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Impact of Fish Feed Formulated with Microalgae Biomass in Experimental Tanks w*ith (Oreochromis Niloticus)* Tilapia Culture

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Abstract

This project aims to evaluate a fish feed developed in CIATEJ with little modification, which included algal biomass and vegetal protein. This product was tested at the laboratory scale and the pilot-scale upscaling was needed for use with impact assessment studies in real ambient conditions. This food has antioxidant capacity due to the inclusion of microalgae. Additionally, it is nutritionally balanced with plant-based protein sources, partially replacing fishmeal. The feed, compared to commercial feed, has a higher protein content (5%) and lower fat content (3%). Evaluating this feed was of great importance to corroborate its consumption, which can reduce oxidative stress, reduce water contamination, and improve the growth rate and quality of the meat in fish.

FCR values of almost 1 were reported here, indicating the more efficient utilization of formulated feed by *Oreochromis niloticus*. The main component of tilapia meat was moisture 74.31%, followed by protein 14.44%, lipids 6.24%, carbohydrates 0.10%, fibre 1.39%, and ash 3.62%. in experimental feed. In our results in fish filate, we found a 12% increase in protein percentage and a 29% decrease in fat percentage compared to the commercial feed, as expected from the higher protein and lower fat formulated feed. The amount of protein increment in fish filet we achieved in 2 months, the experimental diets significantly influenced the content of polyunsaturated fatty acids at 2.4% while saturated fatty acids were 1.3%. Omega 6 and Omega 9 were 2.33% and 1.73% respectively. The polyunsaturated fatty acid/saturated fatty acid ratio was also higher.

Keywords: Fish-feed; Formulation; Tilapia; Microalgae; Extrusion.

Introduction

As aquaculture is the fastest-growing sector of the food industry in 2019, it was expected to be valued at US$ 31.94 billion (https:// www.marketwatch.com) with a growth rate of more than 7.1% between 2020 and 2027. Increased human consumption and health safety factors are now driving forces of the growth of the aquaculture industry. Nile tilapia *(Oreochromis niloticus), is* the second-largest farmed fish in the world. Fish meals are commonly used as a source of nutrients for fish and shrimp in aquaculture systems [1]. However, demand and supply are a problem now due to high volatility in the global market for fish meals and also security in the aquaculture sector [2,3]. This fact, combined with the scarcity and high market price of fishmeal, has encouraged research to replace fish meal with alternative vegetable protein sources [4]. Aquafeed accounts for at least 75–90% of aquaculture's operating costs. Microalgae-based aquafeed is not only environmentally friendly, but it can also be cost-effective with proper optimization of the wastewater use from the same aquaculture industry. Another problem is the water stability of the fish feed, which generates large amounts of waste with high nitrogen content and, when not properly processed, can be deposited in the environment, generating pollutants. This nutrient-rich water can be used for microalgae culture for biomass generation for sustainable aquaculture practices, new protein source generation and increasing water stability in fish feed another important research criteria to avoid this contamination problem. Several previous studies demonstrated that microalgae-based feeds can enhance fish growth and nutritional quality [5] Some algal species like Nannochloropsis sp. and Isochrysis sp. were reported to be used to substitute fish meals in the diet of *Oncorhynchus mykiss*,

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and *Isochrysis sp*. was found efficient for crude proteins, amino acids, lipids, and fatty acids enhancement [6]. Supplementing animal feed with algae enriches livestock products with bioactive components with growth and weight gain, decreases feed consumption, increases immunological response, resistance to illness, and antibacterial and antiviral activity which is more important like less pathogenic attacks and less water contamination [7]. The rapid growth of the algal biotechnology and algal biomass-producing industries at a global scale has driven significant advances in the algal bio-economy and turned algae into an efficient 'cell factory' for food production [7].

However, there are very few complete fish feed formulations with microalgae biomass in the market available yet, although some formulations for feed supplements for ornamental fish is available as commercial products. The cost of microalgal feed remains a key factor for this and is higher than that of conventional feed. Here we tried to evaluate a fish feed formulated with a lower percentage of microalgae biomass inclusion, like 3% which will not contribute towards much cost increment and impact assessment done for 60 days with *Oreochromis niloticus* (TILAPIA) in true experimental conditions. *Oreochromis Niloticus* (tilapia) is a widely cultivated and highly demanded commercial species in Mexico and its impact at small-scale algal protein use will enhance future industrial-scale use in Mexico. Apart from that, this composition has better water stability properties which can reduce water contamination problems at large-scale use.

Methodology

Feed Processing by Extrusion

The formulation of feed was done according to the previous method [8] with 40% humidity, and the conditions of the extrusion were fixed with temperatures 60-80°C (inlet-outlet) at the University of Michoacan. First, we mix the powders with a mixer (Figure 1A), then an extrusion (Figure 1B), with nozzle output of 1.8 millimeters and 2mm cuts to have pellets size 2- 3 mm in size (Figure 1C.). We collected the products and used an oven to dry the products at 65 degrees centigrade for 4 hours and then at 40 degrees for 12 hours to dry maximum moisture from the samples (Figure 1D). Then we carried out a physicochemical analysis and nutritional and microbiological analysis of the feed.

Figure 1: A) Powder mixer; B: Extrusor C: Product with moisture D: Electric oven for product drying.

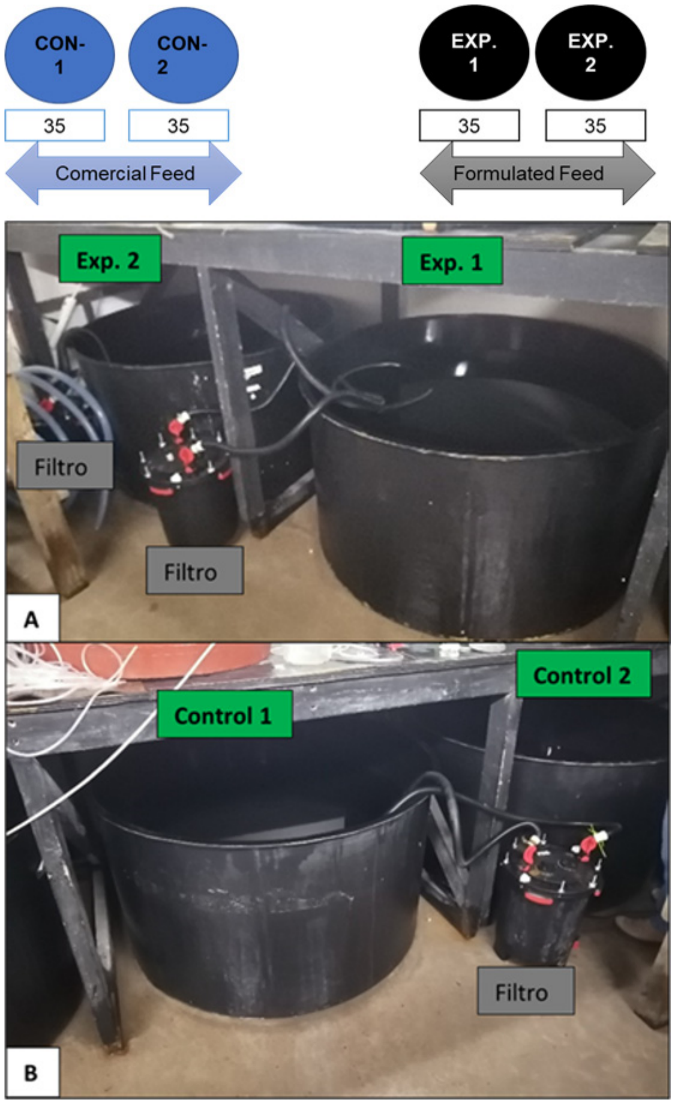

Figure 2: Right A) Tanks with fish to experiment with formulated feed Exp.1 and Exp 2; B) tanks with fish to experiment with commercial feed Control 1 and Control 2.

Determination of the Nutritional and Physicochemical Value of developed Foods

The nutritional analysis of each ingredient was performed with methods of AOAC International 1990 (NMX-F-608, F-083, F-607, F615, F-613), in the laboratory of the Analytical and Metrological Services Unit (USAM) of CIATEJ to determine the content of protein, carbohydrates and lipids. Subsequently, the formulations were performed using the Excel, program, balancing protein, lipid, and carbohydrate levels.

Water Stability Analysis

The method according to Cruz-Suarez et al., [9] was used for the water stability study of the extruded product above. Dry extruded feed (2g) and a commercial feed in triplicate were weighed, which were placed in glass vessels with 100 ml of water. Feeds were kept for 30-50minutes immersing in water with constant agitation (manually). After 50 minutes the dry matter loss was determined by draining the samples in a net; the products were placed in a drying oven at 70°C for 24 hours. The amount of loss with the difference in weight was determined.

Water absorption capacity: It was done according to the procedure of modifying the filtration of the sample by a mesh. Dry feed pellets immersed in water for different time periods were examined. The experiment was carried out in fresh water at room temperature. 1g of pellet of each type of product was

Table 1: Nutritional value of the extruded feed.

Nutritional value	Commercial feed (% of biomass)	Formulated feed (% of biomass)	Method
Humidity	12	6.19 ±0.5	NMX-F-083-1986
Ash	10	16.22 ±0.7	NMX-F-607-NORMEX-2013
Fat	10	8.07 ±0.3	NOM-086-SSA1-1994
Proteina	40	45.84 ±0.6	NMX-F-608-NORMEX-2011
T. Carbohidrate	24	23.68 ±1.1	NOM-501-HCFI/SSA1-2010
Fibre	4	4.48 ±0.7	NMX-F-613-NORMEX-2017
Fungus & Yeast	0	0	NOM-127-SSA1-1994,

Table 2: Growth parameters.

Growth parameters					
		FW	SGR	WG (%)	FCR
Initial week	Cont.	43.64	NA	NA	NA
	Expt.	43.28	NA	NA	NA
2nd week	Cont.	54.065	0.65	23.92	0.91
	Expt.	53.78	**0.70**	**24.26**	0.90
4th week	Cont.	66.64	0.80	23.28	0.91
	Expt.	66.13	**0.82**	**22.96**	**0.92**
6th week	Cont.	80.54	0.92	20.93	0.94
	Expt.	**84.85**	**1.24**	**28.40**	**0.94**
8th week	Cont.	93.57	1.16	16.15	0.98
	Expt.	**107.925***	**1.53***	**27.18**	**1.17***

randomly chosen and the dry weight was determined. Pellets were left submerged for 1, 5, and 10 minutes. After the immersion period, pellets were carefully retrieved and excess water was drained with an absorbent paper. Finally, pellets were reweighted to obtain a weight increase after immersion.

Density: To measure the density of the extruded feed and of a commercial (Cruz-Suarez et al., 2006)feed (control) for the tilapia method was used. 100 ml of fresh water was measured in a graduated cylinder, to which 30 grams of the extruded feed was added; subsequently, the displacement of the water was calculated for volume calculation, and the density was estimated with the following formula:

Density = weight of the sample/volume of the sample (feed)

Evaluation of Fish Feeding with Food Developed in Experimental Tanks

Feeding with fingerling Tilapia fish: A 60-day feeding trial was conducted to assess the suitability of microalgae and soybeans as protein sources in the diet of *Oreochromis niloticus* fingerlings with almost 42 g mean initial body weight. Diets were formulated and tested containing a high protein content of 45%, with 19% vegetable protein replacement.

Experimentation in Tanks at the Michin Aquarium. For the tilapia tanks, feeding was established in 2 tanks with control feed and another 2 tanks with formulated feed, each tank with 35 tilapias. Each tank contains 550 litres of water, 2 filters (1 handmade filter and 1 industrial filter) were installed, aeration was placed through a tube connected to the aeration pipe, and a commercial feeder (feeding 3 times a day).

Obtaining Tilapias and growth measurement: Thirty-five tilapias were placed for each tank obtained from the Michin aquarium industry. After collecting the fish, biometry of weight and length was carried out on each tilapia to maintain an initial record of the experimentation. Each tank was fed 3 times a day, with a JacRossy brand automatic feeder. Until you get the right amount of feed every week. Animals were weighed at the beginning and end of the experiment to determine initial (IW) and final (FW) weights and weight gain. The diets were weighed when they were administered to the animals, to determine the rate of weight gain per day (%W), specific growth per day (SGR), feed conversion ratio (FCR) etc... Performance data were submitted to analysis of variance (ANOVA).

Growth performance parameters: All growth parameters were determined at 2-week intervals. After the feeding trial, growth parameters including weight gain (%WG), length gain (LG), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR), were determined individually using the following equations:

1. FW (g) = (Total fish biomass (g)/ number of fish)

2. DCG = (Final weight – initial weight) /days

3. % WG = ((Final weight – initial weight (g) / initial weight (g)) *100)

4. FCR = Total food consumed (g) - WG (final weight – initial weight (g) / initial weight (g))

5. SR= % of surviving fish = ((final number of fish-initial fish)/ (initial number of fish))*100 6.LR=Length measurements we measure every 15 days.

Results

Feed Scaling up and characterization

The extrusion product was more asymmetric and thus the pellets maintained their size (2.5-3.5 cm) and with a controllable temperature gradient, thus maintaining greater resistance, maintaining its shape, and better stability in water. The velocity of production was 60 kg/hr. There was not much temperature gradient we opted to use not to degrade the functional properties of the algal ingredient used for the product. The temperature range was 60-80°C. Here we got moist extrusion material, and then one electric oven was used to dry the material slowly, and it also gave the good binding ability for better water stability. Then we filtered the feed to get a uniform size between 2.5 to 3.5 was needed for our experiment with Tilapia.

The basic function of fish feed produced through the extrusion process is to kill the microorganisms and improve the quality and digestibility to provide safe fodder for fish. In addition, with a longer and easier storage period, fish feed via an extrusion process, including floating or sinking types.

The commercial fish feed extrusion process refers to cooking the mixture of feed ingredients under high temperature, moisture, and high pressure by means of a fish feed extruder within a short time; as a technological treatment, extrusion can make it possible to process a variety of fish feed ingredients and raw materials with high water content. According to the moisture content of raw materials treatment, the fish feed extrusion process can be divided into two types - dry type extrusion and wet type extrusion.

Determination of the Nutritional, Physicochemical, And Microbiological Value of Developed Foods

Moisture, ash, protein, and lipid levels were not significantly different from commercial feeds. Only the percentage of protein used here is higher is 45%; generally, those used for tilapia from 35 to 40%, and the source of protein is animal protein replaced by 19% by vegetable protein such as isolated soy and protein from microalgae. In the case of food formulated by CIATEJ, less moisture was obtained, and thus contamination and growth of microorganisms were avoided (Table 1).

The increasing demand for fish for human consumption has caused fish farming to become a more intense activity and has increased the need for formulas of hundreds of fish for aquaculture. Tilapias are aquatic organisms with moderate protein requirements. One of the traditional ingredients used to develop protein diets is fishmeal. Despite its high cost, this supply is in high demand for both aquaculture and terrestrial animals. Efforts are underway to search for alternative conventional and unconventional protein sources. The purpose of this study is to review the impact of alternative protein sources, such as microalgae and soybeans to partially replace fishmeal in tilapia feed. In addition, it addresses the challenges facing aquaculture feed regarding the substitution of fishmeal for plant-based proteins.

The top ten producers include three Asian countries like Thailand, the Philippines and Sri Lanka and one North American country (Mexico) [10] Among all protein sources, soybean has become one of the best candidates and potentially of alternative plant protein source to replace FM in practical diet for tilapia due to its balanced amino acid composition. Aquaculture diets are usually the meals resulting after oil removal from the soybean and are the most studied aquaculture diets. However, it has a limiting factor that lacks methionine and contains some anti-nutrients such as trypsin inhibitor, hemagglutinin, and anti-vitamins (Tacon et al., 2009) when included in a higher percentage of the diet. [12] reported a test diet with 75% FM was replaced by SBM, which improved tilapia growth similar to the Fish meal diet. [13] also reported no significant difference in weight gain and feed efficiency of a hybrid tilapia-fed diet containing 35% fishmeal and a soy-protein-based diet. A recent study reported that microalgae-based feeds improve nutritional quality and fish growth (Sarker, Kapuscinski, McKuin, et al., 2020). By using *Nannochloropsis sp.* and *Isochrysis* sp. biomass to substitute fish meals and fish oil in the diet of rainbow trout was found to be effective for increasing digestibility coefficients for crude proteins, amino acids, lipids, and fatty acids [5] Microalgae biomass can be used as a high-value feed for fish and shrimp in sustainable aquaculture [14]. However, developing a balanced diet with microalgae and scaling up at the industrial level was lacking. So here we replaced 19% of animal protein with vegetal protein, including soy and microalgae protein first time in 2017 [8] In this formulation, the protein was higher like 45% and fat was kept lower like 2% compared to the commercial diet. We want to see the algal biomass effect as an antioxidant and other supplements like vitamins and minerals on tilapia growth.

Density: The feed from extrusion had a density of 1.03g/cm^3, a bit higher than commercial feed that is values are very close to those shown by the commercial control of 0.93/cm^3.

Texture: Hardness was measured by TPA texture profile analysis of the control and extruded feeds. Hardness is related to resistance and is the maximum force required to compress/break the feed pillate, generally occurring at the point of maximum compression, each sample was tested 6 times at constant force. The analysis of the texture profile revealed that the extruded feed with microalgae showed higher hardness values due to may be for good agglutinating material used that is gelatin, than the control feed, obtaining 9.77kg/F for the extruded feed and 9.68 kg/F for the control feed. This means that it maintains better stability in water, proposing that a pellet will have high durability if the product is able to relax the force applied on the surface in a non-elastic way.

Texture analyzers are a common method to understand the hardness of the fish feed industry. If it is too hard not easy to digest, in our formulated feed case, it only varies a little not a significant difference from commercial feed.

Water absorption capability: Water absorption capability was shown at different time intervals, which absorbed a greater amount of water as time passed (10-30 min) after immersion. Compared to the control feed, the extruded pellets absorbed less water without biomass disintegration to water, probably due to the higher density of extruded foods (Figure 3A).

The percentage of water absorbed by the extruded feeds after the first 5 minutes of hydration was 37.2% on its initial weight, 80.82% absorption at minute 10, and 100.22% at minute 20. Figure 3 shows the tendency of the different treatments, those absorbed more water and noticeably good amounts like 3.87 to 5.11g. as the time of immersion in water is longer. Water absorption always favours good digestibility of the feed. However, it was found to be a little higher in the case of commercial feed like 35.2 to 127.2%.

Water Stability analysis: For aquatic feeds, important quality factors are the pellet disintegration and nutrient leaching that impacts water contamination. In our experiment, the water stability in water of the feed extruded showed little higher losses of matter 21.6% at 30 min and 22.4 % at 60 min with respect to commercial feed 14.4% after 60 min of immersion in water. As we are thinking about the extrusion processing, we have done it without a temperature gradient one that helps gelatinization of ingredients to increase binding ability, we just may need to improve that next work using different extrusion processes to increase, Still, the values are quite impressive not to contaminate that much of water after one hour of time periods which is enough for the fish population to finish feeding (Figure 3B).

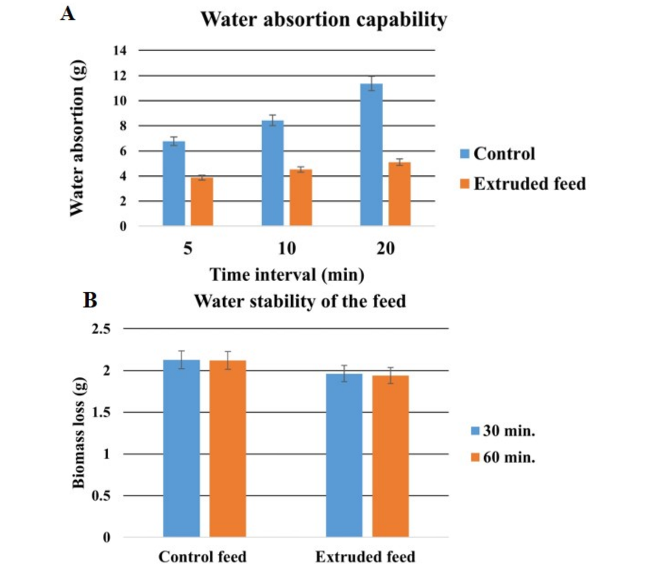

Figure 3: A) Water absorption B) biomass disintegrated to water (water stability) from the feed pellet.

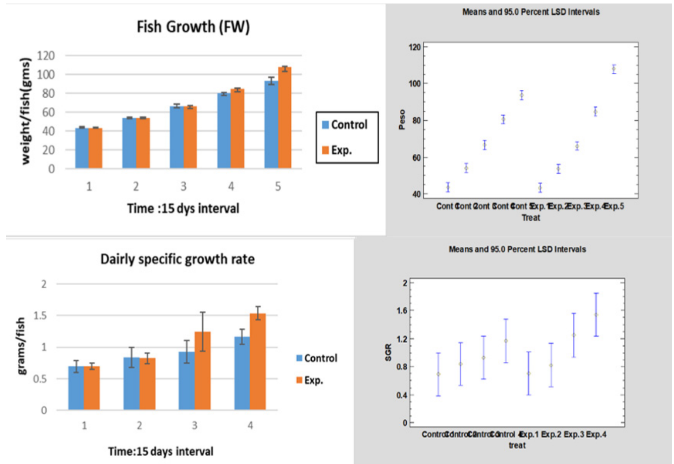

Figure 4: A) Growth analysis during experimental feed (FW), B) Daily-specific growth rate.

Table 3: Nutritional value of the meat (quality).

Nutritional value of the Fish filet	Commercial Feed	Formulated Feed
Humidity	74.31±1.03	74.15±1.05
Ash content	3.62±0.03	4.71±0.03
Fat	**6.24±1.01**	**4.82±1.01**
Proteine	**14.44±1.01**	**16.19±1.03**
Carbohydrates	o.10±0.01	0.10±0.01
Fiber	1.39±1.01	2.13±1.05

Weight and growth measurements

Fish actively consumed all feeds in time, and there was a progressive increase in juvenile growth on both diets. Mean values of growth performance parameters feed utilization efficiency and survival rate data of *O. niloticus* fed different experimental diets are summarized in Table 2. The control diet recorded an average weight gain of 93.57 g, while the Expt. average weight increased by 107.92 g. noticeably higher. For both experimental diets, weight gain was significantly different (P<0.01), the increase with exposure time, registering the maximum values at the end of the experiment (after 2 months) (Figure 4A & 4B).

Under good growing conditions, 1-gram fish are grown in nursery ponds to 1 to 2 ounces (20 to 40 grams) in 5 to 8 weeks and then stocked in nursery ponds. In monosexual grow-out ponds under good temperature regimes, males typically reach a weight of 1/2 pound (200 grams or more) in 3 to 4 months, 1 pound (400 grams or more) in 5 to 6 months and 1.5 pounds (400 grams or more) in 5 to 6 months. (700 grams). grams) in 8 to 9 months. In our practice, daily change was not possible because high water costs were generated because the ammonia concentration was sometimes high. We also found a similar growth rate in 2 months nearly 90-100gms, with starting initial weight at 43-44 gms. A simple one-way ANOVA analysis estimated within the group demonstrated that the P-value of the F-test is less than 0.05 i.e. 0.0001, there is a statistically significant difference between the mean weight of the experimental feed with the commercial feed treatment at the 5% significance level. From the LCD plot, it is clear that the initial control feed was not significantly different from the experimental feed, but in the last two weeks, it has shown a statistical difference in growth, such as greater weight gain with the experimental feed (Figure 4B).

Average Specific Growth Rate: The daily specific growth rates (SGR) showed clear fluctuations ranging from Experimental feed that was 1.53 and in the commercial diet was 1.16 (Table 2 & Figure 5). Tilapia fed an algae diet exhibited better SGR than tilapia fed a commercial diet.

From a simple estimation of one-way ANOVA analysis estimated within the group given that the P value of the F test is less than 0.05, that is, there is no statistically significant difference between the specific growth value with commercial feed and experimental feed, indicating that these pairs do not show statistically significant differences at the 95% confidence level. In just the last week, the experimental feed with specific growth showed statistically significant differences compared to the commercial feed at a 95% confidence level (Figure 5A).

Weight gain Percent (WG%): In the experimental diet, an average weight gain of 27.17g was recorded, while the commercial diet recorded a weight gain of 16.15g (Figure 5A). For both experimental diets, weight gain increased, but it was not significant (P<0.01) with exposure time, recording the maximum values at the end of the experiment (after 2 months).

Since the F-test P-value is greater than or equal to 0.05, there is no statistically significant difference between the mean %W from one TREAT level to another at the 5% significance level. However, the growth range (27 to 28) percentage is notably higher in the case with experimental diet as (Figure 5B).

Feed conversion ratios (FCR): Means of the Feed Conversion Ratio (FCR) of Nile tilapia fed on commercial diets and seaweed diets at the end of the experiments are presented in Figure 5B.

The FCR values were almost similar in the fish fed the commercial diet compared to those fed the microalgae diets during the first month of the experiment.

According to the current results, a (P<0.05) significant difference was recorded between the FCR of fish fed with commercial diet and fish fed with CIATEJ formulation. In general, at the end of the experiment, fish fed with diets of microalgae and vegetable proteins showed the highest FCR compared to those fed with commercial diets.

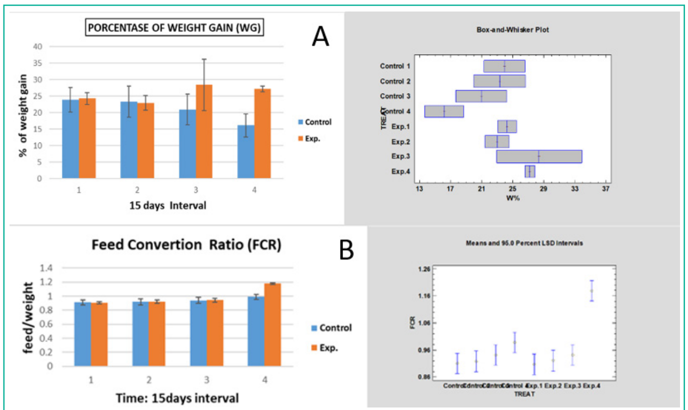

Figure 5: A) Weight Gain Percent (WG%), Graphic 5 B) Feed Conversion Ratios (FCR).

Table 4: Fatty-acid profile.

	Fatty acid profile	CON	EXPT
1	palmítico	2.2±0.01	0.81±0.01
2	esteárico	0.58±0.03	0.31±0.01
3	Oleico	0.38±0.04	1.76±0.04
4	Linoleico	1.66±0.72	2.26±0.72
5	linolénico	0.14±0.01	0.13±0.01
6	**Omega 3**	**0.14±0.01**	**0.21±0.01**
7	**Omega 6**	**1.87±0.06**	**2.33±0.06**
8	**Omega 9**	0.39±0.02	1.76±0.02
9	Saturated fatty acids	2.61±0.08	**1.13±0.08**
10	Monoaaturated fatty acids	0.39±0.06	1.76±0.06
	Polisaturated fatty acids	**1.80±0.05**	**2.4 ±0.05**

The intervals currently displayed are based on Fisher's Least Significant Difference (LSD) procedure. They are constructed so that if two means are equal, their intervals will overlap 95% of the time. In Plot, display the intervals graphically by selecting Plot of Means from the list of plot options. In multiple-range tests, these intervals are used to determine which means are significantly different from others. An asterisk has been placed next to 9 pairs, indicating that these pairs show statistically significant differences at the 95% confidence level. The method currently used to discriminate between means is Fisher's Least Significant Difference (LSD) procedure. With this method, there is a 5% risk of calling each pair of means significantly different when the actual difference equals 0.

Body length gain: The means of length gain was found to be 17.58 ± 1.48 cm for the fed commercial diet (Control Diet) and fish fed with algae formulation (Diet-Expt.) is 18.0 ± 1.01 cm during 2 months of growth period. Length gain was found to be slightly greater but was not significantly (P>0.05) higher in Expt. Dietetics compared to a commercial diet.

Survival rate: No mortality (Table 8) was observed during the entire process time of the experiments, neither in fish fed with commercial diet nor fish fed with algae. (SR=100%).

Growth summary: FCR is a measure of an animal's efficiency in converting feed mass to body mass. The comparisons here were made separately every 15 days during the experiment. FCR values were significantly higher in the microalgae diet than in the fish-fed commercial diet. According to (15) the FCR for fish fed with well-prepared diets ranges between 0.91 and 1.17(16) concluded that FCR 1.19 indicated the most efficient feed utilization by Oreochromis niloticus fingerlings. FCR values of almost 1 were reported here, indicating the more efficient utilization of food by *Oreochromis niloticus.* The results suggested that the microalgae diet promoted Nile tilapia growth and improved nutrient utilization, which is reflected in better overall length gain, weight gain, FCR and SGR. The lower growth rate recorded in the commercial fish-fed diet can be attributed to low feed conversion efficiency. Progressive increases in WG, LG and SGR were observed in microalgae-based diets, recording better growth. Table 2.

There are few previous studies on Spirulina biomass use in fish feed in common carp (Cyprinus carpio) 10% fish meal replacement with Spirulina biomass demonstrated doubled weight gain and in *Pungasius sutchi* feeding with 5% Spirulina supplement enhances significant growth. The survival rate was also higher (100%) when Tilapia (*Oreochromis niloticus*) was fed with 5% dietary *Spirulina platensis*. The inclusion of 10% *S. platensis* resulted in better colouration in Rainbow trout reported by (Aminul Islam et al., 2018). So here the effect of spirulina biomass inclusion rate is evaluated in our work to validate that a small percentage, like 3% biomass inclusion, has a significant effect on tilapia growth within 3 months. Another higher protein factor was concentrated soy protein, contributing to the higher growth rate.

Nutritional value of the meat (quality): The main component of tilapia was moisture 74.15% and 74.31%, followed by protein 16.19% and 14.44%, lipids 4.82% and 6.24%, carbohydrates 0.10% and 0.10%, fibre 2.13% and 1.39%, ash 4.71% and 3.62%. commercial feed and experimental feed, respectively Table 3. These results have a very similar range to previous reports on tilapia fillet fish by Heliyon et al. 2021. In our results, we found a 12% increase in protein percentage and a 29% decrease in fat percentage, as expected from the higher protein, lower fat formulated feed compared to the commercial feed. It is a good amount of protein we achieve in 2 months, you can increase the percentage of protein in fish meat when we feed longer with food formulated with algae. FCR values of almost 1 were reported here, indicating the more efficient utilization of food by *Oreochromis niloticus*.

Fatty acid profile in fish meat: Twelve fatty acids were identified in the fillet, predominantly palmitic (C16:0) and linoleic (C18:2n-6). The experimental diets significantly influenced the content of polysaturated fatty acids by 2.4 %, while saturated fatty acids were 1.3 %. Omega 6 and Omega 9 was 2.33% and 1.76% respectively. Higher polyunsaturated fatty acid/saturated fatty acid ratios compared to control, contributing to a reduced thrombogenic index in sampled fillets. Table 3 & 4.

Discussion

Alternative protein ingredients for feed production in the aquaculture sector have lots of importance due to an increasing gap in the protein supply. Usually, products derived from fish, soybean, wheat, corn, and animal by-products are the major protein source for animal feed (Tacon et al., 2009) Due to the shortage in supply of conventional ingredients, sustainable feed generation is a concern with conventional protein sources, alternative protein sources from crop plants [18], insects [19], single-cell proteins (bacteria, yeast and microalgae) and filamentous fungi [20,21] nowadays has been extensively studied. Microalgae were previously reported as additives in feeds to boost growth, immunity, meat colouration/quality, and other biological activities [22], whereas developing a balanced feed and evaluation of its impact on growth has very few reports. Specifically, microalgae have a great possibility for the next generation marine protein source, due to their potential to replace fishmeal and sustainability as ingredients in aquaculture [23-27]. But the inclusion rate and the feed impact assessment are always key factors as in some cases higher rates of microalgae inclusion in feeds also resulted in negative effects in certain species [26,28-30]**.** So here we specifically evaluated low inclusion rate is beneficial to tilapia growth rate or not; we also found that within a few months, it was having significant effects.

The inclusion of specific algae species is also very important due to their nutritional profile. Previous reports have shown that including small amounts of 2.5e10% [31] and 20% [32] of microalgae in diets has resulted in higher growth performance, feed utilization efficiency and physiological activity of fish. Microalgae-based feeds are better due to phytate content and intestinal inflammation produced by the soybean meal [33].But the use of microalgae-derived feeds at higher inclusion levels also exerts a negative effect on fish growth due to the presence of inhibitory substances in microalgae e.g. tannins, protease and amylase inhibitors etc [31].

Conclusion

We conclude that algae biomass and other plant protein biomass added to tilapia feed nutritionally enriches fish fillets in only 2 months of feeding, significant growth, and is a good choice for commercial production.

Author Statements

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References

1. Olsen RL, Hasan MR. A limited supply of fishmeal: Impact on future increases in global aquaculture production. Trends in Food Science and Technology. 2012; 27: 120–8.
2. Chen F, Leng Y, Lu Q, Zhou W. The application of microalgae biomass and bio-products as aquafeed for aquaculture. Algal Research. 2021; 60: 102541.
3. Jannathulla R, Rajaram V, Kalanjiam R, Ambasankar K, Muralidhar M, Dayal JS. Fishmeal availability in the scenarios of climate change: Inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources. Aquaculture Research. Blackwell Publishing Ltd. 2019; 50: 3493–506.
4. Richter N, Siddhuraju P, Becker K. Evaluation of nutritional quality of moringa (Moringa oleifera Lam.) leaves as an alternative protein source for Nile tilapia (Oreochromis niloticus L.). Aquaculture. 2003; 217: 599-611.
5. Sarker PK, Kapuscinski AR, Vandenberg GW, Proulx E, Sitek AJ. Towards sustainable and ocean-friendly aquafeeds: Evaluating a fish-free feed for rainbow trout (Oncorhynchus mykiss) using three marine microalgae species. Elementa. University of California Press. 2020; 8.
6. Sarker PK, Kapuscinski AR, McKuin B, Fitzgerald DS, Nash HM, Greenwood C. Microalgae-blend tilapia feed eliminates fishmeal and fish oil, improves growth, and is cost viable. Sci Rep. 2020; 10: 19328.
7. Kusmayadi A, Leong YK, Yen HW, Huang CY, Chang JS. Microalgae as sustainable food and feed sources for animals and humans – Biotechnological and environmental aspects. Chemosphere. 2021; 271: 129800.
8. Martínez-Delgado AA, Khandual S, Morales-Hernandez N, Martínez-Bustos F, Vélez-Medi-Na JJ, Nolasco-Soria H. International Journal of Food and Nutritional Science Fish Feed Formulation with Microalgae H. Pluvialis and A. Platensis: Effect of Extrusion Process on Stability of Astaxanthin and Antioxidant Capacity. 2020.
9. Cruz-Suarez LE, Patricia Ruiz-Díaz P, Cota-Cerecer E, Nieto-Lopez MG, Guajardo-Barbosa C, Tapia-Salazar M, et al. Revisión sobre Algunas Características Físicas y Control de Calidad de Alimentos Comerciales para Camarón en México. 2006; 175.
10. El-Sayed AFM. Tilapia culture. Tilapia Culture. CABI Publishing. 2006; 1–277.
11. Feed ingredients and fertilizers for farmed aquatic animals Sources and composition.
12. Jauncey K, Falaye E. The use of meat and bone meal, hydrolyzed feather meal and soybean meal in practical fry and fingerling diets for Oreochromis niloticus. 1983.
13. Violal S, Arieli' Y, Zohar' G. Animal-Protein-Free Feeds for Hybrid Tilapia (Oreochromis niloticusx 0. aureus) in Intensive Culture. Aquaculture. 1988; 75.
14. Camacho-Rodríguez J, Macías-Sánchez MD, Cerón-García MC, Alarcón FJ, Molina-Grima E. Microalgae as a potential ingredient for partial fish meal replacement in aquafeeds: nutrient stability under different storage conditions. J Appl Phycol. 2018; 30: 1049–59.
15. SS de Silva, TA Anderson. Fish Nutrition in Aquaculture. Google Libros.
16. Ogunji JO, Wirth M. Deutscher Tropentag 1999 in Berlin Session: Sustainable Technology Development in Animal Agriculture Effect of Dietary Protein Content and Sources, on Growth, Food Conversion and Body Composition of Tilapia Oreochromis niloticus Fingerlings.
17. M Aminul Islam Professor AK, Mizanur Rahman Professor M, Rafiqul Islam Professor M, Chandra Shaha Assistant Professor D. A Seminar Paper on Use of Spirulina in Fish Culture Course Title: Seminar Course Code: AQC 598. 2018.
18. Kaushik SJ, Hemre GI. Plant proteins as alternative sources for fish feed and farmed fish quality. In: Improving Farmed Fish Quality and Safety. Elsevier Inc. 2008; 300–27.
19. Henry M, Gasco L, Piccolo G, Fountoulaki E. Review on the use of insects in the diet of farmed fish: Past and future. Vol. 203, Animal Feed Science and Technology. Elsevier BV. 2015; 1–22.
20. Barka A, Blecker C. BA Microalgae as a potential source of single-cell proteins. A review. Biotechnol Agron Soc Environ. 2016; 20: 427-436.
21. Jones SW, Karpol A, Friedman S, Maru BT, Tracy BP. Recent advances in single cell protein use as a feed ingredient in aquaculture. Vol. 61, Current Opinion in Biotechnology. Elsevier Ltd. 2020; 61: 189–97.
22. Li J, Fan Z, Qu M, Qiao X, Sun J, Bai D, et al. Applications of Microalgae as Feed Additives in Aquaculture. 2015.
23. Guedes AC, Sousa-Pinto I, Malcata FX. Application of Microalgae Protein to Aquafeed. In: Handbook of Marine Microalgae: Biotechnology Advances. Elsevier Inc. 2015; 93–125.
24. Moomaw W, Berzin I, Tzachor A. Cutting Out the Middle Fish: Marine Microalgae as the Next Sustainable Omega-3 Fatty Acids and Protein Source. Industrial Biotechnology. 2017; 13: 234–43.
25. Sarker PK, Kapuscinski AR, Bae AY, Donaldson E, Sitek AJ, Fitzgerald DS, et al. 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. 2018; 13.
26. Rahman Shah M, Antonio Lutzu G, Alam A, Sarker P, Kabir Chowdhury MA, Parsaeimehr A, et al. Microalgae in aquafeeds for a sustainable aquaculture industry. J Appl Phycol. 2018; 30: 197–213.
27. Tibbetts SM. The Potential for 'Next-Generation', Microalgae-Based Feed Ingredients for Salmonid Aquaculture in Context of the Blue Revolution. In: Microalgal Biotechnology. InTech. 2018.
28. Wang Y, Li M, Filer K, Xue Y, Ai Q, Mai K. Evaluation of Schizochytrium meal in microdiets of Pacific white shrimp (Litopenaeus vannamei) larvae. Aquac Res. 2017; 48: 2328–36.
29. Madeira MS, Cardoso C, Lopes PA, Coelho D, Afonso C, Bandarra NM, et al. Microalgae as feed ingredients for livestock production and meat quality: A review. Vol. 205, Livestock Science. Elsevier BV. 2017; 111–21.
30. Xie S, Wei D, Tan B, Liu Y, Tian L, Niu J. Schizochytrium limacinum Supplementation in a Low Fish-Meal Diet Improved Immune Response and Intestinal Health of Juvenile Penaeus monodon. Front Physiol. 2020; 11: 613.
31. Norambuena F, Hermon K, Skrzypczyk V, Emery JA, Sharon Y, Beard A, et al. Algae in fish feed: Performances and fatty acid metabolism in juvenile Atlantic Salmon. PLoS One. 2015; 10.
32. Younis ESM, Al-Quffail AS, Al-Asgah NA, Abdel-Warith AWA, Al-Hafedh YS. Effect of dietary fish meal replacement by red algae, Gracilaria arcuata, on growth performance and body composition of Nile tilapia Oreochromis niloticus. Saudi J Biol Sci. 2018; 25: 198–203.
33. Grammes F, Reveco FE, Romarheim OH, Landsverk T, Mydland LT, Øverland M. Candida utilis and Chlorella vulgaris counteract intestinal inflammation in Atlantic salmon (Salmo salar L.). PLoS One. 2013; 8: e83213.