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Feeding Whole Thraustochytrid Biomass to Cultured Atlantic Salmon (*Salmo salar*) Fingerlings: Culture Performance and Fatty Acid Incorporation

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Abstract: Replacement of fish oil by 5% thraustochytrid whole cell biomass in diets for Atlantic salmon had no ill effect on fish growth performance, carcass total lipid and total fatty acid content. Carcass fatty acid composition indicated incorporation of the dietary thraustochytrid-derived fatty acids. This was confirmed by compound specific stable isotope analysis (CSIA) which revealed significantly ^{13}C-depleted (δ^{13}C value of −24‰) ω3 long-chain (≥C_{20}) polyunsaturated fatty acids (ω3 LC-PUFAs) in the fingerlings fed the thraustochytrid biomass containing diet, reflecting the highly ^{13}C-depleted glycerol used to grow the thraustochytrid cultures. This finding demonstrates the bioavailability of the ω3 LC-PUFA of the Australian strain thraustochytrid culture (TC) 20 from the whole cell biomass that was partly cultivated on crude glycerol produced during biodiesel manufacturing. This paper demonstrates the value of Australian thraustochytrid strains grown heterotrophically for their wider biotechnological potential including as a source of higher value lipids, in particular the health-benefitting ω3 LC-PUFA, for use in aquaculture and other applications.

Keywords: thraustochytrid; Atlantic salmon; compound specific stable isotope analysis; polyunsaturated fatty acids; aquaculture

1. Introduction

Thraustochytrids are heterotrophic protists found ubiquitously in the marine environment with a wide geographic distribution from polar regions including the Antarctic Peninsula [1] and Iceland [2] to the tropics in Malaysia, India, Australia and other countries [3–6]. It is considered that thraustochytrids play an important role in the marine ecosystem; they can be bacterivores, detritivores and parasites [6–8]. They are classified into the kingdom Chromista, class Labyrinthulomycota and phylum Heterokonta. This phylum also includes the chromophyte algae such as brown algae and diatoms [9–12]. The controversial labelling of thraustochytrids as microalgae was based on whether they lost the capability to photosynthesize, due to the lack of a plastid or any vestiges of photosynthetic apparatus; or the ancestor of the *Stramenopiles* may have been photosynthetic and subsequently lost their plastids in the evolution of multiple lineages [13].

Heterotrophic cultivation of thraustochytrid biomass has well recognized potential in producing a feedstock for biodiesel as well as high-value lipid bio-products such as docosahexaenoic acid (DHA, 22:6ω3), eicosapentaenoic acid (EPA, 20:5ω3), carotenoid pigments and squalene and other non-lipid materials including exopolysaccharide [9,14–17]. The high-value omega-3 long-chain (LC ≥ C_{20})

polyunsaturated fatty acid (ω3 LC-PUFA) containing oils, also termed LC omega-3 oils, have been conventionally sourced from wild harvest fish and, to a lesser degree, from krill and squid fisheries. Alternative sources of LC omega-3 oils from microalgae have been of increasing interest as replacements for fish oil due to the growing concerns related to global food security, plateauing harvest of the wild fisheries and increasing global aquaculture and nutraceutical production that are all reliant on LC omega-3 oils, in addition to the occurrence of high levels of pollutants in some wild-caught fish oils.

A number of studies has demonstrated the potential of thraustochytrids and oils derived from their biomass as a renewable source of essential fatty acids, in particular DHA, for aquafeeds with species such as shrimp, Nile tilapia, rainbow trout and different life stages in Atlantic salmon [18–22]. Studies reported the inclusion of 10%–13% of whole-cell thraustochytrids in salmonid feed generally have no negative effect on growth performance and digestibility [23]. Carter et al. (2003) [23] demonstrated the inclusion of 10% whole-cell thraustochytrid had no detrimental effect on the growth performance, whole-body chemical composition, organ somatic indices or measures of immune function of salmon; but the thraustochytrid-fed fish were more susceptible to diseases and seawater transfer challenges compared to that of fish oil diet.

The technology challenges with respect to the use of thraustochytrids include availability of high biomass and LC omega-3 producing strains, high production costs, and the considerable effort required in scaling up biomass production. As many of the previous studies have used thraustochytrid-derived oil, the aim and novelty of this study was to assess the feasibility of feeding whole-cell Australian thraustochytrid biomass, grown in this case on low-cost crude glycerol sourced from a local biodiesel plant, to Atlantic salmon fingerlings. In addition, we examined the potential use of stable isotope signatures to trace the unique thraustochytrid-derived ω3 LC-PUFA as they bioaccumulated into the fish.

2. Materials and Methods

2.1. Thraustochytrid Biomass Production

Aurantiochytrium sp. TC 20 was maintained at the Australian National Algae Culture Collection, strain number CS-997, GenBank accession number JN675250. Strain isolation information, medium preparation and culturing conditions have been reported previously [3,24]. In brief, the growth medium consisted of 4% (*w*/*v*) pure and crude glycerol (with glycerol content of 16.4 g/L), 0.2% (*w*/*v*) peptone, 0.2% (*w*/*v*) yeast extract, 0.5% (*w*/*v*) monosodium glutamate, 0.2% (*w*/*v*) corn steep liquor, metal solution (1 mL/L) and vitamin solution (1 mL/L) [24]. Cells were harvested after 7 days cultivation in shaking incubators at 25 °C, 150 rpm and pH 7 and centrifuged at 2500 rpm (500× *g*) at 20 °C for 10 min. Cells were resuspended and washed with 10 mL of sterile 3.15% (*w*/*v*) ammonium formate solution to remove salt and freeze dried overnight.

2.2. Salmon Fingerlings Feeding Trial

2.2.1. Diets

The feeding trial was conducted at the CSIRO Agriculture and Food Bribie Island Research Centre. A control diet and a thraustochytrid diet (Table 1) were fed to salmon fingerlings (0.85 g) in triplicate tanks for 70 days. The mashes were mixed without the oil component and extruded through a 24 mm intermeshing, twin-screw extruder (APV MPF 24; APV-Baker, Peterborough, United Kingdom). A 1.5 mm die was used to produce a 2 mm pellet that was dried to approximately 5% moisture and subsequently coarsely ground and sieved into 3 size class pellets (0.5–1.0 mm, 1.0–1.4 mm and 1.4–2.0 mm). Pellets were vacuum infused with their respective oil components. Both diets contained very high levels of fish meal, typically used at the very early stage of hatchery culture, in order to prevent any potential nutrient deficiencies in small fingerlings. Canola oil was used to balance the lipid content in both diets. The control diet contained 1.8% tuna oil to provide a similar level of DHA+EPA

to the test diet which contained 5% (*w*/*w*) of thraustochytrid biomass (TB). The use of tuna oil (TO) was preferentially selected in this study compared to commercially used fish oil in order to deliver a higher ratio of DHA:EPA, providing a closer comparison to the TB fatty acid ratio.

Table 1. Ingredient composition (% of total ingredient mass) and chemical composition of the experimental diets.

Composition (%)	Tuna Oil Control	Thraustochytrid Biomass Test
Fishmeal	79.0	76.0
Wheat flour	7.0	5.6
Vitamin premix	2.0	2.0
Soy lecithin	1.0	1.0
$CaHPO_4$	1.0	1.0
Betaine	1.00	1.00
Mineral premix	0.52	0.52
Stay-C	0.14	0.14
Vitamin E (50%)	0.03	0.03
Astaxanthin	0.01	0.01
Choline chloride	0.50	0.50
Thraustochytrid biomass (TB)	0.0	5.0
% Oil in feed	**Control**	**Thraustochytrid**
Tuna Oil (TO)	1.80	0.0
Canola Oil (CO) (control)	6.0	7.2
Diet Chemical Composition		
Dry matter content (%)	94.1	96.7
Protein (%)	62.6	61.8
Lipid (%)	17.7	18.9
Ash (%)	12.4	12.9
Energy (kJ/g)	22.9	22.9

2.2.2. Experimental Setup, Fish and Feeding

Six experimental tanks (300 L)—three tanks for the TO containing control diet and three tanks for the TB containing experimental feeds—were set-up with 3 L/min flow of continuously aerated freshwater with the water temperature maintained at 16 °C for the duration of the experiment. A total of 100 fish per tank were kept at constant light (24L:0D) under transparent lids to allow some dim light for continuous feeding while avoiding fish escapees. Temperature was measured daily and water quality (dissolved oxygen (DO), pH, NH_3, NO_2^- and NO_3^-) every week. Water quality was kept within an optimum range for this species, mean ± SE: Temp = 16.3 ± 0.64 °C; DO = 7.31 ± 0.29 mg/L; pH = 7.43 ± 0.22; NH_3 = 0.25 ± 0.03 mg/L; NO_2^- = 0.01 ± 0.06 mg/L; NO_3^- = 56 ± 34 mg/L.

The fingerlings were transported from the Saltas Fish Hatchery, Wayatinah, Tasmania, and reared on site with approval from the CSIRO Animal Ethics Committee, approval number A11/2014. The experiment was conducted with approval from the CSIRO Animal Ethics Committee, approval number A6/2016. The approved methodology was used in accordance with the "Australian Code for the Care and Use of Animals for Scientific Purposes", 8th Edition, and complied with all associated legislation and regulations regarding the welfare of animals.

Fish were fed 7 times a day at 6% body weight/day initially, and the delivered feed reduced to 3% BW/day by the end of the experiment. Manual feeding occurred at 08:00, 12:00 and 16:00 and another 4 feeds were delivered automatically daily at 18:00, 21:00, 24:00 and 03:00. Automatic feeders were checked for missed deliveries and reloaded daily with fresh food. Feed consumption could not be measured accurately due to the small pellet size, so it was only assessed visually and recorded daily. Feed conversion ratio (FCR) was calculated based on feed delivered.

Fish were sampled for bulk weights on days 34, 56 and 70 by weighing 30 fish individually to provide size distribution data for each tank. Three individual samples of 30 pooled fish were taken initially and 10 fish were pooled per tank on day 70 for whole body composition analyses.

2.3. Whole Carcass Composition Analyses

Whole fish and feed dry matter content were determined by gravimetric analysis following drying at 105 °C for 16 h. Ash content was determined based on mass change after combustion in a muffle furnace at 550 °C for 16 h. The lipid portion of the samples was extracted according to the method of Folch et al. (1957) [25] to determine crude lipid content. Measurement of total nitrogen content was undertaken using an elemental analyser (Flash 2000 Thermo Fisher Scientific, USA) and data were used to calculate sample protein content based on $N \times 6.25$ [26]. Gross energy was determined by isoperibolic bomb calorimetry in a Parr 6200 oxygen bomb calorimeter (Par Instrument Company, Moline, IL, USA). Carbohydrate was calculated by difference.

Fatty Acid Analyses

The lipid extract was evaporated to dryness under a stream of N_2 gas in a glass test tube in a heat block set at 45 °C. The KOH/MeOH/H_2O (3 mL) was added, and the screw cap fitted test tube was flushed with N_2 gas, sealed tightly, vortexed and placed in a heating block at 100 °C for 1 h. The tubes were removed, cooled and 1 mL of Milli-Q H_2O and 1.8 mL of 4:1 hexane:dichloromethane (DCM) were added. The tubes were vortexed well, then centrifuged at 1107× *g* for 5 min. The top layer was removed with a Pasteur pipette, and the bottom layer was re-extracted twice more with 1.8 mL of 4:1 hexane:DCM, centrifuging in between.

The aqueous fraction from the saponification step was acidified with 500 µL of concentrated HCl. Hexane:DCM 4:1 (3 mL) was added, and the tube was shaken and vortexed well, then centrifuged at 3000 rpm for 5 min. The top, solvent layer was removed with a Pasteur pipette and transferred to a clean test tube, and the aqueous layer was re-extracted twice more with 2 mL of 4:1 hexane/DCM with mixing and centrifuging in between.

The combined solvent layers were blown down to dryness, and 3 mL MeOH:HCl:$CHCl_3$ (10:1:1) were added to the test tube which was then flushed with N_2 gas and closed with a Teflon-lined screw cap. The tube was placed in a heating block at 100 °C for 1 h and then cooled, and the reaction was quenched with 3 mL of Milli-Q water. Hexane:DCM 4:1 (1.8 mL) was added to the test tube which was shaken and vortexed well, then centrifuged at 1107× *g* for 5 min. The upper, solvent layer was removed with a Pasteur pipette and transferred to a vial. The aqueous layer was re-extracted twice more with 1.8 ml of 4:1 hexane/DCM with mixing and centrifuging in between.

Gas chromatography (GC) and GC-mass spectrometry analyses of fatty acid methyl esters (FAME) was performed to confirm component identifications [27]. The $\delta^{13}C$ values of the individual FAME were determined using a Thermo Scientific Trace Ultra gas chromatograph equipped with a BPX70 column (50 m × 0.32 mm × 0.25 µm), coupled via a GC combustion interface and Conflo IV to a Delta V Plus isotope ratio mass spectrometer (Thermo Scientific). Samples were injected at 70 °C, and thereafter the oven temperature was raised to 160 °C at 10 °C/min and, finally, to 255 °C at 4 °C/min where it was held for 10 min.

Oxidation of individual compounds was achieved via a mixture of copper and nickel wires at 980 °C. Each sample was injected three times, bracketed with an isotopically characterized fatty acid mixture to check $\delta\ ^{13}C$ values of the sample. Values were corrected for methylation by analysis of a fatty acid standard derivatized using the same methylation mixture.

The $\delta\ ^{13}C$ values of the pure glycerol and culture medium were determined via a NA1500 CNS analyser (Carlo Erba) coupled to the same Delta V isotope ratio mass spectrometer.

2.4. Statistical Analyses

Differences in culture performance indices among diets were tested by an independent sample *t*-test using NCSS 11. Stable isotope data were analyzed using ANOVA and *t*-tests ($n = 3–9$) in SigmaPlot 11.0 In the process, a normality test (Shapiro-Wilk) and an equal variance test were undertaken, and the power of the performed test was examined each time. A Tukey test was used for all pairwise multiple comparisons. Tank effects were also examined using ANOVA ($n = 3$); there were no significant tank effects detected in the stable isotope data. Principal component analysis (PCA) of the fatty acid profiles was performed using Primer 6 (Figure 1).

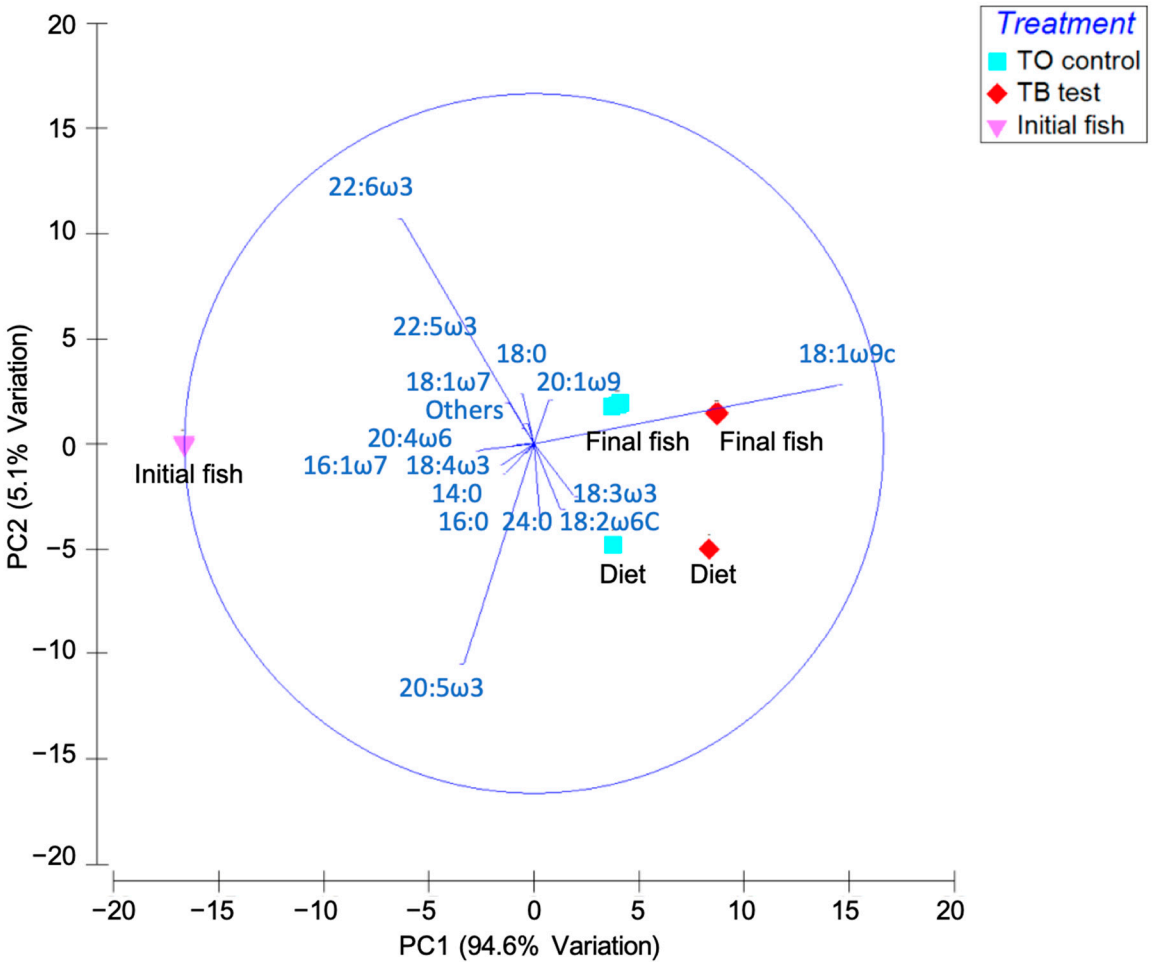

Figure 1. Principal component analysis (PCA) plot of fatty acid (FA) composition (>1% in at least one sample) of diets, the initial fish and the final fish.

3. Results and Discussion

Fish grew 20 fold to reach 15.5 ± 0.47 g on the control diet and 15.8 ± 0.26 g on the thraustochytrid diet at 70 days (Table 2). The feeding trial was stopped at 70 days, as the fish would have more than enough time to turn over the lipid and gain sufficient weight. There were no significant differences in specific growth rate, survival, feed consumption or FCR. Fish macronutrient composition was also similar (Table 2). Table 3 shows the fatty acid composition of the diets, the initial fish and the final fish. No significant differences in culture parameters occurred, and we noted small numerical improvement in growth and FCR when 1.8% tuna oil was replaced by 5% w/w whole thraustochytrid biomass in salmon fingerlings. It is proposed that the higher initial PUFA, including DHA and EPA, was obtained from endogenous reserves during early development and then decreased as fish were subsequently fed.

Table 2. Summary of culture performance and whole fish composition after 70 days of culture. No significant difference was found (*t*-tests, $p > 0.05$).

Culture Performance	Tuna Oil Control	Thraustochytrid Biomass Test
Initial weight (g)	0.8 ± 0.0	0.8 ± 0.0
Final weight (g)	15.5 ± 0.5	15.8 ± 0.3
Body weight gain (fold increase)	18.6± 0.5	18.9± 0.3
SGR (%BW day^{-1})	4.2 ± 0.0	4.2 ± 0.0
Survival (%)	99.7 ± 0.3	99.7 ± 0.3
Feed offered (mg day/fish)	203.1 ± 0.5	200.5 ± 1.0
Final weight coefficient of variation (%)	30.6 ± 2.4	28.6 ± 1.9
Food conversion ratio (FCR)	1.0 ± 0.0	0.9 ± 0.0
Whole Fish Composition (on DM Basis, $n = 3$)		
Dry matter content (%)	25.9 ± 0.5	26.3 ± 0.3
Protein (%)	58.9 ± 0.6	57.8 ± 0.4
Lipid (%)	37.1 ± 0.5	37.8 ± 0.9
Ash (%)	6.2 ± 0.2	5.8 ± 0.4
Energy (kJ/g)	27.7 ± 0.1	27.2 ± 0.2

Table 3. Fatty acid (FA) composition (expressed as % of total FA) of diets, the initial fish and the final fish; fatty acid methyl esters (FAME as mg/g of dry weight) for the initial and final fish.

% Fatty Acid	Tuna Oil Control	SD	Thraustochytrid Biomass Test	SD	Initial Fish	SD	Final Fish-Tuna Oil Control	SD	Final Fish-Thraustochytrid Biomass Test	SD
Saturated Fatty Acids										
14:0	4.3 ^{b}	0.1	3.8 ^{d}	0.0	5.7 ^{a}	0.0	3.9 ^{c}	0.0	3.4 ^{e}	0.0
16:0	14.8 ^{c}	0.3	15.8 ^{b}	0.1	16.8 ^{a}	0.1	14.9 ^{c}	0.1	14.7 ^{c}	0.1
18:0	3.8 ^{d}	0.1	3.1 ^{e}	0.0	4.8 ^{a}	0.0	4.6 ^{b}	0.1	4.1 ^{c}	0.1
24:0	1.6 ^{a}	0.0	1.5 ^{a}	0.0	0.0 ^{b}	0.0	0.2 ^{b}	0.4	0.0 ^{b}	0.0
Subtotal	24.6	0.3	24.1	0.1	27.4	0.1	23.6	0.4	22.1	0.1
Monounsaturated Fatty Acids										
16:1ω7c	4.6 ^{b}	0.0	3.7 ^{d}	0.0	7.5 ^{a}	0.0	4.4 ^{c}	0.0	3.6 ^{d}	0.0
18:1ω9c	31.6 ^{d}	0.1	35.5 ^{b}	0.1	14.7 ^{e}	0.0	32.8 ^{c}	0.2	36.9 ^{a}	0.0
18:1ω7c	2.5 ^{d}	0.0	2.3 ^{e}	0.0	3.0 ^{a}	0.0	2.8 ^{b}	0.0	2.7 ^{c}	0.0
20:1ω9	1.6 ^{c}	0.0	1.6 ^{c}	0.0	1.2 ^{d}	0.0	2.4 ^{b}	0.0	2.5 ^{a}	0.0
Subtotal	40.3	0.1	43.1	0.1	26.5	0.0	42.3	0.2	45.7	0.0
Polyunsaturated Fatty Acids										
18:2ω6	7.5 ^{b}	0.0	8.2 ^{a}	0.0	5.3 ^{e}	0.1	6.3 ^{d}	0.0	7.1 ^{c}	0.0
18:3ω3	3.9 ^{b}	0.0	4.7 ^{a}	0.0	1.0 ^{e}	0.0	3.0 ^{d}	0.0	3.7 ^{c}	0.0
18:4ω3	0.9 ^{b}	0.0	0.7 ^{c}	0.0	1.1 ^{a}	0.0	0.7 ^{d}	0.0	0.6 ^{e}	0.0
20:4ω6	0.6 ^{b}	0.0	0.3 ^{e}	0.0	1.6 ^{a}	0.0	0.5 ^{c}	0.0	0.4 ^{d}	0.0
20:5ω3	7.8 ^{b}	0.0	6.7 ^{c}	0.0	8.7 ^{a}	0.0	3.3 ^{d}	0.0	2.7 ^{e}	0.1
22:5ω3	0.1 ^{c}	0.1	0.2 ^{c}	0.1	2.4 ^{a}	0.0	0.9 ^{b}	0.3	0.9 ^{b}	0.0
22:6ω3	11.0 ^{d}	0.1	9.2 ^{e}	0.1	21.8 ^{a}	0.1	15.1 ^{b}	0.1	13.2 ^{c}	0.1
Subtotal	31.7	0.1	29.9	0.1	41.9	0.1	29.9	0.3	28.6	0.1
FAME mg/g of Dry Weight					708.0	26.3	713.7	9.7	708.0	13.0

Tukey pairwise comparisons: means that do not share a letter are significantly different. Fatty acids present at <1% of total fatty acids in all samples: 15:0, 17:0, 17:1, 19:0, 18:3ω6, 20:0, 20:1ω7, 20:2ω6, 20:3ω3, 22:0, 22:1ω9, 22:4ω6, 23:0, 24:1ω9.

Our fatty acid results, as shown by PCA plot (Figure 1), reveal similar profiles for the fish receiving the two diets. These findings are in agreement with Miller et al. (2007) [19], indicating thraustochytrid lipids can be used to replace fish oil in Atlantic salmon parr diets without detriment to the growth. Sprague et al. (2015) [21] also showed that replacement of fish oil with thraustochytrid biomass in Atlantic salmon post-smolt diet for 133 days at levels of 11% and 5.5% resulted in no negative effects. The authors reported that fish fed with higher dietary thraustochytrid inclusion had similar DHA levels to fish-oil-fed fish. Additionally, Mizambwa (2017) [28] reported that inclusion of *Schizochytrium* spp. biomass (6.25% of the diet) as a source of DHA, for 400 days in Atlantic salmon fish (from 865 g to a slaughter weight of approximately 3 kg), was able to improve final weight, SGR, condition factor and

deposition of DHA in tissues (i.e., fillet and liver) without reducing the quality of fillet in terms of red colour and gaping score [28].

The results of our study and those from previous research combined clearly indicate that whole thraustochytrid biomass can effectively replace fish oil in practical salmon diets over the entire culture cycle (from fry to market). One limitation of our study is the large proportion of omega-3 LC-PUFA provided by the fishmeal component of the diet which could have masked the response of the fish to dietary fatty acids. This aspect was addressed in our study by tracing compound specific carbon isotope ratios for individual fatty acids. Similar techniques were used in Hixson et al. (2014) [29] and Katan et al. (2019) [30]. Nonetheless, future research on fatty acid metabolism from thraustochytrid biomass as a replacement for fish oil will need to be performed in larger fish-fed formulations containing very low proportions of fish meal (<10%) as is now employed in modern diet formulations by the global salmon industry.

The TB diet had the highest proportions of 18:2ω6 and 18:3ω3, reflecting the higher amount of canola oil in the diet. There were also small, although significant, differences in essential fatty acid proportions, especially for 20:4ω6. Figure 2 shows the ratios of individual major fatty acids, calculated as TO control/TB test, in the diets and the salmon fingerlings at the end of the 70 day feeding period. There were no significant differences in the ratios, except for 18:4ω3 ($p = 0.004$) and 20:4ω6 ($p \leq 0.001$). The small and significant differences in fatty acid proportions in the diets were generally mirrored in the final fish composition with one notable exception (Figure 2). There was nearly twice the level of 20:4ω6 in the TO diet than in the TB diet, but the difference was much smaller in the final fish carcass probably because of selective retention and/or desaturation and elongation of 18:2ω6.

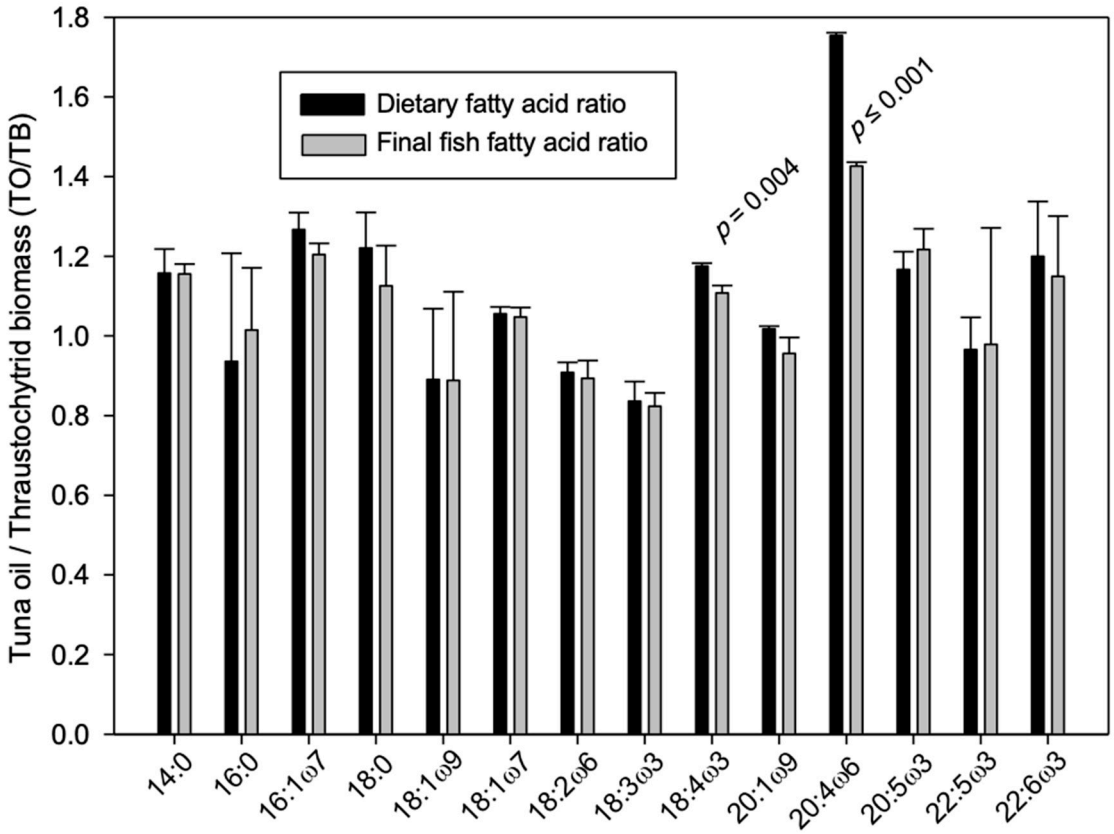

Figure 2. Ratios of individual major fatty acids, calculated as tuna oil (TO) control/thraustochytrid biomass (TB) tests, in the diets and the salmon fingerlings at the end of the 70 day feeding period. The error bars indicate the standard deviation, and there were no significant differences in the ratios, except for 18:4ω3 ($p = 0.004$) and 20:4ω6 ($p \leq 0.001$).

Katan et al. (2019) [30] reported that LC-PUFA synthesis in liver of farmed Atlantic salmon (specifically 20:5ω3 and 20:4ω6) was largely driven by dietary 18:3ω3 and 18:2ω6, even when 20:5ω3 and 22:6ω3 were supplied at levels above minimum requirements. Although replacement of fishmeal with plant-based diets generally does not affect the overall health and growth of the fish, a range of studies have observed a reduced level of LC-PUFA (including DHA and EPA) which indicates the heightened importance of fish oil used in modern fish feed formulations [19,20,29,31].

Compound specific carbon stable isotope ratios were obtained for the TO control diet and the TB diet for the PUFA 18:3ω3 and the LC-PUFA 20:5ω3 and 22:6ω3. For all the selected fatty acids, the TB diet had a ratio which was more depleted in ^{13}C. These results undoubtedly reflect the highly depleted ratios for pure glycerol and the crude glycerol medium with values of −32.9 and −30.1‰, respectively. These depleted ratios were then reflected in the final fish carcass (Figures 3 and 4). All ω3 PUFA and ω3 LC-PUFA showed a diet-tissue discrimination, reflecting the tendency for lighter isotopes to react more rapidly than heavier isotopes.

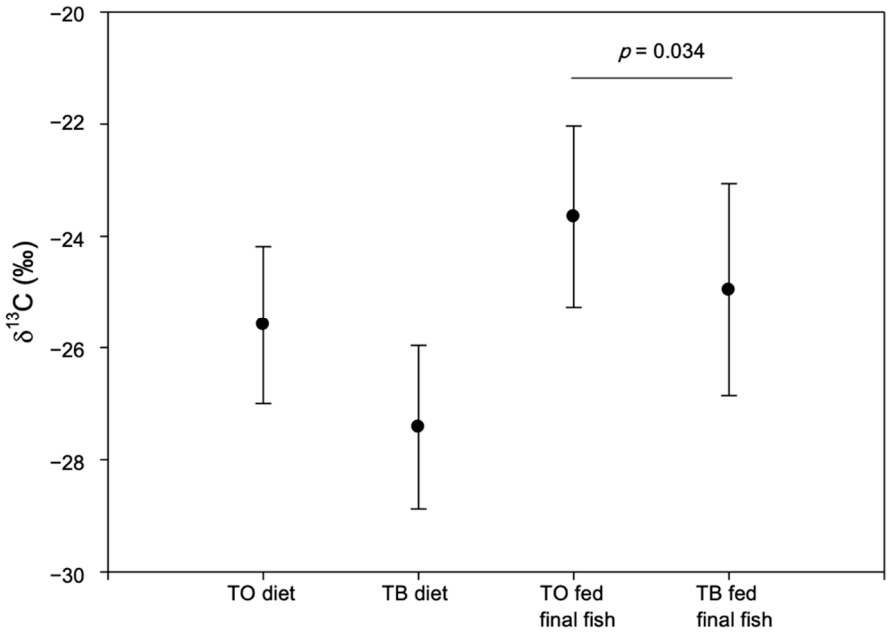

Figure 3. Mean stable isotope ratios of ω3 fatty acids (18:3ω3, 20:5ω3 and 22:6ω3) in the diets and the salmon whole carcass at the end of the 70 day feeding period.

The commercial feasibility of using whole thraustochytrid biomass instead of fish oil in salmon feed depends critically on the production cost and technological developments; reducing processing costs by using the entire biomass product, without the need to separate the oil, has merit. In low fishmeal diets, the use of whole TB would be required at more elevated inclusion rates than trialed in this study, so further assessment of extrusion characteristics, palatability and digestibility needs to be performed to understand if suitable pellets can be manufactured with the right oil profile and the contribution of all macronutrients from this novel ingredient. Our study adds to a growing body of evidence towards the use of thraustochytrid biomass as a promising alternative to fish oil for the entire salmon life cycle. The use of a crude glycerol stream as a carbon source for a heterotrophic production system could not only substantially reduce the cost of commercial production, but also potentially reduce the greenhouse gas emissions of industries including power plants through the sustainable growth and utilization of thraustochytrids-derived LC omega-3 oils. Future research and development on the use of thraustochytrid biomass to replace fish meal, in addition to fish oil, will reduce the reliance of the aquafeed industry on the use of fish meal. Furthermore, such development can also meet the need to substitute fish meal derived from small pelagic fish or forage fish with a more sustainable feedstock such as thraustochytrid biomass. The fish stocks presently being used for fish

meal production can then potentially be used to provide food and nutrients to malnourished people from developing countries [32].

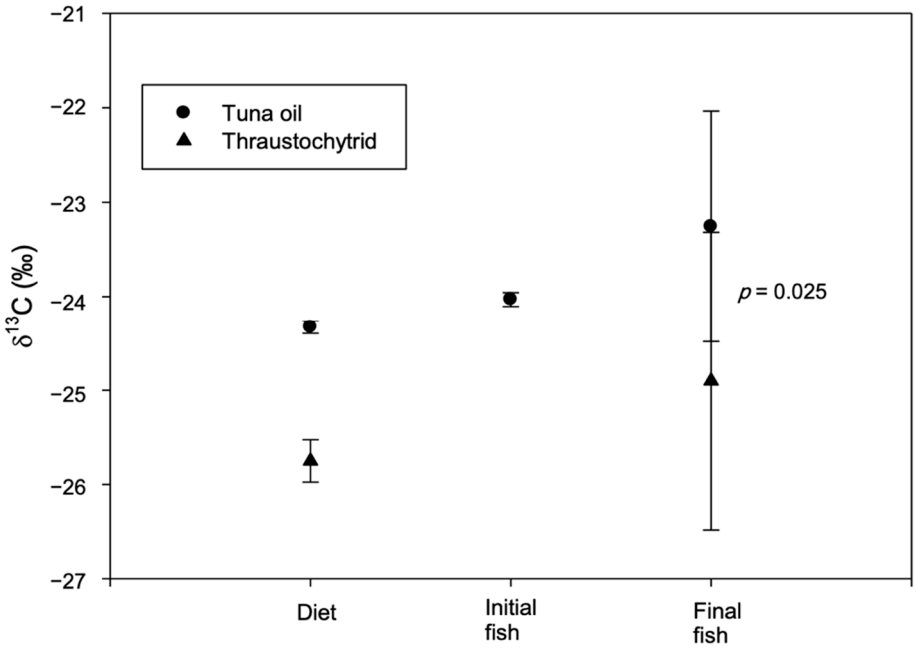

Figure 4. Stable isotope ratios of 22:6ω3 in the diets, the initial fingerlings and the salmon whole carcass at the end of the 70 day feeding period.

In conclusion, heterotrophic cultivation of thraustochytrids has considerable potential in producing a novel sustainable feedstock rich in the health-benefitting LC omega-3 oils. The greatest interest comes from the nutraceutical and aquaculture industries with application in fish meal and fish oil replacement; high DHA content of as much as 14 g/L (40% total fatty acids, cell dry weight at 69 h) can be achieved using our thraustochytrid strains [33]. The application of whole thraustochytrid biomass in aquaculture is likely one of the most cost-effective non-GM approaches to ensure the sustainable growth of aquaculture while improving the quality of farmed fish.

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