NORD University

MASTER THESIS

Deffated microalgae *Nannochloropsis* sp. as a feed ingredient for Atlantic salmon (*Salmon salar*). Effect on growth, feed utilisation, digestibility and physical quality of feed.

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Abstract

The aim of the study was to investigate the effect of defatted biomass of *Nannochloropsis* sp. in diets for Atlantic salmon on growth, feed utilization, nutrient digestibility, whole body and fillet composition. Effect on physical feed quality was also investigated. A 12 week growth experiment, followed by a three week digestibility experiment was carried out using six replicate groups of Atlantic salmon with 214 g start weight kept in sea water. Three diets were designed with a fish meal based control diet and defatted biomass of *Nannochlorpsis* sp. replacing fishmeal in the ratios 10% (low algae) and 20% (high algae). The results showed significant reduction in digestibility of protein and energy for each level of algae in the diet. Retention of lipid and energy was significantly lower in the fish fed the high algae diet compared to the control diet. The high algae group had significantly higher feed intake and feed conversion ratio compared to the other two feeding groups. The weight gain and specific growth rate tended to be reduced at highest algae inclusion. No differences were observed in whole fish proximate composition or fillet composition among the diets. The physical feed quality differed among the diets. Control feed had highest bulk density, pellet weight, fat leaking and water stability at 30 minutes, but lowest hardness compared to the other two groups. Diameter and expansion differed significantly among all three diets. No differences were observed among the diets for pellet durability index, sinking velocity and water stability after 45 min and 60 min.

This research have shown that inclusion of defatted microalgae *Nannochloropsis* sp. should be lower than 20% and closer to 10% in feed for Atlantic salmon. The use of algae in the feed improved physical quality in terms of hardness, durability and fat leakage, but reduced expansion rate and bulk density.

Keywords: Atlantic salmon, Microalgae, Feed ingredients, Growth, Digestibility, physical quality.

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1 Introduction

1.1 Global aquaculture production

In 1988, Food and Agriculture Organization (FAO) introduced a definition of aquaculture to differentiate between aquaculture and capture fisheries:

> „Aquaculture is the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated. For statistical purposes, aquatic organisms which are harvested by an individual or corporate body which has owned them throughout their rearing period contribute to aquaculture, while aquatic organisms which are exploitable by the public as a common property resources, with or without appropriate licences, are the harvest of fisheries“ (FAO, 1988).

The global aquaculture production has shown an average annual growth of 8% during the last three decades. In 1980, aquaculture production was around 7 million tons, growing to approximately 97 million tons in 2013, of which around 70 million tons was food fish. In the same period, wild capture fisheries have remained almost constant, at approximately 90 million tons per year (FAO, 2016). In World Bank report, Fish To 2030, it's estimated that aquaculture food fish production will reach global wild capture fish in 2030. With a growing population in the world, more of our food has to be produced by means of aquaculture. Increasing demand for fish cannot be met by wild capture simply because many fish stocks are already fully exploited, and increased harvest cannot be expected (Bank, 2013).

Farming of salmonids are contributing to the supply high value fish to the marked. The total supply of salmonids to the market was in 2014, approximately 3.5 million tons (Figure 1). Atlantic salmon was dominate species contributing with 2.3 million tons, of which 1.3 million tons were produced in Norway (Fiskeridirektoratet, 2016).

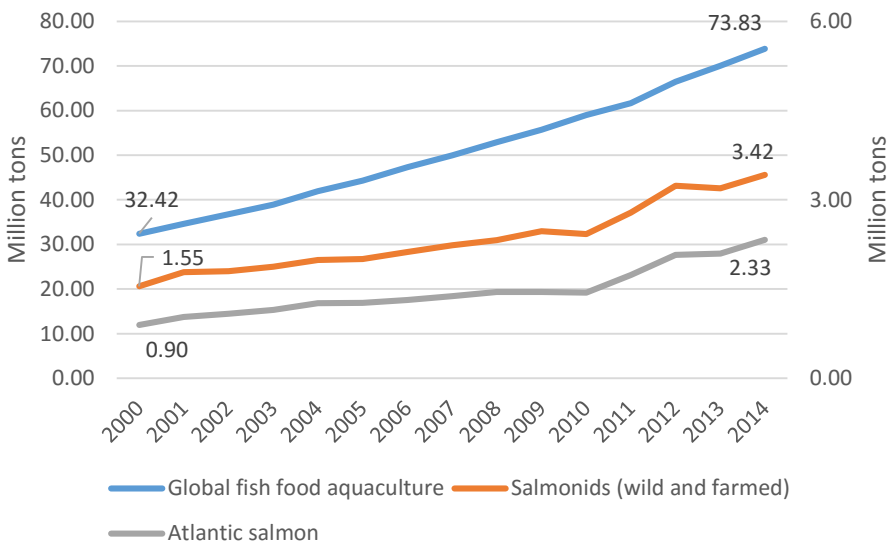

Figure 1. Global aquaculture-, Salmonids- and Atlantic salmon production from 2000-2014 (FAO, 2016).

1.2 Animal feed production

Animal feed production is a big industry. In 2014, approximately 980 million tons of animal feed were produced globally and the distribution among species is shown in Figure 2. Production of feed for poultry, pigs and ruminants dominated with more than 90 % of the total feed production while feed for aquatic animals was only 4 % (Alltech, 2015). To reach the predicted growth in world aquaculture production by 2030, production of aquafeed have to grow substantially.

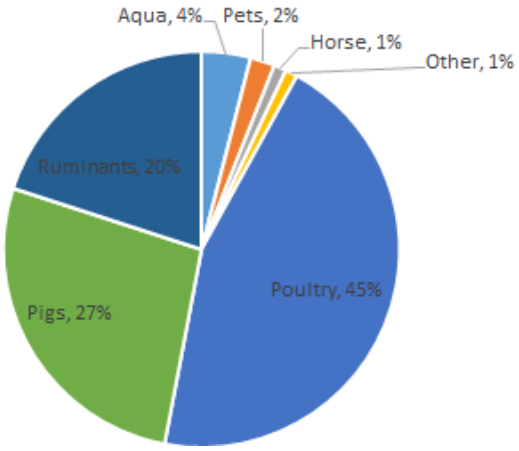

Figure 2. Feed production 2014 by species.

The current numbers of feed mills in the world is estimated to be around 31 thousands (Alltech, 2015). Feed manufacturing technology is a process combining knowledge of technology, chemistry and nutrition to produce a nutritious feed for animal, promoting good growth, animal health and product quality.

Feed technology for salmon feed production has developed substantially since 1980's. Since late 1980's, early 1990's, feed companies started to explore extrusion technology for manufacturing of salmonid feed. The technology was already used for production of food and pet feed. Extrusion process is a high – temperature - short – time process, of which feed ingredients are kneaded into a dough, cooked, and shaped under high moisture and high pressure (Figure 3) (Burnell et al., 2009).

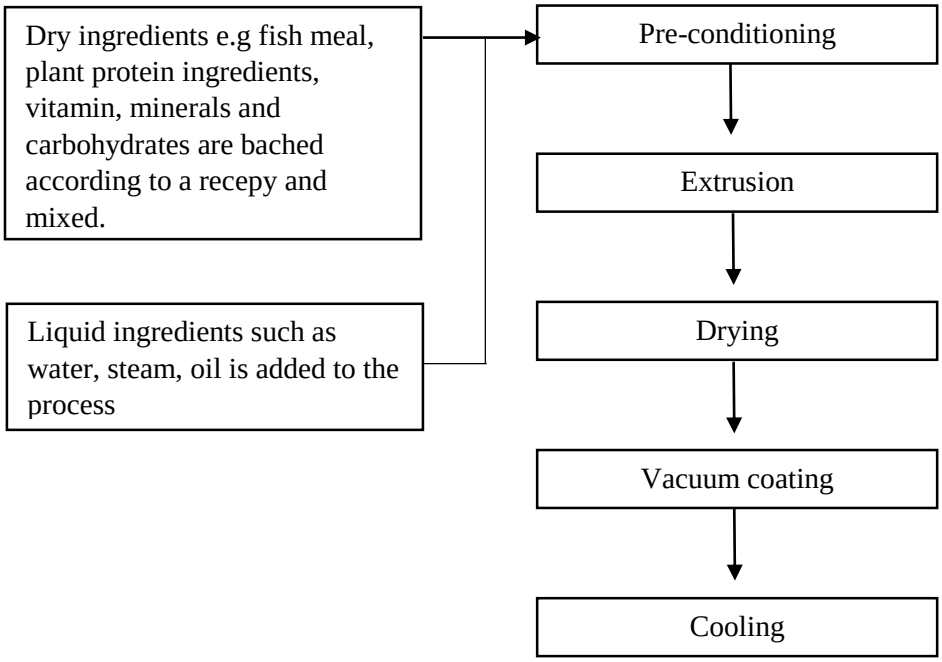

Figure 3. Main steps in production of extruded feed.

Most factories producing salmonid feed are constructed with a system to produce expanded pellets. The thermo-mechanical part of the feed production starts with pre-conditioning of the mixed dry feed ingredients. Main objectives of pre-conditioning is to moisten, heat and soften the ingredients.

Water and steam is added under constant mixing to moisten and heat the mash prior to extrusion. Small extruders with low production capacity (<100 kg/h) usually don't have pre-conditioner. Pre-conditioning can also be left out (by passed) when small feed amounts are produced, and large extruders are down scaled. The dry mix of ingredients is then conditioned in the extruder (Jun, 2012).

In the extruder barrel, the conditioned ingredients are kneaded into a viscous dough, usually for a short time period (5-40 seconds). This process include multiple steps, such as homogenizing, mixing and cooking, under elevated moisture (liquid and steam), pressure and high temperature (125-150°C). In the process, the feed ingredients are conveyed from the inlet to the outlet under constant mixing and sharing. At the outlet, pellets are shaped in a die, and cut into pellets by rotating knives. The instant pressure drop from high to atmospheric pressure, as the dough is forced through the die is flashing off water vapor. As liquid water (under high pressure) is transformed into water gas (low pressure), pellet is expanding. This process leaves small open pores in the pellet. The porous structure is important for production of high energy pellets. Oil and other ingredients that are sensitive for heat process e.g. carotenoids and vitamins can be added post extrusion employing vacuum coating technology. Vacuum coating is used in the production of high energy diets with lipid level up to 40%. Feed pellet are placed in the vacuum coater – an air tight container, where air is sucked out creating a negative pressure. Oil is sprayed onto the pellets under constant mixing. When vacuum is released, oil is pressed into the pellet (Oehme, 2013; Samuelsen, 2015).

Feed manufactures today are commonly combining different raw ingredients to meet the nutritional requirement for the fish species. Ingredients such as starch and protein are activated in the extrusion process and act as a binder in the feed. In presence of heat and water, starch gelatinize and protein denature. Gelatinization of starch is important for expansion of the pellet and for binding (Aarseth, 2004; Glencross et al., 2007). Protein are also important binders in the feed (Sørensen et al., 2009).

Physical quality of feed have been described as how well feed resist different handling without formation of dust or broken particles. Handling, such as conveying, storing in bins and feeding, is causing different impact on the pellet resulting in dust and broken pellets. Fines and small particles are not eaten by the fish and is considered as economical loss with adverse effects on the

environment, feed intake, nutrient digestibility and growth performance (Samuelsen, 2015; Sørensen, 2012; Oehme, 2013).

A wide range of variables affect pellet quality. Manipulating and changing processing condition such as moisture, temperature, retention time or pressure in the extruder, has an effect on pellet quality. Also feed ingredients has a strong impact on physical quality of feed. Plant ingredients often result in more durable pellets than marine ingredients (Sørensen, 2012).

Pellet quality of feed can be assessed by different methods as reviewed by Sørensen (2012). Some important quality parameters of aqua feeds are durability, water stability, sinking velocity, bulk density and oil leakage. Durability test measure feed particles and fines that are created after the feed was exposed by mechanical turbulences. Water stability test is used to investigate leaching rate of dry matter from the pellet. This method can also be used to mimic how feed dissolves in the fish stomach. High water stability minimize loss of nutrients when feed has been soaked in water for long periods. Sinking velocity measure how fast the pellet sink to the bottom. Slow sinking rate gives the fish longer time to catch pellet and minimize loss through water outlet in tanks or pellet drifting away in sea cages. Sinking rate can be adjusted in production process by adjusting bulk density. Bulk density is analysed as mass of feed pellet that is divided by volume. Bulk density is highly correlated with expansion rate. Fat leakage is a measure for oil leaking out of the pellet. Leaking of oil can be a problem in high energy feed.

1.3 Feed ingredients for Atlantic salmon

In aquaculture, feed is the highest single production cost ranging from 40% - 60% depending of species and farming system. Fish meal and fish oil are global commodities, and price fluctuate depending on the availability and demand. This has increased the feed cost for carnivore species that use most of the marine ingredients, such as salmonids, shrimps and marine fish (Figure 4). Other species such as tilapia and carp are less dependent on fishmeal and fish oil (Ytrestøyl et.al., 2015).

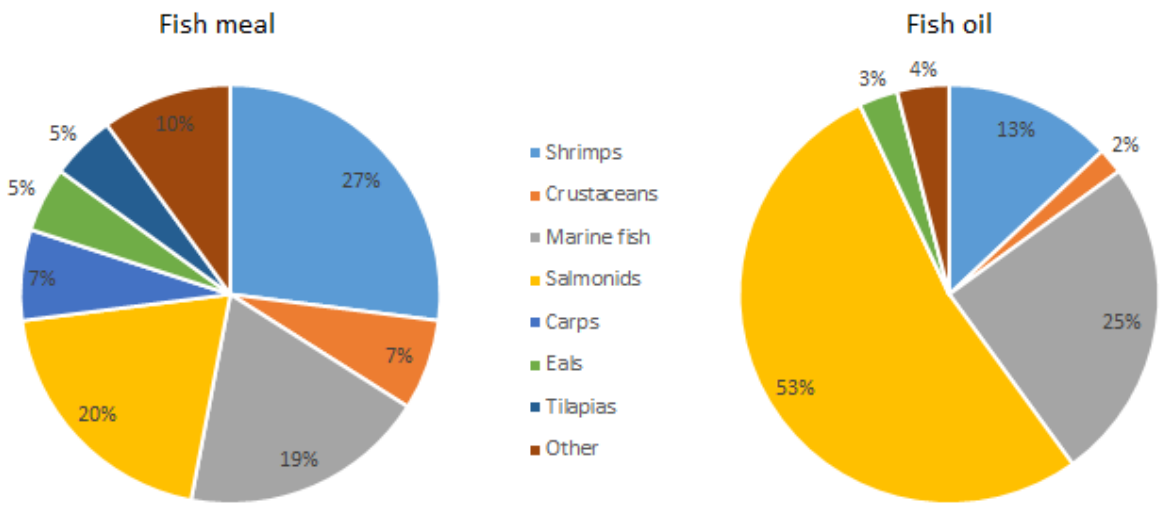

Figure 4. Utilization of fish meal. (left) and fish oil (right) for feed production in aquaculture 2012. Prodution of salmonids consume 20 % of fish meal and 53 % of fish oil, respectively.

Wild fish stocks used for production of fish meal and fish oil are tapped resource. Sustainability of salmonid farming depending on these resource are debated. Wild fish stocks that goes into production of fish meal and fish oil can also be used directly for human consumption to feed the growing world population (FAO, 2012). From 1990 – 2010, fish meal was the dominating protein ingredient in feed for Atlantic salmon, mainly because of steady supply and well balanced amino acid composition. Fish oil was the dominating oil used in salmon feed because of the favorable composition of long chain omega-3 (n-3) polyunsaturated fatty acid (EPA and DHA) and availability on global market (Maisashvili et al., 2015; Tacon et al., 2008; Ytrestøyl et al., 2015). Today, the availability of fish oil for salmon feed is nearly fully exploited. Salmon industry and other emerging market that use EPA and DHA in food, dietary supplements and clinical nutrition have grown substantially in past decade. The supply of fish oil is not increasing with the marked demand. This is creating competition in the raw material marked (ProAlgae, 2013).

Plant ingredients are extensively used in aqua diets today. Inclusion level of fish meal and fish oil in feed ingredients have changed during the past 20-30 years (Figure 5). This change is enabled by extensive research on alternative feed ingredients (Ytrestøyl et al., 2015; Sørensen et al., 2011a). The unsteady supply and fluctating price of fish meal and fish oil has also been a strong motivation to change ingredients composition in feed. However, use of plant ingredients that can be directly consumed by humans are also criticized and considered unsustainable (Torrissen et al.,

2011). There is also a limitation in use of plant ingredients because many of them have low nutritional quality, they are high in fiber and have unbalanced amino acids (AA) and may contain anti-nutritional components (Francis et al., 2001).

There is still a need to evaluate new ingredients from sources that are not used today. New ingredients may have an impact on nutritional and physical quality of feed, fish growth performances, digestibility, feed intake, and nutrient retention (Sørensen et al., 2011a).

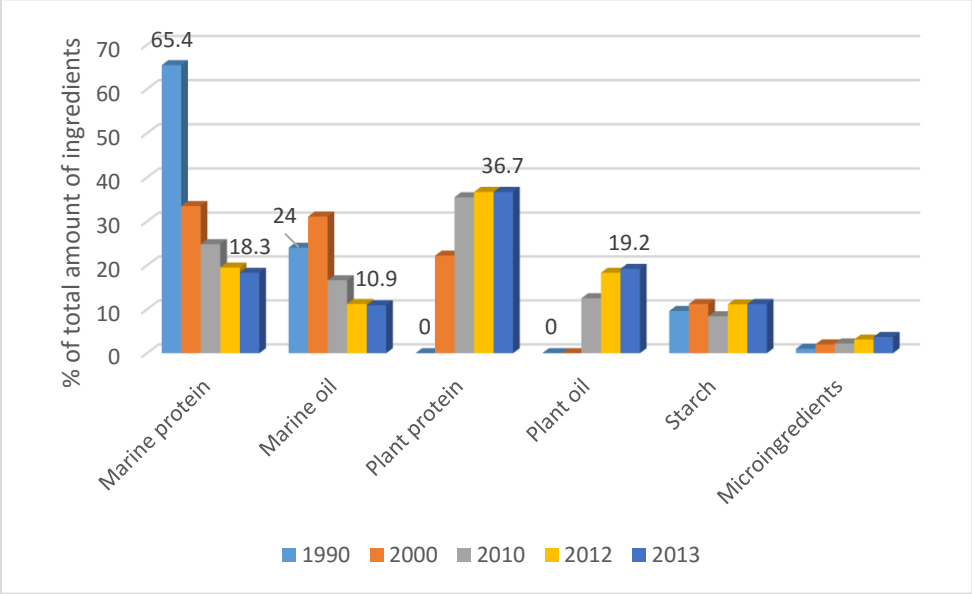

Figure 5. Evolution of salmon feed used in Norway from 1990 to 2013. We see that use of marine-protein and oil have been decreasing and use of alternative like plant- protein and oil have been increasing (Ytrestøyl et.al., 2015).

1.4 Microalgae as feed ingredient

Microalgae's are considered to be promising biomass for a more sustainable future. Microalgae are typically found both in fresh- and marine water. They can be either phototropic, heterotrophic or a mix of these two, called mixotrophic. For production of microalgae, phototrophic algae are consider to be more sustainable and more efficient then heterotrophic. That is because heterotrophic microalgae need organic carbon sources for growth, and that source usually comes from crop plants (Chisti, 2007). Microalgae are single-cell organisms which can live individually,

or in colony with a few other cells. They are different in size and shape and unlike macro algae and land plants they don't growth roots system, stems or leaves. Micro algae have several advantages in comparison to conventional crops. They have higher area productivity and do not need to be grown on arable land, they can be grown in open water or bioreactors, they have no need for pesticides, they have no adverse effect on biodiversity and overall the cultivation is easier. Their use will also contribute to a reduction in CO_2 footprint because they have higher carbon fixation rates than plants (Singh et al., 2011). Micro algae species belonging to *Nannocloropsis sp., Tetraselmis sp., Isochrysis* ISO-T., *H.pluvialis* and *C.vultaris* are also known to have antioxidant activity substance (Goiris et al., 2012).

In recent years, there has been growing interest in production of microalgae to extract the oil and use it for biodiesel. The leftovers, defatted microalgae contain proteins, minerals, vitamin, carotenoids and other bioactive components with antioxidant effects that may be as feed ingredients in aqua diets (Oilgae, n.d.).

For fish in general and carnivore fish in particular, microalgae are promising feed ingredients because of their nutrient profile. However, the chemical composition of microalgae differs among algal species. Hence, differences in bioavailability and nutrient utilization can be expected. Protein content of different algae species varies considerably (10 – 60%). If the algae have low nutrient bioavailability, poor palatability or contain anti-nutritional factors, adverse effects may be observed on the fish. It can lead to reduction in growth and feed performance.

Previous studies with Atlantic salmon fed *Tetraselmis Chlorophyceae* algae meal at 5-10% replacement of fish meal, showed no significant differences in growth performance compared to control diet (Kiron et al., 2012). Similar results was found for common carp and whiteleg shrimp fed with *Nanofrustulum Bacillariophyceae* at 25% and 40% inclusion level, respectively (Kiron et al., 2012). Red macro alga *Porphyra dioica* fed to rainbow trout (*Oncorhychus mykiss)* at 5 % and 10 % inclusion level in feed, had no significant negative effects on growth performance. However, significant lower final weight was observed when 15 % inclusion level was used (Soler-Vila et al., 2009). This indicate that inclusion level of various algae species has to be carefully optimized.

Protein digestibility in mink for the 3 microalgae species *Nannochloropsis oceanica, Phaeodactylum tricornutum and Isochrysis galbana,* where calculated to be 36%, 80% and 19%,

respectively. The authors suggested that *P.tricornutum* had higher digestibility because the cell walls of that algae was more easily digested compared to the other two algae species tested (Skrede et al., 2011). Apparent protein digestibility for Spirulina algae fed to Atlantic salmon and Artic charr were estimated to be 84.7% and 82.2, respectively (Burr et al., 2011). These results suggest that some microalgae are more suitable as feed ingredients for salmon than other.

Before the introduction of novel microalgae into commercial fish diets, research need to evaluate their quality as a feed ingredient as well as the safety. The quality of the ingredient is closely associated with digestibility and bioavailability of nutrients. Experiments have to be carried out to investigate how much of the nutrient is taken up in the digestive tract. Because nutritional quality varies a lot among algae species and processing procedures, different products have to be evaluated separately.

No studies have been reported earlier using defatted microalgae *Nannochloropsis* sp. as feed ingredients for Atlantic salmon. Therefore, the aim of the present study was: (1) to investigate the potential of using defatted microalgae *Nannochloropsis* sp. in feed for Atlantic salmon on growth, feed utilisation and nutrient digestibility and (2) to evaluate effect of alga biomass in feed on physical quality of feed. Based on published literature it was decided to use 10% (low algae) and 20% (high algae) inclusion level of defatted algae to replace fish meal.

2 Materials and Methods

2.1 Fish and experimental setup

A 12 week growth experiment, followed by a 3 week digestibility experiment was carried out at Nord University research station in Bodø, Norway for a period of February to June 2015. The experimental fish was Atlantic salmon (*Salmo salar, L.),* Aqua Gen strain hatched at Cermaq Hopen in Bodø. The fish was held at the research station for approximately 5 months before the start of the experiment. The temperature during these 5 months were average 5-6°C and fish were fed on a commercial diet. A total of 270 fish were used with an initial weight of 215.4 ± 25.8 g and fork length 26.4 ± 1.2 cm. The fish were distributed randomly into 18 tanks, with a water volume of approximately 740-L, with 15 fish in each tank. The photoperiod was 24-hour light during the experiment. Fish were adapted to the experimental units two weeks before the experiment started and before they were introduced to experimental diets. Each experimental tank was connected to a flow-through system supplying sea water to the tanks. Sea water was taken from 250 m depths in Saltfjorden with a salinity of 35‰. Both sea water and dissolved oxygen were recorded once per day in the morning. The water temperature during the experimental period had a mean of 7.1°C ± 0.01, ranging from 6.7°C to 7.4°C. Dissolved oxygen was measured in the outlet, with a mean saturation of 92.6 ± 0.37, ranging between 84% and 99%, Figure 6.

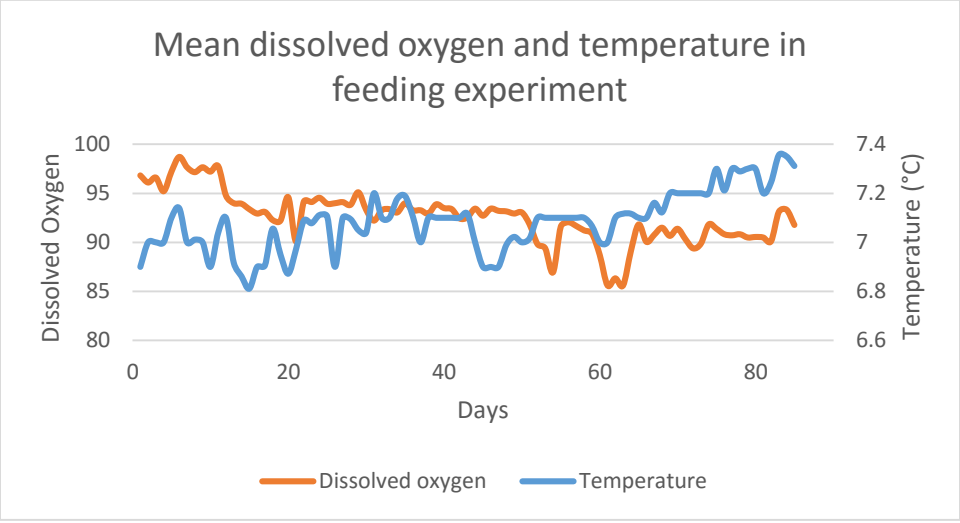

Figure 6. Mean dissolved oxygen and temperature in the experiment.
Oxygen and temperature was recorded through-out the experiment. Temperature and oxygen was inversely related to each other.

2.2 Experimental design and diet

An experiment was conducted to investigate the growth, feed utilisation and nutrient digestibility of feed containing the microalgae *Nannochloropsis* sp. A fish meal based control diet was used. Fish meal was replaced with defatted algae biomass at 10% and 20% in low (L) and high (H) algae groups respectively. Each diet was fed to 6 replicate tanks, 90 fish per treatment. The allocation of experimental feed to the different tanks are shown in Figure 7.

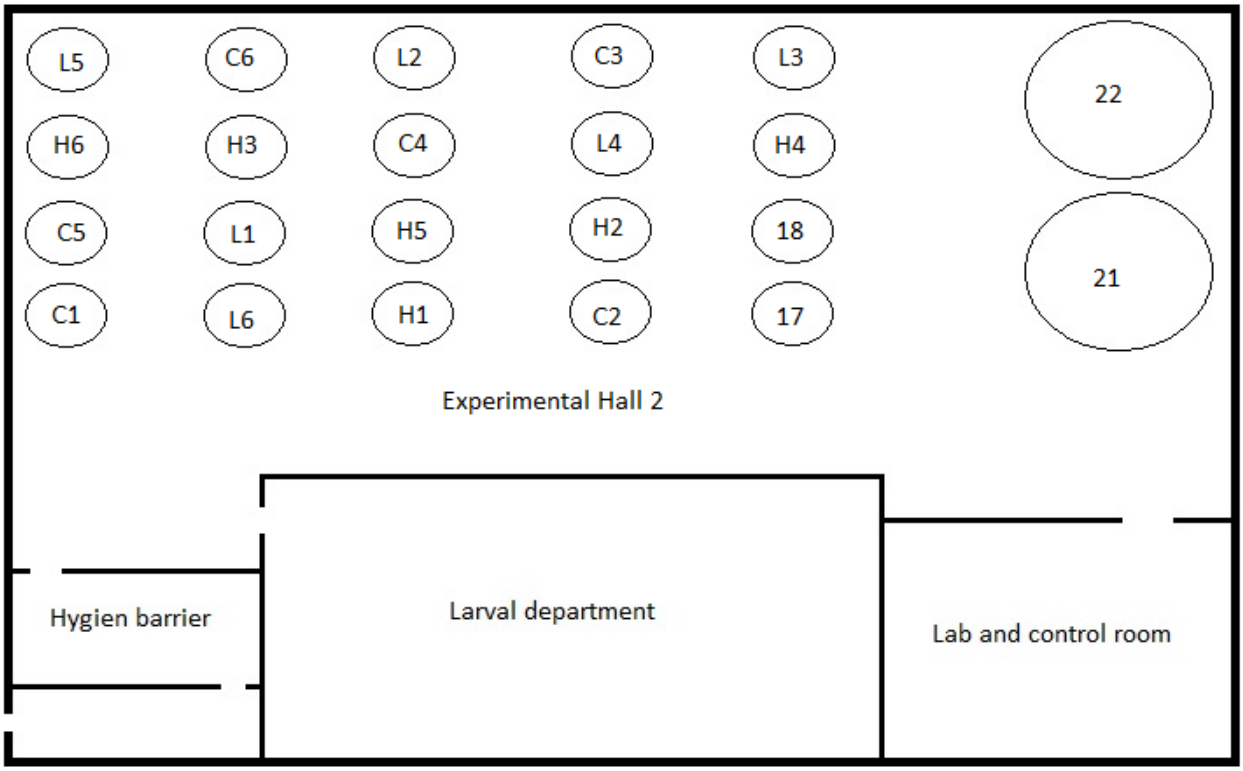

Figure 7. Allocation of feed to the experimental units. Each diet was in 6 replicate tanks and the fish was randomly distributed in 18 tanks for the 3 diet. Mark C is for control, L for low algae and H for high algae group.

The experimental diets were produced at Center for Feed Technology (Fôrtek) at Norwegian University of Life Sciences (NMBU), Ås, Norway. The feeds were extruded in a twin-screw Buhler extruder. The parameters for the extrusion process are shown in Table 1 and extruded pellet are shown in Figure 8.

Table 1. Extruder parameters. The extruder had 5 sections, all with different temperature range from 36°C – 127°C.

	Experimental diet		
Diet code	**Control**	**Low algae**	**High algae**
Die diameter	2 mm	2mm	2mm
Temperature in the extruder (°C)			
Section 1	37 – 38	36	39 – 40.0
Section 2	99 – 106	92– 98	90 – 91
Section 3	126 – 127	124 – 126	121 – 122
Section 4	112	108	106 – 107
Section 5	83 – 91	74	57 – 66
Water to the Extruder (kg)	12	13	13
Die temperature (°C)	95	92	76 - 78
Die pressure (bar)	21	25	30 - 32
SME (Wh/kg)	863 – 914	836 – 845	615 - 702
Torque (Nm)	334 - 351	347 - 366	342 - 381

SME stands for specific mechanical energy.

Figure 8. Visual apparence of the experimental diet after extruder. The color of the control diet was light brown and become ligh green in low algae and dark green in high algae.

The ingredient composition and proximate composition of the experimental diet is shown in Table 2. Proximate composition of defatted algae biomass is shown in Table 3.

Table 2. Ingredients composition and analysed chemical composition of the diets.

Ingredients (g/100g)	**Experimental diets**		
	Control	**Low algae**	**High algae**
Fish meal[1]	69	59	49.5
Algal biomass[2]	0	10	20
Wheat[3]	12	12	12
Wheat gluten[4]	5	5	5
Fish oil[1]	13.5	13.5	13.0
Microingredients[5]	0.5	0.5	0.5
Marker[6]	0.01	0.01	0.01
Diet - Proximate composition (g/100 g)[7]			
Moisture	5.8	5.8	6.4
In dry matter (g/100g)			
Crude protein	56.4	53.5	50.1
Crude lipid	21.1	20.6	20.0
Ash	11.4	12.3	13.3
Energy (KJ g^{-1})	24	24	23

[1] Nordsildmel AS, Norway. [2] Cellana LLC, Kona, Hawaii. [3] Felleskjøp. [4] Gluten Vital, Alimenta AS, Norway. [5] Vitamin and mineral mix is a proprietary formulation of Polarfeed, Leknes, Norway. [6] Metal Rare Earth Limited, Shenzhen, China. [7] Feed was analysed in 4 replicate.

Table 3. Proximate composition of defatted microalgae biomass used in feed.

Defatted microalgae biomass[1]	Chemical composition (g/100g)
Moisture	2.2
In dry matter (g/100g)	
Crude protein	43.0
Crude lipid	2.5
Ash	23.5
Carbohydrate[2]	28.8
Energy KJ g $^{-1}$	19.0

[1] Analyses of 4 replication of defatted algae biomass. [2]Carbohydrates were calculated by differences (100-moisture-crude protein-crude lipid-ash)

2.3 Feeding of fish

In the feeding experiment, fish were fed for 84 days. The Atlantic salmon were fed 2 mm pellets distributed in two meals per day. The two meals were fed in the morning from 08:00-09:00 and in the afternoon from 14:00-15:00, using automatic feeders (Arvo Teck, Finland). Fish were fed 1.2-1.5% of the biomass every day, adjusted on a weekly basis according to the weight gain. Approximately 30 minute before each feeding, all feed traps were flushed to remove faeces from the tank, in alter to minimize contamination of faeces in the collected uneaten feed. All uneaten feed and faeces was collected into a steel wire mesh. Faeces was removed and uneaten feed were frozen at -20°C. Dry matter (DM) of feed waste was determined by oven drying at 110°C overnight. DM was used to calculate DM feed intake. A recovery test was performed in the tanks after the experiment was terminated under the same environmental condition as during experiment (Helland et al., 1996).

2.4 Fish sampling and data collection

All 270 fish were individually weighted and fork length was measured at the beginning and at termination of the experiment.

All fish were anesthetized with Tricaine-sulfonate (MS 222, 100 mg/l) prior to all handling. Fish samples for whole body composition (n= 6) and fillet composition (n= 12) were taken from holding

tanks, anesthetized and stunned. 12 fish were filleted immediately and fillet from both side of two fish packed in bags, and frozen at -20°C. The fish for whole body analyses were packed and frozen at -20°C. 3 days later these samples was transferred to campus in Nord University and stored at -40°C for later analysing of proximate composition.

When the growth experiment was terminated, the experiment continued as a digestibility experiment for 3 weeks. Faeces was collected by stripping (Anderson et al., 1995). Fish were in total stripped 3 times (once per week) to ensure enough faeces for digestibility determination. Faeces from all fish in one tanks were pooled. Freeze dried samples for each tank were subsequently pooled two by two within treatments groups. Consequently, six tanks were reduced to 3 replicates before chemical analyses of faeces and used for calculation of nutrient digestibility. At the end of the experiment, fish was anesthetized with MS 222 and given a percussive stun. Three fish from each tank (n=18 for each diet treatment) was immediately filleted and fillets from both sides of these fish, six fillet in total were packed and frozen at -20°C. 6 fish for whole body analysis from each tank, n=36 for each treatment, total 108 fish was packed six in each bag, and frozen at -20°C. These fillets and whole fish were transferred to main campus three days later and stored in -40°C for later analyses of proximate composition.

2.5 Chemical analysis

Proximate composition of feed, faeces, initial and final whole body as well as fillets were analysed with establishment methods (AOAC – Official Methods of Analysis).

For preparation, fillets were taken out of the freezer and thawn for 3 hours before they were skinned and all bones were taken out. For initial and final fillet composition, fillets were minced to a homogeneous sample using a home food processor (Bosch, Grinder MCM2004/02, Slovenia). These samples were used for analyses of protein, fat, moisture and ash.

Fish from initial and final samplings for whole body composition were taken out from the freezer and thawn for one day before it was cut in 10 cm pieces, minced in an industrial food processor (Foss tecator 2096 homogenizer, Denmark) until it became homogeneous. These samples were used for protein, fat, moisture, ash and energy. For analyses of experimental diets, approximately 100 gr of each diets were homogenized in a food processor (Retsch, Grindomix GM 200, Germany). Finely ground samples were then used for analyses of protein, moisture, ash and

energy. Yttrium and lipid was analysed by Eurofins (Moss, Norway. Faeces were freeze dried (VirTis benchtop U.S) for 72 hour in -76°C with 20 µ Bar and analysed for protein, ash and energy. Yttrium and lipid in freeze dried samples of faeces were analysed by Eurofins.

Initial whole fish were measured in duplicate from individual 6 fish and initial fillet were measured in duplicate from 12 fish, total 4 fillet was pooled from 2 fish. Final whole fish were measured in duplicate from each experimental tanks (n=18), each pooled sample from 6 fish and final fillet were measured in duplicate from each experimental tanks (n=18), each pooled sample from 3 fish, total 6 fillet. Diet samples were measured in 4 replicate per diet. Freeze dried faeces were pooled two by two tank for each diet.

Protein analyses were carried out using Kjeldahl method (Kjeltec Autoanalyser, Tecator, Sweden). Approximately 1 g of homogenised fish samples (0.5 g for diet and 0,3 for faeces) were weighed into a nitrogen free paper and put into a glass tube which were then placed in a fume hood with ceramic bottom. Two Kjeldahl tablets were put in each of the two glass tubes with samples (also in control and blind sample) and 15 ml of concentrated sulphuric acids (H_2SO_4). Glass tube with samples was placed at 420°C and heated for 45 minutes, cooled down for 20 min. Distilled water and 75 ml was mixed with the sample. The glass tube were placed in Kjeltec auto analyser and the amount of N determined. The factor used for calculating protein from nitrogen content was 6.25.

Moisture content were determined by oven drying at 105°C for 20 hours. Approx. 5 g of homogenised fish samples and or diet (1 g for faeces) were weighed in a steel cup. Weigh before and after oven drying was used for calculation of moisture in %.

Lipid content was measured by extracting the fat out of the sample. Homogenised fish and or diet samples approx. 10 g were weighed and transferred to a porcelain cup. 20 g of water and 20 g sodium sulphate was put in the same cup and mixed well together to a dry powder and transferred to a bottle with a 50 g of Ethyl acetate. These bottles were held on shaker for one hour before solution was extracted through a Whatman (41, CAT NO. 1441-150) filter paper and into a glass cylinder. Samples were then pipette to a weighed glass cup and the solution evaporated over water bath until all the water was evaporated. The glass cup with remaining moisture were dried in oven at 105°C for 20 minutes, then dried samples were weighed and used to determination of the lipid content.

Ash content was determined gravimetrically after flame combusting at 550°C for 12 hours. Approximately 5 g of homogenised fish sample and or diet (0.5 g for faeces) were weighed before and then after oven drying and results used for a calculation of ash %.

Energy were measured by bomb calorimetry (IKA c200, GmbH & Co. KG, Germany). Prior to the energy analysis, homogenised fish samples of 1 g (0.5 for faeces) were made in a pelleting press to make pellets. Pellet were then put in bomb calorimetry for analysing and date were recorded.

2.6 Physical quality of diet

Physical quality of experimental diets were evaluated by different methods. These were sinking velocity, durability, texture, bulk density and fat leakage.

2.6.1 Sinking velocity

Sinking velocity was measured as described by Obirikorang et al. (2015) with some modifications. 15 pellet were randomly selected for determination of sinking velocity and the test was run in six replicate. A 50 cm transparent cylinder, 8 cm (width) was used for the test. The cylinder was filled with water (8.5°C and salinity 35‰) from the experimental tank and was changed among the replicate measurements. Sinking velocity was recorded with a Go pro camera over a distance of 15 cm between two marks at 10 cm and 25 cm repertory from the top. Single pellet was dropped from 5 cm height over the water surface. Those pellet that had contact with the wall of the cylinder were excluded from the calculation. Recorded video were imported to a Go Pro Studio software and value given as a pellet sink in cm/sec.

2.6.2 Durability

Dry pellet durability were measured as described by Wolska et al. (2016) with a Ligno Tester (Borregaard LignoTech, Sarpsborg, Norway). Each diet was tested in 3 replicates. Approximately 100 g of dust free feed was placed in the pellet chamber. Air with a temperature of 36°C and a fixed pressure at 80 bar, was used to convey pellets in the air stream through a pipe. Air was blown through the chamber to sift out the dust. Remaining pellets were weighted and pellet durability Index (PDI) were calculated based on remaining pellet and initial pellet and given as %.

Water stability test were conducted as described by Obirikorang et al. (2015). Approximately 1 g of samples were weighted into a pre-weighed histology cassette 3.2 cm * 2.6 cm * 0.5 cm with a

1 mm width * 5 mm length outlet. None of the pellet were under pressure while the cassette were closed. Pellet were randomly chosen and each diet were measured in 3 replicate at different time period which include 15, 30, 45 and 60 minute and temperature of 25°C in each trial run. Each replicate were placed in to a beaker with distilled water. The beakers were placed in a water bath (Julabo SW22, JULABO GMBH, Seelbach, Germany), with a 100 rpm shake per minute. After each trial, the cassettes were taken out of the beaker, water was gently wiped off with a dry paper tissues. The remaining feed pellet residual and cassette were placed in an oven (80°C) and dried for 48 hours. After this process, the feed pellet residual and cassette were weighed to determine the dry matter calculated as water stability %.

2.6.3 Texture

Hardness of the extruded pellet as well as diameter were recorded using a TA-XT2 (Stable Micro Systems Ltd., Surrey, England) analyser. 20 pellet in six replicates for each diet were randomly selected. The feed pellets were placed horizontal and cracked under a cylinder probe SMP/0.5 The cylinder probe were 1.2 cm (width) and speed 0.1 cm with a 60% compression used to determine the hardness of each pellet. Total work in Newton (N) were automatic calculated in the stable micro system computer program. The length of each pellet were manually measured with a micrometer electronical caliper.

2.6.4 Bulk density

Bulk density was measured as a feed that enters a glass cylinder with a diameter of 6 cm and a volume of 290 ml. The feeds were gently poured into the cylinder through a plastic funnel. A ruler was then used to flatten the feed to the rim of the beaker. The analysis were performed in 6 replicate for each diet and bulk density was calculated as a gram of feed per liter.

2.6.5 Fat leakage

Fat leakage was determined by weighing approximately 5 gr of diet into a plastic tray with two layers of Whatman gel blotting paper (Grade GB003, 30x60 cm) in the bottom. This plastic tray with blotting paper and diet was oven dried in 40°C for 24 hours before weighed to determine the fat leakage and calculated as fat leakage in %. All analysis was performed in 6 replicates for each diet.

2.7 Calculation

Effects of the experimental diets on fish performance was calculated as:

Specific growth rate % day^{-1} (SGR) = ((Ln FBW – ln IBW)/D)*100

Where FBW is final body weight (g/fish) and IBW is initial body weight (g/fish) and D is the total days of experiment.

Weight gain (WG) % =(FBW-IBW)*100/IBW

Where FBW is final body weight (g/fish) and IBW is initial body weight (g/fish).

Thermal growth coefficient (TGC) = (FBW$^{1/3}$-IBW$^{1/3}$) / (∑DDG)*100

Where FBW is final body weight (g/fish) and IBW is initial body weight (g/fish) and DDG is daily degree days.

Feed intake (FI) % body weight per day^{-1} = 100*Total feed intake/total experiment days/square root FBW*IBW

Feed Conversion Ratio (FCR) = DFI/WG

Where FI= Dry Feed intake g/fish and WG= Weigh gain g/fish.

Nutrient Retention (NR) % = (FBW * FN (or E) content – (IBW * IN (or E) content) * 100 / N (or E) I

Where FBW is final body weight (g/fish) and IBW is initial body weight (g/fish), FN=Final Nutrient, E=Energy, IN=Initial Nutrient and NI= Nutrient Intake.

Apparent Digestibility Coefficient (ADC) = 1-(marker in feed / marker in feces) * (Nutrient content of feces / Nutrient content of diet)

Marker is Yttrium (III) oxide.

2.8 Statistical analyses

All observations from replicate tanks as well as diet and diet physical quality were analysed statistically using Graphpad Prism 6 (Graphpad Software Inc., La Jolla, CA, USA). One-way Anova was used to analyse the data. The data were checked for normality with Kolmogorov-Smirnov test with Dallal-Wilkinson-Lilliefor P-value and equal variance were checked with Bartlett`s test. Tukey's multiple comparison tests were used to rank significant differences among means. For non-parametric data, the Kruskal–Wallis test was used followed by Dunn's multiple comparison tests.

SPSS version 2.2 from IBM was used for Person's correlation coefficient and Shapiro-Wilk normality and Levene's equal variance in digestibility data as well as Lingno pellet durability where only 3 data was used.

The differences between groups were considered significant at $P < 0.05$ and a tendency if $0.05<P<0.1$.

3 Results

3.1 Fish growth performance

The fish was in good health and no mortality was observed during the growth experiment. In the digestibility experiment one fish in tank L6 was observed with a bleeding eye. Two fish died between 2nd and 3rd stripping. Repeated stripping, in total three times, may have been stressful, resulting in the mortality.

Biometrical data is presented in Table 4 and Figures 9-10. The fish grew from an average initial weight of 215.4 g to a final weight of 419 g, an average 94.5 % in body weight gain. There were no significant differences (P>0.05) in IBW, FBW, TGC or PER among diet groups.

Table 4. Growth and feed utilization of Atlantic salmon offered experimental diets during a 12 week experiment.

	Control	**Low algae**	**High algae**	**P value**
IBW (g)	214.5 ± 13.05	213.8 ± 9.16	217.9 ± 7.66	-
FBW (g)	429.0 ± 29.97	420.2 ± 32.45	407.7 ± 29.58	0.4999
TGC	2.6 ± 0.26	2.5 ± 0.20	2.3 ± 0.20	0.1250
PER	2.2 ± 0.15	2.1 ± 0.14	2.0 ± 0.24	0.2395

Numbers are shown as mean ± SD; n = 6 replicate tanks. Different letters in a row, if any, represent significant differences (P < 0.05) between groups.
IBW – Initial body weight (g)
FBW – Final body weight (g)
TGC – Thermal growth coefficient.
PER - Protein efficiency ratio.

The cumulative feed intake increased during the course of the experiment (Figure 9).

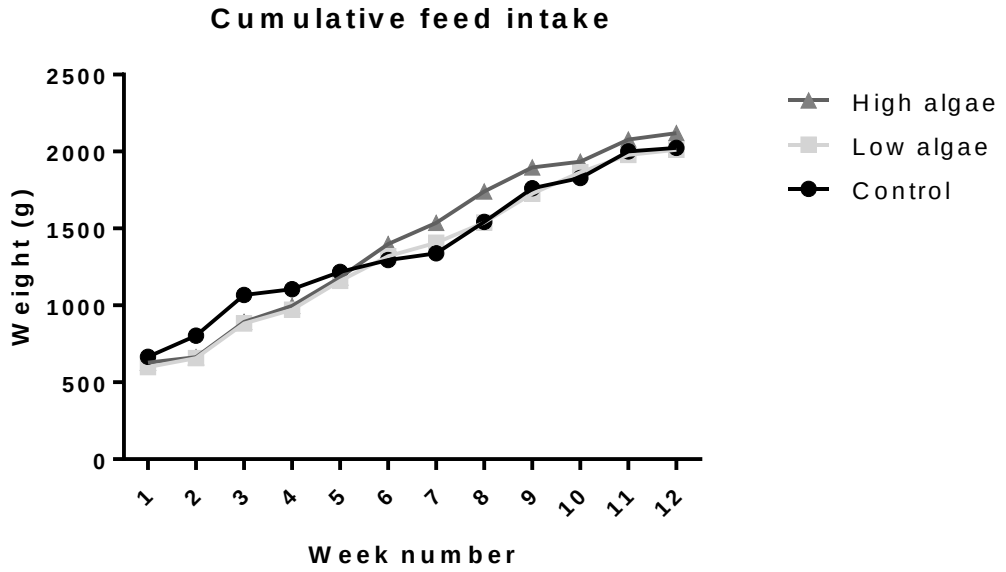

Figure 9. Cumulative feed intake through-out the experiment. Lines are given as cumulative dry matter intake per week per group; n=6 replicate tanks.

The SGR (P = 0.0847) and WG (P = 0.0878) tended to differ among groups Figure 10. The high algae group tended to have the lowest WG and SGR compared to the other two groups. There were significant differences in FI and FCR with a p-values of, 0.0094 and 0.0089, respectively. The two algae fed groups had higher values for FI and FCR compared to control.

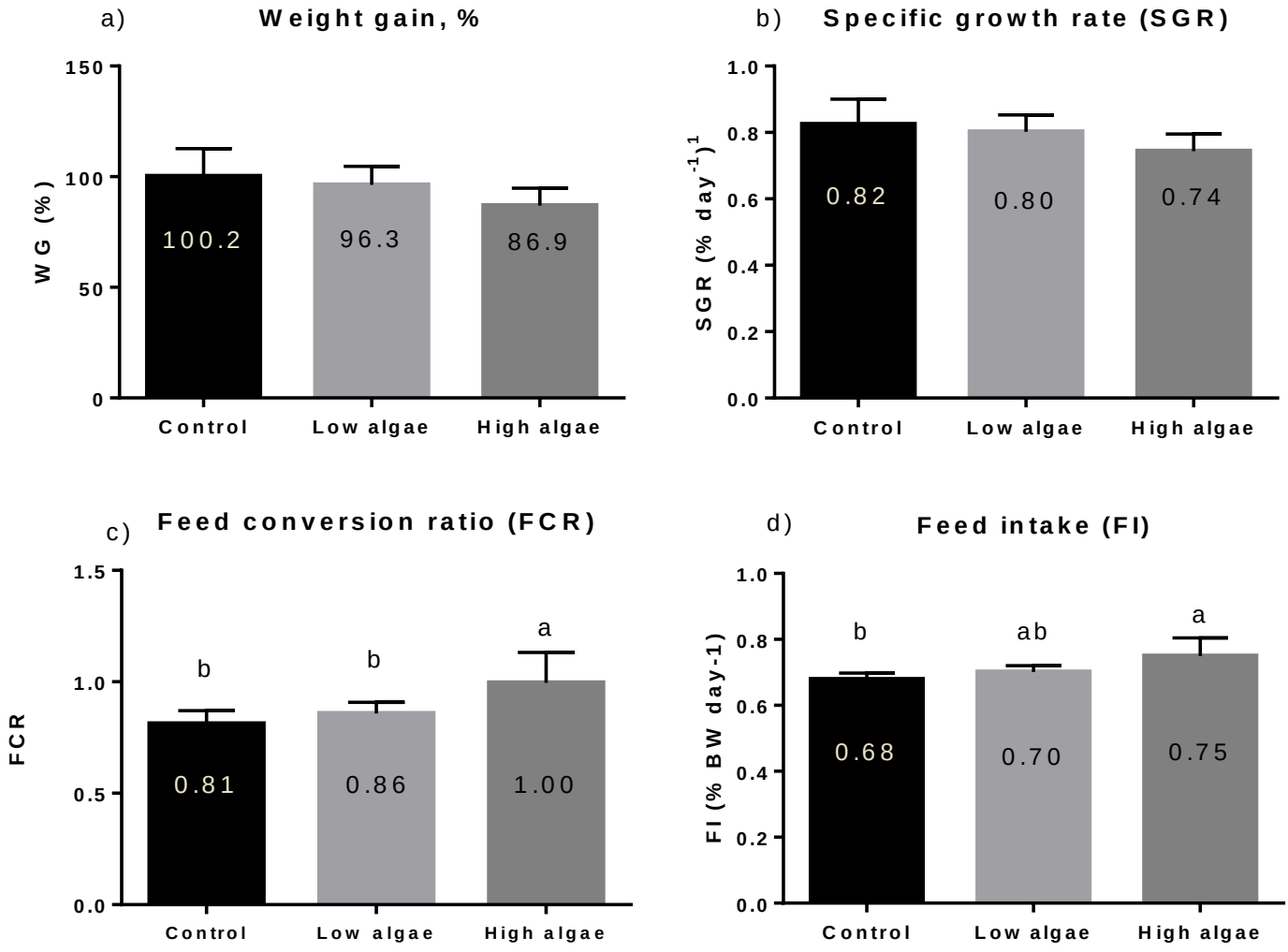

Figure 10. Weight gain (a), Specific growth rate (b), Feed conversion ratio (c) and Feed intake (d) for fish fed control diet and diets with microalgae during the course of 12 week experiment. Numbers are shown as mean; n = 6 replicate tanks. Different letters above column, if any, represent significant differences ($P < 0.05$) between groups.

3.2 Proximate composition of whole fish and fillet

The proximate composition of the whole fish and fillet is given in Table 5. No significant differences were observed among diet groups. However, the moisture and ash content in fish fed high algae feed, tended to be higher than the other two groups with p-values of 0.09 and 0.05, respectively. For all groups, the lipid composition was numerically higher for initial than final whole body and fillet composition.

Table 5. Proximate composition of the whole fish and fillet in Atlantic salmon fed the experimental diets during the course of a 12 week growth experiment.

	Initial	Control	Low algae	High algae	P value
Whole body					
Moisture, g/kg	692.2	686.6 ± 3.59	684.9 ± 4.87	692.2 ± 7.54	0.0909
Per 1000 g dry matter					
Protein	547.1	555.0 ± 10.97	556.3 ± 15.43	562.6 ± 13.81	0.5971
Lipid	373.3	364.0 ± 5.02	362.5 ± 11.72	358.1 ± 11.51	0.5779
Ash	66.9	59.9 ± 1.90	63.2 ± 4.21	64.5 ± 2.79	0.0595
Energy	27.6	27.5 ± 0.19	27.4 ± 0.25	27.3 ± 0.25	0.3370
Fillet					
Moisture, g/kg	742.7	744.3 ± 4.89	742.0 ± 3.50	741.4 ± 3.17	0.4217
Per 1000 g dry matter					
Protein	775.7	788.2 ± 8.48	789.5 ± 24.52	805.7 ± 18.19	0.2166
Lipid	181.8	145.2 ± 17.69	147.9 ± 25.11	149.0 ± 8.82	0.9354
Ash	56.0	55.4 ± 5.34	60.9 ± 6.57	55.3 ± 3.20	0.1356

Numbers are shown as mean ± SD; n = 6 replicate tanks. Different letters in a row, if any, represent significant differences ($P < 0.05$) between groups.

3.3 Apparent digestibility coefficients

The ADCs of dry matter, protein, lipid, ash and energy are shown in Tabel 6. The ADCs of dry matter ranged from 70.7 % (high algae) - 76.0 % (control). The control group had higher ADC of DM than the algae fed fish. The ADCs of energy and protein were significantly different among all groups. The ADC of energy was ranging from 79.2 % (high algae) - 85.9 % (control) and for protein the ADC ranged from 83.4 % (high algae) - 87.9 % (control). Digestibility of lipid ranged

from 87.8 % (high algae) - 92.6 (control). Both algae groups were significantly lower then the control group. The ADCs of ash ranged from 15.5 % (control) - 30.6 % (high algae).

Table 6. Apparent digestibility coefficients (ADCs, %) of dry matter (DM), protein, lipid, ash and energy of diets for Atlantic salmon

	Control	Low algae	High algae	P value
Dry matter	76.0 ± 0.5^{a}	71.6 ± 0.7^{b}	70.7 ± 0.6^{b}	< 0.0001
Protein	87.9 ± 0.2^{a}	85.0 ± 0.7^{b}	83.4 ± 0.3^{c}	< 0.0001
Lipid	92.6 ± 0.4^{a}	88.6 ± 1.0^{b}	87.8 ± 0.5^{b}	0.0003
Ash	15.5 ± 1.9^{b}	20.8 ± 2.9^{b}	30.6 ± 2.0^{a}	0.0006
Energy	85.9 ± 0.5^{a}	81.5 ± 0.9^{b}	79.2 ± 0.4^{c}	< 0.0001

Numbers are shown as mean ± SD; n = 6 replicate tanks. Different letters in a row, if any, represent significant differences (P < 0.05) between groups.

3.4 Nutrient retention

The nutrient retention in whole fish is shown in Table 7. No significant differences were observed among diet groups in protein retention. Retention of lipid was in the range from 53.4 % (high algae) - 66.7 % (control), while energy ranged from 36.7 % (high algae) - 46.0 % (control). For both lipid and energy, significantly highest retention was noted for the control and lowest retention was observed for the high algae fed fish.

Table 7. Retention of protein, lipid and energy in Atlantic salmon fed the experimental feed for 12 weeks.

	Control	Low algae	High algae	P value
Protein retention	39.5 ± 3.35	39.8 ± 1.78	36.2 ± 4.69	0.1852
Lipid retention	66.7 ± 5.12^{a}	64.6 ± 9.87^{ab}	53.4 ± 9.64^{b}	0.0346
Energy retention	46.0 ± 3.34^{a}	43.5 ± 4.40^{ab}	36.7 ± 5.85^{b}	0.0091

Numbers are shown mean ± SD; n = 6 replicate tanks. Different letters in a row, if any, represent significant differences (P < 0.05) between groups.

3.5 Physical quality of diets

The visual appearance of the experimental feed after extrusion was different among feed. The color of control diet was light-brown and the color became light green in low algae and dark green in

high algae diet as shown in Figure 8. The high algae diet appeared to be more oily on the surface of the pellet compared to the other two diets.

Results of the physical quality of diets is shown in Table 8 and Figure 11. No significant differences were observed in pellet durability index, water stability for 45 - 60 min or sinking velocity. Pellet durability index and water stability at 45 and 60 min tended ($P<0.10$) to differ among the feed groups.

Water stability at 15 min (Figure 11) ranged from 91.9 % (low algae) – 93.6 % (control) and significant differences were observed between control and low group. After 30 min (Figure 11), significant differences was observed among control and the two algae diet. Low algae group had numerically lowest water stability after 45 min and 60 min compared to the other two groups, but no significant differences were noted.

Hardness of diets ranged from 21.1 N (control) – 25.3 N (high algae). Significant differences were observed between control and high algae diet. The diameter of pellets were significantly different among all groups with the highest value for the control group (2.1 mm). Length of pellets ranged from 3.8 mm (control) – 4.2 mm (high). The two algae diets were significantly longer than control.

Weigh of the individual pellet ranged from 14.6 mg (high algae) – 15.3 mg (control). The control group was significantly heavier than high algae group.

The bulk density were numerically highest in control 714.8 g compared to low and high algae groups 688.1 g. The control diet had significantly higher bulk density compared to the two algae diets. Expansion rate ranged from 1.3 % (high algae) - 9.4 % (control) and was found to be significant different among all groups.

Fat leakage differed significantly among the diets. Highest leakage was observed for the control, while no differences were noted for the two algae diets.

Table 8. Physical quality of experimental feed.

	Control	Low algae	High algae	P value
Pellet durability index (%)	98.4 ± 0.14	98.7 ± 0.05	98.4 ± 0.95	0.0714
Sinking velocity (cm/sec)	6.8 ± 0.25	6.5 ± 0.22	6.8 ± 0.35	0.1150
Hardness (N)	21.1 ± 0.69^{b}	22.2 ± 0.77^{ab}	25.3 ± 1.47^{a}	<0.0001
Diameter (mm)	2.1 ± 0.02^{a}	2.1 ± 0.02^{b}	2.0 ± 0.04^{c}	<0.0001
Length (mm)	3.8 ± 0.09^{b}	4.1 ± 0.09^{a}	4.2 ± 0.15^{a}	0.0001
Weight (mg)	15.3 ± 0.37^{a}	15.0 ± 0.30^{ab}	14.6 ± 0.36^{b}	0.0121
Bulk density (g L^{-1})	714.8 ± 11.05^{a}	688.1 ± 6.82^{b}	688.1 ± 6.01^{b}	<0.0001
Expansion rate (%)	9.4 ± 0.78^{a}	7.4 ± 0.83^{b}	1.3 ± 1.77^{c}	<0.0001
Fat leakage (%)	2.3 ± 0.78^{a}	0.6 ± 0.16^{b}	0.6 ± 0.37^{b}	<0.0001

Numbers are shown as mean ± SD; n = 6 replicate tanks, except pellet durability index 3 replicates. Different letters in a row, if any, represent significant differences (P < 0.05) between groups.

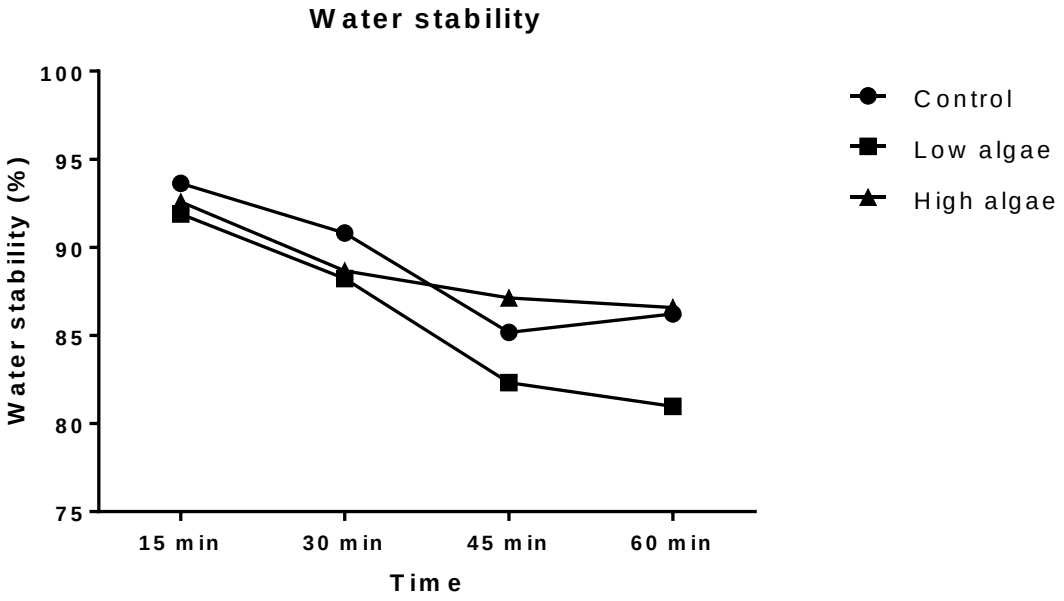

Figure 11. Physical quality of diet. Water stability measured after 15- 30- 45 and 60 min in water bath. Different symbols are given as % of remaining dry matter. At 15 min, significant differences were observed between control and low group. At 30 min, significant differences were observed between control and the two algae diets. No significant differences were noted after 45-60 min, but low algae group had numerically lowest water stability at 45-60 min.

3.6 Correlation among fish growth parameter and physical quality of feed

Correlation data for fish growth parameter and physical quality of feed are shown in Table 9. WG was positively correlated with SGR, PER and TGC and negatively correlated with FCR. FI was positievly correlated with FCR, but negatively correlated with PER.

Diameter of pellet was negatively correlated with FCR, and FI. Hardness where negatively correlated with WG, SGR, TGC but positively correlated with FCR and FI. Lenght was negatively correlated with WG and SGR but positively correlated with FCR and FI. Bulk density was was negatively correlated with FI. Expansion rate was negatively correlated with FCR and FI.

Water stability at 15 min was positively correlated with water stability at 30 min, water stability at 60 min, bulk density and fat leakage. Water stability for 30 min was negatively correlated with length but positively correlated with bulk density and fat leakage. Water stability for 45 min was positively correlated with water stability for 60 min but negatively correlated with durability. Diameter was negatively correlated with hardness, length but positively correlated with weight, bulk density and fat leakage. Hardness was positively correlated with length but negatively correlated with bulk density and expansion. Length was negatively correlated with bulk density, expansion and fat leakage. Weigh was positively correlated with bulk density and expansion. Bulk density was positively correlated with expansion and fat leakage. Expansion was positively correlated with fat leakage.

Table 9. Correlation among growth parameters and physical quality of feed.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	**WG**																			
2	**SGR**	.999**																		
3	**FCR**	-.838**	-.848**																	
4	**PER**	.864**	.873**	-.930**																
5	**TGC**	.987**	.989**	-.845**	.886**															
6	**FI**	-.337	-.347	.777**	-.612**	-.350														
7	**WS15**	.373	.367	-.311	.188	.371	-.179													
8	**WS30**	.180	.171	-.264	.072	.152	-.327	.621**												
9	**WS45**	-.091	-.089	.184	-.175	-.112	.299	.374	.353											
10	**WS60**	-.005	-.007	.190	-.219	-.034	.356	.498*	.454	.889**										
11	**Diameter**	.439	.434	-.540*	.261	.394	-.522*	.208	.438	-.379	-.193									
12	**Hardness**	-.516*	-.519*	.685**	-.439	-.508*	.646**	-.314	-.448	.190	.078	-.832**								
13	**Length**	-.496*	-.488*	.554*	-.352	-.448	.499*	-.456	-.735**	.084	-.101	-.751**	.777**							
14	**Weight**	.283	.283	-.425	.201	.280	-.462	.298	.177	-.313	-.209	.679**	-.445	-.200						
15	**BD**	.223	.220	-.449	.236	.225	-.614**	.607**	.569*	-.034	.085	.518*	-.580*	-.615**	.492*					
16	**SV**	-.074	-.071	.083	-.085	-.045	.141	.233	.119	.196	.273	-.156	.037	.025	.034	.233				
17	**Expansion**	.451	.446	-.555*	.276	.406	-.535*	.225	.436	-.381	-.196	.999**	-.833**	-.756**	.685**	.523*	-.157			
18	**Durability**	.653	.653	-.559	.615	.626	.304	-.541	-.647	-.784*	-.761*	.173	-.168	.126	-.027	-.599	-.277	.167		
19	**Fat L**	.535*	.519*	-.457	.340	.472	-.300	.742**	.666**	.201	.324	.473	-.438	-.669**	.395	.674**	.313	.486*	-.614	

**. Correlation is significant at the 0.01 level (2-tailed)
*. Correlation is significant at the 0.05 level (2-tailed)

WG – Weight gain. SGR – Specific growth rate. FCR – Feed conversion ratio. PER – Protein efficient ration. TGC – Thermal growth coefficient. FI – Feed intake. WS 15-60 – Water stability. BD – Bulk density. SV – Sinking velocity. Fat L- Fat leakage.

4 Discussion

4.1 Fish growth performance

This experiment was carried out to investigate the potential of using a defatted microalgae *Nannochloropsis* sp. in feed for Atlantic salmon. The control diet was based on fish meal, and the two test diets were formulated with algae biomass replacing fish meal at 10% (low algae) or 20% (high algae). The results showed that inclusion of algae biomass (*Nannochloropsis*) tended to reduce WG and SGR at highest inclusion level. The SGR of the Atlantic salmon was 0.79 % of body weight per day, which is in line with a previous growth study with Atlantic salmon of similar size reared at 6°C seawater (Handeland et al., 2008). The present experiment showed lower SGR and TGC compared to a growth study where the yeast *yarrowia lipolytica* was fed to Atlantic salmon (Hatlen et al., 2012), but higher values than Kiron et al. (2012) reported in study with Atlantic salmon fed the microalgae species *Tetraselmis Chlorophyceae* at inclusion level of 5-10%.

The pellet size of the experimental diet was smaller than pellets commercially used for this size of fish (Biomar, 2010). The pellet used in present experiment were 2 mm in size but Biomar recommend 3.5 mm for fish up to 250 g in size. It could be questioned whether suboptimal pellet size has compromised growth of the fish. The effect of pellet size on growth was investigated by (Bailey et al., 2003). The author concluded that Atlantic salmon can accept a range of pellet size. The growth of the fish in the present experiment indicate that pellet size was not a limitation for growth.

The FI and FCR in the present experiment was in line with Hatlen et al. (2012). They observed significant differences in FI and FCR between 10 and 30 % inclusion of *yarrowia lipolytica* in the diet fed to Atlantic salmon. They suggested that increased FI and FCR was due to increased carbohydrates level in the feeds with *yarrowia lipolytica*. Carbohydrates are poorly digested by Atlantic salmon.

In the present study, FI from week 6 and to termination was higher for fish fed the high algae feed, compared the other two groups. The FCR was also highest in the high algae fed group. These findings are also in line with Hatlen et al. (2012), who observed increasing FI and FCR with 20 and 30% inclusion level of the *yarrowia lipolytica*. Fishmeal replacement with the microalgae

Spirulina platensis showed that up to 10% inclusion in feed had no negative effect on growth performance in rainbow trout. The authors even suggested that inclusion of *Spirulina platensis* in feed, improved feed efficiency due to bacteria augmentation in the fish gut (Teimouri et al., 2013).

4.2 Proximate composition of whole fish and fillet

Percentage of whole body protein increase with muscle growth (Shearer, 1994). Protein content increased from start to termination of the experiment in all groups. No significant differences were observed in proximate composition of whole fish or fillet among the feeding groups in the present experiment.

The higher lipid content observed for initial whole fish and final fillet than at termination is in line with Alne et al. (2011). Alne et al. (2011) reported a reduction in the fat content in the muscle during the first spring in sea (May-July) for both S0 and S1. This phenomena is not fully understood but one plausible explanation is that energy demanding processes are taking place.

4.3 Apparent digestibility coefficients

The ADCs for dry matter, protein, lipid and energy decreased with increasing algae biomass in the feed. Though a reduction was noted in ADCs in algae fed groups, the value were in line with other digestibility studies with Atlantic salmon. Hatlen et al. (2012) reported a reduction in digestibility of dry matter, protein, fat and energy with increasing inclusion level of *yarrowia lipolytica* from 10-30% in the diet. At 30% inclusion of *yarrowia lipolytica* the ADC values for dry matter, protein, lipid and energy were 67%, 84%, 94% and 81%, respectively (Hatlen et al., 2012). The authors explained the reduction in digestibility of energy, partly because digestibility of protein and fat was reduced. However, they also suggest that carbohydrate present in the *yarrowia lipolytica* had a negative effect on energy digestibility.

The present results are also in line with Berge et al. (2005) feeding Atlantic salmon (1400 g) with 10-20% bacterial protein meal, in replacement of fish meal. They observed that ADCs of protein, fat and energy tended to be decreased with increasing inclusion of bacterial protein meal in the diet. The reduced ADCs of protein was explained by indigestible bacteria cell walls rendering the protein unavailable for digestive enzymes. The reduction in fat digestion in their study was mainly explained by differences in fatty acid composition in the bacterial protein meal. Lipid in bacteria meal contain mainly 16:0 and 16:1 fatty acids that has reduced digestibility compared to unsaturated fatty acids.

The ADCs of protein for low and high algae fed groups are in line with Burr et al. (2011). They estimated a protein digestibility of 84.7% in *Spirulina* algae fed to Atlantic salmon. Storebakken et al. (1998) reported ADC value of protein 90 %, 81 % and 89 %, respectively, when Atlantic salmon were fed fish meal, soybean meal or bacterial meal in diet. Protein digestibility in algae fed fish were lower in our experiment, however we observed higher energy digestibility than (Storebakken et al., 1998) reported for soybean meal and bacteria meal.

The reduced lipid digestibility noted for the two algae containing test diets may be associated with the low lipid content in the algae compared to the fish meal. Fish meal commonly contain between 5-12% fish oil (EUfishmeal, n.d.), while the defatted *Nannochloropsis* biomass contained only 2.5%. Approximately 50% of the dry matter content of the algae consisted of ash and carbohydrate, 23.5% and 29%, respectively. Content of carbohydrate in the microalgae was not analyzed, but calculated by difference (content of dry matter – crude protein – lipid – ash). The lipid content of the high algae feed was slightly lower than the other two diets in order to keep a constant crude protein : energy ratio. The reduction in the lipid digestibility observed for the two algae containing feeds may also be explained by the increasing carbohydrate level accompanied with increased level of indigestible cellwalls, as earlier suggested by Hatlen et al. (2012). The reduced lipid digestibility most likely also explain the reduction in energy digestibility noted for the two alga containing feed compared to the control diet.

Digestibility of ash show large variation among different feeding studies, also negative values are reported (Aas et al., 2015; Sørensen et al., 2016; Aslaksen et al., 2007). The ADC values of ash is strongly affected by elements in the water. Elements commonly found in high concentration in seawater, such as Na, Ca, Mg show the largest variation in ADC from negative to positive values, compared to other elements, and has a major impact to the overall digestibility of ash (Thodesen et al., 2001; Storebakken et al., 1998). Fish in seawater is drinking water and the gastrointestinal tract has an important role in osmoregulation. Drinking of seawater is partly explaining the variation in ADC for ash in the present experiment. Increasing ADC of ash in the diets containing micro algae may suggest less drinking. The content of ash in the feeds increased with algae biomass inclusion, and corroborated with the higher ash digestibility observed for the algae diets.

4.4 Nutrient retention

The retention of ingested nitrogen was similar across feeding groups and showed no significant differences. The average nitrogen retention was 36 %. That is in line with nitrogen retention reported by Berge et al. (2005) for Atlantic salmon fed with bacterial protein meal. The nitrogen retention in present experiment was lower than Aas et al. (2015) reported for Atlantic salmon fed pellet with different physical pellet quality. The latter author reported retention of nitrogen in the range from 47-51%. However, in the study of Aas et al. (2015), the energy content in the diet was also higher than in the present study. Lipid has a protein sparing effects on protein retention (Halver et al., 2002). As earlier discussed, pellet size and oil content was not optimal in the diet, explaining the lower lipid- and energy retention. Lower retention of oil and energy is also explained by reduction in nutrient digestibility.

4.5 Physical quality of diet

Physical quality differed among the experimental diets. Hardness and length increased, while diameter and pellet weight decreased with increasing algae level in diet. Expansion rate was reduced with algae in diet and correspondingly the bulk density was reduced.

The lower expansion rate and diameter of the two algae diets may be explained by the lower temperature used in the production of these feeds. The extrusion parameter for experimental diet in Table 1 showed that temperature in section 2-5 was lower during production of high algae diet compared to the other two feed production. This could have affected gelatinization of starch in the high algae diet resulting in lower expansion and diameter. It's not possible to draw any conclusions about starch gelatinization because no analyses were performed to determine starch gelatinization. Nevertheless, expansion is important for absorption of oil. The control diet had the highest lipid content and highest expansion, in line with Aas et al. (2015), who reported that lower expansion rate gave less oil absorption. The oil absorption capacity of the experimental diets were not measured in present experiment. However, the oily surface of the high algae feed may suggest a lower oil absorption capacity. More leaking would have been expected if pellets were coated with higher levels of oil. However, less oil was added to this diet to keep a constant protein : energy ratio. Sørensen et al. (2011b) found that fat leaking was associated with low oil absorption capacity, and not with oil level. The higher oil leakage observed for the control diet may be explained by a different microstructure of the diet (Sørensen et al., 2009).

Hardness of pellet in the present study increased when algae was replacing fish meal. The high algae feed had numerically highest braking point at 25.3 N, compared to 21.1 and 22.3 in control and low algae pellets, respectively. High algae diet also had numerically higher moisture. Li (2012) observed higher hardness in feed pellet when moisture in feed increased from 2.5%-5.0% and 5.0%-7.5% followed by a reduction when moisture subsequently increased from 7.5%-10%. Aas et al. (2011a) reported higher pellet hardness than we obtained in the present study. They reported values in the range from 45.7 N – 54.1 N. In that study, the pellet was bigger (12 mm), compression speed and the cylinder probe used to break the pellet was also different. Lack of standardization for the methods used to analyze hardness make comparison between experiments difficult. In a review, Sørensen (2012) reported variation in pellet hardness from 9 N – 82 N, depending on raw ingredients, production process and/or method used to analyse hardness. Some pellet lay horizontal and some vertical when texture are measured, and some report use flat end probe to crack the pellet when other use knives. The hardness of the pellets used in the present experiment is well within the normal range reported earlier.

No significant differences was noted in pellet durability index measured with Ligno tester in present study. The value were generally high for all diets. Ligno durability values are often high. Aas et al. (2011a) reported pellet durability index ranging from 97.5% – 99.3%, while Aas et al. (2014) reported values ranging from 98.1% – 99.2%. Durability can also be measured with other methods for example Holmen tester. Holmen tester gives usually more variation in durability than Ligno tester. However, it is not possible to measure pellet durability in Holmen after the pellet have been coated with oil due to problems with oil leaking in high energy diets using this method.

Water stability for all diets was high after 15 minute shaking in water bath. Overall water stability decreased over time, in line with Aas et al. (2011b). Lowest water stability was noted for low algae pellets after 45-60 min. One explanation to the differences in water stability may be explained by hardness of the pellet. Highest hardness was associated with high water stability, and these findings are in line with (Aas et al., 2011b). High water stability minimize loss of nutrients when pellets are soaked in water.

4.6 Correlation

The negative correlation between diameter and hardness is in line with (Sørensen et al., 2009). The negative correlation between diameter and length may suggest that more expansion take place radial direction. Another explanation is that the cutter speed was adjusted for the more expanded control pellets. Details about the feed production is, however, not known. Diameter was positively correlated with pellet weight and bulk density. Earlier research have reported a negative correlation between diameter/expansion rate and bulk density (Blanche et al., 2004). The bulk density of the present feed was carried out on coated feed, while (Rokey et al., 2014) reported differences in bulk density on coated as well as uncoated feed. Besides, more lipid was added to the control diet, explaining the higher bulk density, though this feed was more expanded. The negative correlation between hardness and bulk density is in line with findings reported by (Sørensen et al., 2009). This is mainly because higher bulk density was noted for the most expanded pellets that also had the highest lipid content.

The positive correlation between hardness and length reflect that the least expanded pellet (the two algae diets) where longer pellet.

Fat leakage was positively correlated to bulk density and water stability, and negatively correlated to length. This observation is explained by the different processing conditions and ingredients used in the control diet.

The correlation between pellet quality, growth and feed utilization was low to intermediate. Overall the significant positive and negative correlations were explained by the pellet characteristics earlier discussed. Overall, pellet quality was affected both by processing conditions and ingredients selection. The design of the experiment does not allow any conclusions to be drawn about possible interaction between nutritional and physical quality of pellets in the present experiment.

5 Conclusion

The aim of the study was to investigate the potential of the defatted microalgae biomass *Nannochloropsis* sp. as a fish meal replacer for Atlantic salmon. A combined growth and digestibility study was designed to investigate the effect of replacing fish meal in the control diet with 10% (low algae) or 20% (high algae) in the test diets. The study parameters used to evaluate differences among the diets were growth, feed conversion rate, digestibility of energy and nutrients as well as whole body proximate and fillet composition. Pellet quality was also analysed in terms of water stability, hardness, sinking velocity, bulk density, expansion rate, fat leakage, weight, length and diameter.

Effect on growth, feed utilisation and digestibility

The results showed that inclusion level of *Nannochloropsis* sp. should be lower than 20%. The highest inclusion level gave a significant reduction in digestibility of dry matter, protein, lipid, energy as well as nutrient retention of lipid and energy. Feed intake, feed conversion ratio and ash digestibility was significantly increased at the highest inclusion level of the *Nannochlorpsis* sp. Weight gain and specific growth rate tended to be reduced at highest inclusion level. Significant negative effects were observed on digestibility of dry matter, protein, lipid and energy already at 10% inclusion level of microalgae in the feed.

Effect on physical quality of feed

Physical quality of feed were significantly affected by incorporation of the microalgae *Nannochloropsis* sp. The microalgae biomass in the feed improved hardness of pellet, fat leakage and increased pellet length but reduced expansion rate, diameter, weight and bulk density.

Based on the findings in this study it can be concluded that incorporation of *Nannochloropsis* sp. biomass should not exceeds 10% in the feed.

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