Digestibility of the defatted microalgae Nannochloropsis sp. and Desmodesmus sp. when fed to Atlantic salmon, Salmo salar

Gong, Y., Sørensen, M., Kiron, V., Huntley, M. & dos Santos Guterres, H. A.

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Digestibility of dry matter, protein, ash and energy of feeds containing defatted microalgae *Nannochloropsis* sp. and *Desmodesmus* sp. fed to Atlantic salmon, *Salmo salar*

YANGYANG GONG [1, 2], HORACIO AMARAL DOS SANTOS GUTERRES [1], MARK HUNTLEY [3,4], METTE SØRENSEN [1,*], VISWANATH KIRON [1]

[1] *Nord University, PB1490, Bodø 8049, Norway;* [2] *Key Laboratory of East China Sea and Oceanic Fishery Resources Exploitation, East China Sea Fisheries Research Institute, China;* [3] *Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, USA*; [4] *Marine Laboratory, Nicholas School of the Environment, Duke University, Beaufort, NC, USA*

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* *Correspondence: Phone: +47 95 04 58 65. Email: 1*

Running title: Digestibility of microalgae in Atlantic salmon

Abstract

The aim of the study was to investigate the apparent digestibility coefficient (ADC) of defatted biomass derived from microalgae *Nannochloropsis* sp. and *Desmodesmus* sp. when fed to Atlantic salmon post-smolts in seawater. Two experiments were carried out to determine the ADC of dry matter (DM), protein, ash and energy. The test diets consisted of a 70:30 mixture of a fishmeal-based reference diet to test ingredient, with yttrium oxide as inert marker. Diets used in experiment one were produced employing a cold pelleting process. Extruded diets were used in experiment two. The results showed that ADC values of DM and energy in the two microalgae differed significantly, while the digestibility of ash and protein in the two algae were similar. The ADC value of DM was 48-63% and 32-47% for *Nannochloropsis* sp. and *Desmodesmus* sp., respectively. The ADCs of energy in *Nannochloropsis* sp. was 61% and for that in *Desmodesmus* sp. was 51%. The ADC of protein was 72-73% and 54-67% for *Nannochloropsis* sp. and *Desmodesmus* sp., respectively. The ADC of ash in *Nannochloropsis* sp. was 36-80% and that in *Desmodesmus* sp. was 41-73%. The results showed that extrusion improved the ADC of DM, protein and ash compared to the cold pelleted diets fed to Atlantic salmon.

Introduction

The aquafeed industry has shifted its preference from fishery-based to plant-based feeds. Soy protein concentrate and rapeseed oil are the main plant ingredients that are used to replace fishmeal and fish oil, respectively (Ytrestøyl *et al.* 2015). Use of plant protein ingredients have limitations compared to fishery-based ingredients, mainly because of anti-nutritional factors and imbalanced amino acid composition (Krogdahl *et al.* 2010). Dose-dependent growth inhibition and intestinal enteritis have been observed in Atlantic salmon that consumed feeds with plant-derived ingredients (Krogdahl *et al.* 2003). The use of plant oils in aquafeeds also compromises the quality of the marketable product due to a less favorable n-6 dominated profile (Bell *et al.* 2001). Furthermore, aberrations in lipid metabolism upon ingestion of plant oils may compromise fish health (Kjær *et al.* 2008; Todorčević *et al.* 2008). Recent research has also shown that salmon fed n-6 fatty acids are more susceptible to viral diseases (Martinez-Rubio *et al.* 2012; Martinez-Rubio *et al.* 2014). There is an urgent need to find novel feed ingredients for the aquafeed industry, which ideally are not used directly as human food.

Microbe-derived ingredients including microalgae, yeasts and bacteria are promising alternative resources that can be used in feeds for carnivorous fish. These ingredients may have high nutritional value and a relatively low impact on the environment (Taelman *et al.* 2013). Microalgae have been used for rearing molluscs and zooplankton as well as larval stages of fish and crustaceans (Brown *et al.* 1997). Furthermore, the favorable fatty acid and amino acid profiles of some microalgae species have spurred research on microalgae-incorporated feeds for farmed fish (Skrede *et al.* 2011; Shields & Lupatsch 2012). Inclusion of 20% and 40% microalgae *Spirulina* sp or 20% *Scenedesmus* in diets for tilapia and gilthead sea bream gave the same growth and protein utilization as fish fed fishmeal-based diet (Olvera-Novoa *et al.* 1998; Vizcaíno *et al.* 2014). A marine microalgae, *Schizochytrium*

74 sp. that replaced 2.5-5% of fish oil in feeds was found to improve the EPA + DHA retention in
75 Atlantic salmon with increasing levels of microalgae (Kousoulaki *et al.* 2016). The latter authors
76 reported no differences in weight gain or feed conversion when *Schizochytrium* sp was added to the
77 diets.
78
79 Some microalgae strains such as *Nannochloropsis* and *Desmodesmus* have been developed for bio-
80 diesel production due to their capacity to accumulate large quantities of lipids (Hu *et al.* 2013;
81 Moody *et al.* 2014). The microalgae biomass after lipid extraction contains protein, minerals and
82 carotenoids as well as bioactive components with health benefits (Yaakob *et al.* 2014; Maisashvili
83 *et al.* 2015). Defatted microalgae-derived biomass has been shown a potential to be used as a feed
84 ingredient in aquafeeds (Kiron *et al.* 2012; Patterson & Gatlin III 2013; Kiron *et al.* 2016).
85
86 Before novel ingredients can be used commercially, they need to be thoroughly tested on the
87 targeted species; to ensure their safety as well as to understand any adverse effects on growth, feed
88 utilization, animal health or product quality. One of the main challenges with the use of microalgae
89 is the high variability in nutrient composition among various strains of microalgae (Lum *et al.* 2013;
90 Tibbetts *et al.* 2015). Evaluation of nutrient digestibility is useful for assessing the value of an
91 ingredient for farmed fish species (Glencross *et al.* 2007). Besides, different processing conditions
92 when feed are produced could also have an impact on nutrient digestibility and overall feed
93 utilization (Sørensen, 2012). Compared to pelleting process, extrusion increased digestibility of dry
94 matter and protein in gibel carp, in particular for diets low in fish meal (Shi *et al.* 2016). The present
95 study was carried out to determine the ADC of dry matter, protein, ash and energy of defatted
96 microalgae-derived biomass in the diets for Atlantic salmon. Two experiments were designed to
97 study the digestibility of *Nannochloropsis* sp. and *Desmodesmus* sp as well as the the two

98 microalgae containing reference diets, produced by use of either cold-pelleting (experiment one) or
99 extrusion technology (experiment two).
100
101 **Material and methods**
102 Two separate feeding trials (employing cold-pelleted and extruded pellets, respectively) were
103 designed to investigate the apparent digestibility coefficients of dry matter, protein, ash and energy
104 of defatted microalgae-derived biomass in the feeds for Atlantic salmon.
105
106 ***Diet formulation and preparation***
107 The test microalgae (*Nannochloropsis* sp. and *Desmodesmus* sp.) were acquired from Cellana
108 (Kona Pilot Facility, Kailua-Kona, Hawaii, USA), under a US Department of Energy funded project
109 Grant DE- EE0003371. Defatted microalgae biomass was collected after oil extraction. The
110 proximate composition of defatted microalgae-derived biomass is presented in Table 1.
111
112 The diets for experiment one (cold-pelleted diets) were produced at the feed laboratory of Nord
113 University, Bodø, Norway. A reference diet (P-CO) was formulated to contain approximately 540 g
114 kg^{-1} protein, 200 g kg^{-1} lipid, and yttrium oxide (0.1 g kg^{-1}) was used as the indigestible inert
115 marker (Table 2). Fishmeal and fish oil were used as the main protein and lipid sources in the
116 reference diet, and gelatinized potato starch was the binder. Two test diets were formulated by
117 mixing 70% of the reference diet and 30% of either *Nannochloropsis* sp. (P-NA) or *Desmodesmus*
118 sp. (P-DE). The feeds were prepared by thoroughly mixing all the ingredients in a mixer (Bear
119 Varimixer RN 20 VL2, A/S Wodschow & Co., Broendby, Denmark). The homogeneous mixture
120 was blended with cold tap water (approximately 40%) to obtain a malleable dough. The dough was
121 cold-pressed through a meat mincer (Sirman TC22 RIO, Sirman SpA, Curtarolo, Italy) to produce
122 spaghetti-like strings, which were dried in an oven (Rational SCC 101, Rational AG, Landsberga,

123 Germany) for 20 h at 35 °C. The dried strings were manually crushed and then sieved to obtain feed
124 pellets at an approximate diameter of 4–5 mm. The finished diets were then vacuum packed and
125 stored at 4°C until use.
126
127 The diets for experiment two (extruded diets) were produced at the Center for Feed Technology
128 (ForTek), Norwegian University of Life Sciences, Ås, Norway. The reference diet (E-CO) was
129 formulated to contain approximately 520 g kg^{-1} protein, 190 g kg^{-1} lipid. In this study also, we used
130 yttrium oxide (0.1 g kg^{-1}) as the indigestible inert marker (Table 3). Fishmeal and fish oil were the
131 main protein and lipid sources in the reference diet, and wheat was used primarily as a binder. A
132 new batch of *Nannochloropsis* sp. biomass was used in experiment two, while *Desmodesmus* sp.
133 came from the same batch as that of experiment one (Table 1). The reference diet and two test diets
134 (E-NA, E-DE) were processed using a twin-screw cooking extruder (BCTG 62/20 D, Bühler, Uzwil,
135 Switzerland). The extruded diets were stored in plastic lined paper bags and shipped to Nord
136 University.
137
138 ***Digestibility trials***
139 The trials were conducted according to the procedures approved by the National Animal Research
140 Authority (Forsøksdyrutvalget, Norway). The digestibility studies were conducted at Mørkvedbukta
141 Research Station (Nord University, Bodø, Norway). Atlantic salmon post-smolts (Aquagen strain,
142 Aquagen AS, Sluppen, Trondheim, Norway) were purchased from a commercial producer (Cermaq
143 Norway AS, Hopen, Norway). Salmon for experiment one was 73 g when the fish arrived at the
144 research station September 2013 and was maintained for approximately 1.5 years before the start of
145 the digestibility experiment. Salmon for experiment 2 was on average 70 grams when fish arrived
146 at the research station in June 2014. Before the experiments started, the fish were fed commercial
147 diets, Skretting Spirit 75 (Skretting, Stavanger, Norway). The size of the pellet was 3-4.5 mm

148 depending on the fish size. Both fish groups were vaccinated with ALPHA JECT micro 6-2
149 (Pharmaq, Oslo, Norway).

150 The fish were kept in 12 1100 L (exp. one) and 9 (exp. two) experimental tanks supplied with
151 seawater drawn from a depth of 250 m from the Saltenfjorden. The fish were reared under 24 h of
152 continuous light condition. Fish were fed by automatic feeders, and throughout the experimental
153 period the daily ration was 1% of the biomass.
154
155 In experiment one, Atlantic salmon with an average weight of approximately 1600 g were
156 used. Groups of 20 fish per tank were kept at ambient temperature. The average water
157 temperature in the tanks was 8.2 °C and the oxygen was above 85% of saturation throughout the
158 experimental period. Fish were randomly assigned to 3, 6 and 3 replicate tanks of the control,
159 *Nannochloropsis* sp. and *Desmodesmus* sp. treatments, respectively. Three experimental diets were
160 used for 21 days i.e. until faeces collection.
161
162 In experiment two, Atlantic salmon with an average body weight of 435.6 g were used. Groups of
163 50 fish per tank were kept at ambient temperature. The water temperature was 5-6 °C, and oxygen
164 saturation was kept above 83%. This study employed triplicate tanks for each dietary treatment. The
165 fish were fed three different diets for 11 days before faecal collection commenced.
166
167 Faecal samples from each fish were collected according to Austreng (1978). Prior to stripping, the
168 fish were anesthetized with 70 mg L^{-1} of tricaine methanesulfonate (MS-222, Argent Chemical
169 Laboratories, Redmond, USA). The ventral caudal area of the anesthetized fish was gently dried by
170 tissue paper before faeces collection. The fish faeces were pooled within tank and freeze dried. For
171 experiment one, faeces from the groups fed *Nannochloropsis* sp. were pooled, two by two tanks.

Number of replicates was consequently reduced from six to three samples and stored at −40 °C prior to chemical analyses.

Chemical analyses

Microalgae, experimental diets and freeze-dried faeces were finely ground by mortar and pestle, and homogenized prior to analyses of dry matter (105 °C for 20 hours) (ISO 6496–1999), crude protein (N × 6.25; Kjeldahl Auto System, Tecator Systems, Höganäs, Sweden) (ISO 5983–1987), crude lipid (Soxtec HT6, Tecator, Höganäs, Sweden) (ISO 6492–1999), ash (incineration in a muffle furnace at 540 °C for 16 h) (ISO 5984–2002), and energy (IKA C200 bomb calorimeter, Staufen, Germany) (ISO 9831–1998). Yttrium was analyzed by inductive coupled plasma mass spectroscopy (ICP-MS) at Eurofins (Moss, Norway) (NS-EN ISO 11885). All the samples were analyzed in duplicate.

Calculations and statistical analysis

The ADC of protein, energy or dry matter of the reference diet and test diets were determined using the following equation (Cho & Slinger 1979):

$$ADC_{nutrient/energy} = [1 - \frac{(Marker_{diet} \times Nutrient_{faeces})}{(Marker_{faeces} \times Nutrient_{diet})}] \times 100$$

$$ADC_{dry\ matter} = [1 - (Marker_{diet} \div Marker_{faeces})] \times 100$$

where $Marker_{diet}$ and $Marker_{faeces}$ represent the marker content (% dry matter) of the diet and faeces, respectively, and $Nutrient_{diet}$ and $Nutrient_{faeces}$ represent the nutrient contents (% dry matter) in the diet and faeces.

194 The apparent digestibility coefficients of the test ingredients were calculated according to the
195 equation of Bureau & Hua (2006) as follows:

196 $$ADC_{ingredient} = ADC_{testdiet} + (ADC_{testdiet} - ADC_{ref.diet}) \times [\frac{0.7 \times Nutrient_{ref.}}{0.3 \times Nutrient_{ingredient}}]$$

197 Where $Nutrient_{ref.}$ represents the nutrient content (% dry matter) of the reference diet and
198 $Nutrient_{ingredient}$ is the nutrient content (% dry matter) of the test ingredient.

199

200 All statistical analyses were performed using SPSS 19.0 software package for Windows. The data
201 were tested for normality (Shapiro-Wilk normality test) and Levene's test of equality. Significant
202 differences among ADC values of diets were determined by the Tukey's HSD test, and significant
203 differences between ADC values of microalgae and ADC values of microalgae between cold-
204 pelleted and extruded process were identified by employing the independent t-test. The differences
205 were regarded as significant when $P < 0.05$.

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Results

The tested microalgae differed in their chemical composition (Table 1), especially in the protein (269-429 g kg^{-1} microalgae) and ash contents (160-233 g kg^{-1} microalgae). Crude lipid content was low; the values were in the range 10-42 g kg^{-1} microalgae. All experimental diets were well accepted by Atlantic salmon and no mortality was noted during the course of the experiments.

Experiment one

The ADCs of DM, protein and ash of the experimental diets and the ADC values for the two microalgae are given in Table 4. The ADC of DM ranged from 64.5% (P-DE) to 77.4% (P-NA), and the values of P-NA and P-DE were significantly ($P < 0.05$) lower compared to that of the P-CO diet. The ADC of protein in the three diets differed significantly ($P < 0.05$) and the values ranged from 81.9% to 88.4%. The ash content of the P-DE and P-NA diets were higher than that of the P-CO diet, although we did not detect statistically significant differences.

The ADC of DM and protein in the microalga *Nannochloropsis* sp. was significantly higher than those of *Desmodesmus* sp. The ADC of ash in the two algae did not differ significantly.

Experiment two

The ADC of DM, protein, ash and energy of the experimental diets and the ADCs of the microalgae *Nannochloropsis* sp. and *Desmodesmus* sp. are presented in Table 5. The results showed no differences in ADC of DM in the diet E-CO (69.2%) and diet E-NA (67.3%). However, the ADC of DM in the E-DE diet (62.8%) was lower ($P < 0.05$) compared to the values of the other two diets. The ADCs of protein in E-NA (82.2%) and E-DE (82.0%) were not significantly different, but these two diets had lower ADCs than that of the E-CO diet (85.6%; $P < 0.05$). The ADCs of ash in E-NA (41.9%) and E-DE (34.6%) were not significantly different, but these two diets had higher ADCs

than that of the E-CO diet (7.9%; $P < 0.05$). Digestibility of energy in the three diets differed significantly, and the values ranged from 75.0% to 83.3%.

The digestibility of DM and energy in the two microalgae *Nannochloropsis* sp. and *Desmodesmus* sp. differed significantly ($P < 0.05$). Although the overall digestibility of *Nannochloropsis* sp. was greater, the digestibility of ash and protein in the two algae were similar ($P > 0.05$).

Cold pelleted vs. extruded feed

Digestibility of DM, protein and ash for *Nannochloropsis* sp. and *Desmodesmus* sp. were affected by processing of the feed (Table 6). The ADC of DM and ash were significantly improved for the extruded *Nannochloropsis* sp., while no differences were noted for the ADC of protein. The extruded *Desmodesmus* sp. showed significantly higher ADC of protein and ash, while no differences were noted between the two processes for digestibility of DM ($P < 0.05$).

Discussion

Two experiments were carried out to determine the ADCs of DM, protein and ash in the defatted microalgae derived from either *Nannochloropsis* sp. or *Desmodesmus* sp. For experiment two, ADC of energy was also calculated. The chemical composition indicates low lipid content of the microalgae. The biomass was obtained after lipid extraction, and this explains the low lipid content of the ingredient. These two microalgae were chosen for biofuel production because they normally can accumulate lipid content up to 50%-60% of the dry biomass (Mata *et al.* 2010; Scott *et al.* 2010; Hu *et al.* 2013). The protein and ash content of the two microalgae varied widely, and the differences in the chemical composition of the microalgae biomass were reflected in the composition of the test diets. The protein content of the test diet ranged from 451 g kg^{-1} to 563 g kg^{-1} DM.

Protein content in the algae ingredient (as well as for the feed and faeces), was calculated based on total N in the sample × 6.25. This conversion factor may not be correct because total N also include nitrogen from non-protein sources such as nucleic acids, amines, glucosamides and N-containing components in the cell walls. Besides, the value is not constant, but it change with species, the growth stage of the algae and the extraction methods used (Lourenço *et al.* 2004; Safi *et al.* 2013). Lourenço *et al.* (2004) reported that protein nitrogen ranged from 59.3-96.8% of total nitrogen, but suggested an N conversion factor of 4.78 as an average across 12 species investigated under different cultivation conditions. A more recent paper investigated the N conversion factor for rigid cell walled microalgae including *Nannochloropsis oculata*, and suggested 6.28 as a conversion factor for whole algae (Safi *et al.* 2013). Because the algae used in the present experiment both have rigid cell walls it was decided to use 6.25 as conversion factor to calculate protein content in microalgae ingredient, feed and faeces content.

292 Digestibility of the protein of the fishmeal-based reference diets P-CO/E-CO were 88.4/85.6% are
293 in line with other studies, though values have been reported in the range 84.7%-92.1% (Grisdale-
294 Helland & Helland 1997; Refstie *et al.* 1998; Krogdahl *et al.* 2004; Sørensen *et al.* 2011).
295 Digestibility of protein and DM showed greater variation between the ingredients than the reference
296 diets. The digestibility of protein in the reference diet P-DE was 3% lower than the P-NA, while the
297 *Desmodesmus* sp. was 26% lower than *Nannochloropsis* sp. in experiment one. For the extruded
298 diets in experiment two, digestibility of protein was similar for the reference diets, while the
299 *Desmodesmus* sp. was approximately 7% lower compared to the *Nannochloropsis* sp. Greater
300 variation in nutrient digestibility for test ingredients compared to reference diets have also been
301 reported in experiments that estimate the nutrient digestibility of plant ingredients in fish (Glencross
302 *et al.* 2004).
303
304 The reduction in DM digestibility for the test diets compared to the reference diets is most likely
305 explained by increased concentration of indigestible cell wall components in the biomass after oil is
306 removed. A number of feeding experiments, performed with salmonids, have indicated that
307 digestibility and bioavailability of nutrients in single cell organisms such as bacteria (Aas *et al.*
308 2006b), yeast (Lee 2002; Aas *et al.* 2006b; Berge *et al.* 2013), and microalgae (Skrede *et al.* 2011),
309 may be impacted by the rigid cell walls (Storebakken *et al.* 2004). Also the differences in DM and
310 protein digestibility observed between *Nannochloropsis* sp. and *Desmodesmus* sp. in the present
311 experiment may be explained by differences in the complex structure of microalgae cell wall as
312 well as different composition of non-starch polysaccharides that may restrict enzymatic digestion
313 (Domozych *et al.* 2012; Scholz *et al.* 2014). Cell walls of green algae are diverse both in terms of
314 morphology and composition of polymers. Some algae have polymers similar to plant
315 carbohydrates, i.e. polymers similar to cellulose, pectins, hemicelluloses, arabinogalactan proteins
316 and lignin (Domozych *et al.* 2012). The cell wall composition of *Nannochloropsis* sp. and

317 *Desmodesmus* sp. used in the present experiment has not been characterized. Assuming that the cell
318 wall morphology and composition of *Nannochloropsis* sp. is similar to those of *Nannochloropsis*
319 *gaditana*, the cell wall has a bilayer structure - a cellulosic inner wall protected by an outer
320 algaenan layer making the cells resistant to enzymatic digestion (Becker 2007; Scholz *et al.* 2014).
321 Members of the *Desmodesmus* have elongate cells of different shape, and the cells are arranged in
322 flat coenobia of two to 32 cells in one row (Komárek & Fott 1983). The cell walls enclosing the
323 coenobia is complex and consist of four sporopollenic cell wall layers (Vanormelingen *et al.* 2007),
324 protecting the cell against enzymatic digestion. In comparison, the cell wall of the cyanobacteria
325 *Spirulina* sp. is made of mucopeptides, and therefore this type of cell wall can be more easily
326 utilized, explaining the higher DM digestibility (82.1%) compared to the values observed for
327 *Nannochloropsis* sp. and *Desmodesmus* sp. in the present experiment (Becker 2007). Effects of
328 different polysaccharides on energy and nutrient digestibility were recently studied in barramundi
329 (*Lates calcarifer*) (Irvin *et al.* 2015). The latter authors reported that cellulose, pectin and lignin had
330 a significant negative effect on digestibility of DM and energy, while digestibility of protein was
331 negatively affected by pectin and lignin. There were also significant interactive effects when
332 different carbohydrates were combined. Though detailed information about carbohydrate
333 composition for the two algae is lacking in the present experiment, composition of the cell walls
334 most likely had a great impact on the digestibility of DM and protein in the present experiment.
335
336 The variations in ADC of protein in the two microalgae were in line with results reported by Skrede
337 *et al.* (2011). In the study of Skrede *et al*. (2011), a dose-response experiment was conducted on a
338 carnivore model-animal, mink, by replacing fishmeal with different microalgae (*Nannochloropsis*
339 *oceanica* / *Phaeodactylum tricornutum* / *Isochrysis galbana*) at three levels 6%, 12% and 24%. The
340 authors found a linear reduction in crude protein digestibility with increasing inclusion levels of the
341 three algae. *N. oceanica* and *I. galbana* caused negative effects on protein digestibility even at 6%

342 inclusion, while only the highest inclusion level of *P. tricornutum* induced negative effects on the
343 digestibility. Based on linear regression, apparent protein digestibility values for *N. oceanica*, *P.*
344 *tricornutum* and *I. galbana*, were 35.5%, 79.9% and 18.8%, respectively. In comparison, the present
345 study obtained protein digestibility values of 72.4% 67.1% for *Nannochloropis* sp. and
346 *Desmodesmus* sp., respectively. These values were however, lower than the protein digestibility of
347 the microalga *Spirulina* sp. 84.7% fed to Atlantic salmon (Burr *et al.* 2011). ADC of protein in the
348 *Nannochloropsis* sp. and *Desmodesmus* sp. biomass was consistent with protein digestibility of
349 some reported seaweeds (65.5%-79.5%) fed to rainbow trout (Pereira *et al.* 2012).
350
351 Digestibility of energy in the reference diet of experiment two is comparable to other studies in
352 Atlantic salmon, reporting values in the range from 73%-88% (Refstie *et al.* 1998; Krogdahl *et al.*
353 2004). The inclusion of both the *Nannochloropsis* sp. and *Desmodesmus* sp. biomass caused a
354 decrease in the test diet ADC values. In line with these findings, Kousoulaki *et al.* (2015) reported a
355 reduction in energy digestibility from 83/84% to 80% when *Scizochytrium* sp. was included from 0
356 - 15% in the diet. In contrast, Sørensen *et al.* (2016) reported no reduction in energy digestibility
357 when *Phaeodactylum tricornutum* was fed to Atlantic salmon at inclusion rates of 3-12% of the diet.
358 The ADC of energy in the two microalgae in the present experiment was lower than the earlier
359 reports on the microalga *Spirulina* sp. (82.5%) fed to Atlantic salmon (Burr *et al.* 2011) but was
360 within the range of values reported for seaweeds (58.0%-72.7%) and most terrestrial plant
361 ingredients (56%-96%) fed to rainbow trout and Atlantic salmon (NRC 2011; Pereira *et al.* 2012).
362
363 The ADC values obtained from the two experiments were different. Overall, ADCs of DM and ash
364 in the two microalgae were higher in the experiment two. In addition, the ADC of protein in the
365 *Desmodesmus* sp. was higher in the experiment two. Digestibility of ingredients can vary between
366 batches Aas *et al.* (2006a). In the two studies, we used the alga *Nannochloropsis* sp. from different

367 production batches, while *Desmodesmus* sp. belonged to the same batch. Ingredient composition
368 was also slightly modified between the two experiments. A pre-gelatinized potato starch was used
369 as binder in experiment one, while wheat and wheat gluten were used in the experiment two. Wheat
370 gluten is a highly digestible protein and with the low inclusion rate used in the present experiment
371 no effects were expected on digestibility of DM, protein or energy (Storebakken *et al.* 2000;
372 Storebakken *et al.* 2015). The different starch sources were chosen in the two experiments because
373 of their different functionality as binders (Sørensen *et al.* 2010). A pre-gelatinized starch was used
374 as a binder for the cold pelleted feed in experiment one because it is soluble in cold water. Wheat
375 and wheat gluten was chosen for experiment two because of their unique properties as binders in
376 high energy feeds. Though digestibility and utilization of starch is affected by numerous factors
377 including botanical origin (Glencross *et al.* 2012; Kamalam *et al.* 2016), no effects were expected
378 on nutrient digestibility in the present experiment because of the modest inclusion level. This is
379 supported by other research reporting no significant differences in nutrient digestibility when olive
380 flounder was fed either wheat or potato starch at 20% inclusion in the diet (Rahman *et al.* 2016).
381
382 Feed manufacturing technology may also explain differences in utilization of diets by Atlantic
383 salmon (Glencross *et al.* 2011; Sørensen 2012). The feeds in experiment one were produced by
384 cold-pelleting, while those in experiment two were prepared by extrusion. Extrusion is a
385 thermomechanical process that combines high temperature (120–130°C), high pressure (20–30 bar)
386 and shear forces (Sørensen 2012), and these processes can disrupt the algal cell walls to increase the
387 extractability and bioavailability of nutrients (McMillan *et al.* 2013; Maehre *et al.* 2015; Shene *et al.*
388 2016). Feeding gilthead sea bream (*Sparus aureta*) extruded feed compared to pelleted feed,
389 improved growth, digestibility of energy and starch (Venou *et al.* 2009). Increased utilization and
390 retention of protein and lipid in the diet retained as body protein and lipid (Venou *et al.* 2009; Shi *et*
391 *al.* 2016), as well as improved feed conversion factor, has also been reported in fish fed extruded

392 compared to pelleted feed (Hilton *et al.* 1981; Venou *et al.* 2009). Research with Atlantic salmon
393 has suggested that extrusion of diets containing *Schizochytrium* sp. is enough to disrupt cell walls
394 and increase bioavailability of nutrients for Atlantic salmon (Kousoulaki *et al.* 2015).
395
396 Different morphology of micro-algae may also explain different effects of extrusion processing.
397 The *Nannochloropsis* sp. is present as single cell, while *Desmodesmus* sp. form colonies of cells
398 cells (Becker 2007; Vanormelingen *et al.* 2007; Yokota & Sterner 2011; Scholz *et al.* 2014).
399 Thermomechanical treatment of the *Desmodesmus* sp. may have helped in tearing apart the cells,
400 improving accessibility of protein digesting enzymes. The greater ADC of protein in the
401 *Desmodesmus* sp. ingredient after extrusion processing could thus be attributed to the morphology
402 of the cells.
403
404 **Conclusions**
405 The nutrient digestibility values of the defatted microalgae-included diets were lower compared to
406 the reference diets. Furthermore, the digestibility values of the two defatted microalgae biomass
407 obtained from biorefinery were different from each other. The digestibility of dry matter, protein
408 and ash in the microalgae seem to be improved in the extruded diets. The defatted microalgae that
409 may become available in the market as a co-product following the extraction of other high-value
410 algal components has a potential value to be used as a feed ingredient for Atlantic salmon.
411
412
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637

Table 1

Proximate composition of test ingredients *Nannochloropsis* sp. and *Desmodesmus* sp. (g kg^{-1})

	Nannochloropsis sp.[a]	*Nannochloropsis* sp.[b]	*Desmodesmus* sp.
Dry matter	978	978	886
Crude protein	429	421	269
Crude lipid	42	25	10
Ash	233	230	160
Energy (kJ g^{-1})	18.8	18.6	16.6

[a] *Nannochloropsis* sp. used in the experiment one

[b] *Nannochloropsis* sp. used in the experiment two

Table 2

Formulation (g kg^{-1}) and proximate composition (g kg^{-1} dry matter) of the reference diet and test diets for experiment one

Ingredients	P-CO	P-NA	P-DE
Fishmeal[a]	753	527	527
Fish oil[a]	120	84	84
Mineral and vitamin premix[b]	7	5	5
Potato starch[c]	120	84	84
Nannochloropsis sp.[d]		300	
Desmodesmus sp. [d]			300
Yttrium oxide (Y_2O_3)[e]	0.1	0.1	0.1
Proximate composition			
Dry matter	963	966	962
In dry matter:			
Crude protein	563	524	485
Crude lipid	211	170	159
Ash	116	151	135
Gross energy (kJ g^{-1})	23.6	22.3	21.9

The abbreviations in the table stand for: P-CO – Cold-Pelleted Reference diet; P-NA –Cold-Pelleted *Nannochloropsis* sp. diet; P-DE –Cold-Pelleted *Desmodesmus* sp. diet

a Bodø Sildoljefabrikk AS, Bodø, Norway

b Proprietary formulation of Skretting Aquaculture Research Center, Stavanger, Norway.

c Swely gel 700, Lyckeby Culiner, AB, Fjälklinge, Sweden

d Cellana, Kona, Hawaii, USA

e Metal Rare Earth Limited, Shenzhen, China

Table 3

Formulation (g kg^{-1}) and proximate composition (g kg^{-1} dry matter) of the reference diet and test diets for experiment two

Ingredients	E-CO	E-NA	E-DE
Fishmeal[a]	703	492	492
Fish oil[a]	120	84	84
Wheat[b]	120	84	84
Wheat gluten[c]	50	35	35
Mineral and vitamin premix[d]	7	5	5
Nannochloropsis sp.[e]		300	
Desmodesmus sp. [e]			300
Yttrium oxide (Y_2O_3)[f]	0.1	0.1	0.1
Proximate composition			
Dry matter	956	927	940
In dry matter:			
Crude protein	545	516	474
Crude lipid	199	186	160
Ash	113	151	135
Gross energy (kJ g^{-1})	23.2	22.0	21.7

The abbreviations in the table stand for: E-CO – Extruded Reference diet; E-NA –Extruded *Nannochloropsis* sp. diet; E-DE –Extruded *Desmodesmus* sp. diet

a Norsildmel AS, Fyllingsdalen, Norway

b Felleskjøpet, Kambo, Norway

c Gluten Vital, Alimenta AS, Hagan, Norway

d Normin AS, Hønefoss, Norway

e Cellana, Kona Hawaii, USA

f Metal Rare Earth Limited, Shenzhen, China

Table 4

Apparent digestibility coefficients (ADCs, %) of dry matter (DM), protein, ash in the reference and test diets, and in the defatted microalgae biomass of experiment one

ADC diets	P-CO	P-NA	P-DE
DM	77.4 ± 0.7[a]	68.5 ± 1.8[b]	64.5 ± 0.5[c]
Protein	88.4 ± 0.4[a]	84.5 ± 0.6[b]	81.9 ± 1.0[c]
Ash	16.5 ± 2.1[a]	25.5 ± 5.7[a]	26.1 ± 5.8[a]

ADC microalgae	*Nannochloropsis* sp.	*Desmodesmus* sp.
DM	47.9 ± 5.8[a]	31.8 ± 1.6[b]
Protein	72.9 ± 2.3[a]	54.1 ± 5.4[b]
Ash	35.7± 12.2[a]	40.6 ± 14.4[a]

The abbreviations in the table stand for: P-CO – Cold-Pelleted Reference diet; P-NA –Cold-Pelleted *Nannochloropsis* sp. diet; P-DE –Cold-Pelleted *Desmodesmus* sp. diet

The data are presented as mean ± SD (n=3)

Different superscript among rows denotes significant differences ($P < 0.05$).

Table 5

Apparent digestibility coefficients (ADCs, %) of dry matter (DM), protein, ash and energy in the reference and test diets, and in the defatted microalgae biomass of experiment two

ADC diets	E-CO	E-NA	E-DE
DM	69.2 ± 1.0[a]	67.3 ± 0.5[a]	62.8 ± 2.7[b]
Protein	85.6 ± 0.2[a]	82.2 ± 0.2[b]	82.0 ± 0.6[b]
Ash	7.9 ± 2.2[b]	41.9 ± 1.2[a]	34.6 ± 4.7[a]
Energy	83.3 ± 0.5[a]	77.4 ± 0.4[b]	75.0 ± 1.3[c]

ADC microalgae	*Nannochloropsis* sp.	*Desmodesmus* sp.
DM	63.1± 1.5[a]	46.9 ± 9.5[b]
Protein	72.4 ± 0.8[a]	67.1 ± 3.1[a]
Ash	79.9 ± 2.6[a]	73.4 ± 11.5[a]
Energy	60.5 ± 1.6[a]	50.9 ± 5.1[b]

The abbreviations in the table stand for: E-CO – Extruded Reference diet; E-NA –Extruded *Nannochloropsis* sp. diet; E-DE –Extruded *Desmodesmus* sp. diet

The data are presented as mean ± SD (n=3)

Different superscript among rows denotes significant differences ($P < 0.05$).

Table 6

Apparent digestibility coefficients (ADCs, %) of dry matter (DM), protein and ash of microalgae for Atlantic salmon fed cold pelleted and extruded diets

	Nannochloropsis sp.		*Desmodesmus* sp.	
	cold pelleted	extruded	cold pelleted	extruded
DM	47.9 ± 5.8[a]	63.1± 1.5[b]	31.8 ± 1.6[a]	46.9 ± 9.5[a]
Protein	72.9 ± 2.3[a]	72.4 ± 0.8[a]	54.1 ± 5.4[a]	67.1 ± 3.1[b]
Ash	35.7± 12.2[a]	79.9 ± 2.6[b]	40.6 ± 14.4[a]	73.4 ± 11.5[b]

The data are presented as mean ± SD (n=3)

Different superscript between rows within microalgae denotes significant differences ($P < 0.05$).