Microalgae *Scenedesmus* sp. as a potential ingredient in low fishmeal diets for Atlantic salmon (*Salmo salar* L.)

Yangyang Gong[a,b§], Tharindu Bandara[a,c§], Mark Huntley[d,e], Zackary I. Johnson[e], Jorge Dias[f], Dalia Dahle[a], Mette Sørensen[a*], Viswanath Kiron[a]

[a] Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway

[b] Key Laboratory of East China Sea Fishery Resources Exploitation, East China Sea Fisheries Research Institute, Shanghai, 200090, China

[c] Department of Animal Sciences, Faculty of Animal Sciences and Export Agriculture, Uva Wellassa University, Sri Lanka

[d] Department of Animal Science, 149 Morrison Hall, Cornell University, Ithaca, NY, 14853, United States of America

[e] Marine Laboratory, Nicholas School of the Environment, Duke University, Beaufort, NC, United States of America

[f] SPAROS Lda, Olhão, Portugal

§ Gong and Bandara have equally contributed to the research and have shared first authorship

Emails of the authors:

Yangyang Gong yangyuang.gong@nord.no

Tharindu Bandara tharinduacademia@hotmail.com

Mette Sørensen mette.sorensen@nord.no

Kiron Viswanath kiron.viswanath@nord.no

Mark Huntley meh333@cornell.edu

Zackary Johnson zackary.johnson@duke.edu

Jorge Dias JorgeDias@sparos.pt

Corresponding author*: mette.sorensen@nord.no

Abstract

Salmonid feeds can be formulated with high quality microalgae to maintain sustainability in the aquaculture industry. But, the suitability of different microalgae species as potential feed ingredients needs to be documented to enable ready acceptance by the farming industry. The aim of the present study is to investigate the potential of the microalga *Scenedesmus* sp. as a major ingredient in low fishmeal feeds of Atlantic salmon. Three feeds were formulated with *Scenedesmus*/fishmeal, at inclusion levels of 0/10, 10/5 and 20/2.5% (CT, SCE 10 and SCE 20, respectively); to investigate the effect of the ingredient on the weight gain, growth rate, feed conversion ratio, nutrient retention and chemical composition and nutrient digestibility in Atlantic salmon. In addition, the physical characteristics of feeds were investigated to assess the impact of the alga-incorporation on the quality of the feeds. Fish (initial average weight of 229 g) in 6 replicate tanks were fed one of the experimental feeds for 65 days. The results showed that fish fed SCE 20 had significantly lower weight gain, specific growth rate, thermal growth coefficient and feed conversion ratio than the CT group, which did not receive the microalga. Furthermore,

the condition factor and protein efficiency ratio of the microalga-fed groups were lower than the CT group. Hepatosomatic and viscerosomatic indices of the groups did not differ significantly. Ash and protein content of whole fish fed SCE 20 were significantly higher, but dry matter, lipid, and energy of this group were lower than either the CT or the SCE 10 group. Retention of lipid and energy of all groups differed significantly, while that of protein was significantly different in the *Scenedesmus*-fed groups. Compared to the CT feed, digestibility of dry matter, protein, and energy in the algal feeds were significantly reduced. The highest fat leakage observed for the feed devoid of the alga and the hardness of the SCE 20 feed points to the better physical stability of the alga-containing feeds. Higher contents of n-3 fatty acids and PUFAs were found in the whole body of fish fed SCE 10. In conclusion, *Scenedesmus* sp. can be incorporated in low fishmeal diets for Atlantic salmon, at inclusion levels below 10%.

Keywords: Microalgae, *Scenedesmus* sp., Atlantic salmon, Apparent Nutrient Digestibility, Feed Conversion Ratio; Fatty Acid Composition

Introduction

Global aquaculture production is increasing year-over-year (FAO, 2018) and to sustain the trend in the future, industry should depend on high quality feed ingredients. Considering sustainability issues and high price of fishmeal and fish oil, the European fish feed industry has reduced its reliance on marine ingredients by employing more plant ingredients (Shepherd et al., 2017; Ytrestøyl et al., 2015). Consequently, feed sustainability measured in terms of fish in:fish out has improved considerably (Bendiksen et al., 2011; Crampton et al., 2010; Sanden et al., 2011; Ytrestøyl et al., 2015). According to Tacon and Metian (2015), more than 70% of the global aquaculture production depends on formulated feeds or feed input. The need for high quality

ingredients will increase with the growth of the aquaculture sector. Therefore, future feed ingredients should be derived from sustainable and scalable sources. Furthermore, the use of plant ingredients should not jeopardize human food security. High levels of plant oils in feeds have changed the lipid profile in the flesh of farmed Atlantic salmon (Sprague et al., 2016). Since 2006, the contents of 18:2n-6 (linoleic acid, LA), 18:3n-6 (γ-linolenic acid, GLA) and C18:1n-9 (oleic acid, OA) has increased while C20:5n-3 (eicosapentaenoic acid, EPA) and C22:6n-3 (docosahexaenoic acid, DHA) have been reduced (Sprague et al., 2016). This reduction in EPA and DHA levels in the fish flesh is raising concerns about the nutritional benefits of Atlantic salmon. As microalgae are primary producers of EPA and DHA in the food web, there is increasing interest for their use in supplementing fish feeds (Kousoulaki et al., 2015; Sørensen et al., 2016).

Microalgae can play a pivotal role in both freshwater and marine aquaculture because they contain high quality protein and can accumulate EPA and DHA. All essential amino acids are present in microalgae, though the level of individual amino acids may vary with growth medium composition and environmental conditions (Brown, 1991; Safafar et al., 2016). Although strain- and species-specific variations in the fatty acid composition are evident, some microalgae may be promising sources of PUFA, especially EPA and DHA (Lang et al., 2011). *Scenedesmus* sp. is a commercially available microalga, and it is grown in photobioreactors. The content of protein, lipid and carbohydrate in *Scenedesmus obliqus* dry matter is in the range 50-56%, 12-14% and 10-17%, respectively (Becker, 2007). Palmitic acid (16:0), OA, LA and α-linolenic acid (18:3n-3, ALA) are the dominant fatty acids in *Scenedesmus* sp. (Tibbetts et al., 2015).

Growth, feed utilization and nutrient digestibility of carnivorous fish fed microalgae depends on the microalgal type (Burr et al., 2011; Gong et al., 2018; Kiron et al., 2016; Vizcaíno et al., 2014) as well as inclusion level (Sørensen et al., 2016; Sørensen et al., 2017). Therefore, the effects of

potential fishmeal replacements have to be evaluated by conducting feeding and digestibility trials with candidate microalgae.

Sørensen et al. (2016, 2017) have already shown the potential of other microalgae as fishmeal replacements in feeds for Atlantic salmon. The aim of the present study was to investigate the weight gain, growth rate, feed conversion ratio, nutrient retention, chemical composition of whole body and nutrient digestibility of Atlantic salmon fed low fishmeal diets where microalga *Scenedesmus* partly replaced fishmeal, a mix of plant protein concentrates and wheat.

Materials and methods

Experimental design and feeds

The feeding trial was approved by the National Animal Research Authority (FDU: Forsøksdyrutvalget ID-5887) in Norway. The animal handling procedures were according to approved protocols.

The test microalgae *Scenedesmus* sp. (5.6% moisture, 45.7% protein, 9.1% fat, 15.8% fiber and 8.3% ash) used in the feeds was cultured in closed photobioreactors, dewatered by centrifugation and spray drying at Algafarm (Pataias, Portugal) and commercialized by Allmicroalgae – Natural Products® (Lisbon, Portugal). The study comprised three experimental diets: a control diet (CT) with a low level of fishmeal (10%) and relatively high levels of soy, pea and potato protein concentrates (1:1:1 blend), wheat gluten and corn gluten as major protein sources; a diet containing 10% *Scenedesmus* and 5% fishmeal (SCE10); and a diet with 20% *Scenedesmus* and 2.5% fishmeal (SCE20) (Table 1). In order to balance the protein, lipid, carbohydrates and energy contents of the feeds, the gradual increase of the microalgae incorporation level was made at the expenses of

fishmeal, but implied also some minor changes on the level of the various plant protein sources and a pronounced reduction of wheat meal. In all diets, the major lipid source was a blend of fish oil and rapeseed oil (1:1). All diets were supplemented with crystalline amino acids (L-histidine and DL-methionine) and inorganic phosphate. Diets contained also 0.02% yttrium oxide as an inert marker for digestibility measurements.

The experimental extruded diets were manufactured by SPAROS LDA (Olhão, Portugal). All powder ingredients were mixed accordingly to the target formulation in a double-helix mixer (model 500L, TGC Extrusion, France) and ground (below 400 μm) in a micropulverizer hammer mill (model SH1, Hosokawa-Alpine, Germany). Diets (pellet size: 3.0 mm) were manufactured with a twin-screw extruder (model BC45, Clextral, France) with a screw diameter of 55.5 mm. Extrusion conditions: feeder rate (78 kg/h), screw speed (235 rpm), water addition (approximately 295 ml/min), temperature barrel 1 (28-31ºC), temperature barrel 3 (118-121°C). Extruded pellets were dried in a vibrating fluid bed dryer (model DR100, TGC Extrusion, France). After cooling, oils were added by vacuum coating (700 mbar, for approximately 50 sec) (model PG-10VCLAB, Dinnissen, The Netherlands). Immediately after coating, diets were packed in sealed plastic buckets and shipped to the research site.

Fish and experimental set up

The experimental fish, Atlantic salmon (*Salmo salar*), were obtained from a commercial producer (Sundsfjord Smolt, Nygårdsjøen, Norway) and maintained at the Research Station, Nord University, Bodø, Norway for approximately 4 months before the start of the feeding trial. At the

start of the experiment, a total number of 450 fish (Initial weight 229 ± 3.8 g, total length 27.0 ± 0.2 cm) (mean ± SD) were randomly allocated to the experimental units (n = 6 tanks per treatment group) .

The feeding experiment was carried out in a flow-through system. In total, 18 circular fiberglass tanks (800 L and 0.9 m deep) were used for the study. Each tank was supplied with 740 L of water pumped from Saltenfjorden, from a depth of 250 m. During the experiment, water flow rate was maintained at 1000 L per hour, and the average temperature and salinity of the rearing water were 7.4 ± 0.1°C and 35 ‰, respectively. Oxygen saturation was always above 85% saturation measured at the water outlet. A 24-h photoperiod was maintained throughout the experimental period.

Feeding regime

The fish were fed ad libitum using automatic feeders (Arvo Tech, Finland); two feedings per day were administered from 08:00-09:00 and 14:00-15:00. The fish was fed 10% in excess based on the feed intake of the previous week. Approximately 30 min before each feeding, all the tanks were flushed to remove faeces from the tanks and minimize the risk of contaminating uneaten feed with faeces. The uneaten feeds were removed from the feed collection traps of each tank shortly after every feed delivery. These leftover feeds were stored at -20°C and the amount gathered weekly was later oven-dried at 110°C to determine the dry matter that was used for calculating the actual feed consumption of the fish.

Fish sampling and data collection

153 At the beginning and end of the experiment, all fish (450) were individually weighed and their total
154 lengths recorded. Before handling, fish were anesthetized using tricainemethanesulfonate (MS 222,
155 160 mg/L). From the initial stock, 6 fish were sampled to assess the initial chemical composition
156 of the fish. Upon termination of the experiment, 6 fish per tank were pooled to assess the final
157 chemical composition. These fish were packed in plastic bags, immediately frozen and kept at -
158 20°C until analyses. The fecal matter from the remaining fish in the tanks was obtained by stripping
159 individual fish. The fish and fecal samples were immediately transferred to -20 °C storage prior to
160 further analyses.

161

162 **Biochemical analyses**

163 The frozen fish samples were thawed for approximately 24 h at 4^0C, and each fish was
164 homogenized using an industrial food processor (Foss Tecator, 2096 homogenizer, Denmark)
165 before analyzing the whole body chemical composition. Frozen fecal samples were freeze dried
166 (VirTis benchtop, U.S.A.) for 72 h at -76°C and at a pressure of 20 bar. The freeze-dried fecal
167 samples from two tanks of a particular feed group were pooled prior to the analysis of their
168 chemical composition. The chemical composition of the feed pellets was also determined.

169 The moisture, protein, ash, and energy contents of the fish, feed and freeze-dried faeces were
170 determined as described below. Total dry matter content was determined by oven drying the
171 samples at 105°C for 24 h until consistent results were obtained (ISO 6496-1999). Protein content
172 was analyzed by using the Kjeldahl method (Kjeltech Auto Analyser, Tecator, Höganäs, Sweden,
173 Crude protein = N × 6.25, ISO 5983–1987). Ash content was measured gravimetrically by
174 combusting the samples using a flame at 550°C for 12-16 h until constant weights were registered

(ISO 5984–2002). Energy content was measured using a bomb calorimeter (IKA, c200, GmbH & Co. KG, Germany) (ISO 9831–1998).

Total lipid content of the fish was determined by the ethyl-acetate extraction method. Eurofins® (Moss, Norway) analyzed the total lipid content of the feed and faeces, employing the Soxhlet method with acid hydrolysis (Soxtec HT 6209, Tecator, Höganäs, Sweden: modified AOAC method 954.020). Fatty acid composition of fish and feed was measured by gas chromatography (GC) of methyl-ester derivatives in the samples. For this, the homogenized samples were lyophilized for 72 h before the lipids were extracted and analyzed in duplicate. Total lipid from the samples was extracted based on the method of Bligh and Dyer (1959). The fatty acid methyl esters (FAMEs) were prepared following the AOCS Official Method Ce 1b-89. FAMEs were separated and quantitated using a Scion 436 GC (Bruker, USA) equipped with a flame ionization detector, a splitless injector and a DB-23 column (Agilent Technologies, USA). Standard mixtures of FAMEs were used for identification and quantitation of common fatty acids in samples (GLC-473, Nu-Chek Prep, Elysian, MN, USA). Yttrium contents in both faeces and feeds were analyzed by Eurofins (Moss, Norway) as described by Sørensen et al. (2016).

Physical quality of feed

The method described by Sørensen et al. (2011) was employed to analyze susceptibility of pellets to leak fat, which may reduce the nutrient quality of feeds during storage or in automatic feeders.

Pellet hardness was determined by using TA-XT2 analyzer (Stable Micro Systems Ltd, Surrey, England). Feed pellets (n = 120) from a particular feed group were randomly selected and their hardness values were determined in 6 replicates (20 pellets per replicate). Each pellet was placed

197 horizontally and hardness was measured using a cylindrical probe (SMP/0.5, 1.2 cm width) at 60%
198 compression rate and at a velocity of 1 mm sec^{-1}. Hardness value was registered in Newtons (N),
199 as the peak force during the first compression.

200 Pellet length was measured using Vernier caliper (Biltema® Art. 16-105). 120 feed pellets from
201 each feed group were randomly selected and analyzed in 6 replicates (20 pellets per replicate).
202 Pellet diameter was measured using a TA-XT2 analyzer (Stable Micro Systems Ltd, Surrey,
203 England).

204 To determine the physical stability of the feeds in water, pellet samples from each feed group were
205 placed into a pre-weighed embedding cassette (M 512 Macrosette™, Simport®, Canada, 40.1 x
206 28.5 x 13 mm). Briefly, 3 g of pellets were incubated in a water bath (Julabo™, SW22, Seelbach,
207 Germany) at 25°C. Four shaking regimes were employed to determine the pellet stability: 100
208 shakings of the cassette per minute over 15, 30, 45 and 60 min. The test was carried out in 6
209 replicates for each treatment. After incubation, cassettes were placed on tissue paper and gently
210 dried and placed in a pre-heated oven at 80°C for 48 h. Residual dry matter weight of each cassette
211 was determined after drying. The weight difference of dry matter before and after incubation,
212 divided by dry matter weight of the feeds before incubation was calculated to determine the pellet
213 stability.

214

215 **Calculations and statistical analysis**

216 Fish growth performance was analyzed using the following equations.

217 $$\text{Weight gain } (\%) = \left(\frac{W_f - W_i}{W_i}\right) \times 100$$

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

$$\text{Specific Growth Rate (\% day}^{-1}) = \left(\frac{\text{Ln}\,(W_f) - \text{Ln}\,(W_i)}{\text{No. of feeding days}}\right) \times 100$$

$$\text{Feed intake (\% BW day}^{-1}) = \frac{\text{Daily feed intake in dry basis (g)}}{\sqrt{W_f \times W_i}} \times 100$$

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

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

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

where T is the temperature in °C and d is time in days.

$$\text{Hepato - somatic index (\%)} = \frac{\text{Liver weight of fish (g)}}{W_f\,(g)} \times 100$$

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

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

where FL (cm) = Fork length of fish

Apparent Digestibility Coefficient (ADC) and nutrient and energy retention were calculated according to following equations.

$$\text{ADC (\%)} = \left[1 - \frac{(\text{marker in feed} \times \text{nutrient in faeces})}{(\text{marker in the faeces} \times \text{nutrient in feeds})}\right] \times 100$$

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

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. Retention of a digested nutrient was calculated based on values for each tank:

$$\text{Nutrient (or Energy) retention efficiency}_{\text{digested}}(\%) = \frac{\text{Nutrient (or Energy) retention}(\%)}{\text{ADC}(\%)} \times 100$$

In this study, tank was used as the experimental unit. Statistical analyses were performed by using R v3.3.1 (R Development Core Team, 2016), employing packages stats v3.5.0 (R Development Core Team, 2016) and dunn.test package (v1.3.5) (Dinno, 2016). Data were checked for normality by the Kolmogorov-Smirnov test. For parametric data, one way analysis of variance (ANOVA) was performed after checking for equal variance using Bartlett's test. Tukey's multiple comparison test was used to identify the significant differences among the means of the 3 groups. For non-parametric data, the Kruskal-Wallis test, followed by Dunn's multiple comparison test, was performed to decipher the significant differences between the groups. A significance level of $p<0.05$ was chosen to indicate the differences.

Results

Chemical composition and quality of pellets

The chemical composition of the feeds (dry matter basis) is given in Table 2.Fatty acid composition of the feeds is provided in Table 3. Palmitic acid in the feed increased with increasing inclusion of

252 *Scenedesmus*. The fatty acids α-Linolenic acid (ALA) and Eicosapentaenoic acid (EPA) were
253 higher in the SCE 20 feed while Docosahexaenoic acid (DHA) decreased with increasing inclusion
254 of the alga.

255 Physical characteristics of the experimental feeds are given in Table 4. The color of the CT feed
256 was light-brown, and those of the alga-incorporated feeds were light (SCE 10) and dark black
257 (SCE 20) (Figure 1). Fat leakage was least from the SCE 20, though this feed appeared to have an
258 oilier surface than the other feeds. Hardness values of the feeds varied from approximately 23 to
259 40 N. The SCE 20 had significantly higher hardness, while no differences were noted between CT
260 and SCE 10. Length of pellets varied from 4.1 to 4.4 mm. The SCE 20 feed had significantly
261 shorter pellets than CT, while SCE 10 tended to be longer than the SCE 20 but shorter than the
262 CT.

263 Results of the pellet stability test are shown in Figure 2. The lowest stability ($P<0.05$) was observed
264 for the CT feed compared with the SCE 10 and SCE 20 at 15, 45 and 60 min. The stability of SCE
265 10 and SCE 20 were not significantly different.

266

267 **Apparent digestibility coefficients (ADC)**

268 Digestibility values of dry matter, lipid, and energy decrease with increasing inclusion level of the
269 alga-fed groups (SCE 10 and SCE 20) were significantly different compared to the CT group
270 (Table 5). Digestibility of protein in fish fed the SCE 20 was significantly lower compared to the
271 CT group, but no significant differences were noted between SCE 10 and the CT group. ADCs of
272 ash of all three groups were negative but increasing the inclusion of microalgae did not make the
273 values significantly different.

Growth performance

The weight gain, growth rate, feed intake, feed conversion ratio, protein efficiency ratio, and condition indices (condition factor and somatic indices) are given in Table 6. The fish grew from an initial average weight of 229.1 g to a final average body weight of 447.0 g during the experimental period of 85 days. Significant reduction in the final mean body weight, weight gain, specific growth rate, and thermal growth coefficient was noted in fish fed the SCE 20, compared to the fish in the CT group. No differences in feed intake were found among dietary treatments. Feed conversion ratios of the fish fed the algae feeds were poorer than the control group. As for the protein efficiency ratio, fish fed the CT feed had higher values than groups fed SCE10 and SCE 20. Condition factor was significantly higher in fish fed the control feed than fish fed the *Scenedesmus*-incorporated feeds. No significant differences were recorded between the hepato-somatic and viscero somatic indices of the three study groups.

Nutrient retention

Retention efficiency of lipid, protein, and energy is given in Table 7. Retention efficiency of lipid in the three feed groups differed significantly, with the highest retention detected in fish fed the CT feed and lowest in those fed the SCE 20 feed. Fish fed the SCE 20 also showed significantly lower retention of protein and energy compared to CT fed groups, while fish fed SCE 10 tended to have values lower than the CT group, but higher than the SCE 20 group. Retention efficiency of digested lipid differed significantly, and the lowest value was found in the SCE 20 group and highest in the CT group. The retention efficiency of digested protein and energy of the SCE 20

group was lower than that of the CT group. No differences in retention efficiency of digested protein and energy were noted for the fish fed CT vs SCE 10.

Chemical composition of fish

The chemical composition of fish from the initial population and those sampled at the termination of the experiment are presented in Table 8. Values from the initial population were excluded from the statistical analysis.

At the end of the experimental period, protein was highest in fish fed SCE 20 and lowest in those fed SCE 10, while the lipid content was significantly lower in fish fed SCE 20 compared with the other two groups. The ash content was significantly lower in the CT fed fish and highest in fish fed SCE 20, and the energy was significantly higher in CT and lowest in fish fed SCE20.

Fatty acid composition of whole body

Fatty acid composition of the whole body is given in Table 9. The saturated fatty acids (ΣSFAs) tended (P=0.092) to decrease with increasing inclusion level of algae in the feeds, though significant reduction was observed for stearic acid, C18:0. Monounsaturated fatty acids were not significantly different among feed groups. Linoleic acid (LA), C18:2 n-6 dominated the n-6 fatty acids and LA was lower in fish fed the CT feeds than those fed the algal feeds, but a significant difference was noted only between the CT and SCE 10. The ALA (P=0.050), EPA (P=0.070) and DHA (P=0.097) were higher in fish fed *Scenedesmus*-containing feeds compared to those on the control feed. This resulted in an overall higher content of Σn-3 FAs and ΣPUFA in the whole body of fish fed algae feeds, though significantly higher content was noted only for those fed SCE 10.

Discussion

Experimental feeds

Most studies performed to investigate the suitability of microalgae for Atlantic salmon have employed high fishmeal feeds (Kiron et al., 2012; Kiron et al., 2016; Kousoulaki et al., 2016; Kousoulaki et al., 2015; Sørensen et al., 2016; Sørensen et al., 2017). The present study was designed to investigate the potential of the microalga *Scenedesmus* sp. in high plant protein–low fishmeal feeds. The fishmeal inclusion level in the control feed of the present experiment was based on an earlier study in which Atlantic salmon grew from 137 g to approximately 400 g on feeds containing 10 or 30% fishmeal (Kousoulaki et al., 2009). Although the authors did not observe any differences in weight gain or feed utilization they emphasized the importance of the quality of the fishmeal when its inclusion level is low (Kousoulaki et al., 2009). Later studies with rainbow trout have shown that marine protein ingredients (krill products) can be incorporated at 5% level, but to avoid negative effects on growth and feed utilization the protein quality must be secured by supplementation of amino acids (Zhang et al., 2012). In the present experiment, we have seen a nonsignificant reduction in growth and feed utilization in SCE 10 compared to the control group. Reducing fishmeal to 2.5% in combination with 20% of the microalga *Scenedesmus* sp. significantly compromised growth and feed utilization compared to fish fed the SCE 20.

Protein content of the microalga was lower while lipid content was comparable to a high-quality fishmeal. To balance the lipid component, fish oil and rapeseed oil were slightly reduced with the incorporation of the microalga in the feed. Palmitic acid, oleic acid, LA and ALA are the dominant fatty acids in *Scenedesmus* sp. (Tibbetts et al., 2015). The fatty acid composition of the

340 experimental feeds was mainly reflected by the composition of fish oil and rapeseed oil, but LA
341 and ALA content were slightly higher in the SCF 20 feeds.

342 The differences in the pellet quality observed in the present study could be due to the ingredients
343 and processing parameters in the extrusion process, as reported by Sørensen (2012). Furthermore,
344 Samuelsen et al. (2018) has indicated that for better extruder performance, feed hardness and
345 durability, the optimal inclusion level of high lipid microalgae such as *Schizochytrium* sp. is 13.2%.
346 Fat leakage was higher in the CT feeds; this can possibly be explained by the microstructure and
347 the ingredient composition of the feed. Earlier studies have indicated that different pellet
348 microstructure is dependent on the feed ingredients (Draganovic et al., 2013; Sørensen et al., 2009).

349 Hardness values observed in the present experiment were higher than those recorded by Morken
350 et al. (2012), but lower than the values reported by Samuelsen et al. (2018). The hardness of the
351 pellets is positively correlated with pellet diameter (Samuelsen et al., 2018). Diameter of the pellets
352 from the different feed types used in the present experiment were similar, but was lower than those
353 employed in other studies, e.g. 8-11 mm (Samuelsen et al., 2018). The hardness of pellets may be
354 affected by the functional components such as carbohydrate fractions, starch source, amount of
355 starch, as well as the type of the plant protein ingredients in the feeds (Sørensen, 2012). Although
356 the starch and non-starch polysaccharides contents were not analyzed in the experimental diets,
357 the content and composition probably varied widely. Increasing the content of non-starch
358 polysaccharides result in harder pellets (Hansen and Storebakken, 2007; Sørensen et al., 2011).

359 The stability of the CT feeds was lower than the SCE 10 and SCE 20 feeds, at all the assessed time
360 points, except for 30 min. Water stability values recorded in the present study were higher than
361 those reported by Aas et al. (2011). Higher pellet stability has been associated with reduced feed

362 intake in rainbow trout (Aas et al., 2011). However, in the present experiment we did not observe
363 any significant differences in feed intake.

364

365 **Apparent digestibility coefficients**

366 In general, with the incorporation of the microalga the ADC values of dry matter, protein and lipid
367 were reduced significantly. The results are in line with findings reported for Nile tilapia
368 (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) fed diets containing 30%
369 *Scenedesmus dimorphus* (Teuling et al., 2017). Overall, the ADC values of protein were lower
370 than those reported for 10 and 20% incorporation of *Desmodesmus* sp. (Kiron et al., 2016) or
371 *Nannochloropsis oceania* (Sørensen et al., 2017) in feeds for Atlantic salmon. Lipid digestibility
372 was also lower in the present study than that reported by Kiron et al. (2016) and Sørensen et al.
373 (2017). Therefore, nutrient digestibility, depends on the microalgal type. The variation in ADC
374 values of protein, lipid and energy of different microalgae species was reported earlier by us and
375 others (Gong et al., 2018; Skrede et al., 2011; Teuling et al., 2017).

376 The microalga used in the present study were centrifuged and spray-dried without any further
377 processing. The cell walls of the alga were assumed to be more intact, in contrast to the oil-
378 extracted microalgae biomass used in the studies of Kiron et al. (2016) and Sørensen et al. (2017).
379 This could be one reason for the lower nutrient digestibility recorded in this study compared to our
380 above-mentioned studies. Teuling et al. (2017) reported that 10 min bead milling of *Scenedesmus*,
381 *Chlorella* and *Nannochloropsis* can disrupt 11-39% of the algal cell walls and significantly
382 increase the soluble protein fraction of the algae, which in turn is likely to improve protein
383 digestibility. Teuling et al. (2019) confirmed that there is a high correlation between nutrient
384 digestibility and the accessibility of nutrients from the microalga *Nannochloropsis gaditana* by

385 Nile tilapia. The authors also observed different effects on cell wall integrity and digestibility by
386 using various pre-treatments. The difference in digestibility of the *Desmodesmus* sp. (Kiron et al.,
387 2016) and *N. oceania* (Sørensen et al., 2017) and the *Scenedesmus* sp. in the present experiment
388 could be attributed to the discrepancies in pretreatment-induced nutrient availability.

389 The negative digestibility of ash may be associated with drinking of seawater (Thodesen et al.,
390 2001). The digestibility value of the hardest feed in the present study, SCE 20 feed decreased
391 further compared to the CT feed. Gastro-evacuation time for pellets with higher value for hardness
392 or water stability will be longer (Aas et al., 2011), and during such circumstances, fish may drink
393 seawater to soften the pellets or prevent dehydration (Sørensen et al., 2016). This results in high
394 intake of elements present in seawater. The ash digestibility values were lower than those reported
395 by Sørensen et al. (2016, 2017).

396

397 **Growth Performance of the fish**

398 There were no mortalities during the course of the experiment and the fish performed well. The
399 present findings suggest that in spite of relatively low levels of fishmeal in the experimental diets
400 (2.5-10%), the overall growth performance and feed utilization were similar to those reported by
401 Kiron et al. (2016), or even better compared to Atlantic salmon of comparable size fed fishmeal-
402 based feeds (Sørensen et al., 2017). However, inclusion of *Scenedesmus* up to 20% in the 2.5%
403 fishmeal diet could not sustain the growth and feed utilization of fish. Feeding Atlantic salmon
404 with 20% *Desomdesmus* sp. (Kiron et al., 2016) or 10% defatted *Nannochloropsis oceania*
405 (Sørensen et al., 2017) had no negative effect on final mean body weight, weight gain, specific
406 growth rate, and thermal growth coefficient – in these studies fishmeal inclusion level was 10%.

On the other hand, weight gain and specific growth rate of Atlantic salmon were negatively affected when fish were fed 11% *Schyzochrytrium* sp. (Sprague et al., 2016) or 12% *Phaeodactylum tricornutum* (Sørensen et al., 2016). The responses, however, also depend on fish size, microalgae species, ingredient and chemical composition of feeds, as well as the nutrient digestibility and physical quality (e.g. hardness) of feeds (Glencross et al., 2007).

Feed conversion ratio recorded in the present experiment was in line with the results of Kiron et al. (2016). Fish fed the SCE10 and SCE 20 feed had significantly higher feed conversion ratio compared with the CT group, but lower than the values reported by both Burr et al. (2012) and Sprague et al. (2015). Poor feed conversion ratio recorded for the SCE 10 and SCE 20 feed may indicate lower bioavailability of nutrients from the microalga compared with the CT feed. However, feed intake of all the study groups was not significantly different, suggesting that incorporation of the microalga had no negative effect on palatability. In contrast to our findings, Palmegiano et al. (2009) reported increased feed intake and improved feed conversion ratio when 70% *Isochryris* sp. was fed to gilthead sea bream (*Sparus aurata*) juveniles.

Condition indices are used to evaluate the general well-being or fitness of fishes (Bolger and Connolly, 1989). Condition indices were not affected in the present study; this result is corroborated by the study of Vizcaíno et al. (2014), in which the authors fed gilthead sea bream (*Sparus aurata*) 12 and 20% *Scenedesmus almeriensis*.

Protein efficiency ratio was significantly lower in the algae-fed fish compared to the fish fed the CT feed. However, values were within the 2-2.7% range reported in other studies in which Atlantic salmon were fed microalgae-incorporated feeds (Kiron et al., 2012; Kiron et al., 2016; Norambuena et al., 2015). The reduced protein efficiency ratio obtained in our study could be due to the low bioavailability of nutrients from the microalgal feeds.

Energy and nutrient retention efficiency

Protein, lipid, and energy retention efficiencies were reduced in fish fed the microalga-containing feed; protein and lipid values in the present experiment were higher than those reported by Sørensen et al. (2016) and Aas et al. (2015). Energy retention efficiency was in line with values (42-50%) reported by Sørensen et al. (2016). The reduced retention of digested lipid and protein from the diet SCE 20 indicates that the utilization of lipid and protein from the microalga might be lower than that from LT fishmeal and other high quality plant ingredients.

Chemical composition of the fish

Earlier studies have reported changes in the chemical composition of fish fed microalgae feeds (Dallaire et al., 2007; Mustafa et al., 1994). Although weight gain, protein efficiency ratio as well as protein retention of fish fed the SCE 20 feed was lower compared to the other study groups, whole body protein content was high in this fish group. As for the whole body lipid content, the apparently higher ($p>0.05$) values observed in fish fed the SCE 10 feeds cannot be explained based on the feed lipid content, as reported by others (Dallaire et al., 2007; Watanabe, 1982). The lower lipid content in fish fed the SCE 20 feed can be explained by lower utilization of energy. Consequently, only marginal differences were observed in whole body energy level of the feed groups. Whole body lipid content of fish in the present study was higher than values (29-32%) reported for Atlantic salmon fed microalgae feed (Kiron et al., 2012; Kiron et al., 2016; Norambuena et al., 2015; Sørensen et al., 2016).

451 The ash content of fish in the present study was in line with the value reported for fish fed with
452 microalgae (Kiron et al., 2016). The non-significant higher whole-body ash values observed in the
453 algae fed fish were noteworthy and suggest improved utilization of the elements in fish fed the
454 algae incorporated feeds.

455 In spite of the low fishmeal level and 50% replacement of fish oil with rapeseed oil, the calculated
456 content of EPA + DHA was 2.6%, 2.7% and 2.0% of the CT, SCE 10 and SCE 20 feeds,
457 respectively. These levels are in the nutritional requirement range recently suggested by Bou et al.
458 (2017a, 2017b) . When Atlantic salmon are fed feeds devoid of fishmeal or fish oil, the requirement
459 of 1% EPA + DHA (National Research Council, 2011) seems to be too low. The significantly
460 increased contents of LA, ALA and EPA in the whole body of fish fed SCE 10 feed points to an
461 improved utilization and deposition of fatty acids. However, higher incorporation of the
462 microalgae did not result in any significant differences in the fatty acids. In salmonid fish, the fatty
463 acid composition of the flesh is closely related to the composition in feed (Sprague et al., 2016;
464 Teimouri et al., 2016). The increased Σn-6 FAs content in whole body of fish fed *Scenedesmus*
465 feed was mainly attributed to the higher content of LA in the feed. The increase in Σn-3 FAs and
466 ΣPUFA observed in fish fed the SCE 10 feeds is also noteworthy. The modest increase in whole
467 body EPA and DHA, in spite of reduced content of DHA in the SCE 10 and SCE 20, may have
468 been stimulated by slightly higher LA and ALA in the microalgae. The pathways are well known
469 for the endogenous production of EPA and DHA from n-3 or n-6 C_{18} PUFA (Tocher, 2015). Earlier
470 studies have shown that substrate-specific acyl elongases and desaturases can be modulated by the
471 dietary fatty acid composition to stimulate the production of EPA and DHA from ALA (Tocher et
472 al., 2003; Zheng et al., 2005). Furthermore, it has been shown that high levels of dietary EPA and
473 in particular DHA reduce endogenous production of EPA and DHA (Bou et al., 2017a; Thomassen

et al., 2012). The CT-fed fish had lower EPA and DHA content while the microalga-fed fish had similar or higher values compared to the initial EPA and DHA content. The tendency of increased EPA and DHA content as well as increased PUFA contents of Atlantic salmon induced by an ingredient such as *Scenedesmus* sp. is favorable from a nutritional point of view.

Conclusion

The present study indicates that incorporation of microalgae *Scenedesmus* sp. of up to 10% in low fishmeal diet did not affect the feed intake, growth and chemical composition of salmon. However, the inclusion of the microalga, particularly at 20% in low fishmeal diets, significantly reduced the digestibility, nutrient retention efficiency and feed conversion ratio in Atlantic salmon. *Scenedesmus* sp. at 10% in the diet improved the total n-3 and PUFA content in salmon. Inclusion of the microalga up to 10% also did not significantly alter the physical quality of the diet.

The microalga *Scenedesmus* has the potential to be used as feed ingredient in diets for Atlantic salmon. However, novel, cost-effective methods for cell wall destruction may be essential for increasing the bioavailability of nutrients.

Declarations

Abbreviations

CT – Control group

DHA - Docosahexaenoic Acid

EPA – Eicosapentaenoic Acid

FAO – Food and Agricultural Organization of the United Nations

IFFO – The Marine Ingredients Association

PUFA – Poly Unsaturated Fatty Acid

SCE 10 – Low alga group

SCE 20 – High alga group

SFA – Saturated fatty acids

Acknowledgments

This study is a contribution from the project "The Marine Algae Industrialization Consortium (MAGIC): Combining biofuel and high-value bioproducts to meet the RFS" led by Zackary Johnson, Duke University. The aquafeed component in the project is led by Kiron Viswanath, Nord University. Ghana Vasanth, Engineer at Nord University, is thanked for her assistance during the study. The authors also acknowledge the support of the staff at the Research Station of Nord University.

Funding

The funding for the study was from the Strategic Research Funds of Nord University and partly from the US Department of Energy (Grant DE-EE0007091) under the project "Marine Algae Industrialization Consortium (MAGIC): Combining biofuel and high-value bioproducts to meet the RFS" awarded to Duke University. Yangyang Gong was financially supported by a fellowship from the China Scholarship Council as well as the funding from East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The funding agencies had no role in the design, analyses or writing of this article.

Availability of data and materials

All the data are presented in the article. Any additional information required from the authors will be available upon request.

Author's contributions

Yangyang Gong: Execution; Investigation; Methodology; Writing original draft

Tharindu Bandara: Execution; Investigation; Methodology; Writing original draft

Mark Huntley: Conception; Project administration; Review and editing

Zackary Johnson: Conception; Project administration; Review and editing

Jorge Dias: Methodology; Review and editing

Mette Sørensen: Conception; Design of experiment; Execution, Writing the manuscript

Viswanath Kiron: Conception; Design of experiment; Execution, Writing the manuscript

Ethical approval and consent to participate

The National Animal Welfare Authority (Mattilsynet) approved the conduct of animal experiment and the animals were handled according to the sanctioned protocols. All persons associated with the project scientifically are authors on the paper and have approved the final version of the manuscript submitted for review. All data gathered during the study formed the basis of this manuscript and is presented in its entirety.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

Aas, T.S., Sixten, H.J., Hillestad, M., Ytrestøyl, T., Sveier, H., Asgard, T., 2015. Feed intake and nutrient digestibility and retention in Atlantic salmon fed diets with different physical quality. NOFIMA, Tromsø.

Aas, T.S., Terjesen, B.F., Sigholt, T., Hillestad, M., Holm, J., Refstie, S., Baeverfjord, G., Rørvik, K.A., Sørensen, M., Oehme, M., Åsgård, T., 2011. Nutritional responses in rainbow trout (*Oncorhynchus mykiss*) fed diets with different physical qualities at stable or variable environmental conditions. Aquac. Nutr. 17, 657-670.

Becker, E.W., 2007. Micro-algae as a source of protein. Biotechnol. Adv. 25, 207-210.

Bendiksen, E.Å., Johnsen, C.A., Olsen, H.J., Jobling, M., 2011. Sustainable aquafeeds: Progress towards reduced reliance upon marine ingredients in diets for farmed Atlantic salmon (*Salmo salar* L.). Aquaculture. 314, 132-139.

Bligh, E.G., Dyer, W.J., 1959. A Rapid Method of Total Lipid Extraction and Purification. Can. J. Biocehm. Physiol. 37, 911-917.

Bolger, T., Connolly, P.L., 1989. The selection of suitable indices for the measurement and analysis of fish condition. J. Fish Biol. 34, 171-182.

Bou, M., Ostbye, T.K., Berge, G.M., Ruyter, B., 2017a. EPA, DHA, and Lipoic Acid Differentially Modulate the n-3 Fatty Acid Biosynthetic Pathway in Atlantic Salmon Hepatocytes. Lipids. 52, 265-283.

Bou, M., Berge, G.M., Baeverfjord, G., Sigholt, T., Østbye, T.-K., Ruyter, B., 2017b. Low levels of very-long-chain n-3 PUFA in Atlantic salmon (*Salmo salar*) diet reduce fish robustness under challenging conditions in sea cages. J. Nutr. Sci. 6, e32.

Bou, M., Berge, G.M., Baeverfjord, G., Sigholt, T., Ostbye, T.K., Romarheim, O.H., Hatlen, B., Leeuwis, R., Venegas, C., Ruyter, B., 2017c. Requirements of n-3 very long-chain PUFA in Atlantic salmon (*Salmo salar* L): effects of different dietary levels of EPA and DHA on fish performance and tissue composition and integrity. Br J Nutr. 117, 30-47.

Brown, M.R., 1991. The amino-acid and sugar composition of 16 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 145, 79-99.

Burr, G.S., Barrowss, F.T., Gaylor, G., Wolters, W.R., 2011. Apparent digestibility of macro-nutrients and phosphorus in plant-derived ingredients for Atlantic salmon, *Salmo salar* and Arctic charr, *Salvelinus alpinus*. Aquac. Nutr. 17, 570-577.

573 Burr, G.S., Wolters, W.R., Barrows, F.T., Hardy, R.W., 2012. Replacing fishmeal with blends of
574 alternative proteins on growth performance of rainbow trout (*Oncorhynchus mykiss*), and
575 early or late stage juvenile Atlantic salmon (*Salmo salar*). Aquaculture. 334-337, 110-116.
576 Crampton, V.O., Nanton, D.A., Ruohonen, K., Skjervold, P.O., El-Mowafi, A., 2010.
577 Demonstration of salmon farming as a net producer of fish protein and oil. Aquac. Nutr.
578 16, 437-446.
579 Dallaire, V., Lessard, P., Vandenberg, G., de la Noüe, J., 2007. Effect of algal incorporation on
580 growth, survival and carcass composition of rainbow trout (*Oncorhynchus mykiss*) fry.
581 Bioresour. Technol. 98, 1433-1439.
582 Dinno, A., 2016. dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums.
583 FAO, 2018. The State of World Fisheries and Aquaculture. Food and Agricultural Organization of
584 the United Nations, Rome.
585 Glencross, B.D., Booth, M., Allan, G.L., 2007. A feed is only as good as its ingredients – a review
586 of ingredient evaluation strategies for aquaculture feeds. Aquacult. Nutr. 13, 17-34.
587 Gong, Y., Guterres, H.A.D.S., Huntley, M., Sørensen, M., Kiron, V., 2018. Digestibility of the
588 defatted microalgae Nannochloropsis sp. and Desmodesmus sp. when fed to Atlantic
589 salmon, Salmo salar. Aquac. Nutr. 24, 56-64.
590 Hansen, J.Ø., Storebakken, T., 2007. Effects of dietary cellulose level on pellet quality and nutrient
591 digestibilities in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 272, 458-465.
592 Kiron, V., Phromkunthong, W., Huntley, M., Archibald, I., Scheemaker, G., 2012. Marine
593 microalgae from biorefinery as a potential feed protein source for Atlantic salmon,
594 common carp and whiteleg shrimp. Aquac. Nutr. 18, 521-531.
595 Kiron, V., Sørensen, M., Huntley, M., Vasanth, G.K., Gong, Y., Dahle, D., Palihawadana, A.M.,
596 2016. Defatted Biomass of the Microalga, *Desmodesmus* sp., Can Replace Fishmeal in the
597 Feeds for Atlantic salmon. Frontiers in Marine Science. 3.
598 Kousoulaki, K., Mørkøre, T., Nengas, I., Berge, R.K., Sweetman, J., 2016. Microalgae and organic
599 minerals enhance lipid retention efficiency and fillet quality in Atlantic salmon (*Salmo*
600 *salar* L.). Aquaculture. 451, 47-57.
601 Kousoulaki, K., Albrektsen, S., Langmyhr, E., Olsen, H.J., Campbell, P., Aksnes, A., 2009. The
602 water soluble fraction in fish meal (stickwater) stimulates growth in Atlantic salmon
603 (*Salmo salar* L.) given high plant protein diets. Aquaculture. 289, 74-83.

Kousoulaki, K., Østbye, T.-K.K., Krasnov, A., Torgersen, J.S., Mørkøre, T., Sweetman, J., 2015. Metabolism, health and fillet nutritional quality in Atlantic salmon (Salmo salar) fed diets containing n-3-rich microalgae. JNS. 4, e24.

Lang, I., Hodac, L., Friedl, T., Feussner, I., 2011. Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biol. 11, 124.

Morken, T., Kraugerud, O.F., SØRensen, M., Storebakken, T., Hillestad, M., Christiansen, R., ØVerland, M., 2012. Effects of feed processing conditions and acid salts on nutrient digestibility and physical quality of soy-based diets for Atlantic salmon (*Salmo salar*). Aquac. Nutr. 18, 21-34.

Mustafa, M.G., Umino, T., Nakagawa, H., 1994. The effect of Spirulina feeding on muscle protein deposition in red sea bream, *Pagrus major*. J. Appl. Ichthyol. 10, 141-145.

National Research Council, 2011. Nutrient Requirement of Fish and Shellfish. The National Academic Press, Washington, DC, pp. 392.

Norambuena, F., Hermon, K., Skrzypczyk, V., Emery, J.A., Sharon, Y., Beard, A., Turchini, G.M., 2015. Algae in Fish Feed: Performances and Fatty Acid Metabolism in Juvenile Atlantic Salmon. PLoS ONE. 10, e0124042.

Palmegiano, G.B., Gai, F., Gasco, L., Lembo, G., Spedicato, M.T., Trotta, P., Zoccarato, I., 2009. Partial replacement of fish meal by T-ISO in gilthead sea bream (*Sparus aurata*) juveniles diets. Italian Journal of Animal Science. 8, 869-871.

R Development Core Team, 2016. R: A language and environment for statistical computing Vienna, Austria: R foundation for statistical computing.

Sørensen, M., 2012. A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. Aquacult. Nutr. 18, 233-248.

Sørensen, M., Morken, T., Kosanovic, M., Øverland, M., 2011. Pea and wheat starch possess different processing characteristics and affect physical quality and viscosity of extruded feed for Atlantic salmon. Aquacult. Nutr. 17, e326-e336.

Sørensen, M., Berge, G.M., Reitan, K.I., Ruyter, B., 2016. Microalga *Phaeodactylum tricornutum* in feed for Atlantic salmon (*Salmo salar*) —Effect on nutrient digestibility, growth and utilization of feed. Aquaculture. 460, 116-123.

635 Sørensen, M., Gong, Y., Bjarnason, F., Vasanth, G.K., Dahle, D., Huntley, M., Kiron, V., 2017.
636 *Nannochloropsis oceania*-derived defatted meal as an alternative to fishmeal in Atlantic
637 salmon feeds. PLoS ONE. 12, e0179907.
638 Safafar, H., Uldall Nørregaard, P., Ljubic, A., Møller, P., Løvstad Holdt, S., Jacobsen, C., 2016.
639 Enhancement of protein and pigment content in two *Chlorella* species cultivated on
640 industrial process water. Journal of Marine Science and Engineering. 4, 84.
641 Samuelsen, T., Oterhals, Å., Kousoulaki, K., 2018. High lipid microalgae (*Schizochytrium* sp.)
642 inclusion as a sustainable source of n-3 long-chain PUFA in fish feed—Effects on the
643 extrusion process and physical pellet quality. Anim. Feed Sci. Technol. 236, 14-28.
644 Sanden, M., Stubhaug, I., Berntssen, M.H., Lie, O., Torstensen, B.E., 2011. Atlantic salmon
645 (*Salmo salar* L.) as a net producer of long-chain marine omega-3 fatty acids. J. Agric. Food
646 Chem. 59, 12697-12706.
647 Shepherd, C.J., Monroig, O., Tocher, D.R., 2017. Future availability of raw materials for salmon
648 feeds and supply chain implications: The case of Scottish farmed salmon. Aquaculture.
649 467, 49-62.
650 Skrede, A., Mydland, L.T., Ahlstrøm, Ø., Reitan, K.I., Gislerød, H.R., Øverland, M., 2011.
651 Evaluation of microalgae as sources of digestible nutrients for monogastric animals. J.
652 Anim. Feed Sci. 20, 131-142.
653 Sprague, M., Dick, J.R., Tocher, D.R., 2016. Impact of sustainable feeds on omega-3 long-chain
654 fatty acid levels in farmed Atlantic salmon, 2006–2015. Scientific Reports. 6, 21892.
655 Sprague, M., Walton, J., Campbell, P.J., Strachan, F., Dick, J.R., Bell, J.G., 2015. Replacement of
656 fish oil with a DHA-rich algal meal derived from *Schizochytrium* sp. on the fatty acid and
657 persistent organic pollutant levels in diets and flesh of Atlantic salmon (*Salmo salar*, L.)
658 post-smolts. Food Chem. 185, 413-421.
659 Tacon, A.G.J., Metian, M., 2015. Feed Matters: Satisfying the Feed Demand of Aquaculture.
660 Reviews in Fisheries Science & Aquaculture. 23, 1-10.
661 Teimouri, M., Yeganeh, S., Amirkolaie, A.K., 2016. The effects of Spirulina platensis meal on
662 proximate composition, fatty acid profile and lipid peroxidation of rainbow trout
663 (*Oncorhynchus mykiss*) muscle. Aquac. Nutr. 22, 559-566.

664 Teuling, E., Schrama, J.W., Gruppen, H., Wierenga, P.A., 2017. Effect of cell wall characteristics
665 on algae nutrient digestibility in Nile tilapia (*Oreochromis niloticus*) and African catfish
666 (*Clarus gariepinus*). Aquaculture. 479, 490-500.
667 Teuling, E., Wierenga, P.A., Agboola, J.O., Gruppen, H., Schrama, J.W., 2019. Cell wall
668 disruption increases bioavailability of *Nannochloropsis gaditana* nutrients for juvenile
669 Nile tilapia (*Oreochromis niloticus*). Aquaculture. 499, 269-282.
670 Thodesen, J., Storebakken, T., Shearer, K.D., Rye, M., Bjerkeng, B., Gjerde, B., 2001. Genetic
671 variation in mineral absorption of large Atlantic salmon (*Salmo salar*) reared in seawater.
672 Aquaculture. 194, 263-271.
673 Thomassen, M.S., Rein, D., Berge, G.M., Østbye, T.-K., Ruyter, B., 2012. High dietary EPA does
674 not inhibit Δ5 and Δ6 desaturases in Atlantic salmon (*Salmo salar* L.) fed rapeseed oil diets.
675 Aquaculture. 360-361, 78-85.
676 Tibbetts, S.M., Melanson, R.J., Park, K.C., Banskota, A.H., Stefanova, R., McGinn, P.J., 2015.
677 Nutritional Evaluation of Whole and Lipid-Extracted Biomass of the Microalga
678 *Scenedesmus* sp. AMDD Isolated in Saskatchewan, Canada for Animal Feeds: Proximate,
679 Amino Acid, Fatty Acid, Carotenoid and Elemental Composition. Current Biotechnology.
680 4, 530-546.
681 Tocher, D.R., 2015. Omega-3 long-chain polyunsaturated fatty acids and aquaculture in
682 perspective. Aquaculture. 449, 94-107.
683 Tocher, D.R., Bell, J.G., Dick, J.R., Crampton, V.O., 2003. Effects of dietary vegetable oil on
684 Atlantic salmon hepatocyte fatty acid desaturation and liver fatty acid compositions.
685 Lipids. 38, 723-732.
686 Vizcaíno, A.J., López, G., Sáez, M.I., Jiménez, J.A., Barros, A., Hidalgo, L., Camacho-Rodríguez,
687 J., Martínez, T.F., Cerón-García, M.C., Alarcón, F.J., 2014. Effects of the microalga
688 *Scenedesmus almeriensi*s as fishmeal alternative in diets for gilthead sea bream, *Sparus*
689 *aurata*, juveniles. Aquaculture. 431, 34-43.
690 Watanabe, T., 1982. Lipid nutrition in fish. Comparative Biochemistry and Physiology Part B:
691 Comparative Biochemistry. 73, 3-15.
692 Ytrestøyl, T., Aas, T.S., Åsgård, T., 2015. Utilisation of feed resources in production of Atlantic
693 salmon (*Salmo salar*) in Norway. Aquaculture. 448, 365-374.

694 Zhang, Y., Øverland, M., Shearer, K.D., Sørensen, M., Mydland, L.T., Storebakken, T., 2012.
695 Optimizing plant protein combinations in fish meal-free diets for rainbow trout
696 (*Oncorhynchus mykiss*) by a mixture model. Aquaculture. 360-361, 25-36.
697 Zheng, X., Torstensen, B.E., Tocher, D.R., Dick, J.R., Henderson, R.J., Bell, J.G., 2005.
698 Environmental and dietary influences on highly unsaturated fatty acid biosynthesis and
699 expression of fatty acyl desaturase and elongase genes in liver of Atlantic salmon (*Salmo*
700 *salar*). Biochim Biophys Acta. 1734, 13-24.

701

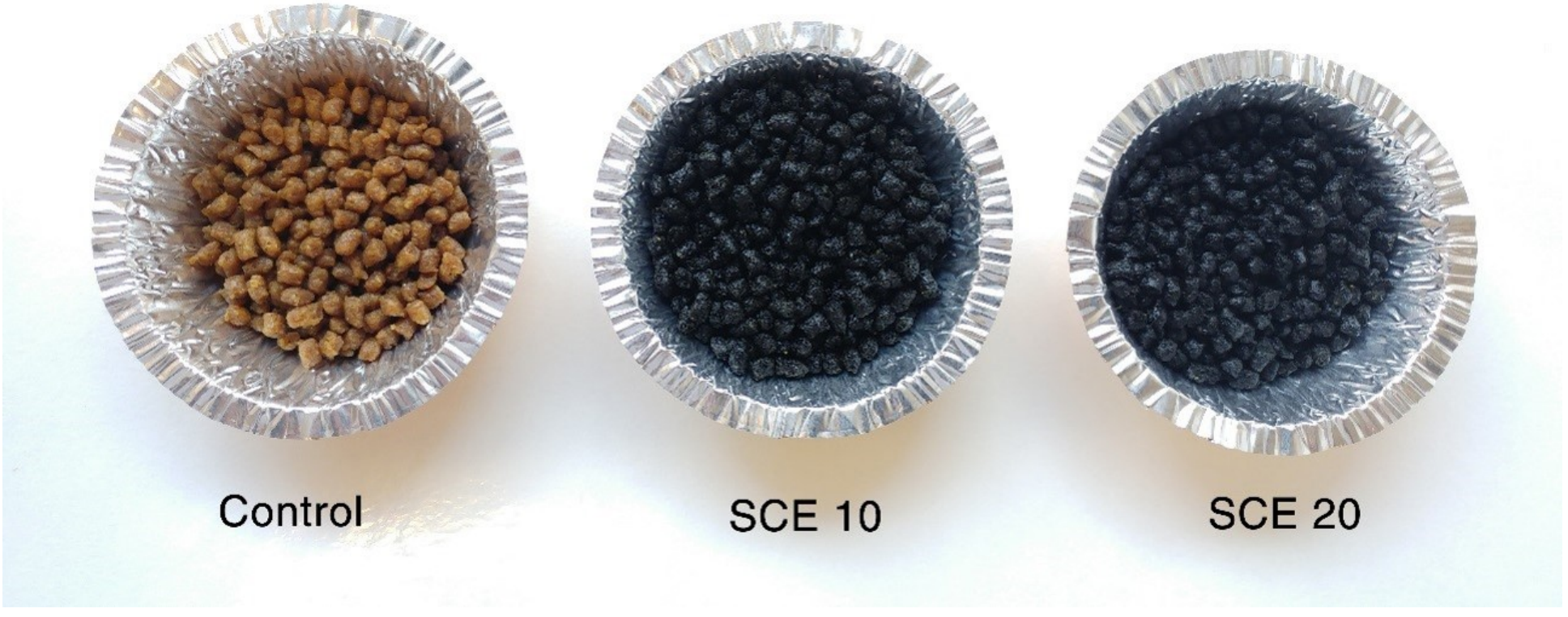
702

Figure legends:

Figure 1: Physical appearance of the three different feeds. Control (CT), SCE 10, SCE 20: low fishmeal control diet, *Scenedesmus* 10% diet, *Scenedesmus* 20% diet, respectively.

Figure 2: Water stability test for CT, SCE 10 and SCE 20 feeds. Control (CT), SCE 10, SCE 20: low fishmeal control diet, *Scenedesmus* 10% diet, *Scenedesmus* 20% diet, respectively. Four shaking regimes were employed to determine the pellet water stability: 100 shakings of the cassette per minute over 15, 30, 45 and 60 minutes. Water stability values are expressed as percentage of dry matter that is retained from the initial dry weight. Error bars depict standard deviations.

713


Control
SCE 10
SCE 20

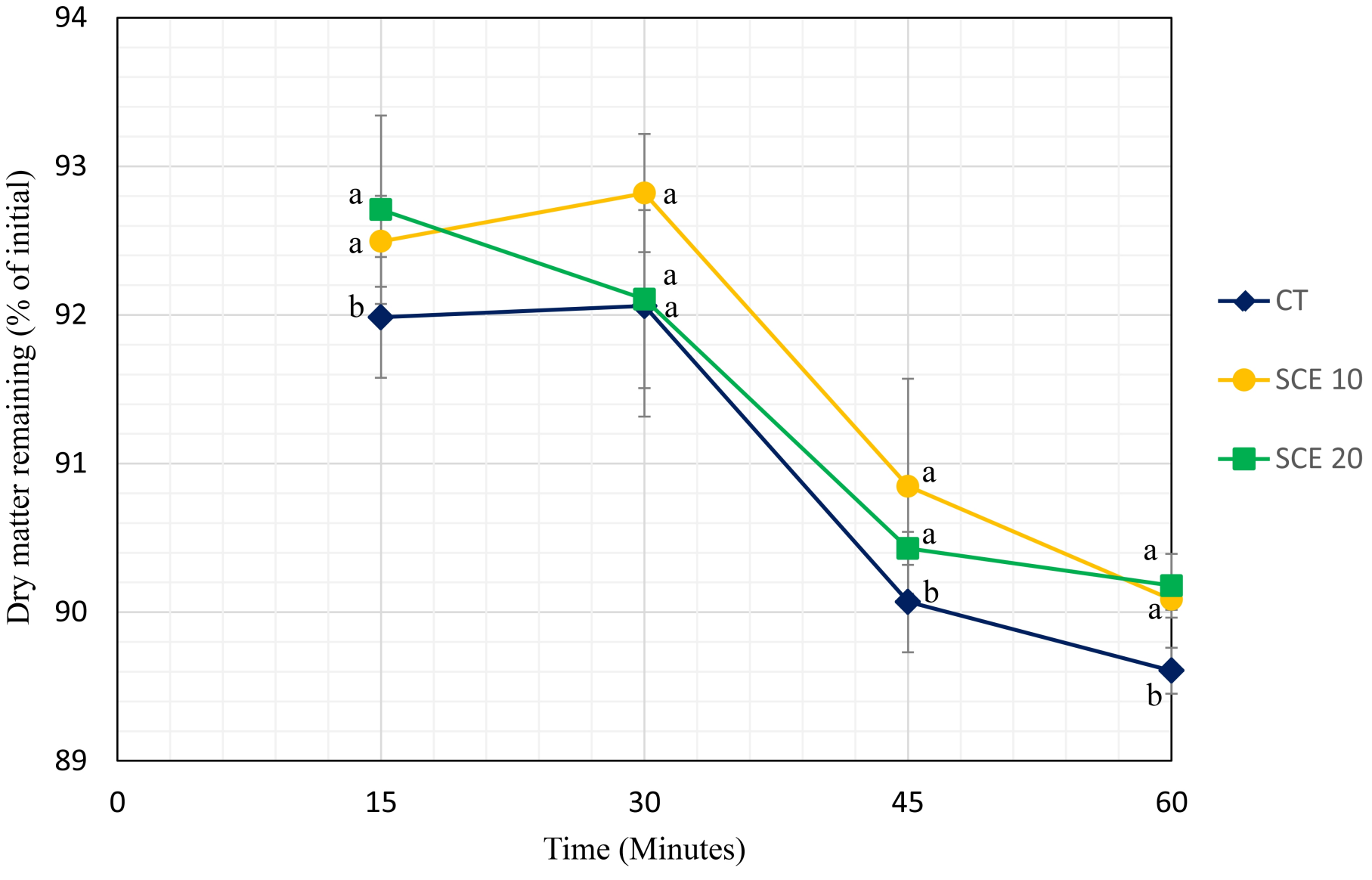

94
93
92
91
90
89
Dry matter remaining (% of initial)
0
15
30
45
60
Time (Minutes)
a
a
b
a
a
a
a
b
a
a
b
CT
SCE 10
SCE 20

Table 1: Ingredient composition (%) of the control (CT), low algae (SCE 10) and high algae (SCE 20) feeds

Ingredients	CT %	SCE 10 %	SCE 20 %
Fishmeal 70 LT FF (SKAGEN) [a]	10.0	5.0	2.5
Scenedesmus sp. – (Allma®) [b]	0.0	10.0	20.0
Soy protein concentrate (SOYCOMIL®) [c]	12.0	11.7	10.9
Pea protein concentrate [d]	12.0	11.7	10.9
Potato concentrate [e]	12.0	11.7	10.9
Wheat Gluten [f]	8.5	8.3	7.7
Corn gluten [g]	7.0	6.8	6.3
Wheat meal [h]	14.5	11.0	7.6
Fish oil (SAVINOR) [i]	10.0	9.8	9.5
Rapeseed oil [j]	10.0	9.8	9.5
Vitamin & Mineral Premix PV01 [k]	1.0	1.0	1.0
Soy lecithin [l]	0.5	0.5	0.5
MCP [m]	2.0	2.0	2.0
L-Histidine [n]	0.1	0.1	0.1
DL-Methionine [o]	0.3	0.3	0.3
Yttrium oxide [p]	0.02	0.02	0.02

a Sopropeche, France

b Allmicroalgae, Portugal

c ADM, The Netherlands

d ROQUETTE Frères, France

e AVEBE, The Netherlands

f ROQUETTE Frères, France

g COPAM, Portugal

h Casa Lanchinha, Portugal

12 i SAVINOR UTS, Portugal

13 j Henry Lamotte Oils GmbH, Germany

14 k PREMIX Lda, Portugal.

15 l Lecico P700IPM, LECICO GmbH, Germany

16 m Fosfitalia, Italy

17 n Ajinomoto Eurolysine SAS, France

18 o Evonik Nutrition & Care GmbH, Germany

19 p Sigma-Aldrich, Spain

20

21

Table 2: Analyzed chemical composition (%) of the feeds

Parameter	CT	SCE 10	SCE 20
Moisture	6.3	6.2	6.9
Dry matter, %			
Protein	49.2	49.3	48.9
Lipid	21.1	22.5	21.0
Ash	5.8	5.6	5.9
Energy (KJ g^{-1})	24.5	24.8	24.9

CT: Control; SCE 10: incorporation of 10% *Scenedesmus* in the diet; SCE 20: incorporation of 20% *Scenedesmus* in the diet. Values are expressed as mean of 4 replicate samples per diet.

25 Table 3 Analyzed fatty acid composition (% of total fatty acids) of the experimental feeds

Fatty acid %	CT	SCE 10	SCE 20
C14:0	3.11	2.90	2.48
C15:0	0.42	0.40	0.53
C16:0	13.05	13.62	14.21
C16:1n-7	3.53	3.43	2.86
C18:0	3.26	3.38	3.87
C18:1n-9	36.46	37.06	36.61
C18:1n-7	3.43	3.48	3.29
C18:2n-6	14.33	14.06	15.08
C18:3n-6	0.35	0.34	0.21
C18:3n-3	4.94	4.69	6.33
C20:0	0.37	0.36	0.65
C20:1n-9	3.45	3.43	1.83
C20:5n-3	3.35	3.29	4.15
C20:4n-6	0.72	0.64	0.50
C22:6n-3	9.12	8.85	7.27
C24:0	0.12	0.08	0.14
Saturates (SFAs)	20.33	20.74	21.88
Monounsaturates (MUFAs)	46.87	47.40	44.59
n-6 PUFAs	15.40	15.04	15.79
n-3 PUFAs	17.41	16.83	17.75

PUFAs	31.81	31.87	33.54

CT: Control; SCE 10: incorporation of 10% *Scenedesmus* in the diet; SCE 20: incorporation of 20% *Scenedesmus* in the diet. Values are expressed as mean value of 2 replicate samples per diet.

SFAs, Saturated fatty acids; MUFAs, Monounsaturated fatty acids; n-6 PUFAs, Omega-6 polyunsaturated fatty acids; n-3 PUFAs, Omega-3 polyunsaturated fatty acids; PUFAs, Polyunsaturated fatty acids

Table 4: Physical characteristics of the experimental feeds

Parameter	CT	SCE 10	SCE 20	p value
Fat leakage (%)	6.2 ± 0.6^{a}	5.3 ± 0.3^{b}	3.9 ± 0.4^{c}	< 0.001
Hardness (N)	22.9 ± 4.8^{b}	22.2 ± 5.0^{b}	39.6 ± 8.1^{a}	< 0.001
Length (mm)	4.4 ± 0.5^{a}	4.2 ± 0.5^{ab}	4.1 ± 0.6^{b}	<0.001
Diameter (mm)	3.0 ± 0.2	3.0 ± 0.1	3.1 ± 0.2	0.4634

CT: Control; SCE 10: incorporation of 10% *Scenedesmus* in the diet; SCE 20: incorporation of 20% *Scenedesmus* in the diet. Fat leakage is expressed as mean ± SD (n=6 replicates). Hardness, length and diameter are reported as an average value of 6 means ± SD, each mean value is an average of 20 pellets. Values in the same row with different superscript letters indicate significant difference ($p<0.05$)

Table 5: Apparent digestibility coefficients (ADC, %) of dry matter, lipid, protein, ash and energy in the experimental feeds

Parameter	CT	SCE 10	SCE 20	p value
Dry matter	67.6 ± 0.8^{a}	62.5 ± 0.2^{b}	54.5 ± 3.1^{c}	<0.001
Lipid	90.9 ± 0.2^{a}	88.1 ± 0.4^{b}	79.4 ± 1.8^{c}	0.001
Protein	82.3 ± 1.1^{a}	77.6 ± 0.9^{a}	69.2 ± 3.4^{b}	< 0.001
Ash	-22.9 ± 8.6	-31.6 ± 8.4	-42.9 ± 7.1	0.061
Energy	77.6 ± 0.4^{a}	72.6 ± 0.1^{b}	63.8 ± 2.5^{c}	< 0.001

CT: Control; SCE 10: incorporation of 10% *Scenedesmus* in the diet; SCE 20: incorporation of 20% *Scenedesmus* in the diet. Values are expressed as mean ± SD (n=6 replicate tanks). Values in the same row with different superscript letters indicate significant difference ($p<0.05$)

Table 6: Weight gain, growth rate, feed conversion ratio, and somatic indices of Atlantic salmon for the experimental period

Parameter	CT	SCE 10	SCE 20	p value
Growth parameter				
Initial body weight (g)	228.4 ± 4.6	230.8 ± 2.2	228.1 ± 4.1	0.418
Final body weight (g)	473.6 ± 47.7^{a}	451.0 ± 23.4^{ab}	416.7 ± 21.8^{b}	0.030
Weight gain (%)	107.1 ± 17.2^{a}	95.4 ± 10.3^{ab}	82.6 ± 7.2^{b}	0.013
Specific growth rate (% day^{-1})	1.12 ± 0.13^{a}	1.03 ± 0.08^{ab}	0.93 ± 0.06^{b}	0.014
Feed intake (% BW day^{-1})	0.86 ± 0.05	0.90 ± 0.04	0.89 ± 0.05	0.363
Feed conversion ratio	0.76 ± 0.09^{c}	0.88 ± 0.04^{b}	0.97 ± 0.04^{a}	<0.001
Protein efficiency ratio	2.69 ± 0.23^{a}	2.36 ± 0.11^{b}	2.13 ± 0.12^{b}	<0.001
Thermal growth coefficient	3.48 ± 0.47^{a}	3.19± 0.27^{ab}	2.8 ± 0.22^{b}	0.015
Condition indices				
Hepato-somatic index (%)	1.6 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	0.781
Viscero-somatic-Index (%)	10.1 ± 1.2	10.4 ± 0.9	11.1 ± 1.4	0.282
Condition factor (g cm^{-3})	1.42 ± 0.04^{a}	1.35 ± 0.02^{b}	1.32 ± 0.03^{b}	<0.001

CT: Control; SCE 10: incorporation of 10% *Scenedesmus* in the diet; SCE 20: incorporation of 20% *Scenedesmus* in the diet; BW, body weight. Values are expressed as mean ± SD (n=6 replicate tanks). Values in the same row with different superscript letters show significant differences ($p<0.05$)

Table 7: Nutrient retention efficiency (%) of lipid, protein and energy (gross) and retention efficiency of the digested nutrients (%) in Atlantic salmon fed the experimental diets

Parameter	CT	SCE 10	SCE 20	p value
Gross				
Lipid	85.7 ± 2.9^{a}	73.0 ± 3.5^{b}	63.0 ± 5.1^{c}	< 0.001
Protein	47.6 ± 3.9^{a}	41.1 ± 2.5^{b}	37.8 ± 2.1^{b}	< 0.001
Energy	49.6 ± 2.7^{a}	43.1 ± 3.7^{b}	36.4 ± 2.1^{c}	< 0.001
Digested				
Lipid	99.2 ± 5.6^{a}	85.4 ± 6.6^{b}	73.2 ± 4.8^{c}	0.020
Protein	62.3 ± 8.7^{a}	54.8 ± 6.8^{ab}	49.8 ± 4.1^{b}	< 0.001
Energy	69.1 ± 4.8^{a}	61.4 ± 7.1^{a}	51.2 ± 4.2^{b}	< 0.001

CT: Control; SCE 10: incorporation of 10% *Scenedesmus* in the diet; SCE 20: incorporation of 20% *Scenedesmus* in the diet. Values are expressed as mean ± SD (n=6 replicate tanks). Values in the same row with different superscript letters indicate significant difference ($p<0.05$)

Table 8: Chemical composition of the whole body (g kg^{-1} dry matter) of Atlantic salmon at the end of the feeding period

Parameter	Initial	CT	SCE 10	SCE 20	p value
Moisture (g kg^{-1})	71.3	68.7 ± 5.6^{ab}	68.5 ± 4.7^{a}	69.3 ± 3.4^{b}	0.017
g kg^{-1} dry matter					
Protein	593.0	556.2 ± 12.3^{ab}	546.4 ± 13.3^{b}	565.6 ± 7.3^{a}	0.032
Lipid	332.6	373.1 ± 8.6^{a}	374.2 ± 7.0^{a}	357.0 ± 4.9^{b}	<0.001
Ash	66.3	56.2 ± 3.3^{b}	58.5 ± 3.2^{ab}	63.7 ± 4.8^{a}	0.012
Energy (KJ g^{-1})	25.8	26.6 ± 0.1^{a}	26.2 ± 0.6^{ab}	26.0 ± 0.2^{b}	0.029

CT: Control; SCE 10: incorporation of 10% *Scenedesmus* in the diet; SCE 20: incorporation of 20% *Scenedesmus* in the diet. Values are expressed as mean ± SD (n=6 replicate tanks). Values in the same row with different superscript letters indicate significant difference ($p<0.05$)

68 Table 9 Fatty acid composition (% of total fatty acids) in fish at the start (initial) and at the end
69 of the feeding period

Fatty acid %	Initial	CT	SCE 10	SCE 20	p value
Saturates (SFAs)					
C14:0	4.06	3.11 ± 0.47	2.96 ± 0.15	3.05 ± 0.23	0.716
C15:0	0.37	0.44 ± 0.03	0.47 ± 0.08	0.43 ± 0.05	0.501
C16:0	12.56	13.79 ± 0.47	13.57 ± 0.26	13.36 ± 0.26	0.123
C18:0	3.18	3.62 ± 0.07[a]	3.45 ± 0.17[ab]	3.33 ± 0.08[b]	0.002
C20:0	0.37	0.42 ± 0.06	0.45 ± 0.13	0.33 ± 0.06	0.087
C24:0	0.11	0.12 ± 0.03	0.12 ± 0.04	0.12 ± 0.03	0.954
ΣSFAs	20.65	21.50 ± 0.86	21.02 ± 0.53	20.61 ± 0.47	0.092
Monounsaturates (MUFAs)					
C16:1n-7	4.29	3.41 ± 0.20	3.22 ± 0.34	3.50 ± 0.12	0.154
C18:1n-9	34.91	37.46 ± 0.89	36.95 ± 0.84	36.88 ± 1.07	0.509
C18:1n-7	3.42	3.45 ± 0.06	3.37 ± 0.10	3.42 ± 0.06	0.217
C20:1n-9	5.16	3.70 ± 0.06	2.95 ± 0.88	3.49 ± 0.15	0.065
ΣMUFAs	47.78	48.03 ± 0.81	46.49 ± 1.97	47.28 ± 1.14	0.204
n-6 PUFAs					
C18:2n-6	13.98	13.95 ± 0.17[a]	14.54 ± 0.42[b]	14.28 ± 0.21[ab]	0.010
C18:3n-6	0.36	0.31 ± 0.11	0.34 ± 0.11	0.36 ± 0.05	0.689
C20:4n-6	0.82	0.63 ± 0.08	0.64 ± 0.06	0.70 ± 0.05	0.183
Σn-6 FAs	15.15	14.89 ± 0.30[a]	15.52 ± 0.38[b]	15.34 ± 0.20[ab]	0.008

n-3 PUFAs					
C18:3n-3	4.49	4.48 ± 0.29[a]	5.18 ± 0.71[b]	4.88 ± 0.25[ab]	0.050
C20:5n-3	3.61	2.91 ± 0.15	3.58 ± 0.76	3.24 ± 0.21	0.070
C22:6n-3	8.32	8.19 ± 0.30	8.22 ± 0.34	8.65 ± 0.48	0.097
Σn-3 FAs	16.42	15.58 ± 0.55[a]	16.97 ± 1.22[b]	16.77 ± 0.89[ab]	0.041
ΣPUFAs	31.57	30.47 ± 0.68[a]	32.49 ± 1.56[b]	32.11 ± 1.00[ab]	0.017
n-3/n-6	1.08	1.05 ± 0.04	1.09 ± 0.06	1.09 ± 0.05	0.202

CT: Control; SCE 10: incorporation of 10% *Scenedesmus* in the diet; SCE 20: incorporation of 20% *Scenedesmus* in the diet. SFAs, Saturated fatty acids; MUFAs, Monounsaturated fatty acids; n-6 PUFAs, Omega-6 polyunsaturated fatty acids; n-3 PUFAs, Omega-3 polyunsaturated fatty acids; PUFAs, Polyunsaturated fatty acids. Values are expressed as mean ± SD (n=6 replicate tanks). Values in the same row with different superscript letters indicate significant difference ($p<0.05$)

Authorship factsheet

Yangyang Gong: Execution; Investigation; Methodology; Writing original draft

Tharindu Bandara: Execution; Investigation; Methodology; Writing original draft

Mark Huntley: Conception; Project administration; Review and editing

Zackary Jonson: Conception; Project administration; Review and editing

Jorge Dias: Methodology; Review and editing

Mette Sørensen: Conception; design of experiment; Execution, Writing the manuscript

Viswanath Kiron: Conception; design of experiment; Execution, Writing the manuscript