DOI: 10.1002/aff2.164

ORIGINAL ARTICLE

AQUACULTURE, FISH and FISHERIES Open Access WILEY

Effects of micro-algae dietary oil replacement on growth, omega — 3 deposition and gut microbiome composition of Nile tilapia (*Oreochromis niloticus*)

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Funding information
Swansea University PhD scholarship, Grant/Award Number: SMARTAQUA

Abstract

Microalgae offer a sustainable source of omega — 3 fatty acids that can replace fish oil in aquafeeds, but the nutritional benefits are not always clear, particularly when microalgae are used as complete oil replacements in starter feeds. We compared the survival, growth, omega — 3 deposition and composition of the gut microbiota of juvenile mixed-sex Nile tilapia (*Oreochromis niloticus*) that had been fed over a 3-month period on six isonitrogenous, isolipidic and isocaloric aquafeeds that varied only on the contribution of fish oil, soya oil and microalgae (*Schizochytrium*) oil as lipid sources. Survival was not affected by diet, but fish fed a diet where the entire oil component (5%) was replaced by microalgae oil grew twice as fast as fish fed plant oil or a mixture of plant and fish oil. Dietary omega — 3 content was strongly correlated with omega — 3 deposition in the fish fillet. Complete replacement of fish oil by plant oil caused a significant decrease in the abundance of Peptostreptococcaceae and an increase in the abundance of Aeromonadaceae which is often associated with an inflammatory response in the fish gut. In contrast, when fish and soya oil in the reference diet were replaced by 100% microalgae oil, an increase in Mycobacteriaceae was observed. Our study indicates that *Schizochytrium* oil can be used to improve the growth of Nile tilapia and increase its omega — 3 content without any of the detrimental effects on the gut microbiome typically associated with some plant oil replacements.

KEYWORDS
algae, aquaculture, feed, growth, tilapia

1 | INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is the second most widely farmed fish worldwide (Prabu et al., 2019) and the main source of fish food for millions of people, particularly in developing countries (Fitzsimmons et al., 2011). The reasons of its success lie in its fast growth, hardiness and herbivorous diet, which makes tilapia feeds relatively cheap (da Silva Dias et al., 2020; Köprücü & Özdemir, 2005). However, although farmed tilapia can help alleviate food insecurity and reduce the risk of malnutrition (Bene & Heck, 2005; Gupta, 2006), they are typically fed a high percentage of plant ingredients (El-Sayed, 1999), which results in low omega — 3 content (Osibona et al., 2009) and diminishes their nutri-

tional value (Karapanagiotidis et al., 2006), impacting those countries where access to healthy food is most needed. Improving the nutritional value of Nile tilapia is, therefore, a priority for many developing countries (Bhujel & Suharman, 2021; Hasselberg et al., 2020).

An adequate dietary intake of eicosapentaenoic (EPA, 20:5*n* − 3) and docosahexaenoic (DHA, 22:6*n* − 3) long-chain polyunsaturated fatty acids (FAs) (omega − 3 for short) is essential for the regulation of many metabolic pathways (Hussein et al., 2019), the adequate functioning of the retina and growth of neural tissue (Lauritzen et al., 2016; Politi et al., 2001), the development of cognition (Liu et al., 2020; Sinn et al., 2012; Zhou, Ding, et al., 2018) and the control of the inflammatory response (Alfaddagh et al., 2018). Omega − 3 present in aquafeeds are deposited in the fish fillet and are then transferred to human consumers (Sissener, 2018), offering possibilities for improving the diet of millions of people through fish farming.

Like many tropical freshwater fish, Nile tilapia convert *n* − 6 and *n* − 3 PUFAs to longer chain highly unsaturated FAs, and their diet is typically supplemented with plant oils under culture (Lim et al., 2011). To make tilapia more nutritious, aquafeeds can be enriched with fish oil (Kris-Etherton et al., 2002), but this is costly (Rana et al., 2009) and increasingly unsustainable (Chuenpagdee et al., 2005), as fish oil is obtained from depleted wild stocks (FAO, 2020). Fish oil is often replaced by plant oils, but these do not have the same omega − 3 content or composition of fish oil (Pickova & Mørkøre, 2007) and can have adverse effects on fish health, including intestinal inflammation and changes in genes involved in the regulation of intestinal transport mechanisms and metabolic pathways (Booman et al., 2018; Kiron et al., 2020).

Unicellular microalgae and cyanobacteria represent sustainable sources of omega − 3 FAs for human and animal nutrition (Sarker et al., 2018; Sarker, Kapuscinski, Vandenberg, et al., 2020; Shah et al., 2018). The nutritional profile of some microalgae is comparable to that of fish oil without any of the sustainability drawbacks (Lum et al., 2013). An alternative to the use of fish oil in aquafeeds, therefore, would be to use oil extracted from microalgae rich in omega − 3, which are more sustainable than fish oil and more nutritious than plant oil (Shah et al., 2018). Microalgae could be used to fortify the diets of farmed fish and increase the nutritional value of fish food (Ryckebosch et al., 2014; Sarker et al., 2018).

Among the microalgae currently being cultivated, *Schizochytrium* sp. (not a true algae but an obligate marine heterotroph) is the only one whose oil is commercially available for incorporation into fish diets (Tocher et al., 2019). *Schizochytrium* possess two important qualities that make it attractive for use in aquafeeds: Its oil is rich in omega − 3 – up to 40%–45% DHA acid and 10% EPA acid (Fedorova-Dahms et al., 2011), and unlike other microalgae which require specific carbon sources to grow, *Schizochytrium* can thrive on agriculture by-products (Carr, 2017) and even wastewater from fish farming (Jung & Lovitt, 2010), which facilitates its culture. High production costs are the main factor limiting the uptake of microalgae in aquafeeds (Ansari et al., 2021), but recent advances in algal biotechnology have made microalgae production more cost-effective (Saratale et al., 2022).

Recent studies have shown that the omega − 3 content of Nile tilapia can be enhanced by the inclusion of *Schizochytrium* (dos Santos et al., 2019; Sarker et al., 2018; Sarker, Kapuscinski, et al., 2016; Sarker, Kapuscinski, McKuin, et al., 2020), but there are uncertainties regarding the optimal level of algal inclusion and the form of administration, as most studies have used whole microalgae rather than microalgae oil, which may contain anti-nutrients and affect the digestibility of FAs and nutrient uptake (Teuling et al., 2019).

The amount of omega − 3 in the diet can alter the composition of commensal bacteria in humans (Menni et al., 2017) and mice (Davis et al., 2017), but whether the same happens in fish is not known. Inclusion of microalgae in aquafeeds can deliver multiple benefits (Shah et al., 2018). Previous studies have shown that the inclusion of *Schizochytrium* in the diet does not impact on fish welfare (Emery et al., 2016) and could improve growth and the omega − 3 content in the fish fillets (Sarker, Gamble, et al., 2016; Watters et al., 2013).

Here, we assessed the effects of varying the amount and sources of dietary oil (microalgae, fish and plant) on omega − 3 deposition, growth, survival and composition of the gut microbiome of Nile tilapia while keeping other dietary ingredients constant. We carried our study from first feeding onwards, as this is the time when the fish microbiome is most plastic and most likely to be affected by changes in the diet and by bacterial colonization history (Uren Webster, Rodriguez-Barreto, et al., 2020). Both fish and microalgae oil are rich in omega − 3 FAs, but microalgae oil has a higher DHA:EPA ratio than fish oil, which enabled us to assess the effects of different FA profiles on tilapia weaning and subsequent performance.

2 | MATERIALS AND METHODS

2.1 | Experimental design and sampling

We obtained 3 days old, mixed sex Nile tilapia (Silver strain) from a certified supplier, originating from three half-sib families (one male, three females). Fish were housed in 18 × 25 L plastic tanks. Rearing conditions and water quality were monitored daily/weekly and maintained within optimal conditions for Nile tilapia (El-Sayed, 2013): temperature 27–28°C, unionized ammonia < 0.05 mg/L, dissolved oxygen > 4.0 mg/L, pH 7.5–8, salinity < 2 ppt, photoperiod 12D:12 L.

Eighteen 25 L opaque plastic tanks (40 L × 31 W × 23 H cm), each containing 90 fish were assigned to 6 experimental diets in triplicate (Figure 1) using a random number generator and connected to a recirculating aquaculture system (TMC System 1000 M, Rickmansworth WD3 5SX). Mean initial size (total length) was 8.5 ± 0.72 mm; mean initial mass (wet weight) was 24 ± 4.4 mg. Stocking density was maintained at 3.6 fish/L (<2.2 g/L) throughout the duration of the experiment, which is within the recommended values to maintain high welfare (Pedrazzani et al., 2020).

Fish were fed to satiation three times per day. Particle size was increased every 3 weeks to match fish growth, starting with 200 μm and increasing to 600, 800 and 1200 μm. Dietary composition was maintained constant over the 3 months of the study.

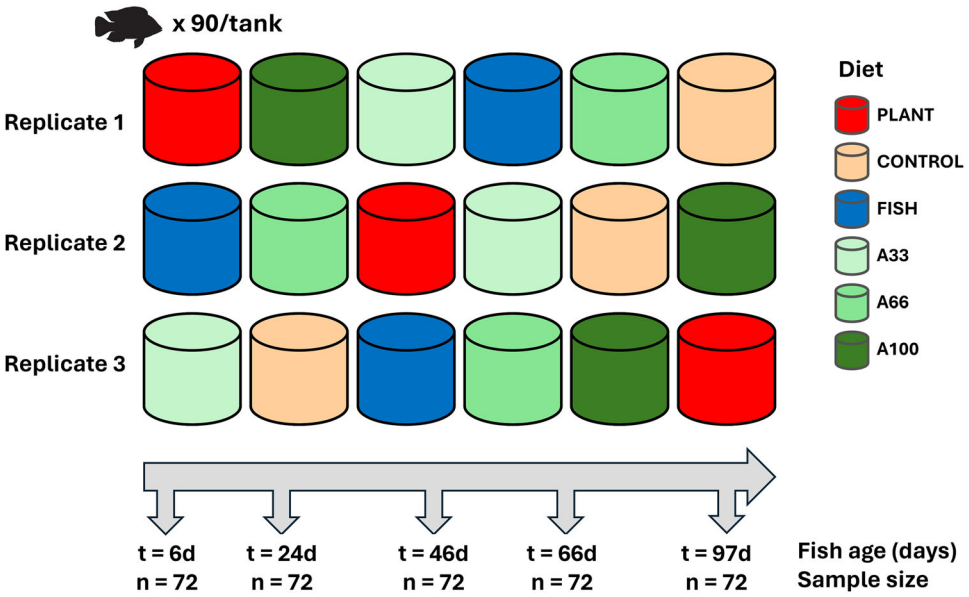

FIGURE 1 Schematic representation of the experimental design and timing of sampling.

Four fish per tank (12 per dietary group) were humanely sacrificed using an overdose of phenoxyethanol and sampled at day 3, and then every ~3 weeks at days 21, 43, 63 and 94. Total sample size was $n = 60$ per diet ($n = 360$ in total). The whole gut of the fish was dissected using aseptic techniques and preserved in RNA-later (QIAGEN N.V.) at −23°C. Total length and weight were recorded for growth analysis, and Fulton's condition factor (K) was calculated as a measure of body condition (Bolger & Connolly, 1989).

2.2 | Dietary formulation

Six isonitrogenous (mean protein = 32%, SD = 1.2), isolipidic (mean lipid = 16% SD = 0.7) and isocaloric diets (mean = 19.36 kJ/g, SD = 0.07) were formulated (Table 1), taken into account the nutritional requirements for the species (FAO, 2022) and in line with other algae feeding studies in tilapia (Sarker, Kapuscinski, McKuin, et al., 2020; Stoneham et al., 2018). Protein sources were maintained constant and consisted of fish meal, soya bean meal and line seed meal, and we only varied the contribution of fish oil, soya oil and *Schizochytrium* oil as the lipid sources. Dietary oils were mixed with the rest of the feed ingredients and made into pellets using a feed mill, as in Teuling et al. (2017).

All diets had the same protein to energy ratio (PE = 16–17 g/MJ) which is within the recommended 13–26 g/MJ optimal range for the species (Konnert et al., 2022). A reference (control) diet included 50% salmon oil and 50% soya oil and was formulated as in previous studies (Teuling et al., 2017, 2019). The other five experimental diets were formulated to replace the control diet with increasing proportions (33% – A33, 66% – A66, 100% – A100) of oil from the microalgae *Schizochytrium* sp. (Henry Lamotte Oils GmbH) or with 100% fish oil (Fish) or 100% plant oil (Plant). Unlike some other studies, we used *Schizochytrium* oil (guaranteed to contain at least 40% DHA acid omega – 3, C22:6) instead of the whole microalgae to make it comparable with the other dietary oils. The proximate analysis (macronutrients) of the six diets is given in Table 2.

2.3 | Fatty acid analysis

An FA profile of the different diets was carried out by Sciantec Analytical (Cawood Scientific). Total fat and omega – 3 content (EPA + DHA) of Nile tilapia muscle at the end of the trial (day 94) were analysed by a commercial laboratory (Campden BRI). Total fat content in the fish muscle (gr fat/100 g muscle) was determined using the Weibull-Stoldt method with test reference TES AC-536 (UKAS). Omega – 3 content was determined as FA methyl esters using test reference TES-AC-090 (UKAS) and is reported as a percentage of omega – 3 FAs in the muscle fat to make it more comparable to the FA results.

2.4 | 16sRNA library preparation and sequencing

DNA was extracted from 72 gut samples collected on days 21 and 94, using the DNeasy PowerSoil PowerLyzer kit (Qiagen), according to the manufacturer's instructions. 16S rRNA libraries were prepared using the Earth Microbiome primers 515F and 806R (Walters et al., 2016) to target the V4 hypervariable region, based on the Illumina Metagenomic Sequencing Library Preparation protocol (Illumina) using Platinum Hot Start Taq Polymerase (Invitrogen). All libraries were purified using AMPure XP magnetic beads (Beckman Coulter), indexed using Nextera XT indices (Illumina), multiplexed in equimolar concentrations and sequenced using an Illumina MiSeq platform (2 × 300 bp). Two extraction blanks were prepared and sequenced alongside the gut samples. The demultiplexed 16s sequences were denoised and

TABLE 1 Formulation (g/kg) of the six experimental diets for juvenile Nile tilapia.

	Diet					
Ingredient	Control	Fish	Plant	A33	A66	A100
Maize	134	134	134	134	134	134
Wheat	200	200	200	200	200	200
Wheat bran	80	80	80	80	80	80
Wheat gluten	125	125	125	125	125	125
Line seed meal	125	125	125	125	125	125
Fish meal	141	141	141	141	141	141
Soya bean meal	135	135	135	135	135	135
Fish oil	25	50	0	16.75	85	0
Soya oil	25	0	50	16.75	85	0
Schizochytrium oil	0	0	0	16.5	33	50
Vitamin and mineral premix	10	10	10	10	10	10
Total	1000	1000	1000	1000	1000	1000

TABLE 2 Proximate analysis (macronutrients) of the diets used in the feeding trial.

	Diet					
	Control	Fish	Plant	A33	A66	A100
Crude fibre (%)	1.9	2.3	2.1	2.4	3.0	2.7
Dry matter (%)	92.0	85.0	91.1	87.3	89.2	83.7
Ash (%)	5.0	4.6	5.3	4.7	4.7	4.7
Total oil (%)	17.19	15.62	16.79	16.04	15.76	15.22
Crude protein (%)	33.6	30.9	32.7	31.9	32.5	30.5
CH (%)	44.21	48.88	45.21	47.36	47.04	49.58
Energy (kJ/g)	20.25	18.98	20.20	19.29	18.92	18.53
PE ratio (g/MJ)	16.59	16.28	15.84	16.07	17.17	16.46
FFE extract. Fat (%)	3.8	4.2	4.6	3.9	4.9	28.6
MUFA (%TFA)	26.14	30.52	21.90	24.57	20.97	18.80
PUFA (%TFA)	55.97	50.64	60.53	56.37	57.42	57.66
SAFA (%TFA)	15.80	15.81	16.08	16.39	17.71	18.50
UFA (%TFA)	2.09	3.03	1.49	2.67	3.90	5.04

Abbreviations: CH, carbohydrates; FFA, free fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids; TFA, total fatty acids; UFA, unidentified fatty acids.

clustered with DADA2 (Callahan et al., 2016) within QIIME 2 2020.2 (Bolyen et al., 2018). The primers were removed by trimming the first 19 bp from all the reads, then we trimmed the forward reads at position 280 bp, and the reverse reads at position 240 bp after inspecting the quality profiles. The forward and reverse reads were then merged (removing the non-matching pairs) and screened for chimera and amplicon sequence variants (ASVs) assigned. Taxonomy was assigned to ASVs using a custom trained classifier (Bokulich et al., 2018) against the SILVA 132 database (Quast et al., 2012), following the removal of mitochondrial, eukaryote and chloroplast sequences, resulting in 7792,427 good-quality sequences. Reads were subsampled at an equal depth (sampling depth = 3364), prior to the calculation of metrics of alpha diversity (Shannon diversity, Chao1 richness) and beta diversity (Bray–Curtis dissimilarity) within QIIME based on ASV-level assignment. Additionally, read counts were obtained for family-level analyses within QIIME.

2.5 | Statistical analysis

R version 4.03 (R Core Team, 2020) was used for all analyses. To assess the effects of diet and time on various response variables, generalised linear mixed effects modelling was employed with the *lme4* (Bates et al., 2015) and *lmerTest* (Kuznetsova et al., 2017) packages, using tank identity as a random factor to account for lack of statistical independence. A Gaussian family link was used to model changes

in fish size, condition factor and microbiome alpha diversity (Shannon diversity, Chao1 richness), a binomial link to model survival, and a Poisson link to assess relative bacterial counts (reads). The *anova* command and the Likelihood Ratio Test (LRT) were used to determine the significance of random and fixed factors against a null model with no predictors. Starting with a full model containing all main effects, the *step* and *drop1* functions were used to arrive at a minimal adequate model based on changes in the Akaike Information Criterion (Crawley, 2013). The *vegan* package (Oksanen et al., 2017) was used to analyse variation in beta diversity, and non-metric multidimensional scaling ordination was employed to visualise Bray–Curtis dissimilarities at days 21 and 94. Multivariate statistical analysis of the gut microbial community was performed by PERMANOVA using *adonis* in the *vegan* package using time since first feeding, diet and fish size as predictors. The *Deseq2* package (Love et al., 2014) was used to identify significant differences in pairwise amplicon sequence variants (ASVs) comparisons across diet treatments using adjusted probabilities to correct for multiple comparisons ($p_{adj} < 0.05$).

3 | RESULTS

3.1 | Variation in fatty acid composition of experimental diets

Analysis of FAs revealed substantial differences in the abundance of omega – 3 PUFA in the experimental diets (Table 3). The amount of EPA was highest in the fish diet (1.42%) and lowest in the plant diet (0.62%). DHA was particularly high in the microalgae diets and increased with the level of algal oil replacement, being highest in the 100% algae (13.92%) and lowest in the plant diet (0.35%). The DHA:EPA ratio varied almost ×30-fold, from 0.56 for the plant diet to 16.77 for the 100% algae replacement. The plant diet, on the other hand, had the highest amount of linoleic acid C18:2 (*n* – 6) and polyunsaturated FAs. The ratio of *n* – 3 to *n* – 6 PUFAs ranged from 0.68 for the plant diet to 1.85 for the complete microalgae replacement.

3.2 | Effects of diet on survival

Mean survival at the end of the ~3-month feeding trial was 85% (SE = 3.18). Tank identity had a significant effect on survival (chi-square = 52.37, df = 1, $p < 0.001$) with survival ranging from 55% to 99% across tanks. However, survival did not differ across diets once variation between tanks was statistically accounted for (chi-square = 10.61, df = 7, $p = 0.156$).

3.3 | Effects of diet on growth

Growth parameters, including initial and final sizes, Fulton's body condition factor (K) and specific growth rates (SGR) of fish under different diets are shown in Table 4. Tank identity did not influence variation in fish weight (chi-square = 0.245, df = 1, $p = 0.620$), and therefore, a linear model was used to examine growth in weight instead of a mixed effect model. Weight gain depended on time since first feeding ($F_{1,330} = 621.16$, $p < 0.001$), diet ($F_{5,330} = 13.01$, $p < 0.001$) and the interaction between time and diet ($F_{5,330} = 10.91$, $p < 0.001$) as fish fed on the full algal diet (A100) grew much faster than fish fed on the control or plant diets (Figure 2). The final weight at the end of the experiment differed significantly between diets ($F_{5,66} = 5.08$, $p < 0.001$). Post hoc pairwise comparisons (Turkey HSD) indicated that fish fed the full 100% algae diet were significantly larger (mean = 3.36 g ± 0.416 SE) than fish fed the plant diet (mean = 1.57 g ± 0.249 SE; $p = 0.003$), the control diet (mean = 1.65 g ± 0.201 SE; $p = 0.005$) or the 66% algae diet (mean = 1.96 g ± 0.277 SE; $p = 0.035$). No significant difference in Fulton's body condition factor (*K*) was detected between diets at the end of the trial ($F_{5,66} = 1.22$, $p = 0.310$).

3.4 | Relationship between dietary omega – 3 content and deposition in the fish fillet

The omega – 3 content of the fat in the fish fillet was closely linked to the omega – 3 content in the diet ($F_{1,4} = 30.08$, $p = 0.005$; $R^2_{adj} = 0.853$; Figure 3). Fish fed the 100% algae diet, which had the highest omega – 3 content (mean = 14.75%), accumulated the highest amount of omega – 3 in the fillet (mean = 28.52%), whereas fish fed the plant diet, which was poorest in omega – 3 (mean = 0.97%), had the lowest omega – 3 content (mean = 15.30%). Inspection of regression coefficients indicates that a 1% increase in omega – 3 in the diet increased the omega – 3 content of the fat in the fish fillet by 0.86% (SE = 0.158).

3.5 | Temporal variation in alpha and beta diversity of the gut microbiome

A marked loss of alpha microbial diversity was detected from day 21 to day 94 (Figure 4), but this was unrelated to diet (Shannon diversity $p = 0.267$; Chao1 richness $p = 0.588$) or to growth in weight (Shannon diversity $p = 0.668$; Chao1 richness $p = 0.683$). Time elapsing since first feeding was the only significant predictor of alpha microbial diversity in the gut of Nile tilapia, as the microbiome of fish became less diverse over time (Figure 4a – Shannon diversity: Time estimate = –0.023, SE = 0.002, $t_{121.58} = -10.92$, $p < 0.001$; Figure 4b – Chao1 richness: Time estimate = –0.307, SE = 0.113, $t_{121.79} = -2.79$, $p = 0.007$). Analysis of beta diversity using PERMANOVA on Bray–Curtis dissimilarity distances reached a similar conclusion. Time since first feeding was a good predictor of beta diversity ($F_{1,127} = 224.30$, $p = 0.001$), whereas diet ($F_{5,127} = 1.358$, $p = 0.201$) and fish size ($F_{1,127} = 0.964$, $p = 0.870$) had no significant effect.

3.6 | Variation in the composition of the gut microbiome

The most abundant ASVs in the gut microbiome of Nile tilapia changed substantially over time (Figure 5). At day 21, five ASVs (two from the

TABLE 3 Fatty acid (FA) profile (%) of the six experimental diets tested in juvenile Nile tilapia.

Fatty acid	Diet					
	Control	Fish	Plant	A33	A66	A100
C08:0 Caprylic acid	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
C10:0 Capric acid	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
C11:0 Undecylic acid	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
C12:0 Lauric acid	<0.05	<0.05	<0.05	<0.05	<0.05	0.05
C13:0 Tridecylic acid	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
C14:0 Myristic acid	0.82	1.20	0.48	0.81	0.75	0.71
C14:1 Myristoleic acid	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
C15:0 Pentadecanoic acid	0.09	0.12	0.07	0.09	0.10	0.10
C15:1 Pentadecylic acid	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
C16:0 Palmitic acid	10.25	10.17	10.58	11.00	12.34	13.32
C16:1 Palmitoleic acid	0.99	1.42	0.59	0.94	0.82	0.74
C17:0 Heptadecanoic acid	0.12	0.13	0.11	0.12	0.11	0.10
C17:1 Heptadecenoic acid	0.21	0.23	0.20	0.17	0.20	0.17
C18:0 Stearic acid	4.02	3.83	4.15	4.02	3.78	3.60
C18:1 Oleic acid	23.67	26.55	20.82	22.42	19.52	17.66
C18:2 Linoleic acid	29.63	23.47	35.87	27.02	23.50	20.11
C18:3 Linolenic acid	23.75	22.96	23.41	24.04	23.29	22.37
C18:4 Stearidonic acid	0.20	0.31	0.09	0.18	0.17	0.17
C20:0 Arachidic acid	0.26	0.25	0.27	0.26	0.26	0.26
C20:1 Gadoleic acid	0.91	1.59	0.29	0.77	0.43	0.23
C20:4 Arachidonic acid	0.12	0.19	0.11	<0.05	0.11	0.12
C20:5 Eicosapentaenoic acid (EPA)	1.03	1.42	0.62	0.95	0.86	0.83
C22:0 Behenic acid	0.24	<0.05	0.29	<0.05	0.21	0.24
C22:1 Erucic acid	0.36	0.73	<0.05	0.27	<0.05	<0.05
C22:4 Adrenic acid	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
C22:5 Docosapentaenoic acid	0.24	0.49	0.08	0.23	0.22	0.14
C22:6 Docosahexaenoic acid (DHA)	1.00	1.80	0.35	3.95	9.27	13.92
C24:0 Lignoceric acid	<0.05	0.09	0.12	0.09	0.13	0.12
n – 6:*n* – 3 ratio	1.13	0.88	1.47	0.92	0.70	0.54
n – 3:*n* – 6 ratio	0.88	1.14	0.68	1.09	1.43	1.85
DHA:EPA ratio	0.97	1.27	0.56	4.16	10.78	16.77
Free FA extracted fat	3.80	4.20	4.60	3.90	4.90	28.60
Monounsaturated FA	26.14	30.52	21.90	24.57	20.97	18.80
Polyunsaturated FA	55.97	50.64	60.53	56.37	57.42	57.66
Saturated FA	15.80	15.81	16.08	16.39	17.71	18.50
Unidentified FA	2.09	3.03	1.49	2.67	3.90	5.04

family Enterobacteriaceae, two different *Aeromonas* spp. and one *Pseudomonas* spp.) dominated the bacterial gut community of Nile tilapia, accounting for ~60% of the total abundance. In contrast, at day 94, the gut microbiome was dominated almost exclusively (90%) by five different ASVs, consisting of two different *Cetobacterium* spp., one *Romboutsia* spp., one from the family Roseiflexaceae and one from the family Enterobacteriaceae (Figure 5).

3.7 | Effect of diet on the gut microbiome composition

At the end of the 3 months feeding trial, 63 pairwise diet comparisons for ASV abundance were statistically significant after controlling for multiple tests (Table 5). Most of the differences in bacterial communities were between the fish fed the plant and the three microalgae

TABLE 4 Survival and growth parameters (mean ± SD) of Nile tilapia under different experimental diets compared to controls.

Diet	Survival rate (%)	Initial BW (g)	Final BW (g)	Initial length (mm)	Final length (mm)	SGR (%BW/day)	*K*
Control	81.3 ± 23.13	0.029 ± 0.002	1.651 ± 0.201	10.78 ± 0.32	45.2 ± 2.26	4.48 ± 0.259	1.79 ± 0.149
Fish	90.7 ± 23.13	0.029 ± 0.003	2.750 ± 0.326	10.56 ± 0.38	55.5 ± 2.57	5.04 ± 0.140	1.61 ± 0.060
Plant	86.2 ± 4.07	0.028 ± 0.002	1.570 ± 0.249	10.63 ± 0.28	44.8 ± 2.90	4.43 ± 0.270	1.74 ± 0.091
A33	86.7 ± 15.38	0.029 ± 0.002	2.789 ± 0.415	10.78 ± 0.28	53.3 ± 2.90	5.06 ± 0.101	1.84 ± 0.183
A66	96.0 ± 1.33	0.028 ± 0.003	1.955 ± 0.277	11.11 ± 0.48	48.6 ± 2.49	4.68 ± 0.131	1.71 ± 0.094
A100	68.9 ± 7.81	0.025 ± 0.001	3.360 ± 0.416	10.00 ± 0.24	58.2 ± 3.04	5.20 ± 0.447	1.71 ± 0.085

Abbreviation: SRG, specific growth rates.

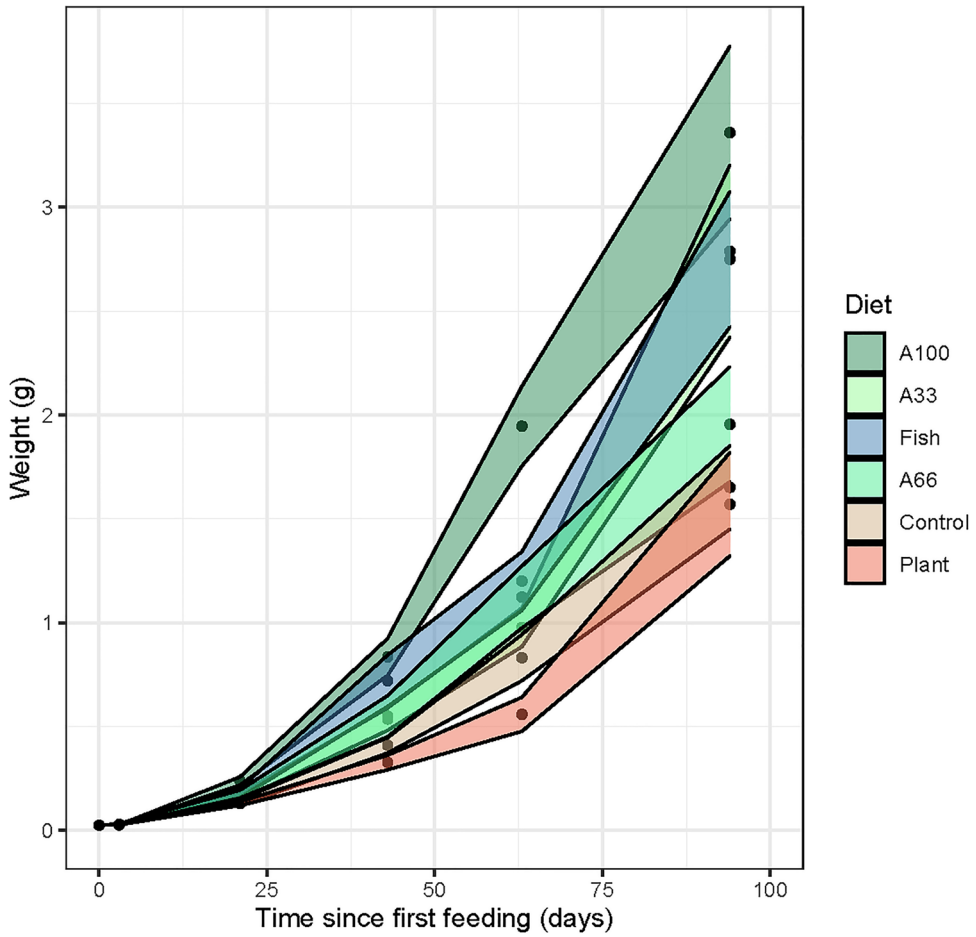

FIGURE 2 Weight gain (g, mean ± SE) of Nile tilapia fed six experimental diets that varied only in the origin of dietary oil (plant, fish, algae) over a 94-day feeding trial. The control (reference) diet had 50% plant oil and 50% fish oil.

diets. Across all diets, the ASVs that differed the most in abundance belonged to three bacterial families: Aeromonadaceae, Peptostreptococcaceae and Mycobacteriaceae (Figure 5). Therefore, specific targeted tests were performed at family level. The overall abundance of Aeromonadaceae differed significantly between diets (LRT, chi-square = 12.65, npar = 5, $p = 0.027$) and increased with fish weight (LRT, chi-square = 21.72, npar = 1, $p < 0.001$). Pairwise Tukey contrasts with Bonferroni adjustment for multiple comparisons indicated that Aeromonadaceae was highest among fish fed plant oil and lowest among fish fed algal and fish oils (Figure 6a). The abundance of Peptostreptococcaceae also differed significantly between diets (LRT, chi-square = 22.02, npar = 5, $p < 0.001$) and varied with fish weight (LRT, chi-square = 40.49, npar = 1, $p < 0.001$). Pairwise comparisons revealed that Peptostreptococcaceae was highest among fish fed algal and fish oils, increasing with the level of algal oil inclusion, and was lowest among fish fed plant oil ($p < 0.001$; Figure 6b). Mycobacteri-

TABLE 5 Number of ASVs that differed significantly in abundance ($p_{adj} < 0.05$) across pairwise diet comparisons at the end of the feeding trial (day 94).

Diet	Diet					
	Plant	Fish	Control	A33	A66	A100
Plant	-	-	-	-	-	-
Fish	13	-	-	-	-	-
Control	2	0	-	-	-	-
A33	7	0	1	-	-	-
A66	13	1	1	1	-	-
A100	10	3	4	4	3	-

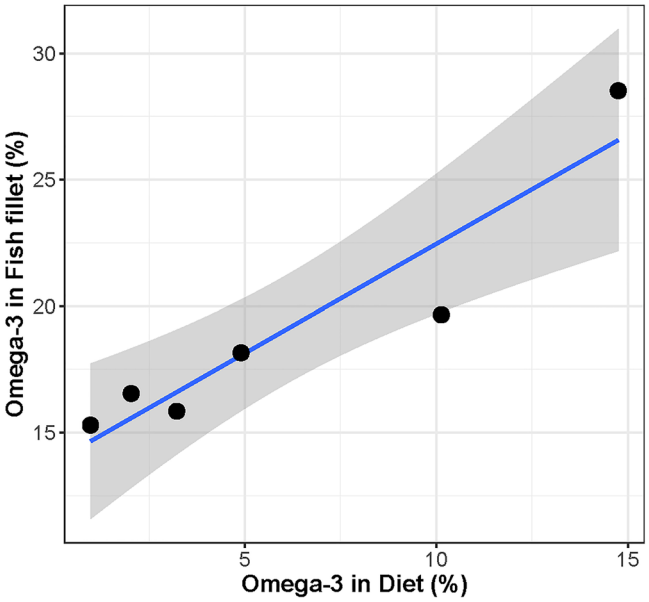

FIGURE 3 Relationship between dietary omega – 3 content and omega – 3 deposition in the fat of the fish fillet. Each point represents a pooled sample of three fish for each experimental diet and the grey envelope the 95 CI.

aceae abundance varied with diet (LRT, chi-square = 11.42, npar = 5, $p = 0.044$) and fish weight (LRT, chi-square = 59.04, npar = 1, $p < 0.001$). They were most abundant among fish enriched with the 100% microalgae oil and least abundant among fish fed the control diet ($p < 0.05$; Figure 6c).

4 | DISCUSSION

Our study indicates that the type of dietary oil consumed from first feeding through the first 3 months of development had a marked effect on the growth, omega – 3 fillet content and composition of the gut microbiome of Nile tilapia. Tilapia raised on a diet where all the oil originated from the microalgae *Schizochytrium* grew twice as fast as fish fed with plant oil or a mixture of plant and fish oil, and as fast as fish raised on 100% fish oil or 33% algal oil (Figure 2; Table 4). A strong positive relation was found between omega – 3 content in the diet and

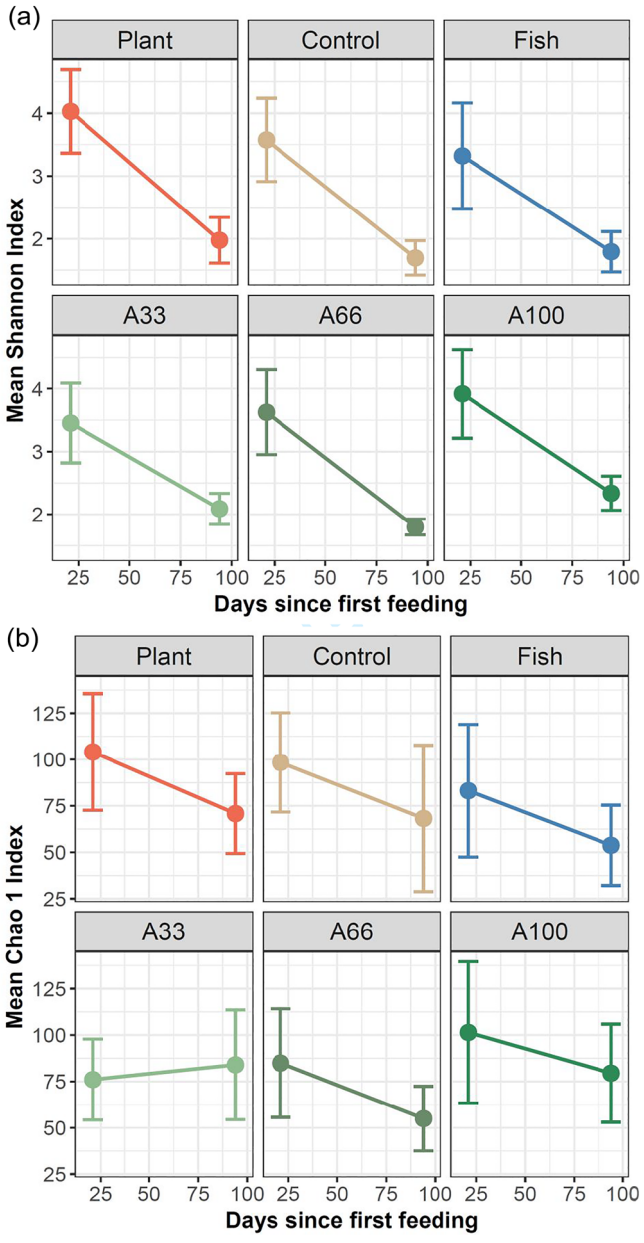

FIGURE 4 Changes in microbiome alpha diversity of Nile tilapia fed on six different diets at day 21 and day 94 of the feeding trial: (a) Shannon diversity and (b) Chao 1 richness.

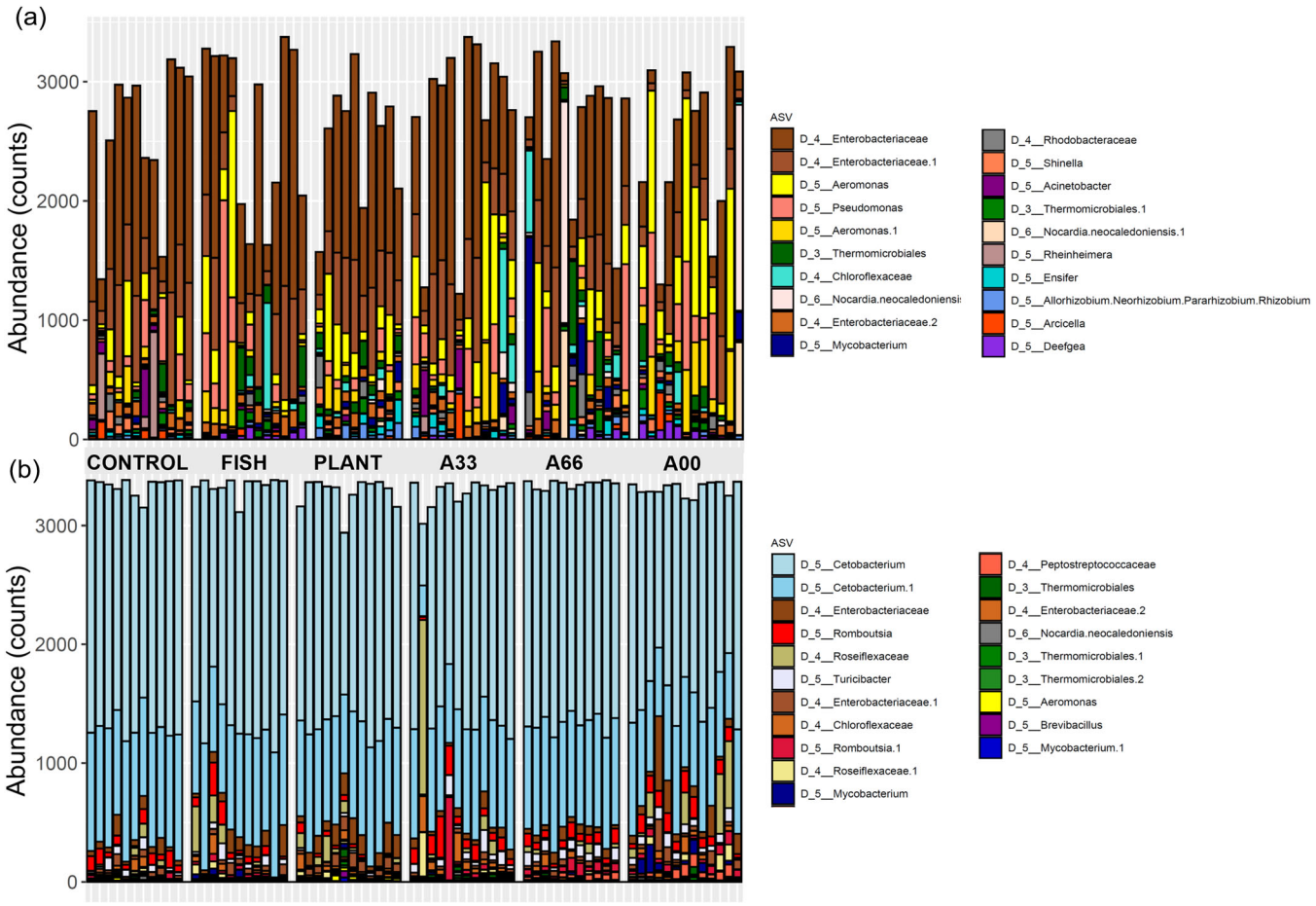

FIGURE 5 Relative abundance (read counts) of the 20 most frequent ASVs at day 21 (top, a) and day 94 (bottom, b) of Nile tilapia fed on six different diets since first feeding. Each bar represents the ASVs of an individual with a level of classification denoted by D_3 (Order), D_4 (Family), D_5 (Genus) and D_6 (Species).

the omega − 3 content of the fish fillet. Thus, tilapia fed a diet with 100% algal oil had almost twice as much omega − 3 in the fat of the fish fillet than fish fed plant oil. A 1% increase in omega − 3 in the diet resulted in a ~0.9% increase in omega − 3 in the fish fillet, suggesting that there is considerable scope for making Nile tilapia more nutritious using microalgae, as indicated in other studies (dos Santos et al., 2019; Sarker, Kapuscinski, et al., 2016; Sarker, Kapuscinski, McKuin, et al., 2020; Stoneham et al., 2018).

Several studies have shown improved growth of Nile tilapia fed whole-cell microalgae (Magbanua & Ragaza, 2024; Trevi et al., 2023), but little is known about the growth benefits of using microalgae oil as a lipid source. The incorporation of the marine microalgae *Nannochloropsis oculata* (whole cell) in tilapia feeds resulted in a significant increase in feed conversion ratio compared to controls (Sarker et al., 2018). Tilapia fed aquafeeds supplemented with whole-cell *Chlorella vulgaris* grew faster (SGR = 1.5%–1.7% bw/day) than controls (Abdel-Tawwab et al., 2022), as did tilapia fed with whole-cell *Schizochytrium* sp., growth rates being 0.87%/day (Sarker, Kapuscinski, McKuin, et al., 2020) and 3.5%/day (Sarker, Kapuscinski, et al., 2016). In our study, Nile tilapia fed on 100% *Schizochytrium* oil replacement grew at a very high rate (SGR = 5.20%/day) but direct comparisons with previous studies are probably unwarranted, as our fish were substantially smaller and would have grown at a faster rate.

No difference in survival was found between diets, which all showed high survival (mean = 85%) in line with values reported for commercial hatcheries of Nile tilapia (Boyd et al., 2005). We cannot tell if the fish fed the 100% algal diet grew faster because they had better assimilation or because they had consumed more food. The fish in our study were fed to satiation three times a day, and although we did not notice any obvious difference in feeding activity, it is possible that a *Schizochytrium*-rich diet may have had better palatability for a facultative herbivorous fish like Nile tilapia (Levina et al., 2021). Previous studies on Nile tilapia have shown low palatability for *Spirulina platensis* (Allen, 2016) but high palatability for *Nannochloropsis oculate* (Sarker et al., 2018), and this is an area that merits further study.

The early life stage is a critical period for the development of the gut microbiota, with potentially long-lasting consequences for vertebrate health (Tamburini et al., 2016). Previous work on the effects of the microalgae *Schizochytrium* on the gut microbiome of fish focused on older individuals, whose microbiome may have already been established (de Souza et al., 2020; Katerina et al., 2020; Kousoulaki et al., 2020; Lyons et al., 2017; Sagaram et al., 2021), as first feeding induces

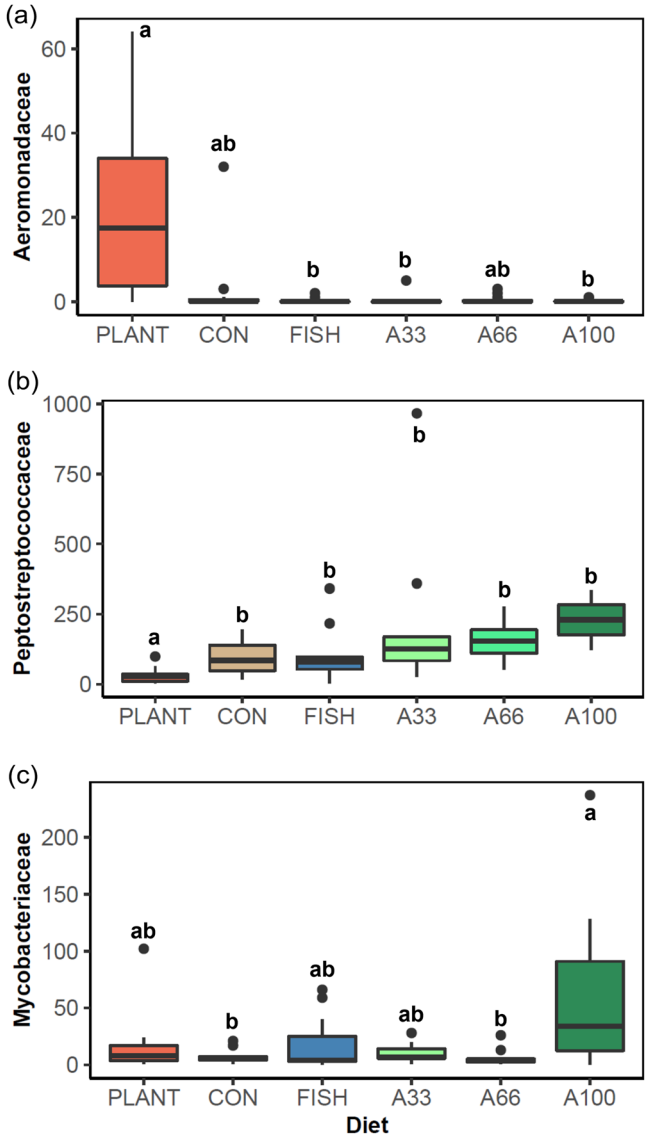

FIGURE 6 Bacterial families that differed in relative abundance (read counts) across pairwise diet comparisons at the end of the feeding trial (day 94): (a) *Aeromonadaceae*; (b) *Peptostreptococcaceae*; (c) *Mycobacteriaceae*. Diets that differ significantly in read counts at $p_{adj} < 0.05$ have different subscripts.

a substantial change and maturation of the gut microbiota (Ingerslev et al., 2014). The early-rearing environment can have a marked effect on the gut microbiome of Nile tilapia (Deng et al., 2021; Deng et al., 2022). By conducting our study from first feeding, we were able to reveal the role of diet in the early development of the gut microbiome without the confounding effects of variation in microbial colonization history. Our results show that the microbiome of Nile tilapia changes rapidly over the first months of life, becoming less diverse as fish develop. Three weeks after first feeding, the gut microbiome of Nile tilapia was dominated by Enterobacteriaceae, *Aeromonas* spp. and *Pseudomonas* spp., as reported previously for several species, including Nile tilapia (Giatsis et al., 2015), pikeperch (*Sander lucioperca*) (Dulski et al., 2018), yellowtail kingfish (*Seriola lalandi*) (Wilkes Walburn et al., 2019) and white cachama (*Piaractus brachypomus*) (Castañeda-Monsalve et al., 2019).

As the fish develop and begin to feed, the gut microbiome becomes less diverse and stabilises (Giatsis et al., 2015; Ingerslev et al., 2014). Consistent with this, we observed a reduction in diversity and richness, and a significant shift in community composition towards a gut microbiome dominated by Fusobacteriaceae, Peptostreptococcaceae and Enterobacteriaceae 3 months after first feeding. A high abundance of Fusobacteriaceae in the gut microbiome of juvenile fish has been reported previously for Nile tilapia (Adeoye et al., 2016) and other warm water fish inhabiting both freshwater and brackish environments (Castañeda-Monsalve et al., 2019; Nayak et al., 2020), and it has been proposed that this may form the core microbiome of non-carnivorous fish (Nayak et al., 2020). Fusobacteria are able to degrade complex dietary fibres through anaerobic fermentation (Vital et al., 2014) and may confer benefits to omnivorous and herbivorous hosts (Castañeda-Monsalve et al., 2019; Martin-Gallausiaux et al., 2018).

The tendency for the fish gut microbiome to become less diverse, and presumably more stable, over time does not appear to be contingent on a particular diet, as it was observed in our study across six diets that varied widely in oil source and omega − 3 content. Diet had no significant effects on alpha microbiome diversity, as reported by others (Adeoye et al., 2016), but it altered the composition of the microbiome of Nile tilapia in important ways. In particular, fish fed microalgae diets displayed a high relative abundance of bacteria belonging to the family Peptostreptococcaceae and a low incidence of bacteria belonging to the family Aeromonadaceae. A recent study has shown that the FA composition of the diet can alter the abundance of potentially pathogenic bacteria as well as the flexibility and thickness of the intestinal membraned of Nile tilapia, affecting its ability to assimilate and digest food (Agbohessou et al., 2024).

The family Aeromonadaceae not only dominates the microbiota of many freshwater fish (Butt & Volkoff, 2019; Kashinskaya et al., 2018; Shang et al., 2021) but also includes a large number of opportunistically pathogenic species, such as *Aeromonas hydrophila*, *Aeromonas salmonicida* and *Aeromonas veronii*. An increase in the abundance of *Aeromonas* spp. has been associated with acute stress (Uren Webster, Consuegra, et al., 2020) and compromised health in Atlantic salmon (Wang et al., 2018). Here, we observed a marked increase in the relative abundance of *Aeromonas* spp. and the family Aeromonadaceae in tilapia fed the 100% plant (soya) oil, which is consistent with a stress response. Nutritional stress resulting from feeding on diets containing only vegetable oil has previously been found to impair growth in Nile tilapia (Betiku et al., 2016), cause intestinal inflammation and increase susceptibility to disease in other fish species (Booman et al., 2018; Coronado et al., 2019; Kiron et al., 2020). These findings suggest that the use of vegetable oils, which are rich in linoleic acid (C18:2 – *n* − 6) but naturally deficient in omega − 3, promotes the growth of bacteria typically associated with gut disorders in many vertebrates (Villamil et al., 2018), including humans (David et al., 2014) and fish (Austin & Austin, 2016; Zhou, Ringø, et al., 2018).

In contrast, inclusion of the microalgae *Schizochytrium* in the diet appears to be beneficial, improving growth not only in Nile tilapia as

our study and other studies have shown (de Souza et al., 2020; dos Santos et al., 2019; Sarker, Gamble, et al., 2016) but also in other species, such as seabream (Ganuza et al., 2008), channel catfish (Li et al., 2009) and Atlantic salmon (Kousoulaki et al., 2016).

The abundance of Peptostreptococcaceae also differed significantly between fish fed different diets, being lowest among fish fed plant oil and highest among fish fed algal oil, increasing with the level of algal oil inclusion. The administration of plant-based oils has been reported to lower the abundance of Peptostreptococcaceae in Atlantic salmon (Egerton et al., 2020; Hartviksen et al., 2014), and the same appears to be true for Nile tilapia. Peptostreptococcaceae seems to thrive in anaerobic environments rich in saturated and unsaturated straight-chain C12–C19 FAs (Gerritsen et al., 2018; Li et al., 2018). Further studies on the potential relation between Peptostreptococcaceae abundance and omega − 3 content seem warranted, as are studies that elucidate the functional role of this family in the fish gut microbiota and how it may influence fish health.

The third bacterial family that differed significantly among fish fed different diets was Mycobacteriaceae, a family common in the microbiota of many fish (Francis-Floyd, 2011), including tilapia (Manrique et al., 2019). Mycobacteriaceae was most abundant in fish fed the 100% microalgal oil diet, but whether this confers any benefits is uncertain. As for the Peptostreptococcaceae family, some members of the *Mycobacterium* genus are also potentially pathogenic (Hashish et al., 2018; Vega-López, 2020), which serves to emphasize the dangers of drawing conclusions at family level. The mechanisms that promote the growth of some bacteria in the fish gut and the suppression of others are not clear (Ringø et al., 2010), but understanding responses to dietary change at species (or even strain) level are typically necessary to predict effects on gut health (Gentile & Weir, 2018).

5 | CONCLUSIONS

Our study shows that a diet rich in vegetable oil, particularly in linoleic acid (C18:2 (*n* − 6), was associated with a decrease in decrease in Peptostreptococcaceae and an increase in Aeromonadaceae, commonly associated with intestinal inflammation in the fish gut, whereas a *Schizochytrium* diet rich in omega − 3 and a high DHA:EPA ratio promoted the proliferation of Mycobacteriaceae and resulted in faster growth and higher omega − 3 deposition in the fish fillet. Taken together our results indicate that *Schizochytrium* oil could serve as a replacement of fish and plant oil and enhance growth. Without diet fortification, tilapia may not provide enough *n* − 3 PUFAs for humans (Strobel et al., 2012; Young, 2009) and result in PUFA *n* − 3:*n* − 6 imbalance (Alam et al., 2014). Although our study did not grow fish to market size, it confirms the dietary omega − 3 benefits of using microalgae – reviewed by Trevi et al. (2023), and suggests that *Schizochytrium* oil could be used for the production of more nutritious and sustainable Nile tilapia without impacting on gut health.

AUTHOR CONTRIBUTIONS

Sergio Trevi: Conceptualization; formal analysis; investigation; writing—original draft. **Tamsyn Uren Webster**: Conceptualization; formal analysis; supervision; writing—review and editing. **Sofia Consuegra**: Conceptualization; funding acquisition; project administration; resources; supervision; writing—review and editing. **Carlos Garcia de Leaniz**: Conceptualization; formal analysis; funding acquisition; project administration; resources; supervision; visualization; writing—original draft.

ACKNOWLEDGEMENTS

We are grateful to Paul Howes, Rebecca Stringwell and CSAR technicians for help with the study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest

FUNDING INFORMATION

Funding for this research was provided by a PhD scholarship to ST and the ERDF SMARTAQUA project.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analysed during the current study are available in the Figshare repository https://doi.org/10.6084/m9.figshare.22903550.v1. The statistical outputs and R code used for the analysis are given in Supplementary Material (Table S1).

ETHICS STATEMENT

The study was conducted following approval by Swansea University's Animal Welfare and Ethical Review Body (Permit SU-Ethics-Student-300919/1210). The results of the study are reported according to the ARRIVE guidelines for studies involving live animals https://arriveguidelines.org.

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PEER REVIEW

The peer review history for this article is available at: https://publons.com/publon/10.1002/aff2.164.

REFERENCES

Abdel-Tawwab, M., Mousa, M.A., Mamoon, A., Abdelghany, M.F., Abdel-Hamid, E.A., Abdel-Razek, N. et al. (2022) Dietary *Chlorella vulgaris* modulates the performance, antioxidant capacity, innate immunity, and disease resistance capability of Nile tilapia fingerlings fed on plant-based diets. *Animal Feed Science and Technology*, 283, 115181.

Adeoye, A.A., Yomla, R., Jaramillo-Torres, A., Rodiles, A., Merrifield, D.L. & Davies, S.J. (2016) Combined effects of exogenous enzymes and probiotic on Nile tilapia (*Oreochromis niloticus*) growth, intestinal morphology and microbiome. *Aquaculture*, 463, 61–70.

Agbohessou, P.S., Mandiki, R., Mes, W., Blanquer, A., Gérardy, M., Garigliany, M.M. et al. (2024) Effect of fatty acid-enriched black soldier fly larvae meal combined with chitinase on the metabolic processes of Nile tilapia. *British Journal of Nutrition*, 131, 1–16.

Alam, M.A., Moniruzzaman, S.M., Akter, S., Sarker, B.S. & Minar, M.H. (2014) Omega-3 and 6 fatty acid imbalance found in tilapia feed in various

tilapia farms of Southeastern region of Bangladesh. *World Journal of Zoology*, 9, 227–234.

Alfaddagh, A., Elajami, T.K., Saleh, M., Elajami, M., Bistrian, B.R. & Welty, F.K. (2018) The effect of eicosapentaenoic and docosahexaenoic acids on physical function, exercise, and joint replacement in patients with coronary artery disease: A secondary analysis of a randomized clinical trial. *Journal of Clinical Lipidology*, 12, 937–947.e932.

Allen, K. (2016) Evaluating *Spirulina* as a protein source in Nile tilapia (*Oreochromis niloticus*) grow-out diets. In: *Department of biology*. Kingston, Ontario, Canada: Queen's University. pp. 87.

Ansari, F.A., Guldhe, A., Gupta, S.K., Rawat, I. & Bux, F. (2021) Improving the feasibility of aquaculture feed by using microalgae. *Environmental Science and Pollution Research*, 28, 43234–43257.

Austin, B. & Austin, D.A. (2016) Aeromonadaceae representative (*Aeromonas salmonicida*). In: *Bacterial fish pathogens*. Singapor: Springer. pp. 215–321.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.

Bene, C. & Heck, S. (2005) Fish and food security in Africa. *NAGA World Fish Center Quarterly*, 28, 8–13.

Betiku, O.C., Barrows, F.T., Ross, C. & Sealey, W. (2016) The effect of total replacement of fish oil with DHA-Gold® and plant oils on growth and fillet quality of rainbow trout (*Oncorhynchus mykiss*) fed a plant-based diet. *Aquaculture Nutrition*, 22, 158–169.

Bhujel, R. & Suharman, I. (2021) Food and nutrition security in the context of COVID-19 and the potential role of tilapia aquaculture. In: IOP conference series: Earth and environmental science. Bristol, England: IOP Publishing. pp. 012023.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R. et al. (2018) Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome*, 6, 90.

Bolger, T. & Connolly, P.L. (1989) The selection of suitable indexes for the measurement and analysis of fish condition. *Journal of Fish Biology*, 34, 171–182.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F. (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37(8), 852–857.

Booman, M., Forster, I., Vederas, J.C., Groman, D.B. & Jones, S.R.M. (2018) Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar*) and Chinook salmon (*Oncorhynchus tshawytscha*) but not in pink salmon (*O. gorbuscha*). *Aquaculture*, 483, 238–243.

Boyd, C.E., McNevin, A.A., Clay, J. & Johnson, H.M. (2005) Certification issues for some common aquaculture species. *Reviews in Fisheries Science*, 13, 231–279.

Butt, R.L. & Volkoff, H. (2019) Gut microbiota and energy homeostasis in fish. *Frontiers in endocrinology*, 10, 9.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13, 581.

Carr, M. (2017) Algal biotechnology is just beginning to blossom. *Industrial Biotechnology*, 13, 221–222.

Castañeda-Monsalve, V.A., Junca, H., García-Bonilla, E., Montoya-Campuzano, O.I. & Moreno-Herrera, C.X. (2019) Characterization of the gastrointestinal bacterial microbiome of farmed juvenile and adult white Cachama (*Piaractus brachypomus*). *Aquaculture*, 512, 734325.

Chuenpagdee, R., Degnbol, P., Bavinck, M., Jentoft, S., Johnson, D., Pullin, R. et al. (2005) Challenges and concerns in capture fisheries and aquaculture. In: *Fish for life: Interactive governance for fisheries*. Amsterdam, the Netherlands: Amsterdam University Press. pp. 25–40.

Coronado, M., Solis, C.J., Hernandez, P.P. & Feijóo, C.G. (2019) Soybean meal-induced intestinal inflammation in Zebrafish is T cell-dependent and has a Th17 cytokine profile. *Frontiers in Immunology*, 10, 610.

Crawley, M.J. (2013) *The R book*. Chichester, UK: John Wiley & Sons.

da Silva Dias, P., Pereira, E.M., Barbieri, P.A., Ferreira, R.L., Júnior, R.D.A.S., da Silva Loiola, A. et al. (2020) Alternative Ingredients for Nile tilapia (*Oreochromis niloticus*). Bibliographical review. *Brazilian Journal of Development*, 6, 5767–5785.

David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E. et al. (2014) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505, 559–563.

Davis, D.J., Hecht, P.M., Jasarevic, E., Beversdorf, D.Q., Will, M.J., Fritsche, K. et al. (2017) Sex-specific effects of docosahexaenoic acid (DHA) on the microbiome and behavior of socially-isolated mice. *Brain, Behavior, and Immunity*, 59, 38–48.

de Souza, F.P., de Lima, C.S., Urrea-Rojas, A.M., Suphoronski, S.A., Facimoto, C.T., Júnior, J.D.S.B. et al. (2020) Effects of dietary supplementation with a microalga (*Schizochytrium* sp.) on the hemato-immunological, and intestinal histological parameters and gut microbiota of Nile tilapia in net cages. *PLoS One*, 15, e0226977.

Deng, Y., Kokou, F., Eding, E.H. & Verdegem, M.C.J. (2021) Impact of early-life rearing history on gut microbiome succession and performance of Nile tilapia. *Animal Microbiome*, 3, 81.

Deng, Y., Verdegem, M.C.J., Eding, E. & Kokou, F. (2022) Effect of rearing systems and dietary probiotic supplementation on the growth and gut microbiota of Nile tilapia (*Oreochromis niloticus*) larvae. *Aquaculture* 546, 737297.

dos Santos, S.K.A., Schorer, M., Moura, G.D.S., Lanna, E.A.T. & Pedreira, M.M. (2019) Evaluation of growth and fatty acid profile of Nile tilapia (*Oreochromis niloticus)* fed with *Schizochytrium* sp. *Aquaculture Research*, 50, 1068–1074.

Dulski, T., Zakęś, Z. & Ciesielski, S. (2018) Characterization of the gut microbiota in early life stages of pikeperch *Sander lucioperca*. *Journal of Fish Biology* 92, 94–104.

Egerton, S., Wan, A., Murphy, K., Collins, F., Ahern, G., Sugrue, I. et al. (2020) Replacing fishmeal with plant protein in Atlantic salmon *(Salmo salar)* diets by supplementation with fish protein hydrolysate. *Scientific Reports* 10, 4194.

El-Sayed, A.-F.M. (1999) Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. *Aquaculture* 179, 149–168.

El-Sayed, A.-F.M. (2013) Tilapia feed management practices in sub-Saharan Africa. In: *On-farm feeding and feed management in aquaculture. FAO Fisheries and Aquaculture Technical Paper*. Rome: FAO. pp. 377–405.

Emery, J.A., Norambuena, F., Trushenski, J. & Turchini, G.M. (2016) Uncoupling EPA and DHA in fish nutrition: Dietary demand is limited in Atlantic salmon and effectively met by DHA alone. *Lipids* 51, 399–412.

Food and Agriculture Organization (FAO). (2020) *The state of world fisheries and aquaculture, 2020*. Rome: Food & Agriculture Organization of the United Nations. Fisheries Department.

Food and Agriculture Organization (FAO). (2022) Oreochromis niloticus. In: *Cultured aquatic species information programme*. Rome: FAO Fisheries and Aquaculture Department.

Fedorova-Dahms, I., Marone, P., Bauter, M. & Ryan, A. (2011) Safety evaluation of DHA-rich algal oil from *Schizochytrium* sp. *Food and Chemical Toxicology* 49, 3310–3318.

Fitzsimmons, K., Martinez-Garcia, R. & Gonzalez-Alanis, P. (2011) Why tilapia is becoming the most important food fish on the planet. In: Liu, L. & Fitzsimmons, K. (Eds.) Better science, better fish, better life proceedings of the ninth international symposium on tilapia in aquaculture (ISTA 9). Wallisellen: ISTA, pp. 1–8.

Francis-Floyd, R. (2011) *Mycobacterial infections of fish*. Stoneville: Southern Regional Aquaculture Center USA.

Ganuza, E., Benítez-Santana, T., Atalah, E., Vega-Orellana, O., Ganga, R. & Izquierdo, M. (2008) *Crypthecodinium cohnii* and *Schizochytrium* sp. as potential substitutes to fisheries-derived oils from seabream (*Sparus aurata*) microdiets. *Aquaculture* 277, 109–116.

Gentile, C.L. & Weir, T.L. (2018) The gut microbiota at the intersection of diet and human health. *Science* 362, 776–780.

Gerritsen, J., Umanets, A., Staneva, I., Hornung, B., Ritari, J., Paulin, L., Rijkers, G.T., de Vos, W.M. & Smidt, H. (2018) *Romboutsia hominis* sp. nov., the first human gut-derived representative of the genus *Romboutsia*, isolated from ileostoma effluent. *International Journal of Systematic and Evolutionary Microbiology* 68, 3479–3486.

Giatsis, C., Sipkema, D., Smidt, H., Heilig, H., Benvenuti, G., Verreth, J. et al. (2015) The impact of rearing environment on the development of gut microbiota in tilapia larvae. *Scientific Reports* 5, 18206.

Gupta, M. (2006) *Challenges in sustaining and increasing fish production to combat hunger and poverty in Asia*. NAGA, 29, 4–10.

Hartviksen, M., Vecino, J.L.G., Ringø, E., Bakke, A.-M., Wadsworth, S., Krogdahl, Å. et al. (2014) Alternative dietary protein sources for Atlantic salmon (*Salmo salar* L.) effect on intestinal microbiota, intestinal and liver histology and growth. *Aquaculture Nutrition* 20, 381–398.

Hashish, E., Merwad, A., Elgaml, S., Amer, A., Kamal, H., Elsadek, A. et al. (2018) Mycobacterium marinum infection in fish and man: Epidemiology, pathophysiology and management; a review. *Veterinary Quarterly* 38, 35–46.

Hasselberg, A.E., Aakre, I., Scholtens, J., Overå, R., Kolding, J., Bank, M.S. et al. (2020) Fish for food and nutrition security in Ghana: Challenges and opportunities. *Global Food Security* 26, 100380.

Hussein, J., Attia, M.F., El Bana, M., El-Daly, S.M., Mohamed, N., El-Khayat, Z. et al. (2019) Solid state synthesis of docosahexaenoic acid-loaded zinc oxide nanoparticles as a potential antidiabetic agent in rats. *International Journal of Biological Macromolecules* 140, 1305–1314.

Ingerslev, H.-C., von Gersdorff Jørgensen, L., Strube, M.L., Larsen, N., Dalsgaard, I., Boye, M. et al. (2014) The development of the gut microbiota in rainbow trout (*Oncorhynchus mykiss*) is affected by first feeding and diet type. *Aquaculture* 424, 24–34.

Jung, I. & Lovitt, R. (2010) Integrated production of long chain polyunsaturated fatty acids (PUFA)-rich *Schizochytrium* biomass using a nutrient supplemented marine aquaculture wastewater. *Aquacultural Engineering* 43, 51–61.

Karapanagiotidis, I.T., Bell, M.V., Little, D.C., Yakupitiyage, A. & Rakshit, S.K. (2006) Polyunsaturated fatty acid content of wild and farmed tilapias in Thailand: Effect of aquaculture practices and implications for human nutrition. *Journal of Agricultural and Food Chemistry* 54, 4304–4310.

Kashinskaya, E.N., Simonov, E.P., Kabilov, M.R., Izvekova, G.I., Andree, K.B. & Solovyev, M.M. (2018) Diet and other environmental factors shape the bacterial communities of fish gut in an eutrophic lake. *Journal of Applied Microbiology* 125, 1626–1641.

Katerina, K., Berge, G.M., Turid, M., Aleksei, K., Grete, B., Trine, Y. et al. (2020) Microalgal *Schizochytrium limacinum* biomass improves growth and filet quality when used long-term as a replacement for fish oil, in modern salmon diets. *Frontiers in Marine Science* 7, 57.

Kiron, V., Park, Y., Siriyappagouder, P., Dahle, D., Vasanth, G.K., Dias, J. et al. (2020) Intestinal transcriptome analysis reveals soy derivative-linked changes in Atlantic salmon. *Frontiers in immunology* 11, 3013.

Konnert, G.D.P., Gerrits, W.J.J., Gussekloo, S.W.S., Schrama, J.W. (2022) Balancing protein and energy in Nile tilapia feeds: A meta-analysis. *Reviews in Aquaculture*. 14, 1757–1778.

Köprücü, K. & Özdemir, Y. (2005) Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 250, 308–316.

Kousoulaki, K., Berge, G.M., Turid, M., Aleksei, K., Grete, B., Trine, Y. et al. (2020) Microalgal *Schizochytrium limacinum* biomass improves growth and filet quality when used long-term as a replacement for fish oil, in modern salmon diets. *Frontiers in Marine Science* 7, 57.

Kousoulaki, K., Mørkøre, T., Nengas, I., Berge, R. & Sweetman, J. (2016) Microalgae and organic minerals enhance lipid retention efficiency and fillet quality in Atlantic salmon (*Salmo salar* L.). *Aquaculture* 451, 47–57.

Kris-Etherton, P.M., Harris, W.S. & Appel, L.J. (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 106, 2747–2757.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H. (2017) lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software* 82, 1–26.

Lauritzen, L., Brambilla, P., Mazzocchi, A., Harsløf, L., Ciappolino, V. & Agostoni, C. (2016) DHA effects in brain development and function. *Nutrients* 8, 6.

Levina, A.D., Mikhailova, E.S. & Kasumyan, A.O. (2021) Taste preferences and feeding behaviour in the facultative herbivorous fish, Nile tilapia *Oreochromis niloticus*. *Journal of Fish Biology* 98, 1385–1400.

Li, M.H., Robinson, E.H., Tucker, C.S., Manning, B.B. & Khoo, L. (2009) Effects of dried algae *Schizochytrium* sp., a rich source of docosahexaenoic acid, on growth, fatty acid composition, and sensory quality of channel catfish *Ictalurus punctatus*. *Aquaculture* 292, 232–236.

Li, X., Liu, G., Liu, S., Ma, K. & Meng, L. (2018) The relationship between volatile fatty acids accumulation and microbial community succession triggered by excess sludge alkaline fermentation. *Journal of Environmental Management* 223, 85–91.

Lim, C., Yildirim-Aksoy, M. & Klesius, P. (2011) Lipid and fatty acid requirements of tilapias. *North American Journal of Aquaculture* 73, 188–193.

Liu, J.-H., Wang, Q., You, Q.-L., Li, Z.-L., Hu, N.-Y., Wang, Y. et al. (2020) Acute EPA-induced learning and memory impairment in mice is prevented by DHA. *Nature Communications*, 11, 1–15.

Love, M., Anders, S. & Huber, W. (2014) Differential analysis of count data–the DESeq2 package. *Genome Biology*, 15, 10–1186.

Lum, K.K., Kim, J. & Lei, X.G. (2013) Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. *Journal of Animal Science and Biotechnology*, 4, 53.

Lyons, P.P., Turnbull, J.F., Dawson, K.A. & Crumlish, M. (2017) Effects of low-level dietary microalgae supplementation on the distal intestinal microbiome of farmed rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* 48, 2438–2452.

Magbanua, T.O. & Ragaza, J.A. (2024) Selected dietary plant-based proteins for growth and health response of Nile tilapia *Oreochromis niloticus*. *Aquaculture and Fisheries* 9, 3–19.

Manrique, W.G., Figueiredo, M.A.P., Charlie-Silva, I., de Andrade Belo, M.A. & Dib, C.C. (2019) Spleen melanomacrophage centers response of Nile tilapia during *Aeromanas hydrophila* and *Mycobacterium marinum* infections. *Fish & Shellfish Immunology* 95, 514–518.

Martin-Gallausiaux, C., Béguet-Crespel, F., Marinelli, L., Jamet, A., Ledue, F., Blottière, H.M. et al. (2018) Butyrate produced by gut commensal bacteria activates TGF-beta1 expression through the transcription factor SP1 in human intestinal epithelial cells. *Scientific Reports* 8, 1–13.

Menni, C., Zierer, J., Pallister, T., Jackson, M.A., Long, T., Mohney, R.P. et al. (2017) Omega-3 fatty acids correlate with gut microbiome diversity and production of *N*-carbamylglutamate in middle aged and elderly women. *Scientific Reports* 7, 1–11.

Nayak, S., Al Ashhab, A., Zilberg, D. & Khozin-Goldberg, I. (2020) Dietary supplementation with Omega-6 LC-PUFA-rich microalgae regulates mucosal immune response and promotes microbial diversity in the zebrafish gut. *Biology* 9, 119.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P., O'hara, R. et al. (2017) Vegan: Community ecology package. MASS. (R package. Version).

Osibona, A., Kusemiju, K. & Akande, G. (2009) Fatty acid composition and amino acid profile of two freshwater species, African catfish (*Clarias gariepinus*) and tilapia (*Tilapia zillii*). *African Journal of Food, Agriculture, Nutrition and Development* 9, 608–621.

Pedrazzani, A.S., Quintiliano, M.H., Bolfe, F., Sans, E.C.D.O. & Molento, C.F.M. (2020) Tilapia on-farm welfare assessment protocol for semi-intensive production systems. *Frontiers in Veterinary Science* 7, 606388.

Pickova, J. & Mørkøre, T. (2007) Alternate oils in fish feeds. *European Journal of Lipid Science and Technology* 109, 256–263.

Politi, L., Rotstein, N. & Carri, N. (2001) Effects of docosahexaenoic acid on retinal development: Cellular and molecular aspects. *Lipids* 36, 927–935.

Prabu, E., Rajagopalsamy, C.B.T., Ahilan, B., Jeevagan, I.J. & Renuhadevi, M. (2019) Tilapia—An excellent candidate species for world aquaculture: A review. *Annual Research & Review in Biology* 31, 1–14.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2012) The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* 41, D590–D596.

R Core Team (2020) *R: A language and environment for statistical computing v. 4.0.3*. Vienna, Austria: R Foundation for Statistical Computing.

Rana, K.J., Siriwardena, S. & Hasan, M.R. (2009) *Impact of rising feed ingredient prices on aquafeeds and aquaculture production*. Rome: Food and Agriculture Organization of the United Nations (FAO).

Ringø, E., Olsen, R., Gifstad, T., Dalmo, R., Amlund, H., Hemre, G.I. et al. (2010) Prebiotics in aquaculture: A review. *Aquaculture Nutrition* 16, 117–136.

Ryckebosch, E., Bruneel, C., Termote-Verhalle, R., Goiris, K., Muylaert, K. & Foubert, I. (2014) Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil. *Food Chemistry* 160, 393–400.

Sagaram, U.S., Gaikwad, M.S., Nandru, R. & Dasgupta, S. (2021) Microalgae as feed ingredients: Recent developments on their role in immunomodulation and gut microbiota of aquaculture species. *FEMS Microbiology Letters* 368, fnab071.

Saratale, R.G., Ponnusamy, V., Sirohi, R., Piechota, G., Shobana, S., Dharmaraja, J. et al. (2022) Microalgae cultivation strategies using cost–effective nutrient sources: Recent updates and progress towards biofuel production. *Bioresource Technology*, 361, 127691.

Sarker, P.K., Gamble, M.M., Kelson, S. & Kapuscinski, A.R. (2016) Nile tilapia (*Oreochromis niloticus*) show high digestibility of lipid and fatty acids from marine *Schizochytrium* sp. and of protein and essential amino acids from freshwater *Spirulina* sp. feed ingredients. *Aquaculture Nutrition*, 22, 109–119.

Sarker, P.K., Kapuscinski, A.R., Bae, A.Y., Donaldson, E., Sitek, A.J., Fitzgerald, D.S. et al. (2018) Towards sustainable aquafeeds: Evaluating substitution of fishmeal with lipid-extracted microalgal co-product (*Nannochloropsis oculata*) in diets of juvenile Nile tilapia (*Oreochromis niloticus*). *PLoS One* 13, e0201315.

Sarker, P.K., Kapuscinski, A.R., Lanois, A.J., Livesey, E.D., Bernhard, K.P. & Coley, M.L. (2016) Towards sustainable aquafeeds: Complete substitution of fish oil with marine microalga *Schizochytrium* sp. improves growth and fatty acid deposition in juvenile Nile tilapia (*Oreochromis niloticus*). *PLoS One* 11, e0156684.

Sarker, P.K., Kapuscinski, A.R., McKuin, B., Fitzgerald, D.S., Nash, H.M. & Greenwood, C. (2020) Microalgae-blend tilapia feed eliminates fishmeal and fish oil, improves growth, and is cost viable. *Scientific Reports* 10, 1–14.

Sarker, P.K., Kapuscinski, A.R., Vandenberg, G.W., Proulx, E., Sitek, A.J. & Thomsen, L. (2020) Towards sustainable and ocean-friendly aquafeeds: Evaluating a fish-free feed for rainbow trout (*Oncorhynchus mykiss*) using three marine microalgae species. *Elementa: Science of the Anthropocene*, 8, 1–16.

Shah, M.R., Lutzu, G.A., Alam, A., Sarker, P., Kabir Chowdhury, M.A., Parsaeimehr, A. et al. (2018) Microalgae in aquafeeds for a sustainable aquaculture industry. *Journal of Applied Phycology*, 30, 197–213.

Shang, S., Ren, J., Wang, J., Xin, S., Xia, J. & Tang, X. (2021) High-throughput sequencing reveals significant diversity in the gut microbiomes of humpback (*Chanodichthys dabryi*) and crucian carp (*Carassius carassius*). *Biologia* 76, 655–662.

Sinn, N., Milte, C.M., Street, S.J., Buckley, J.D., Coates, A.M., Petkov, J. et al. (2012) Effects of n-3 fatty acids, EPA v. DHA, on depressive symptoms, quality of life, memory and executive function in older adults with mild cognitive impairment: A 6-month randomised controlled trial. *British Journal of Nutrition* 107, 1682–1693.

Sissener, N.H. (2018) Are we what we eat? Changes to the feed fatty acid composition of farmed salmon and its effects through the food chain. *Journal of Experimental Biology* 221, jeb161521.

Stoneham, T.R., Kuhn, D.D., Taylor, D.P., Neilson, A.P., Smith, S.A., Gatlin, D.M. et al. (2018) Production of omega-3 enriched tilapia through the dietary use of algae meal or fish oil: Improved nutrient value of fillet and offal. *PLoS One* 13, e0194241.

Strobel, C., Jahreis, G. & Kuhnt, K. (2012) Survey of n- 3 and n-6 polyunsaturated fatty acids in fish and fish products. *Lipids in Health and Disease* 11, 144.

Tamburini, S., Shen, N., Wu, H.C. & Clemente, J.C. (2016) The microbiome in early life: Implications for health outcomes. *Nature Medicine* 22, 713–722.

Teuling, E., Schrama, J.W., Gruppen, H. & Wierenga, P.A. (2017) Effect of cell wall characteristics on algae nutrient digestibility in Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarus gariepinus*). *Aquaculture* 479, 490–500.

Teuling, E., Wierenga, P.A., Agboola, J.O., Gruppen, H. & Schrama, J.W. (2019) Cell wall disruption increases bioavailability of *Nannochloropsis gaditana* nutrients for juvenile Nile tilapia *(Oreochromis niloticus*). *Aquaculture* 499, 269–282.

Tocher, D.R., Betancor, M.B., Sprague, M., Olsen, R.E. & Napier, J.A. (2019) Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: bridging the gap between supply and demand. *Nutrients* 11, 89.

Trevi, S., Uren Webster, T.M., Consuegra, S. & Garcia de Leaniz, C. (2023) The benefits of microalgae replacement in fish nutrition: A meta-analysis. *Scientific Reports* 13, 2208.

Uren Webster, T.M., Consuegra, S. & Garcia de Leaniz, C. (2020) Early life stress causes persistent impacts on the microbiome of Atlantic salmon. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics* 40, 100888.

Uren Webster, T.M., Rodriguez-Barreto, D., Castaldo, G., Gough, P., Consuegra, S. & Garcia de Leaniz, C. (2020) Environmental plasticity and colonisation history in the Atlantic salmon microbiome: A translocation experiment. *Molecular Ecology* 29, 886–898.

Vega-López, F. (2020) Mycobacterium marinum infection: Fish tank granuloma. In: *Hunter's tropical medicine and emerging infectious diseases*. Amsterdam: Elsevier. pp. 569–570.

Villamil, S.I., Huerlimann, R., Morianos, C., Sarnyai, Z. & Maes, G.E. (2018) Adverse effect of early-life high-fat/high-carbohydrate ("Western") diet on bacterial community in the distal bowel of mice. *Nutrition Research* 50, 25–36.

Vital, M., Howe, A.C. & Tiedje, J.M. (2014) Revealing the bacterial butyrate synthesis pathways by analyzing (meta) genomic data. *MBio* 5, e00889-00814.

Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A. et al. (2016) Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. *Msystems* 1, e00009–00015.

Wang, C., Sun, G., Li, S., Li, X. & Liu, Y. (2018) Intestinal microbiota of healthy and unhealthy Atlantic salmon *Salmo salar* L. in a recirculating aquaculture system. *Journal of Oceanology & Limnology*, 36, 414–426.

Watters, C.A., Rosner, L.S., Franke, A.A., Dominy, W.G., Klinger-Bowen, R. & Tamaru, C.S. (2013) Nutritional enhancement of long-chain omega-3 fatty acids in tilapia (*Oreochromis honorum*). *Israeli Journal of Aquaculture*, 65, 1–7.

Wilkes Walburn, J., Wemheuer, B., Thomas, T., Copeland, E., O'Connor, W., Booth, M. et al. (2019) Diet and diet-associated bacteria shape early microbiome development in yellowtail kingfish (*Seriola lalandi*). *Microbial Biotechnology* 12, 275–288.

Young, K. (2009) Omega-6 (n-6) and omega-3 (n-3) fatty acids in tilapia and human health: A review. *International Journal of Food Sciences and Nutrition* 60, 203–211.

Zhou, M.-M., Ding, L., Wen, M., Che, H.-X., Huang, J.-Q., Zhang, T.-T. et al. (2018) Mechanisms of DHA-enriched phospholipids in improving cognitive deficits in aged SAMP8 mice with high-fat diet. *The Journal of Nutritional Biochemistry* 59, 64–75.

Zhou, Z., Ringø, E., Olsen, R.E. & Song, S.K. (2018) Dietary effects of soybean products on gut microbiota and immunity of aquatic animals: A review. *Aquaculture Nutrition* 24, 644–665.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Trevi, S., Uren Webster, T.M., Consuegra, S. & Garcia de Leaniz, C. (2024) Effects of micro-algae dietary oil replacement on growth, omega – 3 deposition and gut microbiome composition of Nile tilapia (*Oreochromis niloticus*). *Aquaculture, Fish and Fisheries*, e164. https://doi.org/10.1002/aff2.164