Delivering a nutritionally enhanced tilapia fillet using a pre-harvest phase omega-3 thraustochytrids protist enriched diet

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Short Communication-

Delivering a nutritionally enhanced tilapia fillet using a pre-harvest phase omega-3 Thraustochytrids protist enriched diet

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Running Title: Enhancing omega-3 fatty acid content in farmed tilapia

Keywords: Tilapia; Thraustochytrids; Human health, Omega-3 enhancement, Sustainable aquaculture production.

Abbreviations: **DHA,** Docosahexaenoic acid; **EPA**, Eicosapentaenoic acid; **EFA**, Essential fatty acid; **MUFA**, Monounsaturated fatty acids; **n-3**, Omega-3; **n-6**, Omega-6; n-9, Omega-9; **LC-PUFA**, long-chain polyunsaturated fatty acid, **PUFA**, Polyunsaturated fatty acids; **SFA**, Saturated fatty acids.

Abstract

Nile tilapia (*Oreochromis niloticus*) offers an affordable food source to many low-income consumers. However, farmed tilapia has drawn much criticism over the low omega-3 (n-3) and high omega-6 (n-6) lipid levels. Subsequently, it has been questioned whether it is truly healthy food. This study fed tilapia with a specialised 'finishing' diet with the inclusion of commercial Thraustochytrids protist biomass and oil before the harvestable fish size. The fish were fed with two different dietary regimes over 6 weeks. One was a commercially available tilapia feed used as a reference. The second diet is composed of an exclusive oil source from Thraustochytrids protist (HI-n3). The results show that HI-n3 had significantly increased the fillet n-3 content by 400 % in comparison to commercial diet (COM) after Week 6 of feeding. Specifically, docosahexaenoic acid (DHA, n-3) content was the attributing fatty acid for the n-3 increase. This was particularly evident when DHA was expressed as a percentage of total lipid content. The n-3: n-6 ratio increased in tilapia fed with the HI-n3 diet attributed to the DHA accumulation. The investigation shows that it is possible to favourably 'lipid tailor' tilapia before harvest.

Practical applications

The practical application of this technique is to enrich farmed tilapia with a high dietary omega-3 (n-3) Thraustochytrids protist oil source for a short-term period before harvesting. It was an objective that the fillet product would be more functional in its nutritional content by supplying more than just high-quality protein for consumers. This would have paramount implications for low-income consumers, where high n-3 oil foods are not readily available or affordable (e.g., landlocked nations). Furthermore, tilapia is widely consumed in China and Southeast Asian countries but is also promoted as a high nutritional value food source in the western hemisphere. This investigation advocates the ability to change the image of this fish species by a simple dietary manipulation. In an era of elevated intake of n-6 fatty acids food sources, n-3 rich fish is a vital balance to counter this negative trend in human health.

1 Introduction

The aquaculture industry today produces over 51 % of global seafood production and is set for further expansion to meet a rising global human population expected to be 9.3 billion by 2050 [1].

This is in response to the growing demand for seafood because of its high biological value in proteins, trace elements, and essential fatty acids that is seen as part of a healthy diet. The presence of high amounts of omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA) in fish and fishery products has been shown to mitigate inflammation, immunological neurological and dysfunction, and obesity in humans [2, 3].

Oily marine fish such as farmed salmon, sea trout, and sea bass are iconic species for consumers in supplying dietary n-3 LC-PUFAs. However, freshwater fish farming is growing rapidly in tropic and subtropic regions and accounts for the largest proportion of farmed fish production [4]. In particular, the Nile tilapia (*Oreochromis niloticus*) is a significant freshwater and brackish farmed finfish species for America, Asia, but more so as an affordable food source in the African continent. In 2018, greater than 5 million tonnes of farmed Nile tilapia were produced globally, with a worth of over USD 7.5 billion [1]. Tilapia provides an affordable high-quality protein for consumers due to their high-density farming, water quality tolerance, simple life cycle, and limited farming practices needed [5]. As such, tilapia is considered a suitable species for sustainable aquaculture to meet future food demands.

Nile tilapia are omnivorous with a long intestine and their fundamental nutritional requirements are less demanding than many farmed carnivorous species (e.g., salmon and trout) [6,7]. As such, there is little requirement for commercial diets to have significant amounts of marine-derived ingredients (e.g., fishmeal and oil) to supply the major nutrient requirement, or to support efficient growth rates. Tilapia *per se* do not require LC-PUFA lipids of the n-3 series as an essential biological requirement.

As such, Nile tilapia typically requires more n-6 fatty acids than n-3 [8]. Subsequently, the nutritional demands of Nile tilapia raised at optimal temperature can be largely met by more economic plant-derived oils, such as rapeseed, cereals, soya, and palm oils [9]. Consequently, this has led to a very low or even negligible n-3 fatty acid profile in farmed tilapia muscle fillet. In recent years, this has been highlighted as a matter of public health concern regarding the nutritional value of farmed tilapia to the consumer and this has attracted a negative image of tilapia as an intensively farmed fish [10].

For many commercial fish diets, the supply of n-3 LC-PUFAs is typically from marine fish oils. This is now seen as increasingly unsustainable due to the finite production of fish oil from wild capture fisheries, but more importantly, the economic feasibility of using this expensive high-value oil in low-value tilapia fish [11]. In the present study, a feed trial was conducted to validate whether tilapia could be fortified using a short-term feeding regime at preharvest sized tilapia (>350 g). Rather than using fish oil to achieve this, this study employed a high n-3 LC-PUFA based enrichment diet using cultured Thraustochytrids biomass and oil from a non-genetically modified strain (Megatech Research GmbH, Baar, Switzerland). These microorganisms are fungus-like protists that are heterotrophic and can be fed on a number of waste carbon sources, e.g., food waste, seaweed, brewery and agriculture waste [12]. When cultured at optimal conditions, Thraustochytrids protist possesses a predominately docosahexaenoic acid (C22:6 n-3, DHA) lipid profile compared to fish oil, which has a more diverse fatty acid profile.

2 Materials and methods

2.1 Experimental design and diet composition

A 6-week feeding trial was conducted using 3000 L tanks within a recirculating aquaculture system which was maintained at 26 ±1.0 °C. Farm-raised red Nile tilapia (*Oreochromis niloticus*, Fishgen Ltd, Llanelli, UK) were randomly distributed into two tanks at a stocking density of 50 fish per tank (~350 g). Two diet regimes were used in the present study with a commercial diet (COM, crude protein 40.67 %) selected based on the designated formulation as a tilapia grower stage. The second diet was a Thraustochytrids enrichment diet (HI-n3, crude protein 41.54 %, Supplementary Table 1) with over three-fold higher n-3 levels than the commercial diet (Table 1). Furthermore, the HI-n3 test diet

was formulated to have almost twice the level of lipid (21.00 g 100 g^{-1}) as the commercial diet (12.65 g 100 g^{-1}, Supplementary Table 2). This dietary strategy was to create a 'wash out effect' that would replace the existing high omega 6 fatty acids in the fish with omega-3 fatty acids. The HI-n3 diet had the inclusion of Thraustochytrids meal and oil (Megatech Research GmbH, Barr, Switzerland), which was formulated and produced by the Aquaculture and Nutrition Research Unit (ANRU), Carna Research Station, National University of Ireland Galway, Ireland. The diet was extruded through a PM-80 (Bottene, Vicenza, Italy) using a 3 mm pellet die. Diets were air-dried at 40 °C for 48 hrs and stored in airtight containers. The design of the diet was formulated with the Thraustochytrids protist biomass and oil as an effective substitution of fish and plant oils (Supplementary Table 1). The nutritional composition of the commercial and experimental diets is presented in Supplementary Table 2.

This investigation was undertaken for a total period of 6-weeks and before the start of the trial, fish were grown to size using the COM diet. All fish appeared healthy throughout the feeding trial period and readily accepted the diets. Tilapia were fed daily to approximately 3 % body weight per day in two rations at 1000 and 1500 hr (GMT+1) to satiation [6]. The feeding rate was assigned to be the same and a continuum for both the regular commercial and the experimental diets as in keeping with standard guidelines for feeding tilapia of this weight class [13]. Water parameters were recorded and maintained at pH 7.4, dissolved oxygen >5.50 mg L^{-1} (Polaris, OxyGuard International A/S, Farum, Denmark), ammonia <0.35 mg L^{-1}, nitrite <0.06 mg L^{-1}, and nitrate <11.8 mg L^{-1} (Hach Lange DR3900, Little Island, Ireland) in the RAS over the experimental period. These conditions are deemed optimal for tilapia culture [14]. At the conclusion of the feed study, three fish were sampled for their fillet and liver samples from each dietary group. Three fish were also sampled at weeks 2 and 4 to assess the change in the fatty acid profile in HI-n3 treatment group. The investigation

conformed to European guidelines (Directive 2010/63/EU) for the care and welfare of animals for scientific purposes and local ethical standards of husbandry.

2.2 Nutritional analysis

Three fish were randomly removed from each tank at bi-weekly intervals and processed for analysis. Tilapia were filleted laterally, and each side was used and combined into one pooled sample. Tilapia livers were also removed from each fish and all samples were subsequently stored at -80 °C until analysis. The proximate compositional analysis for both commercial tilapia diets and test diet formulation were conducted according to standard AOAC 2002 protocols and values obtained were expressed as g 100 g^{-1} (Supplementary Table 2). The proximate composition of the fish fillet and liver was carried out using the same protocol. Fatty acid was extracted from the fillet and livers samples and transmethylated [15]. Samples were then measured for fatty acid composition using gas chromatography coupled with a flame ionisation detector (GC-FID 6850, Agilent Technologies, Little Island, Ireland). Known external amounts of external and internal standards (Nu-Chek Prep, Minnesota, USA) were used to identify and quantify the fatty acid of interest in the samples.

2.3. Statistical analysis

Datasets are expressed as mean values and where applicable, their corresponding standard error of the mean. A one-way permutational multivariate analysis of variance (PERMANOVA, $p<0.05$, 9999 permutations, Bray-Curtis similarity index) was carried out on the overall change in the fatty acid profile of the fish fillet using PAST4 software (https://past.en.lo4d.com). Fatty acids of interest (e.g., DHA and EPA) were analysed using one-way analysis of variance (ANOVA) and statistical differences

were discerned using the posthoc Tukey test ($p<0.05$). The time effect of the algal enrich diet had on n-3 and n3:n-6 fillet fatty acid profile was calculated using minitab software (v16). A principal component analysis (PCA) was used to interpret and visualise the overall fatty acid profiles in the fish fillets.

3 Results and discussion

There is considerable interest in promoting fish as a source of n-3 fatty acids in the human diet, with the emphasis on n-3 LC-PUFA (i.e., eicosapentaenoic acid, EPA and DHA) [2]. This class of lipids has been linked to mitigating various health conditions in humans, including anti-inflammatory, reducing cardiovascular disease risk, lowering blood cholesterol, and decreasing the risk of stroke and dementia [16,17]. Consumption of oily marine fish such as sardines, mackerel, and salmon are recommended as a source of LC-PUFA n-3 fatty acids. The European Food Safety Authority (EFSA) has been suggested that the consumption of one to two portions of ~150-300 g of fish per week would meet the nutritional requirement in adults [18].

The goal of this study is to deliver a more nutritious farmed tilapia for consumers through the enhancement of the fatty acid profile using a DHA enriched diet (Table 1). The enrichment feeding trial showed that the HI-n3 diet had raised the fillet n-3 content (23.07 mg g^{-1}) and n3:6 ratio (1.66) by over threefold at week 6 when compared to the commercial tilapia diets (7.33 mg g^{-1}, Table 2). This increase in n-3 in the fillets is attributed to the rising level of DHA, while other mean n-3 fatty acids remained relatively static ($p>0.05$, one-way ANOVA). It is evident in the study that the COM feed had the least amount of DHA in the fillet, and in comparison, to the HI-n3 treatment group at

the same sampling point (week 6), HI-n3 had >560 % higher DHA content (3.23 and 18.14 mg g^{-1}, respectively). This is much higher than typical levels reported in contemporary salmon fillets [19]. The n-3 EPA content in fish fed with the reference diet was significantly higher ($p<0.05$, one-way ANOVA) compared to the other three diets and reflected the higher EPA supplied by this diet. The amounts of major n-6 PUFA deposition in the fish fillet (mg g^{-1} fillet) were not significantly different between the diets ($p>0.05$, one-way ANOVA). The results have also shown that the high Thraustochytrids protist enriched diet (HI-n3) can greatly reduce the total of n-6 level by nearly 25 % in the fillet muscle. The decrease can mainly be attributed to the lowering of linoleic acid levels that are often associated with plant-derived oils The feeding of the HI-n3 diet to tilapia dramatically changes the levels over the 6-week leading to an effective shift due to the n-3 enhancement capacity of this diet. One-way PERMANOVA carried out on the fillet fatty acid composition showed a significant difference between the dietary treatments and over time ($p = 0.0287$, $f = 2.209$). This was presented in the spatial distinction observed on the PCA plot, confirming the differences among the dietary treatments. The first two axes represented 98.14 % of the total data variance (Figure 1).

The changes in the fatty acid profile can also be confirmed by the total n-3 content and n-3: n-6 ratio increases in the liver (>5.31 and >6.98 fold higher, respectively) when compared to COM dietary treatment at week 6 (Table 3). This reflected the dietary lipid profile of n-3 LC-PUFA's ingested; confirming the hepatic storage over time attaining a progressively higher level over 2-6 weeks of feeding compared to fillet concentrations.

In another study, *Schizochytrium* spp. (part of the Thraustochytrids) was used as an n-3 enricher in tilapia [20]. However, this study fed the tilapia for a longer period (i.e., 8 weeks over 6 weeks), and started the feed trial with smaller fish (~160 g). In contrast, the present investigation started with

much larger fish (~350 g), which are closer to the typical harvestable weight. In addition, the current study showed that feeding for 2 weeks with the HI-n3 diet was capable of doubling the mean n-3:n-6 ratio compared to the tested commercial diet, and nearly triple the ratio after 4 weeks of feeding. Therefore, the strategy employed in the present study is more cost-effective and commercially feasible when used as a 'finishing diet' before harvest. This is particularly important given that the cost associated with using Thraustochytrids (e.g., *Schizochytrium* and *Aurantiochytrium* species) oils are much higher than the cheaper and n-6 fatty acid rich plant-derived oils. Using the HI-n3 time series fillet fatty acid data and COM acting as a baseline, it is possible to express the total n-3 fatty acid increase as $y = 0.3755x + 8.2993$ ($R^2 = 0.974$). For n-3: n-6 ratio, the increase can be expressed as $y = 0.0294x + 0.5074$ ($R^2 = 0.971$).

The dietary enrichment strategy of using high n-3 was to attain an end-product lipid profile, which meets the recommended nutritional guidelines for human health. The use of nearly double the lipid content in the test diet to the commercial diet (21.00 vs 12.65 g $100g^{-1}$) was to create a 'wash out' effect that had no impact on the overall fillet lipid content. While the use of Thraustochytrids protist was similar to previous Nile tilapia feed studies such as those carried out by Sarker et al. [20] and dos Santos et al. [22], the impact on carcass composition from the increased lipid content warrants further investigation. Regardless, even if there was a negative effect on fish growth performance, feed utilisation metrics, and carcass composition, the impact would be negligible. This is due to the Thraustochytrids rich diet being specifically intended for use prior to the harvest phase, and at a such short proposed feeding period (i.e., 2-6 weeks). The enrichment strategy of using high n-3 was to attain an end-product lipid profile that meets the recommended nutritional guidelines for human health since tilapia, when fed with commercial plant-based diets containing plant-based oil, do not meet this recommended nutritional requirement. Furthermore, it has been shown in another study

that rainbow trout fed with *Schizochytrium* spp. (30 % inclusion, similar to *Aurantiochytrium* sp.) was more effective at digesting DHA at the lower water temperature of 8 °C than 15 °C [23]. This temperature difference can potentially be explained by the higher number of double bonds and carbon chain length giving DHA more fluidity at lower temperatures. Therefore, making DHA easier to digest and cross the intestinal membrane barrier compared to other fatty acids, e.g., saturated, and monosaturated fatty acids [24]. As such, further studies are warranted in understanding whether this difference in Thraustochytrids protist lipid digestibility at different water temperatures can substantially change as well in tilapia and have economic and consumer impact, i.e., optimum rearing is 28 °C. Certainly in another study on Nile tilapia, it was reported that dietary DHA derived from *Aurantiochytrium sp*. (up to 4 % inclusion level) had improved growth performance indicators (e.g., weight gain (18 % increase), specific growth rate (6 % increase), and FCR (8 % decrease)) when raised at suboptimal temperatures, i.e., 22 °C [25]. In our study tilapia were reared at a marginally lower temperature than optimum for the species (26 °C) and the presence of DHA in the enrichment diets would be deemed beneficial according to other studies in this area on tilapia held at much lower temperatures [26].

It should be noted that the level of total n-3 LC-PUFAs (i.e., EPA & DHA) supplied by a portion of Atlantic salmon fillet would be far higher than in tilapia fillet due to the salmon inherent higher lipid content. A consumer would, therefore, need to consume more tilapia than salmon, in order to meet the same n-3 intake. Nevertheless, the enhancement of n-3 LC-PUFAs in tilapia would have a significant impact on landlocked, inland regions, Middle Eastern, Far-East Asian, and African nations, where the consumption of farm-raised tilapia is substantially higher than Atlantic salmon and other oily fish due to its affordability and availability. Such an increase in n-3 intake would also reduce incidences of premature birth and other health issues in expectant mothers and newborn infants in

low-income countries [27]. This technology should not just be confined to tilapia but further developing in other low-value fish species such as African catfish (*Clarias gariepinus*), snakehead (*Channa* sp.), carp (*Cyprinus* sp.) that common farmed in low economic nations. Overall, the implementation of biofortifying farmed fish with Thraustochytrids would meet the United Nation's Sustainable Development Goals: 2) Zero Hunger, 3) Good Health and Wellbeing, and 14) Life Below the Water.

4. Conclusion

There is much criticism of farmed fish in particular tilapia possessing low n-3/n-6 ratio than their wild counterparts. This has the potential of causing inflammatory responses and health-related issues in humans. The current study has shown that it is possible to raise the n-3 fatty acid content and raising the overall n-3/n-6 ratio in tilapia fillets through a short term 'lipid tailoring' feeding strategy.

When used at the later stages in the production cycle of farmed tilapia (i.e., pre-harvest), the cultured Thraustochytrids protist biomass and oil were almost able to raise the n-3/n-6 ratio by double in the fillet after 2 weeks, and triple after 6 weeks of feeding when compared to the commercial diets. This approach would offer the farmer an economic approach to produce a unique healthy tilapia product with a higher product value (i.e., n-3 LC-PUFA). This dietary Thraustochytrids protist n-3 enhancement concept could also be applied to both broodstock male and female tilapias. Where the dietary enhancement could improve the nutritional status and quality of the milt and ova in terms of fecundity, fertilisation rate, hatchability of fertilised eggs, and subsequent larval robustness. Other potentials of using high Thraustochytrids protist supplementation could be directed towards enhancing n-3 fatty content in other commercially important affordable farmed fish species such as catfish (e.g. *Clarias* sp. and *Pangasius* sp.) and carp (e.g. *Cyprinus* sp.). Or to

reverse the declining low-level n-3 content in farmed salmon and trout compared to their wild counterparts. Apart from the obvious use for lipid modulation, the Thraustochytrids biomass may be used as a sustainable alternative for plant-based proteins such as soya bean meal in compounded diets at all stages of fish production due to its excellent protein and amino acid characteristics.

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Conflict of interest

This study was funded by Megatech Research GmbH, which is the producer of the thraustochytrids. The co-author Tom Brudenell-Bruce is the CEO of Megatech Research GmbH.

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Figure Legend

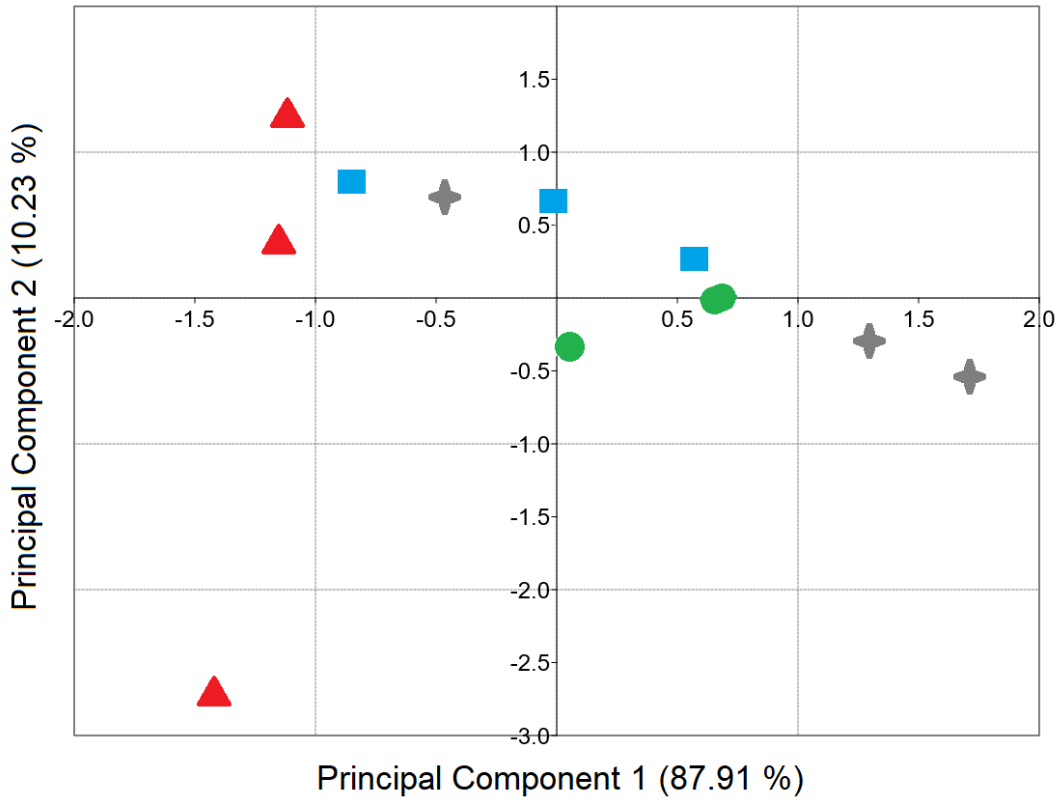

Figure 1: Principal component analysis: Score biplot of the first and second principal component on the Nile tilapia (*Oreochromis niloticus*) fillet fatty acids composition fed with the different test diet and different sampling points. Component 1 and 2 explains 98.14 % of the sample variation. ▲, commerical reference diet, COM; ■, week 2, ●, week 4, ✚, week 6 HI-n3 diet.

Table 1: Fatty acid composition of the test diets used in the Nile tilapia (*Oreochromis niloticus*) feed trial (% of the total lipid).

	COM	HI-n3
14:0	1.15	7.81
16:0	11.68	19.90
18:0	3.05	1.08
20:0	0.43	0.09
22:0	0.26	<0.01
24:0	0.14	<0.01
Σ SFA	**16.71**	**28.88**
16:1n-9	0.18	<0.01
16:1n-7	2.43	4.32
18:1n-9	42.15	4.59
18:1n-7	3.05	6.52
20:1n-11	<0.01	<0.01
20:1n-9	1.22	0.13
20:1n-7	<0.01	<0.01
22:1n-11	0.32	<0.01
22:1n-9	0.50	<0.01
24:1n-9	0.25	<0.01
Σ MUFA	**50.10**	**15.56**
18:2n-6	20.11	5.23
18:3n-6	0.06	0.14
20:2n-6	0.11	<0.01
20:3n-6	0.08	0.07
20:4n-6	0.51	0.32
22:4n-6	0.11	<0.01
22:5n-6	0.28	7.30
Σ n-6 PUFA	**21.26**	**13.06**
18:3n-3	6.20	0.88
18:4n-3	0.27	0.28
20:3n-3	<0.01	<0.01
20:4n-3	0.12	0.44
20:5n-3	1.97	1.50
22:5n-3	0.30	0.29
22:6n-3	2.41	38.57
Σ n-3 PUFA	**11.27**	**41.96**
n3:n6	**0.53**	**3.21**
Σ PUFA	**32.53**	**55.02**

	COM	HI-n3		
		Week 2	*Week 4*	*Week 6*
14:0	2.45 ±0.42	2.92 ±0.40	3.51 ±0.05	3.67 ±0.53
16:0	17.03 ±1.99	14.00 ±0.21	15.20 ±0.70	13.97 ±0.17
18:0	8.56 ±3.18	3.96 ±0.71	3.97 ±0.32	3.93 ±0.23
20:0	0.62 ±0.00	0.43 ±0.07	0.41 ±0.00	0.35 ±0.02
22:0	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00
24:0	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00
∑ SFA	**28.66 ±5.59**	**21.30 ±1.39**	**23.09 ±1.07**	**21.91 ±0.95**
16:1n-9	3.70 ±0.67	3.33 ±0.43	3.34 ±0.06	3.38 ±0.24
16:1n-7	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00
18:1n-9	38.57 ±0.92	31.00 ±3.15	26.90 ±1.63	25.30 ±4.53
18:1n-7	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00
20:1n-11	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00
20:1n-9	1.93 ±0.23	1.93 ±0.22	1.62 ±0.06	1.52 ±0.32
20:1n-7	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00
22:1n-11	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00
22:1n-9	<0.01 ±0.00	0.91 ±0.09	0.79 ±0.23	0.68 ±0.00
24:1n-9	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00
∑ MUFA	**44.20 ±1.83**	**37.17 ±3.89**	**32.65 ±1.97**	**30.88 ±5.10**
18:2n-6	13.67 ±1.52	13.47 ±0.79	12.53 ±0.30	11.93 ±0.95
18:3n-6	0.76 ±0.00	0.43 ±0.01	0.35 ±0.09	0.36 ±0.00
20:2n-6	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00	0.68 ±0.00
20:3n-6	<0.01 ±0.00	0.39 ±0.02	0.24 ±0.00	0.40 ±0.00
20:4n-6	0.88 ±0.21	0.60 ±0.04	0.73 ±0.12	0.55 ±0.04
22:4n-6	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00
22:5n-6	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00
∑n-6 PUFA	**15.31 ±1.74**	**14.88 ±0.85**	**13.85 ±0.51**	**13.92 ±0.99**
18:3n-3	3.34 ±0.20	2.61 ±0.20	2.34 ±0.06	2.18 ±0.29
18:4n-3	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00
20:3n-3	<0.01 ±0.00	0.40 ±0.05	0.34 ±0.00	0.38 ±0.00
20:4n-3	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00	<0.01 ±0.00
20:5n-3	<0.01 ±0.00	0.44 ±0.06	0.52 ±0.01	0.49 ±0.15
22:5n-3	1.16 ±0.04	1.45 ±0.08	1.71 ±0.05	1.89 ±0.34
22:6n-3	3.23 ±0.30	9.58 ±3.06	14.93 ±0.84	18.14 ±5.40
∑n-3 PUFA	**7.73 ±0.54**	**14.49 ±3.45**	**19.84 ±0.96**	**23.07 ±6.18**
n3:n6	**0.50**	**0.97**	**1.43**	**1.66**
∑ PUFA	**23.04 ±2.28**	**29.37 ±4.30**	**33.70 ±1.47**	**36.99 ±7.17**

Table 2: Fatty acid composition in the Nile tilapia (*Oreochromis niloticus*) muscle fillets over the feed trial period (% of the total lipid, ± SE, *n*=3).

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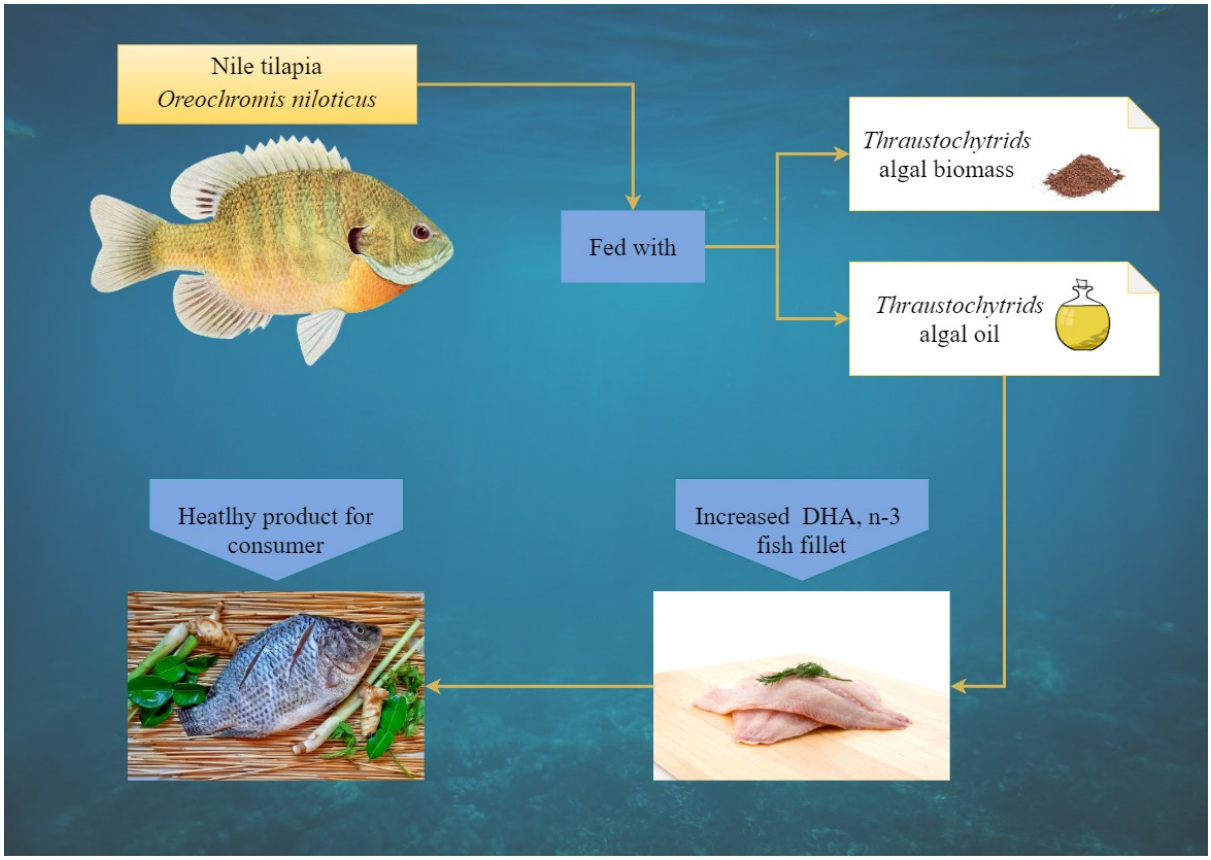
Table 3: Fatty acid composition in the liver of Nile tilapia (*Oreochromis niloticus*) over the feed trial period (% of the total lipid, *n*= 5 pooled samples).

	COM	**HI-n3**		
		Week 2	*Week 4*	*Week 6*
14:0	2.38	3.07	<0.01	4.89
16:0	17.00	15.20	18.50	14.10
18:0	8.80	4.68	5.66	4.75
20:0	0.39	<0.01	<0.01	<0.01
22:0	0.11	<0.01	<0.01	1.11
24:0	<0.01	<0.01	<0.01	<0.01
∑ SFA	**26.30**	**19.88**	**24.16**	**19.96**
16:1n-9	3.13	3.61	3.09	3.39
16:1n-7	<0.01	<0.01	<0.01	<0.01
18:1n-9	37.60	35.70	35.50	18.70
18:1n-7	<0.01	<0.01	<0.01	<0.01
20:1n-11	<0.01	<0.01	<0.01	<0.01
20:1n-9	1.61	2.27	<0.01	<0.01
20:1n-7	<0.01	<0.01	<0.01	<0.01
22:1n-11	<0.01	<0.01	<0.01	<0.01
22:1n-9	0.41	<0.01	<0.01	<0.01
24:1n-9	<0.01	<0.01	<0.01	<0.01
∑ MUFA	**42.87**	**41.58**	**38.59**	**22.09**
18:2n-6	11.40	15.20	15.90	9.64
18:3n-6	0.41	<0.01	<0.01	<0.01
20:2n-6	0.54	<0.01	<0.01	<0.01
20:3n-6	<0.01	<0.01	<0.01	<0.01
20:4n-6	0.40	<0.01	<0.01	<0.01
22:4n-6	<0.01	<0.01	<0.01	<0.01
22:5n-6	<0.01	<0.01	<0.01	<0.01
∑n-6 PUFA	**12.75**	**15.20**	**15.90**	**9.64**
18:3n-3	2.44	3.07	<0.01	1.63
18:4n-3	<0.01	<0.01	<0.01	<0.01
20:3n-3	0.34	<0.01	<0.01	<0.01
20:4n-3	<0.01	<0.01	<0.01	<0.01
20:5n-3	0.19	<0.01	<0.01	<0.01
22:5n-3	0.80	1.34	<0.01	2.58
22:6n-3	1.94	7.61	12.90	26.10
∑n-3 PUFA	**5.71**	**12.02**	**12.90**	**30.31**
n3:n6	**0.45**	**0.79**	**0.81**	**3.14**
∑ PUFA	**18.46**	**27.22**	**28.80**	**39.95**



Nile tilapia (*Oreochromis niloticus*) fed with a finishing diet supplemented with omega-3 rich *Thraustochytrids* has resulted in a 400 % increase in omega-3 docosahexaenoic acid (DHA) when compared to a commercial control diet. This has implications on producing an affordable and healthy fish product for consumers.