MASTER THESIS

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Evaluation of the microalgae *Scenedesmus* sp. as a potential feed ingredient for Atlantic salmon(*Salmo salar*)

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Abbreviations

FAO - Food and Agricultural Organization of the United Nations

SSB - Statistik Sentralbyrå (Statistic Norway)

FKD – Fiskedirektoratet (Directorate of Fisheries Norway)

IFFO – The Marine Ingredients Organization

NRC - Norwegian Research Council

NOFIMA- The Norwegian Institute of Food, Fisheries and Aquaculture Research

ISO - International Organization for Standardization

AOAC – Association for Analytical Communities

EPA - Eicosapentaenoic acid

DHA - Docosahexaenoic acid

CT - Control group

SCE 10 – Low algae group

SCE 20 – High algae group

Abstract

Availability of high quality protein and lipid ingredients to support future growth of Atlantic salmon farming is a challenge. Carnivore salmonids depends on high quality ingredients to support high growth rate, good health, high product quality at the same time as the environmental footprint is kept low. In recent years, fishmeal and fish oil is to a large extent replaced with plant derived ingredients in feeds for salmonids. Plant ingredients in carnivore fish diets have nutritional limitations and sustainability is also debated. Single cell organisms such as microalgae, yeast and bacteria has gained increasing interest as sustainable ingredients in diets for salmonids. The aim of the present study was to investigate the potential of using the microalgae *Scenedesmus* sp. in low fish meal diets for Atlantic salmon. Three diets were formulated with microalgae *Scenedesmus* sp. incorporated at 0, 10 and 20%. Effect of inclusion level were studied on growth, feed utilization, nutrient digestibility and physical quality of feed. Fish with an initial average weight of 229.1 ± 1.5 g were fed the experimental diets in 6 replicates in a 65-days combined growth and digestibility trial. The results showed that fish fed with 20% *Scenedesmus* sp. in the feed had significantly lower final mean body weight, weight gain, specific growth rate, thermal growth coefficient and feed conversion rate than the control group. Both microalgae fed groups showed lower condition factor and protein efficiency ratio compared to the control. Hepasomatic index and viscerosomatic index did not differ among the groups. Proximate composition of whole fish showed that fish fed the 20% incorporation of microalgae in the feed had significantly higher ash and protein content but lower content of dry matter, lipid and energy. Retention of lipid differed among all groups while retention of protein and energy was significantly lower at 20% incorporation of algae in the feed. Compared to the control, digestibility of dry matter, protein and energy was significantly different among all the three dietary treatments. Non-significant negative values were recorded for digestibility of ash, and the values became more negative with more algae in the feed. Physical characteristics of the feeds showed highest fat leakage from the control group. Highest pellet hardness and lowest pellet length was recorded for the diet with 20% of microalgae. Pellet diameter was similar among feed groups. Water stability of the algae included diets were significantly higher than that of the control group. In conclusion, *Scenedesmus* sp. can be used at 10% inclusion in diets for Atlantic salmon without negative effect on nutrient retention, digestibility, growth of fish and physical quality of feeds.

Keywords: Microalgae, *Scenedesmus* sp., Atlantic salmon, Digestibility, Physical quality

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1 Introduction

1.1 Global Aquaculture: A brief overview

Aquaculture can be defined as farming of aquatic organisms under controlled conditions. The aim of aquaculture production is to increase the production by means of regular stocking, controlled feeding, breeding programs and protection from diseases and predators (FAO, 1988). Global aquaculture production, both marine and inland aquaculture, has shown a steady increase since 1950 (FAO, 2016). In 2014, the global total aquaculture production was 73.8 million tonnes (FAO, 2016). Carps, tilapias, and salmonids was the major farmed species in 2014. The growth in global aquaculture production is significant to meet the increasing demand of seafood in spite of declining world capture fishery productions. In broader perspective, aquaculture production has contributed to livelihood development and increased food security all around the world.

1.2 Production of Atlantic salmon and sustainability

Aquaculture production of Atlantic salmon (*Salmo salar*) is dated back to 19th century (FAO, 2004). More than 50% of the global market for Atlantic salmon is constituted by farmed salmon (Solar, 2009). In 2014, total production of farmed Atlantic salmon was 2.3 million tons. Although this figure is relatively small compared with the production volume of other species, Atlantic salmon is a high value product and well paid in many markets around the world. Substantial improvements of the production technologies, hatcheries, value addition and higher market prices have driven Atlantic salmon production to competitive level against other fish species produced at lower cost. Concurrent with increased production, consumer awareness has increased. Salmon produced at high ethical standards and lowest ecological footprint are important concerns among consumers (Verbeke et al., 2007). Farming of Atlantic salmon is sustainable compared to production of terrestrial meat. Carbon foot print of farmed salmon is 2.9 kg of carbon per kg of edible product (Winther et al., 2009). This figure is significantly lower than terrestrial meat production of poultry, pork and beef (Figure 01). Atlantic salmon is also superior to terrestrial monogastrics in utilization of nutrients in feed (protein, lipid and energy) (Ytrestøyl et al., 2015).Other sustainability indicators such as Fish In Fish Out Ratios (FIFO) as well as Forage Fish Dependency Ratio (FOFDR) have also shown great improvement during the last two decades (Ytrestøyl et al., 2015).

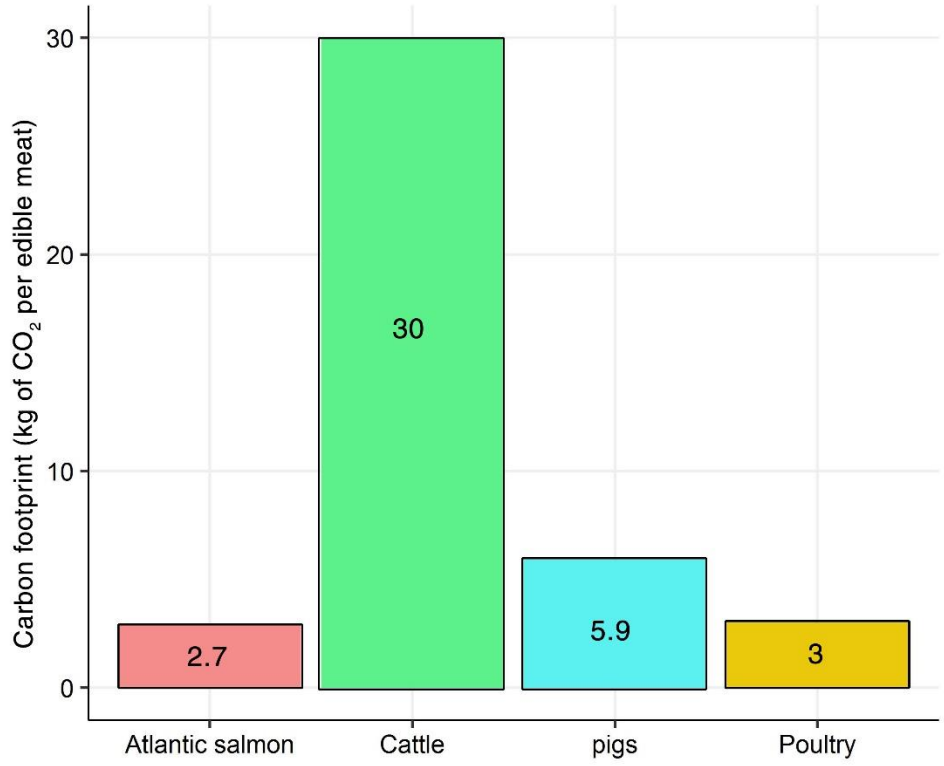

Figure 01: Carbon footprint of Atlantic salmon, cattle, pigs and poultry productions (own elaboration based on data by Winther et al. (2009))

1.3 Atlantic salmon production in Norway

Norwegian salmonid farming is dated back to late 1960s - early 1970s (Ford, 1984). Production of Atlantic salmons in Norway began as a means of livelihood support for rural communities, in face of declining capture fishery production. Several decades later, Atlantic salmon production became one of the premier industries in Norway.

In 2015, total Atlantic salmon production in Norway was 1.3 million tones where three companies collectively share more than 1 million tons (SSB, 2016). Norway is world largest Atlantic salmon producer, with more than 54% of global market, followed by Chile, UK, and Canada with market shares of 26%, 7 % and 6 % in 2015, respectively (Figure 02). Success story of Norwegian Atlantic salmon production can be attributed to several key technological developments. The most important developments were implementation of family selection breeding programs, development of nutritious high quality feed, vaccines and disease prevention, improved farm management and access to high quality smolt for on-growth (Liu et al., 2011). The development of the Norwegian aquaculture industry is also characterized by

research and innovation fostered by a strong collaboration between researchers and the industry (Ford, 1984).

Norwegian Atlantic salmon production is considered as one of the most sustainable aquaculture practice compared to elsewhere (FKD, 2009). Strong regulations paying attention to sustainability issues, fish welfare, escapees, pollution, disease prevention and feed resources has contributed to a sustainable development of Norwegian Atlantic salmon production.

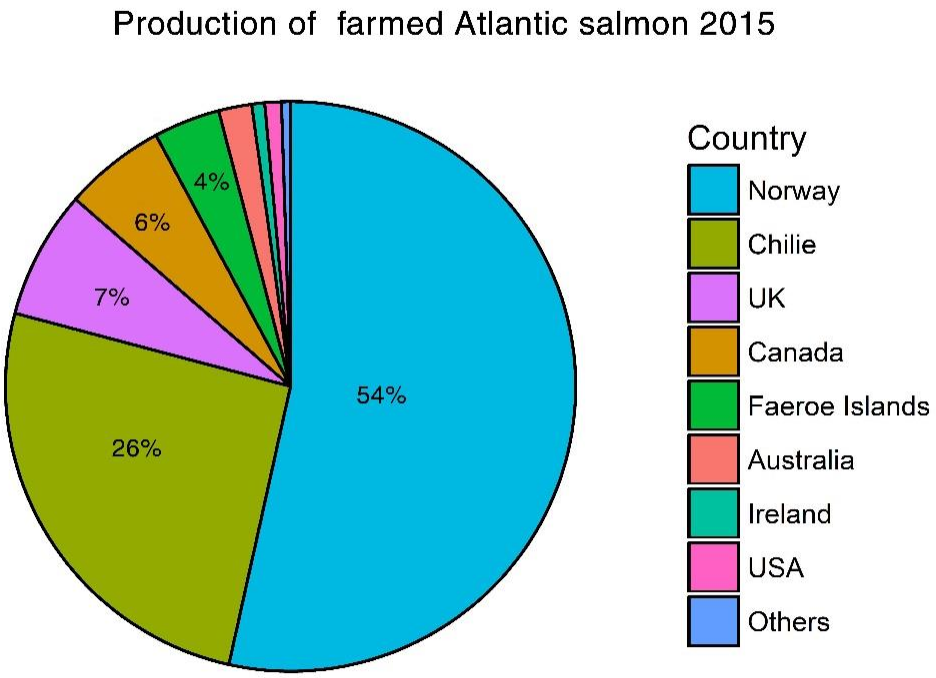

Figure 02: Production of farmed Atlantic salmon in 2015 (FAO, 2015)

1.4 Historical development of Atlantic salmon diets

Fish feed technology in Norway is dated back to early 1970s where first pelleted feed was introduced to salmonids aquaculture (Talbot and Rosenlund, 2002). Pelleted feeds had many advantages compared to feeding with raw fish. Introduction of extruded fish feed in 1980s significantly changed the salmon feed industry. Extrusion processing is the dominating practice for production of Atlantic salmon feeds (FAO, 2004). Extruded fish feed has several beneficial characteristics compared to the conventional steam pelleted feeds. The pellets are more resistant to wear and tear, heat labile anti-nutritional factors are inactivated (such as lectins), and the process efficiently inactivate microorganisms such as the bacteria *Salamonella* (Levic and Sredanovi, 2010). Most of these favorable characteristics are results of extrusion processing and conditions used during extrusion. During the extrusion process, pre-conditioned feed materials are heated and kneaded throughout the barrel. The material is eventually pushed through the die and cut by rotating knifes as the outlet of the extruder (Levic and Sredanovi, 2010). In this process the feed mix is subjected to higher temperature over a relatively short time. The process is gelatinating starch and denaturing protein, both important for binding of the pellet and the final structure. Final quality of the extruded feed is determined by temperature along with other parameters (e.g. time, ingredient composition). Later on, in early 1990s, introduction of vacuum oil impregnation allowed production of high energy salmonid diets (Figure 03). Use of high -energy diets has contributed to improve protein utilization, growth performance, increase digestibility of energy and improve feed conversion ratios in farmed Atlantic salmon.

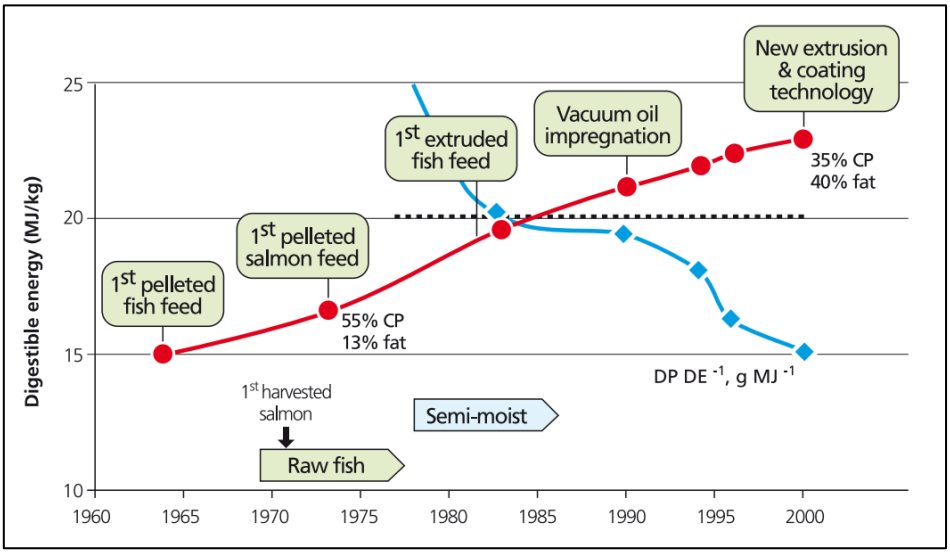

Figure 03: Historical development of feeds in Norwegian salmon industry in relation to digestible energy and inclusion levels (Talbot and Rosenlund, 2002).

1.5 Physical quality of the feed

High utilization of the feed resources in aquaculture practices are important to ensure profitability, improved feed utilization and health of the cultured species. In production perspective, aquatic feeds should ensure reduced wastage, minimum water pollution and efficient utilization by the aquatic animals.

Physical quality of feeds is defined as "ability of processed feed, either pelleted or granulated to withstand handling without creating excessive amount of fines" (Sørensen, 2009). Physical quality characteristics in feeds for salmonids are usually described in terms of hardness, bulk density, pellet length and diameter, sinking velocity, expansion rate and fat leakage.

1.5.1 Hardness

Pellet hardness resembles the resistance of pellets for external pressures. This includes resistance to break when stored in bins, crushing pellets in screw conveyor (Kaliyan and Vance Morey, 2009). In production perspective, pellets should be sufficiently hard to prevent breaking into small particles and fines. Low hardness may increase the water pollution and feed wastage by creating small particles and fines. However, too high pellet hardness may cause increased retention time and thus reduced gastrointestinal evacuation rate. This may in turn reduce feed intake and result in poor growth in aquatic animals. Measurement of pellet hardness may differ by targeted species and type of instrument. In fish feeds, hardness can be measured by texture analyzer (TA-XT2®, Model 1000 R; SMS Stable Micro Systems, Blackdown Rural Industries, Surrey, UK).

1.5.2 Pellet length and diameter

Pellet size (length and diameter) is an important parameter that decides the ingestion of feeds by fish. Pellets larger than the gape width of fish will not be ingested. Optimal size of the feed pellets has been investigated for various fish species such as Atlantic Char (Linnér and Brännäs, 1994), Atlantic Salmon (Wañkowski, 1979). These latter authors have suggested that pellets should be 2% of the fish length and 25-50% of gape width, respectively. Smith et al. (1995) pointed out that fish can adjust their feeding behavior according to pellet size.

Measurement of pellet size can be done by Vernier caliper.

1.5.3 Pellet durability index

Pellet durability index measures the stress on pellets when they are delivered by pneumatic devices. Different durability measurements devices are used for testing the durability. These include, Holmen durability index, Tumbling box tester, Ligno durability and DORIS durability measurement.

1.5.4 Bulk density

Bulk density is the weight of a certain volume of pellet. Bulk density is correlated to the sinking velocity of the pellets and is highly correlated to the degree of expansion in extrusion process. Rockey and Huber (1994) and Glencross et al. (2011) found that bulk density may vary between 500-550 gl^{-} and 320-400 gl^{-} for sinking and floating diets, respectively.

1.5.5 Fat leakage

Fat leakage from feed pellets results in lower energy content and different nutritional profile. Oil absorption capacity is the main governing factor for fat leakage from pellets. Fat leakage is related to the ingredient composition and processing conditions (Øverland et al., 2007, Sørensen et al., 2011b). Øverland et al. (2007) and Sørensen et al. (2010) have reported two different methods for determining the fat leakage from pellets.

1.6 Feed ingredients in aquaculture

Intensive aquaculture depend on external feed supplies and is currently account for 50% of total aquaculture productions (FAO, 2016). Fish needs to be provided a nutritionally balanced feed, produced by combining different ingredients to meet nutrient requirement.

Feed resources in aquaculture practices include both compounded aqua feeds and natural food resources (Tacon and Metian, 2008). In fish farming, compounded aqua feeds represent the largest operating cost (Tacon and Metian, 2008). Production of a compound feed includes careful selection of feed ingredients from sustainable resources at lowest possible cost. The fish feed industry is constantly searching for new ingredients to reduce dependence on a few resources. In global aquaculture practices, a wide range of ingredients are used.

1.6.1 Fish meal and fish oil

Fish meal and fish oil are key ingredients in aqua diets, though there has been a trend to replace these ingredients with plant based ingredients (Ytrestøyl et al 2015). The preference for using fish meal and fish oil in fish feed is mainly because of their high protein content, balanced amino acid profile, unique fatty acid composition (supplier of EPA, DHA) and unique amino acids (e.g. Taurine), and palatability. Fish meal also enhance fish growth and improve fish health significantly (Miles and Chapman, 2015). In addition, higher palatability of fish meal ensures efficient feed intake. The absence of carbohydrates and anti-nutritional factors ensures good digestion and absorption of nutrients, high growth performance and even health promoting effects through immunomodulatory activity (Miles and Chapman, 2015). High nutrient digestibility and retention of nutrients contribute to reduced water pollution and has a positive effect of the marine environment.

Use of fish meal and fish oil in aqua diets are limited by their availability and price. The availability in the future is not expected to improve (Figure 04). Fish meal is often produced from small pelagic fishes (Anchovy, Pollock, Menhaden), that is not commonly used for human consumption. A key challenge is that majority of the world's fish resources are fully exploited, limiting a future growth of fish meal production. The largest producer of fish meal and fish oil is Peru. In 2007, export of Peruvian anchovy derived fish meal and fish oil shared 41% and 30.6% of global fish meal and fish oil, respectively (Tacon et al., 2011). However, these fisheries strongly fluctuate, amongst others with the natural phenomena El-Niño, causing marked reduction in harvesting of anchovy. This in turn reduce fish meal and oil production. There is also an increasing trend that small pelagic fishes are used directly for human

consumption. Consequently, the increasing competition for marine resources should only be met by smart use and increased use of fish by products in production of fish meal and fish oil. (Olsen and Hasan, 2012).

The marine ingredients used by Norwegian salmonid farming include fish meal produced from forage fish such as herring, mackerel and blue whiting. Catch quotas regulate fishing on these species. The fish meal composition may vary from year to year depending on the catch quotas of different species. The fish meal and fish oil producers have now started to use marine by products for production of fish meal and fish oil. Consequently, the use of marine by products has increased from 29700 tonnes in 2008 (Chamberlain, 2011) to 137800 tonnes in 2013 (Olafsen et al., 2014). In 2013, about 25% of the fish meal and fish oil used by Norwegian salmon farmers were derived from by-products (Ytrestøyl et al., 2015).

Novel marine ingredients from lower trophic levels (zooplankton) is also gaining more interest. Ingredients from the lower trophic level includes zooplankton from both Arctic and Antarctic waters. Although availability of zooplanktons is high, inadequate processing and harvesting technology undermines their wide scale usage. Antarctic krill (*Euphausia superba*) has a great potential to replace fish meal in aqua diets. Partial inclusion (25%) of krill meal has improved the growth response and nutrient digestibility (Olsen et al., 2010) in Atlantic salmon. However, as described by Sørensen et al. (2011a) presence of fluoride and copper may limit the inclusion level. Also, sustainability issues are the major barriers for wide scale use of krill meal in Atlantic salmon production.

The price of fish meal and fish oil heavily depends on availability. Increasing competition has resulted increased prices. The aqua feed industry has met this challenge with replacing fish meal and fish oil with alternative feed ingredients.

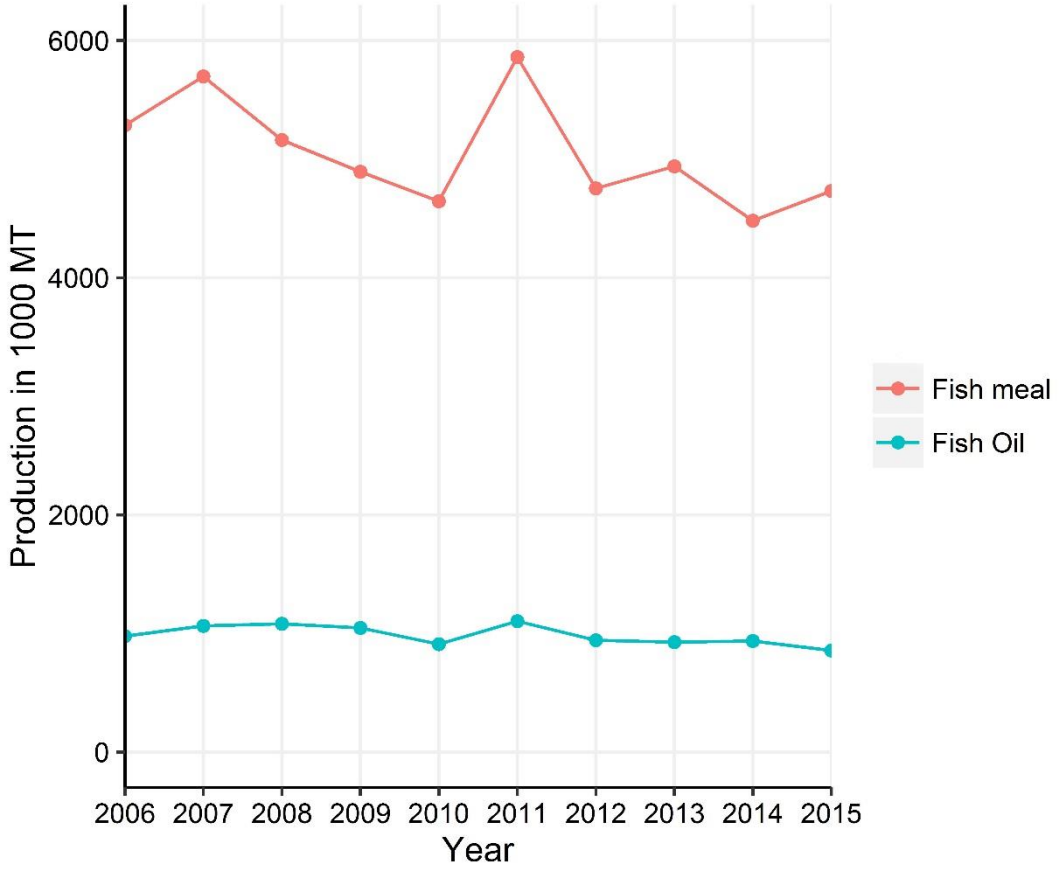

Figure 04: Production of fish meal and fish oil from 2006-2015 (IFFO, 2016)

1.6.2 Alternative feed ingredients in aquaculture

Shortage of fish meal and oil to meet the demands of a growing aquaculture industry has stimulated research and innovations in the aqua feed sector. A vast range of ingredients including plant ingredients, terrestrial animal byproducts, microbial ingredients, insect based ingredients and genetically modified ingredients are used in aqua-diets worldwide. Tacon et al (2011) have given an overview over the major ingredients used in diets for various fish species. An updated overview over the most commonly used ingredients in production of Atlantic salmon was also reported by Ytrestøyl et al (2015) and Shepherd et al. (2017).

1.7 Feeds for salmonids aquaculture

A steady growth in production of Atlantic salmon has increased the demand for feed, which is expected to increase to 3,672,00 tonnes by 2020 (Tacon and Metian, 2008). The salmonid diets are globally as well as in Norway dominated by plant ingredients. Replacement of fish meal with plant ingredients has been the main trend in Norwegian salmon feed (Figure 05) during the last 15-20 years. Major plant ingredients in Norwegian aquaculture includes soybean meal, pea protein concentrate, wheat gluten and sunflower expeller. Fish oil is mainly replaced with rapeseed oil.

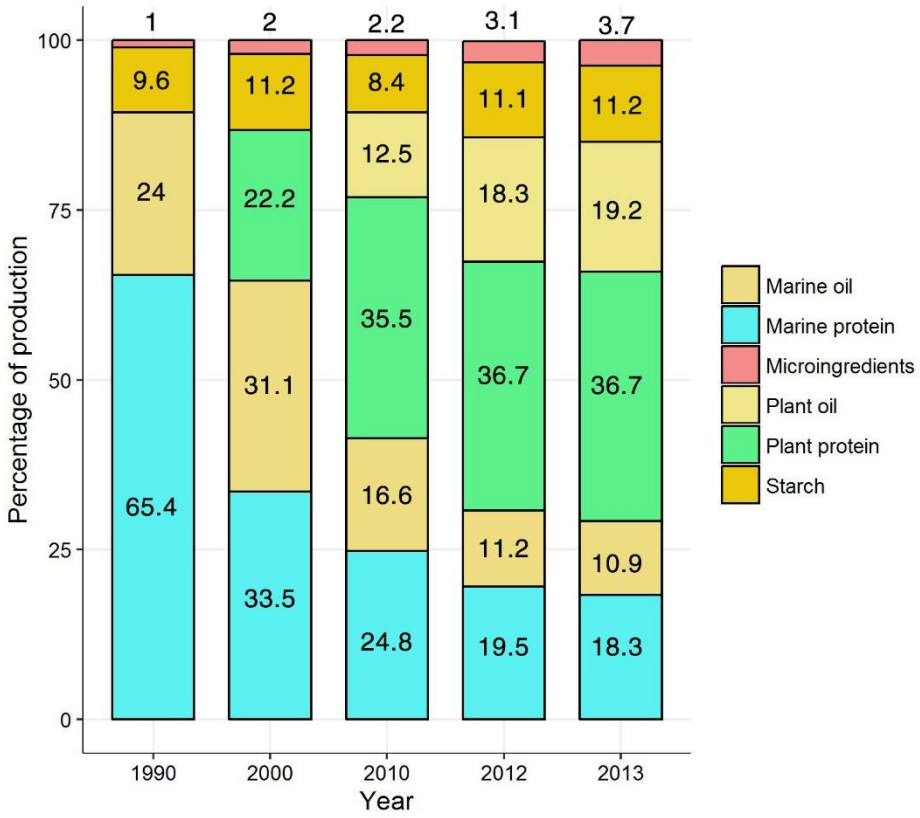

Figure 05: The main ingredients used in production of Norwegian salmon feed. The major trend is a reduction in using of marine protein and oil ingredients and increased use of plant derived ingredients (Adapted from Ytrestøyl et al. (2015))

1.7.1 Plant ingredients for Atlantic salmon feeds

Plant derived ingredients are advantageous compared to fish meal and fish oil because of their abundant availability and favorable price. The inclusion level of plant ingredients in fish diets, however, may be restricted by content of anti-nutritional factors, imbalanced amino acid profiles, lower amount of protein content and higher amount of carbohydrates. The plant oils with the greatest potential to be used in aqua diets are high in polyunsaturated fatty acids. The main disadvantage with plant oil compared to fish oil is the lack of EPA and DHA. There is an increasing concern about the long-term effects of low levels of these fatty acids on fish health (Rosenlund et al., 2016, Sissener et al., 2016).

Digestibility of plant ingredients varies with inclusion level, origin, anti-nutritional factors and processing methods. Studies have shown that inclusion of solvent extracted soybean meal (20%-30%) cause reduced digestibility of lipids in salmonids (Refstie et al., 2000, Storebakken et al., 2000). Use of solvent extracted soy bean meal is also associated with morphological changes in the distal intestine of salmonids. In contrast, inclusion of wheat gluten at 50% level has increased protein digestibility of Atlantic salmon without any morphological changes in the distal intestine (Storebakken et al., 2000). Digestibility of lipids is particularly higher in canola/rapeseed oil compared to other varieties such as palm oil and sunflower oil. This may be a result of favorable n-3/n-6 ratio in canola oil compared to other plant oils (Sørensen et al., 2011a).

Incorporation of plant ingredients in salmonid diets and related health and growth response have been investigated by various authors (Król et al., 2016, Skov et al., 2012, Van den Ingh et al., 1996). Król et al. (2016) concluded that salmonids without any evolutionary adaption to plant based diets, show adverse effects on health and growth when they are fed plant ingredients in the diet. These responses may be caused by anti-nutritional factors, imbalanced fatty acids and amino acid profiles. Anti-nutritional factors may interfere with digestion, absorption, metabolism and consequently also utilization of feed. In addition to that, higher incorporation of plant oils has demonstrated morphological and histopathological changes, and that may have negative effects for gut health in Atlantic salmon (Moldal et al., 2014).

Although plant ingredients may have specific limitations for use at high inclusion level, modern processing technology such as enzymatic treatment, solvent purification and extrusion technology can be used to eliminate anti-nutritional factors. Plant breeding can also be used to develop plant varieties low in anti-nutritional factors and improved quality for carnivore fish.

Other methods such as air classifications and fractionation can be used to produce protein concentrates.

1.7.2 Other alternative feed ingredients in Atlantic salmon diet

Terrestrial animal byproducts include, blood meal, meat and bone meal, poultry byproduct meal and feather meal. Animal by products are used all over the world in different aquaculture practices. However, these rendered products have higher variability in nutritional composition and lower digestibility. Also, nutritional composition of these products is highly depending on the processing conditions.

Use of rendered animal products in Atlantic salmon productions, are dated back to 1930s. However, their use was limited in certain parts of the world (e.g. North America) in late 1980s, mainly because of digestibility issues (Bureau, 2006). Significant improvements in the production technology have overcome these issues in last decades. However, in 2001 the use of animal by-products was banned in the European Union to avoid spreading of Bovine Spongiform Encephalopathy (BSE). Only since 2013, animal by-products derived from poultry and swine, including feather meal and blood products, were released from the ban and can now be used in animal feeds in Europe.

1.7.2.1 Poultry by product meal

Poultry by product meal consist of various by products from poultry industry and may include necks, gizzards, heads, underdeveloped eggs and clean intestines. Nutritional composition of poultry by products may vary according to substrate and processing conditions. Usually poultry by product meal contains more saturated fatty acids and non-essential amino acids compared to fish oil and fish meal, respectively.

Only a few studies have investigated the potential of poultry by product meal in diets for Atlantic salmon. (Hatlen et al., 2015) observed a protein digestibility of poultry byproduct meal of 77% when constituting 50% of the protein in the feed. (Hatlen et al., 2015)suggested that the low protein digestibility was explained by suboptimal-processing conditions (high temperature) or sub optimal nutrient compositions. Other studies with salmonids fed poultry by-product meal reported higher protein digestibility (Bureau et al., 1999, Cheng and Hardy, 2002, Dong et al., 1993). Saturated fatty acids limit the use of poultry meal in feeds for cold water aquaculture. For this this reason, only the protein fraction has a potential while the lipid fraction should be limited (Skrede et al., 2011).

1.7.2.2 Blood meal

Blood meal is considered as a cost effective and highly digestible protein source for aquaculture species. Fresh animal blood from slaughter houses can be produced into various products such as whole blood, hemoglobin and plasma meals. Common practices to produce blood meal include solar drying, ring drying, spray drying and oven drying methods.

The different processing methods has a huge impact on nutritional quality of blood meal. Heat damage in the production of blood meal reduces the bioavailability of nutrients. Spray drying is the preferred drying method, resulting in higher nutrient digestibility and improved nutrient bioavailability compared to other drying methods.

Amino acid profile of blood meal usually comprised with higher content of lysine, valine and leucine (NRC, 1993, Sørensen et al., 2011a) . Blood meal contain high amount of iron (Fe) which act as a pro-oxidant, that may reduce pigmentation of Atlantic salmon (Rørvik et al., 2003). However, low inclusion, 4.1% of the diet, showed positive effects on growth rate and immunity in Atlantic salmon parr (Gisbert, 2013). Use of blood meal can also prevent cataract in Atlantic salmon, largely due to its high content of histidine (Breck et al., 2003).

1.7.2.3 Bacterial protein meal

In recent years, significant attention has been given to the bacterial meal as feed ingredient for Atlantic salmon. Proximate composition of the bacterial meal depends upon the type of bacteria, processing conditions and substrate conditions (Sørensen et al., 2011a). Spray dried bacterial meal contains 70% of crude protein and 10% crude lipids (Aas et al., 2006). Amino acid composition of the bacterial meal resembles fish meal except it's higher tryptophan and lower lysine concentrations (Skrede et al., 1998). Composition of fatty acids are dominated by C 16:0 (49%) and C 16:1 (n-7) (36%) (Øverland et al., 2010). Studies with Atlantic salmon fed bacterial protein meal showed increased growth up to an inclusion of 36% of the diet (Aas et al., 2006, Berge et al., 2005). Romarheim et al. (2011) has also reported that bacterial meal produced from natural gas can prevent the soybean induced enteritis in Atlantic Salmon. One of the problem associated with bacterial protein meal is, reduced bioavailability of nutrient due to rigid cell walls. High content of nucleic acids, up to 10% of the dry matter, may also have negative effects on fish physiology by elevating uric acid level in plasma (Aas et al., 2006).

1.8 Microalgae in Aquaculture

Microalgae play a pivotal role in both fresh water and marine aquaculture. Microalgae consist of large number of microscopic unicellular algae inhabited in both fresh water and marine water. Though culturing of microalgae can be dated back to late 1800s to early 1900s, first microalgae culturing was stared in 1910 for aquaculture purposes (Del Campo et al., 2007). Since then culturing of microalgae for aquaculture purposes has gained growing interest. In recent years, development of the bio-fuel technology also influenced wide scale use of microalgae as a novel aquafeed ingredient (Gong et al., 2017).

In larval fish nutrition both prepared micro algal feeds and/or fresh microalgae play significant role. Larval stages of marine bivalves, marine gastropods (abalone, conch), and several species of shrimp and zooplanktons depend on the microalgae. Higher nutritional profile, antioxidant activity, high growth rate and appropriate size of microalgae contributed to this popularity in larval aquaculture (Roy and Pal, 2015). Several species of microalgae are used for start feeding in hatcheries, such as *Isochrysis* sp., *Chaetoceros* sp. *Teraselmis* sp. and *Pavlova* sp. These species can be used alone or in mixes. Microalgae such as *Pavlova* sp. and *Isochrysis* sp. are fed to zooplankton in order to enrich them with DHA. These enriched zooplanktons are then used as for start feeding of fish larvae.

1.8.1 Nutritional profile of microalgae

Microalgae contain a wide range of chemical compounds such as carbohydrates, lipids, proteins, vitamins and other various bioactive components. Nutritional composition may vary depending on the various culture conditions, species of concern, different strains, shape and size, digestibility and biochemical composition.

1.8.1.1 Amino acid composition

Amino acids in fish feed are important for protein synthesis, feed utilization, growth, stress response, immunity and survival (Li et al., 2009). Amino acid composition of most of the microalgae are in line with fish meal and other dietary sources of proteins (Table 01). Studies with a large array of microalgae have shown that amino acid pattern are quite similar, though minor differences are present, in particular for the sulfur containing amino acids methionine and cysteine (Brown, 1991, Roy and Pal, 2015). Moreover, most of the amino acid composition of algae follow the same pattern as other aquatic animals such as oyster and shrimps (Brown,

2002). This suggest that microalgae can be a good source of amino acids for different aquaculture species.

Table 01: Amino acid composition of several microalgae compared with fish meal

	C. gracilis [1]	*N. Oceania* [2]	*Isochrysis galbana* [2]	*Phaeodactylum tricornutum* [2]	N. closterium [1]	High quality fish meal [1]
Lysine	6.3	4.8	3.1	4.2	5.7	6.8
Methionine	2.4	1.8	2.5	2.0	1.6	2.5
Tryptophan	1.6	1.7	2.5	1.3	1.4	0.7
Threonine	4.5	3.6	4.6	3.7	5.5	3.5
Valine	5.9	4.6	6.1	4.6	6.2	4.0
Isoleucine	5.8	3.5	5.1	3.8	5.0	3.7
Leucine	7.2	6.7	9.2	6.2	8.1	6.2
Phenylalanine	6.7	3.9	5.7	4.2	6.9	3.3
Arginine	6.6	4.9	4.1	4.4	6.6	5.4
Histidine	2.4	ND	ND	ND	1.4	ND

ND – Not Determined

[1] Brown (1991)

[2] Skrede et al. (2011)

1.8.1.2 Fatty acid composition

Fatty acid profile of microalgae consists of saturated, monounsaturated and polyunsaturated fatty acids (PUFA). Fatty acid composition may differ among different algal species (Table 02). Variations of the culture conditions (Temperature, nutrient concentrations and light) affects cell cycle, lipid class composition and membrane fluidity of microalgae which in turn decide fatty acid composition (Napolitano, 1999). However, Brown (2002) pointed out that despite these changes there is correlation between different algal taxa and fatty acid composition. Microalgae may be a promising source of PUFA in fish diets. Compared with other micro algal classes, Chlorophytes (e.g. *Scenedesmus* sp.) usually has deficiency in PUFA while eustigmatophytes (e.g. *Nanochloropsis* sp.) and cryptomonads (e.g. *Chryptomonas* sp.) are rich in PUFAs (Brown, 2002).

Table 02: Fatty acid composition of selected microalgae types (Napolitano, 1999)

	Oscillatoria sp.	***Scenedesmus sp.***	***Cladophora sp.***	***Navicula sp.***	***Chryptomonas sp.***	***Peridinium sp.***
14:0	2.1	1.4	0.5	3	5	6.9
16:0	18.5	12.7	39.3	16	10	28.8
16:1ω7	22.6	-	1.3	31	9	1.6
C16 PUFA	-	-	0.5	17	1	-
18:0	1.6	0.6	0.3	1	8	0.6
18:1ω9	1.9	11.6	12.9	2	16	29
18:1ω7	0.5	0.4	1.1	-	-	-
18:2ω6	11.4	12.9	10.7	1	26	0.5
18:3ω6	-	1.3	0.7	-	-	0.1
18:3ω3	24.6	22.2	12.2	1	5	0.2
18:4ω3	-	3.3	-	-	-	5.2
20:0	-	0.2	0.3	-	-	-
20:1ω9	-	0.3	0.6	-	-	-
20:4ω6	-	2.8	-	-	-	2.0
20:5ω3	-	-	2.3	26	-	7.8
22:5ω3	-	-	-	-	-	-
22:6ω3	-	-	0.02	-	-	11

1.8.1.3 Other biochemical components from microalgae

Microalgae are also rich in other types of biomolecules such as pigments, and nutraceutical compounds. Pigments such as astaxanthin, lutein, and beta carotenoids are important for skin and flesh coloration of rainbow trout and salmonids (Del Campo et al., 2007, Sommer et al., 1992). Lutein has ability to prevent the cataracts and enhance the immunity by antioxidant activity in farmed species (Yaakob et al., 2014). Other compounds such as beta 1-3 glucan from microalgae has initiated host defense mechanisms and improved both specific and non-specific immunity of various fish species such as Rohu (Misra et al., 2006, Sahoo and Mukherjee, 2001) and rainbow trout (Skov et al., 2012). Presence of non-protein sulphonic acid taurine, distinguish microalgae from other land based plant ingredients. Taurine rich

dietary ingredients enhance growth and survival, reduce susceptibility for diseases in carnivore fish (Salze and Davis, 2015).

Vitamin content of microalgae is different among species. Extensive review by Brown and Miller (1992) showed that this variation has largely observed in ascorbic acid of microalgae. Increase reproductive performance, reduce oxidative damage, disease resistance showed by incorporation of ascorbic acid into salmonid feeds (Sandnes et al., 1984). In this perspective, ascorbic acid rich algae such as *Chaetoceros gracilis, T. pseudonana* has higher potential in incorporating into aquatic animal diet.

1.8.2 Microalgae in formulated fish diets

The potential of the different strains of microalgae in formulated fish diets has been experimented in various studies. Digestibility of different strains of microalgae in finfish species has shown various results. Studies with Atlantic char (*Salvelinus alpinus*) and Atlantic salmon (*Salmo salar*) fed with *Spirulina* sp. at 30% inclusion level showed protein digestibility of 82% and 84.7%, respectively (Burr et al., 2011). However, 6% inclusion of *Phaeodactylum tricornutum* at in Atlantic salmon diet (Sørensen et al., 2016) showed protein digestibility of 90%. For the carnivore mink protein digestibility of the microalgae *Nannochloropsis oceanica, Phaecodctylum tricornutum* and *Isochrysis sp*. was estimated to 35.5%, 79.9% and 18.8%, respectively (Skrede et al., 2011)

Incorporating algae into fish feed may also have positive effects on nutrient utilization and the growth performance of the fish. In a study, Olvera-Novoa et al. (1998) found that 20% inclusion of *Spirulina maxima* in feed for *Oreochromis mossambicus* increased feed utilization, growth performance and nutrient utilization. Tibaldi et al. (2015) also reported that diets with freeze dried *Isochrysis* sp. at 20% level enhanced the protein efficiency ratio, and specific growth rate in European sea bass. On the contrary, Inclusion of 11% -15% inclusion level of *Schyzochytrium* sp. in feed for Atlantic salmon (Kousoulaki et al., 2015, Sprague et al., 2015) showed significant growth reduction and feed utilization. These findings imply that inclusion level, type of algae and fish species has influenced on the results. Therefore, introduction of microalgae into commercial fish diets should be subjected to careful evaluation of the particular microalgae species in the target fish species. Experiments should be designed to determine digestibility, nutrient retention and effect on fish growth and health when incorporating microalgae into fish diets.

Several studies have investigated the potential of *Scenedesmus* sp. as a fish feed ingredient (Vizcaíno et al., 2014, Tartiel et al., 2008). However, no previous studies have reported the effect of *Scenedesmus* sp. as a feed ingredient for Atlantic salmon. Therefore, the present study hypothesis that incorporation of microalgae *Scenedesmus* sp. in diets for o Atlantic salmon diet has no negative effects on growth, nutrient digestibility and nutrient retention. The aims of the study were 1) to investigate the feasibility of using microalgae *Scenedesmus* sp.in feed for Atlantic salmon on growth, feed utilization and nutrient digestibility and 2) to evaluate the effect of inclusion level of *Scenedesmus* sp. on physical quality of the feed.

2 Material and Methods

2.1 Fish and experimental setup

The feeding experiment was carried out at the Mørkvedbukta research station, Nord University, Bodø, Norway in a flow-through system. In total 18 circular fiber glass tanks (800 l and 0.9 m deep) were used. Each tank was supplied with 740L of water pumped from Saltfjorden at a depth of 250 m. During the experiment, water flow rate was maintained at 1000L per hour. Salinity of the water was 35 ‰. Water temperature and of dissolved oxygen content was measured every morning. Consistent 24-hour photoperiod was maintained throughout the experimental period.

This experiment was approved by the National Animal Research Authority (FDU, ID-5887 in Norway). Experimental fish of Atlantic Salmon (*Salmo Salar*) was obtained from Sundsfjord Smolt, Nygårdsjøen, Norway and maintained at the research station for approximately four months prior to starting the experiment. At the start of experiment, a total number of 450 fish with initial weight 228.44 ± 5.87 g and initial total length 26.98 ± 0.15 cm were randomly distributed to the experimental units (n=25 fish per tank).

2.2 Experimental diets

The experimental diets were produced at Sparos LDA®, Olhão, Portugal. Microalgae *Scenedesmus* sp. used in the diets was produced in closed photobioreactors at allma®. Proximate composition of the *Scenedesmus* sp. used in diet is presented in Table 03. Three experimental diets were optimized to contain low fish meal and a mixture of soy protein concentrate, pea protein concentrate and potato concentrate in 1:1:1 ratio (Table 04). Fish oil and rapeseed oil were used as lipid source in 1:1 mix. The experimental diets were formulated to contain 1) 10% fish meal and no microalgae. (CT), 2) 5% fish meal and 10% microalgae (SCE 10) and 3) 2.5% fishmeal and 20% microalgae (SCE 20). The plant protein mix, wheat gluten and lipid sources were slightly reduced with increasing incorporation of microalgae to balance protein, lipid, carbohydrates and energy.

Table 03: Proximate composition of *Scenedemus* sp. used in the feeds.

Nutrient	Chemical composition (per 100g)
Carbohydrates	15.6 g
Dietary Fiber	15.8 g
Fat	9.1 g
Protein	45.7 g
Ash	8.3 g
Moisture	5.6 g
Energy	358 kcal

Table 04: Ingredient composition of the three different diets

Ingredients	CT %	SCE 10 %	SCE 20 %
Fishmeal 70 LT FF (SKAGEN)	10.000	5.000	2.500
Scenedesmus sp. – (allma®)	0.000	10.000	20.000
Soy protein concentrate (SOYCOMIL®)	12.000	11.750	10.900
Pea protein concentrate	12.000	11.750	10.900
Potato concentrate	12.000	11.750	10.900
Wheat Gluten	8.500	8.300	7.700
Corn gluten	7.000	6.850	6.350
Wheat meal	14.500	11.000	7.650
Fish oil (SAVINOR)	10.000	9.800	9.550
Rapeseed oil	10.000	9.800	9.550
Vitamin & Mineral Premix PV01	1.000	1.000	1.000
Soy lecithin - Powder	0.500	0.500	0.500
MCP	2.080	2.080	2.080
L-Histidine	0.100	0.100	0.100
DL-Methionine	0.300	0.300	0.300
Yttrium oxide	0.020	0.020	0.020
Total	100.00	100.00	100.00

2.3 Experimental Design

The experiment was planned as a combined growth performance and nutrient digestibility study and lasted for 65-days. Study parameters were growth, feed utilization and nutrient digestibility. Each of the diets (CT, SCE 10 and SCE 20) were fed to fish in 6 replicate tanks and 25 fish per tank (Figure 06).

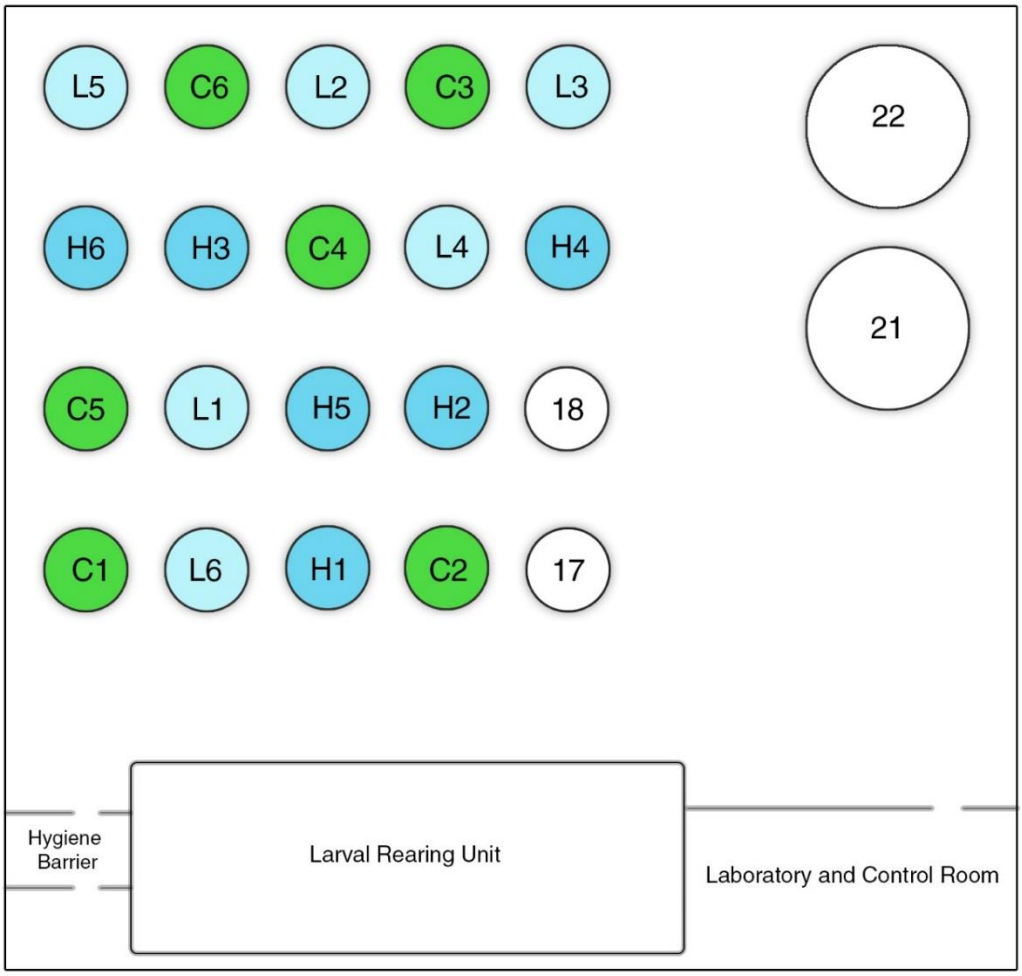

Figure 06: Organization of tanks and allotment of feed. Each of the treatment groups consisted 6 replicates (C: Control, L: Low algae diet, H: High algae diet). Tanks No. 17, 18, 21 and 22 were not used in this study. Tanks were arranged in above pattern to avoid confounding error.

2.4 Feeding regime of fish

The fish were fed *ad libitum* using automatic feeders (Arvo Tech, Finland), administrated in two feedings per day, from 0800-0900 in the morning and 1400-1500 in the afternoon. Feeding rate was increased once per week during the course of the experiment, based on the average feed intake of the previous week, targeting 10-15% waste feed. Approximately 30 minutes

before each feeding, all the tanks were flushed in order to remove faeces from the tanks and minimize the risk of contaminating uneaten feed with faeces. After each feeding, remaining feeds from each tank were collected to the steel wire mesh. Uneaten feed materials were separated from the faeces and stored at -20 °C. At the end of each week, all collected uneaten feed materials from each tank were oven dried at 110 °C overnight to calculate dry matter content.

2.5 Fish Sampling and data collection

At the beginning and end of the experiment, all the 450 fish were individually weighted and total length was recorded. Before handling, fish were anesthetized by using Tricaine-methanesulfonate (MS 222, 100 mg/L).

At the beginning of experiment, 6 fish were anesthetized and sacrificed by a percussive stunning to the head. All 6 fish were stored at -20 °C for chemical analyzes.

At the end of trial, 6 fish for analyses of whole body proximate composition were taken out from each tank (n=36 for each treatment; total 108 fish). Sampled fish were anesthetized and sacrificed by giving percussive stunning to the head. The fish were packed in plastic bags and immediately stored at -20 °C for chemical analyses.

Faeces were collected from all the remaining fish in the tank. Fecal matter was obtained from individual fish by stripping, and pooled per tank to ensure enough material for chemical analysis. Immediately after termination of experiment, all fish and fecal samples were transferred to the main campus at Nord University and stored at -20 °C.

2.6 Sample preparation

The fish samples were taken out from the freezer and thawed for approximately 24 hours before the analysis of whole body proximate composition. Each fish was cut into approximately 10 cm pieces, and homogenized by using industrial food processor (Foss tecator, 2096 homogenizer, Denmark). Feed samples (100 g from each feed) for proximate analysis were homogenized by food processor (Retsch, Grindomix, GM 200, Germany). Both homogenized feeds and fish were analyzed for protein, crude fat, ash, moisture and energy.

Samples of faeces were freeze dried (VirTis benchtop, U.S.A.) for 72 hours at -76°C with 20 bar. After freeze drying, faeces was pooled within feed group, reducing number of replicates from six to three prior to the analysis of chemical composition. The freeze dried faeces were then analyzed for protein, moisture, ash and energy.

Proximate chemical composition was analyzed on individual fish (n=6) or pooled samples (n = 6 fish per tank) on fish samples from start and termination of the experiment, respectively. Feeds were analyzed in 4 replicates per diet.

2.7 Proximate chemical analysis of whole fish, faeces and feeds

2.7.1 Moisture analysis

Total dry matter content was determined by using oven drying of respective samples at 105 °C for 24 hours to constant weight (ISO 6496-1999). Approximately 5 g of the homogenized fish samples (5 g for feeds and 1g for wet faeces) were weighted into the steel cups. Percentage of moisture was calculated as the weight difference, before and after drying oven.

2.7.2 Lipid content

Approximately 10 g of homogenized fish sample was weighted into a porcelain cup and 20 g of $NaSO_4$ was added to the same cup. Samples were ground together to a dry powder and transferred into a bottle with 50 ml of Ethyl Acetate. Bottles were placed on shaker for one hour and supernatant of each bottle was filtered through Whatman filter paper (41, CAT NO. 1441-150) to a measuring cylinder. 20 ml of the sample were pipetted to weighted glass cup and evaporated on water bath until all the water was removed. Glass cups were placed in the oven at 105°C for 30 minutes until all moisture was removed. Weight of the dried samples were used to determine the crude lipid content of the samples.

2.7.3 Protein analysis

Protein analyses were carried out by using Kjeldahl method (Kjeltech Auto Analyser, Tecator, Höganäs, Sweden). Approximately 1g of the homogenized fish samples (0.3 g for faeces and 0.5 g for feeds) were weighed into the Nitrogen free paper and then it was transferred into glass tubes. For each tube two Kjeldahl tablets were inserted. Under the fume hood 15 ml of concentrated H_2SO_4 acid was added to each of the glass tube. All the glass tubes were placed on preheated (420 °C) hotplate over 45 minutes and cooled down for 30 minutes. 75 ml of Distilled water was added to each of the tube. Glass tubes were placed on Kjeltec Auto Analyzer for determining of Nitrogen. Crude protein content was calculated by using factor 6.25 (ISO 5983–1987).

2.7.4 Ash content

Ash content of the samples were measured gravimetrically by use of flame combusting at 550 °C for 12-16 hours until constant weight (ISO 5984–2002). Approximately 5 g of homogenized fish sample (5 g of feeds and 0.5 g of faeces) were used for determination of the ash content. Ash percentage in each sample was determined by weight difference before and after oven drying.

2.7.5 Energy content

Energy content was measured by bomb calorimetry (IKA, c200, GmbH & Co. KG, Germany) (ISO 9831–1998). Approximately 0.5g of homogenized fish sample or faeces were pelleted and placed on calorimetry. Energy released from each pellet was recorded.

2.7.6 Yttrium content

The content of Yttrium was analyzed by Eurofins® as described in (Sørensen et al., 2016). Ashed samples of faeces were dissolved in HCl and HNO_3 acid by heating and finally dissolved in 5% HNO_3 acid. Yttrium amount was then detected by ICP-AEF, Opima 3000 V (Perkin Elmer, USA) (NS-EN ISO 11885).

2.7.7 Lipid content of faeces and feeds

Total lipid content of the freeze-dried feeds and faecal samples were analyzed by Eurofins® by Soxhlet method with acid hydrolysis (Soxtec HT 6209, Tecator, Höganäs, Sweden modified; AOAC method 954.020).

2.8 Physical quality of diet

2.8.1 Fat leakage

Approximately 5 g of each diet were weighed into plastic tray with two layers of Whatman gel blotting paper (Grade GB003, 30 × 60 cm) covering the bottom. Plastic tray with blotting paper and diet was heated in the oven at 40 °C for 24 hours. Final weight of the diet was recorded in order to calculate the fat leakage. Each diet group were analyzed in 04 replicates.

2.8.2 Hardness

Feed pellets (N = 20) from each diet were randomly selected and hardness was analyzed in 6 replicates. Hardness was analyzed by using TA-XT2 (Stable Micro Systems Ltd, Surrey, England) analyzer. Each pellet from each diet group were placed horizontally and cracked by

using cylindrical probe. (SMP/0.5, 1.2 cm width) at 60% compression rate and 1mm sec^{-1}. Hardness of the pellets were automatically recorded by the TA-XT2 program in Newton (N).

2.8.3 Length

Length of the pellets were analyzed by using Vernier caliper (Biltema® Art. 16-105) in mm. 20 feed pellets from each diet were randomly selected analyzed in 06 replicates for length of the diet.

2.8.4 Water stability

Approximately 3g sample of pellets from each diet were placed into pre-weighted embedding cassette (M 512 Macrosette™, Simport®, Canada). Each cassette has dimensions of 40.1 x 28.5 x 13 mm. Test were carried out in 6 replicates for each treatment. Cassettes that include the pellets were placed on 1L beaker and 300 ml of distilled water was added. Beakers were then incubated in a water bath (Julabo™, SW22, Seelbach, Germany) at 25 °C and subjected to 100 shakings per minute over 15, 30, 45 and 60 min respectively. After incubation, cassettes were placed on paper tissues and gently dried. All the cassettes were then placed on pre-heated oven at 80 °C for 48h. Residual dry matter weight of each cassette were determined after drying. Water stability was calculated as weight difference of dry matter before and after incubation, divided by dry matter weight of the feeds before incubation.

2.9 Calculations

Fish growth performance was calculated based on following equations:

$$\text{Weight gain (\%)} = \left(\frac{W_f - W_i}{W_i}\right)\times 100$$

where, W_f=final body weight of fish (g/fish) and W_i=initial body weight of fish (g/fish)

$$\text{Specific Growth Rate (\% day}^{-}) = \left(\frac{\text{Ln }(W_f) - \text{Ln }(W_i)}{\text{No. of days}}\right)\times 100$$

$$\text{Food conversition ratio (FCR)} = \frac{\text{Total feed intake in dry basis (g)}}{\text{Weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain}}{\text{Total protein ingeted (g)}}$$

$$\text{Thermal growth coefficient (TGC)} = \frac{(W_f)^{1/3} - (W_i)^{1/3}}{(T\times t)} \times 1000$$

where T is temperature in °C and t is time in days.

$$\text{Hepato} - \text{somatic index} = \frac{\text{Liver weight of fish (g)}}{W_f(g)}$$

$$\text{Viscero} - \text{Somatic Index} = \frac{\text{Visceral Weight (g)}}{W_f(g)} \times 100$$

$$\text{Condition factor} = \frac{W_f(g)}{FL^3} \times 100$$

where FL= Fork length of fish

$$\text{Lipid \%} = \frac{10300\times\text{Lipid (g)}}{((40 - 2.17\times\text{lipid (g)})\times\text{Sample weight (g)}}$$

Apparent Digestibility Coefficient (ADC),

$$\text{ADC} = [100 - \left[\frac{\%\ indicator\ in\ feed}{\%\ indicator\ in\ faeces} - \frac{\%\ indiacator\ in\ faeces}{\%\ nutrient\ in\ feeds}\right]] \times 100$$

$$\text{Nutrient/Energy retention} = \frac{(W_f \times N_f\ (\text{or}\ E_f\ ) - W_i \times N_i\ (\text{or}\ E_i\ )}{\text{NI (or EI)}} \times 100$$

Where N_f=final nutrient content of the body; N_i=initial Nutrient content of the body, E_i= Initial Energy content of the body, E_f=Final Energy content of the body, NI=Nutrient intake or EI= Energy Intake

2.10 Statistical analysis

Statistical analyses were performed by using R™ v3.3.1 (R Development Core Team, 2016) statistical program. Data were checked for normality by Kolmogorove-smirnov test. For parametric data, one way analysis of variance (ANOVA) was performed with Bartlett's test for equal variance. Tukey's multiple comparison test were used to identify significant difference among the means. For non-parametric data Kruskal-Wallis test was performed followed by Dunn's multiple comparison test. Correlation analysis was performed by using Pearson correlation coefficient in R™ v3.3.1 (R Development Core Team, 2016). Graphs were generated by using ggplot2 package (Wickham, 2009).

The difference between treatment was considered significant at $p<0.05$ and $0.05<p<0.01$ considered as tendency.

3 Results

3.1 Growth Performance, nutrient and feed utilization

The overall growth performance, nutrient retention and feed utilization data are shown in Table 05. The fish grew from an initial average weight of 229.1 g to a final mean body weight of 447.0 g. Significant reduction of the final mean body weight, weight gain, specific growth rate, protein efficiency ratio and thermal growth coefficient were noted in fish fed the SCE 20, compared with CT group. Fish fed the SCE 10 tended to have lower final mean body weight and specific growth rate compared with those fed CT, but the values were higher than fish fed SCE 20. Though no significant differences were noted, fish fed the SCE 10 had lower weight gain, thermal growth coefficient and poorer feed conversion ratio compared to those fed CT feed. Feed conversion ratio was significantly poorer for groups fed SCE 20 compared with fish fed the CT. Condition factor was significantly higher in fish fed the CT diet than fish fed the alga incorporated diets. No significant difference in hepato-somatic index and viscero somatic index were recorded among groups.

Table 05: Growth performance, nutrient and feed utilization of Atlantic salmon for experimental period

Parameter	CT	SCE 10	SCE 20	p value
Growth parameter				
Initial mean body weight (g)	228.4 ± 4.61	230.8 ± 2.22	228.1 ± 4.10	0.4184
Final mean body weight (g)	473.6 ± 47.74^{a}	451.0 ± 23.39^{ab}	416.3 ± 22.12^{b}	0.0292
Weight gain (%)	107.1 ± 17.22^{a}	95.4 ± 10.32^{a}	82.4 ± 7.43^{b}	0.0124
Specific growth rate (% day)	1.1 ± 0.13^{a}	1.0 ± 0.08^{ab}	0.9 ± 0.05^{b}	0.0143
Feed conversion ratio	0.7 ± 0.07^{b}	0.8 ± 0.04^{b}	0.9 ± 0.04^{a}	0.0002
Protein Efficiency ratio	2.6 ± 0.23^{a}	2.3 ± 0.11^{b}	2.1 ± 0.10^{b}	0.0001
Thermal growth coefficient	3.4 ± 0.48^{a}	3.1 ± 0.27^{a}	2.8 ± 0.22^{b}	0.0154
Somatic Indices				
Hepato-Somatic index	1.5 ± 0.14	1.5 ± 0.15	1.5 ± 0.16	0.7809
Viscero-Somatic-Index	10.0 ± 1.23	10.4 ± 0.94	11.1 ± 1.38	0.2817
Condition factor	1.4 ± 0.04^{a}	1.3 ± 0.02^{b}	1.3 ± 0.03^{b}	0.0001

Values are expressed as mean ± SD (n=6 replicates). Values in the same row with different superscript letters show significant differences ($p<0.05$)

3.2 Proximate composition of the whole fish

The proximate composition of fish from the initial population and experimental groups sampled at termination of the experiment are presented in Table 06. Values from the initial population were excluded from the statistical analysis, but the numerical values show that dry matter content increased during the course of the experiment. In the dry matter, both lipid and protein increased during the course of the experiment, but the lipid content increased more than protein.

At termination of the experiment, proximate composition showed significant differences in protein, lipid ash and energy among the feeding groups (Table 06). The protein was highest in fish fed SCE 20 and lowest in those fed SCE 10, while CT ranked in between. Content of lipid was significantly lower in fish fed SCE 20 compared with the other two groups, while no differences were observed between fish groups fed SCE 10 and CT. The content of ash was significantly lower in the CT fed fish and highest in fish fed SCE 20, while fish fed SCE 10 ranked in between. Content of energy was significantly highest in CT and lowest in fish fed SCE20.

Table 06: Proximate composition of the whole fish on a dry matter basis

Parameter	Initial	CT	SCE 10	SCE 20	p value
Dry matter (g/kg)	287.3	312.6 ± 5.57^{ab}	315.4 ± 4.72^{a}	306.7 ± 3.37^{b}	0.0164
per 1000 g in dry matter basis					
Protein	542.7	556.2 ± 12.25^{ab}	546.4 ± 13.29^{b}	565.6 ± 7.32^{a}	0.0322
Lipid	332.5	373.1 ± 8.62^{a}	374.2 ± 6.98^{a}	357.0 ± 4.90^{b}	0.0010
Ash	66.2	56.2 ± 3.25^{b}	58.4 ± 3.16^{ab}	63.7 ± 4.84^{a}	0.0117
Energy	25.7	26.6 ± 0.12^{a}	26.2 ± 0.60^{ab}	26.0 ± 0.18^{b}	0.0327

Values are expressed as mean ± SD (n=6 replicates). Values in the same row with different superscript letters indicate significant difference ($p<0.05$)

3.3 Nutrient retention

Retention of lipid, protein and energy is shown in Table 07. Retention of lipid differed significantly among all three dietary groups, with highest retention in fish fed CT and lowest in those fed SCE 20. Fish fed the SCE 20 showed significantly lower retention of protein and energy compared to CT fed group, while fish fed SCE 10 tended to be lower than CT and higher than SCE 20.

Table 07: Nutrient retention of the Atlantic salmon at the end of 65 day feeding trial

Parameter	CT	SCE 10	SCE 20	p value
Lipid	85.7 ± 2.85^{a}	71.6 ± 2.64^{b}	62.9 ± 5.15^{c}	< 0.0001
Protein	48.5 ± 3.87^{a}	40.9 ± 2.48^{ab}	38.4 ± 1.50^{b}	0.0007
Energy	49.6 ± 3.08^{a}	45.0 ± 2.60^{ab}	38.9 ± 1.72^{b}	< 0.0001

Values are expressed as mean ± SD (n=6 replicates). Values in the same row with different superscript letters indicate significant difference ($p<0.05$)

3.4 Apparent digestibility coefficients

ADC of dry matter, lipid, protein, ash and energy are presented in Table 08. Digestibility of dry matter, protein and energy was significantly different among all the three dietary treatments. Highest ADC's for dry matter, protein, energy and ash were noted for the CT feed and lowest values were noted for SCE 20. For ADC of lipid, the lowest value was observed for fish fed SCE 20 and highest value was noted for CT group. ADC of ash showed negative values for all three groups. The values became more negative with higher inclusion of microalgae in the feed, however, no significant differences were observed among dietary treatments.

Table 08: Apparent digestibility coefficients (ADC %) of dry matter, lipid, protein, ash and energy in the Control feed (CT), low algae (SCE 10) and high algae (SCE 20) feed

Parameter	CT	SCE 10	SCE 20	p value
Dry matter	67.5 ± 0.89^{a}	62.6 ± 0.17^{b}	54.7 ± 3.10^{c}	0.0005
Lipid	90.7 ± 0.24^{a}	89.4 ± 0.04^{a}	79.4 ± 1.41^{b}	0.0036
Protein	83.6 ± 0.45^{a}	79.2 ± 0.10^{b}	71.5 ± 1.95^{c}	< 0.0001
Ash	-13.7 ± 3.10	-16.5 ± 0.54	-25.9 ± 8.64	0.2964
Energy	77.9 ± 0.37^{a}	72.9 ± 0.09^{b}	63.9 ± 2.50^{c}	< 0.0001

Values are expressed as mean ± SD (n=6 replicates). Values in the same row with different superscript letters indicate significant difference ($p<0.05$)

3.5 Feed quality

3.5.1 Proximate composition of the feed

The proximate chemical composition of the feeds are presented in Table 09. The SCE 20 had slightly lower dry matter compared to that of CT and SCE 10. Protein content was on average 49% and no significant differences were noted among the feeds. The lipid content ranged from 21-22%, with the significantly highest value for SCE 10. Ash content differed significantly among all three feeds with highest value for SCE 20 and lowest value for SCE 10. No difference in protein level was observed among experimental feeds. Ash content was significantly different among treatments. Significantly higher energy content was recorded in SCE 10 and SCE 20 compared with CT feed.

Table 09: Proximate composition of the feeds based upon dry matter basis

Parameter	CT	SCE 10	SCE 20	p value
per 100 g in dry matter basis				
Dry matter	93.7 ± 0.01^{a}	93.7 ± 0.09^{a}	93.0 ± 0.06^{b}	0.0010
Protein	49.2 ± 0.18	49.3 ± 0.30	48.9 ± 0.22	0.1368
Lipid	21.0 ± 1.16^{ac}	22.5 ± 0.33^{a}	20.9 ± 0.16^{c}	0.0208
Ash	5.7 ± 0.01^{b}	5.6 ± 0.01^{c}	5.9 ± 0.02^{a}	< 0.0001
Energy	22.9 ± 0.05^{b}	23.2 ± 0.17^{a}	23.1 ± 0.04^{a}	0.0191

Values are expressed as mean ± SD (n=4 replicates). Values in the same row with different superscript letters indicate significant difference ($p<0.05$)

3.5.2 Physical characteristics of experimental feeds

Physical characteristics of the experimental feeds are shown in Table 10. Color of the experimental feeds changed from light-brown (CT feed), light-black (SCE 10) to dark black (SCE 20) (Figure 07). Fat leakage differed significantly among all the three feeds. Though the SCE 20 feed had the least leakage, this feed appeared to have a more oily surface compared with CT and SCE 10. Hardness varied from approximately 23 N to 40 N. The SCE 20 had significantly highest hardness, while no differences were noted between CT and SCE 10. Length of pellet varied from 4.3 mm to 4.1 mm. The SCE 20 feed had significantly shorter pellet than CT, while SCE 10 tended to be longer than the SCE 20 but shorter than the CT. There were no significant differences in pellet diameter among the experimental feeds.

Table 10: Physical characteristics of the experimental feed

Parameter	CT	SCE 10	SCE 20	p value
Fat leakage (%)	6.2 ± 0.58^{a}	5.3 ± 0.25^{b}	3.9 ± 0.40^{c}	< 0.0001
Hardness (N)	22.9 ± 4.78^{b}	22.2 ± 5.00^{b}	39.6 ± 8.07^{a}	< 0.0001
Length (mm)	4.3 ± 0.53^{a}	4.2 ± 0.45^{ab}	4.1 ± 0.59^{b}	0.0001
Diameter (mm)	3.0 ± 0.18	3.0 ± 0.13	3.1 ± 0.22	0.4634

Values are expressed as mean ± SD (n=6 replicates). Values in the same row with different superscript letters indicate significant difference ($p<0.05$)

Figure 07: Physical appearance of the three different feeds

3.5.3 Water Stability test

Results of the water stability test are shown in Figure 08. CT feed showed significantly lower water stability compared with the SCE 10 and SCE 20 at 15, 45 and 60 minutes. No significant variation in the water stability of SCE 10 and SCE 20 was recorded during the experimental period. However, SCE 10 has the highest water stability compared to the other two feeds.

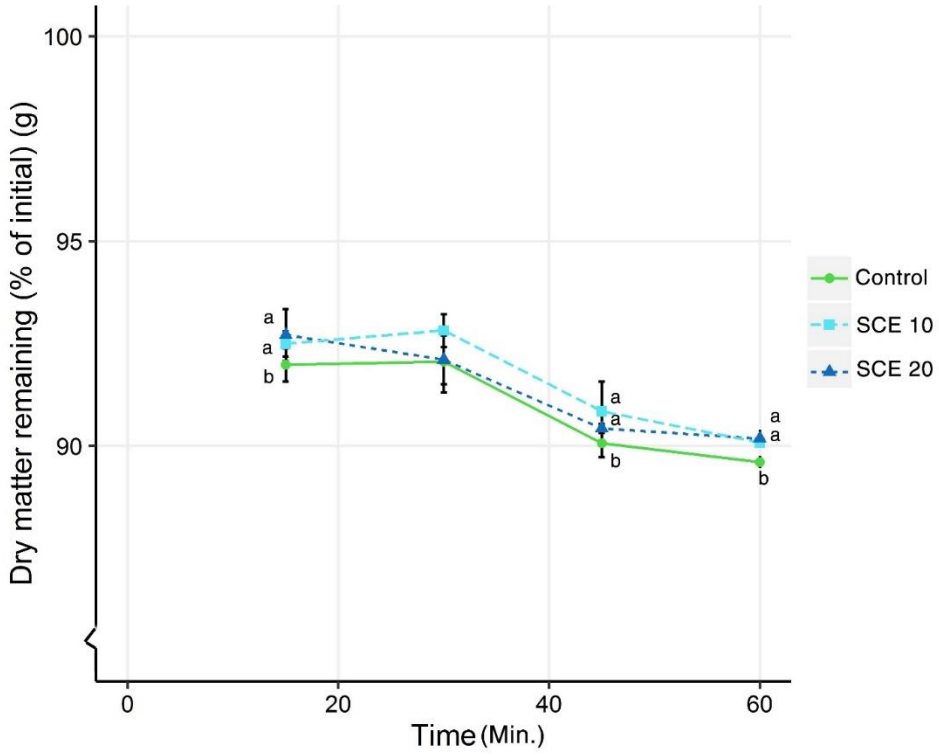

Figure 08: Water stability test for CT, SCE 10 and SCE 20 feeds.

3.6 Correlation

Correlation coefficients between fish growth parameters and physical quality of the feeds are shown in Table 11. There was a significant positive correlation between fat leaking and weight gain, thermal growth coefficient as well as specific growth rate. Significant negative correlation was observed between feed conversion ratio and weight gain. No significant correlation found among water stability at 15,30,45, 60 minutes, pellet hardness, pellet diameter, feed conversion ratio as well as feed intake.

Table 11: Correlation among physical quality of the feeds and growth parameters of Atlantic salmon

Variables		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	WG	-													
2	SGR	1.00*	-												
3	FCR	-1.00*	-0.99	-											
4	PER	0.99	0.97	-0.99	-										
5	TGC	1.00*	1.00*	-0.99	0.98	-									
6	Diameter	-0.36	-0.41	0.30	-0.20	-0.39	-								
7	Hardness	-0.87	-0.89	0.83	-0.77	-0.88	0.78	-							
8	Length	0.98	0.96	-0.99	0.99	0.97	-0.14	-0.73	-						
9	FL	1.00*	1.00*	-0.99	0.97	1.00*	-0.42	-0.90	0.96	-					
10	WS 15	-0.96	-0.95	0.98	-0.99	-0.95	0.09	0.70	-1.00	-0.94	-				
11	WS 30	-0.03	0.03	0.09	-0.19	0.01	-0.92	-0.48	-0.25	0.04	0.29	-			
12	WS 45	-0.43	-0.38	0.49	-0.58	-0.40	-0.69	-0.08	-0.62	-0.37	0.66	0.91	-		
13	WS 60	-0.92	-0.89	0.94	-0.97	-0.90	-0.04	0.60	-0.98	-0.89	0.99	0.42	0.75	-	
14	FI	0.59	0.63	-0.54	0.44	0.62	-0.97	-0.91	0.39	0.64	-0.35	0.79	0.48	-0.22	-

*correlation is significant at 0.05 level (2-tailed)

(WG – Weight gain, SGR – Specific Growth Rate, FCR – Feed Conversion Ratio, PER – Protein Efficiency Ratio, TGC – Thermal Growth Coefficient, FI – Feed Intake, FL – Fat Leakage, WS 15 - Water Stability at 15 minutes, WS 30 - Water Stability at 30 minutes, WS 45 - Water Stability at 45 minutes, WS 60 – Water Stability at 60 minutes)

4 Discussion

4.1 Growth performance of the fish

Results obtained in the present work showed that 10% replacement of fish meal with *Scenedesmus* sp. had no negative effect on final mean body weight, weight gain, specific growth rate and thermal growth coefficient. These findings are in line with other studies feeding Atlantic salmon feeds with 11% *Spirulina* sp. (Burr et al. (2012), or 10% defatted *Nannochloropsis oceania* Sørensen et al. (pers. comm.). Other studies have reported negative effects on weight gain and specific growth rate when Atlantic salmon were fed diets with *Schyzochrytrium* sp. at 11% inclusion (Sprague et al., 2015) or *Phaedactylum tricornutum* at an inclusion rate of 12% (Sørensen et al., 2016). Consequently, the significant reduction of final mean body weight, specific growth rate and thermal growth coefficient for fish fed 20% inclusion of the *Scenedesmus* sp. was not unexpected and is also in line with other studies (Dallaire et al., 2007). The latter authors reported reduced specific growth rate in rainbow trout (*Oncorhynchus mykiss*) fed diets with 25% replacement of fish meal by consortium of microalgae (*Scenedesmus* sp., *Chlamydomonas* sp.). However, specific growth rate of the present experiment was in line with Kiron et al. (2016) who was feeding Atlantic salmon feeds with defatted microalgae *Desmodesmus* sp. replacing 10% and 20% of the fish meal, respectively. These results suggest that relationship between growth parameters and inclusion level of microalgae depends on fish species, inclusion level, palatability and processing conditions of microalgae (Tibaldi et al., 2015).

Fish fed SCE 20 had significantly higher feed conversation ratio compared with the CT group, but lower than the values reported by both Burr et al. (2012) and Sprague et al. (2015). Feed conversion ratio recorded in the present experiment was in line with Kiron et al. (2016). Higher feed conversion ratio recorded in SCE 20 may indicate that poor nutrient bioavailability of microalgae compared with CT feed. No significant difference was observed in feed intake among the dietary treatments suggesting that incorporation of the microalgae into the feed had no negative effects on palatability. In contrast to our findings, Palmegiano et al. (2009) reported increased feed intake and improved feed conversion ratio when *Isochryris* sp. fed to gilthead sea bream (*Sparus aurata*) juveniles at 70% inclusion level.

Somatic indices are used to evaluate the general well-being or fitness of the fishes (Bolger and Connolly, 1989). Somatic indices were not affected by the diets in the present study. These observations are in line with Vizcaíno et al. (2014), who reported no effect of 12% and 20% incorporation of microalgae *Scenedesmus almeriensis* in diets for gilthead sea bream (*Sparus aurata*) on hepatosomatic index or viscero-somatic index. However, Kiron et al. (2016) observed a reduced hepatosomatic index and viscero-somatic index (1.3% and 8.2% respectively) when Atlantic salmon was fed 10% and 20% defatted microalgae *Desmodesmus* sp in the feed. The reduction in condition factor for the algae fed groups in the present study, was concomitant with the reduced weight gain of fish. However, condition factor in fish can be affected by several factors in addition to nutritional status, such as sex, age and seasonal variations (Mazumder et al., 2016).

Protein efficiency ratio was reduced at the highest inclusion level of algae, but was within the 2%-2.7%, range reported by other authors, in studies with microalgae in feeds for Atlantic salmon (Norambuena et al., 2015, Kiron et al., 2016, Kiron et al., 2012). In our experiment, reduced protein efficiency ratio might be attributable to low bioavailability of nutrients from the microalgal feeds.

4.2 Proximate composition of the fish

Changes in proximate composition of fish fed microalgae diets, have been reported in earlier studies (Dallaire et al., 2007, Mustafa et al., 1994). The higher whole body protein content in fish fed SCE 20 coincidence with lowest weight gain, protein efficiency ratio as well as protein retention, pointing to reduced utilization of protein in the feed. This is also in line with Shearer (1994) who found increased whole body protein content with higher growth of the fish.

Whole body lipid content of fish in the present experiment was higher than values reported in other experiments with microalgae incorporated into diets for Atlantic salmon (Kiron et al., 2012, Kiron et al., 2016, Norambuena et al., 2015, Sørensen et al., 2016). The latter authors showed that whole body lipid content ranged from 29%-32%. The numerically higher ($p>0.05$) whole body lipid content observed in fish fed SCE 10 was not explained by lipid content in feeds, as reported by other authors (Dallaire et al., 2007, Watanabe, 1982). Neither can the lipid content in fish fed SCE 10 be explained by higher feed intake as no differences were noted among dietary groups. More likely, the lower lipid content in fish fed SCE 20 can be explained by lower utilization of energy. Consequently, only marginal differences were observed in whole body energy level among the dietary groups.

The ash content was in line with other reported values on fish fed with microalgae (Kiron et al., 2016). The non-significant numerically higher whole body ash values observed in the algae fed fish were noteworthy. However, the values may have been confounded with elements present in the gastrointestinal tract. The negative digestibility values indicated differences in drinking rate. The fish were not starved before sampling because faeces had to be collected for digestibility determination. In future, whole fish proximate analysis should be analyzed using starved fish or without the gastrointestinal tract.

4.3 Apparent digestibility coefficients

The reduction in digestibility coefficients of dry matter, lipid, protein and energy are in line with other studies with Atlantic salmon fed microalgae in the feed (Gong et al., 2017, Kiron et al., 2016, Skrede et al., 2011, Sørensen et al., 2016). The reduction in digestibility of protein and dry matter, can be explained by the indigestible rigid cell walls of microalgae. In the present study, cell wall characteristics of experimental *Scenedesmus* sp. was not studied. Microalgae *Scenedesmus* sp. grow in colonies of four to eight cells. Species differ in cell morphology and can be identified by colony morphology (Guiry and Guiry, 2017). Outer cell wall ornamentation of the *Scenedesmus* sp. varies considerably (Staehelin and Pickett-Heaps, 1975). Outermost layer of the *Scenedesmus pannonicus* consisted of cellulose warts, spikelet and fine bristles (Staehelin and Pickett-Heaps, 1975). Regardless of the species, terminal cells in the colony of the *Scenedesmus* sp. bears curved spines. These characteristics of cell wall may also affect the nutrient digestion in Atlantic salmon.

The outer cell wall of *Scenedesmus* sp. is made up by a chemically inert biological polymer sporopollenin (Staehelin and Pickett-Heaps, 1975). The inner cell wall is made up by hemicellulose and cellulose polymers (e.g. *Scenedesmus obliques*; Voigt et al. (2014)) as well as pectin (e.g. *S. pannonicus*;Staehelin and Pickett-Heaps (1975). Studies with shrimp has shown that both sporopollenin and cellulose reduced digestibility of dry matter and protein when *Scenedesmus* sp. were incorporated into the diet (Sardinha et al., 2016). Based on the biochemical composition of the cell walls of *Scenedesmus* sp., it may be expected that cell walls had an adverse effect on nutrient digestibility and nutrient utilization in the experimental feeds.

ADC of protein in the algae fed groups in the present experiment was lower than earlier reported by Sørensen et al. (2016). The latter authors reported protein digestibility of 90% and 89% with an incorporation of *Phaeodactylum tricornutum* in Atlantic salmon diet at 3% and 6%, respectively.

However, our observation was in line with Skrede et al. (2011) who estimated a protein digestibility of 80% in mink fed *Phaeodactylum tricornutum.* In contrast to our findings, Burr et al. (2011) reported 85% protein digestibility when fish meal was replaced with *Spirulina* sp. at an inclusion rate of 30% of the diet. Digestibility of protein is affected both by fish species, inclusion level and algae species. In addition, digestibility values of protein is greatly affected by faecal collection method (Storebakken et al., 1998). Great care should be taken when comparing protein digestibility values across different experiments. In addition, Spyridakis et al. (1989) pointed out that use of anesthetics to minimize the stress, has induced spontaneous defecation which may alter the ADC of nutrients in fish.

Digestibility of ash in the present experiment showed negative values. These values were lower than those reported by Sørensen et al. (2016) using *Phaeodactylum tricornutum* as a fish meal replacer in Atlantic salmon diets. Negative ash digestibility can be explained by drinking rate. Higher drinking rate may be associated with physical characteristics of feeds, such as higher hardness of the pellets (Sørensen et al., 2016). In the present experiment, pellet hardness was significantly higher in fish fed with SCE 20. Pellets with higher hardness and higher water stability has longer gastro-evacuation time in order to dissolve in the stomach (Aas et al., 2011). Drinking of sea water to maintain osmoregulation but may contribute to negative digestibility of ash (Thodesen et al., 2001).

ADC of energy was within the range of values reported for seaweeds (58%-73%) and most of the terrestrial plant ingredients (56%-96%) (as reviewed by Gong et al.,2017). However, Digestibility of the energy in the present experiment was lower than the values recorded by Sørensen et al. (2016) and Burr et al. (2011) when microalga *P.tricornutum* and *Spirulina* sp. fed to Atlantic salmon respectively.

Hua and Bureau (2009) reported that apparent digestibility of dietary lipid was inversely related to the proportion of saturated fatty acids in the feeds. Fatty acid composition of the feeds was not analyzed in the present study. However, according to published literature fatty acid composition in *Scenedesmus obliquus* is dominated by C 16:0 fatty acids (40%) (Mandal and Mallick, 2009, Tang et al., 2011). The reduction in digestibility of fatty acids is more pronounced with reduction of water temperature in rainbow trout (*Oncorhynchus mykiss*) (Ng et al., 2003). The increasing content of saturated fatty acids in the diets with microalgae may have contributed to a reduction in

lipid digestibility. Though water temperature was only modest in this experiment, it was constant across the tanks and is therefore not explaining the differences in lipid digestibility.

4.4 Nutrient retention

Retention of protein in the present experiment was higher than the values observed by Sørensen et al. (2016) and Aas et al. (2015). Energy retention values in the present experiment was in line with values reported by Sørensen et al. (2016) (42%-50%). However, average lipid retention of the present experiment was higher than that of Sørensen et al. (2016) and Aas et al. (2015). The latter authors reported lipid retention values in the range of 55%-69% and 43%-47%, respectively. However, in our study retention of protein, lipid and energy was reduced with increasing level of microalgae in the feed.

4.5 Physical characteristics of experimental diets

The differences in pellet quality in the present experiment may have been affected by both ingredients and processing parameters in the extrusion process as earlier reported by Sørensen (2012). However, extrusion parameters were not available and it is not possible to draw any conclusions about the effect of extrusion processing parameters on the physical quality of pellets. The discussion has to be limited to effects of ingredients on pellet quality. The highest fat leakage from CT feed may be explained by the microstructure of the diet. Earlier studies have shown that fish meal based feeds have a different microstructure than the pellets based on plant ingredients (Sørensen et al., 2009). It can therefore be expected that microstructure of the pellets in the present study differed, affecting fat leakage. There appears to be a relationship between fat leakage and oil absorption capacity (Sørensen et al., 2011b). The latter authors reported that, pellets with low oil absorption capacity had higher fat leakage. Though oil absorption capacity was not measured in our study, the SCE 20 pellets had more oily surface compared to the other feeds. This observation suggests a lower oil absorption capacity of SCE 20. More oil leakage would be expected if pellets were coated with higher amount of oil. The higher fat leakage from CT diet may also be attributed to suboptimal processing conditions (Sørensen et al., 2011b)

Pellet length was significantly smaller in SCE 20 pellets. However, no significant difference among pellet diameter was recorded in the present study.

Hardness values recorded in the present experiment was higher than those recorded by Morken et al. (2012), but lower than the values recorded by Oehme et al. (2012). Hardness of the pellets is positively correlated by the pellet diameter (Oehme et al., 2012). Pellet diameter used in the present experiment were similar among the experimental diets, but was lower than pellets used in other studies (10-11mm) (Oehme et al., 2012, Aas et al., 2011). Hardness of pellets may be affected by functional components in the ingredients, such as carbohydrate fractions. Increased carbohydrate content with incorporation of microalgae in the feeds may explain the higher hardness of SCE 20 pellets (Sørensen et al., 2011b, Hansen and Storebakken, 2007). The SCE 20 also had the highest moisture level compared to that of other diets. Draganovic et al. (2011) reported that reduced moisture content in the extruder had a positive effect on hardness of the pellets.

Results of the water stability test indicates that CT diet had lowest water stability compared with SCE 10 and SCE 20 at all treatment times, except for 30 min. Water stability values recorded in the present experiment were higher than that of recorded by the Aas et al. (2011). Higher water stability of the pellets is associated with less leaching of nutrients in the water.

4.6 Correlation coefficient among growth parameters and physical characteristics

Several studies have reported effects of physical quality of pellets on feed intake, nutrient utilization and growth of the fish (Aas et al., 2011, Baeverfjord et al., 2006, Obaldo et al., 2000). In our study, there were no significant relationship in between physical quality of feeds and feed intake of fish. The negative correlation ($p>0.05$) between the feed intake and pellet diameter as well as feed intake and pellet hardness, was mainly explained by SCE 20 pellets, which had the highest diameter as well as higher hardness concurrent with the lowest feed intake. Studies in poultry have shown that larger pellet diameter resulted in lower feed intake, as the animal was fed to satiation at lower energy intake (Lundblad et al., 2011). However, in the present study pellet diameter was similar among feeds and had minor impact on feed intake.

In contrast to the other studies (Aas et al., 2015, Baeverfjord et al., 2006), no significant difference was noted between water stability and feed intake in fish. Li (2012) pointed out that less variation in the experimental units could detect significant changes in the feed intake and water stability. However, water stability of the present experiment showed lower variation than the values recorded by both Aas et al. (2011) and Baeverfjord et al. (2006).

Though large variation was observed for hardness among the experimental diets in the present study, no significant effects were noted on feed intake and growth. These findings are in line with other published literature on salmonids (Aas et al., 2011, Oehme et al., 2012). In contrast, Glencross et al. (2011) reported that incorporation of lupins into rainbow trout diets cause higher pellet hardness and increased feed intake. These contradictive results indicate that pellet hardness is not a good measure for feed intake and pellet water stability in Atlantic salmon and rainbow trout diets.

The significant correlation between fat leakage and growth of fish may be explained by higher growth of fish fed CT diet. The CT diet also had the greatest fat leakage. However, no fat leakage was noted during the course of experiment. Consequently, the fat leakage data had no practical implication.

5 Conclusion

The present study indicates that incorporation of microalgae *Scenedesmus* sp. upto 10% level did not cause any adverse effect on fish growth, feed conversion ratio, hepato-somatic and viscero somatic indices. However, higher inclusion of microalgae cause significant changes in digestibility, nutrient retention and proximate composition of Atlantic salmon. Inclusion of microalgae up to 10% also did not significantly altered the physical quality of the diet. Novel, cost effective methods for cell wall destruction in single cell protein are essential for increasing the bioavailability of nutrients. More research is needed to understand the effect of extrusion parameters on physical quality of *Scenedesmus* sp. incorporated diets.

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