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Local fish feed ingredients for competitive and sustainable production of high-quality aquaculture feed

LIFF

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1. Executive summary

Highlights:

- ***Mussel meal*** **produced from undersized mussels, grown specifically to reduce the overload of nutrients in certain areas or grown in co-culture in areas of finfish farming can be efficiently used to replace fishmeal in fish feed.**
- ***Seaweed*****, an abundant resource in the Nordic countries, can be used in fish feed in significant amounts if care is taken of high content of arsenic content and if the price of seaweed products is compatible.**
- ***Microalgae*** **as feed resource are still in early stages of development and can, in near future, be regarded as very interesting sources of protein and lipid in aquaculture feeds.**
- **The new ingredients tested in this project are innovative alternatives for future development of sustainable, environmental friendly and economic development of aquaculture in the Nordic countries.**

While wild fisheries have stagnated, aquaculture has experienced 7-9% growth per annum for the last two decades for meeting the increased demand for fish for the growing population in the world. This growth has been supported by increased use of plant raw materials, but a broader spectrum of raw materials will be needed for the future growth of aquaculture.

There is a strong political and societal interest in the Nordic countries and Europe to expand aquaculture production. The major drivers for this policy are to be able to provide consumers with high-quality, locally produced fish products to create rural jobs in times of declining fisheries. Also to decrease the dependency on import of fish or feed raw material from Asia, Africa and America.

Nordic finfish aquaculture is currently firmly focused on species such as Atlantic salmon, rainbow trout, Arctic char, and to a lesser degree also Atlantic cod and Atlantic halibut. Also, warm water species such as Tilapia have drawn certain attention where tempered water is available. The preferred feed for these species has been based on industrial fishmeal and oil as major ingredients. In the search of alternative feed sources it is important to identify local feed sources in order to minimize transportation and thereby limit the carbon footprint of feed production. The new raw materials should be sustainable, improve ecosystem cycling and optimal for fish welfare, growth and product quality.

The main objective of the project was to test new local raw materials for aquaculture feed and to implement those into the production chain, thereby:

- moving the Nordic aquaculture industry towards a more competitive and sustainable production, focusing on efficient and responsible use of local feed sources,
- identifying novel fish feed ingredients optimizing the use of marine raw materials,
- creating added value through the use of new marine raw materials such as mussel meal, seaweed and microalgae,
- decreasing the dependency on fishmeal and fish oil as fish feed ingredients,
- lowering carbon footprint of aquaculture production, and
- establishing a user driven diversified "green growth" in aquaculture production of high quality fish products.

The raw materials tested in the present study were mussel meal, seaweed and microalgae.

Mussel meal was selected based on the following:

- Undersized mussels are not used for human consumption.
- Mussels are grown specifically to reduce the overload of nitrogen and phosphorous in certain areas such as the Baltic region.
- Mussels are grown in co-culture in areas of finfish farming in order to close the nutrient loop in the production area.

Using these sources for production of mussel meal as ingredient for fish feed utilizes by-products from existing mussel industries and mussels otherwise not suitable for human consumption, thus ensuring sustainability. The mussel meal used in this study was produced from fished mussels from southern Danish waters (as this was the only raw material available). The mussels were steamed open and only the meat was used for the production. The chemical analyses of the mussel meal revealed the following:

- The essential amino acid requirements of fish are expected to be covered with use of blue mussel meal. The blue mussel meal contains high levels of some free amino acids with possible positive attractant properties.
- The gonads are high in glycogen content and the level of glycogen therefore varies with season.
- DHA and EPA counted for 16 and 17% of the fatty acids. This makes the blue mussel a very good source for these important fatty acids.
- All of the heavy metals were present, but the concentration was lower than the upper limit for feed materials. However, it will be important for the use of blue mussel meal in fish feed that the levels of heavy metals are monitored, as the heavy metal levels in the environment will be reflected in the mussel.

The mussel meal was replacing fishmeal in diets for Rainbow trout. Overall, the fish fed mussel meal based diet performed really well with regard to digestibility, growth, feed conversion and nutrient retention. *Growth performance* was slightly lower compared to fish fed fishmeal based diets, but only when the fish were fed in a restrictive manner, i.e. when they were forced to utilise the protein as efficiently as possible. This effect was observed whether the inclusion level of fishmeal or mussel meal was 50% or 15%. These small performance differences for restrictively fed fish was possibly due to a slightly lower methionine level in mussel meal, resulting in marginally lower fat digestibility and lower protein retention. However, when the fish were fed *ad libitum*, the mussel meal diet resulted in an almost identical performance and the small negative effect of methionine limitation was eliminated by a higher feed/protein intake. In a commercial fish farm, the feeding strategy would not normally be as restrictive as the one used in the present study, i.e. under normal or practical circumstances mussel meal could fully replace fishmeal, at least from a nutritional point of view. *Feed attractant properties* of mussel meal appeared similar as for fish meal, but since rainbow trout are generally not picky, it cannot be out-ruled that mussel meal may have a more positive effect in other species.

Overall assessment is that the rainbow trout thrived well on all three diets, showing active endocrine growth stimulation and rapid growth, not only when fed ad lib, but even on a restricted ration. In those terms, mussel meal, either as the sole protein source (MM diet) or as a partial protein source (FMM diet) appears to be a good replacement for fish meal.

Interestingly, mussel meal had a distinct effect on *filet colour*, which may or may not be a problem, depending entirely on what the consumers may think or like. It could possibly be promotional for specific products (i.e. organic, environmentally friendly etc.) if presented the right way, but if presented with more conventional product it could also be a drawback whereas the consumers of portion size trout expect it to have more or less white flesh. The outcome of a small *taste test* was very positive. Everyone in the "taste panel" preferred the mussel meal fed fish, both due to taste but also due to a firmer texture. This quite distinct effect of mussel meal therefore deserves further investigation.

Seaweed is a widely available but underutilised Nordic bio resource, a heterogeneous group with different nutrient composition. Seaweed has been used for human consumption and is known as a healthy food supplement providing the necessary amino acids, beneficial polysaccharides, fatty acids, antioxidants, vitamins and minerals.

Effluents from land as well as production of fish in sea cages results in discharges of nutritional salts and organic materials into the environment. Production of macro algae (and mussels) results in the intake and elimination of these nutritional salts and organic material from the aquatic environment. This provides the potential for a life cycle which is beneficial from a sustainability perceptive, where algae (and mussels) and fish for consumption are farmed in what is known as a multi-trophic aquaculture. Limited information is available on the use of seaweed as ingredients in fish feed. The aim of the present study was to examine the effect of two different types of seaweed products available in the open market; a seaweed powder 1 of brown algae (LAM) and a seaweed powder 2 mixture of several European brown species (OHT).

Chemical analyses reviled the following:

- Both powder types contained 8-10% protein and <1.5% lipid. The main part of the seaweed powders was ash (minerals) together with an unanalysed rest that probably consisted of different polysaccharides.
- Both powder types contained levels of total and inorganic arsenic above upper limit for feed materials. The powders can therefore not be used in fish feed. Removal of arsenic compounds can be accomplished by precipitation, adsorption or cementation in process solutions.

Inclusion of the seaweed products did not have significant effects on neither *growth nor feed utilization* in Tilapia or Arctic char. The *mineral and vitamin content* of the seaweed powders are not competitive with the mineral and vitamin premix commonly used, with the exception of a slight effect of using the OHT product at the current market prices of ingredients used in the feed formulation. The lipid content in the experimental diets is a bit lower in LAM15 (10.2%) than LAM5 (10.6%) and likewise in OHT15 (10.5%) and OHT5 (10.8%).

It may be concluded that even though seaweed is found in abundance in the Nordic countries, it has low nutrient densities. Its use in diets for Tilapia and Arctic char will therefore fully depend on its marked price and the present indicative prices do not make the use of these types of seaweed profitable.

There has been an increasing interest in the use of **microalgae** as ingredient in aquaculture feed during the last years. Microalgae could serve as protein and in particular a lipid source for fish, in addition to the presence of possible bioactive compounds in the algae biomass. Some microalgae are also capable of *de novo* synthesis of the essential fatty acids EPA and DHA. Over the years, a number of studies have aimed at optimising microalgae production, mainly with the aim of using the oil fraction of the algae for production of biofuel. The defatted biomass from the microalgae used for this purpose could then serve as a protein raw material in animal feed. The nutritional value of the defatted biomass is similar to fishmeal regarding the content of essential amino acids. It is also rich in vitamins and minerals, along with possible unique bioactive compounds. Whole microalgae could also be of interest as they are natural sources of the essential fatty acids EPA and DHA. Recent studies show that such a defatted biomass from microalgae can replace some of the corn and soybean used in diets for pigs, broilers and laying hens.

The intension of the project was test different types of **microalgae** in diets for Tilapia. A considerable effort was put into finding commercial microalgae products, but it appears that there is very limited availability of microalgae in the quantities necessary for testing in fish feed. Several companies stating that they are developing microalgae for fish feed, were contacted but none of them had any product ready for testing in trials with fish. The types of microalgae available in the free market cost approximately $ 40 per kg and are therefore far from being feasible to use as a significant raw material in formulation of fish diets. This search revealed the fact that the work on developing the algae into compatible raw material into practical diets for fish still has a long way to go. Most of the development is still at lab scale stage and only a handful of products have appeared in the market.

Hence, the products are still priced in such a way that they are far too expensive to compete with other sources of nutrients in fish diets. However, five species of microalgae produced by two Icelandic laboratories were analysed and test formulated into diets based on that analyses in order to establish the quantities required for fulfilling the nutritional needs of the fish.

2. Results from the project

2.1 Mussel meal

In recent years, feed costs have been increasing. Moreover, oil prices are increasing transport costs and causing environmental concerns.

Mussel meal may be an unique local alternative ingredient in fish feed due to its nutritional characteristics that are similar to those of fish meal, with an adequate amino acid profile and additionally, a source of astaxanthin. Furthermore, mussel meal obtained from "environmental friendly mussel production" is a highly innovative and novel step towards sustainable and environmentally friendly finfish aquaculture. The mussels remove nitrogen and phosphate from the water by filtering nutrient particles and microscopic organisms, converting non-food into food. The aquaculture envisage future nitrogen-neutral fish production by adjacent farming of mussels absorbing the nitrogen discharged from fish metabolism and other sources in the ocean. The mussels, mainly undersized mussels not used for human consumption and mussels grown specifically to reduce the overload of nitrogen and phosphorous in certain areas such as the Baltic region and as co-culture in areas of finfish farming, should be used for production of mussel meal as ingredient for fish feed, thereby closing the nutrient loop. By using mussel meal in fish feed, nitrogen and phosphate is eco-cycled while the mussel shells may be used for poultry feed, thereby contributing to lowering the carbon footprint of the production.

2.1.1 Processing of Blue mussel meal (SWEDEN)

Odd Lindahl, The Royal Swedish Academy of Sciences, Sweden

The mussel meal used was produced in June 2013 at the pilot plant for production of mussel meal, situated in Ellös on the Swedish west coast about 100 km north of Gothenburg. This pilot plant was operated by the project "Pilot Plant for the Production of Mussel Meal" having the Swedish Rural Economy and Agricultural Societies as project owner. The project was financed by The Swedish Agriculture and Environmental Agencies, Rural Developmental programs in Västra Götaland, Öster Götland and Kalmar and finally also by the Västra Götaland Region. Running time of the mussel meal production project was from 2010 to 2014.

The mussel meal was produced using fished mussels from southern Danish waters as raw material. The mussels were steamed open and the meat and shells were separated at the Royal Frysk GmbH factory close to the island of Sylt in NW Germany. The mussel meat were sorted into A-grade quality for human consumption (seafood market) and B-grade quality which were frozen in 5 kg bags to be used as fish food in aquariums or other similar uses. B-grade frozen mussel meat was used for the production of mussel meal.

For drying a wood chips heated rotating drum-dryer was used designed by AB Torkapparater in Stockholm. The capacity of the small pilot dryer was about 300 - 400 kg of mussel meat per 24 hours, which resulted in 60 - 80 kg dried mussel meat (ca 20% of the steamed wet weight). As a mean ca 5 % of the weight of the fresh mussels can be dried to mussel meat, but this may vary with the meat content of the live mussel (Lindahl et al., 2005). The corresponding original amount of fresh mussels used for the production for the LIFF project could thus be estimated to about 4000 kg.

During the drying process, the temperature was slowly raised so that the mussel meat at the end reached a temperature of at least 80 - 85 °C for 30 minutes or more. The mussel tissue had then turned into 10 - 20 mm rather hard particles. After cooling, this material was grinded using a small grain mill and then poured into 25 kg sacks. Before closing the sacks, samples were taken for testing on the occurrence of Salmonella. Finally, the mussel meal was sent by truck to Skretting ARC in Stavanger, Norway, together with a certificate that no Salmonella had been detected.

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Lindahl O., Hart R., Hernroth B., Kollberg S., Loo L.-O., Olrog L., Rehnstam-Holm A.-S., Svensson J,. Svensson S. and Syversen U. 2005. Improving marine water quality by mussel farming – A profitable measure for Swedish society. Ambio, Vol. 34, No. 2: 131-138.

Link about the project (in Swedish):
http://www.youtube.com/watch?v=Gm15NvS_maA&feature=youtu.be

2.1.2 Nutritional content in blue mussel meal (NORWAY)

Ann-Cecilie Hansen, NIFES -The National Institute of Nutrition, Norway

Summary

The essential amino acid requirements of fish are expected to be covered with use of blue mussel meal. The blue mussel meal contains high levels of some free amino acids with possible positive attractant properties.

- The level of glycogen varies with season, as the gonads have high level of glycogen.
- DHA and EPA counted for 16 and 17% of the fatty acids. This makes the blue mussel a very good source for these important fatty acids.
- All heavy metals were present, but were under the upper limit for feed materials. However, it will be important for the use of blue mussel meal in fish feed that the levels of heavy metals are monitored, as the heavy metal levels in the environment will be reflected in the mussel.

The material

The mussel meal in this study was processed from mussels (*Mytilus edulis*) coming from the south- western part of the Baltic. The mussels were fished during the winter/spring 2012. In the Royal Frysk Muscheln GmbH mussel processing plant, the mussels were steamed, meat and shells were separated, the meat was sorted and the second grade was frozen and filled into 5 kg plastic bags, which later was used to produce the mussel meal.

The mussel meal was produced by the Pilot Project for Processing Mussel Meal, situated in Ellös on the Swedish west coast. When processed the mussel meat was dried in a rotating dryer at around 85 °C until dry. After cooling and grinding, the meal was put into 25 kg sacks.

Analytical methods

The meal was analyzed for proximal composition; dry matter was determined gravimetrically after drying at 104 C for 24h, total nitrogen with a nitrogen element analyzer (LECO FP-528; LECO Corporation, St. Joseph, MI, USA) and calculated as Nx6.25, lipid gravimetrically after acid hydrolysis and extraction with di-ethyl ether and ash gravimetrically after combustion at 540 C for 16h. Starch was analyzed using an enzymatic method described by Hemre et al. (1989). Amino acids were determined after hydrolysis of the protein with 6 M hydrochloric acid, derivatised with phenylisothocyanate (PICT), and analyzed in a Waters HPLC amino acid analyzer system using L- norlaucine as the internal standard. Minerals were determined using ICP-MS after complete digestion in nitric acid after cooking in microwave oven for 1h. Sterols were analyzed by: extraction of lipids with di-ethyl ether, saponification of fatty acids, extraction of sterols and separated by GLC and detected by flame ionization. Vitamin K was analyzed by a newly developed method method using HPLC-, vitamin C was analyzed by HPLC after acid extraction and vitamin B12 was analyzed microbiologically using Lactobacillus delrucecki spp. lactis (ATCC4797) (Mæland et al., 2000). Astaxanthin was analysed by HPLC after chloroform/ methanol extraction.

Results and discussion

The blue mussel meal had a relatively high level of protein, although the level was slightly lower than herring fish meal (66 vs. 72%) (Table 1). The amino acid composition was approximately the same as fish meal, the exceptions being methionine, lysine and isoleucine being a slightly lower in mussel meal than in fish meal (Table 2). However the essential amino acid requirements of fish are expected to be covered with use of blue mussel meal. Mussels are known to have high level of some free amino acids which they use in osmoregulation (Duinker et al., 2001). Especially the level of taurine and glycine is much higher in the blue mussel meal than in fish meal. These are free amino acids believed to have positive attractant properties for fish.

The glycogen level of the blue mussel meal was 10 % (Table 1). The level of glycogen vary grately through the season, as the gonads have high level of glycogen (Figure 1) (Hovgaard et al., 2001). The difference in protein level in the blue mussel meal reflects the relatively high glycogen level compared to fish meal, and the protein level in the blue mussel meal will co-vary with the glycogen level, and thereby by season.

The fat level was 8.8% of the dry meal, the same as for fish meal (Table 1). The cholesterol level in the blue mussel meal was 2157 mg/kg compared to 7660mg/kg in pacific herring fish (Table 3) (NRC, 2011). Several unknown peaks were detected (Figure 2), and are probably sterols typical for mussels, one of them being trans-22-dehydrocholesterol (Murphy et al., 2002), giving a total sterol level of 7008mg/kg (8% of total lipid), however if fish can utilize these sterols is unknown (Nina Liland, pers. com.). DHA and EPA counted for 16 and 17% of the fatty acids (Table 4). In Pacific herring oil the DHA and EPA level is 4.8 and 8.1% respectively. This makes the blue mussel a very good source for these important fatty acids.

The level of the vitamin C, K and B12 are shown in Table 1 together with the level of astaxanthin. The level of vitamin C was below the limit of quantification. In the literature blue mussel are known to be a good source of vitamin B12, and the analysis show that this is true with vitamin B12 content at the same level as in fish meal. Observations, of feeding mussel meal to laing hens, suggest high level of pigment even though the content of astaxanthin is low. The method used does not analyze esterified astaxanthin, which may be the main pigment in blue mussel. The total level of vitamin K was high and the analysis of the different forms of menaquinones (MK-forms) show a special pattern very different from fish meal, dominated by MK-11 (Figure 3).

The ash level was 9%, approximately the same level as for fish meal. Mineral analysis showed that most of the ash consisted of phosphor (P) and potassium (K). All the minerals measured, except magnesium (Mg) and selenium (Se) were lower in blue mussel meal than in fish meal (Table 5). The unwanted heavy metals were all present, but were under the upper limit for feed materials (EU directive 2002/32/EC). However, it will be important for the use of blue mussel meal in fish feed that the levels of heavy metals are monitored, and that they are harvested in an aria with low pollution. Mussels accumulate metals effectively and heavy metal levels in the environment will be reflected in the mussel. The level of total arsenic was high, as you will find in all sea food. Arsenic will mostly be in the not toxic organic form arsenobetaine (Sloth, 2004). However no speciation analysis was done.

Table 1. Macronutrient level (%) and selected micronutrients (mg/kg) in 92% dry blue mussel meal and herring fish meal

Nutrient	Blue mussel meal	Fish meal, herring *
Protein	66.0	72
Lipid	8.8	8.4
Ash	9.1	10.4
Glycogen	10.0	--
Dry matter	95.0	92.0
Moisture	5.0	8.0
Rest**	1.1	--
Vitamin B_{12}	0.55	0.4
Vitamin C	<0.2	
Astaxanthin	0.74	
Total vitamin K	1.1	

*NRC, 2011. **Rest =100% - (%moisture- %lipid- %protein- %glycogen- %

Table 2. Level of total and free amino acid (%) in 92% dry blue mussel meal and herring fish meal

	Total amino acids, blue mussel meal	Total amino acids, fish meal, herring *	Free amino acids, blue mussel meal	Free amino acids, fish meal **
Indispensable amino acids				
Valine	3.04	3.26	0.02	0.19
Histidine	1.28	1.53	0.04	0.18
Leucine	4.63	4.69	0.02	0.23
Threonine	3.08	2.49	0.31	0.10
Arginine	4.78	3.73	0.05	0.12
Lysine	5.19	7.30	0.31	0.19
Methionine	1.64	2.20	0.11	0.05
Isoleucine	2.83	3.64	0.01	0.10
Phenylalanine	2.52	2.68	0.01	0.11
Dispensable amino acids				
Taurine	1.32		1.47	0.70
Alanine	3.44		0.31	0.05
Proline	2.57		nd	0.05
Tyrosine	2.49		0.04	0.06
Serine	3.32		0.10	0.05
Glycine	3.90		0.62	0.10
Aspartic acid	6.84		0.18	0.02
Glutamic acid	8.84		0.31	0.18

*(NRC, 2011) ** (Duinker *et al.*, 2004)

Table 3. The level of sterols (mg/kg) in 92% dry blue mussel meal

Sterol	Blue mussel meal
Cholesterol	2157
Brassicasterol	1691
Campesterol	1041
Campestanol	38
Stigmasterol	166
Sitosterol	130
Sitostanol	60
Stigmasta-dienol	33
Stigmast-enol	2
d-7-avenasterol	2
Sum unknown	1687
Sum Phytosterols	**4851**
Sum Cholesterol	**2157**
Sum total	**7008**
% sterols of total lipid	8

Table 4. The level of fatty acids (% of fatty acids) in 92% dry mussel meal

Fatty acids	Blue mussel meal
<12:0	<0.1
14:0	4.1
15:0	0.4
16:0	15.2
17:0	0.7
18:0	2.8
Total saturated	**23.2**
16:1n-9	0.6
16:1n-7	8.1
18:1n-9	1.5
18:1n-7	2.4
20:1n-11	0.9
20:1n-9	2.4
20:1n-7	1.2
Total mono sat.	**17.2**
16:2n-4	0.5
16:4n-3	0.4
18:2n-6	0.9
18:3n-6	<0.1
20:4n-6	2.2
18:3n-3	0.6
18:4n-3	2.3
20:5n-3 EPA	17.2
20:2n-6	0.5
20:4n-3	2.2
22:5n-3	0.9
22:6n-3 DHA	16.1
24:6n-3	0.1
Sum unsat.	**43.2**
Sum EPA + DHA	**33.3**
Sum n-3	**38.5**
Sum n-6	**4.1**
n-3/n-6	**9.3**

*(NRC, 2011)

Table 5. Minerals (mg/kg) and heavy metals (mg/kg) in 92% dry blue mussel meal and in herring meal. The upper limit for heavy metals in feed ingredients and feed are also given (mg/kg)

Mineral	Blue mussel meal	Fish meal. herring*	Upper limit in feed ingredient/feed (mg/kg)**
Phosphor (P)	9550	16700	
Calcium (Ca)	3760	22000	
Sodium (Na)	1890	5900	
Potassium (K)	6210	10800	
Magnesium (Mg)	1800	1400	
Iron (Fe)	300	114	
Selenium (Se)	3.17	1.95	
Heavy metals			
Total arsenic (As)	9.75		10/4
Cadmium (Cd)	1.21		2/1
Total mercury (Hg)	0.07		0.5/0.2
Led (Pb)	1.22		10/5

*(NRC, 2011) **EU directive 2002/32/EC

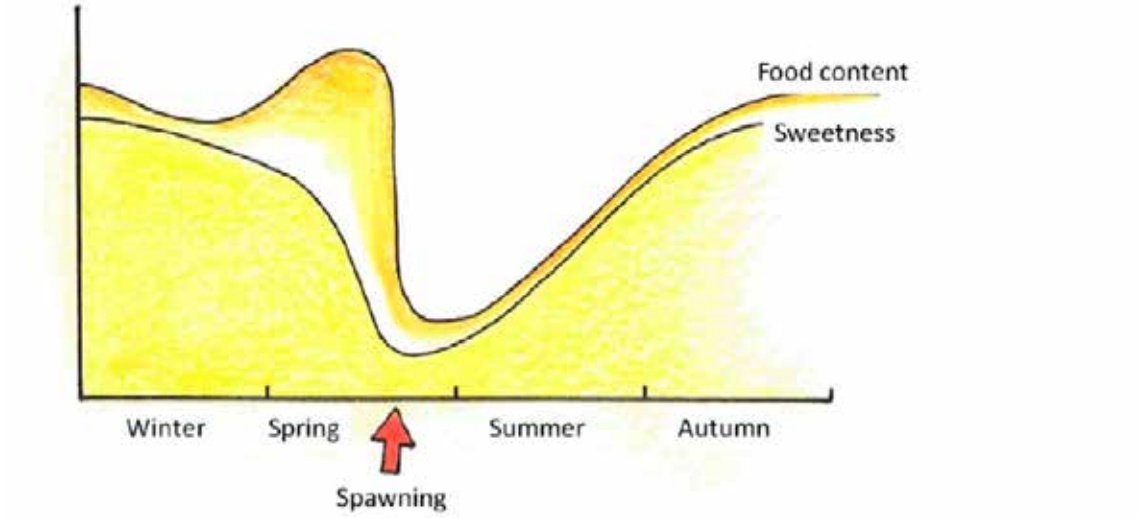

Figure 1. A principal figure of food content and sweetness (glycogen level) of mussels (modified from Hovgaard at al. (2001))

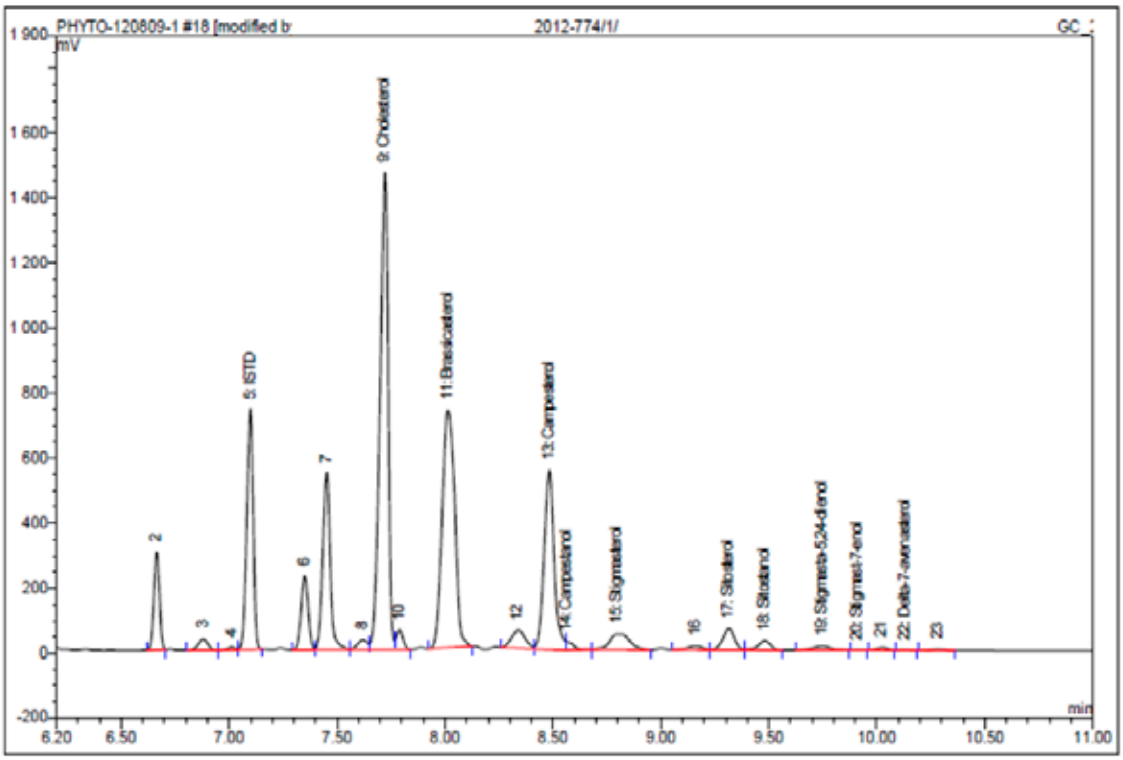

Figure 2. Chromatogram of sterols in blue mussel meal. Unknown peaks that probably are sterols are peak: 6, 7 and 12. Peak 12 is probably trans-22-dehydrocholesterol.

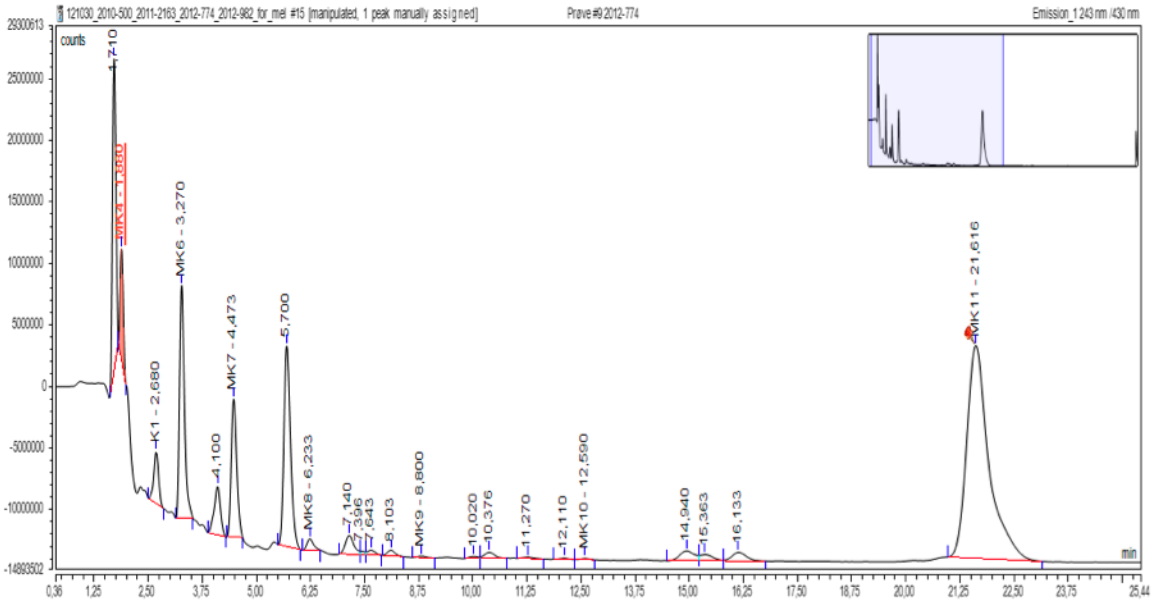

Figure 3. Chromatogram of MK forms in blue mussel meal.

Acknowledgment

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2.1.3 Substitution of fishmeal with mussel meal in rainbow trout diets (DENMARK)

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Abstract

One of the new raw materials to be tested in the project: *Local fish feed ingredients for competitive and sustainable production of high-quality aquaculture feed*, was mussel meal. This report summarises the trials performed on rainbow trout in 2012-2014 at DTU-Aqua, Hirtshals and the most important outcome of these. The purpose of the work in Hirtshals was to evaluate mussel meal from a nutritional point of view only. The mussel meal was produced by Odd Lindahl (The Royal Swedish Academy of Sciences) and the feed formulated and produced by Skretting ARC, Stavanger. Raw materials were analysed by NIFES, Bergen and DTU-Aqua, Hirtshals and all the remaining analyses for the study was performed in Hirtshals. This report includes results not previously shown, but also a comparison of the two years. The trials were designed to evaluate mussel meal as a protein source in fish diets, or specifically in rainbow trout diets. This was done by combining diets optimized for evaluating protein quality with study design (restrictive and *ad libitum* feeding). Overall, the fish fed mussel meal based diet performed really well with regard to digestibility, growth, feed conversion and nutrient retention. Growth performance were slightly lower compared to fish fed fishmeal based diets, but only when the fish were fed in a restrictive manner, i.e. when they were forced to utilise the protein as efficiently as possible. This effect was observed whether the inclusion level of fishmeal or mussel meal was 50% (2012) or 15% (2013). These small performance differences for restrictively fed fish was possibly due to a slightly lower methionine level in mussel meal, resulting in marginally lower fat digestibility and lower protein retention. However, when the fish were fed *ad libitum*, the mussel meal diet resulted in an almost identical performance and the small negative effect of methionine limitation was eliminated by a higher feed/protein intake. In a commercial fish farm, the feeding

strategy would not normally be as restrictive as the one used in the present study, i.e. under normal or practical circumstances, mussel meal could fully replace fishmeal, at least from at nutritional point of view. Feed attractant properties of mussel meal appeared not to be any better than of fish meal, but since rainbow trout are generally not picky, it cannot be out-ruled that mussel meal may have a more positive effect on other species.

Interestingly, mussel meal had a distinct effect on filet colour, which or may not be a problem, depending entirely on what the consumers may think or like. It could possibly be promotional for specific products (i.e. organic, environmentally friendly etc.) if presented the right way, but if presented with or as more conventional product it could also be a drawback.

Studies conducted in 2012 at DTU-Aqua, Hirtshals

1) Two digestibility studies
2) Ammonia and urea excretion following a single meal
3) Growth study A, fish fed to satiation
   a. Blood and tissue (gut/liver) sampled analysed by GU (physiological effect parameters).
   b. Ussing chamber studies on gut epithelia (physiological effect parameters), performed by GU.
   c. Filet colour analysed by chromameter
4) Growth study B, fish fed in a restrictive/iso-energetic manner.
   a. Fish sampled for nutrient retention
   b. Blood (plasma) and tissue sampled to be analysed by GU (physiological effect parameters).

Studies conducted in 2013/14 at DTU-Aqua, Hirtshals

Growth study C, including:

Study C-1; restrictive feeding (1.1%)

i. Fish sampled for nutrient retention, beginning and end of trial
ii. Faeces stripped at the end of experiment for analysis of nutrient digestibility
iii. Blood (plasma) sampled to be analysed by GU (physiological effect parameters).

Study C-2;* ad libitum *feeding

iv. Ussing chamber studies on gut epithelia (physiological effect parameters), performed by GU.
v. Blood and tissue (gut/liver) sampled analysed by GU (physiological effect parameters).
vi. Fish sampled for nutrient retention, beginning and end of trial
vii. Faeces stripped at the end of experiment for analysis of nutrient and energy digestibility

Diets

Feed formulation

The diets was formulated and produced by Skretting ARC, Stavanger and raw materials analysed by NIFES, Bergen and DTU - Aqua, Hirtshals. Year 1 diets are named FM-1, FM:MM-1 and MM-1 whereas year 2 diets are named FM-2, and MM-2. Yttrium oxide was added to all diets. Formulation is shown in Table 6.

Year 1 diets were formulated to include a large proportion of either fishmeal or mussel meal or a mixture of both and to the largest extent possible avoid other protein sources. This was done in order to minimize the influence of other protein sources thereby increasing the chance for linking possible effects to mussel meal inclusion. It should be noted however that both whole wheat (9-17%) and soya protein (13-20%) are included in the formulation (Table 6.). Three diets were produced with either 50% fishmeal (FM -1), 50% mussel meal (MM-1) or 25% of each (FM-MM-1). As the protein content of fishmeal (72%) and mussel meal (66%) differed slightly, fishmeal contributed with 83% or 41% to total protein in the FM-1 and FM:MM-1 diet respectively, whereas mussel meal contributed with 79% and 38% in the MM-1 and FM:MM-1 diet. Soy protein concentrate was added (12-14%) as the only other significant protein source. The added oil consisted of almost equal amounts of fish oil and rapeseed oil (10-12% of each). The diets were formulated to have a relatively low digestible protein to digestible energy ratio (DP/DE), in order to maximize protein utilisation.

Table 6. Diet formulation for the experimental diets including formulated values for crude protein, crude fat and digestible energy

Raw material	Unit	FM-1 2012	FM:MM-1 2012	MM-1 2012	FM-2 2013	MM-2 2013
Mussel meal	%	0.0	25.1 (38%)	50.2 (79%)	0	16 (24.5%)
Fish meal, Nordic	%	50.0 (83%)	25.0 (41%)	0.0	15 (26%)	0
Wheat, whole	%	17.2	13.1	9.1	14	13
Wheat gluten	%	-	-	-	13	15
Fishoil	%	10.0	11.0	11.9	19	20
Rapeseed oil	%	10.0	11.0	11.9	5	5
Premixes	%	0.3	1.3	2.4	3	3
Soya protein concentrate	%	12.5	13.3	14.1	20	17
Soybean meal extracted	%	-	-	-	10	10
Water change	%	0.0	0.2	0.4	1	1
Sum	%	100	100	100	100	100
Formulated values						
Crude protein	%	43.1	43.1	43.1	40	40
Crude fat	%	27.4	28.1	28.8	28	28
DE	MJ/kg	20.3	20.3	20.3		

2012 results revealed that the protein quality of mussel meal was similar to fishmeal and could replace fishmeal. Year 2 reference diet (FM-2) diets was formulated to resemble a commercial trout diet with a low inclusion level of fishmeal, whereas in the mussel meal diet, fishmeal was fully substituted with mussel meal. Two diets were produced containing either 15% fishmeal (FM-2) or 16% mussel meal (MM-2). The protein content of the year 2 mussel meal was slightly lower (63.7%), and mussel meal contributed with 24.5% of total protein in the MM-2 diet, whereas the fishmeal contributed with 26% of total protein in the FM-2 diet. The remaining protein sources in the diets were soya protein concentrate, soybean meal extracted and wheat gluten. The added oil was fish oil (~20%) and rapeseed oil (5%).

Feed analyses

Gross energy was measured by bomb calorimeter (IKA C7000). Furthermore, the diets were analysed for dry matter and ash (NMKL, 1991), crude protein (ISO, 2005), (protein=6.25×Kjeldahl nitrogen), and crude fat (Bligh and Dyer, 1959 (modified to fish feed). Nitrogen free extract (NFE) was calculated as: dry matter% - protein% - fat%-ash%. The results are shown in Table 7 and shows that the measured values for crude protein and fat are in accordance with the formulated.

Table 8. Amino acid composition given as g/100g feed, all 5 diets are shown. 2012 diets were analysed by NIFES and 2013 by DTU-Aqua

Amino acid content g/100 g feed	FM-1 2012	FM:MM-1 2012	MM-1 2012	FM-2 2013	MM-2 2013
Essentiel					
ALA	2.45	2.22	2.00	1.76	1.63
ARG	2.64	2.66	2.76	2.39	2.42
HIS	0.84	0.86	0.83	0.90	0.91
ILE	1.63	1.66	1.70	1.58	1.52
LEU	3.09	2.94	2.83	3.02	2.95
LYS	3.22	3.01	2.98	2.64	2.72
MET	1.00	0.95	0.86	0.90	0.86
PHE	1.48	1.55	1.64	1.86	1.91
THR	1.70	1.75	1.79	1.42	1.43
VAL	1.97	1.91	1.82	1.71	1.60
Non essentiel					
ASP	3.99	4.04	4.18	3.36	3.27
GLU	6.43	6.12	6.00	9.66	10.05
GLY	2.25	2.29	2.22	1.72	1.69
HYP	0.27	0.26	0.22	0.16	0.14
PRO	1.88	1.81	1.71	2.71	2.89
SER	2.06	2.00	2.07	1.94	2.02
TAU	0.31	0.42	0.52	0.10	0.19
TYR	1.23	1.35	1.40	1.41	1.49

Statistical analysis
2012: One-way ANOVA was used for the statistical comparison, followed by post hoc analysis (Holm-Sidak) for the multiple comparisons, i.e. diet was the only factor

2013: For the 2013 data, a two-way ANOVA was chosen, i.e. diet and feeding ration were the two factors. The statistical analysis results shown are the p-values indicating whether diet and/or feeding ration resulted in significant differences. Also shown is the p-value for interaction, i.e. whether e.g. the effect of diet depended on diet ration. This means that the p-values for e.g. diet includes both restricted and *ad libitum* fed fish. However, a significant p-value for e.g. diet does not necessarily mean that significant effects were observed for both restrictively and *ad libitum* fed fish if analysed separately. Post-hoc test could be performed in order to elucidate these differences, but in the present report it was chosen to focus on the overall effects of diet and feeding ration.

Digestibility studies
In 2012, two digestibility studies were performed; one starting at the same time as the growth study and one starting at the end, using fish from the growth study, i.e. fish that had been fed the diets for 9 weeks. As described in the Year 1 result report, the studies were performed in a modified Guelph digestibility system, which allows for collection of all uneaten pellets and collection of all produced faeces. The apparent digestibility coefficient (ADC) of macronutrients was subsequently calculated using the direct method (Jobling, 1994, 2001), using the equation: ADCi = (Ci−Fi)/Ci; where i = protein, lipid, NFE or DM, C = consumed amount of i, and F = faecal loss of i. The results from the first two digestibility studies are shown in Table 9 and Table 10.

In 2013/14, nutrient digestibility was assessed from stripped faeces, using the indirect or indicator method. This part of the study took place after ending of the growth study, i.e. in immediate continuation of the final weighing and sampling fish for gross composition. The fish were fed in the evening the day before the day of stripping and over a time period of approximately 4 hours. Furthermore, feeding was not started at the same time-point for all tanks, but in a staggered manner, securing a comparable time period from the end of feeding to the start of stripping for all tanks. All fish in each tank were stripped and the faeces pooled into one sample per tank. Faeces from restrictively and *ad libitum* fed fish were analysed and evaluated separately. The stripped faeces was frozen at -80°C and freeze dried for later analyses of nutrients, energy and Yttrium. Apparent digestibility coefficients were calculated from nutrient and yttrium content in feed and faeces respectively, using the equation:

$$\text{ADC (N)} = 100 - 100 \left(\frac{\%Y \text{ in } feed}{\%\, Y \text{ in } faeces} \times \frac{\%\, N \text{ in } faeces}{\%\, N \text{ in } feed} \right)$$

where Y is yttrium and N is nutrients or energy.

Digestibility in the 2012-studies

Table 9. Apparent digestibility coefficients; ADC 2012 trials. (Mean ± SD, N=6). Significant differences are indicated by different letters (One-way ANOVA). As energy content was not measured on faeces, the digestible energy in DP/DE was estimated from digestible protein, fat and NFE values and using energy content values of 23.7 MJ/kg, 39.6 MJ/kg and 17.2 MJ/kg for protein, fat and NFE respectively.

ADC (%) 2012	FM-1 (2012) Study 1	FM:MM-1 (2012) Study 1	MM-1 (2012) Study 1	FM-1 (2012) Study 2	FM:MM-1 (2012) Study 2	MM-1 (2012) Study 2
Nitrogen	93.2±0.50	94.0±0.83	94.0 ± 0.69	92.3±0.45^{a}	91.9±0.17^{a}	93.0±0.55^{b}
Fat	94.9±0.33^{a}	93.8±0.86^{b}	92.4 ± 0.38^{c}	92.9±0.62	92.9±0.46	92.6±0.58
NFE	64.0±2.02	66.0±3.17	65.4 ± 2.61	61.3±3.89	60.1±1.49	62.3±3.04
Dry matter	86.0±0.81	87.0±1.34	86.8 ± 0.80	84.9±1.06	84.5±0.17	85.8±0.8
Phosphorus	63.3±0.95^{a}	73.5±1.53^{b}	82.1 ± 0.50^{c}	64.9±1.44^{a}	70.4±1.08^{b}	79.0±0.84^{c}
Ash	53.5±2.25^{a}	59.0±3.78^{b}	62.3 ± 2.57^{b}	55.0±1.72^{a}	54.4±1.42^{a}	59.1±2.14^{b}
DP/DE	18.70	18.53	18.43	18.80	18.49	18.37

Digestibility in the 2013/2014-studies

Table 10. Apparent digestibility coefficients; ADC 2013 trial (Mean ± SD, N=3). Results from the Two-way ANOVA are shown on the right columns. *Energy digestibility was calculated from measured energy content of feed and faeces.DP/DE values were calculated for each diet/ration group from measured protein and energy digestibility values.**

ADC (%) 2013	FM-2 (2013) Restrictive	MM-2 (2013) Restrictive	FM-2 (2013) Ad libitum	MM-2 (2013) Ad libitum	P Diet Two way Anova	P Ration Two way Anova	P Interact. Two way Anova
Nitrogen	92.8±0.5	93.5±0.4	92.3±0.8	93.1±0.3	0.029	0.168	0.860
Fat	97.1±0.2	95.6±0.3	96.2±0.8	95.0±0.5	0.002	0.031	0.586
NFE	41.4±2.0	43.8±0.8	39.2±2.2	40.5±2.7	0.162	0.051	0.629
Dry matter	80.9±0.9	81.6±0.7	79.7±0.7	80.5±0.7	0.196	0.062	0.883
Phosphorus	63.3±0.3	72.8±1.2	60.6±1.2	70.7±1.0	<0.001	0.003	0.649
Ash	79.1±1.5	81.8±1.8	77.4±1.3	81.4±0.3	0.003	0.219	0.434
Energy*	89.3±0.2	89.6±0.4	88.8±0.8	88.9±0.3	0.535	0.085	0.653
DP/DE**	19.11	19.01	19.10	19.08			

ADC – Nitrogen

The apparent digestibility of protein/nitrogen showed a slight increase with mussel meal inclusion, cf. Table 10. This effect was not significant at the first trial in 2012, but the second trial performed at the end of the growth study showed significant effect of mussel meal inclusion at 100% substitution. A small but significant positive effect of mussel meal inclusion was also observed in 2013/14 on faeces stripped at the end of the growth trial, i.e. fish which had been acclimatized to the feed for several weeks. *Ad libitum* feeding had no significant effect on nitrogen digestibility.

ADC – Fat.

In the first trial in 2012, the apparent fat digestibility decreased significantly and a "dose dependent" manner with increased mussel meal inclusion, but at the second trial this effect was no longer observed with almost identical ADC values, cf. Table 9. In 2013, the inclusion of mussel meal caused a significant decrease in fat digestibility, although the absolute difference between the FM-2 and MM-2 diet was not as pronounced as between the FM-1 and MM-1 diet, cf. Table 10. *Ad libitum* feeding resulted in a significantly lower fat digestibility, although the differences were small.

ADC – NFE (nitrogen free extract) and dry matter (DM)

Mussel meal inclusion had no significant effect on NFE or dry matter digestibility in neither 2012 nor 2013, cf. Tables 9 and 10. However, the overall NFE digestibility decreased from about 60-65% in the 2012 study to approximately 40% in the 2013 study. Correspondingly, DM decreased from about 85-87% in 2012 to approximately 80% in 2013, likely due to the increased inclusion of vegetable ingredients. *Ad libitum* feeding led to a slightly lower digestibility of both NFE and DM, but no significant effect was observed, cf. Table 10.

ADC – phosphorus and ash

Inclusion of mussel meal led to a significant increase in phosphorus and ash digestibility both years and furthermore, showed a clear dose dependent response in 2012, cf. Tables 9 and 10. This clear effect in both parameters was probably due to phosphorus being added to the mussel meal diets in order to optimize for phosphorus and to obtain equal levels. As phosphorus was added as free phosphorus, the digestibility was expected to be higher. *Ad libitum* feeding led to a significantly lower digestibility for phosphorus, but not ash.

ADC – energy (only 2013)

Energy digestibility was measured on stripped faeces only and calculated from the measured energy content of feed and freeze dried faeces. No effect of diet was observed, and although *ad libitum* feeding caused a slight decrease in digestibility, it was not significant (Table 10).

Digestible protein/digestible Energy (DP/DE)
DP/DE values were calculated for both years, but in 2012 DE had to be estimated from the amount of digestible nitrogen, fat and NFE and their corresponding energy values. However, the used energy values for protein, fat and NFE are general or average values and do not necessarily truly represent the energy value of faeces components in this particular study. In 2013 ADC energy was measured directly from energy content of faeces and feed and consequently the calculated DP/DE is more correct. Either way, the calculated DP/DE values for the two years, correspond well, and are in accordance of the values that was aimed for beforehand (Tables 9 and 10).

Overall, the digestibility results from the two studies in 2012 and the study in 2013 corresponded well and show only minor effects of mussel meal inclusion. The lower methionine content in the mussel meal may possibly have been the caused by lower lipid digestibility in the two mussel meal diets. Methionine supplementation even at very low level has been found to increase lipid digestibility (Nordrum et al., 2000; Espe et al., 2011). This was linked to methionine induced taurine production, increasing the level of taurine-conjugated bile salts, which constitute most of the total bile salts in rainbow trout and play a central role in the digestion and uptake of lipids (Romarheim et al., 2008; Espe et al., 2011).

It should be noted that the indirect method may slightly overestimate digestibility (especially for fat), whereas the direct method might slightly underestimate digestibility. Furthermore, fish size affect digestibility, with larger fish resulting in lower ADC values. This could contribute to differences between the 2012 and 2013 studies, but also to differences between restrictive and *ad libitum* fish in the 2013 trial, where the final average fish size, at the time of stripping, differed (Table 11).

Growth trials
In 2012, two parallel growth trials (A & B) were performed in two different tank systems, with *ad libitum* and restrictive feeding respectively. In 2013, only one growth trial (C) was performed, but both restrictive (C-1) and *ad libitum* (C-2) feeding was included. Feeding fish in a relatively restrictive manner made it easier to evaluate differences in protein quality as restrictive feeding in combination with a relatively low DP/DE value "forced" the fish to utilise the protein efficiently. Using *ad libitum* feeding on the other hand allows for an evaluation of the maximal growth potential of the diets. Growth trial A was performed in the modified Guelph tank system (flow through), allowing for very accurate feed loss recording. Growth trials B and C were performed in recirculated system consisting of 12 (~900 L) fiberglass tanks, equipped with swirl separators for collection of uneaten pellets. Although feed loss was recorded in all trials/tanks, the two *ad libitum* trials were used to evaluate differences in feed attractant properties of fish meal and mussel meal, respectively. Fish were sampled for physiological markers in all growth trials, as described in previous reports, and in growth trial A also for chromameter analysis of filet colour. Chromameter analysis was not performed in the 2013 trial.

Overall condition of fish

The average start and end weight as well as condition factor of fish in all three trials is shown in Table 11. The start weight of the different diets groups was very similar within the same trial, but the 2012 fish were relatively small in order to perform the growth study during a period with high growth rates. Likewise, initial condition factors were very similar within the same trial, but increased with time and as expected more in *ad libitum* fed fish.

Table 11. Average weight and condition factor at the start and end of all growth trials

Growth trial / Diet / ration	Avg weight start	Condition Factor	Avg weight end	Condition Factor
2012 - A / FM-1/ ad libitum	56.5	1.31	292	1.44
2012 - A / FM:MM-1/ ad libitum	58.4	1.30	299	1.44
2012 - A / MM-1 / ad libitum	58.1	1.29	286	1.42
2012 - B / FM-1 / restrictive	48.6	1.21	179	1.33
2012 - B / FM:MM-1 / restrictive	48.1	1.20	174	1.33
2012 - B / MM -1 / restrictive	48.1	1.20	174	1.34
2013 - C / FM – 2 / restrictive	204	1.25	460	1.45
2013 - C / MM – 2 / restrictive	204	1.27	449	1.46
2013 - C / FM – 2 / ad libitum	206	1.27	578	1.58
2013 - C / MM – 2 / ad libitum	207	1.26	568	1.55

Specific growth rates (SGR) and feed conversion ratio (FCR)

Results for the SGR and FCR for the two 2012 trials and the 2013 trial is shown in Table 12 and Table13, respectively. Overall, all three growth studies worked out well, with high performance and generally little variation among tanks. Only the tank based results is shown here, as the results for the individually pit-tagged fish did not affect the conclusion. SGR was lower and FCR higher in the 2013 study, likely a consequence of using larger fish growing less. However, the three studies revealed only minor differences between diet groups. When the fish where fed in a restrictive manner (2012-B), small, but significant differences between fish meal and mussel meal protein quality were revealed, resulting in significantly lower SGR and higher FCR for the MM-1 diet group. The results also showed that the "mixed group"; FM:MM-1 actually showed a slightly better performance with time compared with the FM-1 diet group.

No significant differences were found among *ad libitum* fed fish, indicating that differences in protein quality were only revealed when the fish were forced to utilise the protein efficiently by restrictive feeding and by a relatively low DP/DE.

Table 12. SGR (%/day) based on tank biomass gain and FCR (kg feed/kg biomass gain) in the two 2012 studies on *ad libitum* and restrictively fed fish. Significant differences (One-way ANOVA) between groups are shown by different letters. N=3 for the A-study carried out in 2012 and N= 4 for the B-study carried out in 2012.

Growth trial Diet ration	2012 - A FM-1 ad libitum	2012 - A FM:MM-1 ad libitum	2012 - A MM-1 ad libitum	2012 - B FM-1 restrictive	2012 - B FM:MM-1 restrictive	2012 - B MM -1 restrictive
SGR (%/day) period 1	3.48±0.20	3.49±0.14	3.46±0.05	2.26±0.03^{a}	2.21±0.04^{ab}	2.19±0.01^{b}
SGR (%/day) period 2	2.57±0.13	2.63±0.20	2.57±0.21	2.01±0.02^{a}	2.01±0.01^{a}	1.96±0.02^{b}
SGR (%/day) period 3	2.14±0.09	2.28±0.05	2.01±0.19	1.71±0.02^{a}	1.73±0.01^{a}	1.67±0.02^{b}
FCR (kg/kg) period 1	0.71±0.02^{a}	0.67±0.02^{b}	0.68±0.01^{ab}	0.74 ± 0.01^{a}	0.75 ± 0.01^{ab}	0.77 ± 0.01^{b}
FCR (kg/kg) period 2	0.73±0.03	0.68±0.04	0.69±0.03	0.74 ± 0.01^{a}	0.73 ± 0.00^{a}	0.76 ± 0.01^{b}
FCR (kg/kg) period 3	0.83±0.02	0.77±0.02	0.80±0.05	0.75 ± 0.00^{a}	0.73 ± 0.01^{b}	0.77 ± 0.01^{c}

Table 13. SGR (%/day) based on tank biomass gain and FCR (kg feed/kg biomass gain) in the 2013 study including both *ad libitum* and restrictive feeding. P-values of a performed Two-way ANOVA analysis using diet and ration as the two factors are given. N=3. *SGR and FCR (start to end) could be estimated as no fish were removed from the tanks during the trial

Growth trial Diet ration	2013 - C FM-2 Restrictive	2013 - C MM-2 Restrictive	2013 - C FM-2 Ad libitum	2013 – C MM-2 Ad libitum	P Diet Two way Anova	P Ration Two way Anova	P Interaction
SGR (%/day) period 1	1.23±0.01	1.19±0.01	1.81±0.03	1.77±0.11	0.304	<0.001	0.994
SGR (%/day) period 2	1.16±0.02	1.13±0.02	1.25±0.05	1.22±0.08	0.337	0.016	0.811
SGR start to end*	1.19±0.02	1.16±0.02	1.53±0.04	1.49±0.02	0.041	<0.001	0.753
FCR (kg/kg) period 1	0.89±0.01	0.92±0.00	0.91±0.00	0.93±0.01	0.002	0.008	0.285
FCR (kg/kg) period 2	0.99±0.02	1.01±0.02	1.07±0.02	1.06±0.04	0.805	0.003	0.256
FCR (kg/kg) start–end*	0.95±0.01	0.97±0.07	1.00±0.01	1.00±0.02	0.229	0.003	0.176

In 2013, where the fishmeal and mussel meal inclusion were reduced to ~15%, the performed two-way ANOVA on SGR (start-end) revealed a significant negative effect of mussel meal inclusion and also on the FCR for the first period. However, one-way Anova analysis did not reveal any significant differences among diet groups. Feed ration had a major impact, as *ad libitum* feeding caused (as expected) a considerably higher SGR, but also a significantly lower FCR for both diet groups.

Protein efficiency ratios (PER), protein retention (PR), biological value (BV) and energy utilisation

The parameters were calculated as follows:

PER (kg/kg) = kg biomass (BM) gain/kg protein intake.

$$PR.(\%) = \frac{BM(end)x\ protein\ content\ (end) - BM\ (start)x\ protein\ content\ (start)}{protein\ intake} x\ 100$$

$$BV = \frac{BM(end)x\ protein\ content\ (end) - BM\ (start)x\ protein\ content\ (start)}{protein\ intake\ x\ ADC\ (protein)} x\ 100$$

$$ER\ (\%) = \frac{BM(end)x\ energy\ content\ (end) - BM\ (start)x\ energy\ content\ (start)}{energy\ intake} x\ 100$$

Gross composition of fish were in 2012 analysed on whole fish, where in 2013, the stomach and gut were removed as the stomach and gut contained considerable amount of feed from the feeding a few hours before (late feeding due to stripping).

2012 study (see Table 14)

PER values were all approximately 3 kg/kg and revealed a generally high protein quality for all diets. Minor, but significant differences were found, notably a significantly lower PER for MM-1 diet group in the second and third growth period. As observed for SGR and FCR, the FM:MM-1 group tended to show the best performance, and significantly better than the two other diet groups in the third period.

PR values of approximately 50% also indicates an overall high quality of protein, however, the MM-1 diet group showed a significantly lower protein retention compared to both FM-1 and FM:MM-1, indicating a poorer utilisation of proteins/amino acids. No significant differences were observed between the FM-1 and FM:MM-1 diet groups.

BV value takes into account the digestibility of the protein, i.e. shows the utilisation of absorbed amino acids. The BV results shown in table 14 are calculated by use of digestibility study 1 results (Table 9), but an average of the two ADCN values could also be used. Due to the slightly higher nitrogen digestibility of the MM-1 diet compared to the FM diet the relative difference between these diet groups became larger. The FM-1 and FM:MM-1 diet group did not show significant differences.

Table 14. Protein Efficiency Ratio (kg biomass gain/kg protein intake), protein retention (PR; %), biological value (BV; %) and energy retention (%) based on tank biomass gain in the 2012 study (only restrictive feeding). Significant differences (One-way ANOVA) between groups are shown by different letters. N=4 for all groups. *see text for comment.

Growth trial Diet ration	2012 - B FM-1 restrictive	2012 - B FM:MM-1 restrictive	2012 - B MM -1 restrictive	P-value One-way Anova
PER (kg/kg) – Period 1	3.10 ± 0.06	3.04 ± 0.05	3.00 ± 0.02	0.051
PER (kg/kg) – Period 2	3.11 ± 0.04^{ab}	3.16 ± 0.02^{a}	3.05 ± 0.04^{b}	0.009
PER (kg/kg) – Period 3	3.06 ± 0.02^{a}	3.12 ± 0.03^{b}	3.00 ± 0.03^{c}	<0.001
PR. (%) – Period 1	50.3 ± 1.4	50.2 ± 0.5	48.3 ± 1.3	0.069
PR. (%) – Period 2	49.6 ± 0.6^{a}	50.3 ± 0.4^{a}	48.7 ± 0.5^{b}	0.003
PR. (%) – Period 3	49.6 ± 0.4^{a}	49.6 ± 0.7^{a}	47.9 ± 0.5^{b}	0.004
BV (%) – Period 1	53.9 ± 1.5	53.4 ± 0.5	51.4 ± 1.4	0.04
BV (%) – Period 2	53.2 ± 0.6^{a}	53.6 ± 0.4^{a}	51.8 ± 0.5^{b}	0.002
BV (%) – Period 3	53.2 ± 0.5^{a}	52.7 ± 0.8^{a}	51.0 ± 0.6^{b}	0.002
Energy ret. (%) – Period 1	51.7 ± 2.2	52.9 ± 2.5	52.0 ± 3.9	0.851
Energy ret. (%) – Period 2	52.8 ± 8.9*	54.6 ± 3.1	52.3 ± 5.6	0.866
Energy ret. (%) – Period 3	54.5 ± 3.3*	52.2 ± 3.6	50.8 ± 3.2	0.341

ER revealed no significant differences between diet groups, partly due to generally higher variability among tanks. Unfortunately, fish sampled from one of the FM-1 tank at the end of growth period 2 showed considerably lower fat and energy content, compared to the other tanks in the same diet group, and appeared not to be a representative subsample. This resulted in an overall lower energy retention at the end of growth period 2 and higher energy retention after growth period 3 in the FM-1 diet group. If this one tank was excluded, energy retention would be 56.7±5.5 % after period 2 (instead of 52.8%) and 52.9±1.3 after growth period 3. However, excluding this one tank did not change the statistical outcome. Energy retention is in general more prone to higher variability among tanks compared to nitrogen retention. Nitrogen content varies very little among individual fishes and hence it is fairly easy to collect a representative sub-group from a tank. Fat and hence energy content of individual fishes varies more making it more difficult to sample a truly representative subsample from a tank.

2013 study (see Table 15)

PER, PR and BV were all slightly lower than in the 2012 study. This may again be attributed to the use of larger fish, but performance was nevertheless satisfying. Mussel meal inclusion caused a slight decrease in PER, especially in the restrictively fed fish, but a significant effect of diet was only observed after the first period. No significant effect was observed, when the entire period was considered. Similar to the 2012 study, mussel meal inclusion led to a significantly lower PR and BV, but only in restrictively fed fish. Interestingly, PR and BV were actually slightly higher in the MM-2 fish compared to the FM-2 fish when they were fed *ad libitum*, however, no significant effect was observed. Due to these "opposite" effects, the two-way *ANOVA* analysis revealed a significant interaction, i.e. the effect of diet was dependent on ration and hence no overall diet effect was observed. However, performing one-way *ANOVA* analysis revealed the differences caused by diet. As for SGR and FCR, *ad libitum* feeding caused clear significant effects with lower PER, PR and BV, but the one-way *ANOVA* on PR and BV revealed only an *ad libitum* effect for the FM-2 diet. Similar to 2012, no significant differences were observed for energy retention.

Table 15. Protein Efficiency Ratio (kg biomass gain/kg protein intake), protein retention (PR; %), biological value (BV; %) and energy retention (%) based on tank biomass gain in the 2013 including both restrictive and *ad libitum* feeding. P-values of a performed Two-way ANOVA analysis using diet and ration as the two factors are given. N=3. * Due to significant interaction for PR and BV, the data were also analysed by one-way ANOVA. Significant differences found by this method is shown as different letters.

Growth trial Diet ration	2013 - C FM-2 Restrictive	2013 - C MM-2 Restrictive	2013 - C FM-2 Ad libitum	2013 – C MM-2 Ad libitum	P Diet Two way Anova	P Ration Two way Anova	P Inter- action
PER (kg/kg) : Period 1	2.69±0.03	2.62±0.01	2.62±0.00	2.59±0.04	0.009	0.007	0.243
PER (kg/kg) : Period 2	2.43±0.06	2.38±0.05	2.23±0.04	2.28±0.09	0.956	0.003	0.811
PER (kg/kg) : start–end	2.53±0.04	2.48±0.03	2.43±0.02	2.41±0.06	0.479	0.003	0.161
PR, (%) : start – end	45.8±1.23^{a}	43.9±0.70^{b}	40.7±0.66^{c}	42.0±0.43^{bc}	0.589	<0.001	0.009*
BV (%) : start – end	49.4±1.37^{a}	47.0±0.94^{b}	44.1±0.60^{c}	45.1±0.34^{bc}	0.225	<0.001	0.011*
Energy retention (%)	52.2±1.2	51.5±3.1	53.2±2.1	52.6±1.0	0.596	0.381	0.973

Nitrogen excretion as a measure of protein utilisation

Following digestibility study 1 in 2012, the fish were fed a fixed, daily ration of 1.7% of the start biomass in each tank for five days to ensure that excreted ammonia was generated from a well-defined and constant amount of feed. On the sixth day, water supply was turned off and water sampled every hour for the next 24 hours, and analysed for ammonia nitrogen (TAN) and urea. Figure 4 illustrates the accumulated excretion of TAN or urea-N for each of the dietary treatment groups during 24 h, and normalised to the intake of digestible protein. Overall, TAN excretion from the different experimental groups followed the same pattern throughout the experiment: FM<FM:MM<MM. After 24 hours, TAN excretion ranged from 37.3±1.7 mg N g^{-1} digestible protein intake in the FM group to 39.8±1.5 mg N g^{-1} digestible protein intake in the MM group, but no significant differences were observed at this point. Significant differences were detected, but primarily during the first 14 hours. In contrast, urea-N excretion was not significantly affected by diet.

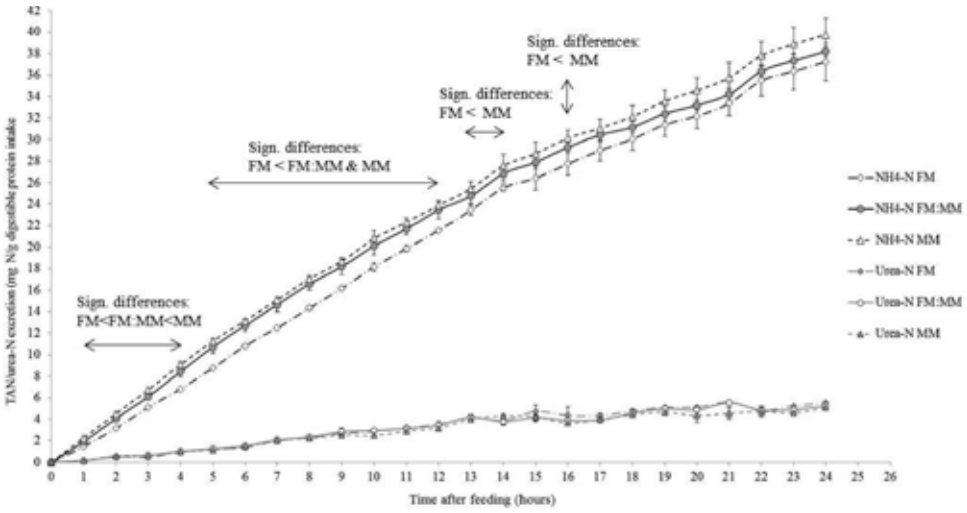

Figure 4. Accumulated TAN and Urea-N excretion, normalized to digestible protein intake

Ammonium is the primary by product of protein catabolism (Kajimura et al., 2004), and it is generally believed that amino acids fed in excess of what the synthesis apparatus can utilise will be de-aminated and result in increased ammonia excretion. Furthermore, deficiency of only one amino acid may also lead to increased nitrogen excretion, as the level of this amino acid sets the limit for protein synthesis and the remaining amino acids, will then effectively be in excess (Green and Hardy, 2008). In the present study, methionine could possibly be limiting for protein synthesis, leading to a decrease in protein retention in the MM group combined with an increase in TAN excretion (Figure 4). The study shows that TAN excretion may be used as a fast screening method for evaluating feed protein quality.

Mussel meal as feed attractant

Mussel meal as feed attractant was evaluated by recording feed loss in particular from *ad libitum* fed fish. Feed intake per kg biomass was calculated by estimating tank biomass in between weighing, by using the SGR for each tank, and by using the equation: $\ln W_{dayX} = (SGR/100)\times 1+\ln W_{day(X-1)}$, where SGR is the SGR calculated for each growth period, and W_{day-X} is the biomass on the day of interest. From recorded daily feed intake and estimated daily biomass, the daily feed intake per biomass (as % of biomass) was calculated. The results for the 2012 *ad libitum* trial and the 2013 growth trials are shown in Figures 5 and 6, respectively.

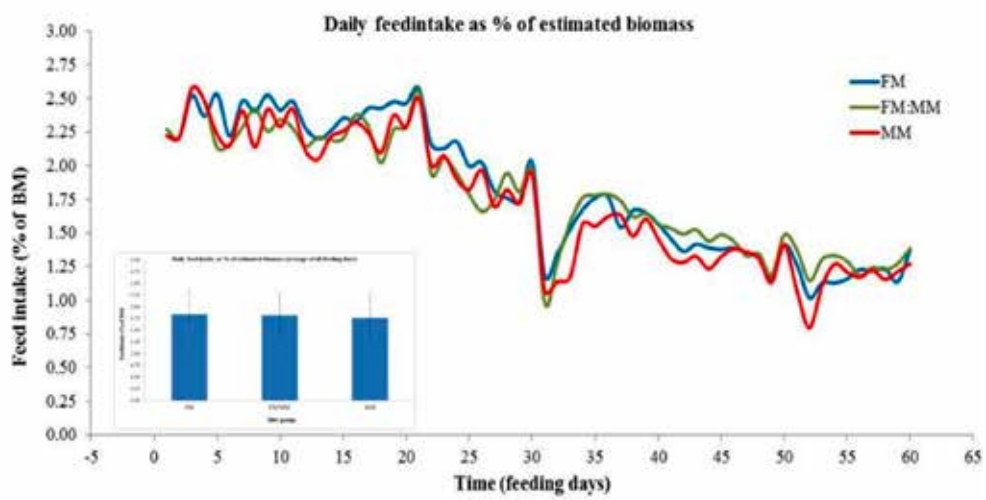

Figure 5. Study-A in 2012. Daily feed intake shown as per cent of estimated body mass. Only mean values are used to create the line, N=3 for each line. The inserted figure shows the average daily feed intake for the entire period

As expected, the daily feed intake decreased gradually with increasing body size from approximately 2.25-2.5% of BM at the beginning of 2012-A trial to about 1.25 % of BM at the end. In the 2012 trial with restrictive fed fish the final feeding ration was 1.3% of expected biomass and as the final weight of these fish last year was slightly lower than the start weight in the present study, the restrictive feeding ration was set at 1.1%. Initially the *ad libitum* feeding ration was based on the previous *ad libitum* study and set at 1.6%. However, during the first 1-2 weeks this had to be increased to 1.9%, a level which was maintained for the rest of this growth period. In the second growth period, the restrictive ration remained at 1.1%, but the *ad libitum* ration gradually was decreased to 1.3% because of increasing feed loss. The actual feed intake over time as percentage of biomass in the 2013 trial is shown in Figure 6.

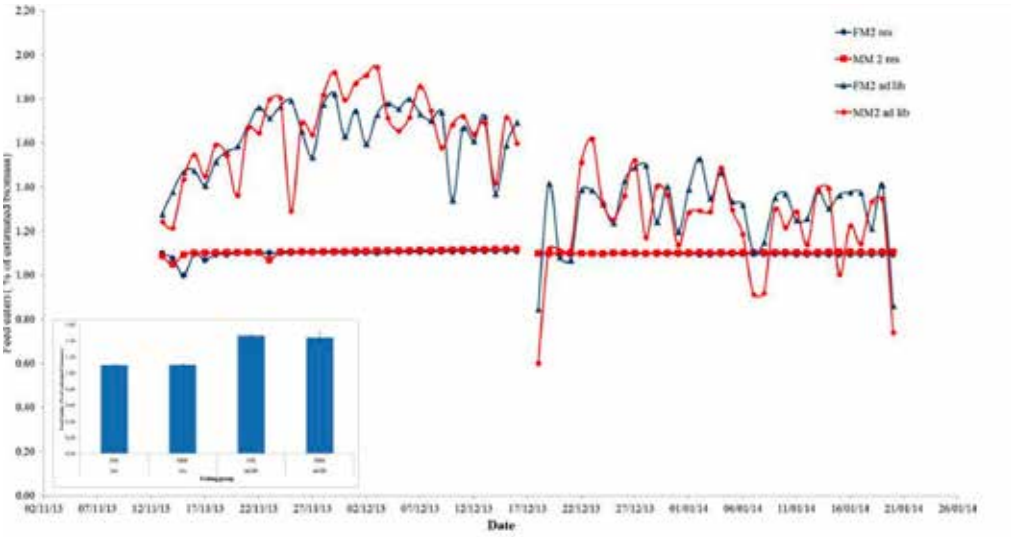

Figure 6. Trials carried out in 2013. Daily feed intake shown as per cent of estimated body mass. Only mean values are used to create the line, N=3 for each line. The inserted figure shows the average daily feed intake for the entire period

Figure 6 shows that in both *ad libitum* trials, feed intake was never constant but fluctuating from day to day, probably indicating that when the fish are fed to satiation they cannot maintain a high continuous feed intake, but need to empty the intestines to some extent in between. This "wavy" eating pattern is even partly masked in the figure as the three tanks within each diet group did not always follow the same eating pattern and increased variation within each diet group. The average feed intake for the entire growth trial was approximately 1.8% of biomass for all diet groups in 2012, i.e. mussel meal had no overall effect on feed intake compared to fishmeal in the present study. In 2013, the overall average feed intake was 1.1% for the restrictively fed FM2 or MM2 tanks and 1.47% and 1.44% for the *ad libitum* fed FM2 and MM2 tanks, respectively. No significant differences were found between diet groups. However, it is not known whether mussel meal could have a more positive effect on diets for other species such as salmon which are more "picky" than trout.

Effect of mussel meal on filet colour

As shown in the picture below (Figure 7), mussel meal had a clear effect on filet colour in the 2012A study, and furthermore, colour seemed to show a dose dependent increase with higher mussel meal inclusion. The colour differs from salmon colour, i.e. more orange than red/pink. The shown figure was taken 3 weeks before the end of trial. Pictures were taken at the end of trial as well, but unfortunately did these not turn out well and did not display the real difference in colour. Mussel meal inclusion also induced distinct colouration of the filet, even at the relatively low inclusion level. It is difficult to tell from the picture, but the degree of colouration appeared to match the 2012 study, i.e. a less dense colour than the lowest inclusion level (25%) in 2012, but still a distinct colour change.

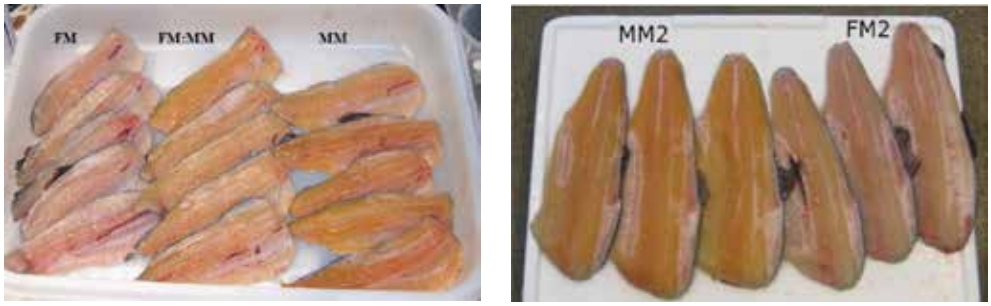

Figure 7. The picture on the left side shows filets sampled after 6 weeks in the 2012 trial and on the right side filets at the end of the 2013 trial.

Conclusion

Overall, all trials worked really well, i.e. the fish ate well, very low mortality and no tanks were "outliers", resulting in little variation among tanks. The results are clear; nutrient digestibility of mussel meal is generally high, at the level of fish meal, although the lower methionine level may lead to slightly lower fat digestibility. On the other hand, protein digestibility appears to be at least as good as for fishmeal.

Obviously, only restrictive feeding and a relative low dietary protein content compared to a commercial diet or a relatively low DP/DE lead to differences in performance in trout fed fishmeal or mussel meal diets, respectively, - with the fishmeal fed fish performing better in terms of growth rate, feed conversion and protein retention. Again, it appears that methionine limitation is the likely candidate for this difference. However, in commercial diets with only a small inclusion level of fishmeal/mussel meal and a high proportion of plant-protein, it is likely that the diets would have to be supplemented with methionine anyway.

As soon as the fish were fed *ad libitum*, these small difference between fish meal and mussel meal were evened out, i.e. when the fish were not forced to utilise the protein as efficiently as possible, as proteins were fed in excess, it was no longer possible to see differences between the diets. In a commercial fish farm, the fish would under normal circumstances not be fed as restrictively as in the present study, and in this case, mussel meal could from a nutritional point of view fully replace fishmeal in either type of diet.

Feed attractant properties of mussel meal/meat have been tested in some studies with positive outcome, but in the present study we saw no indications of this. However, it is important to keep(have) in mind that rainbow trout are not real picky eaters, and are willing to eat diets with even high proportion of plant ingredients. Salmon might have been a better species for this particular test. Also, in 2012, the reference diet was based on fishmeal, and could probably not be more attractant for trout at least, i.e. it would have been almost impossible to formulate a diet with higher feed attractant properties. The 2013 diet included more plant proteins, but still high quality and quite purified plant proteins.

The distinct colour change/increase in fish fed mussel meal diet may or may not be a problem, depending entirely on the consumers preferences. The colour is quite different from the well-known salmon colour, but disappears almost completely after cooking. It could possibly be promotional for specific products (i.e. organic, environmentally friendly etc.) if presented the right way, but if presented as more conventional product it may also be a drawback, as the consumers at least with portion size trout expect it to have more or less white flesh.

Finally, at the end of the 2012 study we did a small taste test with filets from fishmeal and mussel meal fed fish and the outcome was very positive. Everyone in the "tastepanel" preferred the mussel meal fed fish both due to taste but also due to a better firmer texture for the mussel meal fed fish. This quite distinct effect of mussel meal is something which deserves to be investigated further.

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2.1.4 Effect of Mussel meal on fish physiology (SWEDEN).

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We have participated in three studies on rainbow trout conducted at DTU-Aqua, Hirtshals during autumn 2012 and winter 2013/2014.

Thrandur Björnsson (ThB) and Kristina Snuttan Sundell (KSS) participated in the detailed design of the experimental series during the spring of 2012.

KSS and Linda Hasselberg Frank (LHF) then participated directly in the main sampling of the Growth Study A (GSA) during October 21-25, 2012. LHF then also participated directly in the main sampling of Growth Study B (GSB) on November 6, 2012 and finally, KSS and LHF participated directly in the main sampling of the Growth Study C (GSC) during January 18-21, 2014.

The GSA, GSB and GSC studies were performed as described in the report by Bodil K. Larsen, DTU-Aqua, Hirtshals, September 2014. Here, we present data which have not been reported before, i.e. plasma GH and IGF-I data from the GSA and GSB and the data from the GSC as a whole.

Methods

Sampling for Growth Study C (GSC)

GSC included two feeding regimes (*ad lib* and restricted ration) and two diets (fish-meal based diet (FM) and mussel-meal based diet (MM).

Fish from the *ad lib* feeding groups were anaesthetized using Aquacalm™, a metomidate hydrochloride (12.5 mg/L) and weight and length of each fish was recorded. 12 FM-fed fish and 12 MM-fed fish were sampled for blood from the caudal vein using heparinized syringe and needle. The blood was centrifuged at 10 000 g for 5 min and plasma was transferred to three 0.5 ml microtubes and frozen at -80°C for further analysis. After blood sampling, the fish were quickly killed with a sharp blow to the head and intestinal segments for physiological analyses in Ussing chambers dissected out. One anterior and one posterior intestinal segment was also taken and fixed in 5 mL 4% paraformaldehyde for further processing and microscopic analysis. The livers were dissected out and weighed for assessment of liver-somatic index (LSI) and muscle tissue (Norwegian Quality Cut) was taken, wrapped in aluminum foil and frozen at -80°C for further analysis.

Fish from the restricted feeding groups were sampled in the same manner as the *ad lib* groups, but no Ussing chamber studies were conducted on these fish.

Plasma hormone radioimmunoassay (RIA) analyses

Plasma growth hormone (GH) levels were measured in a specific salmonid GH RIA, which is a three days competitive assay with a primary antibody against recombinant chum salmon GH. Insulin-like growth factor I (IGF-I) levels were measured in extracted plasma with a RIA procedure using anti-Barramundi IGF-I antibody from GroPep Bioreagents (Australia). Radioactivity was counted using a Wallac 1470 gamma-counter. Cortisol was measured in plasma using a competitive RIA with cortisol antibodies purchased from Guildhay Ltd. (Guildford, Surrey, UK). [2, 6, 7-^{3}H]-Cortisol from Amersham (Buckinghamshire, UK) was used as a tracer and standard were prepared from hydrocortisone. Radioactivity was determined in a beta-counter (Wallac 1409 Liquid Scintillation Counter). Standard curves and plasma levels of GH, IGF-I and cortisol were calculated using the software AssayZap (BioSoft, USA).

Ussing chamber studies on gut epithelia

The intestine, from the last pyloric caeca to the anus, was carefully removed and opened longitudinally, divided in a proximal and a distal part and thereafter washed and placed in ice-cold salmon Ringer solution continually gassed with air. The serosa was peeled off the intestinal segments before mounted into Ussing chambers. 4-ml of Ringer solution were added to each side of the intestinal epithelium and the preparations allowed 60 minutes for stabilization of the electrical parameters before the start of the experiment. The intestinal area of exposure was 0.75 cm^2. Oxygenation and stirring were ensured by an air-lift on both sides of the intestinal segments. The temperature in the Ussing chambers was kept at 10ºC by the use of cooling mantles. The electrical parameters; transepithelial resistance (TER), short-circuit current (SCC) and transepithelial potential (TEP) were measured every five minutes throughout the experimental period (150 min) as a continuous monitoring of preparation viability and integrity. The paracellular permeability of the intestinal epithelium was further assessed as the apparent permeability (P_{app}) of the hydrophilic marker ^{14}C- mannitol. Amino acid transport was measured by addition of ^{3}H-L-lysine.

The experiment started (t = 0) by renewing the Ringer solution on the serosal side while the Ringer solution on the mucosal side was replaced with Ringer solution containing ^{14}C-mannitol (spec. act. 0.04 MBq ml^{-1}) and 0.5 mM L-lysine (unlabeled) together with ^{3}H-L-lysine (spec. act. 0.13 MBq ml^{-1}). For assessment of P_{app} and L-lysine tranport, 50 µl of the serosal Ringer was sampled after 10, 15, 20, 50, 80, 85 and 90 min.

Radioactivity was assessed in a liquid scintillation counter using a dual label protocol ($^{14}C/^{3}H$) ((Wallac 1409 Liquid Scintillation Counter) after adding 5 ml of Optiphase High Safe II (Wallac, Turku, Finland) P_{app} was calculated using Equation (1):

(1) $P_{app} = dQ/dT \times 1/AC_o$
(2) L-Lysine = $dQ/dT \times 1/A$

where dQ/dT is the appearance rate of the molecule in the serosal compartment of the Ussing chamber, A is the area of intestinal surface exposed in the chamber and C_o is the initial concentration on the mucosal side.

Statistics

Data were tested for homogeneity of variances by using Levene's test and then analyzed using one-way ANOVA followed by Student-Newman-Keuls post hoc test to investigate differences between treatment groups. Data from the Ussing chamber experiment were analyzed in a mixed linear model with feed as main factor as well as factor tank nested within feed. All data are expressed as mean ± SEM and $P \leq 0.05$ is regarded as significant. The statistical analysis was performed using SPSS 19.0 software.

Results

Growth Study A (GSA) - ad lib *feeding*

Plasma growth hormone (GH) levels were measured at the onset of the study (week 0) and after 3, 6 and 9 weeks of *ad lib* feeding of the three test diets: fish-meal based diet (FM; ●), mussel-meal based diet (MM; ●), or a diet containing equal amount of fish- and mussel-meal (FMM; ●). Plasma GH levels decreased significantly in all groups during the first 6 weeks after which they stabilized (Figure 8). At week 6 and 9, the MM-fed fish had significantly higher plasma GH levels than the two other diet groups.

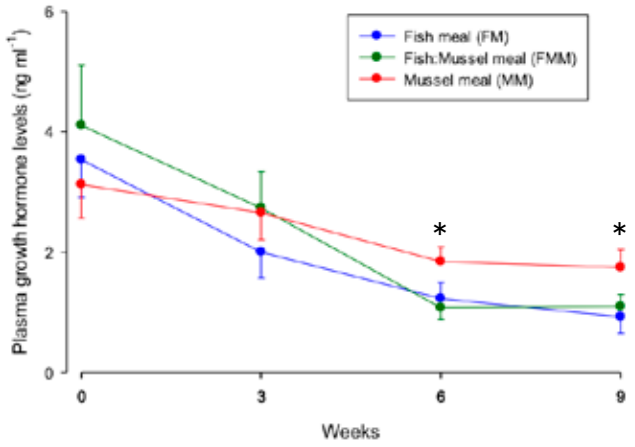

Figure 8. Plasma growth hormone (GH) levels of rainbow trout fed fish-meal based diet (FM; ●), mussel-meal based diet (MM; ●), or a combination fish/mussel-meal based diet (FMM; ●). The fish were fed the diets *ad lib* for 9 weeks. All data are expressed as mean ± SEM (n= 12). *indicates a statistically significant difference between the MM group and the others, at the P<0.05.

Plasma insulin-like-growth factor I (IGF-I) levels were measured at the onset of the study (week 0) and after 3, 6 and 9 weeks of *ad lib* feeding of the three test diets: fish-meal based diet (FM; ●), mussel-meal based diet (MM; ●), or a diet containing equal amount of fish- and mussel-meal (FMM; ●). Plasma IGF-I levels increased significantly in all groups throughout the 9 week feed trial (Figure 9). The FM-fed fish had significantly lower plasma IGF-I levels than the two other diet groups during weeks 3 through 9.

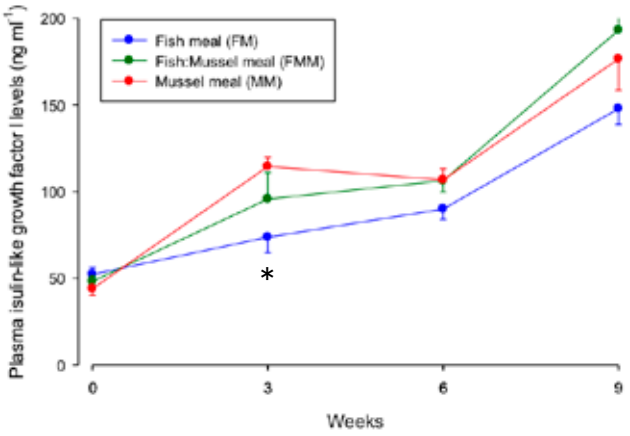

Figure 9. Plasma insulin-like growth factor I (IGF-I) levels of rainbow trout fed fish-meal based diet (FM; ●), mussel-meal based diet (MM; ●), or a combination fish/ mussel-meal based diet (FMM; ●). The fish were fed the diets *ad lib* for 9 weeks. All data are expressed as mean ± SEM (n= 12). *indicates a statistically significant difference between FM group and the others, at the P<0.05.

Growth Study B(GSB) - restricted feeding

Plasma growth hormone (GH) levels were measured at the onset of the study (week 0) and after 3, 6 and 10 weeks of restricted feeding of the three test diets: fish-meal based diet (FM; ●), mussel-meal based diet (MM; ●), or a diet containing equal amount of fish- and mussel-meal (FMM; ●). Plasma GH levels decreased significantly in all groups throughout the 10-week feeding trial without any significant differences among groups (Figure 10).

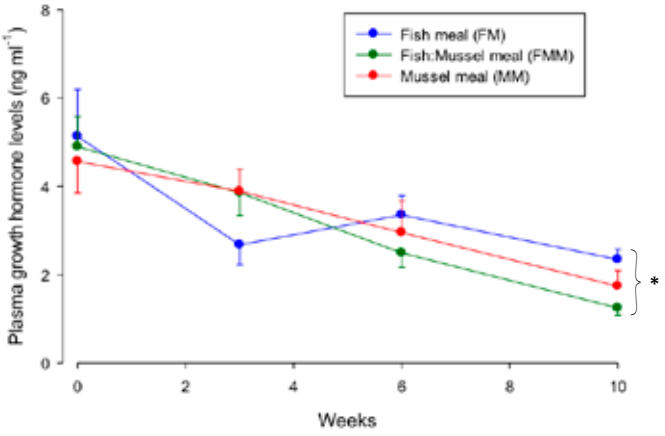

Figure 10. Plasma growth hormone (GH) levels of rainbow trout fed fish-meal based diet (FM; ●), mussel-meal based diet (MM; ●), or a combination fish/mussel-meal based diet (FMM; ●). The fish were rationed the diets for 10 weeks. All data are expressed as mean ± SEM (n= 12). *indicates a statistically significant difference with time, at the P<0.05.

Plasma insulin-like-growth factor I (IGF-I) levels were measured at the onset of the study (week 0) and after 3, 6 and 10 weeks of restricted feeding of the three test diets: fish-meal based diet (FM; ●), mussel-meal based diet (MM; ●), or a diet containing equal amount of fish- and mussel-meal (FMM; ●). Plasma IGF-I levels increased significantly in all groups throughout the 10-week feed trial without any significant differences among groups (Figure 11).

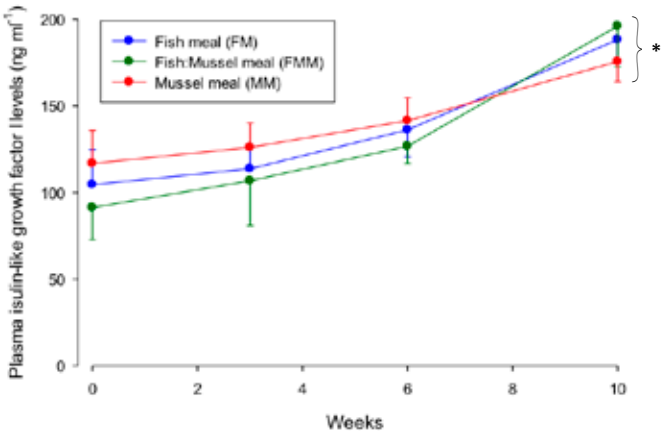

Figure 11. Plasma isnulin-like growth factor I (IGF-I) levels of rainbow trout fish-meal based diet (FM; ●), mussel-meal based diet (MM; ●), or a combination fish/mussel-meal based diet (FMM; ●). The fish were rationed the diets for 10 weeks. All data are expressed as mean ± SEM (n= 12). *indicates a statistically significant difference with time, at the P<0.05.

Growth Study C GSC) - ad libitum *feeding*

The average weight, length, condition factor (CF) and liver somatic index (LSI) from the 24 fish sampled at the end of trial is shown in Figure 12. No statistically significant differences were observed between groups.

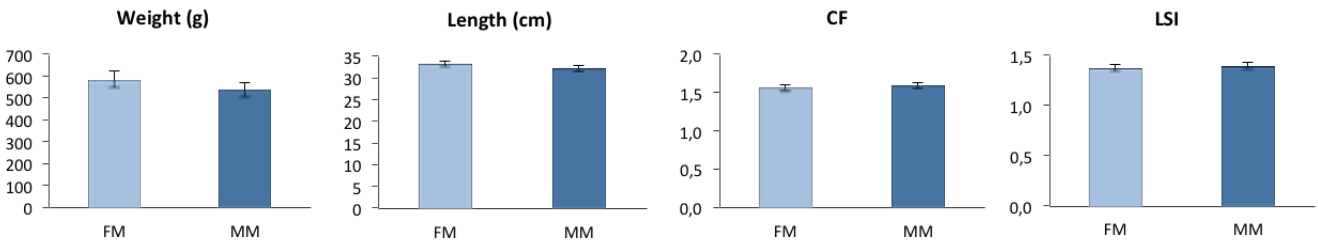

Figure 12. The average weight, length, condition factor (CF) and liver somatic index (LSI) for the FM and MM diet groups in Growth Study C – *ad lib* feeding. All data are expressed as mean ± SEM (n=12).

Growth Study C – restrictive feeding

The average weight, length, condition factor (CF) and liver somatic index (LSI) from the 24 fish sampled at the end of trial is shown in Figure 13. No statistically significant differences were observed between groups, except for the length where MM were significantly shorter than FM.

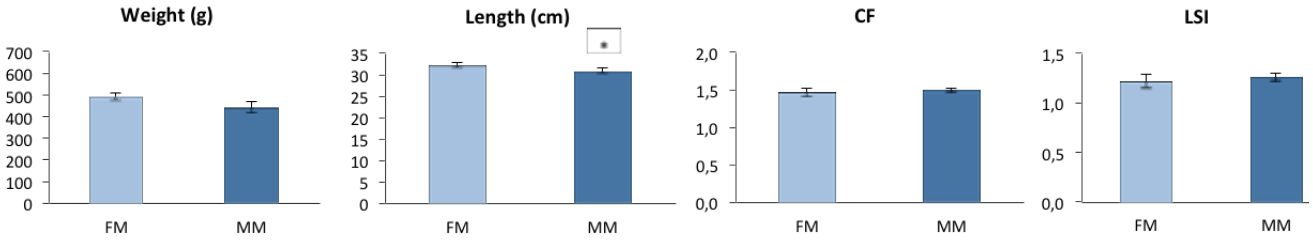

Figure 13. The average weight, length, condition factor (CF) and liver somatic index (LSI) f for the FM and MM diet groups in Growth Study C – restrictive feeding. All data are expressed as mean ± SEM (n=12). *indicates a statistically significant difference at the P<0.05.

Plasma GH and IGF-I results from Growth Study C

The plasma GH levels as well as the plasma IGF-I levels were similar in the FM and MM *ad lib* fed groups, while a diet-based difference in plasma GH levels is seen in the groups given restricted rations, with GH levels elevated in the MM-fed fish (Figure 14).

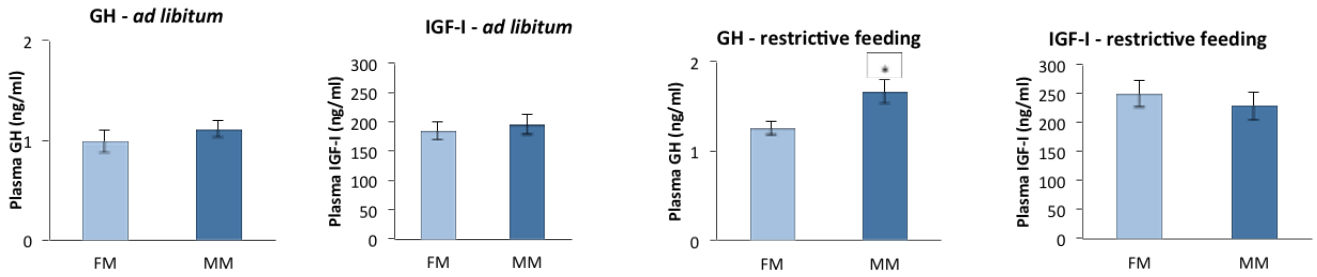

Figure 14. Plasma GH and IGF-I levels for the FM and MM diet groups in Growth Study C, measured with a competitive radioimmunoassay (RIA). All data are expressed as mean ± SEM (n= 12). *indicates a statistically significant difference at the P<0.05.

Plasma cortisol levels

Plasma cortisol levels varied among the experimental groups, most apparent in the feed-restricted group, where plasma cortisol levels were significantly elevated in the FM-group (Figure 15).

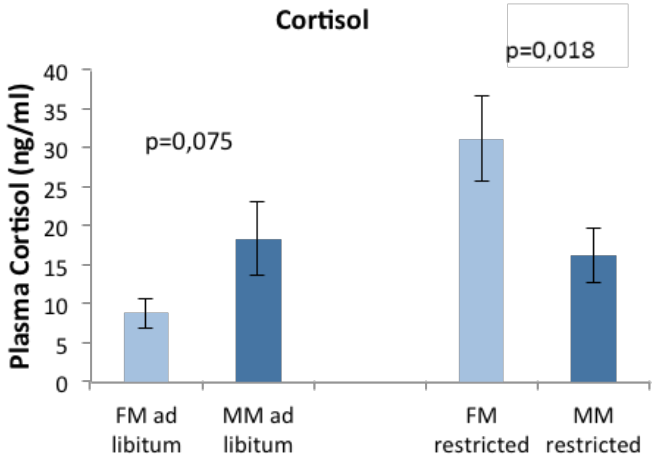

Figure 15. Plasma cortisol levels for the two diet groups FM and MM in Growth Study C, measured with a radioimmunoassay (RIA). All data are expressed as mean ± SEM (n= 12).

Intestinal physiology

No effects of MM diet could be observed for TER, while in the distal intestine, the Papp for mannitol was increased in the MM group (Figure 16).

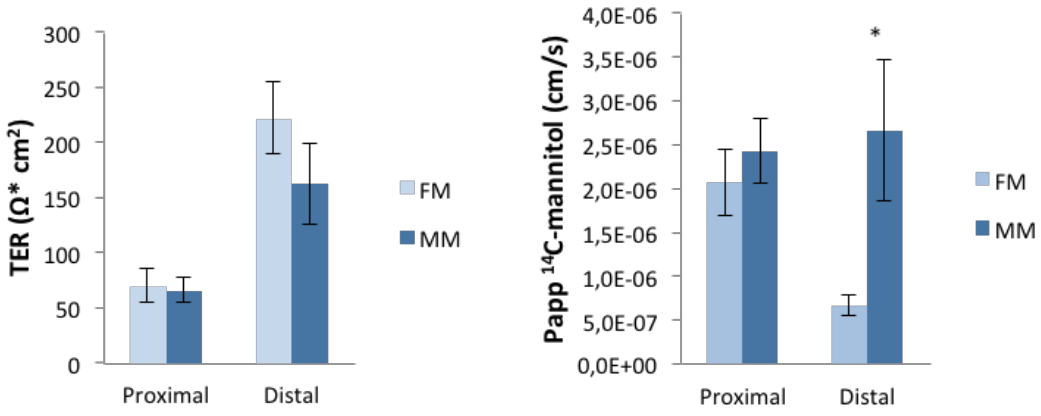

Figure 16. Intestinal epithelial integrity measured as transepithelial electrical resistance (TER) and apparent permeability (Papp) of the hydrophilic marker molecule mannitol. All data are expressed as mean ± SEM (n=12). *indicates a statistically significant difference at the p<0.05 level.

No diet effects were observed for the active transport mechanisms in the intestine (Figure 17), and neither were any effects found of nutrient transport across the intestinal epithelium (Figure 18).

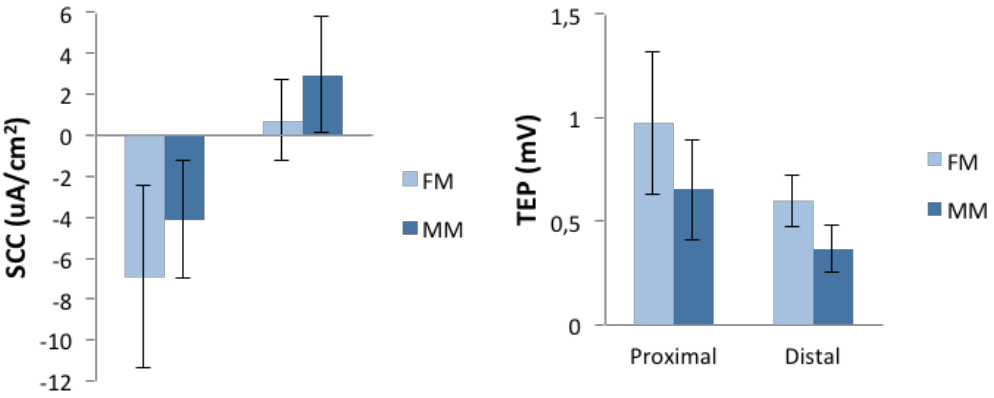

Figure 17. The active transport activities of the intestinal epithelium measured as short circuit current (SCC) and transepithelial potential difference (TEP). No statistical differences were observed. All data are expressed as mean ± SEM (n=12).

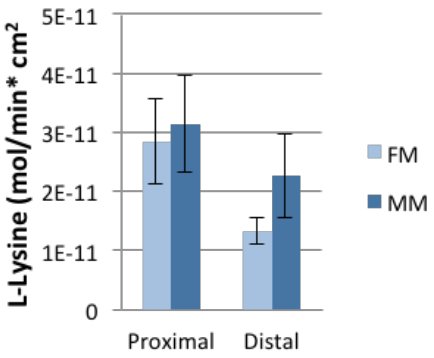

Figure 18. Nutrient transport, measured as transepithelial L-lysine flux rate. All data are expressed as mean ± SEM (n=12).

Discussion

GH and IGF-I are the two main endocrine regulators of growth in fish as other vertebrates, and analysis of the plasma levels of these hormones gives important information about the activity level of growth stimulation.

In GSA and GSB, where the three diet combinations FM, MM and FMM were tested, the most striking result is that there's a general decrease in plasma GH levels throughout both trials, concurrent with an increase in plasma IGF-I levels. Thus, in both trials, the IGF-I:GH ratio increases significantly from the experimental start to finish.

A high IGF-I: ratio, i.e. relatively high IGF-I levels and low GH levels, is an indication of good growth conditions. This is due to the fact that when muscle mass increases rapidly, this increases that amount of GH-receptors available for GH binding, and it is thus a mechanism which causes a decrease in plasma GH levels. As GH binding to its receptors causes a release of IGF-I, both from the liver and the musculature, IGF-I levels increase. In contrast, starving, non-growing fish have high GH levels and low IGF-I levels in plasma, and thus a relatively low IGF-I:GH ratio.

In both trials, the fish were transferred to a novel environment at the beginning of the trial; relatively tall, transparent Perspex cylinders. It is possible that successful acclimation to this environment has decreased stress levels with time and allowed greater physiological focus on growth, allowing increased endocrine growth stimulation through higher IGF-I:GH ratio.

In the sister report from DTU, the growth data for the fish in feeding trials A (GSA) and B (GSB) has been summarized in the two tables below (Tables 16 and 17).

Table 16. Average weight and condition factor at the start and end of all growth trials

Growth trial / Diet / ration	Avg weight start	Condition Factor	Avg weight end	Condition Factor
2012 - A / FM-1/ ad libitum	56.5	1.31	292	1.44
2012 - A / FM:MM-1/ ad libitum	58.4	1.30	299	1.44
2012 - A / MM-1 / ad libitum	58.1	1.29	286	1.42
2012 - B / FM-1 / restrictive	48.6	1.21	179	1.33
2012 - B / FM:MM-1 / restrictive	48.1	1.20	174	1.33
2012 - B / MM -1 / restrictive	48.1	1.20	174	1.34
2013 - C / FM – 2 / restrictive	204	1.25	460	1.45
2013 - C / MM – 2 / restrictive	204	1.27	449	1.46
2013 - C / FM – 2 / ad libitum	206	1.27	578	1.58
2013 - C / MM – 2 / ad libitum	207	1.26	568	1.55

Table 17. SGR (%/day) based on tank biomass gain and FCR (kg feed/kg biomass gain) in the two 2012 studies on *ad libitum* and restrictively fed fish. Significant differences (One-way ANOVA) between groups are shown by different letters. N=3 for the A-study carried out in 2012 and N= 4 for the B-study carried out in 2012.

Growth trial Diet ration	2012 - A FM-1 ad libitum	2012 - A FM:MM-1 ad libitum	2012 - A MM-1 ad libitum	2012 - B FM-1 restrictive	2012 - B FM:MM-1 restrictive	2012 - B MM -1 restrictive
SGR (%/day) period 1	3.48±0.20	3.49±0.14	3.46±0.05	2.26±0.03^{a}	2.21±0.04^{ab}	2.19±0.01^{b}
SGR (%/day) period 2	2.57±0.13	2.63±0.20	2.57±0.21	2.01±0.02^{a}	2.01±0.01^{a}	1.96±0.02^{b}
SGR (%/day) period 3	2.14±0.09	2.28±0.05	2.01±0.19	1.71±0.02^{a}	1.73±0.01^{a}	1.67±0.02^{b}
FCR (kg/kg) period 1	0.71±0.02^{a}	0.67±0.02^{b}	0.68±0.01^{ab}	0.74 ± 0.01^{a}	0.75 ± 0.01^{ab}	0.77 ± 0.01^{b}
FCR (kg/kg) period 2	0.73±0.03	0.68±0.04	0.69±0.03	0.74 ± 0.01^{a}	0.73 ± 0.00^{a}	0.76 ± 0.01^{b}
FCR (kg/kg) period 3	0.83±0.02	0.77±0.02	0.80±0.05	0.75 ± 0.00^{a}	0.73 ± 0.01^{b}	0.77 ± 0.01^{c}

Although Table 17 shows SGR to decrease throughout both feeding trials, it is important to note that this is the nature of this parameter. Under normal conditions, larger fish have lower SGR than smaller fish as SGR is the rate of growth expressed in % growth per day. In absolute terms, i.e. the increase in muscle mass (Table 16), the GSA fish increased from 56-58 g at the onset to 286-299 g at the end of the trial, or more than 5-fold over the 9-week period. During the first 3-week period (0-3 weeks), the average increase in muscle mass was 57 g whereas it was 105 g during the last 3-week period (6-9 weeks).

The fish in GSB, given a restricted ration, increased 3.7-fold over the 10-week period. During the first period 0-3 weeks, the average increase in muscle mass was 30 g whereas it was 63 g during the last period, 6-10 weeks.

Although there are some observed differences, e.g. in GSA, the fish on MM diet have somewhat higher plasma GH levels than the other groups, and the fish on the FM diet have lower IGF-I levels than the other groups, in terms of the development of the IGF-I:GH ratio, all three diet groups appear to have similar status of endocrine growth stimulation.

The assessment of the intestinal epithelial integrity using the Ussing chamber technique, in GSC, revealed no major effects on the active transport mechanisms as indicated by SCC and TEP. The transport rate of L-lysine was unaffected in the major nutrient transporting intestinal region, i.e. the anterior region. The tendency towards decreased TER in the distal region in combination with higher diffusion rate of ^{14}C-mannitol may indicate that MM may cause a certain degree of disturbance of the intestinal barrier, resulting in a leakier epithelia in this intestinal region. This, however, does not seem to have any significant negative effect on the health and welfare of the fish since the growth of the fish were similar in both diet groups.

Thus, an over-all assessment is that the rainbow trout thrived well on all three diets, showing active endocrine growth stimulation and rapid growth, not only when fed *ad lib*, but even on a restricted ration. In those terms, mussel meal, either as the sole protein source (MM diet) or as a partial protein source (FMM diet) appears to be a good replacement for fish meal.

2.2 Seaweed

Seaweed is a heterogeneous group with different nutrient composition. Seaweed has been used for human consumption through ages and is known as a healthy food supplement providing necessary amino acids, beneficial polysaccharides, fatty acids, antioxidants, vitamins and minerals. Seaweed is mainly found in the oceans in the temperate zones of the world (optimum temperature 3 - 20˚C). In many parts of the world, including the Nordic countries, there is limited utilisation of the seaweed resources. Hence, seaweed is a widely available but underutilised Nordic bio resource. In Asia however there is a long tradition of utilising seaweed as food and the market is actually much bigger than harvesting natural sources can cover giving room for extensive cultivation of seaweed (annual production several millions of tons).

Production of fish results in discharges of nutritional salts. Production of macro algae and mussels results in the intake and elimination of these nutritional salts. This provides the potential for a bio-cycle, which is beneficial from a sustainability perceptive, where algae, mussels and fish for human consumption are farmed in what is known as a multi-trophic aquaculture.

Limited information is available on the use of seaweed as ingredients in fish feed. The aim of this study is to examine the effect of two different types of available in the open market.

2.2.1 Nutritional content in seaweed powder (NORWAY)

Ann-Cecilie Hansen, NIFES -The National Institute of Nutrition, Norway

Summary

- Two seaweed powders were tested for nutrient content and level of heavy metals
- The tested seaweed powders contained 8-10% protein and <1.5% lipid. The main part of the seaweed powders tested was ash (minerals) and an unanalysed rest part probably consisting of different polysaccharides
- The *Laminaria digitata* powder can be a source for the fatty acid EPA
- **Both seaweed powders had levels of total and inorganic arsenic above upper limit for feed materials from seaweed, and the studied powders can therefore not be used in fish feed** (even though the content of total arsenic as well as inorganic arsenic was high in both types of seaweed tested in the experiment, analysis of arsenic in the fillets of the Arctic charr showed that all samples had lower concentrations of arsenic than the detectable level of 0.4 µg/kg, see page 64).

The seaweed powder 1 was produces of the brown algae *Laminaria digitata* (fingertare in Norwegian) produced by Thorverk, Iceland. The seaweed powder 2 was produced by Ocean Harvest Technology (OHT), Ireland and was a mixture of several European brown species (but no *Laminaria* or *Ascophyllum*).

Analytical methods

The meal was analyzed for proximal composition; dry matter was determined gravimetrical after drying at 104 C for 24h, total nitrogen with a nitrogen element analyzer (LECO FP-528; LECO Corporation, St. Joseph, MI, USA) and calculated as Nx6.25, lipid gravimetrically after acid hydrolysis and extraction with di-ethyl ether and ash gravimetrically after combustion at 540 C for 16h. Starch was analyzed using an enzymatic method described by Hemre et al. (1989). Amino acids were determined after hydrolysis of the protein with 6 M hydrochloric acid, derivatised with phenylisothocyanate (PICT), and analyzed in a Waters HPLC amino acid analyzer system using L- norlaucine as the internal standard. Minerals were determined using ICP-MS after complete digestion in nitric acid after cooking in microwave oven for 1h. Sterols were analyzed by: extraction of lipids with di-ethyl ether, saponification of fatty acids, extraction of sterols and separated by GLC and detected by flame ionization. Fatty acids were determined by GCL and vitamin C and vitamin A (tokoferol and tokotrienol) with HPLC.

Results and discussion

The seaweed powders had a protein level of 8 and 10% (Table 18). The level of amino acids was low reflecting the protein content in the powders (Table 19). Also the level of free amino acids (FAA) was low (Table 20), however there were differences between the two seaweed products; *Laminaria digitata* hawing more FAA than the OHT powder. Seaweed will therefore probably not have the expected attractant properties as other seafood ex. blue mussel. The two seaweed powders contained 0.3 and 2.6% glycogen respectively (Table 18). The literature describes a range of polysaccharides in seaweed, probably not detected in the method used for glycogen analysis (Holdt & Kraan, 2011). These compounds are probably reflected in the rest part in Table 18, consisting of 53 and 30 % of the two powders.

The total lipid level was below the limit of quantification (<1.5 %) (Table 18). The cholesterol levels in the two seaweed powders were approximately the same: 68.0 mg/kg and 50.5 mg/kg, respectively (Table 21), much lower than in fish meal as you can expect. The dominant phytosterol was sitosterol in both powders; however the level in the *Laminaria digitata* powder had much higher level than in the OHT powder (Table 21). The level of EPA was very different in the two powders where EPA counted for 11.6 % of the fatty acids in the *Laminaria digitata* and only 0.7 % of the fatty acids in the seaweed powder from OHT (Table 22). The level of DHA was low in both powders: for 0.9 % for *Laminaria digitata* and 0,6% for the powder from OHT (Table 22). In fish oil (anchovy) EPA is 17% of the fatty acids while the level of DHA is 8.8%. From these results *Laminaria digitata* can be a source for EPA.

The ash level was 28.9% in *Laminaria digitata* and 44.1% in the OHT powder (Table 18). This is a much higher level than in fish meal. Mineral analysis showed that the dominant mineral in *Laminaria digitata* was magnesium (Mg) while in the OHT powder the dominant minerals were Mg together with potassium (K). The OHT powder had higher level of phosphorus than *Laminaria digitata*, otherwise the mineral levels were the same. Both powders had higher or the same mineral levels as fish meal (Table 23). As the ash level was such high in the OHT powder this must be considered more a mineral additive than a protein source.

For the unwanted heavy metals they were all present, and mercury (Hg), cadmium (Cd) and led (Pb) were under the upper limit for feed materials (EU directive 2002/32/EC). However, the Pb level in the OHT powder being below the upper limit for feed materials it was high (7.18 mg/kg). The level of total arsenic was high and above the upper limit for feed materials in *Laminaria digitata*. High total arsenic levels are normal for seaweed and other seafood. In seaweed, arsenic will mostly be in the organic forms of arsenosugars, which are not acute toxic, and its therefore special legislation regarding upper limit for seaweed. However the level of inorganic arsenic was also very high, especially in *Laminaria digitata* (28.3 mg/kg), and both powders was above upper limit for inorganic arsenic for feed materials (2 mg/kg) (EU directive 2002/32/EC). It will be important for the use of seaweed in fish feed that the levels of heavy metals are monitored, and that they are harvested in an area with low pollution. Seaweed accumulates metals effectively and heavy metal levels in the environment will be reflected in the algae.

Table 18. Average macronutrient level (%) and vitamin level (mg/kg) in seaweed powder and herring fish meal.

Nutrient	*Laminaria digitata*	OHT	Fish meal, herring *
Protein	8.1	10.2	72
Lipid	<1.5	<1.5	8.4
Ash	28.9	44.1	10.4
Glycogen	0.3	2.6	--
Dry matter	92	89	92
Moisture	8	11	8
Rest**	53.2	30.6	--
Vitamin A	na	<0.05	--
Vitamin C	na	<0.2	--
Thiamine	na	0.6	--

*(NRC, 2011)
**Rest =100% - (%moisture- %lipid- %protein-%glycogen- % ash)
na = not analysed

Table 19. Level of indispensable and dispensable amino acid (%) in seaweed powder from two producers and in herring fish meal.

Indispensable amino acids	*Laminaria digitata*	OHT	Fish meal, herring *
Valine	0.35	0.41	3.26
Histidine	0.12	0.08	1.53
Leucine	0.41	0.48	4.69
Threonine	0.32	0.19	2.49
Arginine	0.29	0.36	3.73
Lysine	0.39	0.27	7.30
Methionine	0.12	0.13	2.20
Isoleucine	0.25	0.30	3.64
Phenylalanine	0.29	0.34	2.68
Dispensable amino acids			
Taurine	0.01	0.01	
Alanine	0.72	0.49	
Proline	0.32	0.35	
Tyrosine	0.18	0.19	
Serine	0.41	0.38	
Glycine	0.50	0.41	
Aspartic acid	0.86	0.83	
Glutamic acid	0.82	1.20	

*(NRC, 2011)

Table 20. Free amino acids (mg/g) in seaweed powder from two producers.

Amino acid	*Laminaria digitata*	OHT
Taurine	0.30	0.09
Aspartic acid	0.52	0.31
Threonine	0.15	nd
Serine	0.13	nd
Glutamic acid	0.79	0.45
Glutamine	0.44	nd
Glycine	0.24	nd
Alanine	3.00	0.24
Phosphoethanolamine	nd	0.02
Ammonium	0.05	0.31

nd = not detected

Table 21. The analysed level of sterols (mg/kg) in seaweed powder from two producers.

Amino acid	*Laminaria digitata*	OHT
Brassicasterol	51.1	4.4
Campesterol	94.3	17.8
Campestanol	0.5	1.9
Stigmasterol	5.1	9.0
Sitosterol	690.6	228.4
Sitostanol	17.8	48.3
Stigmasta-dienol	2.6	1.4
d-7-avenasterol	0	8.6
Sum fytosterol	**862.1**	**319.8**
Sum CHOL	**68.0**	**50.5**
Sum total	**930.1**	**370.4**

Table 22. The analysed level of fatty acids in seaweed powder used from two producers and in anchovy fish oil (% of fatty acids).

Fatty acids	*Laminaria digitata*	OHT	Fish oil, anchovy
<C12	<0.1	<0.1	--
14:0	6	4.6	7.4
16:0	19.2	52.3	17.4
18:0	1.8	2.3	4.0
Total sat	28.8	61.5	34.6
16:1	3.9	4.3	10.5
18:1n-9	22	8.7	11.6**
20:1	0.5	0.4	1.6
22:1	<0.1	<0.1	1.2
Total mono	27.7	17.4	24.9
18:2 n-6	5.5	5.6	1.2
18:3 n-6	0.5	0.2	0.1
20:4 n-6	5.4	2.7	0.1
18:3 n-3	5.6	1.5	0.8
18:4 n-3	7.5	0.8	3.0
20:5 n-3 EPA	11.6	0.7	17.0
22:5 n-3	0.1	0.1	1.6
22:6 n3 DHA	0.9	0.6	8.8
n3:n6 ratio	2.3	0.4	24.0
Total n-3	26.1	3.9	27.4

*(NRC, 2011)
** sum all 18:1

Table 23. Minerals (mg/kg) and heavy metals (mg/kg) in seaweed powder from two producers and in herring fish meal. The upper limit for heavy metals in feed ingredients and feed are also given (mg/kg).

Mineral	*Laminaria*	OHT	Fish meal	Upper limit in feed material
Iodine (I)	4300	230		
Sodium (Na)	30300	35000	16700	
Potassium (K)	26100	26800	22000	
Magnesium (Mg)	51900	27200	5900	
Phosphor (P)	6610	15500	10800	
Iron (Fe)	2060	1150	1400	
Selenium (Se)	0.193	0.098	1.95	
Heavy metals				
Total arsenic (As)	**67.3**	24.9		40***
Inorganic arsenic	**28.3**	**11.3**		2
Cadmium (Cd)	0.33	1.06		2
Total mercury (Hg)	0.014	0.009		0.5
Lead (Pb)	0.25	**7.18**		10

*(NRC, 2011)
**EU directive 2002/32/EC
***Upon request of the competent authorities, the responsible operator must perform an analysis to demonstrate that the content of inorganic arsenic is lower than 2 mg/kg

Acknowledgment

Tanks to the technicians at NIFES for excellent work with the analysis.

References:

Holdt, S.L. & Kraan, S. (2011) Bioactive compounds in seaweed: functional food applications and legislation. *J Appl Physiol*, **23**, 543-597.

NRC (2011) Nutrient requirement of fish and shellfish. (Hardy, R.W. ed. National Academy Press, Washington D.C. USA.

2.2.2 Use of different types of seaweed in diets for Tilapia (ICELAND)

Jón Árnason, Matís ohf., Stefanía Karlsdóttir, Matorka ehf.. Iceland

Introduction

There has been an interest in testing seaweed as an ingredient in diets for fish. The nutrient content of seaweed is in many respects different from the mainstream raw materials currently used in fish feed. In particular it is low in protein but high in total carbohydrates (in particular fibre) and minerals. It has been claimed that seaweed also possesses various bioactivity, due partly to considerable content of phenols.

Two types of Seaweed meals were tested:

- Kelp (*Laminaria digitata*) meal made by Thorverk Ltd. in Iceland (LAM)
- Meal from mixed seaweed produced by Ocean Harvest Technology in Ireland (OHT)

Materials and methods

Fish type: Tilapia from the strain of Íslensk matorka with an average weight of 72 grams was allocated into 15 x 200 L tanks giving an average biomass of 2 kg per tank (initial density 10 kg per m^3). The experiment was carried out in three replicates, in the facilities of Matorka at Fellsmúli

Water: Fresh water in a flow through system

Temperature: 24 - 28°C

Feeding: the fish was fed to apparent satiation two times per day. The feed offered to each tank was registered. After the afternoon feeding all uneaten feed was removed and stored in a freezer for later estimate of eaten feed.

Diets: Extruded diets with two inclusion levels of each of the seaweed products tested (LAM, OHT). Formulation of the different diets and their chemical content is shown in Table 24.

Table 24. Composition of the diets used in the experiment

Diet nr.	2988	2990	2989	2992	2991
Type	Control	Lam 5	Lam 15	OHT 5	OHT 15
Raw materials %:					
NSM FM 68,16	23,3	24,6	37,9	24,0	36,1
Wheat	12,6	8,0	8,0	8,0	8,0
SOYA 47/5 Brasil	20,0	20,0	7,0	20,0	10,0
Rape seed meal DK	20,0	18,2	10,0	18,8	10,0
Lam. digitata	0,0	5,0	15,0	0,0	0,0
Mixed Sea weed OHT	0,0	0,0	0,0	5,0	15,0
Corn gluten meal	20,0	20,0	18,0	20,0	16,7
Fish oil	3,1	3,3	3,1	3,2	3,2
Laxa premix	1,000	1,000	1,000	0,999	0,999
Chemical composition %:					
Water	6,0	6,0	6,0	6,0	6,0
Dry matter	94,0	94,0	94,0	94,0	94,0
Protein	46,0	48,0	49,0	45,0	46,0
Lipid	11,5	10,6	10,2	10,8	10,5
Ash	6,8	9,8	10,1	10,1	12,3
Glycogen	12,9	11,0	11,7	10,9	11,1
Rest	16,8	14,6	13,0	17,2	14,1

Results

The weight development of the fish, as average of three replicate tanks per treatment, is shown in Table 25.

Table 25. Fish weight development during the 48 day experiment

Diet nr.	2988	2990	2989	2992	2991
Treatment	Control	LAM 5	LAM 15	OHT 5	OHT 15
Weight development:					
Initial biomass	2,03	2,00	2,00	2,00	2,02
Biomass day 48	4,27	4,65	4,66	4,47	4,76

The growth and SGR was similar in all groups but a variation between the replicates within treatments was observed (Figures 19 and 21). Figure 20 and Figure 22 show the feed conversion ratio (FCR). No marked effect of effect of treatment was observed, neither on fish weight nor SGR or FCR.

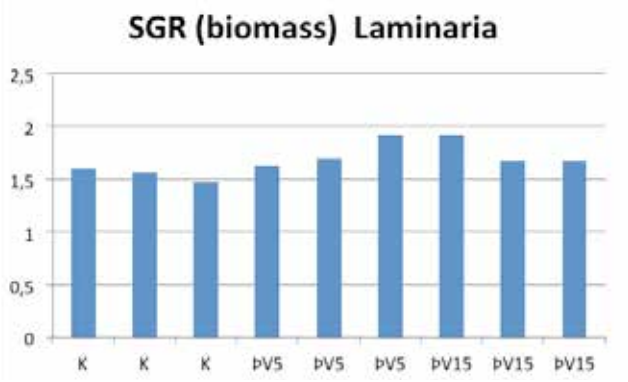

Figure 19. SGR of biomass in tanks fed diets containing different amounts of kelp meal. K = Control fed traditional feed, ÞV indicate 5% (Þv5) and 15% (Þv15) inclusion levels of the LAM kelp meal in the diets.

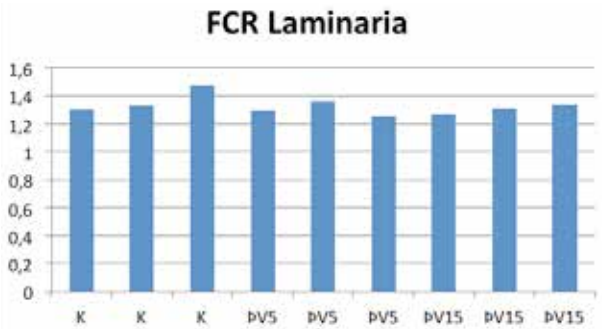

Figure 20. Feed conversion ratio (FCR) in tanks fed diets containing different amounts of kelp meal. K = Control fed traditional feed, ÞV indicate 5% (Þv5) and 15% (Þv15) inclusion levels of the LAM kelp meal in the diets.

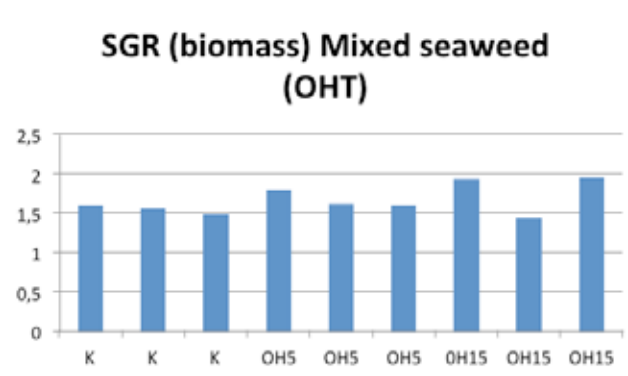

Figure 21. SGR of biomass in tanks fed diets containing different amounts of mixed seaweed meal (OHT). K = Control fed traditional feed, 5 (OH5) and 15 (OH15) indicate 5 and 15% inclusion levels of the OHT kelp meal in the diets.

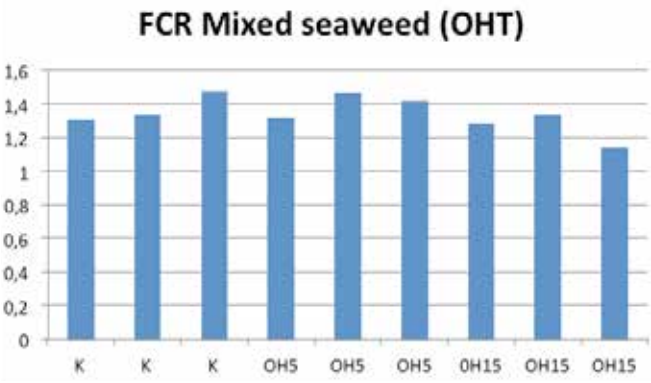

Figure 22. Feed conversion ratio in tanks fed diets containing different amounts of mixed seaweed meal (OHT). K = Control fed traditional feed, 5 (OH5) and 15 (OH15) indicate 5 and 15% inclusion levels of the OHT kelp meal in the diets.

Discussion and conclusion

The results show that inclusion of the seaweed products tested did not have significant effect on neither growth nor feed utilization in Tilapia. When looking at the effect of the inclusion of the seaweed products on the diet optimisation it can be seen that the mineral and vitamin content in the seaweed meals are not competitive with the mineral and vitamin premix, with the exception of a slight effect when using the OHT product (see Table 24.).

Even though seaweed is found in abundance, in the Nordic countries, it has low nutrient densities (see separate report on the chemical composition of seaweeds) and therefore its use as raw material in diets for tilapia fully depends on its marked price. The present indicative prices from the meal suppliers however do not make the use of these types of seaweed profitable.

2.2.3 Use of different types of seaweed in diets for Arctic charr (ICELAND)

Jón Árnason, Matís ohf., Iceland

Introduction

There has been an increasing interest in using seaweed as an ingredient in diets for fish. The nutrient content of seaweed is in many respects different from the mainstream raw materials currently used in fish feed. In particular it is low in protein but high in total carbohydrates (in particular fibre) and minerals. It has been claimed that seaweed also possesses various bioactivity, due partly to considerable content of phenols.

Two types of Seaweed meals were tested:

- Kelp (*Laminaria digitata*) meal made by Thorverk Ltd. in Iceland (LAM)
- Meal from mixed seaweed produced by Ocean Harvest Technology in Ireland (OHT)

Materials and methods

Fish type: 825 Arctic charr from Íslensk matorka with an average weight of 270 grams were allocated into 15 x 700 L tanks giving an average biomass of 14,8 kg per tank (initial fish density of 10 kg per m^3).

Diets: Extruded diets with two inclusion levels of the two seaweed products, LAM and OHT. Formulation of the different diets and their chemical content is shown in Table 26. The experiment was carried out in three replicates, in the facilities of Matís at Keldnaholt.

Water: Fresh water in a recirculation system

Temperature: 9°C

Feeding: The fish was fed to apparent satiation, partly by automatic feeders and partly by hand feeding two times per day. The feed offered to each tank was registered. All uneaten feed was removed (before feeding in the morning), the number of uneaten pellets counted and the weight calculated to dry pellet according to the average weight of dry pellets. The dry weight of uneaten feed was then subtracted from the feed offered to each tank.

Table 26. Composition of the diets used in the experiment

Diet nr.	2988	2990	2989	2992	2991
Type	Control	Lam 5	Lam 15	OHT 5	OHT 15
Raw materials %:					
NSM FM 68,16	23,3	24,6	37,9	24,0	36,1
Wheat	12,6	8,0	8,0	8,0	8,0
SOYA 47/5 Brazil	20,0	20,0	7,0	20,0	10,0
Rape seed meal DK	20,0	18,2	10,0	18,8	10,0
Lam. digitata	0,0	5,0	15,0	0,0	0,0
Mixed Sea weed OHT	0,0	0,0	0,0	5,0	15,0
Corn gluten meal	20,0	20,0	18,0	20,0	16,7
Fish oil	3,1	3,3	3,1	3,2	3,2
Laxa premix	1,000	1,000	1,000	0,999	0,999
Chemical composition %:					
Water	6,0	6,0	6,0	6,0	6,0
Dry matter	94,0	94,0	94,0	94,0	94,0
Protein	46,0	48,0	49,0	45,0	46,0
Lipid	11,5	10,6	10,2	10,8	10,5
Ash	6,8	9,8	10,1	10,1	12,3
Glycogen	12,9	11,0	11,7	10,9	11,1
Rest	16,8	14,6	13,0	17,2	14,1

Results

Fish growth, Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR)

The weight development of the fish, as average per treatment is shown in Figure 23.

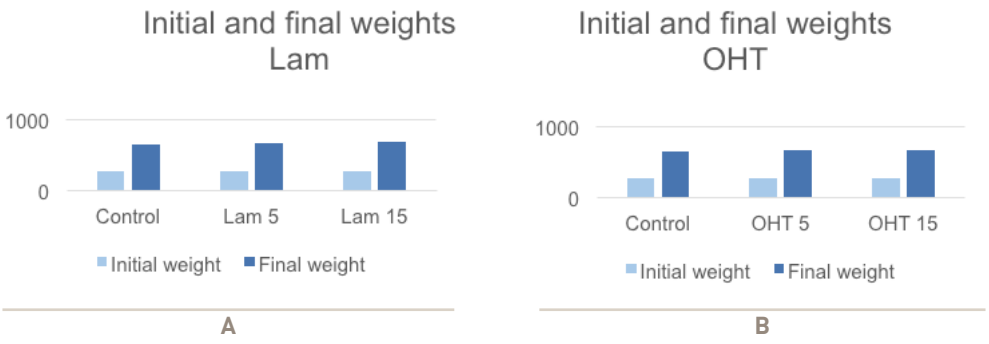

Figure 23. Average initial and final weight of the fish in the growth trial (A: *Laminaria digitata*; B:Ocean Harvest Technology: Mixed Seaweed meal)

The growth and SGR is similar in all groups but there is variation between the replicates within treatments (Figures 24 and 26). Figure 25 and Figure 27 show the feed conversion ratio (FCR). No marked effect of effect of treatment on either fish weight or SGR or FCR were observed.

Figure 24. SGR of fishfed different amounts of the LAM kelp meal in 5% and 15% inclusion levels.

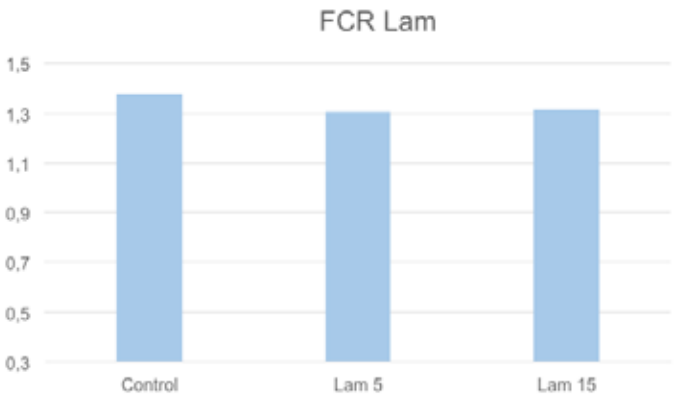

Figure 25. Feed conversion ratio (FCR) of fish fed different amounts of the LAM kelp meal in5% and 15% inclusion levels.

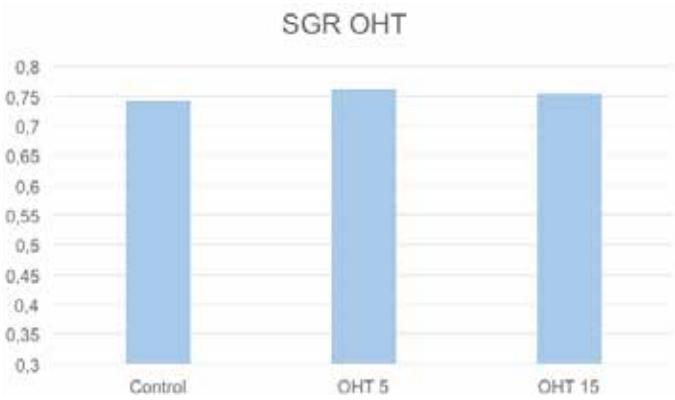

Figure 26. SGR of fish fed different amounts of mixed seaweed meal (OHT) in 5% and 15% inclusion levels.

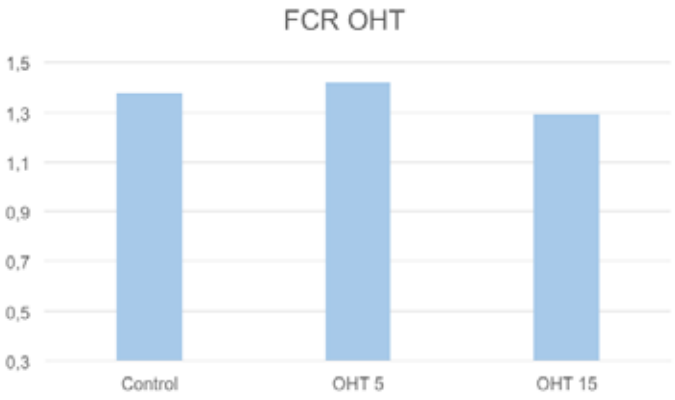

Figure 27. Feed conversion ratio of fish fed different amounts of mixed seaweed meal (OHT) in 5% and 15% inclusion levels.

Composition of fish

No effect of different diets on the nutritional composition of the filets related to treatment were observed (Table 27.).

According to the report on the chemical composition from NIFES, the content of total Arsenic as well as inorganic arsenic was high in both seaweed types tested in the experiment. Analyses of arsenic in the filets of the Arctic charr however showed that all samples contained arsenic lower than the detectable level of 0.4 µg/kg.

Table 28 shows characterization of the lipid in the filets. There seems to be some effect of the seaweed inclusion on the content of the fatty acids EPA and DHA, and the ratio between n-3 and n-6 fatty acids, but these effects were not consistent.

Table 27. Nutrient composition of the filets as a resultant of different types and inclusion of seaweed.

Diet nr.	2988	2990	2989	2992	2991
Diet	Control	LAM 5	LAM 15	OHT 5	OHT 15
As is:					
DM%	29,9	29,5	30,6	31,5	29,0
CP%	20,7	21,0	20,0	20,7	21,0
Total lipid %	6,6	6,7	7,9	8,5	5,9
Ash %	2,6	4,3	3,4	2,9	3,5
In DM:					
CP%	69,2	71,1	65,3	65,6	72,4
Total lipid %	32,1	31,7	39,5	41,3	27,9
Ash %	39,4	64,7	42,9	34,0	60,2

Table 28. Characterization of the lipid content of filets after feeding different types and inclusion levels of the seaweed products tested.

Diet nr.	2988	2990	2989	2992	2991
Diet	Control	LAM 5	LAM 15	OHT 5	OHT 15
SUM sat.%	20,8	20,5	21,1	20,5	20,9
SUM.mono.sat. %	50,5	49,2	49,9	50,6	49,3
EPA %	3,3	3,6	3,7	3,3	3,7
DHA %	9,4	10,0	9,9	8,8	10,5
SUM. EPA+DHA %	12,7	13,6	13,6	12,1	14,2
n-3 / n-6 ratio	1,9	1,9	2,3	1,8	2,2

Conclusion

The results show that inclusion of the seaweed products tested did not have significant effect on neither growth nor feed utilization in Arctic charr. The use of these products in feed for Arctic charr will depend upon the price of the raw material whereas it is rather low in its contribution of nutrients into the feed formulation.

2.4 Microalgae (ICELAND)

Jón Árnason, Matís ohf. ICELAND

Interest in the use of micro algae as feed ingredient in aquaculture feed has been increasing the last years, as protein- and in particular, lipid source in addition to the presence of possible bio-active compounds in the algae biomass. Over the years, a number of research studies has been done in the field of optimising microalgae production, mainly with the aim of using the oil fraction of the algae for production of biofuel. The defatted biomass from the microalgae could then be used as a raw material in animal feed. The nutritional value of the de-fatted biomass is similar to fishmeal, meaning that it contains all the essential amino acids. It is also rich in vitamins and minerals along with its unique bioactive compounds. Whole microalgae could also be of interest as ingredients in fish feed as they are natural sources of the essential fatty acids EPA and DHA.

Resent research also show that such a defatted biomass from microalgae can replace some of the corn and soybean used in diets for pigs, broilers and laying hens.

The intention in this project was test different types of micro algae in diets for fish (tilapia). A considerable effort was put into finding some commercial algae, but the harvest of that was very poor, as it appears that there is a very limited availability of microalgae in the quantities necessary for testing in fish feed. Several companies stating that they are developing microalgae products for fish feed, were contacted but none of them had any product ready for testing in growth trials with fish. The types of micro algae available in the free market cost about $ 40 per kg and are therefore far from being feasible to use as a significant raw material in formulation of fish diets.

Materials and methods

Microalgae for the studies.

Five different types of microalgae were collected for the investigation, two from the Blue Lagoon in Grindavík, Iceland and three types cultivated at the laboratory of the University of Akureyri and Matís ohf. in Akureyri, Iceland. However, the quantities obtained were considered too small for testing in growth trials for fish as intended.

Composition

The composition of macronutrients was analysed in dried samples of the microalgae.

Diet formulation

The micro algae were formulated as raw materials into start feed using WinMix linear optimisation programme, to fulfil the nutrient requirement of the tilapia.

Results

Nutrient composition

The nutrient composition of the obtained samples of micro algae biomass is shown in Table 29.

Table 29. Nutrient composition of micro algae

	Percentage of dry matter (DM)			
	Protein	Lipid	Ash	"rest"
From Blue lagoon:				
1BL freeze dried	19,4	23,8	30,1	26,7
2 BL dry	43,5	1,2	20,3	35,0
From Akureyri (freeze dried algae):				
Chlorella sp.	6,5	4,5	76,4	11,3
Nannochloris sp.	21,4	4,4	NA	NA
Pheodactylum tricornutum	30,0	3,1	57,5	15,1

There was a considerable variation in the nutrient content amongst the different microalgae. The crude protein content varyied between 6,5-43,5% and the lipid content ranged from 1,2% to 23,8%. In summary, it is obvious that the nutritive value of different microalgae can vary considerably.

Diet formulation

Example of formulations with similar nutrient compositions with different types of microalgae are presented in Table 30.

Table 30. Formulation of diets with micro algae

Raw materials / Diet	Control	1BL freeze dried	2 BL dry	*Chlorella* sp.	*Nanochloris* sp.	*P. tricornutum*
Inclusion %						
Wheat	39,7	33,9	36,0	32,9	34,2	34,9
Fish meal	15,8	16,4	16,9	16,2	16,5	16,7
Soya meal	10,0	10,0	10,0	10,0	10,0	10,0
Canola meal	20,0	20,0	20,0	20,0	20,0	20,0
Corn gluten meal	13,5	12,3	9,2	13,9	12,0	10,8
Micro algae	0,0	5,5	5,5	5,5	5,5	5,5
Fish oil	0,0	0,8	1,4	0,6	0,9	1,1
Premix	1,0	1,0	1,0	1,0	1,0	1,0
Chemical composition %						
DM	89,0	89,0	89,0	90,0	89,6	89,5
Crude protein	35,0	35,0	35,0	35,0	35,0	35,0
Lipid	5,9	7,8	7,2	6,6	6,9	7,0
Ash	5,9	7,5	7,0	9,9	5,9	8,9
Starch	27,9	24,3	25,0	23,9	24,3	24,6

As seen in Table 30, all microalgae products are included at the same concentration in order to test if there are some positive nutritive effects of the inclusion of the different algae masses. The inclusion of algal mass was conducted based on the possible use of the algae with the lowest nutrient content, i.e. *Chlorella* sp. produced in Akureyri, Iceland.

Discussion

The effort of finding different micro algae to be tested in this project revealed the fact that the work on developing the microalgae into compatible raw material in practical diets for fish still has a long way to go. Most of the development is still only in the lab scale stage and only a handful of products have appeared in the market. Furthermore, these products are still priced in such a way that they are far too expensive to be able to compete with other sources of nutrients. The variability in nutrient content of the different micro algae tested was analysed and the algae test formulated into diets based on that analyses. The nutritionally poorest algae could not be incorporated as more than 5,5 percent in diets for Tilapia if necessary nutrient supply should be adequate to meet the minimum needs for start feeding of the species. However, it was no point in testing these samples in trials with fish, while one is awaiting further choices of micro algae for evaluation of the nutritional quality.

3. Carbon footprint of novel diets

The new raw materials tested in this project are all locally available within the Nordic countries and therefore the need of transportation should be significantly reduced compared to the commercial feeds used in today's aquaculture. This is important, as transportation of raw materials over long distances is adding a considerable part to the carbon footprint (and price) in present feed production.

Another characteristic of the new raw materials tested in the project grow on and utilize components resulting in the carbon footprint and other environmental factors of other activities such as agriculture and other human activities creating "pollution" of the environment.

Yet another characteristic of the new raw materials is for example that they are based on the utilisation of resources that are currently under- or un-utilized. By substituting classical ingredients from industries that cause high environmental impacts, such as crop production and fisheries, with raw materials tested in this project, a considerable reduction in carbon footprint could be reached.

Originally a separate work package in the LIFF project was intended to evaluate the effect of the new raw materials on the carbon footprint of Nordic aquaculture by use of Live Cycle Assessment (LCA) analyses. This work was planned to be carried out by a Canadian collaborator (Dr. Andre Dumas at the Coastal Zones Research Institute in New Brunswick) and financed by Coastal Zones Research Institute and official Canadian funding. However, the financing of this part of the project failed due to lack of funding for the project in Canada and therefore Dumas had to withdraw from participation in the project.

4. Innovation process

The innovation angel in the present process was to test out novel raw materials in feed for fish.

Integration of local raw materials as ingredients in fish feeds would be a new approach in sustainable aquaculture including a significant reduction in the carbon footprint of the production.

A Nordic Innovation supported network project done by the group in 2010-2011 pointed out the main opportunities of using locally produced raw materials in the Nordic countries. These include use of rapeseed, barley, microalgae, seaweed, mussel meal, squid meal, starfish meal, single cell proteins, fungus, invertebrates and use of remnants from one fish species to another.

This project focused on utilization of mussel meal, seaweed and microalgae in fish feeds.

Mussel meal is a unique alternative ingredient in fish feed due to its nutritional characteristics, similar to those of fish meal regarding amino acid profile.

Further mussel meal obtained from "environmental mussel production" is a highly innovative and novel step towards sustainable and environmentally friendly finfish aquaculture. The mussels remove nitrogen and phosphate from the water; they live in, by filtering nutrient particles and microscopic organisms, converting non-food into food. Adjacent farming of mussels absorbing the nitrogen discharged from fish metabolism, in fish farms, envisage future nitrogen neutral fish production. Mussels can also be used to reduce the present biological loud characterizing areas such as the Baltic Sea. The mussels could in turn be used as a raw material for mussel meal production. In addition undersized mussels, from present production for human consumption not used for human consumption, should be used for production of mussel meal.

Using mussel meal in fish feed, nitrogen and phosphate is eco-cycled and thus closing the nutrient loop, while the mussel shells may be used for poultry feed, contributing to further lowering the carbon footprint of the production.

Seaweed and microalgae are major natural resources of feed ingredients. Seaweed has been used for human consumption, known as a healthy food supplement providing necessary amino acids, beneficial polysaccharides, fatty acids, antioxidants, vitamins, minerals and possible bio active compounds. Much less information exists on the use of seaweed as an ingredient into fish feed.

Microalgae can also become a sustainable substitute for fish oil and fish meal due to its content of essential amino acids and the essential fatty acids EPA and DHA. Microalgae can be produced under controlled conditions in the effluents from aquaculture productions providing a natural circulation of N, P, C and other matter.

The innovative angel of the project is:

- The new feed formulations increase the possibility of using local ingredients in aquaculture feed.
- All the raw materials evaluated can contribute to eliminate biological load from other human activities and turn them into valuable feed ingredients.
- The new ingredients tested lead to increase the sustainability of Nordic aquaculture and can help reducing the carbon footprint in Nordic aquaculture
- The findings from the project have also been presented for the feed industry both within the project to Skretting AS, as well as informally to others, from the aquaculture industry, outside the project as part of consultancy. The comments from the industry are positive but for the time being the materials tested are not commercially available. The industry will surely follow the development of alternative ingredients in the future.
- All participants will take active part in relevant national and international conferences and workshops where results from the project will continue to be communicated and disseminated even though the project has come to an end.
- Participants in project have been contacted by different stakeholders in the Nordic aquaculture and marine industries, about the findings of the project. This indicates an interest of use of local ingredients into fish feed to increase sustainability, if the cost of the solutions are economically feasible.

None of the participants in the project had any experience in innovative processes but they were very satisfied with the way the project created value for them in their practical situations.

Table of abstract

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Title:

Local fish feed ingredients for competitive and sustainable production of high-quality aquaculture feed, LIFF

Abstract:

Studies were conducted to evaluate Blue mussel meal, seaweed meal and dried micro algae as ingredients in fish feed. The blue mussel meal was tested as replacement for fishmeal in feed for Rainbow trout. Two different commercial types of seaweed meal were tested at 0%, 5% and 15% inclusion in diets for Arctic charr and Tilapia. Five different types of micro algae were analysed for nutrient content and formulated into a feed meant for Arctic charr.

Blue Mussel meal was found to be comparable to fish meal as protein source in feed for Rainbow trout without having any effect on growth, feed utilisation or physical parameters. No negative effects of incorporating as much as 15% of neither of the two seaweed meals on growth and feed utilisation of the experimental fish were observed. There was considerable variation in the nutrient content in the different micro-algae collected and there was only room for 5,5% of the one, with the lowest nutrient content, in a start feeding formulation for Tilapia.
As a conclusion of the project it can be stated that from a nutritional point of view, both Blue mussel meal Seaweed meal are interesting and sustainable macro ingredients in future fish feed in the Nordic countries. These raw materials are locally available in the Nordic countries and therefore have a potential in lowering the carbon footprint of fish feed in the region. The evaluation of available micro algae for use as feed constituents showed that the development of the micro algae industry still has a long way to go before they represent a real alternative as raw material in fish feed production.

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Local fish feed ingredients for competitive and sustainable production of high-quality aquaculture feed

LIFF

The present project aimed at evaluating the possibilities of increasing the use of local resources in feed production for the growing aquaculture industries in the Nordic countries.

The results from the project show that there are possibilities to develop new raw materials within the Nordic countries that can substitute those presently used in fish feed and thereby increase the sustainability of Nordic aquaculture.

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