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Extrusion processing improves rainbow trout digestibility of microalgal *Nannochloropsis oculata* co-product biomass for more sustainable aquaculture diets

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ABSTRACT

Aquaculture feeds include fishmeal (FM) and fish oil (FO), derived from wild-caught marine fish such as anchovy and sardines, and pose ecological, food security, and economic drawbacks for sustainable aquaculture, the world's fastest-growing agricultural sector. Protein-rich microalgal co-product is a promising alternative to unsustainably sourced fishmeal in aquaculture diets. Microalgal co-product is a defatted biomass left over after extracting omega-3-rich oil for human nutraceuticals and crude oil for fuels. In this study, we report the first evaluations of nutritive values and digestibility of raw, extrusion- and enzymatic-processed co-product of the marine microalga *Nannochloropsis oculata* (*N. oculata* co-product). Results allowed us to evaluate the feasibility of using processed biomass in aquafeed for rainbow trout (*Oncorhynchus mykiss*), an important model for all salmonid aquaculture. Extrusion processing temperature (90 °C vs 127 °C) influenced the nutritional value of *N. oculata*. Protein and energy levels were significantly higher in co-product from both extrusion temperature treatments than in non-extruded raw co-product. Essential amino acid levels did not differ between extrusion temperatures, except that methionine was significantly lower in coproduct from 127 °C extrusion processing than from 90 °C extrusion processing. The protein level in extrusion processed co-product was not significantly influenced by pre-cooking. We detected the highest digestibility of crude protein, energy, most of the amino acids, and omega 3 polyunsaturated fatty acids (n3 PUFA) in extruded (90 °C) *N. oculata* co-product ingredient. Nutrient digestibility of enzymatic processed *N. oculata* co-product were mostly the same as extrusion processing or lower for certain nutrients. Overall, extrusion processing of *N. oculata* at 90 °C provided the best outcome for digestible protein, and yielded an excellent source of digestible protein, amino acids, and long chain omega-3 profile for rainbow trout and which could be an alternative to replace fishmeal in rainbow trout diets.

1. Introduction

Aquaculture, the fastest growing food sector globally (8 % average production increase/yr. for 1970–2014), now produces half of all fish for human consumption, as global capture fisheries have reached or exceeded their sustainable limits and plateaued at ~96 million tonnes/yr. [1,2]. Aquaculture–also the world's most efficient protein generator–is projected to keep rising to produce 109 million metric tons in 2030 [3,4]. Aquaculture, thus, plays a key role in solving a grand challenge: feeding >9 billion people by 2050 [3,5–7].

The production of industrial aquaculture feeds is likewise expected to increase, with 73.15 million tonnes of compound feeds projected to be used by 2025 [8]. Aquaculture is expanding and it is expected to continue to grow for the foreseeable future and the shortage of fishmeal (FM) and fish oil (FO) for use in aquafeed will cause in a limit on aquaculture production in the future if goals to lessen their use in feeds are not met [9]. Aquaculture feed used 18.3 % of captured fish from the ocean in 2017 for FM and FO [10]. Fed aquaculture needs to reduce dependence on fishmeal (FM) and fish oil (FO) inclusion in feeds for its growth to achieve a sustainability transition. Approximately 16.9 million of the 29 million tonnes of forage fish (such as herrings, sardines, and anchovies) caught globally each year are currently used for

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aquaculture feed [11]. Globally, 50–60 % of FM and 90 % of FO go into aquafeeds [3,12–14].

Aquafeed industry is seeking good ingredients that can complement FM and FO use in aquaculture diet. Alternative microalgal ingredients could be potential to fill the raw material gap. Reducing FM and FO dependence in aquafeeds is critical for carnivorous fish like farmed salmonids (rainbow trout and Atlantic salmon), aquaculture's largest global FM and FO user. Farmed salmonids —primarily Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*)— used approximately 24–30 % of FM and 50–60 % of FO destined for aquafeeds. We focused the research presented here on evaluating readily available processing methods to improve nutrient digestibility of the novel alternative microalgal ingredient, *N. oculata* co-product, by rainbow trout as an important model for all farmed salmonids. High performing microalgal feed can lead to improved protein transfer efficiency and reductions in food waste to the environment, as well as reducing the total volume of food required and the nutrients and greenhouse gas emissions directly associated with feed ingredients and feed production [17–20]. Prices for FM and FO have more than doubled during the 2000s and have been steadily increasing over the last 10 years [10]. Rising costs, food security concerns, and the necessity of reducing the levels of FM and FO in aquafeeds provide strong impetus to develop novel aquafeed ingredients that have high digestibility and comparable nutritional values [15,21,22]. Increasing attention has focused on marine microalgae for salmonids feeds because of their good amino acids and fatty acid profiles [21,23–26].

Recent research showed that marine microalga *Nannochloropsis* sp. has potential for aquaculture feeds as a rich source of EPA as well as other nutrients such as protein, amino acids, and as a good source of minerals [20,23,25–30]. We also found that converting the microalgae industry's large volumes of *Nannochloropsis oculata* co-product produces a cost viable and environmentally beneficial ingredient for tilapia aquafeed [20,31]. There is limited studies available focusing on the effect of the incorporation of *Nannochloropsis* sp. on nutrient digestibility, growth, and feed utilization of trout and Atlantic salmon [23,25,27–29]. There is limited documentation available about the use of *Nannochloropsis* sp. in rainbow trout aquaculture feed. We previously reported that the digestibility of essential amino acids in the whole cells of *Nannochloropsis* sp. in rainbow trout diet was <90 % [25]. Moreover, nutritional feeding experiment conducted with rainbow trout and Atlantic salmon have shown that it is possible to include in the feed a maximum of 10 % of *Nannochloropsis* sp. biomass without negative effects on growth [23,27,32]. The literature, however, lacks data on digestibility of *N. oculata* co-product by rainbow trout, but having these data is the first step in designing a sustainable diet that includes co-product to substitute for fishmeal and fish oil. Quantifying whether microalgal ingredients have good nutritive values and high digestibility for aquaculture species is key to reduce feed costs, minimize negative environmental impacts (including phosphorus and nitrogen eutrophication emissions), and improve the FCR of aquafeeds [20,25,31,33].

Prior research results suggested the need to assess if extrusion or enzymatic processing might improve *N. oculata* co-product nutritive quality and digestibility in fish [20,31,34]. A primary challenge is that cell wall rigidity of microalgae can reduce nutrient digestibility of microalgal ingredients [35]. For example, the rigid cell wall of *Nannochloropsis gaditana* is made up of two layers including an inner cellulose-based layer and an outer algaenan-based layer [36]. The outer algaenan-based layer is very rigid and resistant to enzymes and chemicals, making *Nannochloropsis* cell walls complex and difficult to rupture [37]. These indigestible, complex non starch polysaccharides remain in *Nannochloropsis* co-product, mostly associated with the rigid cell walls leftover after oil extraction from whole cells. Clearly, such complex polysaccharides should be kept at low levels in trout feeds because they inhibit the digestibility of nutrients and energy [28,38]. Thus, further extrusion processing of the microalgal ingredient into concentrates, disruption of cell walls, and may be needed to increase both the protein and the energy digestibility of microalgal co-product ingredients. Also, it is important to evaluate extrusion processing temperature on the nutritional value of microalgal co-product to reduce antinutritional factors, improving digestibility without denaturing nutrient value. In the present study we compared nutritional values between low (90 °C) and high extrusion temperature (127 °C) with or without precooking microalgal co-product.

Promising research is emerging on enzymes (xylanases, glucanases, cellulases) that hydrolyze non-starch polysaccharide (NSP) into products available for bacteria as prebiotics or for the fish as digestible nutrients [39]. Also, exogenous proteases augment endogenous peptidases by increasing protein digestibility and hydrolyzing proteinaceous anti-nutritional factors such as lectins and trypsin inhibitors in terrestrial animals [40–42]. Exogenous enzymes are already widely used in cereals, grain, legumes and oilseed meals diets of fish and terrestrial animals [40,41,43,44]. The digestive tracts of monogastric animals, such as rainbow trout, lack any appreciable NSP enzyme activity [45]. Thus, treating under-utilized co-product with NSP enzymes could enhance digestibility and utilization of nutrients by fish. In the past, we outlined the need to better understand how anti-nutrients in *N oculata* co-product limit inclusion rates in aquafeeds and to identify practical steps to improve nutrient digestibility to achieve higher replacement levels [31]. After extracted lipid from *N. oculata* the leftover co-product seems to elevate these anti-nutrient levels including NSP in *N. oculata* co-product biomass, which resulted lower digestibility and growth of when fed raw co-product diet [31]. Towards this goal, this study investigated whether inclusion of one or more NSP and protease enzymes in *N. oculata* co-product diet enhances nutrient digestibility in rainbow trout.

This study aimed to find an effective way to enhance nutrient digestibility of *N. oculata* co-product to help achieve wide use of this ingredient. Developing tractable and affordable methods to increase digestibility of microalgal ingredients will also improve FCRs and reduce feed costs and nutrient loads in fish culture effluents, while also helping drive algae-based aquafeeds towards cost-competitiveness with conventional feed [20,33]. In this study, we developed a new protein meal by extrusion and enzymatic processing *N. oculata* co-product. Then, we determined the nutrient digestibility in rainbow trout of raw *N. oculata* co-product, enzyme-treated and extrusion processed co-product and test diets to evaluate feasibility of using them in aquafeed for rainbow trout.

2. Materials and methods

2.1. Experimental design and methods of processing of N. oculata co-product

2.1.1. Extrusion processing of N. oculata co-product

We compared four extrusion processing treatments of *N. oculata* co-product (Fig. 1a A): High thermal extrusion without precooking (127 °C, no pre-cooking), Low thermal extrusion without precooking (90 °C, no pre-cooking), High thermal extrusion with precooking (127 °C, with pre-cooking), Low thermal extrusion with precooking (90 °C, with pre-cooking). *N. oculata* co-product was processed at the Kapuscinski-Sarker Lab space in Natural Sciences II (University of California, Santa Cruz CA, USA) using a single-screw extruder (TT-100 tabletop lab scale extruder from Akron Tool and Die, Akron Ohio, USA). The co-product was exposed to an average target temperature in the barrels of either 90 °C or 127 °C, and passed through the extruder for 18 s exposure. In pre-cooking treatments, the co-product was pre-cooked as a dry mash with the same single-screw cooking extruder with an 18 s exposure to 90 °C in the extruder barrel. After extrusion processing in all treatments, the co-product was ground to prepare it for use. For all treatments, the pressure varied at the die head with an average of 1.04 ± 0.18 PSI depending on diet moisture and screw speed. In order to achieve the appropriate barrel retention time (18.3 ± 0.17 s) the screw speed was adjusted according to the consistency of the mash. The average motor RPM speed was 35.5–40.8 % for higher moisture mash (>30 %) and

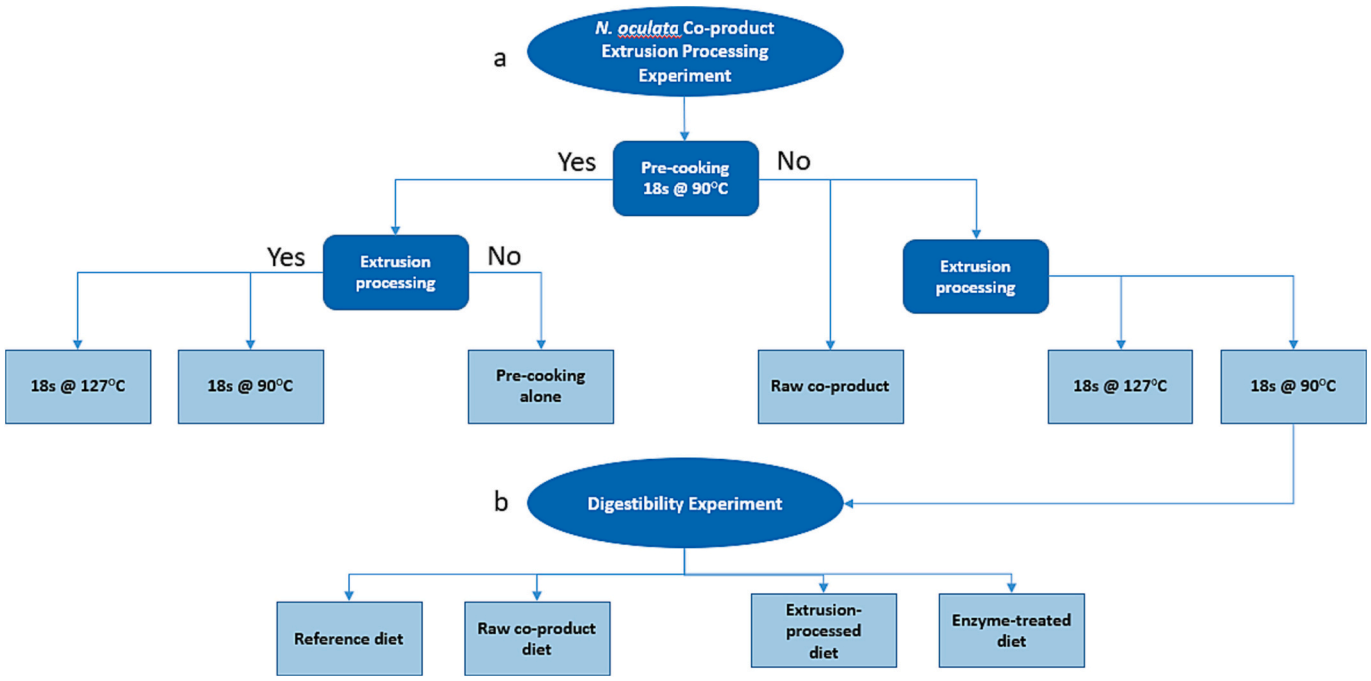

Fig. 1. Experiments and process-flow diagram of the study. The dark blue ovals represent the two experiments conducted in the study (a) and (b). Under experiment (a), the dark blue boxes represent production process of co-product, and the light blue boxes represent treatment groups from the production process: High thermal extrusion without pre-cooking (127 °C, no pre-cooking), Low thermal extrusion without pre-cooking (90 °C, no pre-cooking), High thermal extrusion with pre-cooking (127 °C, with pre-cooking), Low thermal extrusion with pre-cooking (90 °C, with pre-cooking), Pre-cooking alone, and Raw co-product. Under experiment (b), the light blue boxes represent the diets tested in the digestibility experiment. The extrusion processed diet included the low thermal with no pre-cooking extrusion-processed co-product. This co-product was chosen from treatments analyzed in (a) because it showed the best results. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

40.8–50 % for lower moisture mash (<25 %). For each treated and ground material, 3 samples were then collected for chemical analysis. For the work, Qualitas Health, Inc. donated us a *N. oculata* co-product, leftover from the company's large-scale production of human supplements. Our recent digestibility and growth experiments showed the promise of using *N. oculata* whole cells in tilapia [20,25,31]. We also found that Qualitas Health's under-utilized, defatted co-product has consistent quality and higher protein (50 %), methionine (1 %), and lysine (2.7 %) content than its whole cells; a good amount of remaining EPA (28 %); and low ash content (8.6 %) [31]. Thus, in this study pursues a more economically viable path: convert under-utilized protein-rich *N. oculata* co-products of industrial microalgae production, available in large quantities, into value-added aquafeed ingredients.

2.1.2. Enzymatic processing of N. oculata co-product

We investigated one enzymatic processing treatment, informed by prior experiments in our lab (unpublished data). We used a combination of three enzymes (xylanase-glucanase-protease) and determined appropriate doses for rainbow trout [44]. We treated the co-product with a combination of these enzymes at the same doses used in our previous experiment, which gave best results with this combination [44]: a fungal mono-component enzyme as endo-1,4—xylanase (Xylanase RONOZYME® WX L, DSM Nutritional Products, USA) at 208 mg/kg; Glucanase (a fungal, multi-component enzyme comprising endo-1,3(4)–glucanase as the main activity, β-Glucanase, RONOZYME® VP (L), DSM Nutritional Products, USA) at 67 mg/kg; and Protease (serine protease enzyme; DSM Nutritional Products, USA) at 228 mg/kg. Enzymes are stable under the low acidity pepsin conditions in the gastric environment. These products are also designed for pelleted feeds and retain good stability in the upper ranges of 180-200F for 30 s in the conditioner (personal communication, DSM).

2.2. Determination of N. oculata co-products digestibility

2.2.1. Dietary experimental design and methods

The four treatments in the digestibility experiment compared the digestibility of raw, enzyme-treated and one type of extrusion processed *N. oculata* (90 °C, no pre-cooking), as well as a reference diet alone (Table 1b). Results from the extrusion processing experiment guided selection of the 90 °C processed co-product for this digestibility experiment because 127 °C processed co-product showed depressed levels of methionine. Treatments were evaluated by determining the apparent digestibility coefficients (ADCs) of protein, lipid, energy, and amino acids, in the microalgae and test diets. We first prepared a nutritionally complete, high-quality reference diet (Table 1) and then combined it with microalgal biomass at a 7:3 ratio (as is basis) to produce three test diets (raw co-product, extrusion processed co-product meal, enzymatic processed co-product) following the standard apparent digestibility protocol [25,31,33,46,47]. We then included an indigestible marker, yttrium oxide, from Thermo Scientific, Waltham MA, USA, in the basal diet at 1.0 % as a digestion indicator [48]. We mixed the micro-ingredients and then slowly added them to the macro-ingredients to ensure a homogeneous mixture. We thoroughly mixed and steam-pelleted the ingredients using a California Pellet Mill, dried the pellets in a forced-air oven (22 °C, 24 h), sieved them, and stored at −20 °C. The proximate composition, energy, and amino acid profiles of the three test ingredients (microalgae) and three test diets used in the digestibility experiment were determined as described in Section 2.2.3 (results provided in Tables 2 and S1). Chemical composition of fishmeal ingredient used in this experiment for the digestibility experiment provided in Table S2.

Table 1
Ingredient composition of the reference diet and microalgal co-product test diets for the digestibility experiment.

Ingredient (g/kg)	Diet			
	Reference	70 % Ref + 30 % *raw N. oculata*	70 % Ref + 30 % extruded *N. oculata*	70 % Ref + 30 % enzyme-treated *N. oculata*
Fish meal	300	210	210	210
Corn gluten meal	170	119	119	119
Wheat meal	174	121.8	121.8	121.8
Soybean meal	129	90.3	90.3	90.3
Wheat gluten	100	70	70	70
Vitamin/mineral premix	5	3.5	3.5	3.5
Fish oil	112	78.4	78.4	78.4
Yttrium oxide	10	7	7	7
N. oculata raw co-product	0	0	0	300
N. oculata extruded co-product	0	300	0	0
N. oculata enzyme-treated co-product	0	0	300	0

2.2.2. Fish, feeding and feces collection

Prior to the digestibility trial, we randomly allocated juvenile rainbow trout with an average weight of 25.0 ± 0.9 g in 757 L rectangular tanks (16 fish/tank, four tanks/dietary treatment, total 256 trout for 16 tanks) of fresh water recirculating aquaculture systems (RAS) at the University of California, Santa Cruz CA, USA. Fish were fed the reference diet for 7-days to acclimate them to lab-made pellets before we began feeding test diets. During the trial we employed appropriate restricted pair feeding to supply the same quantity of dietary nutrients (feed) to all groups [25,33,49,50]. Reference and test diets were administered two times daily between 0930 and 1700 h. We monitored dissolved oxygen, dissolved oxygen saturation, temperature, and pH daily using a handheld YSI 1020Pro multiparameter meter, and ammonia, nitrite, nitrate, and alkalinity weekly using a benchtop YSI 9500 spectrophotometer. Conditions were maintained to within limits recommended for rainbow trout. Overall water quality conditions were

Table 2
Proximate, energy, amino acid and fatty acid profiles of the reference and three test diets (enzyme, raw and extrusion processed at 90 °C) for the digestibility experiment.

Nutrient	Diet			
	Reference	Raw	Processed	Enzyme
Proximate composition				
Moisture	11.61 ± 0.08	11.06 ± 0.1	8.25 ± 0.05	14.46 ± 0.05
Protein	40.19 ± 0.09	44.26 ± 0.06	45.75 ± 0.14	43.25 ± 0.09
Fat	14.56 ± 0.19	11.73 ± 0.09	11.96 ± 0.07	10.61 ± 0.11
Fiber	1.51 ± 0.18	1.69 ± 0.2	1.64 ± 0.1	1.4 ± 0.13
Ash	11.22 ± 0.25	11.43 ± 0.2	11.47 ± 0.1	10.86 ± 0.17
Carbohydrates	22.42 ± 0.09	21.52 ± 0.31	22.57 ± 0.27	20.82 ± 0.23
Calories	3376.33 ± 25.18	3240.67 ± 9.84	3350.67 ± 3.84	3095.33 ± 14.44
Essential amino acids				
Methionine	0.52 ± 0.3	0.7 ± 0.4	0.75 ± 0.43	0.61 ± 0.35
Cystine	0.36 ± 0.21	0.39 ± 0.22	0.4 ± 0.23	0.35 ± 0.2
Lysine	1.6 ± 0.92	2.12 ± 1.22	2.18 ± 1.26	2.02 ± 1.16
Phenylalanine	1.79 ± 1.03	2.07 ± 1.2	2.12 ± 1.22	1.99 ± 1.15
Leucine	3.21 ± 1.86	3.69 ± 2.13	3.77 ± 2.18	3.57 ± 2.06
Isoleucine	1.37 ± 0.79	1.72 ± 0.99	1.59 ± 0.92	1.63 ± 0.94
Threonine	1.35 ± 0.78	1.71 ± 0.99	1.73 ± 1	1.56 ± 0.9
Valine	1.62 ± 0.94	2.14 ± 1.24	2.02 ± 1.16	1.99 ± 1.15
Histidine	0.86 ± 0.49	0.92 ± 0.53	0.96 ± 0.55	0.88 ± 0.51
Arginine	2.37 ± 1.37	2.65 ± 1.53	2.7 ± 1.56	2.55 ± 1.47
Glycine	3.58 ± 2.06	3.43 ± 1.98	3.58 ± 2.07	3.23 ± 1.86
Aspartic	2.95 ± 1.71	3.54 ± 2.04	3.73 ± 2.15	3.39 ± 1.96
Serine	1.93 ± 1.11	2.25 ± 1.3	2.4 ± 1.39	2.2 ± 1.27
Glutamic	8.52 ± 4.92	8.16 ± 4.71	8.66 ± 5	7.91 ± 4.56
Proline	3.69 ± 2.13	3.24 ± 1.87	3.57 ± 2.06	3.28 ± 1.89
Hydroxyproline	0.88 ± 0.51	0.64 ± 0.37	0.64 ± 0.37	0.51 ± 0.29
Alanine	2.5 ± 1.44	2.86 ± 1.65	3.01 ± 1.74	2.69 ± 1.55
Tyrosine	1.28 ± 0.74	1.49 ± 0.86	1.54 ± 0.89	1.45 ± 0.84
Tryptophan	0.27 ± 0.16	0.42 ± 0.24	0.43 ± 0.25	0.38 ± 0.22
Fatty acid fractions (% of total fatty acids)				
Total saturated	24.06 ± 0.11	20 ± 0.07	19.83 ± 0.22	18.16 ± 0.07
Total monounsaturated	63.24 ± 0.3	50.27 ± 0.11	50.41 ± 0.42	45.19 ± 0.19
Total polyunsaturated	37.66 ± 0.07	30.11 ± 0.11	29.94 ± 0.58	27.24 ± 0.14
Total ω3 LCPUFA	18.96 ± 0.06	15.14 ± 0.08	14.91 ± 0.3	13.34 ± 0.08
Total ω6 LCPUFA	1.22 ± 0.03	1.3 ± 0.01	1.27 ± 0.04	1.19 ± 0.02
ω3/ω6 PUFA	1.49 ± 0.01	1.46 ± 0.01	1.41 ± 0.01	1.36 ± 0.01
ω3/ω6 LCPUFA	15.55 ± 0.36	11.67 ± 0.1	11.74 ± 0.16	11.23 ± 0.15
20:4ω6/20:5ω3	0.07 ± 0	0.09 ± 0	0.09 ± 0	0.09 ± 0

PUFA, polyunsaturated fatty acids; LCPUFA, long chain polyunsaturated fatty acids.

as follows; average water temperature 15.4 °C, pH 8.6, dissolved oxygen 8.7 mg/L, total ammonia nitrogen 0.2 mg/L, nitrite nitrogen 0.1 mg/L, and nitrate nitrogen 26.8 mg/L.

We collected fish fecal samples daily, once before the morning feeding and once before the afternoon feeding from a radial flow settler (installed between the culture tank outflow and sump tank inflow) designed to collect intact fecal matter at its bottom. Uneaten feed pellets and residues were siphoned out of the radial flow settler after each feeding to prevent contamination. We gently withdrew intact solid fecal pellets from a separate collection basin using pipettes and placed samples in a 50 mL Falcon tube (BD Falcon™). We allowed fecal samples to settle in the falcon tube to ensure the solid fecal samples fully settled in the bottom of the tube and then removed the supernatant water from the top using pipette, and then froze them at −20 °C. We pooled fecal samples from every collection from each specific tank during the experiment. At the end of the experiment, we lyophilized, finely ground, and stored samples at −20 °C for proximate and amino acid analysis.

2.2.3. Chemical analysis and calculations

Chemical analysis and digestibility calculations were based on standard methods described in our recent articles [25]. Three types of samples (microalgal co-products, diets and feces) were analyzed for proximate composition, crude fiber, gross energy, and amino acid profiles. We sent these three types of samples to New Jersey Feed Laboratory, Inc. (Ewing, NJ, USA) for the following types of analysis [51]: moisture (Association of Official Analytical Chemists, AOAC, 1995, no 930.15), crude protein (AOAC 990.03), lipid (AOAC 920.39), ash (AOAC 942.05), crude fiber (AOAC 1978.10), energy (automated oxygen bomb calorimeter), amino acids (high-performance liquid chromatography, HPLC analysis, via AOAC methods 994.12, 985.28, 988.15, and 994.12) and fatty acids (fatty acids methyl esters, FAME analysis, via AOAC method 963.22).

We calculated apparent digestibility coefficients (ADC) for macronutrients, amino acids, fatty acids and energy of the test and the reference diets using the standard method as described by [46,51]:

$$ADC = 1 - (F/D \times D_i/F_i)$$

where: D = % nutrient (or kJ g^{-1} gross energy) of diet; F = % nutrient (or kJ g^{-1} gross energy) of feces; D_i = % digestion indicator (yttrium oxide) of diet; F_i = % digestion indicator (yttrium oxide) of feces.

We calculated the apparent digestibility of the microalgae as test ingredients using the equation by [45,52]:

$$ADC_{test}\ \text{ingredient} = ADC_{\text{test diet}} + \left(\left(ADC_{\text{test diet}} - ADC_{\text{ref.diet}}\right) \times \left(0.7 \times D_{ref}/0.3 \times D_{\text{ingredient}}\right)\right)$$

where: D_{ref} is the percentage of nutrient or kcal/g gross energy in the reference diet, and $D_{ingredient}$ is the percentage of nutrient or kcal/g gross energy in the ingredient.

3. Statistical analysis

For the extrusion-processing experiment, we conducted a two-way analysis of variance (ANOVA) of macronutrients and of essential amino acids in co-product from the 2 by 3 factorial treatment design. For the digestibility experiment, we conducted one-way analysis of variance (ANOVA) of apparent digestibility coefficients for macronutrients, fatty acids and amino acids in the reference and test diets, as well as for test ingredients. For all analyses, when ANOVA results showed $p < 0.05$, indicating significant differences, we compared the treatment means using Tukey's test of multiple comparisons with 95 % level of significance. Data were expressed as the mean with ±SE of four replicates. We carried out statistical analyses using the IBM Statistical Package for the Social Sciences (SPSS) program for Windows (v. 22.0, USA).

4. Results

4.1. Extrusion processing

Extrusion processing had numerous effects on the proximate compositions and energy values of the co-product ingredient (Table 3). Most parameters were higher in both extruded temperature treatments compared to the non-extruded treatment. Interaction effects between extrusion and cooking treatments were not detected among the macronutrient values except for fat. Dry matter ($p < 0.001$), protein levels ($p < 0.001$), and energy levels ($p < 0.001$) significantly increased by extrusion processing at both temperatures compared to non-extruded raw co-product but were not affected by the cooking ($p > 0.05$). Fat content was significantly lower in both extrusion processing temperatures than non-extruded raw co-product (<0.001). Fat levels also showed significant cooking and interaction effects and were higher in cooking than non-cooking ($p < 0.001$; Extrusion * cooking, $p = 0.01$). Fiber content was not affected either by extrusion ($p = 0.09$) or cooking ($p = 0.9$). Carbohydrate ($p < 0.001$) and ash ($p < 0.001$) content were higher in both extrusion processing temperatures than non-extruded raw co-product. Additionally, carbohydrate content was higher after cooking treatment compared to raw co-product ($p = 0.004$).

With the exception of methionine ($p = 0.01$), levels of all essential amino acids ($p > 0.05$) did not differ between extrusion treatments (Table 4). Methionine was significantly affected by extrusion. Methionine levels were significantly lower in the 127 °C extrusion processed treatment than in both the 90 °C extrusion processed and raw co-product. We detected approximately 0.06 % methionine loss due to high temperature extrusion processing at high temperature (127 °C).

The cooking treatment affected levels of several essential amino acids (Table 4). We detected methionine to be higher in the cooking treatment compared to the non-cooked treatment (0.01). Arginine ($p < 0.001$) and histidine ($p < 0.001$) levels were significantly lower in the cooking than the non-cooking raw co-product.

Table 3
Effect of extrusion processing and pre-cooking on macro nutrients of *N. oculata* co-product (%, mean ± standard error). Values are mean and standard error of three replicate samples of treated co-product. Mean values not sharing a superscript letter in the same column differ significantly ($P < 0.05$) from Tukey's HSD test.

	Dry matter	Protein	Fat	Carb[1]	Fib[1]	Ash	Energy
Extrusion							
No (raw)	85.55 ± 0.28^b	52.54 ± 0.12^b	2.24 ± 0.1^a	17.25 ± 0.27^b	0.86 ± 0.09	13.52 ± 0.0^b	2602.67 ± 5.33^b
90	90.5 ± 0.63^a	55.78 ± 0.38^a	2.0 ± 0.16^b	18.51 ± 0.19^a	0.69 ± 0.05	14.2 ± 0.1^a	2744.67 ± 21.9^a
127	90.34 ± 0.66^a	55.59 ± 0.41^a	1.8 ± 0.06^b	18.79 ± 0.12^a	1.04 ± 0.17	14.16 ± 0.12^a	2720 ± 22.69^a
Pre-cooking							
Yes	88.93 ± 0.84	54.69 ± 0.56	1.82 ± 0.07^b	18.48 ± 0.2^a	0.86 ± 0.08	13.94 ± 0.14	2684.11 ± 25.12
No (raw)	88.66 ± 0.99	54.58 ± 0.61	2.21 ± 0.09^a	17.89 ± 0.32^b	0.87 ± 0.12	13.98 ± 0.13	2694.11 ± 27.35
p value							
Extrusion	<0.001	<0.001	<0.001	<0.001	0.09	<0.001	<0.001
Pre-cooking	0.7	0.79	<0.001	0.004	0.9	0.7	0.66
Extrusion * cooking	0.7	0.2	0.01	0.12	0.07	0.77	0.61

[1] Abbreviations refer to carbohydrate (Carb) and fiber (Fib).

Table 4
Effect of extrusion processing and pre-cooking on essential amino acids of *N. oculata* co-product (%, mean ± standard error). Values are mean and standard error of three replicate samples of treated co-product. Mean values not sharing a superscript letter in the same column differ significantly ($P < 0.05$) from Tukey's HSD test.

	Methionine	Lysine	Phenylalanine	Leucine	Isoleucine	Threonine	Valine	Histidine	Arginine	Threonine
Extrusion										
No	0.94 ± 0.03[a]	3.02 ± 0.16	2.59 ± 0.14	4.53 ± 0.26	2 ± 0.16	2.68 ± 0.16	2.77 ± 0.2	0.89 ± 0.09	3.13 ± 0.22	0.7 ± 0.0
90	0.99 ± 0.02[a]	2.96 ± 0.03	2.54 ± 0.03	4.51 ± 0.05	2.06 ± 0.04	2.59 ± 0.05	2.68 ± 0.05	0.84 ± 0.04	2.96 ± 0.1	0.7 ± 0.0
127	0.88 ± 0.03[b]	2.88 ± 0.04	2.48 ± 0.03	4.41 ± 0.07	1.94 ± 0.06	2.53 ± 0.04	2.55 ± 0.06	0.79 ± 0.03	2.88 ± 0.08	0.7 ± 0.0
Pre-cooking										
Yes	0.97 ± 0.02[a]	2.87 ± 0.03	2.46 ± 0.02	4.37 ± 0.04	1.97 ± 0.02	2.49 ± 0.02	2.56 ± 0.03	0.75 ± 0.01[b]	2.78 ± 0.06[b]	0.7 ± 0.0
No	0.9 ± 0.02[b]	2.61 ± 0.09	2.61 ± 0.09	4.6 ± 0.16	2.03 ± 0.11	2.71 ± 0.1	2.83 ± 0.16	0.92 ± 0.06[a]	3.2 ± 0.12[a]	0.7 ± 0.0
p value										
Extrusion	0.01	0.58	0.59	0.85	0.77	0.52	0.35	0.3	0.36	0.61
Pre-cooking	0.01	0.15	0.1	0.23	0.64	0.06	0.11	<0.001	<0.001	0.06
Extrusion * cooking	0.35	0.96	0.27	0.66	0.87	0.5	0.27	0.19	0.49	0.78

4.2. Digestibility of macronutrients, energy, amino acids, and long-chain omega 3 fatty acids in test N. oculata ingredients

Regarding the calculated digestibility of microalgal ingredients, we did not detect significant differences between raw, enzyme-treated, and extruded co-product of *N. oculata* for the ADC of crude protein, ash, and energy (Table 5).

The ADCs of essential amino acids in test *N. oculata* co-product ingredients are summarized in Table 5. With the exception of methionine ($p = 0.04$), the ADCs of individual essential amino acids were not significantly different between raw, enzyme-treated and extruded co-product ($p > 0.05$). The highest ADC value of methionine was detected in the extruded co-product (86.3 %) compared to the raw (83.1 %) and enzyme-treated coproduct (77.2 %). The highest ADC value of lysine and phenylalanine was found in extruded co-product (>89.0 %) compared with raw and enzyme-treated co-product, though differences were not significant.

The ADC of EPA was significantly higher in the extruded *N. oculata* co-product ingredient compared with the raw and not different from the enzyme-treated ($p = 0.04$). We did not detect DHA in the *N. oculata* co-product (Table 5). Finally, we found better ADCs of total n3 PUFA and n3 LC PUFA, in extruded and enzyme-treated co-product ingredient compared with the raw *N. oculata* coproduct ingredient ($p = 0.03$).

4.3. Digestibility of macronutrients, energy, amino acids, and long-chain omega-3 fatty acids in diets

We determined the ADCs of macronutrients, energy, and amino acids in the test diets which are summarized in Table 6.

The ADCs of macronutrients (protein and lipid) and energy contents in the Ref diet were significantly higher than in all *N. oculata* co-product test diets ($p < 0.001$). We did not find significant differences between test diets for the ADC of crude protein. We compared the protein digestibility results from both current and previously published studies (Table S3).

The ADCs of macronutrients and energy levels were not significantly different between the fish-fed raw *N. oculata* coproduct diet, enzyme-treated diet and extrusion-processed diet. We did not find significant differences between diets for the ADC of ash ($p = 0.4$).

Table 6 reports the ADC of essential amino acids for test diets. The ADCs of all essential amino acids in the test diets were highly digestible. Methionine and tryptophan also did not differ between the reference diet, the raw and extruded co-product diet. ADCs of lysine and cystine were not affected by the experimental diets ($p > 0.05$).

The ADCs of long-chain fatty acids, 20:5n3 EPA, n3 PUFA, and n3 LC PUFA in the co-product diets and Ref diets were highly digestible overall (Table 6). We detected significantly lower 20:5n3 EPA ($p < 0.001$), n3 PUFA ($p < 0.008$), and n3 LC PUFA ($p < 0.009$) in *N. oculata* raw diets than Ref and extruded co-product diets. The ADC of 20:5n3 EPA was not significantly different between fish fed the extruded *N. oculata* diet (90.0 %) and reference diet (93.3 %). Extruded diet showed better digestibility of EPA than the enzyme-treated diet (87.3 %) and the raw diet (83.9 %). We did not detect significant differences between extruded and enzyme-treated *N. oculata* co-product diets and reference diet for the ADC of n3 PUFA, and n3 LC PUFA.

5. Discussion

Results from this study suggest that extrusion pre-processing of *N. oculata* co-product, biomass leftover after oil extraction from whole cells, rendered intracellular nutrients more accessible for digestion by rainbow trout. The study demonstrates that with rainbow trout there is either similar or less influence of enzymatic processing than extrusion processing on the macronutrient, essential amino acids, and fatty acids digestibility of *N. oculata* co-product.

5.1. Extrusion processes and biochemical composition of N. oculata co-product ingredient

Extrusion processing exposes feed materials to high temperature, high pressure and strong shear force over a short period of time, which can inactivate some antinutritional factors and thus improve nutritional value and digestibility in ingredients. Despite recognition that extrusion processing affects the nutritional value of traditional feed ingredients (soy and corn), data on how extrusion processing affects microalgal sources are very limited [48,53,54]. Extrusion processing of crop ingredients (soybean meal, soy protein concentrate) has shown enhanced nutrient digestibility and therefore growth performance of the fish [48,55,56]. Microalgae have recently attracted significant interest as a sustainable source of nutrients for the feed industry but are being held back because microalgae processing technology has not matured yet. Microalgae has recently attracted a significant interest as a sustainable source of nutrients for the feed industry but is being held back because microalgae processing technology has not matured yet. Consequently, recent papers have reported the level of microalgae inclusion in aquafeeds (10–33 %) to avoid negative effects on nutrient digestibility and to obtain a good feed conversion ratio (FCR) [20,37,57–59].

We observed, in this study, significantly higher nutritional values for most measured parameters between raw, unprocessed *N. oculata* co-product and co-product that were processed using either a high (127 °C) or low (90 °C) extrusion temperature. Treatment with either extrusion temperature significantly increased protein and energy levels

Table 5
Apparent digestibility coefficients (%, mean ± standard error, n = 4) of nutrients in test ingredients (enzyme-treated, raw, extrusion processed at 90 °C) for rainbow trout.

Nutrient	*N. oculata* ingredients[#]				
	Raw	Extrusion processed	Enzyme-treated	*P*-value	F-value
Proximate composition					
Crude protein	81.39 ± 0.59	83.47 ± 3.28	82.34 ± 0.92	0.76	0.27
Lipid*	−165.58 ± 1.33	−95.68 ± 51.26	−64.36 ± 28.56		
Ash	53.12 ± 1.35	43.36 ± 9.3	55.47 ± 2.51	0.31	1.3
Energy	71.93 ± 1.15	76.47 ± 2.66	74.95 ± 0.51	0.36	1.12
Essential amino acids					
Methionine	83.12 ± 0.95^{ab}	86.32 ± 3.24^{a}	77.19 ± 1.69^{b}	0.04	4.51
Lysine	88.55 ± 0.67	89.12 ± 3.38	87.63 ± 0.8	0.87	0.13
Phenylalanine	85.42 ± 0.99	89.06 ± 1.77	84.59 ± 1.03	0.08	3.27
Leucine	85.79 ± 0.94	87.74 ± 2.33	85.28 ± 1.12	0.53	0.66
Isoleucine	85.82 ± 1.55	86.02 ± 2.53	84.94 ± 1.12	0.90	0.09
Threonine	83.51 ± 0.89	85.5 ± 2.68	83 ± 1.74	0.63	0.47
Valine	83.31 ± 0.72	83.92 ± 2.81	81.97 ± 1.16	0.74	0.30
Histidine	84.39 ± 2.14	83.09 ± 2.94	86.83 ± 1.2	0.50	0.73
Arginine	89.48 ± 0.6	90.2 ± 2.24	89.4 ± 0.82	0.90	0.09
Tryptophan	81.86 ± 1.42	84.99 ± 4.3	77.75 ± 2.3	0.26	1.52
Cystine	81.07 ± 3.02	87.73 ± 6.01	77.26 ± 1.44	0.22	1.77
Fatty acid fractions (% of total fatty acids)*					
**Total n3 PUFA	21.83 ± 4.93^{b}	65.33 ± 13.18^{a}	54 ± 9.93^{a}	0.03	5.0
20:5n3 EPA	63.54 ± 2.82^{b}	78.95 ± 4.79^{a}	74.3 ± 2.97^{ab}	0.04	4.71
Total n3 LCPUFA	28.02 ± 4.7^{b}	67.45 ± 12.11^{a}	57.15 ± 9.06^{a}	0.03	5.14

Docosahexaenoic acid; ND, not detectable (<1 % of total fatty acids).

[#] Mean values not sharing a common letter in the same row differ significantly as determined by Tukey's HSD test, P < 0.05; both letters appearing together means no difference.

[*] Estimated apparent digestibility values of the test ingredients can occur negative or higher than 100 % in experiment due to experimental error or due to endogenous loss and the excretion via the intestine. In that case, digestibility should round either 0 (for negative) or 100 (for >100) (Glencross et al. 2007).

[**] n3PUFA, omega 3 polyunsaturated fatty acids; EPA, eicosapentaenoic acid; n3LCPUFA, omega 3 long chain polyunsaturated fatty acids.

of *N. oculata* compared to raw co-product. Methionine levels were higher in biomass extruded at the low temperature but were lower in biomass extruded at the high temperature, compared with the raw, no statistical differences were observed. This suggested overheating at 127 °C had damaged methionine levels and could reduce nutritional value of co-product. Prior studies reported that overheating of fish meal protein during drying increased the crosslinking between proteins and reduced digestibility of nearly all amino acids, especially methionine and cysteine [60–62]. Both methionine and cysteine are sulfur amino acids, but the sulfur atom of cysteine that is present in the side chain is involved in the formation of reactive sulfhydryl group, and cysteine can be easily oxidized to form cysteine dimer containing disulfide bridge [63]. Results from this study indicated that maintaining extrusion temperature at approximately 90 °C for a relatively short period of time (18 s) avoided potential heat-induced methionine damage in the *N. oculata* co-product.

Precooking results showed that it doesn't improve overall co-product nutritional quality. Specifically, pre-cooking alone, regardless of extrusion temperature, was not sufficient to change the macro nutrient and energy levels in co-product except for lipid value. Extrusion processing resulted lower level of lipid content in *N. oculata* co-product because the lipid might be released from cells due to the high thermal extrusion but it reduces might slip within the extruder barrel. Also, another reason for the lower lipid level might be due to the formation of complexes with amylose or protein [64]. Although there was a slight (significantly) improvement of methionine level in precooked *N. oculata* co-product, the histidine and arginine levels were decreased. It might be the fact that the methionine level was merely relatively higher in the cooked co-products due to the loss of other amino acids like arginine and histidine. Thus, pre-cooking should be avoided in microalgal co-product processing to avoid heat-induced damage most of the amino acids. An interaction of extruder temperature by precooking on almost all nutritional values was not observed in this study with the exception of lipid value. Overall, extrusion processing without precooking provides the best overall improvement in co-product nutritional quality.

5.2. Digestibility differences between reference, extrusion processed and enzyme-treated *N. oculata* co-product diets and ingredients

The results of our digestibility experiment suggest that overall *N. oculata* co-product is an excellent source of digestible protein, amino acids, 20:5n3 EPA, omega 3 (n3) PUFA and n3 LC PUFA for rainbow trout, and showed enhance digestibility via extrusion processing. The results suggest extrusion processed *N. oculata* co product could be used an alternative to replace fishmeal in rainbow trout diets. We detected that the crude protein digestibility of the co-product test diets (raw, extrusion processed and enzyme-treated co-product) was close to 90 %. Although we did not find significant differences between test diets for the ADC of crude protein (ranged from 89.1 to 89.8 %), the raw co-product had numerically lower value (89.1 %) than extruded (89.8 %) and enzyme-treated co-product (89.3 %). We also detected the lowest ADC value of energy in the raw *N. oculata* co-product diets (73.0 %) compared with the Ref (82.5 %), enzyme-treated co-product diet (76.2 %) and the extruded co-product diet (75.4 %), but there was no significant difference between the co-product test diets. We detected that the crude protein digestibility of the co-product test diets (raw, extrusion processed and enzyme-treated co-product) was close to 90 %. The observed ADC of crude protein and energy in the *N. oculata* co-product diet was higher than the protein (85.0 %) and energy (75.2 %) digestibility of *N. oculata* whole cells diet reported in a prior study [25]. The ADC of protein and energy in *N. oculata* diets was consistent with protein digestibility of *Nannochloropsis* sp. raw co-product fed to Atlantic salmon [28].

In terms of the calculated digestibility of the *N. oculata* as test ingredients, we also detected the lowest ADC of crude protein and energy (with no statistical differences) in the raw co-product (81.4 % and 71.9 %) compared to the extruded (83.5 and 76.47 %) and the enzyme-treated co-product (82.3 % and 74.9 %). The values were higher than those for protein (69.3 %) and energy (62.1 %) of whole cells of *Nannochloropsis* sp. fed to rainbow trout [25]. The digestibility of protein in extruded *N. oculata* was consistent with the in vitro protein digestibility

Table 6
Apparent digestibility coefficients (%, mean ± error, $n = 4$) of nutrients in test diets (Reference, enzyme, unprocessed, extrusion processed at 90 °C) for rainbow trout.

Nutrient				Diet digestibility[#]		
	Reference	Raw	Extrusion processed	Enzyme-treated	P-value	F-value
Proximate composition						
Protein	93.8 ± 0.4[a]	89.1 ± 0.3[b]	89.8 ± 1.1[b]	89.3 ± 0.4[b]	<0.001	11.71
Lipid	86.2 ± 1.6[a]	67.1 ± 1.7[b]	73.0 ± 3.5[b]	75.3 ± 1.2[b]	<0.001	13.47
Ash	60.1 ± 1.7	56.9 ± 0.4	52.4 ± 3.6	57.9 ± 1.8	0.14	2.20
Energy	82.5 ± 1.0[a]	73.1 ± 0.9[b]	75.4 ± 2.5[b]	76.2 ± 0.8[b]	0.004	7.82
Essential amino acids						
Methionine	91.1 ± 0.1[a]	87.6 ± 0.3[ab]	90.1 ± 0.5[a]	84.5 ± 0.3[b]	<0.001	8.95
Lysine	92.2 ± 0.2	90.4 ± 0.2	92.1 ± 0.5	89.9 ± 0.2	0.27	0.14
Phenylalanine	94.5 ± 0.1[a]	90.7 ± 0.2[b]	92.2 ± 0.4[b]	90.4 ± 0.2[b]	<0.001	11.02
Leucine	94.7 ± 0.1[a]	91.1 ± 0.2[b]	92.6 ± 0.4[b]	90.8 ± 0.2[b]	0.001	10.69
Isoleucine	93.8 ± 0.1[a]	90.4 ± 0.3[b]	91.3 ± 0.5[b]	89.9 ± 0.2[b]	0.005	7.12
Threonine	92.4 ± 0.2[a]	88.1 ± 0.3[b]	90.0 ± 0.6[b]	87.7 ± 0.4[b]	0.004	7.52
Valine	92.6 ± 0.2[a]	88.2 ± 0.2[b]	89.6 ± 0.5[b]	87.6 ± 0.3[b]	0.001	10.07
Histidine	94.4 ± 0.3[a]	91.6 ± 0.1[b]	91.5 ± 0.5[b]	91.6 ± 0.2[b]	0.01	4.87
Arginine	94.6 ± 0.2[a]	92.6 ± 0.2[b]	93.5 ± 0.4[a]	92.6 ± 0.1[b]	0.02	4.66
Tryptophan	93.7 ± 0.1[a]	87.7 ± 0.5[ab]	91.0 ± 0.9[a]	85.6 ± 0.7[b]	0.004	7.86
Cystine	91.6 ± 0.2	88.2 ± 0.6	91.8 ± 0.8	86.6 ± 0.5	0.059	3.27
Fatty acid fractions (% of total fatty acids)[*]						
Total n3 PUFA	90.3 ± 0.8[a]	81.0 ± 1.1[b]	88.3 ± 0.8[a]	85.6 ± 0.1[ab]	0.008	6.38
20:5n3 EPA	93.3 ± 0.6[a]	83.9 ± 0.9[b]	89.9 ± 0.7[a]	87.3 ± 0.1[ab]	0.001	9.96
Total n3 LCPUFA	89.9 ± 0.9[a]	80.3 ± 1.2[b]	87.9 ± 0.8[a]	85.1 ± 0.1[ab]	0.009	6.22

[#] Mean values not sharing a common letter in the same row differ significantly as determined by Tukey's HSD test, $P < 0.05$; both letters appearing together means no difference.

[*] n3PUFA, omega 3 polyunsaturated fatty acids; EPA, eicosapentaenoic acid; n3LCPUFA, omega 3 long chain polyunsaturated fatty acids.

of *N. granulata* [34] (Table S3). Previous research also showed that extrusion increased the digestibility of energy in soybean meal from 79 % to 82 % in vivo in rainbow trout (*Oncorhynchus mykiss*) [65]. In this study, we did not determine soluble fiber content in extruded co-product but previously it has been indicated that increasing the soluble fiber content might help to improve fiber digestibility and thus increase digestible energy [48]. Although prior research showed that compared to a pelleted diet, an extruded diet using terrestrial ingredients improved feed utilization via improving the digestibility of starch [53,66], energy [53], and protein [67,68], need further research on how soluble fiber could be an increase in the extruded microalgal diets which could ultimately enhance the nutrient digestibility and feed utilization.

The ADCs of all essential amino acids in the test diets were high overall (>90 %). The three essential amino acids methionine, arginine, and tryptophan digestibility did not differ between reference and extruded diet. Also, the raw co-product did not differ, at least for methionine and tryptophan. In terms of the calculated digestibility of the *N. oculata* as test ingredients, with the exception of methionine, the ADCs of all individual essential amino acids were not significantly different between raw, enzyme-treated and extruded co-product. Previous research reported that overheating fish meal during drying reduced digestibility of nearly all amino acids [61]. The highest digestibility of methionine was detected in extruded co-product but the values were only different between the extruded and enzyme-treated. Our prior study, we found the digestibility value of methionine (69.8 %) was lower in whole cells of *Nannochloropsis* sp. fed to rainbow trout [25]. Previously it has been reported the effect of temperature on changing the sulfhydryl groups, cysteine-methionine, how temperature could impact methionine digestibility in rainbow trout feed [60]. The highest ADC value of lysine and phenylalanine was found in extruded co-product (>89.0 %) compared with raw and enzyme-treated co-product. Similarly, our previous study detected lower digestibility of lysine (72.6 %) and depressed ADCs of essential amino acids (59–75 %) in whole cells of *Nannochloropsis* sp. [25] compared with results for extrusion processed *N. oculata* co-product in the current study.

The 20:5n3 EPA EPA-rich *N. oculata* showed a high ADC for total omega 3 fatty acids both in test diets and individual ingredients. The ADC of 20:5n3 EPA was not significantly different between fish fed the extruded *N. oculata* diet (89.9 %) and reference diet (93.3 %). And the extruded *N. oculata* diet showed better digestibility of 20:5n3 EPA than the raw *N. oculata* diet (83.9 %), but significant difference was not detected between extruded and enzyme-treated diet (87.3 %). In terms of calculated digestibility of ingredients, the ADC of EPA was significantly better in the extruded *N. oculata* co-product ingredient (78.9 %) than in the raw (63.5 %) and enzyme-treated co-product (74.3 %). We found significantly higher ADCs of total n3 PUFA and n3 LC PUFA, in extruded co-product ingredient than raw and enzyme-treated *N. oculata* co-product ingredients. Test ingredient digestibility results for omega fatty acids including EPA suggest that *N. oculata* co-product would be a good candidate for EPA supplementation in trout diet formulation. We detected high digestibility for 20:5 n-3 EPA for *N. oculata* extruded co-product (78.9 %%), higher than our previously reported EPA digestibility (69.4 %) for the whole cells of *Nannochloropsis* sp. The reduction in digestibility of methionine and omega fatty acids including EPA in the raw *N. oculata* versus extrusion processed and enzyme-treated *N. oculata* may be attributable to known resistance of the complex cellulosic algal components of the unprocessed co-product to digestive enzymes, potentially inhibiting digestion by trout [36].

6. Conclusion

This study demonstrates that extrusion processing has a slight improvement in the nutritional value of *N. oculata* co-product over raw co-product. Extrusion processing at 90 °C provided the best outcome for digestible protein, given that methionine was significantly lower in high extrusion processing temperature at 127 °C than in low extrusion processing temperature at 90 °C and non-extruded raw co-product. Overall, extrusion processing of *N. oculata* at 90 °C yielded an excellent source of digestible protein, amino acids, and long chain omega-3 profile for rainbow trout and which could be an alternative to replace fishmeal in rainbow trout diets. The next step to understand the nutritional feasibility of replacing ocean-derived fishmeal with this extrusion processed material in trout diets is to test different feed inclusion levels of marine *N. oculata* co-product (replacing fishmeal protein) to determine effects on percent survival, growth performance, feed efficiency, and particularly, maintenance of flesh quality and muscle fatty acid composition of rainbow trout. The next step to understand the nutritional feasibility of replacing FM with this *N. oculata* co-product material in trout diets is to

test different feed inclusion levels of marine *N. oculata* co-product (replacing FM protein and supplementing as a source of major omega 3 polyunsaturated fatty acids, notably for 20:5n3 EPA) to determine effects on percent survival, growth performance, feed efficiency, and particularly, maintenance of flesh quality and muscle fatty acid composition of rainbow trout.

CRediT authorship contribution statement

Conceived and designed the experiments: Pallab K. Sarker; Performed the experiments: Devin Fitzgerald, Connor Greenwood; Pablo Nocera, Kira O'Shelski, Benjamin Lee, Abel Mkulama, Sofie Andrade, Diego Gonzalez Orcajo, Lydia Warkaw; Analyzed the data: Pallab K. Sarker, Benjamin Schoffstall; Contributed reagents/materials/analysis tools: Pallab K. Sarker, Devin Fitzgerald; Wrote-original draft: Pallab K. Sarker; Wrote-review & editing: Pallab K. Sarker, Anne R. Kapuscinski, Benjamin Schoffstall, Devin Fitzgerald.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data generated for this study are included in the article and Supplementary tables.

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Appendix A. Supplementary data

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