CHAPTER -1: INTRODUCTION

Algae form the basis of the entire aquatic food chain and promote the production of renewable resources from fishing at a rate of 100×10^6 ton per year. As a photosynthetic organism, unicellular microalgae play a significant role in aquatic food web. Passing of energy from the base to higher tropic levels comes from autotrophic phytoplankton which are a marvelous source of nutrients as well as several bioactive compounds like carotenoid, antioxidant, fatty acid, peptide, enzymes, and so on (Anand et al., 2014). However, algal species absorb carbon di-oxide including other nutrient ions which have detrimental effect on aquaculture as well as natural environments (Badawy et al., 2008). Most recently, biofuel production from microalgae has grabbed the world's attention, therefore this technology possesses very strong positive impression in mercantile approaches (Anand et al., 2014). Besides, there are many others application of these phototrophic micro-organisms in human and animal food, health, and cosmetic industry. Fishmeal is very common ingredient of fish feed. About 5037 thousand tones fishmeal is used in 2017 in aquaculture industry (FAO, 2018)). Now the increasing evident is showing that such continued consumption of this natural resource will unsustainable both environmentally and economically. Thus, an alternative feed ingredient must be used to supply complete nutritional value. Some plant proteins, commonly used in aquaculture, are lack of certain amino acids such as lysine, threonine, methionine, and tryptophan, where microalgae found to contain all the amino acids essential for animal growth, survivability, and reproduction (Brown et al., 1997). Marine microalgae found to provide alternative source of aqua feed ingredients with an improved environmental sustainability and essential human health well-being.

The aquaculture is a rapidly growing sector and tremendously increasing its production. Various species of potential microalgae have been using frequently in this sector because of their huge nutritious values, where *Chlorella*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Tetraselmis*, *Scenedesmus*, *Skeletonema*, and *Thalassiosira* are the most commonly used genus. They grow rapidly in adverse culture conditions with a wide tolerance range of temperature, light and nutrients, which frequently vary in the farm. The selection of microalgae for massculture must be done on the basis of a good nutrient composition and an absence

of toxins that might be transferred into the food chain (Velichkova et al., 2012). The main purpose of microalgae massculture is to use as feed for potential aquatic animals. Currently 30% of the world algal production is being used as an ingredient of animal feed (Sharma et al., 2013) but the live use in aquaculture is also incorporated to larval rearing of fish, mollusks, and crustaceans (Hattab et al., 2014). Other than that, microalgae also contain numerous bioactive compounds such as pigment components which are responsible as the main source of coloration of different fish species (Becker, 2007).

Many marine microalgae species are rich in DHA or EPA; omega-3 fatty acids (Walsh andDoren, 2015). Some researchers are predicting that microalgae will quickly become a cost competitive component with other feed ingredients like fishmeal due to ongoing technological improvements that lowered the production costs for microalgae (Holland, 2016). Likewise, microalgal co-product, the protein-rich biomass and left over after extracting oil for biofuel and nutraceuticals products, is increasingly available and may be used as a low-cost replacement for conventional protein ingredients in tilapia feeds (Adarme et al., 2012). Some microalgae strains are recognized as excellent sources of proteins, carbohydrates, lipids, and vitamins for food and feed additives since last 40 years. Various species of macro and micro algae have been used to formulate fish feed for feeding trials with different fish species (both freshwater and marine) to assess their nutritional value. As a result, some algae have been found to be beneficial such as *Chlorella* or *Scenedesmus,* when fed to Tilapia (Castro et al., 2009); *Chlorella*, when fed to Korean rockfish (Christaki et al., 2011, Dahman et al., 2019); or a *Nannochloropsis Isochrysis* combination, when fed to Atlantic Cod (Walker et al., 2009, 2010). Therefore, microalgae have tremendous potentiality as nutrient source for both terrestrial and aquatic animals. Various marvelous characteristics have made different algae strains very much suitable as major source of feed ingredient for fish, especially tilapia. The omnivorous feeding habit of tilapia fish provides an immense advantage to accept algae as feed. Tilapia is a kind of fish which can survive in low water quality, low dissolve oxygen, high level of ammonia where other fishes generally can't survive (Walsh, 2000). The hardy nature and convenient management have made this species very much suitable to be selected as an experimental species in different feeding trial with algae so that possible outcomes can be easily analyzed with minimum risk.

A research was needed to determine if less expensive feed ingredients can replace fish meal in fish feed. Thus, this study was designed to use microalgae as a cost-effective source of nutrition in fish feed.

1.1 Objectives of the study:

The study was conducted to achieve the following objectives:

- To observe the growth performance and survival rate of juvenile Tilapia (*Oreochromis niloticus*), fed with different microalgae diets
- To evaluate the water quality parameters of juvenile Tilapia (*Oreochromis niloticus)* culutre tanks, fed with different microalgae diets
- To compare nutritional composition of juvenile Tilapia (*Oreochromis niloticus),* fed with different microalgae diets

CHAPTER -2: REVIEW OF LITERATURE

2.1 General Attributes of Microalgae

Microalgae are unicellular, prokaryotic or eukaryotic microscopic organisms found in both fresh and marine habitat (Durma, 2007). Microalgae range from 5µm to more than 100mm in length (Becker, 2007). Some microalgae species are found as good sources of nutrition for both human and fish. For their immense chemical diversity, microalgae give a wide range of application as food and feed additives of both human and fish. As they show some resistant against some bacteria and virus, they are highly potential for pharmaceutical industries. Valuable substances such as nutraceuticals, carotenoids, phycocyanin, poly unsaturated fatty acid (PUFA), and the whole biomass can be utilized in feed and food industry (El-Sayed, 2006).

2.2 Importance of Microalgae as Fish Feed

The use of microalgae based on its bioactive chemical component has developed rapidly in recent years with application in areas such as fruit, pharmaceutical, cosmetics, aquaculture, and horticulture (Enas et al., 2020). Microalgae play an essential role in aquatic food chain and are popularly used in rearing of aquatic animals like mollusks, shrimps, and fish at different growth stages (Ekubo and Abowei, 2011). These microscopic organisms are the primary food sources for most aquatic organisms and have an important role in aquaculture development since it is the desired food for early growth stages of many commercially important aquatic animals (Gerber et al., 2016). There is huge attention as a possible alternative protein source for cultured fish, particular in tropical and subtropical developing countries where algae production rates are high and their higher protein, vitamins and essential fatty acids contents (Badawy et al., 2008) which can be used to improve the color, flavor and quality of flesh. Data concerning chemical composition of algae give the basic information of the nutritive potential of the algae biomass (Brown et al., 1997). Nearly all the microalgae are producing unique natural chemicals such as antioxidants, carotenoids, fatty acids, enzymes, polymers, peptides, toxins and sterols (Dahman et al., 2019). They contain high number of vitamins, essential amino acids, minerals, and fatty acid and carotenoid pigments for aquatic animals (Ju et al., 2008; Guroy et al., 2011; Gomez et al., 2016). The combinations of suitable algal species can provide a well-balanced diet as they are potential source of vitamins which

facilitates the larval development (Howe et al., 2006; Ju et al., 2012). Therefore, they are essential for larvae nutrition and can be fed either as direct live consumption or indirectly within formulated diets. In most cases, the algae biomass is used as feed or feed supplement. Live algae also improve the water quality. To achieve good growth performance, they must be of an appropriate size for ingestion, e.g., from 1 to 15 μm for filter feeders; 10 to 100 μm for grazers (Webb & Chu, 1983; Lorenz et al., 2000; Jeffrey et al., 2017;) and readily digested. They are also used as feed for zooplankton. Also, they can be induced to accumulate substantial amounts of lipids, leading to high oil yield (Ibrahim and El-Zanfaly., 1980; Fon-Shing et al., 2016). Also, they can be used for feed additives, pharmaceutical and nutraceutical purpose (Kaya and Kaptaner, 2016). But as the microalgae has extremely potent natural antioxidants available and pivotal role in supporting the immune system among other medicinal properties against the detrimental effects of both pathogens and pollutants, therefore, it is highly recommended to make these biomolecules available within food and feed purposes (Howe et al., 2006; Santhosh and Singh, 2007; Guillerme et al., 2017).

2.3 Characterization of the Microalgae Species

2.3.1 *Tetraselmis chuii*

Tetraselmis sp. are unicellular flagellates with elopside to ovoid cell shape, slightly flattened cells with motile, and it usually grows 10 μm long x 14 μm wide (Pfening et al., 2011). It has four flagella that are equal in length, a medium to thick cell wall and it moves in straight line while swimming with the body rotating and the flagella at the forward end. This species reproduces by binary fusions and sexual reproduction has never been observed, the motile cells become non-motile when cell divisions occur, or in some species the non-motile cell attached to the surface. The cell divisions occur during the dark period with the energy that collected during the day in form of carbohydrate storage, while the separation of cells is induced by the onset of the light (Regan, 1988). *Tetraselmis chui*, *Tetraselmis suecica*, and *Tetraselmis tetrahele*, are widely used as a feed in aquaculture because they are easy to culture. Now a days, euryhaline strains of *Tetraselmis* that can grow under a wide range of salinity have gained preference as potential, sustainable sources of lipid forbiofuels (Fon-Sing and Borowitzka, 2016)

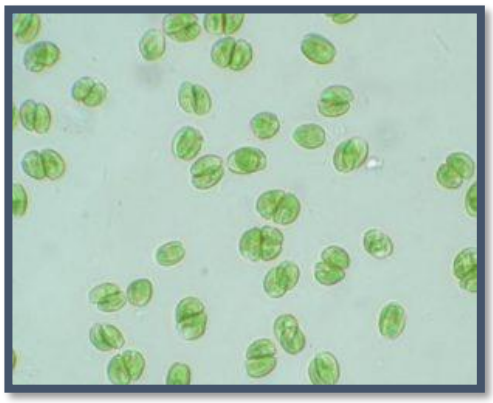

Figure 1: *Tetraselmis Chuii*

2.3.2*Nannochloropsis sp.*

Nannochloropsis sp. is a microalga that is related to diatoms and brown algae and living in freshwater and seawater (Andersen et al., 1998; Sukenik et al., 2009) These unicellular algae are 2-5 microns in diameter and spherical or oblong in shape (Kagan & Matulka. 2015). *Nannochloropsis* species are planktonic, unicellular with either 2–4 μm diameters subspherical or 3-4 × 1.5 μm cylindrical cells (Patterson and Galtin, 2013) that provide a yellow-green chloroplast with the main pigments being chlorophyll a and the xanthophylls, violaxanthin and vaucheriaxanthin (Toguyeni et al., 1997). As the genus appears to lack meiosis thus it never participates in sexual reproduction (Patil et al., 2007). *Nannochloropsi*s is well appreciated for feeding rotifers in fish hatcheries because of their nutritional value and higher biochemical composition

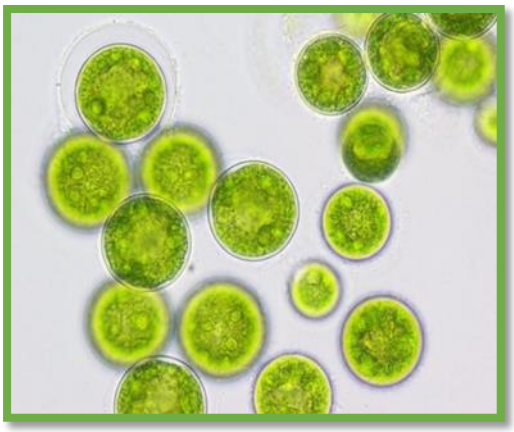

Figure 2: *Nannochloropsis chuii* (Source:Enas et al., 2018)

2.4 Biology and Ecology of *Oreochromis niloticus*

A species of tilapia, a cichlid fish, *Oreochromis niloticus* is commercially known as Nile tilapia, mango fish, niloticus, or boulti. The word, "Tilapia" is a derivative of an African Bushman word simply meaning fish. Fishes belonging to the family Cichlidae is referred to as Tilapias which is an extremely hardy fish tolerating wide range of water parameter such as hardness, pH, temperature, and also dissolved oxygen. This species has their tolerance to hypoxia and will survive in low oxygen levels with high biochemical oxygen demand (BOD). Also, Tilapia is a hardy, prolific, fast growing tropical fish, and it can survive on a diversity of food (Richmond, 2015).

Figure1: *Oreochromis niloticus* (Source: Naylor et al., 2018)

2.4.1 Biological Features

Generally, Nile tilapias are brownish or grayish in color, sometimes with indistinct banding on their body, and the tail is vertically striped. During breeding, males become reddish, especially on their fins (Nico et al., 2019). Sometimes they are confused with the blue tilapia (*O. aureus*), which doesn't have the striped tail pattern and in dorsal fin they have a red edge that is gray or black in Nile tilapia. They can also be separated by meristic. The length of Nile tilapia can reach up to 60 cm (Froese et al., 2015) and weight can exceed 5 kg (11 lb). As average of tilapia, males reach a larger size and grow faster than females. Nile tilapia can live for long time, almost more than 10 years (Nico et al., 2019).

2.4.2 Habitat and Distribution

The freshwater cichlid, Nile tilapia is native to the Nile River basin and the south-western Middle East (Trewavas, 1983; Daget et al., 1991). Mostly for farming purposes, they have been introduced in all continents (more than 50 countries) except

Antarctica (Sheikhzadeh et al., 2012). It was introduced in Bangladesh from Thailand in 1954 (Santhosh and Singh, 2007; Shields et al., 2012). Mostly the natural habitat of Nile tilapia are different freshwater bodies like ponds, lakes, canals, streams, rivers and ranging from sea level to an altitude of 1,830 m (; Froese et al., 2015; Snoeks et al., 2018). As they are euryhaline species, so can also be found in brackish water, but is unable to survive long-term in full salt water (Froese et al., 2015). They can tolerate water temperatures between 8 and 42 °C (46 and 108 °F), although typically above 13.5 °C (56.5 °F) with some variations depending on the population. Although they can survive in a wide range of temperature, generally breeding only occurs in 24 °C (Nico et al., 2006).

2.4.3 Reproductive Biology

Nile tilapias are maternal mouth-brooders. Typically, the male build nest on breeding purpose where mass spawning of a brood occurs. Then the male fertilized the eggs and after that the fertilized eggs are picked up by the female in their mouth even after hatching. Female tilapias can produce several hundred to several thousand young per spawn. They breed within months after birth. Moreover, the younger Nile tilapia leads to high birth and turnover rates.

In the presence of other females, the female tilapia exhibit shortened inter-spawning intervals. Those female tilapias that abandon their young to the care of a male gain this advantage of increased inter-spawning periods. The reason of this reproductive mechanism is to increase the advantage of females allowing them more opportunities to spawn as they don't have to take care of the young ones anymore (Tacon, 1996). In case of males, the more dominant males get the reproductive advantages as they have higher levels of the gonadotropic hormone. Thus, for larger sperm production more successful males are selected. Also, dominant males have both the best territory in terms of resources and the greatest access to mates (Pfenig et al., 2011). Visual communication between mates both stimulates and modulates reproductive behavior between partners such as courtship, spawning frequency, and nest building (Castro et al., 2009).

2.4.4 Feeding Habit

The Nile tilapia is mostly herbivore, but with omnivorous tendencies, especially when young (Froese et al., 2015). Depending on their feeding behavior, they mostly feed on

phytoplankton and algae, and in some populations other macrophytes also an important feed source (Snoeks et al., 2018). Some other recorded food items are detritus and aquatic insect larvae (Froese et al., 2015), including those of mosquitoes (BBC, 2007). However, outside of their native range, it often becomes invasive, threatening more localized species (Froese et al., 2015). Similar to trout and salmon, Nile tilapia typically feeds during day time showing that light is a main factor in their feeding activity. As they have high reproduction rate, overpopulated culture will require night feeding to provide back up in competition among individuals for necessary nutrients. Based on a recent study it was observed that size dimorphism between the sexes is depended on food conversion efficiency rather than the amounts of food consumed. That's why, the male grows larger than the female consuming same amount of food because of their higher efficiency of converting food to body weight (Toguyeni et al., 1997).

2.4.5 Nutritional Profile

Tilapia has become a popular food item, mainly because of its high nutritional value, mild taste, and low expense relative to other finfishes. In America, tilapia has been appeared in top 10 seafood list (2002) and 5th in 2008 (NFI, 2010). Tilapia contains high protein, phosphorus, potassium, vitamin B-12, and is low in fat and saturated fat, omega-3 fatty acids, calories, carbohydrates, and sodium.

2.4.6 Culture Conditions

Production of tilapia is conducted either in pond systems (extensive culture) or in cages and tanks (intensive culture). Tilapia can be cultured in both fresh and salt water in tropical and subtropical regions, but culture can be constrained in temperate climates where indoor culture system is the only way of production (Lowry et al., 1951, Mahmoud et al., 1967). Optimal growing temperatures for tilapia are typically between 22° C (72° F) and 29° C (84° F) but in temperatures greater than 22° C (72° F), spawning normally occurs. Most tilapia species are unable to survive at temperatures below 10° C (50° F), and growth is poor below 20° C (68° F). Under good growing conditions (Swanson et al., 2012), at an age of 5/6 months and at a weight of 150/200 g (0.33/0.44 lb) they reach sexual maturity.

2.4.7 Global Production and significance of Nile Tilapia

The Nile tilapia is well-suited in wide range of trophic and ecological variations, as well as its adaptive life history characteristics make them a potential culture species worldwide (Trewavas, 1983). Therefore, it has been greatly introduced for aquaculture and sport fishing (Toshio et al., 2002; Walker et al., 2009) and is now found in every country in the tropics. Commercially, after carps, tilapia is the second most important group of wild-captured fish, 851 million tons of wild capture in 2018 (FAO, 2020). Tilapia culture has been popular since 1990s and currently it is one of the world's second most common group of farmed fish species, with a commercial production of 6031 million tons, corresponding to an estimated value of $11.7 billion (FAO, 2020). In 2020, Nile tilapia (*Oreochromis niloticus*) culture alone was ranked first among the most cultured species in the world, with a total aquaculture production of 6.031 million tons (FAO, 2020). Nile tilapia represents approximately 87.6% of total global tilapia production (FAO, 2020). In 2010, it is anticipated that global Nile tilapia production will reach nearly 15 million tones, with a market value of nearly $20 billion (FAO, 2020). China is by far the largest consumer and producer (about 46% of global production) of tilapia, with a production estimated at 1.62 million tons in 2018, up from 1.45 million tons in 2017 (FAO, 2018). Other main producing countries of farmed tilapia (2017–2018 data) are Indonesia (122,2741 tones), Egypt (105,1444 tones), Bangladesh (344,784 tones) Brazil (317,080 tones), and Philippines (277, 006 tones) (FAO GLOBEFISH, 2018). Projections indicate that Indonesia is most likely to rival China in tilapia production within the next decade (FAO GLOBEFISH, 2018).

Bangladesh has enormous possibility for tilapia farming due to its rapid growth rate and high market value, thus become one of the most widespread culturable species in Bangladesh (ADB, 2005; Sarker et al., 2018). Production of tilapia, for home or local consumption and for export, has risen enormously in the last few decades. A significant number of farmers in the rural area have been involved in tilapia farming due to its high productivity. Therefore, for viable and sustainable aquaculture, it is urgent need to identify the important and less expensive components, growth and survivability influencing ingredients can be used in culture system.

CHAPTER- 3: MATERIALS AND METHODOLOGY

This study was conducted in two phases. In the first phase, two selected marine microalgae species were cultured. Dried biomass of selected microalgae was kept in refrigerator for further use during feed preparation. In the second phase, growth, survival rate, and nutritional profile of tilapia larvae were determined.

3.1 Study area and Collection of Microalgae Species

In this study selected microalgae *Tetraselmis chuii*, and *Nannochloropsis sp.* were collected from laboratory of live feed research corner, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University. Using Conway culture medium, the pure *T. chuii*, and *Nannochloropsis* culture were maintained at 28 ppt salinity (Khatoon et al., 2018). To maintain pure and healthy stock culture sub-culturing of selected microalgae was done every two weeks. Pure seed culture of all microalgae species was maintained at 30 ppt salinity in Conway medium with a 24 hours photoperiod.

3.2 Seawater collection

Water was collected from the nearest sea beach (Sagorika sea beach, Kattoli, Chattogram) during high tide in Bay of Bengal. Collected seawater was brought in laboratory and was kept overnight to settle solid particle in water. Water was filtered using a Whatman GMF Circles 4.7cm paper using vacuum pump. Filtered water were autoclaved at 115ºC for 15minutes. Filtered and sterilized water was used to prepare media for microalgae culture.

3.3 Media Preparation

Three stock solutions (macronutrients, trace metal solutions, and vitamins) were prepared by dilution of the chemical composition in water as shown in Table 1. The Conway medium was prepared by adding Solution A, Solution B, and Solution C with a volume of 1 ml, 0.5 ml, and 0.1 ml, respectively, into 1 L of filtered and sterilized sea water of 28 ppt salinity.

Table 1: Chemical composition of Conway medium (Tompkins et al., 1995)

Constituents	Quantities
Solution A- Macronutrients	
Sodium nitrate ($NaNO_3$)	20g
Ferric chloride ($FeCl_3$)	1.3g
Boric acid (H_3BO_3)	33.4g
EDTA(b), di-sodium salt	45g
Manganese chloride (MnCl2, 4H2O)	0.36g
Sodium di-hydrogen orthophosphate (NaH2PO4, 2H2O)	20g
Distilled water	1000ml
Solution B- trace metal	
Zinc chloride (ZnCl2)	4.2g
Cobaltous chloride (CoCl2, 6 H2O)	4.0g
Ammonium molybdate ((NH4)6Mo7O24, 4H2O)	1.8g
Cupric sulphate (CuSO4, 5H2O)	4.0g
Distilled water	1000ml
Acidify with HCl to obtain a clear solution	
Solution-C Vitamins	
Vitamin B1	200mg
Vitamin B12	10mg
Distilled water	1000ml

3.4 Culture of Microalgae

Microalgae were cultured in water of 28 ppt salinity at 23 °C temperature with 1600 lux light intensityin 24 hours of photoperiod. Filtered aeration was provided throughout the culture period. The initial cell density was 1×10^5 cells ml^{-1} for each treatment. Growth experiment for each microalgal species was carried out with three replicates until death phase.During the growth curve experiment, growth parameters in terms of cell density and optical density were measured daily. All experimental cultures for biomass collection were harvested in stationary phase as determined by the growth experiment previously. *T. chuii* and *Nannochloropsis sp*were harvested on

Day 8 harvested by centrifugation (HERMLE Z 206A High-Speed).Microalgae were centrifuged at 5000 rpm for 5 min after washing twice with sterilize distilled water. Then sample were freeze dried using Labconco Freezone 4.5 and kept at -20 ℃ until further analysis.

3.5 Mass Culture of Microalgae

Mass cultures of selected potential species were done in large scale in 20 L transparent plastic jar using Conway medium. The cultures were gradually scaled up from an initial starter culture volume of 20 ml to 20 L. Initially, 20 ml of microalgae stock cultures were mixed with 30 ml medium in each 50 ml conical flask (total culture volume 50 ml). Applying batch cultures, the volumes were increased to 250, 500 ml, 1 L, and gradually to 20 L. The cultures were transferred during their exponential phase of growth. Once the cultures reached their stationary phase, all cells were harvested by centrifugation at 5000 rpm for 5 min (using HERMLE Z 206A) followed by washing twice with sterilize distilled water. After that the samples were freeze dried using Labconco Freezone 4.5 and kept at -20 ℃ until further procedure. Mass culture was done continuously until adequate amount of microalgal biomass was obtained for the feeding experiment.

3.6 Feed Formulation for *Oreochromis niloticus* larvae

Six treatment diets were formulated to contain different percentages of microalgae, as the replacement of fishmeal at 25 %, 50 %, and 75 %, while in control diet no replacement was made. The oven-dried microalgae biomass was grinded into fine particles (diameter of 0.4 – 0.5 mm) using a mortar and pestle and then kept them -20 ℃ in Labconco Freezone 4.5 refrigerator until further use

Table2: Feed formulation for the experimental diets

Ingredients	**Control**	**Treatment**					
		25% replacement of fish meal		50% replacement of fish meal		75% replacement of fish meal	
		T25	N25	T50	N50	T75	N75
Commercial fish	68.71	51.53	51.53	34.35	34.35	17.17	17.17

meal							
Tetraselmis chuii (dried powder)	0	17.178		34.35		51.53	
Nannochloropsis (dried powder)	0	0	17.178		34.35		51.53
Wheat flour	9.43	9.43	9.43	9.43	9,43	9.43	9.43
Corn flour	9.43	9.43	9.43	9.43	9.43	9.43	9.43
Rice bran	9.43	9.43	9.43	9.43	9.43	9.43	9.43
Vitamin mixture	1	1	1	1	1	1	1
Mineral mixture	1	1	1	1	1	1	1
Molasse	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100	100

3.7 Proximate Analysis of the Formulated Feed

Table 3: Proximate composition of experimental diets

Proximate	**Control**	**Treatment**					
		T25	N25	T50	N50	T75	N75
Protein	40	37	35.03	35.9	32.45	33.24	29.31
Lipid	12.11	15.13	11.17	13.19	10.12	16.17	11.34
Carbohydrate	19.33	23.22	21.43	24.19	25.18	25.34	26.25

3.7.1 Protein Analysis

According to Lowry et al., (1951) protein was analyzed. For every sample, 5–6 mg of freeze-dried microalgae was taken and made into 25 ml solution by mixing with distilled water. From the 25 ml of sample prepared, 0.5 ml was taken from each sample for protein analysis. Prior to that, Reactive 1 (1 % Potassium sodium tartarate tartrate) and Reactive 2 (2 g of Sodium carbonate in 100 ml of 0.1N NaOH) were prepared. Mixed reagent preparation was done by adding 1 ml of Reactive 1 to 50 ml Reactive 2.Then, 0.5 ml of sample was added with 0.5 ml of 1 N Sodium hydroxide and it was kept in 100 ℃ water bath for 5 minutes. It was then cooled in a water bath and 2.5 ml of the prepared mixed reagent was added 10 minutes after cooling. The

mixed solution was added with 0.5 ml of Folin reagent and was kept in dark places for 30 minutes. The absorbance of the mixed solution was taken with spectrophotometer (UV-1601, Shidmadzu) at the wavelength of 750 nm.

3.7.2 Lipid Analysis

Based on Marsh and Weinstein (1966) the lipid analysis was conducted by the sulphuric acid-charring method, following the carbonization method using tripalmitin as the standard after extracting lipids according to the method of Bligh and Dyer (1959). The samples were extracted from 4.5 ml of chloroform: methanol (1:2) and it was then centrifuged at 10000 rpm for 10 minutes. After separating the supernatant from the biomass, 1.5 ml of chloroform and 1.5 ml of distilled water was added and the sample was centrifuged again to separate two phases. After centrifugation, the polar phase was removed with a pipette and evaporated under vacuum with a water bath at 35 °C. The dryer side was solubilized in 1 ml chloroform. Then, 3 aliquots of 200 μL each was taken from this solution and transferred into test tubes and the solvent was evaporated again. When completely dried, 2 ml of concentrated sulphuric acid was added. The carbonization process was carried out at 200 °C for 15 min, then the tubes were cooled and 3 ml of water was added into each tube. The optical density was measured at 375 nm wavelength.

3.7.3 Carbohydrate Analysis

Carbohydrate analysis was conducted based on the method of Dubois et al., (1956). For each sample, 5–6 mg was taken and made into 25 ml solution by mixing with distilled water. Prior to the analysis, 5 % phenol solution and concentrated sulphuric acid was prepared. Samples were analyzed by adding 1 ml of 5 % phenolic solution and 5 ml of concentrated sulphuric acid. The optical density was measured at 488nm wave length using spectrophotometer (Shimadzu UV-1601, Japan).

3.8 Feeding Experiment

Twenty-one black 5 L plastic bucket were used in this experiment. The tanks were washed thoroughly and soaked in 10 mg L^{-1} chlorine overnight to prevent any disease transfer through the rearing containers. Then the tanks were rinsed intensively with running tap water until the smell of chlorine was gone and they were placed to sun dry. Before stocking, each tank was filled with 4L filtered UV treated freshwater.

Tanks were not arranged randomly as the feeding trials were carried out in a closed hatchery with a uniformly distributed light source of the cool fluorescent light in the hatchery. *Oreochromis niloticus* larvae, at age of day 3, were obtained from a commercial hatchery and conditioned for 1 day. Five days old larvae were stocked at a density of 30larvae L^{-1} according to the standard stocking density of tilapia by FAO. Constant aeration was provided and the photo period was maintained according to the ratio of 12 h light: 12 h dark in each culture tank. The tilapia was fed four times per day at 7:30 am, 10:30 am, 1:30 pm, and 4:30 pm at the rate of 20% of their body weights. The Tilapia larvae were fed with the formulated feed mixed with different algae at 0 %, 25 %, 50 %, and 75 %. All treatments were done with triplicates and the experiment was conducted for a period of 22 days. Nearly 10 % of culture volume was siphoned out daily to maintain water quality. At the end of the feeding trials, the survived Tilapia from each tank were counted and weighed to estimate mean survival for the treatment and control groups.

3.9 Physicochemical Analysis

Water temperature, pH, and dissolved oxygen in the culture tanks were measured daily using the thermometer, pH meter, and DO meter, respectively. Total ammonia nitrogen, nitrite, and phosphate phosphorous were determined on alternate days following the method of Parsons et al., (1984). Thirty ml of water sample was collected from each tank every alternate day and filtered using a Whatman GMF Circles 4.7cm to conduct the analysis.

3.9.1 Total Ammonium Nitrogen (TAN)

Ten ml of water sample was placed in test tube. Then, 0.4 ml of phenol solution (20 g of analytical grade phenol dissolved in 200 ml of 95 % v/v ethyl alcohol) and 0.4 ml of sodium nitroprusside (1 g sodium nitroprusside dissolved in 200 ml of MiliQH2O) were added to the sample. After that, 1 ml oxidizing solution was added to start the reaction. The oxidizing solution was prepared by mixing 100 ml of alkaline reagent (100 g of sodium citrate and 5 g of sodium hydroxide dissolved in 500 ml of MiliQH2O) and 25 ml of sodium hypochlorite solution. The tubes were covered with parafilm and incubated at room temperature (20 - 27 ºC) for 1 hour before it was measured at 640 nm with Shimadzu spectrophotometer (Shimadzu UV-1601, Japan).

3.9.2 Nitrite (NO_2-N)

0.2 ml of Sulfanilamide solution was added to the test tube containing 10 ml of water sample. The sulfanilamide solution was prepared by dissolving 5g of sulfanilamide in a mixture of 50 ml of concentrated hydrochloric acid and diluted to 500 ml with MiliQH2O. Then, after 8 min, 1 ml of NED reagent (0.5 g of the N-(1-napthyl)-ethylenediamine dihydrochloride dissolved in 500 ml of MiliQH2O) was added to the tube and was mixed immediately. One hour afterwards extinction was measured at 543 nm with Shimadzu spectrophotometer (Shimadzu UV-1601, Japan).

3.9.3 Soluble Reactive Phosphorus (PO_4-P)

One ml of mixed reagent was added to the test tube containing 10 ml of water sample. Mixed reagent was prepared by mixing 100 ml of 0.02 M ammonium molybdate, 250 ml sulfuric acid, 100 ml of 0.31 M ascorbic acid, and 100 ml of 0.002 M potassium antimonyl-tartrate. After 5 min and preferably within the first 2-3 hours, extinction was measured at 885 nm by using Shimadzu spectrophotometer (Shimadzu UV-1601, Japan).

3.10 Survival and Growth Analysis of Tilapia Fry

The specific growth rate (SGR) was calculated from the body weight based on the formula of Ricker (1990):

$$SGR = (\ln wf - \ln wi/Dt) \times 100$$

Where, lnwfis the natural logarithm of the weight at time (t), and lnwi is the natural logarithm of the initial weight of the tilapia larvae.

The weight of the larvae was taken at initial (day 4) and when the larvae reached the day 26 stage. Tilapia larvae's survival was determined at the end of the experiment.

3.11 Statistical Analysis

The null hypothesis was that there were no differences of water quality, growth survival rate and nutritional composition between the stress treatments (T25, T50. T75, N25, N50, N75) and the control. The alternative hypothesis that there were no differences of water quality, growth survival rate and notional composition between the stress treatments (T25, T50. T75, N25, N50, and N75) and the control. Mean and standard error of mean were calculated in MS Excel. Obtained data was analyzed by

using one- way analysis of variance (ANOVA) as well as Tukey and Duncan multiple comparisons test.

CHAPTER- 4: RESULTS

4.1 Specific Growth Rate and Survival Rate of Juvenile Tilapia

Specific growth rate varied significantly ($p < 0.05$) among the different treatments (Figure 4). The highest specific growth rate was observed with the treatment T75(10.41±0.11 %), while the lowest specific growth rate was recorded for the treatment N75(8.90±0.09%). However, the treatment T50, T75, and N50 didn't vary significantly ($p < 0.05$), and were similar to the control treatment.

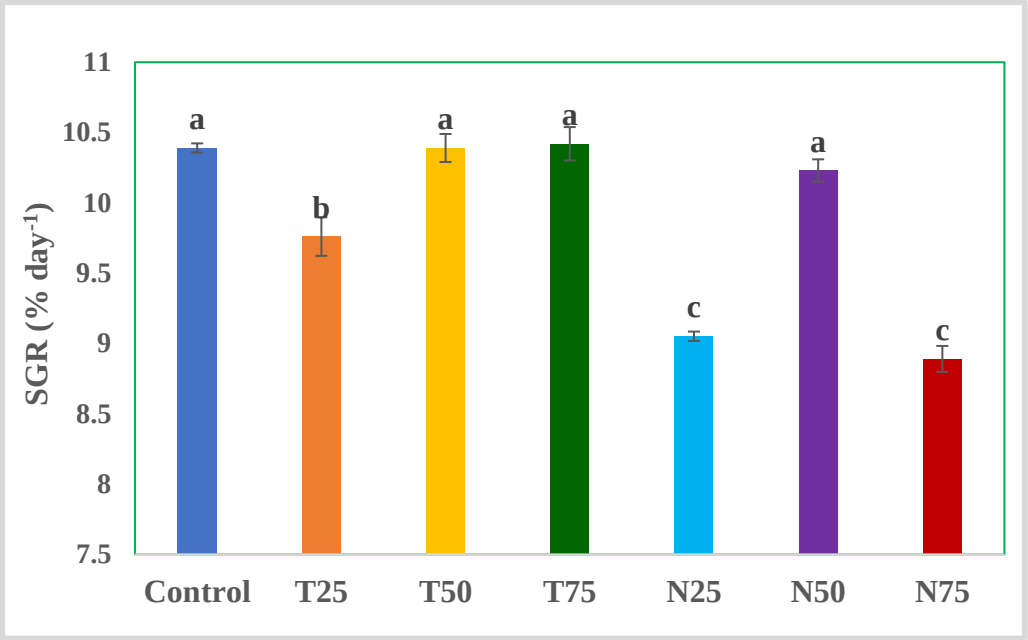

Figure4: SGR (% day^{-1}) of juvenile Tilapia (*Oreochromis niloticus*) in different treatments. T25, T50, and T75 are the replacement of fishmeal with *Tetraselmis* sp. at 25%, 50%, and 75%, respectively; N25, N50, and N75 are the replacement of fishmeal with *Nannochloropsis* sp. at 25%, 50%, and 75%, respectively. Values with different letters are significantly different ($p < 0.05$).

Survival rate of juvenile Tilapia was also significantly ($p < 0.05$) different among the treatments as well as varied from the control treatment (Figure 5). Among the all treatments, the highest survival rate was observed for the treatment T25 (96.11±2.2%), while control feed (79.67±1.95%) showed lowest survivability of juvenile Tilapia.

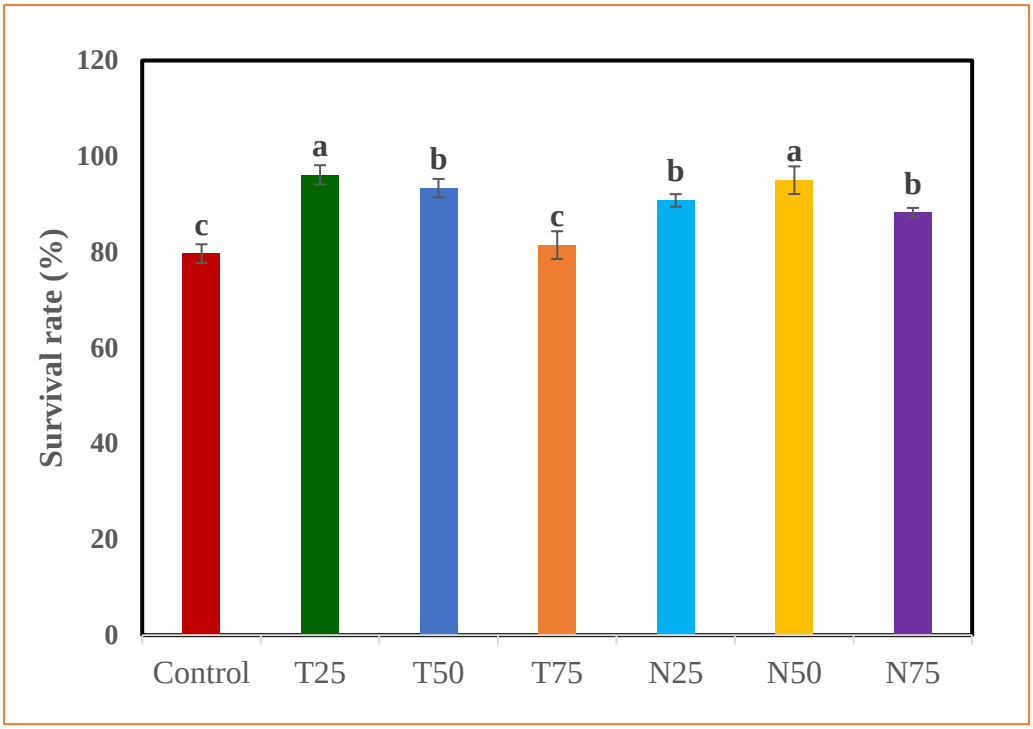

Figure 5: Survival (%) of juvenile Tilapia (*Oreochromis niloticus*) in different treatments. T25, T50, and T75 are the replacement of fishmeal with *Tetraselmis* sp. at 25%, 50%, and 75%, respectively; N25, N50, and N75 are the replacement of fishmeal with *Nannochloropsis* sp. at 25%, 50%, and 75%, respectively. Values with different letters are significantly different ($p < 0.05$).

4.2 Proximate Composition of Microalgae and Juvenile Tilapia

Protein, lipid, and carbohydrate of *Nannochloropsis* sp. and *Tetraselmis* sp. were determined in dry weight basis. Table 4 shows the proximate composition of these two microalgae. The variation in the protein percentages caused the variation of the protein percentages of formulated feed (Table 3, 4).

Table 4: Proximate composition of dried biomass of *Nannochloropsis* sp. and *Tetraselmis chuii* used to replace fish meal in diets for Tilapia (*Oreochromis niloticus)* fry.

Microalgae	**Protein%**	**Lipid%**	**Carbohydrate%**
Nannochloropsis sp.	49±2.87	25±0.29	22±1.62
Tetraselmis chuii	57±2.27	19±1.29	17±0.99

Protein, lipid, and carbohydrate content of juvenile Tilapia (*Oreochromis nilotica)* was determined at the end of the experiment (Figure 6). Most of the proximate composition of juvenile Tilapia, culture in different treatment, were significantly different when compared to the control treatment ($p < 0.05$, Figure 3). However, protein and lipid were significantly different in juvenile Tilapia with different treatments, while carbohydrate didn't vary among the juveniles cultured in different treatments ($p < 0.05$, Figure 3). In this study, among all the treatments, the highest protein in juvenile Tilapia was observed under T75 (27.36 ± 0.377 %), while the lowest was observed under N25 *(*17.41 ± 0.42 %) On the other hand, the highest lipid content in juvenile Tilapia was found under T25 (27.4±0.87%) while the lowest lipid was observed under T75 (19.36±0.47%).

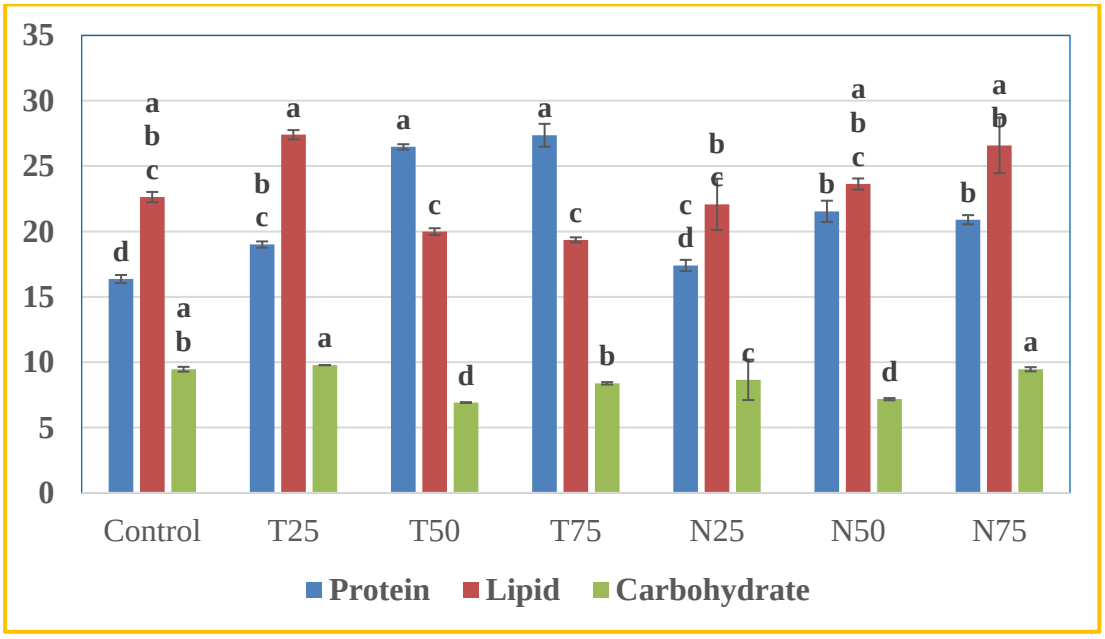

Figure 6: Proximate composition (dry weight basis) of juvenile Tilapia (*Oreochromis niloticus*) in different treatments. T25, T50, and T75 are the replacement of fishmeal with *Tetraselmis* sp. at 25%, 50%, and 75%, respectively; N25, N50, and N75 arethe replacement of fishmeal with *Nannochloropsis* sp. at 25%, 50%, and 75%, respectively. Values with different letters within each series are significantly different ($p < 0.05$).

4.3 Water Quality of the Culture Tanks

Dissolve oxygen, temperature, and pH of juvenile Tilapia culture water tank under different treatments didn't vary significantly; range of dissolve oxygen, water temperature, and pH are shown in Table 5. Total ammonia nitrogen (TAN), nitrite-nitrogen, and soluble reactive phosphorus significantly ($p < 0.05$) varied among the different experiment compared to control treatment. Different TAN was observed in T25 (0.17–0.35mg/L), T50 (0.11–0.34mg/L), T75 (0.22–0.33mg/L), N25 (0.16–0.40mg/L), N50 (0.17–0.40mg/L), andN75 (0.15–0.46 mg/L) which is shown in Figure7. Similarly, different NO_2-N were observed in T25 (0.47–0.67 mg/L), T50 (0.42–0.89mg/L), T75 (0.31–0.89mg/L), N25 (0.39–0.92mg/L), N50 (0.46–0.83mg/L), and N75 (0.36–0.86 mg/L) which is shown in Figure 8. Soluble reactive phosphorus also varied in T25 (0.12–0.21mg/L), T50 (0.12–0.22mg/L), T75(0.02–0.24 mg/L), N25 (0.11–0.41mg/L), N50 (0.12–0.22mg/L), and N75 (0.10–0.23mg/L) which is shown in Figure 9.

Table 5: Dissolve oxygen, water temperature, and pH recorded in different juvenile Tilapia culture tans under different treatments.

Treatments	**DO (mg/L)**	**Temperature^{o}C**	**pH**
Control	4.7–5.6	25.1–27.3	7.1–8.9
T25	4.9–5.9	25.6–27.8	7.4–8.7
T50	4.6–5.4	24.9–27.7	7.6–8.9
T75	4.9–5.7	25.1–28.1	7.2–9.1
N25	4.8–5.8	25.3–27.5	7.5–9.2
N50	4.9–5.7	25.2–27.3	7.6–8.6
N75	4.4–5.9	24.9–27.4	7.4–8.2

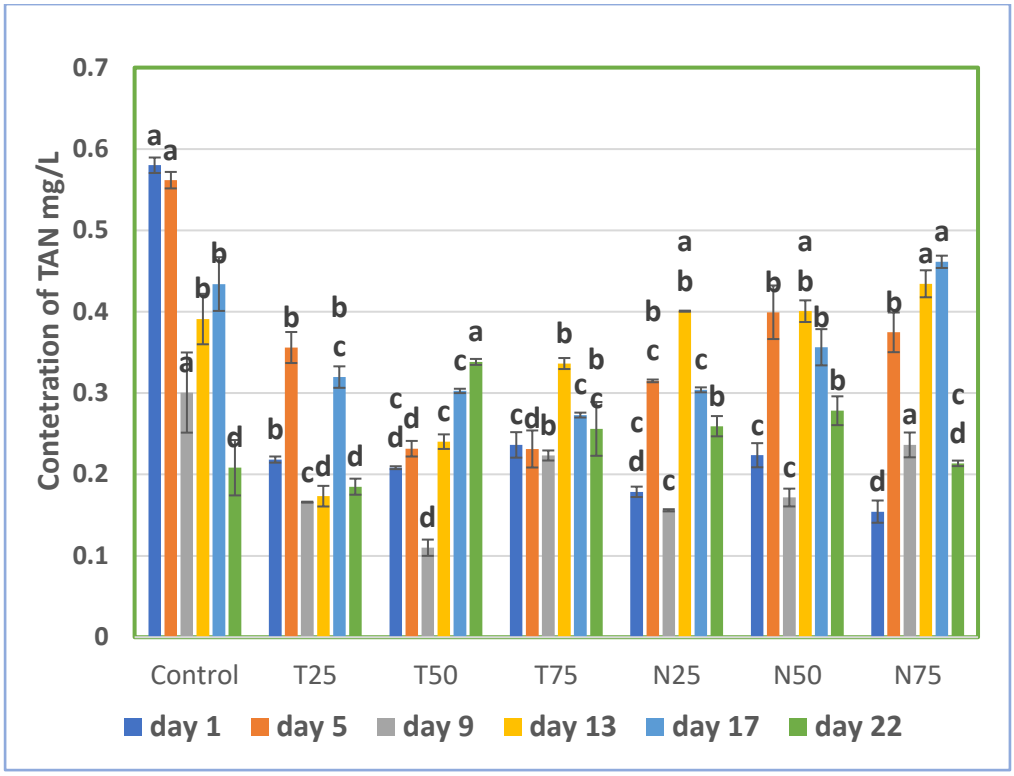

Figure 7: Total ammonia nitrogen (mg/L) of juvenile Tilapia culture tanks during the feeding experiment. T25, T50, and T75 are the replacement of fishmeal with *Tetraselmis* sp. at 25%, 50%, and 75%, respectively; N25, N50, and N75 are the replacement of fishmeal with *Nannochloropsis* sp. at 25%, 50%, and 75%, respectively. Values with different letters within each series are significantly different ($p < 0.05$).

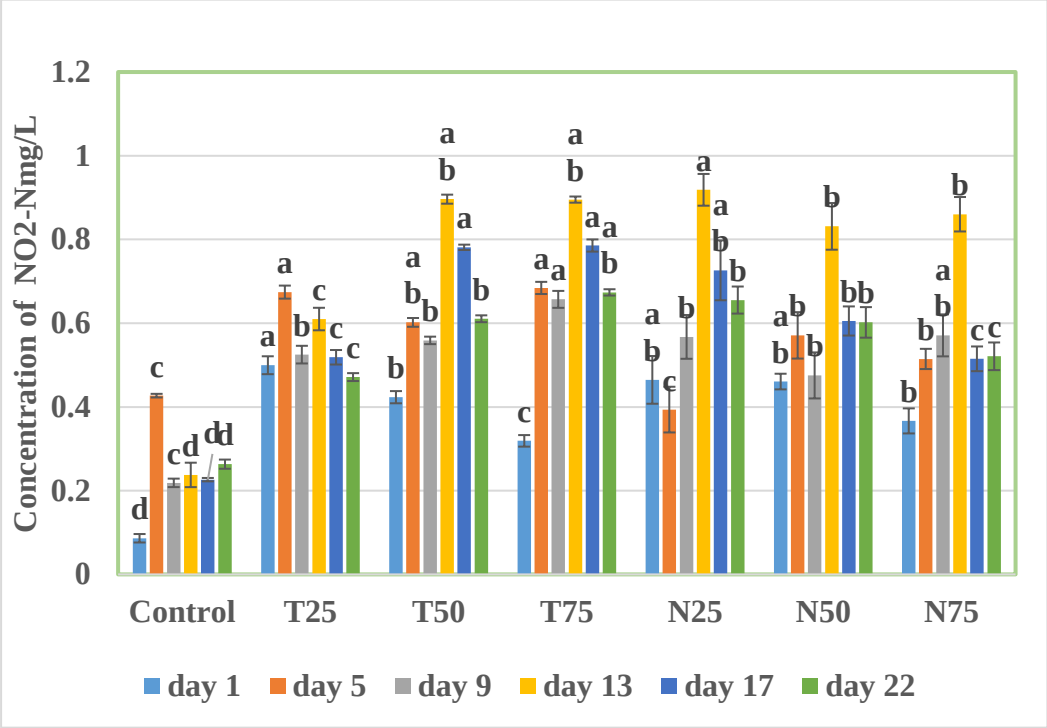

Figure 8: Nitrite -nitrogen (mg/L) of juvenile Tilapia culture tanks during the feeding experiment. T25, T50, and T75 are the replacement of fishmeal with *Tetraselmis* sp. at 25%, 50%, and 75%, respectively; N25, N50, and N75 are the replacement of fishmeal with *Nannochloropsis* sp. at 25%, 50%, and 75%, respectively. Values with different letters within each series are significantly different ($p < 0.05$).

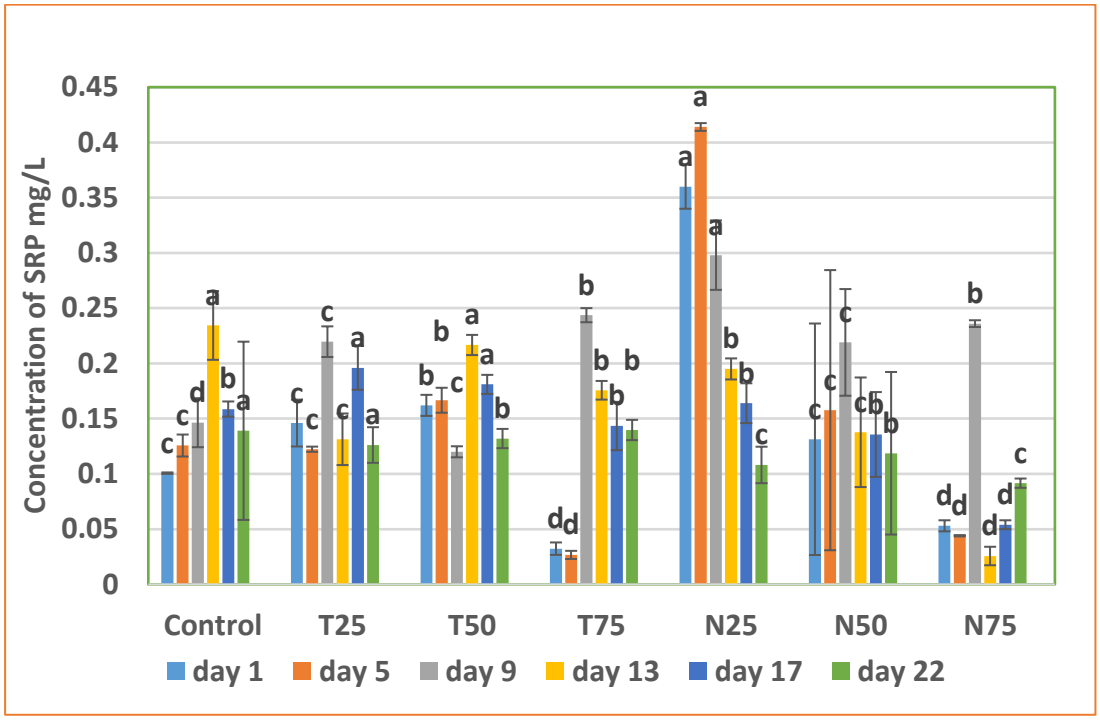

Figure 9: Soluble reactive phosphate (mg/L) of juvenile Tilapia culture tanks during the feeding experiment. T25, T50, and T75 are the replacement of fishmeal with *Tetraselmis* sp. at 25%, 50%, and 75%, respectively; N25, N50, and N75 are the replacement of fishmeal with *Nannochloropsis* sp. at 25%, 50%, and 75%, respectively. Values with different letters within each series are significantly different ($p < 0.05$).

CHAPTER- 5: DISCUSSION

The food inevitability of fish is associated to their activity and optimum environmental conditions, mainly water temperature, pH, alkalinity, hardness, total nitrogen, and ammonia (Brown et al., 2002). In tilapia culture reliance is deliberately increasing on the use of different substitute sources of protein in the feed formulation in order to decrease feed production costs. Many researchers suggested different cost-effective protein sources for the preparation of feed to achieve optimum growth of fish (Tacon and Metian, 2009; Kaushik and Troell, 2010; Radhakrishnan et al., 2016). Commercial fish farming depends upon a balanced feed prepared from quality feed ingredients (Naylor et al., 2009; Yaakobet al., 2014). Microalgae are very important in aquaculture industry and have been used widely as live feeds for larval or juvenile crustaceans and finfish. For all growth stages of bivalve mollusks (e.g., oysters, scallops, clams and mussels), for larval/early juvenile stages of abalone, crustaceans and some fish species, microalgae are used as live feed and for zooplankton used in aquaculture food chains at large. Microalgae act as the primary producer for the entire aquatic food chain which makes it a very important food source in aquaculture, especially during the culture of shrimp, fish and mollusks(Muller-Feuga, 2000). Microalgae species are used in this study due to their importance as feed for both larval and growth stages of bivalves, shrimp and certain fish species in aquaculture (Guedes and Malcata, 2012). In this study, *O. niloticus* was fed with a control feed with a replacement of 25, 50% and 75% of fish meal with two marine microalgae species, *Tetraselmis chuii* and *Nannochloropsis* sp. as sources of protein.

5.1 Specific Growth and Survival Rate of Tilapia

Sinhaet al., (2004) found that fishmeal could be totally replaced with plant protein sources without any adverse effect on the growth of tilapia. In this study, *Oreochromis niloticus* performed best with the 75% replacement of fish meal by using *Tetraselmis chuii* compared to the other feed, that is related with the result of Kiron et al.,(2012) who reported weight gain in Atlantic salmon (*Salmo salar*) fed an experimental diet in which whole and lipid-extracted algal meals replaced 5 or 10 % of dietary protein from fishmeal with *Tetraselmis*, but 50% replacement of fish meal by *Nannochloropsis* showed similar specific growth rate with the control. The

comparison of means indicate that the fish supplied with 75% replacement of fish meal by *Nannochloropsis* showed lowest specific growth rate, that is almost alike with the result of Sarker et al., (2018) which indicated that, when *O.niloticus* were fed with different levels of *Nannochloropsis oculata* (33, 66 and 100 %) it increases their growth performance with the increase of algae percentage in fish diets compared to control diet, except 100% replacement. Best SGR was recorded in tilapia fed with 75% replacement of fish meal by *Tetraselmis chuii* in this experiment.

The results of survival rate of different treatments were surprising. The highest survival rate was found in T25% which was 96.11%, and the lowest in fish with fed with control feed (79.67%). *Oreochromis niloticus* showed better survival rate with fed of microalgae containing feed. Microalgae were reported to enhance growth effects (Sinha et ai., 2008) and have many health benefits for aquatic animals, such as having immune enhancing (Jean-Baptiste and Katoh, 1994), anti-inflammatory or phagocytosis (Jensen et al., 2001), and anti-viral (Jean-Baptiste, 1996) properties. The inclusion of microalgae in the diets was also considered to be essential for the effective use of nutrients by fish, and the improved growth rate was associated with improved physiological conditions such as protein assimilation, lipid metabolism, liver function, stress response and high survival rate (Hough et al.,1995).

5.2 Proximate Composition of Microalgae and Juvenile Tilapia

In this study, diet with *Tetraselmis chuii* and *Nannochloropsis* showed various percentage of lipid content where T25% and N75% showed higher lipid content. After the proximate analysis of fish meal (which was used as the main protein source in this study), *Tetraselmis* and *Nannochloropsis*, higher lipid percentage was found in *Nannochloropsis* (14.07 ± 0.13 %) followed by *Tetraselmis* (13.87 ± 0.45 %) and fish meal (11.21 ± 1.12 %). According to the Mohammed (2017) microalgae provide higher amount of lipid and different kind of fatty acid on diet. There are also diversified use of the supply of fatty acids and vitamins for fish in aquaculture systems (Hamasaki and Gapasin et al., 1998; Yakoob et al., 2014). In general, mixed-microalgal diets provide more balanced nutrition (e.g., lipids, proteins, carbohydrates and essential fatty acids) than single-microalgal diets and thus are more likely to meet the nutritional requirements of larval/juvenile bivalves produced in hatcheries (Whyte

et al., 1990; Fujii et al., 2010). With the increasing of microalgae in diet, the nutrition level in the fish body is increasing as their digestibility.

Various studies suggest that herbivores require comparatively less amounts of proteins compared to carnivores (Toguyeni et al.,1997). *T. chuii* and *Nannochloropsis sp.* were selected in the study as the microalgae, for the tilapia larvae has been shown to contain a sufficient level of protein, lipid and carbohydrate for growth and survival of aquaculture organisms. From the result it was found that, Nile Tilapia fry which was fed with *Tetraselmis chuii* in 75% replacement of fish meal had greater protein content than all other treatments that is strongly correlate with the result of Hassan et al., (2020), where diet supplemented with the highest inclusion level (50%) of dried *T. chuii* resulted in highest protein content in Nile Tilapia (Hassan et al., 2020). In case of *Nannochloropsis*, fry also showed higher protein content where fish meal was replaced at 50% by *Nannochloropsis sp*. than the control that is almost similar with the result of Sorensen et al., (2017) which concluded that *Nannochloropsis* are able to influence high growth and protein content in Nile tilapia in replacement of 30% of fish meal. In the present study among all the treatments replacement of 75% fish meal by using *Tetraselmis chuii* and replacement of 50% fish meal by using *Nannochloropsis* had higher protein content than all other treatments. Sarker et al., (2020) found high protein content in Atlantic cod in 40% replacement of fish meal with *Nannochloropsis* defatted biomass with the *Scizochytrium.*

5.3 Water Quality of the Culture Tanks

Throughout the experiment, water quality parameters, both chemical and physical were in optimal range. Dissolve oxygen level was found in all treatments ranged from 4.4 mg/L to 5.9 mg/L during the entire study period of 22 days. According to Riche and Garling (2003), the preferred DO for optimum growth of Tilapia is above 5 mg/L. Other researchers have proved that Tilapia can tolerate condition of high oxygen super saturation of up to 40 mg/L (Tsadik and Kutty, 1987). Water pH level was ranged from 7.1 to 9.1 mg/L in all the treatments. BEAR (1992) reported a pH range of between 6.5 and 9.0 mg/L as optimum for growth of Tilapia. Temperature was found between 25.4 to 26.8 mg/L in all the treatments. There were no significant differences in temperature among the treatments. Temperatures between 20°C and 36°C have been reported by various researchers as the suitable for Tilapia culture.

According to Kausar and Salim (2006), for instance, the preferred temperature range for optimum tilapia growth in ponds is between 25°C and 27°C. FAO (2011) reported the preferred temperature ranges of between 31°C and 36°C, while Ngugiet al., (2017) gave a range of between 20°C and 35 °C as ideal for Tilapia culture.

Microalgae have a high capacity for extracting inorganic nutrients from waste water, according to Sorenson et al., (2017). The principal method for extracting algal nutrients from wastewater is their incorporation into cell biomass (Bigh et al., 1999). When the ammonia oxidation rate exceeds the rate of nitrite oxidation and the denitrification phase of heterotrophic bacteria under anaerobic conditions, nitrite accumulates in water (Boyd and Tucker, 1998). Increased levels of TAN and NO_2-N with culture time are significant factors that affect the health, survival, and growth of tilapia larvae.

Total ammonia nitrogen significantly varied with the culture days and treatments (Figure 7). BFAR (1992) also reported ammonia levels of between 0.02–0.05 mg/L as the optimum for tilapia growth. In the present study, ammonium concentrations in the all treatments from 0.16mg/L to 0.46mg/L may not be considered a problem because it has been reported that in *Oreochromis niloticus* growing system, even with frequent water exchange, ammonium concentrations may increase up to 1.5mg/L (Chen and Tu, 1991). The ammonium concentrations in this study never exceeded 1.5 mg/L (safe concentration to Tilapia). This indicates that during the study period the harmful nitrogenous waste was effectively removed by phytoplankton and microbial activity (Shilo and Rimon, 1982; Diab and Shilo, 1988).

Nitrite-nitrogen also varied significantly with the culture days and treatments (Figure 8). Nitrite concentrations never exceeded the unsafe level of 1.0 mg/L at any time during the study period although there was an abrupt increase in day 14 in the treatments. Nitrate reached a maximum concentration of 0.91 mg/L in 13^{th} day and this concentration is also not higher than the maximum acceptable concentration of 1.0 mg/L. In the present study control had significantly lower levels of soluble reactive phosphorus than the treatments with significant differences among culture period (Figure 9). The soluble reactive phosphorus levels reported from tilapia culture ranged from 0.016 to 0.082 mg/L. The result of phosphorus from treatments are lower than the levels of 0.061–0.093 mg/L reported by Ekubo et al., (2010) and the

maximum recommended level of 0.2 mg/L for aquaculture (Boyd and Tucker, 1998). Higher levels of soluble reactive phosphorus in culture systems are indications of pollution. The soluble reactive phosphorus levels recorded in this study were lower than the recommended value for aquaculture (Boyd and Tucker, 1998).

CHAPTER 6: CONCLUSIONS

Addition of microalgae within fish diets led to an antioxidant enhancement with slight growth performance improvement and gives fish a great immunity against bacterial infection and increase the survival rate. In this respect, a considerable amount of research with several fish species has been conducted on the use of meals derived from whole cell or lipid-extracted microalgae, alone or in combination with agricultural plant-protein or animal-protein products in fish diets, showing that partial, and sometimes complete replacement of fish meal is possible. This study showed the better survival rate in fish fed with microalgae while their water quality is not good as control tanks water. Nutritional composition also proved the higher proximate value in fish after microalgae used as main protein source. So, the result of this study provides evidence of two potential microalgae that can be used as a replacement of fish meal to enhance growth, survival rate, immunity and nutritional composition of cultivable fish like tilapia. Furthermore, result of this study provide a basis for the expansion of fishmeal-free feeds for tilapia with two different marine microalgae *Tetraselmis chuii* and *Nannochloropsis sp* as protein source. This finding needs further investigations like on-farm growth trials for large scale commercial farming to develop more cost-effective diets using such microalgae as a replacement of fishmeal.

CHAPTER -7: RECOMMENDATIONS AND FUTURE PROSPECTS

The purpose of this study was to determine an alternative protein source for fish as main protein source is fish meal which will reduce the cost of feed. Although a qualitative approach was followed to explore the objectives of the research, there are some limitations of the study which can minimize by following recommendation:

- Mass culture of microalgae should be done in a cost-effective way for commercial purpose.
- There should be a separate place or lab for microalgae culture to avoid contamination.
- Biomass of microalgae should be dry in optimal temperature to reduce protein and lipid degradation.
- There should maintain optimum range of physical and chemical parameter for both micro algae and fish culture.

Future perspectives of this study may include-

- Impact of lipid enriched microalgae on gonadal development of different fishes.
- Comparison of the impact of *Tetraselmis chuii* and *Nannochloropsis sp* on different fishes.

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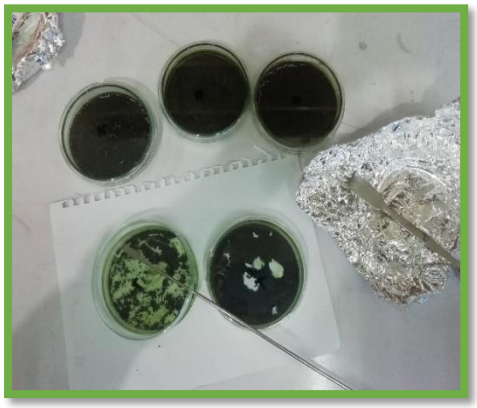
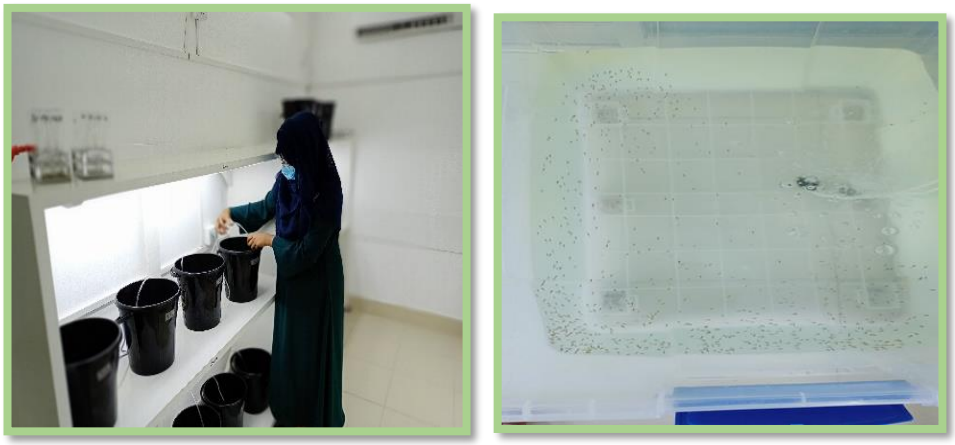
Appendices



Apendix A : Mass Culture of Selected Microalgae and Biomas Production

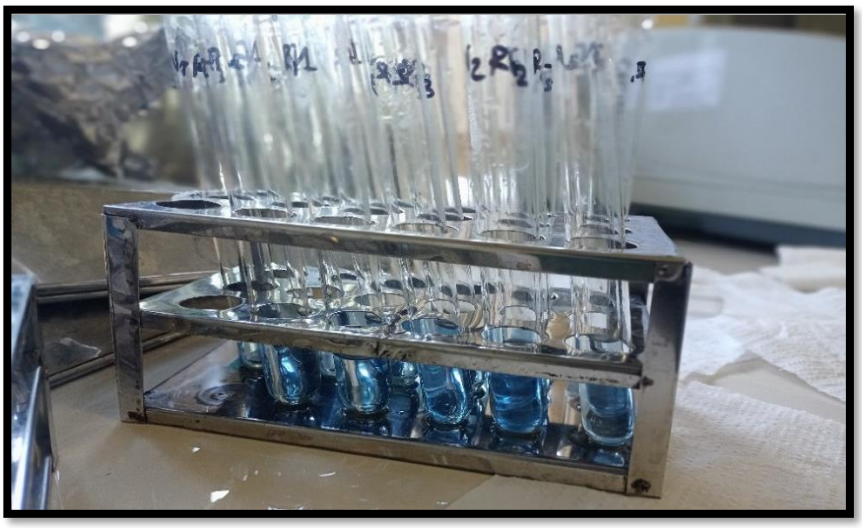
Apendix B: Feed Preparation for *Oreochromis niloticus*



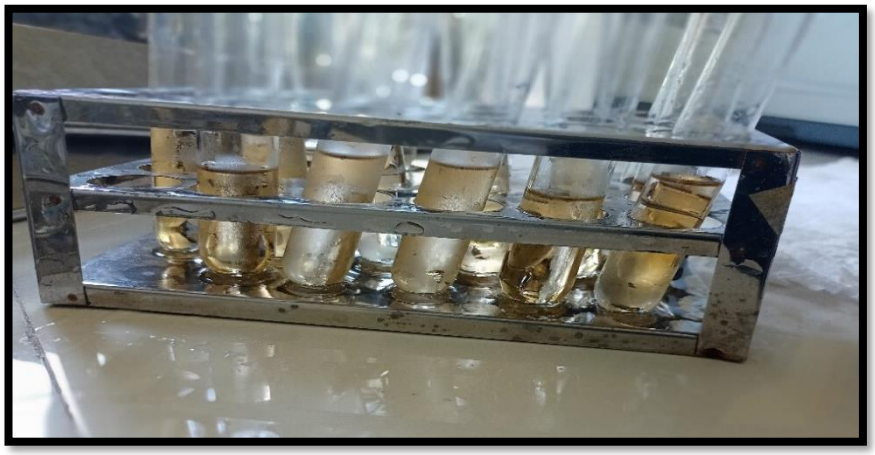
Apendix C: Experimental set up

Appendix D: Determination of Survival rate of Fish

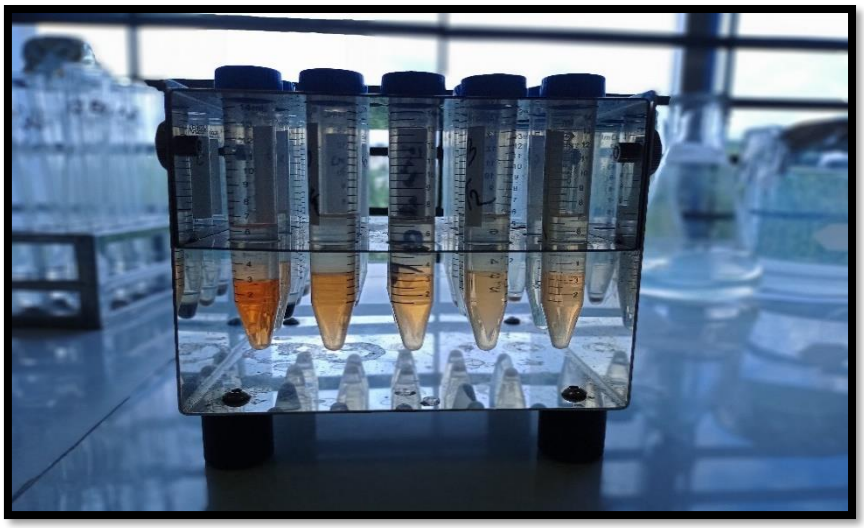
Apendix E: Determination of Water Quality Parameter



Apendix F: Determination of Protein Content



Apendix G: Determination of Carbohydrate Content



Apendix H: Determiantion of Lipid Content

Appendix I: One-way Analysis of Variance examining the growth of *Oreochromis niloticus* after the microalgae used as fish diet

ANOVA

SGR

	SS	Df	MS	F	Sig.
Between Groups	7.745	6	1.291	50.395	.000
Within Groups	.359	14	.026		
Total	8.104	20			

SGR

	VAR00001	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	6.00	3	8.8951		
	4.00	3	9.0510		
	1.00	3		9.7567	
	5.00	3			10.2223
	7.00	3			10.3824
	2.00	3			10.3859
	3.00	3			10.4131
	Sig.		.885	1.000	.763

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix J: One-way Analysis of Variance examining the survival rate of *Oreochromis niloticus* after the microalgae used as fish diet

ANOVA

Survival

	SS	Df	MS	F	Sig.
Between Groups	758.889	6	126.481	9.551	.000
Within Groups	185.407	14	13.243		
Total	944.296	20			

Appendix K: One-way Analysis of Variance examining the water quality of treatment tanks of *Oreochromis niloticus* after the microalgae used as fish diet

ANOVA

TAN

	SS	Df	MS	F	Sig.
Between Groups	.103	6	.017	92.930	.000
Within Groups	.003	14	.000		
Total	.105	20	.		

ANOVA

SRP

	SS	Df	MS	F	Sig.
Between Groups	.065	6	.011	7.800	.001
Within Groups	.019	14	.001		
Total	.084	20			

ANOVA

NO_2-N

	SS	Df	M S	F	Sig.
Between Groups	.067	6	.011	9.200	.000
Within Groups	.017	14	.001		
Total	.084	20			

Appendix L: One-way Analysis of Variance examining the protein content of *Oreochromis niloticus* after the microalgae used as fish diet.

ANOVA

Protein

	SS	Df	MS	F	Sig.
Between Groups	297.358	6	49.560	47.200	.000

Within Groups	14.700	14	1.050		
Total	312.058	20			

Protein

	VAR00001	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	7.00	3	16.9667			
	4.00	3	17.4000	17.4000		
	2.00	3		19.9667	19.9667	
	6.00	3			20.9333	
	5.00	3			21.5667	
	1.00	3				26.4667
	3.00	3				27.3667
	Sig.		.998	.092	.504	.925

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix M: One-way Analysis of Variance examining the lipid content of *Oreochromis niloticus* after the microalgae used as fish diet

ANOVA

Lipid

SS	Df	MS	F	Sig.

Between Groups	168.276	6	28.046	8.257	.001
Within Groups	47.553	14	3.397		
Total	215.830	20			

Lipid

	VAR0000		Subset for alpha = 0.05			
	1	N	1	2	3	4
Tukey HSD[a]	3.00	3	19.3333			
	2.00	3	20.0000			
	4.00	3	22.0667	22.0667		
	7.00	3	22.4333	22.4333	22.4333	
	5.00	3	23.6333	23.6333	23.6333	
	6.00	3		26.5667	26.5667	
	1.00	3			27.4000	
	Sig.		.131	.105	.061	

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix N: One-way Analysis of Variance examining the carbohydrate content of *Oreochromis niloticus* after the microalgae used as fish diet

ANOVA					
Carbohydrate					
	S S	Df	MS	F	Sig.
Between Groups	20.506	6	3.418	2.395	.084
Within Groups	19.980	14	1.427		
Total	40.486	20			

Brief Biography of the Author

Jinat Afruj is the youngest daughter of Habibur Rahman and Mrs Fatema Begum, was born and Grown up in Narsingdi. She has completed Dakhil from Jameya-E-Qusamiya Kamil Madrasha and HSC from Narsingdi Science College. She has also achieved her BSc degree in Fisheries from Chattogram Veterinary and Animal Sciences University. She is now a candidate of Master's degree of the same institute from the Department of Aquaculture. She has expertise on both field and laboratory works. She has done many farms works in Cox's Bazar district and microalgae laboratory research. Internship in Bangladesh's various fisheries related organizations and also in University Malaysia Terengganu, UMT is her advanced qualification besides academic study. She has a lot of experience on co-curricular activities. She has immense interest in research areas include, microalgae, fish breeding, microbiology, fish genetics, bio-floc technology, fish disease, ecology, and advanced aquaculture technologies. She is determined to make her a competent researcher and wants to reach in the apex of research world of fisheries in the world.