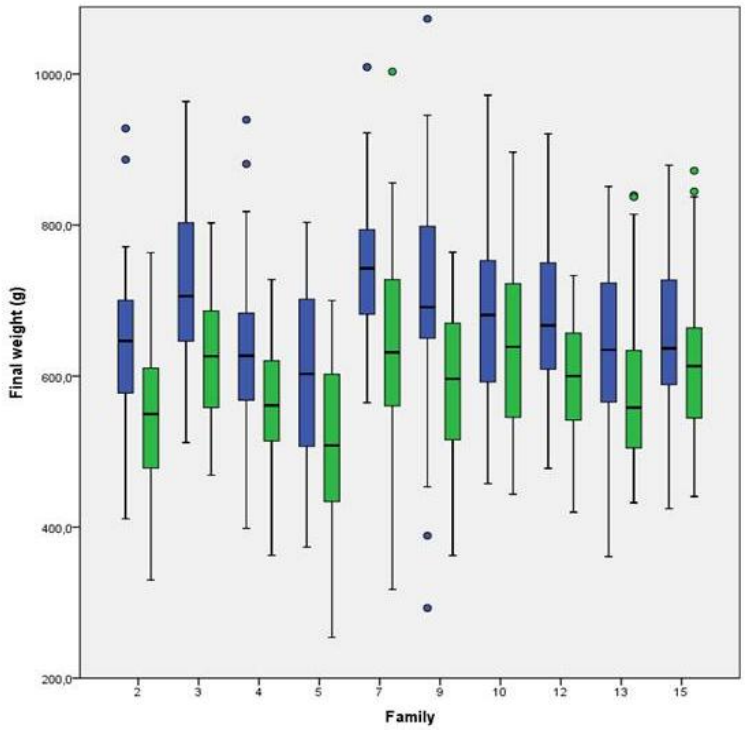

Figure 6. Box-plots of the final weight (g) in December of the ten sibling groups fed the Baltic Blend (green) and the control feed (blue).

3.1.3. Flesh colour

Astanxanthin levels in fillet samples were analysed (n=10+10). No significant differences were detected in astaxanthin levels in the fillets between the two different treatments (Figure 7) (Student t-test, t (15) =0.74, p=0.468).

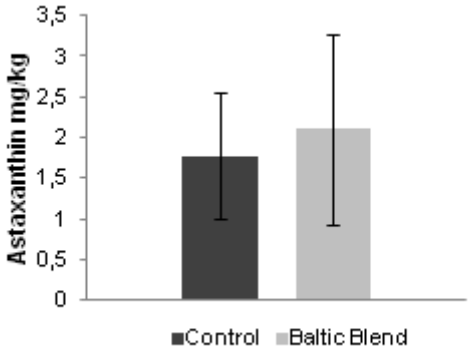

Figure 7. Astaxanthin levels (mg/kg) in fillets from A. charr fed control or Baltic Blend feed (n= 10+10). Student t-test, t (15) =0.74, p=0.468.

3.1.4. Fat content of fillet

The fat content of the fillet (n= 10+10) did not differ significantly in fillet samples from Arctic charr given the two different feeds (Figure 8) (Student t-test, t (14) =1.64, p=0.124).

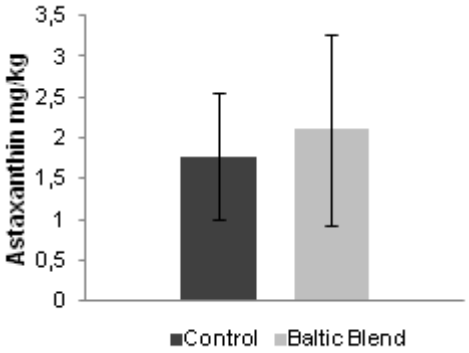

Figure 8. Fat content (%) of fillets from A. charr fed control and Baltic Blend feed (n=10+10) (Student t-test, t (14) =1.64, p=0.124).

3.1.5. Sensory evaluation

The fish fed Baltic Blend was rated as similar as or even better than the fish that were given the control feed in the sensory evaluation. The respondents were asked to grade from 1-9 how they liked the fish, one (1) was not tasteful/very bad and nine (9) very tasteful/very good. The Baltic Blend got an average score of 6.35 and the control 6.19 (Figure 9).

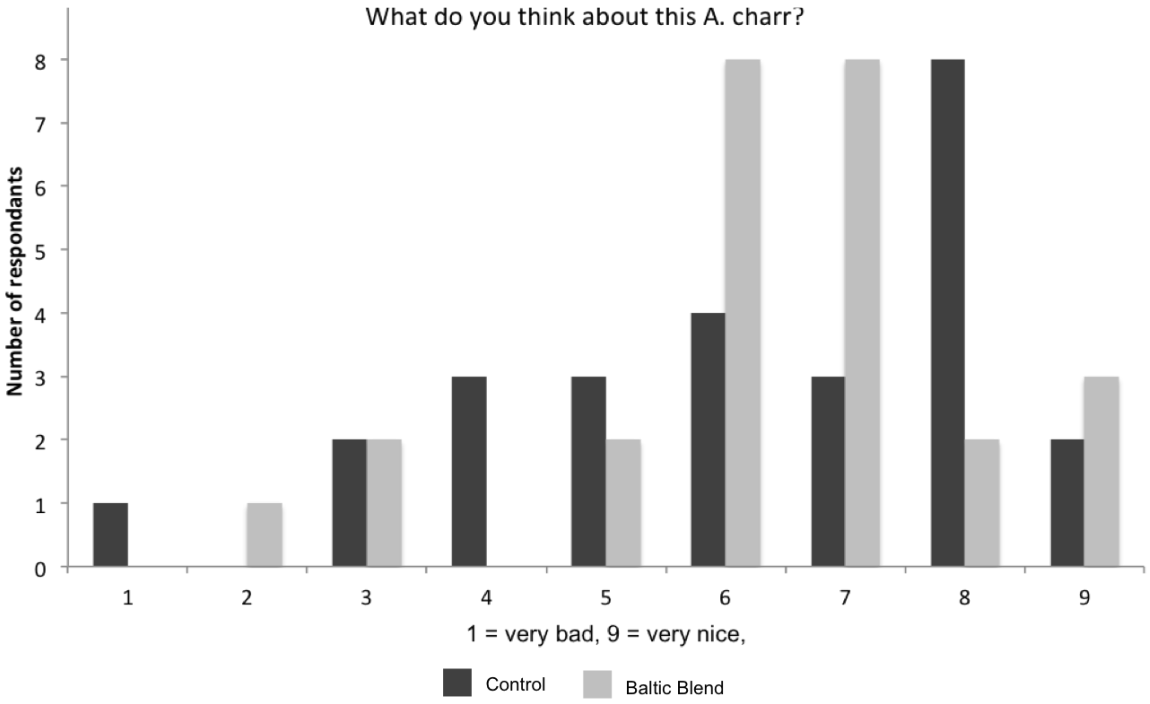

Figure 9. Results from the sensory evaluation of fillet from Arctic charr fed the Baltic Blend and the control feed show that the fish fed Baltic Blend (average score 6.35) was considered as good as the fish fed the control feed (average score 6.19). N= 26.

The panel was also asked to rate the two pieces of fish they had been served in different categories; appearance (A), flavour (F), texture (T) and odor (O). Each of the four categories held five to seven

adjectives to rate between 0-100. Zero indicated no recognition of the adjective and 100 full recognition (Figure 10). With exceptions for (A) white and (O) metallic that were significantly different between the two pieces of fish (Student-t, (A) white, t (43) =2.41, p=0.02; (O) metallic, t (34) =2.11, p=0.042). The ratings of all other adjectives were similar for both treatments.

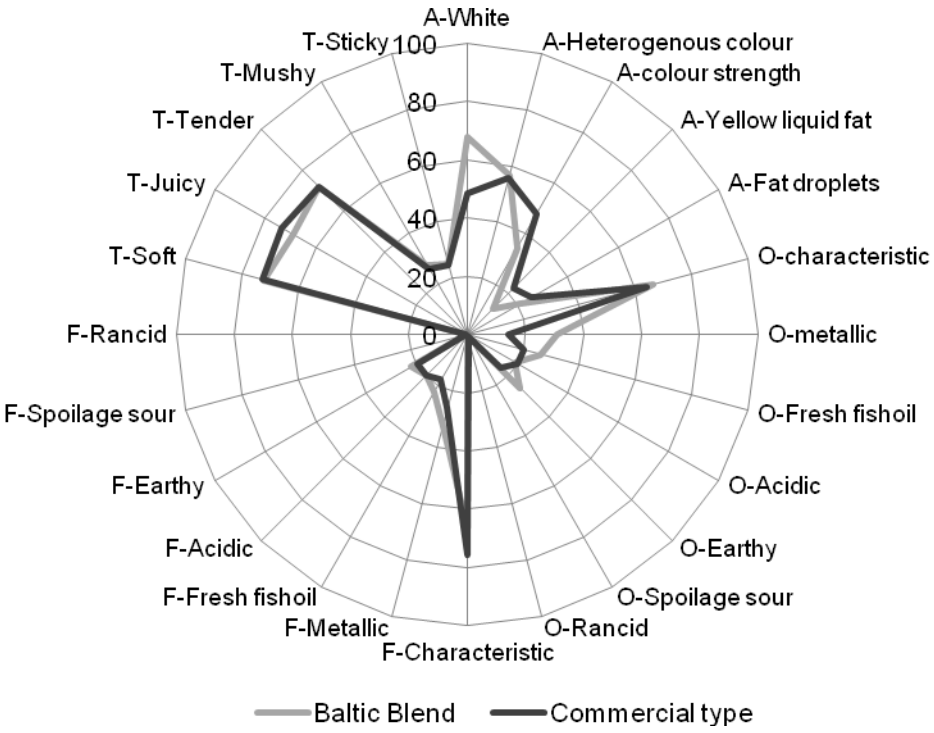

Figure 10. Sensory evaluation of Arctic charr fed the Baltic Blend and a control feed respectively. The prefix A is for appearance, O for odour, F for flavour and T for texture.

3.2. Feed preference test

In the feed preference test Arctic charr showed a clear preference for the Baltic Blend. All fish except one ate with a good appetite and had a mean growth rate of 1.84 % day^{-1} (S.D. ± 0.56) including the 10 day period of acclimatization. The single non-eating fish was excluded from the analysis resulting in 15 fish left in the analysis. Fish did show a preference for the novel feed with ingredients from the Baltic Sea already the second day of the trial. On day ten when positions of the feeds were changed there was no self-selected difference between the consumption of the two feeds. After day eleven a clear preference for the Baltic Blend once again became clear. During the total preference test period there was a significant preference for the Baltic Blend (ANOVA repeated measurement, p=0.046 but no effect of time p=0.09) (Figure 11).

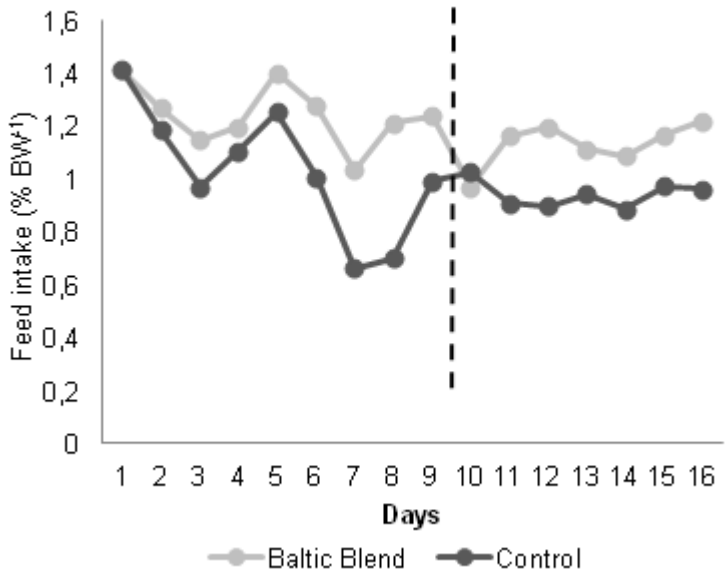

Figure 11. Fish preference between Baltic Blend and a control feed. The black dotted line indicates that feeds swapped place. Arctic charr show preference for the Baltic Blend. (Repeated Measure ANOVA $p<0.05$) n= 15.

3.3. Baltic Sea fishmeal and fish oil evaluation

No mortality or other problems occurred during the duration of the trial.

3.3.1. Lipids

The dietary lipids did not vary in the experimental diets, though the commercial control diet differed in its lipid content (see table 4 in methods). The levels of different pollutants are given in table 5. The fatty acid profiles did not vary between the fish of the different experimental dietary groups either in liver or muscle. A few individual fatty acids showed slight differences (see Appendix).

3.3.2. EROD activity

The group fed fishmeal and purified fish oil with sesamin showed the highest level of EROD activity, which was expected and therefore considered as a negative control in this study. When the feeds composed with the oil and fish meal alterations were statistically analyzed (excluding the commercial feed and the sesamine feed), the largest response by detoxification system was obtained, were compared defatted fish meal and purified fish oil had strongest CYP 450 enzyme 1A activity.

3.3.3. Gene expression

Expressions of the chosen genes PPAR-α, PPAR-β, PPAR-γ, GHR-I, IGF-I, IGF-II did not show any effects between the experimental dietary treatments.

4. Discussion

Results are promising despite the slightly reduced growth for Arctic charr fed the Baltic Sea-sourced feed. Both groups reached an acceptable slaughter size. The ingredients from the Baltic Sea are of little or no value for direct human consumption and suitable for farming of Arctic charr. Separate studies on the protein sources mussel meal and yeast respectively show no growth-reductions in Arctic charr (Lundh et al aquabest report in prep). With slight recipe adjustments in the Baltic Blend, the nutrient utilization and growth of fish could potentially increase.

The protein sources from the Baltic Sea depend on industrial processes that in this pilot-face of product development lack infrastructure. However, large geographical areas in the Baltic Sea have within the scope of Aquabest been targeted as potential areas for blue mussel farming (Lindahl 2013). Large-scale processes for meat extraction are also under development. Harvest of rich populations of sprat and herring in the Baltic Sea could remove more than 1000 tons of phosphorous and 7000 tons of nitrogen (Kiessling 2009). Also, mussels remove significant amounts of nitrogen and phosphorus from the water column. Microbes are promising protein sources for fish and not suitable for human consumption. Ideally, the microbial biomass can grow on anthropogenic waste-materials. More research is in progress to develop a fast-growing microbe with a stable and suitable amino-acid profile for inclusion in fish feeds (Olstorpe et al aquabest report in prep). As for the present, yeast is the most suitable available microbe option on the market.

Arctic charr has shown to be a suitable target species for partial or even total fish oil replacements (Pettersson, Pickova et al. 2009). Though total fish oil replacements might adventure the, for the consumer, important high levels of Omega 3 fatty acids. It is for this reason important to monitor the fatty acid profile in the flesh. Luckily mussel meal contributes with omega 3 and with the current replacement level of 1:1 fish oil: rapeseed oil these levels are more than sufficient for the consumers.

The slight growth reductions in the long-term trial may be compensated by the gain of using sustainable ingredients provided the production costs of a Baltic Blend type of feed can be kept to a market value. The nutrient loop described as the Robin Hood model would generate positive ecosystem effects for the targeted ecosystems. Such positive effects would not only gain the environment but can potentially also improve the general public's attitudes towards the Swedish fish farming industry.

The long-term trial generated a large number of different samples from which food safety; flesh quality, genetic effects and fish welfare can be evaluated. Liver, blood, muscle, hart and feces were sampled, from which the outcomes will be presented in separate Aquabest reports. It is a mandatory requirement that the quality of farmed fish for human consumption is not reduced by using a fish feed based on ingredients that normally are not used in diets for carnivorous fish. The ingredients, such as single cell organisms, mussels and plant oils must be evaluated before they and the feed can be used at a commercial scale (Kiessling, 2009). The unique combination of sustainable raw materials in the Baltic Blend hold a great capacity on which we will know even more as all tissue analysis from this study fall into place.

The sensory evaluation of Arctic charr fed the two different feeds was successful. The panel showed similar preference for the two fish. Performing a sensory evaluation where one of the specimens mirror a commercially sold product can often be to the new products disadvantage. The person performing the tasting can easily favour a product whose characters can be recognized, as how the product should taste. Despite this the fish that had been fed the Baltic Blend got high acceptance from the sensory panel. The panel experienced a difference in the flesh colour after cooking. There was,

however no significant difference in astaxanthin content with respect to diet. This difference experienced by the test panel may depend on other pigments in the mussel meal than astaxanthin.

The feed preference study suggests a positive response by the fish itself to the new Baltic Blend diet. A possible explanation for the fish preference is the inclusion of blue mussels, (*Mytilus edilus*) in the feed. Mussels may function as a taste attractant for salmonids.

The Baltic Blend feed was slightly more oily, a possible result from the structure of the yeast cells, decreasing the pellets absorption abilities. This could clearly be seen in the farming environment during the long-term trial where the water surfaces in the tanks were somewhat oily in contrast to the tanks where control feed was given. The oiliness might also contribute to the fish feed preference in the preference experiment.

Regarding the Baltic Sea fishmeal and fish oil evaluation, Trattner (2008a) has reported that rainbow trout fed with sesamin had a higher level of EROD activity, and Wagner (2013) proved furthermore that in vitro sesamin acts as a mechanism-based inhibitor of EROD in both Atlantic salmon and common carp. The EROD activity inducted by CYP 1A in liver is often used as a biomarker of presenting xenobiotic compounds in fish. Therefore, because of pollutants in the diets of DFM+PFO, DFM+CFO, FM+PFO and FM+CFO, they have a higher level of EROD activity compared with the control group, but the effect of pollutants is still lower than that of sesamin.

By comparison of EROD activity induced by FM and FO, it can be seen that DFM induced a higher level of EROD activity than FM and there is no significant differences between CFO and PFO. Based on our knowledge, it is reported that lipid-soluble antioxidants can act a role of defense on contaminant-stimulated oxidative damage in aquatic organisms. Therefore, it is assumed that lipid-soluble antioxidants, such as tocopherol and carotenoids, were excluded during the process of de-fatted FM and FO purification. This causes a significantly higher level of EROD activity in the group of DFM than CFM, and non-significantly higher level of EROD activity in the group of CFO than PFO.

The conclusion of the pilot study on Baltic Sea fishmeal and fish oil is that other compounds not measured in the protein part of the feed (the fish meal) have affected the results the most. The defatted fish meal is lower in lipid content and most likely some change in the chemistry of especially membrane lipids (phospholipids) has an effect on the results in this study. To reveal the factor, studies of antioxidants and structures will be needed in a future.

In conclusion, the results from these studies are promising. The diet based on ingredients that are of little or no value as human food may be harvested to produce a high quality exclusive product like the Arctic charr for the human table market. In addition, nutrients are added to the ultraoligotrophic waters were Arctic charr are farmed which increases the production of wild fish (Eriksson, Alanärä et al. 2010). This nutrient loop could support a limited farming industry like the Arctic charr, which is beneficial for the public opinion and the development of a sustainable farming industry.

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5. Appendix

Additional tables from the Baltic Sea fishmeal and fish oil evaluation trial

Table 1. Lipid content (% of wet weight) and FA profile (% of total identified FA) in white muscle, expressed in means ± standard deviation (8-10 fish)

	Control	DFM+PFO	DFM+CFO	CFM+PFO	CFM+CFO	CFM+PFO+S	*p*
Lipid (%)	2.07 ± 0.78	1.96 ± 0.41	2.32 ± 1.79	2.78 ± 0.96	2.61 ± 1.80	2.54 ± 1.09	
Fatty acids							
14:0	2.90 ± 0.58^{b}	3.54 ± 0.25^{ab}	3.74 ± 0.75^{ab}	4.10 ± 0.35^{a}	3.67 ± 0.70^{ab}	3.80 ± 0.78^{a}	
16:0	15.5 ± 0.97^{b}	16.3 ± 0.61^{ab}	16.6 ± 1.13^{ab}	16.3 ± 0.86^{ab}	17.2 ± 0.77^{a}	16.8 ± 0.80^{a}	*
16:1n-7	5.49 ± 1.09	6.05 ± 0.68	6.08 ± 1.37	6.79 ± 0.70	5.89 ± 1.24	6.15 ± 0.99	
18:0	2.13 ± 0.11^{a}	1.78 ± 0.09^{b}	1.75 ± 0.14^{b}	2.00 ± 0.08^{a}	2.02 ± 0.13^{a}	2.01 ± 0.11^{a}	#
18:1n-9	22.5 ± 3.60^{a}	17.8 ± 1.64^{bc}	17.2 ± 3.20^{bc}	19.7 ± 1.46^{ac}	17.4 ± 2.74^{bc}	18.0 ± 2.36^{bc}	
18:1n-7	2.48 ± 0.23	2.43 ± 0.10	2.37 ± 0.29	2.49 ± 0.18	2.36 ± 0.31	2.37 ± 0.29	
18:2n-6	7.05 ± 1.87	3.04 ± 0.36	3.02 ± 0.61	3.49 ± 0.51	2.74 ± 0.36	3.35 ± 0.42	*
18:3n-3	1.91 ± 0.49^{a}	1.43 ± 0.13^{ab}	1.39 ± 0.28^{ab}	1.41 ± 0.13^{ab}	1.33 ± 0.22^{b}	1.38 ± 0.23^{ab}	
18:4n-3	1.31 ± 0.16^{a}	1.63 ± 0.13^{ab}	1.66 ± 0.40^{b}	1.66 ± 0.15^{ab}	1.54 ± 0.25^{ab}	1.63 ± 0.30^{b}	
20:1n-9	3.07 ± 0.68^{b}	3.63 ± 0.33^{ab}	3.53 ± 0.65^{ab}	4.34 ± 0.30^{a}	4.13 ± 0.72^{a}	4.13 ± 0.77^{a}	#
20:4n-3	3.50 ± 0.94^{c}	4.35 ± 0.37^{abc}	4.12 ± 0.78^{bc}	5.24 ± 0.38^{a}	4.95 ± 0.87^{ab}	5.00 ± 0.78^{ab}	#
20:5n-3	6.48 ± 0.93^{b}	7.47 ± 0.44^{a}	7.68 ± 0.71^{a}	6.64 ± 0.29^{b}	7.28 ± 0.88^{ab}	7.06 ± 0.60^{ab}	#*
22:5n-3	1.43 ± 0.14	1.42 ± 0.07	1.44 ± 0.09	1.43 ± 0.08	1.39 ± 0.06	1.40 ± 0.11	
22:6n-3	19.6 ± 5.59	24.2 ± 2.75	24.6 ± 6.54	19.2 ± 2.66	23.0 ± 5.7	21.8 ± 5.75	#
SAFA	21.1 ± 0.49^{c}	22.1 ± 0.48^{b}	22.6 ± 0.59^{ab}	22.9 ± 0.89^{ab}	23.4 ± 0.52^{a}	23.2 ± 0.83^{a}	#*
MUFA	35.8 ± 4.98	32.9 ± 2.72	32.0 ± 5.73	36.6 ± 2.34	32.9 ± 5.14	33.8 ± 4.67	
PUFA	43.1 ± 4.72	45.0 ± 2.45	45.4 ± 5.34	40.5 ± 2.42	43.7 ± 5.17	43.0 ± 5.02	#
n3	34.3 ± 6.04	40.6 ± 2.63	41.0 ± 5.65	35.7 ± 2.49	39.6 ± 5.33	38.4 ± 5.02	#
n6	8.83 ± 1.99^{a}	4.40 ± 0.36^{bc}	4.43 ± 0.57^{bc}	4.82 ± 0.60^{b}	4.03 ± 0.26^{c}	4.66 ± 0.44^{b}	*
n3/n6	4.22 ± 1.69^{c}	9.30 ± 1.11^{ab}	9.45 ± 2.04^{ab}	7.51 ± 1.19^{b}	9.90 ± 1.75^{a}	8.30 ± 1.31^{ab}	*

Abbreviations: *CFM* crude fish meal; *DFM* defatted fish meal; *CFO* crude fish oil; *PFO* purified fish oil; *S* sesamin.
Different superscript letters indicate significant differences within six groups ($P < 0.05$), one way ANOVA
* indicates significant differences observed between PFO and CFO groups
# indicates significant differences observed between DFM and CFM groups

Table 2. Hepatic total lipid (%) and fatty acid composition (% total FA) in the phospholipid fraction of fish fed with the experimental diets for 9 weeks.

	CONTROL	*DFM-PFO*	*DFM-FO*	*FM-PFO*	*FM-FO*	*FM-PFO+S*	*FO/FM*	*P*[1]
Lipids (%)	7.72 ± 0.88	8.50 ± 0.80	7.26 ± 0.71	8.21 ± 1.22	8.82 ± 1.27	9.55 ± 1.60		0.760
Fatty acids (%)								
14:0	0.75 ± 0.14	1.23 ± 0.08	1.15 ± 0.09	1.11 ± 0.09	1.06 ± 0.12	1.12 ± 0.16		0.101
15:0	0.20 ± 0.03^{a}	0.38 ± 0.04^{b}	0.35 ± 0.03^{b}	0.26 ± 0.03^{ab}	0.28 ± 0.02^{ab}	0.29 ± 0.03^{ab}	#	0.005
16:0	18.78 ± 1.11	19.95 ± 1.21	19.54 ± 1.11	18.90 ± 1.25	17.47 ± 1.22	19.82 ± 1.62		0.743
16:1 n-7	1.37 ± 0.40	1.86 ± 0.13	1.89 ± 0.14	1.92 ± 0.15	1.87 ± 0.16	2.18 ± 0.22		0.228
17:0	0.27 ± 0.01	0.34 ± 0.03	0.30 ± 0.03	0.28 ± 0.03	0.28 ± 0.03	0.30 ± 0.02		0.600
18:0	7.91 ± 0.85	6.07 ± 0.43	5.52 ± 0.47	6.76 ± 0.69	5.80 ± 0.56	6.21 ± 0.60		0.105
18:1 n-9	19.9 ± 1.27	17.3 ± 0.78	18.7 ± 1.22	16.8 ± 0.82	17.0 ± 1.30	20.6 ± 1.26		0.094
18:1 n-7	3.20 ± 0.22	3.88 ± 0.24	3.98 ± 0.29	3.70 ± 0.26	3.64 ± 0.27	4.06 ± 0.39		0.350
18:2 n-6	4.68 ± 0.34^{b}	1.38 ± 0.06^{a}	1.71 ± 0.32^{a}	1.45 ± 0.16^{a}	1.28 ± 0.03^{a}	1.72 ± 0.28^{a}		< 0.001
18:3 n-3	0.38 ± 0.03	0.40 ± 0.03	0.44 ± 0.09	0.35 ± 0.03	0.37 ± 0.02	0.40 ± 0.06		0.826
18:4 n-3	0.41 ± 0.10	0.48 ± 0.05	0.53 ± 0.04	0.58 ± 0.04	0.69 ± 0.06	0.61 ± 0.07	#	0.057
20:0	0.28 ± 0.04	0.23 ± 0.02	0.23 ± 0.03	0.26 ± 0.03	0.23 ± 0.03	0.25 ± 0.02		0.854
20:1 n-9	2.77 ± 0.37^{a}	4.92 ± 0.35^{b}	4.74 ± 0.41^{b}	4.45 ± 0.45^{ab}	5.10 ± 0.49^{b}	5.08 ± 0.52^{b}		0.004
20:2 n-6	1.01 ± 0.13^{b}	0.51 ± 0.02^{a}	0.51 ± 0.03^{a}	0.45 ± 0.03^{a}	0.50 ± 0.02^{a}	0.45 ± 0.02^{a}		< 0.001
20:4 n-6	3.64 ± 0.25	3.28 ± 0.30	2.88 ± 0.30	2.50 ± 0.25	2.78 ± 0.40	2.37 ± 0.31		0.057
20:5 n-3	5.07 ± 0.46	6.33 ± 0.51	5.99 ± 0.45	6.49 ± 0.51	6.83 ± 0.56	5.48 ± 0.58		0.179
22:1	0.63 ± 0.22^{a}	1.54 ± 0.11^{b}	1.60 ± 0.13^{b}	1.56 ± 0.14^{b}	1.88 ± 0.24^{b}	1.70 ± 0.09^{b}		< 0.001
22:5 n-3	1.18 ± 0.11	0.88 ± 0.08	0.88 ± 0.06	0.96 ± 0.08	1.10 ± 0.10	0.84 ± 0.10		0.059
22:6 n-3	27.0 ± 2.59	29.0± 2.26	28.8 ± 2.38	31.0 ± 2.86	31.7 ± 2.77	26.3 ± 3.03		0.660
SAFA	27.9 ± 1.80	28.1 ± 1.63	27.0 ± 1.53	27.5± 1.99	25.0 ± 1.54	27.9 ± 2.19		0.811
MUFA	27.8 ± 2.12	29.7 ± 1.41	31.2 ± 1.80	28.6 ± 1.61	29.74± 2.27	33.9 ± 2.18		0.323
PUFA	44.2 ± 2.99	42.2 ± 2.85	41.8 ± 2.98	44.0± 3.39	45.3 ± 3.50	38.2 ± 3.99		0.717
n-3	33.7 ± 3.09	37.0 ± 2.81	36.7 ± 2.83	39.43± 3.40	40.6 ± 3.35	33.6 ± 3.70		0.541
n-6	10.57 ± 0.63^{b}	5.20 ± 0.28^{a}	5.13 ± 0.41^{a}	4.54 ± 0.20^{a}	4.67 ± 0.42^{a}	4.58 ± 0.48^{a}		< 0.001
n-3/n-6	3.36 ± 0.51^{a}	7.29 ± 0.68^{b}	7.43 ± 0.61^{b}	8.85 ± 0.87^{b}	9.16 ± 1.09^{b}	7.52 ± 0.85^{b}		< 0.001
n-3/18:3 n-3 [2]	90.37 ± 6.49	95.6 ± 7.21	95.1 ± 10.1	115.8 ± 10.9	116.5 ± 6.40	88.2 ± 7.85		0.147
n-6/18:2 n-6 [3]	1.29 ± 0.07^{a}	2.83 ± 0.29^{b}	2.33 ± 0.30^{ab}	2.48 ± 0.44^{ab}	2.64 ± 0.32^{b}	1.91 ± 0.33^{ab}		0.003

Each value represents the mean value ± S.E.M. (standard error mean) of data from 8-10 fish.
[1] *P* values result from analysis of variance. Different superscript letters in each row indicate significant differences among dietary treatments (Tukey' test, *P*< 0.05).
[2] Includes 18:4 n-3, 20:5 n-3, 22:5 n-3, 22:6 n-3.[3] Includes 20:2 n-6, 20:4 n-6.

Table 3. Hepatic total lipid (%) and fatty acid composition (% total FAME) in the triacylglycerol fraction of fish fed with the experimental diets for nine weeks.

	CONTROL	*DFM-PFO*	*DFM-FO*	*FM-PFO*	*FM-FO*	*FM-PFO+S*	*FO/FM*	*P*[1]
Lipids (%)	7.72 ± 0.88	8.50 ± 0.80	7.26 ± 0.71	8.21 ± 1.22	8.82 ± 1.27	9.55 ± 1.60		0.760
Fatty acids (%)								
14:0	2.03 ± 0.19^{a}	2.93 ± 0.09^{b}	3.35 ± 0.18^{b}	3.39 ± 0.07^{b}	3.11 ± 0.12^{b}	2.94 ± 0.17^{b}		<0.0001
14:1	0.14 ± 0.01	0.17 ± 0.01	0.19 ± 0.02	0.19 ± 0.02	0.18 ± 0.01	0.16 ± 0.01		0.147
15:0	0.17 ± 0.05	0.23 ± 0.01	0.26 ± 0.02	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.02		0.198
16:0	9.38 ± 0.67	8.92 ± 0.32	10.08 ± 0.54	10.34 ± 0.54	10.13 ± 0.49	9.91 ± 0.26		0.307
16:1 n-7	8.46 ± 0.58	8.96 ± 0.26	9.36 ± 0.42	10.13 ± 0.55	9.88 ± 0.49	9.14 ± 0.35		0.135
18:0	2.22 ± 0.21^{b}	1.70 ± 0.06^{ab}	1.69 ± 0.10^{a}	1.89 ± 0.10^{ab}	2.12 ± 0.35^{ab}	1.96 ± 0.08^{ab}		0.039
18:1 n-9	42.1 ± 1.77^{b}	32.0± 0.72^{a}	30.23 ± 0.82^{a}	31.9 ± 0.92^{a}	31.9 ± 0.86^{a}	32.1 ± 0.87^{a}		< 0.001
18:1 n-7	4.18 ± 0.12	4.03 ± 0.08	4.13 ± 0.17	4.02 ± 0.10	4.23 ± 0.12	4.21 ± 0.16		0.767
18:2 n-6	8.04 ± 0.74^{b}	2.79 ± 0.18^{a}	2.94 ± 0.35^{a}	2.88 ± 0.29^{a}	2.28 ± 0.22^{a}	2.64 ± 0.27^{a}		< 0.001
18:3 n-3	1.36 ± 0.12^{b}	1.11 ± 0.04^{ab}	1.14 ± 0.08^{ab}	1.08 ± 0.06^{ab}	0.95 ± 0.06^{a}	0.99 ± 0.08^{a}		0.013
18:4 n-3	0.57 ± 0.09^{a}	1.25 ± 0.07^{b}	1.28 ± 0.06^{b}	1.28 ± 0.05^{b}	1.66 ± 0.08^{c}	1.46 ± 0.06^{bc}	* / #	< 0.001
20:1 n-9	4.60 ± 0.15^{a}	6.20 ± 0.08^{ab}	6.45 ± 0.08^{b}	6.13 ± 0.18^{ab}	6.81 ± 0.16^{b}	6.72 ± 0.26^{b}	*	< 0.001
20:2 n-6	1.12 ± 0.15^{b}	0.56 ± 0.01^{a}	0.57 ± 0.02^{a}	0.49 ± 0.02^{a}	0.53 ± 0.02^{a}	0.52 ± 0.02^{a}	#	0.002
20:3 n-3	0.20 ± 0.02	0.24 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01		0.396
20:3 n-6	1.06 ± 0.11^{b}	0.19 ± 0.01^{a}	0.19 ± 0.01^{a}	0.17 ± 0.01^{a}	0.17 ± 0.01^{a}	0.17 ± 0.01^{a}	#	< 0.001
20:4 n-6	0.53 ± 0.01^{b}	0.44 ± 0.03^{ab}	0.47 ± 0.03^{ab}	0.42 ± 0.04^{ab}	0.41 ± 0.02^{ab}	0.40 ± 0.02^{a}		0.028
20:5 n-3	3.61 ± 0.52^{a}	5.17 ± 0.17^{b}	5.04 ± 0.37^{b}	4.65 ± 0.27^{ab}	4.20 ± 0.25^{ab}	4.34 ± 0.27^{ab}	#	0.015
22:1	2.74 ± 0.54^{a}	6.09 ± 0.22^{b}	6.17 ± 0.28^{b}	6.08 ± 0.27^{b}	6.02 ± 0.24^{b}	6.18 ± 0.30^{b}		< 0.001
22:5 n-3	1.39 ± 0.11^{a}	2.43 ± 0.07^{b}	2.19 ± 0.11^{b}	2.02 ± 0.11^{b}	2.05 ± 0.12^{b}	2.16 ± 0.10^{b}	#	< 0.0001
22:6 n-3	6.02 ± 0.68^{a}	13.94 ± 0.59^{b}	13.21 ± 0.62^{b}	11.87 ± 0.75^{b}	12.22 ± 0.61^{b}	12.83 ± 0.44^{b}	#	< 0.001
24:1	0.38 ± 0.03^{a}	0.76 ± 0.04^{b}	0.80 ± 0.04^{b}	0.71 ± 0.04^{b}	0.71 ± 0.03^{b}	0.74 ± 0.04^{b}		< 0.001
SAFA	13.7 ± 0.95	13.8 ± 0.39	15.4 ± 0.69	15.9 ± 0.58	15.6 ± 0.81	15.0 ± 0.41		0.106
MUFA	62.5 ± 1.53^{b}	58.2 ± 0.79^{ab}	57.4 ± 1.12^{a}	59.2 ± 0.99^{ab}	59.2 ± 1.12^{ab}	59.2 ± 0.81^{ab}		0.030
PUFA	23.7 ± 1.64	28.0 ± 0.75	27.3 ± 1.11	25.0 ± 1.12	24.7 ± 1.15	25.7 ± 0.70	#	0.073
n-3	13.1 ± 1.40^{a}	24.1± 0.69^{b}	23.1 ± 0.94^{b}	21.0 ± 1.01^{b}	21.32± 0.93^{b}	22.0 ± 0.51^{b}	#	< 0.001
n-6	10.64 ± 1.00^{b}	3.96 ± 0.18^{a}	4.17 ± 0.37^{a}	3.92 ± 0.32^{a}	3.37 ± 0.26^{a}	3.71 ± 0.29^{a}		< 0.001
n-3/n-6	1.34 ± 0.24^{a}	6.17 ± 0.29^{b}	5.84 ± 0.41^{b}	5.65 ± 0.41^{b}	6.50 ± 0.29^{b}	6.16 ± 0.42^{b}		< 0.001
n-3/18:3 n-3 [2]	8.80 ± 0.74^{a}	20.9 ± 0.81^{b}	19.8 ± 1.18^{b}	18.8 ± 1.02^{b}	21.8 ± 0.91^{b}	22.1± 1.48^{b}		< 0.001
n-6/18:2 n-6 [3]	0.32 ± 0.02^{a}	0.44 ± 0.03^{ab}	0.46 ± 0.04^{b}	0.39 ± 0.03^{ab}	0.50 ± 0.03^{b}	0.43 ± 0.04^{ab}		0.005

Each value represents the mean ± S.E.M. of data from 8-10 fish.
[1] *P* values result from analysis of variance. Different superscript letters in each row indicate significant differences among dietary treatments (Tukey' test, *P*< 0.05).
[2] Includes 18:4 n-3, 20:3 n-3, 20:5 n-3, 22:5 n-3, 22:6 n-3.
[3] Includes 20:2 n-6, 20:3 n-6, 20:4 n-6

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Table 4. Lipid classes (% of total identified lipid classes) in white muscle of fish fed with the experimental diets (Means ± standard deviation, n=9-10)

Lipid classes	Control	DFM+PFO	DFM+CFO	CFM+PFO	CFM+CFO	CFM+PFO+S	*P*
MAG	0.027^{ab} ± 0.009	0.027^{ab} ± 0.006	0.039^{a} ± 0.017	0.029^{ab} ± 0.004	0.020^{bc} ± 0.007	0.015^{c} ± 0.008	
TAG	0.698 ± 0.125	0.756 ± 0.028	0.707 ± 0.087	0.760 ± 0.040	0.741 ± 0.064	0.743 ± 0.069	
PL	0.156 ± 0.093	0.127 ± 0.015	0.134 ± 0.041	0.115 ± 0.022	0.154 ± 0.037	0.150 ± 0.040	
ST	0.126^{a} ± 0.029	0.088^{b} ± 0.012	0.109^{ab} ± 0.031	0.095^{ab} ± 0.020	0.090^{b} ± 0.017	0.092^{b} ± 0.029	
FFA	-	-	3.7 ± 0.022	-	-	-	

Different superscript letters indicate significant differences between the six groups ($P < 0.05$)
Abbreviation: *MAG* Monoacylglycerol, *TAG* Triacylglycerol, *PL* Phospholipid, *ST* Sterols and *FFA* free fatty acids

Table 5. Hepatic gene expression (Cq) of fish fed with the experimental diets for nine weeks.

	CONTROL	*DFM-PFO*	*DFM-FO*	*FM-PFO*	*FM-FO*	*FM-PFO+S*	*P*[1]
PPAR-α	0.75 ± 0.14	1.23 ± 0.08	1.15 ± 0.09	1.11 ± 0.09	1.06 ± 0.12	1.12 ± 0.16	*0.475*
PPAR-β	0.20 ± 0.03	0.38 ± 0.04	0.35 ± 0.03	0.26 ± 0.03	0.28 ± 0.02	0.29 ± 0.03	*0.068*
PPAR-γ	18.78 ± 1.11	19.95 ± 1.21	19.54 ± 1.11	18.90 ± 1.25	17.47 ± 1.22	19.82 ± 1.62	*0.637*
GHR-I	2.77 ± 0.39	3.31 ± 0.60	3.80 ± 0.32	3.23 ± 0.22	3.72 ± 0.42	3.89 ± 0.23	*0.208*
IGF-I	3.09 ± 0.45	3.26 ± 0.70	3.83 ± 0.32	3.82 ± 0.20	4.47 ± 0.27	3.70 ± 0.25	*0.223*
IGF-II	3.14 ± 0.32	3.11 ± 0.56	3.49 ± 0.32	3.46 ± 0.11	4.09 ± 0.11	3.40 ± 0.19	*0.102*

Each value represents the mean ± S.E.M. of data from 6 fish.
[1] *P* values result from analysis of variance. Different superscript letters in each row indicate significant differences among dietary treatments (Tukey' test, $P < 0.05$).

Table 6. Metabolites with significant variations in the white muscle of CFM+PFO and CFM+CFO groups

Metabolites	Concentration (Means ± Sdev. umol/g) CFM+PFO	CFM+CFO	*P*-value[1]	NMR signal (ppm)	VIP (ci)[2]	Content*
ADP	6.929 ± 0.370	8.300 ± 1.974	0.001		0.91 (0.42)	+
Dimethylamine	0.000 ± 0.000	0.010 ± 0.019	0.0171		-	+
Formate	4.427 ± 0.454	3.301 ± 1.069	0.0003		1.29 (0.33)	-
Glucose	1.451 ± 0.292	0.964 ± 0.267	0.0021		1.80 (0.98)	-
Glutamine	0.417 ± 0.114	0.289 ± 0.099	0.0186		1.55 (1.51)	-
Niacinamide	0.231± 0.097	0.418 ± 0.118	0.0023		2.11 (1.18)	+
Pyruvate	0.041 ± 0.069	0.253 ± 0.026	0.0011		3.56 (0.74)	+
Taurine	3.491 ± 1.214	2.213 ± 0.869	0.0057		1.88 (1.38)	-
Phosphatidylcholine				3.38	3.97 (2.33)	+
Unsaturated FA				5.40	2.78 (1.97)	+
Phospholipid				3.83	2.54 (1.50)	+
Polyunsaturated FA				2.86	2.49 (1.73)	+
Phosphatidylcholine/ethanolamine				4.00	1.05 (0.98)	+

[1]P-value from the analysis with ANOVA, Bonferroni post-hoc test (n=10)
[2] VIP (ci) from the analysis with OPLS-DA with data after log-transformation
* (+): Higher levels in CFM+CFO diet
(-): Lower levels in CFM+CFO diet

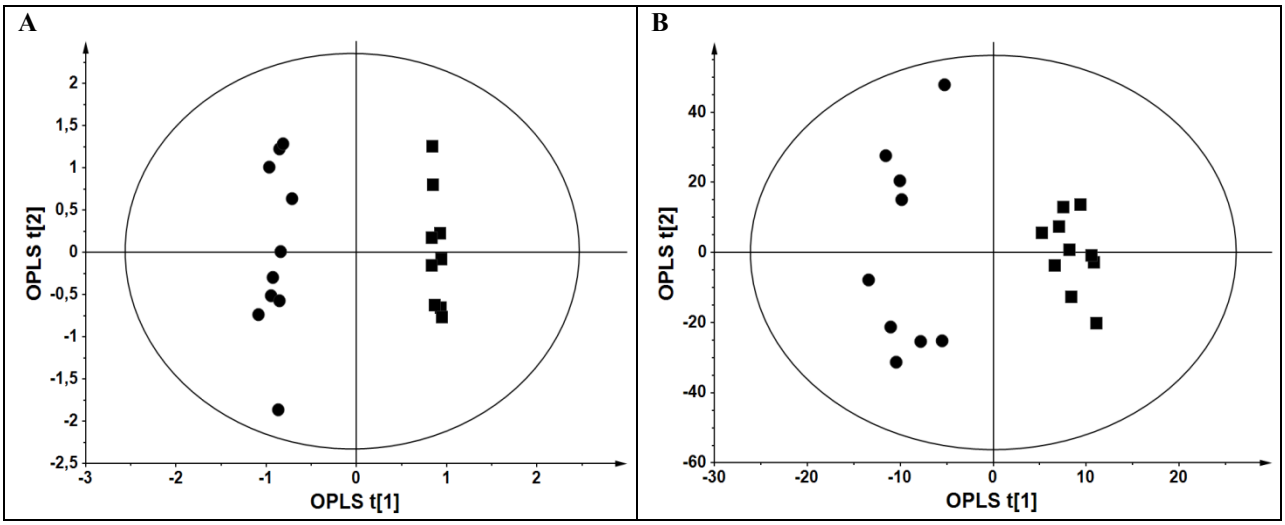

Figure 1. Orthogonal partial least squares-discriminate analysis (OPLS-DA) score plots from ^{1}H NMR spectrum profiles of white muscle extracts from the fish groups of CFM+PFO ● and CFM+CFO ■ (A) OPLS-DA score plot of aqueous white muscle extracts. The total explained X variation was 63.6%. Of this, 11.5% was predictive which was responsible for the class separation between two groups in the OPLS model; and 52.1% was structured. (B) OPLS-DA score plot of white muscle chloroform-phase extracts. The OPLS-DA model was established using one predictive (8.95%) and 5 orthogonal components (76.8%). The models were significant by checking with CV-ANOVA (P<0.05).

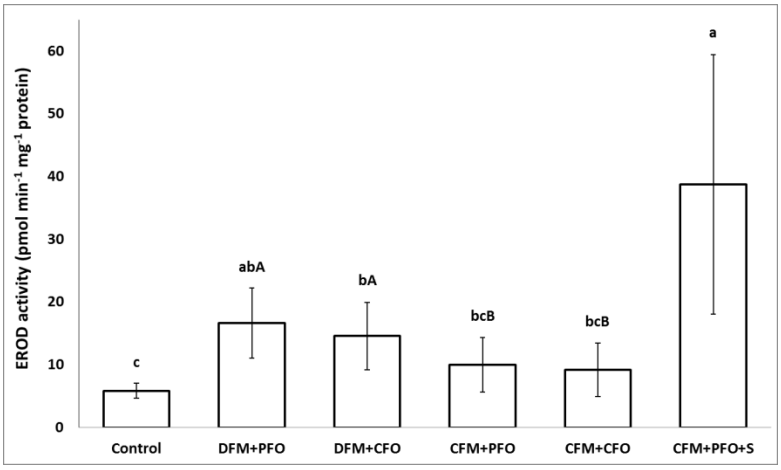

Figure 2. Values on the activity of ethoxyresorufin-*O*-deethylation (EROD) in liver samples (pmol min^{-1} mg^{-1} protein) of fish fed with the 6 experimental diets.
Different small letters indicate significant differences between the 6 groups (P < 0.05, n=6)
Different capital letters indicate significant differences between the 4 groups: DFM+PFO, DFM+CFO, CFM+CFO, and CFM+PFO (P < 0.05, n=6).