Growth Performance and Fatty Acid Profile of Nile Tilapia

Oreochromis niloticus(Linnaeus, 1758) fed with different Phytoplankton

A thesis submitted to the Department of Fisheries, University of Dhaka in partial fulfillment of the requirement for the degree of Master of Science (MS) in Fisheries

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31 January 2016

Dedicated

To

My beloved Parents

(Md. Robiul Islam & Mst. Aktara Begum)

<u>Certificate</u>

This is to certify that the research study entitled **"Growth Performance and fatty acid profile of Nile Tilapia *Oreochromis niloticus* (Linnaeus, 1758) fed with different Phytoplankton"** was done by **Md. Babul Hossain,** Roll No-4206, Registration No. Ha-2380, MS Session: 2013-14, under our supervision.

This is further to certify that it is an original work and suitable in partial fulfillment of the requirements for the degree of **Master of Science (MS) in Fisheries** from University of Dhaka, Dhaka-1000, Bangladesh.

We wish every success in his life.

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Abstract

Tilapia is one of the most important aquaculture fish species and it is popularly cultured in all over Bangladesh. The fish is widely consumed by the people for their rich protein content and higher fatty acids. The objective of the present study was to estimate the growth performance, survival rate, fatty acids profiles and fecundity of Nile tilapia. To estimate the effects of different phytoplankton on growth performance and fatty acids composition of Nile tilapia (*Oreochromis niloticus*) an experiment was conducted for twenty four weeks in concrete small pond located in BCSIR (Bangladesh Council of Scientific and Industrial Research), Dhaka. This study was conducted during the month of May to November, 2015. The fatty acids profile was done in Biological Research Division, Zoology section, BCSIR, Dhaka.

The growth performance of Nile tilapia was found to be maximum with *Chlorella* and lowest with commercial feed that is used as control among four treatments. It can be stated that the growth performance of Nile tilapia could be improved by *Chlorella* and *Spirulina* with supplementary feed wheat bran on the basis of Condition factor (k), ADG, SGR (%) and FCR. The findings of the investigation showed thatCondition factor found in treatment T3 with chlorella ($1.73\pm0.02^{)}$ was significantly higher than that of T1-control ($1.56\pm0.02^{)}$ at 24 weeksculture period. Specific growth rate of treatment T3 with *Chlorella* (11.93 ± 0.28) was significantly differ than treatment T4 with mixed feed (0.77 ± 0.14) at 24 weeks of rearing.

Spirulina has the best proximate composition for human nutritional interest and thus it was found to be better option to produce healthy fish for human consumption. Use of different phytoplankton such as *Spirulina*, *Chlorella* and *Azolla* influence the fatty acid profiles of Nile tilapia. Findings also showed that the amount of Eicosapentaenoic acid in treatment T3 with *Chlorella* (1.83 ± 0.22) is highly significant than other treatments. The highest amount of Docosapentaenoic acid is found in treatment T3 with Chlorella (2.30 ± 0.07) and lowest in treatment T1 with control (0.88 ± 0.46).

The findings of this research suggest that raising Nile tilapia with *Chlorella* and *Spirulina* in ponds could help in better growth performance and improve fatty acid composition of Nile tilapia.

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List of Abbreviations and Symbols

ADG	Average Daily Gain
ADHD	Attention-deficit Hyperactivity Disorder
ANOVA	Analysis of Variance
BCSIR	Bangladesh Council of Scientific and Industrial Research
BW	Body Weight
BC	Before Christ
DHA	Docosahexaenoic Acid
DoF	Department of Fisheries
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FAMEs	Fatty Acids Methyl Esters
FCR	Feed Conversion Ratio
GDP	Gross Domestics Product
GC	Gas Chromatography
IUCN	*International Union for Conservation of Nature*
H_2O	*Water*
GIFT	Genetically Improved Farmed Tilapia
FAO	*Food Agricultural Organization*
PUFA	*Poly Unsaturated Fatty acids*
UNICEF	United Nations Children's Fund
SPSS	Statistical package for the social sciences
SEM	Standard Error of Mean
TDS	Total Dissolved Solid
IUPAC	International Union of Pure and Applied Chemistry
LA	Linoleic Acid
NaOH	Sodium Hydroxide
K	Condition Factor
SGR	Specific Growth Rate
TL	Total Length
µl	Micro liter
±	Plus-Minus
%	Percentages

<	Less than
g	Gram
cm	Centimeter
mi	miles
P^h	Power of Hydrogen

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Chapter 1

Introduction

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1.1 Significance of Fisheries in Bangladesh

Bangladesh is a riverine country.Bangladesh is blessed with numerous natural gifts and about 230 rivers are the best gifts of nature. 230 rivers including tributaries flow through the country constituting a waterway of total length around 24,140 kilometers (15,000 mi). It's not that the river is only resource of water for fishes in our country, it covered with vast recourses of water excluding river like tributaries,estuaries,long cost lines, haors, beels, Baors, wetland, floodplains etc.Fisheries sector of Bangladesh has been playing a very significant role and deserves potential for future development in Bangladesh. Fish production in Bangladesh is increasing rapidly. Recently advanced technologies are applied and new species introduction in aquaculture.

Bangladesh has tremendous fisheries potential. There is a total of 264 species of fresh water fin fishes, 65spp of prawn and shrimp (Hossain,1985). Inland open water is the major source of production in the country, where open water capture fisheries contribute 28.07% and inland culture fisheries contributes 55.15% of the total production during 2013-14 (DOF,2015).Though Bangladesh has vast water body but the production of fish through aquaculture is low compare to other many country of the world. Bangladesh has gained fourth position in inland open water and fifth position in inland culture fisheries in the world(FAO,2014). Fisheries contribute 3.69% of total GDP and about 22.60% of agricultural GDP. Fisheriesprovides about 60% of animal protein in the daily diet of people of Bangladesh (DOF,2015).

1.2 Current status of fisheries in Bangladesh

Declining of wild fish capture with the increasing of population it is become very important to culture of fishes in control condition. At first time pond culture is begun for fresh water fish culture but with in time for the increasing of demand fish culture is started in every water body like lakes, river, bills, haor, baor, ditch etc. At present fish culture is become very popular in marine area. Fisheries sector of Bangladesh can be broadly divided into four major sub-sectors: Inland capture or open water fisheries, inland culture or closed water fisheries, marine industrial fisheries and marine artesian fisheries. Open water fishery is a self-sustaining system although human interventions

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have significantly deteriorated its healthy and productivity recently. Culture fishery on the other hand is primarily an economic venture managed by private individuals and farms. The inland fisheries contribute about 83.22% of total catch and the remaining 16.78% comes from the marine fisheries (DoF, 2013-14). The inland aquatic Bangladesh is rich in faunal diversity containing 266 species of freshwater and brackish water fin fishes. There are 54 species of fin fishes as threatened in Bangladesh, among these 12 species are vulnerable. Now a days, many exotic species such as silver carp, catfish, tilapia, pangas, piranha etc.The brakish water Shrimp culture has been encouraged as it earns valuable foreign currency.

Table 1: Capture water resources are showing in the following table.

Inland Fisheries	Water Area (ha)	Production (m. ton)	Percentages (%)
1.Riiver and Estuaries	853,863	167373	4.72
2.Sundarbans	1777700	18366	0.52
3.Beels	114161	88911	2.51
4.Kaptai Lake	68800	8179	0.23
5.Floodplains	2695529	712976	20.09
Subtotal	3910053	995805	28.07

Table 2: Culture water resources are following in the table.

Inland Fisheries	Water Area (ha)	Production (m. ton)	Percentages(%)
1.Ponds	371309	1526160	43.01
2.Streams	130488	193303	5.45
3.Baors	5488	6514	0.18
4.Shrimp Farming	275274	216447	6.10
5.Pen Culture	6775	13054	0.37
6.Cage Culture	7	1447	0.04
Subtotal	789341	1956925	55.15

Sources: Fish catch static's of Bangladesh, DoF, 2013-14.

1.3 Current status of fisheries in the world

Tilapia has developed into the second most important cultured freshwater fish, behind the carp. Tilapia production is growing exponentially with the global output standing at 2.5 million tonnes annually, and has therefore, been dubbed as the twenty-first century's most culturable fish (Shelton and Popma,2006, Fitzsimmons, 2010). The world's total tilapia production in 2010 was 3.49 million tons (FAO,2012). The last three decades have seen significant developments in farming of tilapias worldwide. They are being farmed in about 85 countries worldwide. Egypt has been expanding its culturing industry in recent years and is now producing 200000 tons (FAO, 2008). Currently, tilapia is farmed commercially in almost 100 countries worldwide, with over 98 percent of the production occurring outside their original habitats (FAO, 2011).

Global inland waters capture production reached 11.6 million tonnes in 2012. Although its upward trend seems continuous, its share in total global capture production does not exceed 13 percent."Inland waters" remains the most difficult subsector for which to obtain reliable capture production statistics. Several countries in Asia, the continent that accounts for two-thirds of the global total, are believed to either under- or over-estimate their inland water catches. The total catch reported by India is very variable and that from Myanmar has increased 4.3 times in a decade (FAO, 2014).

Table 3: Ten major countries producing highest inland water fish population.

Position	Country	Continent	Production,2012 (Tonns)
1.	China	Asia	22,97,839
2.	India	Asia	14,60,456
3.	Myanmar	Asia	12,46,460
4.	Bangladesh	Asia	9,57,095
5.	Cambodia	Asia	4,49,000
6.	Uganda	Africa	4,07,638
7.	Indonesia	Asia	3,93,553
8.	United Republic of Tangania	Africa	3,14,945
9.	Nigeria	Africa	3,12,009
10.	Brazil	Americas	2,66,042
World total			1,16,30,T320

Source: (FAO, 2012)

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1.4Origins of tilapia species

More than half of the world population depends on fish as a principal source of animal protein (Corpei, 2001). Tilapia is a group of "Cichilid" fish native to African countries. In the early days of 20th century, tilapias were wild fish in the great lakes and rivers of that continent. In the central African countries, farming of tilapias in ponds was introduced after Second World War. After that the tilapia species were spread over most of the tropical and sub tropical countries of the world. In recent years, commercial farming of several species of tilapia has become a common practice in aquaculture throughout several regions of the world such as Bangladesh. Although the important natural tilapia genetic resources are in Africa, the major aquaculture industries at present are in Asia. China, Thailand, Philippines, Indonesia, Sri Lanka are the major tilapia producing countries in Asia. A total of about 70 species of tilapia have been so far listed as native to Africa. Only a few species are suitable and popular for farming in ponds and other culture systems, which include Nile tilapia (*Oreochromisnilotica*), Blue tilapia(*O. aureus*), Mozambique tilapia(*O. mossambicus*), Three spotted tilapia(*O. andersonii*), Longfin tilapia(*O. macrochir*), Galilee tilapia*(Sarotherodongalilaeus)*, Blackchin tilapia(*S. melanotheron*) and Redbelly tilapia(*Tilapia zillii*). There are also some genetically improved strains such as Geneticallly Improved Farmed Tilapia (GIFT), red tilapia strains and hybrids. Above all *O. niloticus* has been recognized as the prime domesticated species for farming in wide range of aquaculture systems from simple waste-fed to intensive culture system.

Above all *O. niloticus* has been recognized as the prime domesticated species for farming in wide range of aquaculture systems from simple waste-fed to intensive culture systems. Tilapia is considered suitable for culture, because of their high tolerance to adverse environmental conditions, their relatively fast growth and resistance to disease, excellent quality of its firmly textured flesh and finely appetizing fish to the consumers (Corpei, 2001).

Tilapia, once considered a low value fish, only suitable for the ethnic market, has in recent times gained wider consumer acceptance and is now considered an attractive menu item in chain restaurants.It remains to   be seen whether the **"food fish of the 21st century"**will surplus production of the carps in aquaculture during the new millennium.

The commonly farmed mouth-brooding tilapias are normally highly prolific. Males grow bigger than females when it comes to tilapia.Tilapia is a popular food fish and many species can easily be cultivated in ponds. It has been an important source of protein Africa and the Levant for thousands of years and the Ancient Egyptians cultivated tilapia in ponds along the Nile. The fish even has its very own hieroglyph.

1.5 Introduction of tilapia species in Bangladesh

Mosambic tilapia (*Oriochromismossambicus*) was first introduced from Thailand into Bangladesh In 1954. During the 1970's a renewed interest in tilapia culture developed in some Asian countries including Bangladesh with introduction of Nile tilapia, *O. niloticus*. Nile tilapia (*O. niloticus)* is one of the fish species of great economic importance. In 1974, the Chitralada strain of Nile tilapia, a promising farmed species, was introduced into Bangladesh from Thailand through UNICEF. In 1988, a red mutant Under the Dissemination and Evaluation of Genetically Improved Tilapia in Asia project of world Fish Center, another promising Genetically Improved Farmed Tilapia(GIFT) stain, a synthetic stain of O. tilapia was brought into Bangladesh from the Asian Institute of Technology,Bangkok,Thailand. In July 1994 another promising Genetically Improved Farmed Tilapia(GIFT) stain, a synthetic stain of *O. niloticus*, was introduced from the Philippines under the Dissemination and Evaluation of Genetically Improved Tilapia in Asia project of world Fish Center.

1.6 History and future Tilapia

1.6.1 History of Tilapia

In 4,000 year ago one of the oldest examples of tilapia farming is a bas-relief found in old Egyptian tomb depicting tilapias held in ponds. The Nile tilapia in the Ancient Egyptians the fish was of such great importance to them that it was given its own hieroglyph. The hieroglyph is now number K1 on Gardiner's Sign List, a list of common Egyptian hieroglyphs compiled by British Egyptologist Sir Alan Gardiner. When used as a logogram, this hieroglyph represented a Nile tilapia. When used as a determinative (ideogram), it could signify not only Nile tilapia, but flathead mullets as well. Just like the Nile tilapias, flathead mullets were important food fishes in Ancient Egypt. The Ancient Egyptians were not the only ones who appreciated tilapia. Tilapia has been, and still is, an important food fish for a lot of different groups living in Africa and the Levant. The Greek are known as tilapia fans and Aristotle is believed to have named it

Tilapia niloticus (fish of the Nile) in 300 BC. The tilapia is a part of Christian mythology since the fish caught by the apostle Peter in Matthew 17:27 is believed to have been a tilapia. In English, tilapia species with a certain spotted pattern are commonly referred to as "St. Peter's fish". According to legend, the dark spots on the fish were caused by the fingerprints of the apostle. The name St. Peter's fish is also used for John Dory fish, *Zeus faber*, but this is a marine species and the story in the bible revolves around the Sea of Galilee, which is a freshwater lake in Israel. The Sea of Galilee is home to the tilapia *Sarotherodongalilaeus*.*Sarotherodongalilaeus* has been fished by local fishermen in the Sea of Galilee for thousands of years.

1.6.2 Future of Tilapia

Tilapia could play an important role here, since it can be farmed instead of caught from the wild. Several projects have also been launched where destitute families around the world are encouraged to farm tilapias in their own backyard to feed themselves and possibly sell surplus fish at local markets.

In Latin America, larger commercial farms exporting fresh tilapia to the US market have provided job opportunities for the local population. Tilapia farms exporting frozen tilapia are also an important source of income for many countries in South East Asia. Tilapia farms can be a way for tropical countries to take advantage of their warm climate, since tilapia can be grown year round as long as the water temperature is high enough.

It should be noted that farmed tilapia comes with its own set of problems. Tilapia farms can pollute surrounding waters with organic waste and there is also the risk of tilapias escaping and disturbing the native ecosystem. Tilapia can also bring new pathogens to the local fish populations. One advantage with tilapia however, is that it can be farmed in enclosed systems that prevent pollutions from entering the environment and reduce the risk of escapes. Unlike salmon, another popular food fish, tilapia doesn't need to be farmed in the ocean.

Another important aspect of tilapia farming is that many of the most popular tilapia species do not need to be fed protein rich food. Farmed salmon is typically fed meal produced from fish and other marine organisms. Farming salmon will therefore always increase the demand for other fish, and this can actually contribute to over fishing instead of help solving the problem. The ability to survive on detritus, algae and similar also

make tilapia an ideal choice for small substance farmers that could not afford to purchase fish meal to feed their tilapia.

1.7Fat and fatty acids

Fats, especially vegetable oils, contain an essential fatty acid called linolenic acid that the body cannot make for itself.The amount of linolenic acid required in small and is easily obtained from the foods we commonly eat, especially vegetables and fish. It also appears that linolenic acid, a second fatty acid, is probably essential in humans (Nettleton *et al.*, 1984; Holman *et al.* 1982). Omega-3 fatty acids in fish oils are necessary for optimum healthy heart (Titus *et al.* 1982; Oliw*et al.*1983).Most fishes are relatively low in total fat and relatively high in its proportion of polyunsaturated fatty acids. This feature gives fish a clear health advantage (Nettleton, 1985).Fats are made of different kinds of fatty acids that in turn differ in the amount and arrangement of the carbon and hydrogen atoms they contain. There is still a great to learn about how the body processes different fatty acids, but it seems clear that some fatty acids are more beneficial for health than others (Nettleton, 1985). In particular, polyunsaturated fatty acids have been shown to be more favorable for healthy blood lipid levels than saturated fats. In many people, achieving a better blood lipid pattern can lower the chances of heart attack or stroke (Grundy*et al.* 1982). Fishes have been used in diets designed to prevent and treat cardiovascular disease, one of the leading causes of mortality in today's world. The best ways to achieve a healthy blood lipid pattern are to eat less fat in total to limit the amount of saturated fats consumed and to keep cholesterol intake below 300mg per day (Emst, 1985).

Fish liver oil differs in fatty acids and composition from the oil in flesh. Sincethe liver contains a great deal of fat, however, the liver becomes an important source of polyunsaturated fatty acids (PUFA).Thus consuming whole fish can greatly the amount of polyunsaturated fatty acids from fish there are two outstanding features about the fat in fish; the total amount is very low in most varieties and fat is rich in polyunsaturated fatty acids having more than four double bonds. The predominant polyunsaturated fatty acids in fish oil are the omega-3 fatty acids having either five or six double bonds. There are about seven omega-3 fatty acids in fish oils of which to predominate (Ackman, 1982). Fish oils are protective against heart disease, stroke and possibly diabetes and other diseases as well (Goodnight *et al.* 1982; Bang *et al.* 1980).The implications from the observations among Greenland Eskimos is that fish oils are protective against heart

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disease, stroke and possibly diabetes and other diseases as well (Goodnight *et al.* 1982; Bang *et al.* 1980).

Eicosapentaenoic (EPA) and Docosahexaenoic (DHA) acids are more important. There are about seven omega-3 fatty acids in fish are derived from the phytoplankton in the food chain that fish eat. Mounting scientific evidence shows that as well as helping our hearts, a diet rich in omega-3s is beneficial in diabetes, depression, Attention-deficit Hyperactivity Disorder (ADHD), cancer and Alzheimer's disease. And there's also strong evidence that omega-3s counter inflammatory diseases such as rheumatoid arthritis and Crohn's disease. Omegas are types of polyunsaturated fatty acids. They are called "essential" fatty acids because the body needs them but can't manufacture them, so we have to get them from our diet.

1.8 Significance of Phytoplankton in Aquaculture

Phytoplankton consists of chlorophyll bearing organisms e.g. *Microcystis, Volvox, Eudorina, Oscillatoria*, etc. Live food organisms contain all the nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids (New, 1998) and hence are commonly known as "living capsules of nutrition". Providing appropriate live food at proper time play a major role in achieving maximum growth and survival of the young ones of finfish and shellfish. Live food organisms include all plants (phytoplankton) and animal (zooplankton) lives grazed upon by economically important fishes. Phytoplanktons are generally eaten by zooplankton. Thus, phytoplankton forms the basis of the food chain. Live foods are able to swim in water column and are constantly available to fish and shellfish larvae are likely to stimulate larval feeding response (David, 2003).

Spirulina is a cyanobacterium (blue-green algae) that can be consumed by humans and other animals. There are two species, *Arthrospira platensis* and *Arthrospira maxima*. *Arthrospira* is cultivated worldwide; used as a dietary supplement as well a whole food; and is also available in tablet, flake and powder form. It is also used as a feed supplement in the aquaculture, aquarium and poultry industries. Dried spirulina contains about 60% (51–71%) protein. It is a complete protein containing all essential amino acids, though with reduced amounts of methionine, cysteine and lysine when compared

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to the proteins of meat, eggs, and milk. It is, however, superior to typical plant protein, such as that from legumes.

Chlorella is a genus of single-cell green algae belonging to the phylum Chlorophyta. It is spherical in shape, about 2 to 10 μm in diameter, and is without flagella. *Chlorella* contains the green photosynthetic pigments chlorophyll-a and -b in its chloroplast. Through photosynthesis, it multiplies rapidly, requiring only carbon dioxide, water, sunlight, and a small amount of minerals to reproduce. Legumes are a significant source of protein, dietary fiber, carbohydrates and micronutrients, including folate, thiamin, manganese, magnesium, potassium and iron, such as for cooked black bean. Legumes are also an excellent source of resistant starch which is broken down by bacteria in the large intestine to produce short-chain fatty acids used by intestinal cells for food energy.

Azolla (mosquito fern, duckweed fern, fairy mossand water fern) is a genus of seven species of aquatic ferns in the family Salviniaceae. They are extremely reduced in form and specialized, looking nothing like other typical ferns but more resembling duckweed or some mosses. *Azolla* is rich in proteins, essential amino acids, vitamins and minerals.

Microalgae have an important role in aquaculture as a means of enriching zooplankton for feeding fish and other larvae. In addition to providing protein (essential amino acids) and energy, they provide other key nutrients such as vitamins, essential polyunsaturated fatty acids (PUFA), pigments and sterols, which are transferred through the food chain. It is obviously agreed that the production of live food organisms continues to be a very important first step in intensification of aquaculture, both horizontally as well as vertically.

1.9 Objectives of the Study

The overall objective of the present study was to evaluate the growth performances and fatty acid composition in Nile tilapia (*Oreochromis niloticus*) by intake of different compound feeds.

The specific objectives were-

- ❖ Culture of tilapia fry with different Phytoplankton and plant derivatives.
- ❖ Study of growth performance such as Condition factor (K),Average Daily Gain (ADG), Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) of Tilapia.
- ❖ To observe the effect of commercials feed on growth in comparison to natural feed.
- ❖ To estimate the fatty acid Profiles of Nile tilapia after rearing with these natural and commercial feed components.

CHAPTER 2

MATERIALS AND METHODS

2.1 Specimen selected for the present study

The selected Specimen fish is *Oreochromis niloticus* (Linnaeus, 1758) locally known as "Tilapia". Tilapia is the common name for nearly a hundred species of cichlid fish from the tilapiine cichlid tribe. Tilapia are mainly freshwater fish inhabiting shallow streams, ponds, rivers and lakes and less commonly found living in brackish water.

Classification

Kingdom	Animalia
Phylum	Chordata
Sub-phylum	Vertebrata
Class	Actinopterygii
Order	Perciformes
Family	Cichlidae
Genus	*Oreochromis*
Species	*O. niloticus*

Plate 1: *Oreochromis niloticus*

2.2 Collection of samples

Fry of Tilapia (*Oreochromis niloticus*) were collected from Jahane Nijam Bohumukhi Matsha Hatchery, Tepra, Manikganj. Live fish were collected in May 2015 and carried in oxygenated bags with sample water.

2.3 Laboratory of investigation

The experiment was carried out in the wet laboratory at Zoology Section, Biological Research Division, Bangladesh council of Scientific and Industrial Research (BCSIR, plate 2).

2.4 Experimental Design

Ponds are situated near to the office building where five small concrete ponds are used for the experiment. Each of the small ponds is ten feet in length and six feet in width and

depth is about two and one-fourth feet. All the small ponds are filled up with tap water and labeled according to experimental design. Each of the ponds is filled up with tap water in the quantity of about 650 liters. In total 100 fingerlings of tilapia are stocked in all of the small ponds and each of the small ponds containing about 250 fingerlings. After bringing the fry first acclimatized for sometimes in clean water. Then fish were released slowly into the pond. No feed give on first day. From the second day of stocking, feed given at a rate of 25% body weight of the fish. Feed are supplied regularly on the basis of body weight of the fish. Feed quantity increment in every subsequent six week is done by the percentages of 7%, 5% and 4% respectively. Treatment-1 (small pond 1)) are feeding with the commercial feed as Control,Treatment-2 (small pond 2) are feeding with the *Spirulina* (Phytoplankton),Treatment-3 (small pond 3)are feeding with the *Chlorella* (Phytoplankton),Treatment-4 (small pond 4) are feeding with the mixed feed (*Azolla*,*Chlorella*,*Spirulina*and *Lemna*), respectively.

Plate 2: An Experimental setup of Aquaculture small concrete ponds for Tilapia Culture in BCSIR (Bangladesh Council of Scientific and Industrial Research), Dhaka.

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2.5 Materials and Instruments

a) Beaker (2L, 1L)

b) P^H meter

c) Light intensity meter

d) DO meter

e) Thermometer

f) Digital electric balance

g) Conductivity meter

h) Bowls

i) Measuring steel scale

j) Petridish

k) Filter paper

l) Small ponds

m) Multiplugs

n) Conical Flask

o) Tilapia Fry (Fishes)

p) Live *spirulina* (Phytoplankton)

q) Live *Chlorella* (Phytoplankton)

r) Live *Aaolla* (Phytoplankton)

s) Commercial feed

t) Moringa oleifera

u) Wheat bran

v) Oven

2.6 Types of Feed

Table 4: Four treatments were used in the experiment. These are as follows:

Treatments	Feeds	Supplementary feeds
T_1	Commercial feed	
T_2	*Spirulina*	Wheat bran and *Moringa oleifera*
T3	*Chlorella*	Wheat bran and *Moringa oleifera*
T4	Mixed feed (*Spirulina, Chlorella, Azolla&Pistea*)	Wheat bran and *Moringa oleifera*

Wheat bran and *Moringa oleifera* used as supplementary feed in treatments.

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A: P^h meter

B: DO meter

C: Light intensity meter

D: Digital Thermometer

E: Electric balance

F: Conductivity meter

Plate 3: Showing the Digital P^H Meter (A) Digital DO meter (B), Light Intensity Meter (C), Digital Thermometer (d), Electric balance (E) and Coductivity meter (F).

A: Comercial Feed

B: Spirulina

C: Chlorella

D: Azolla and Pistea

E: Wheat Bran

F: *Moringa Oleifera*

Plate 4:Different types of feeds are used in four treatments.

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2.7Measurements of biological and Physio-chemical parameters and Maintenances

Tilapia can however survive in a wide range of pH, temperature, dissolved oxygen, and feeds on a variety of food items in water (Balirwa, 1998; Njiru, *et. al.,*, 2006). The physico-chemical parameters including water temperature, dissolved oxygen (DO), P^H, light intensity and conductivity content were measured carefully by the help several modern devices such as DO meter, P^H meter, Light intensity meter and conductivity meter for Three times per a week. Growth of the sample was measured after 6 weeks interval. Lengths and widths of the fingerlings were measured with measuring scale and weighing with electric balance. 10 samples were measured randomly at a time. After recording lengths and weights, the fingerlings were slowly released into the labeled pond water. Feed were supplied as per experimental design. The small pond was also cleaned per week and clean water was supplied in each of the aquariums. Growth (lengths and weights) of the fingerlings was measured consequently one time at the interval of 6 weeks. These data were recorded carefully and correctly in the record note book during the every measuring period. Feeding rate was maintained in everyday. Everyday feed were given in the every small pond according to feeding rate mentioned above. Experiment was continued up to 24 weeks.

2.8 Growth Recording

2.8.1 Fish sampling Procedure

Sampling was accomplished at the 6 weeks, 12weeks, 18 weeks and 24 weeks of the experimental period. Prior of weighing, the fishes were caught with a fine mesh size net and their individual length and weight were recorded to the nearest centimeter and nearest gram respectively.After 24 weeks of rearing or at the termination of the experiment the final length (cm) and weight (g) of the individual fish were carefully recorded. A steel measuring scale was employed for measuring the lengths. The total body weight of individual fish was determined by a sensitive electronic balance.

2.8.2 Analysis of experimental data

Experimental data which collected during the growth recording were used to determine the following growth parameters.

Condition Factor (K %)

Condition factor is the volume of fish relative to its length and taken to mean the well-being degree of an individual fish in respect to its habitat where it lives so as to understand how well a given habitat supports life of an individual in terms of nutritional requirements and other environmental conditions (Weatherly, 1987). The condition factor is used in studies of fisheries biology to indicate the well-being degree of fish in the environment in which they live and to verify if they make good use of the foods available (Weatherly, 1987). Sexual differences, age, changes in seasons, gonad maturity levels, nutritional levels and maturity of fishes can influence the condition factor (K) value (Lagler, 1952; Kotos, 1990).

This is the factor through which condition of the fish is expressed in numerical terms i.e. degree of plumpness or flatness is usually estimated as the condition factor. It was calculated by the following formula as suggested by Hile (1936).

$K = (W/ L^3) \times 100$

Where,

K= Condition factor

W= Body weight in grams

L= Body length in centimeters

Average Daily Gain (ADG, g/day)

Average daily gain means the increase of body weight per day. It was calculated by the following formula as suggested by Jones (1967).

$$ADG = \frac{(\text{Mean final fish weight} - \text{Mean initial fish weight})}{\text{Time } (T2 - T1)}$$

Where

T_2= Final time

T_1= Initial time

Specific Growth Rate (SGR %)

SGR Mean the percentage of body weight increase per day. Specific growth rate was calculated by the following formula as suggested by Hopkins (1992).

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SGR (%) = $\frac{(\ln W_T - \ln w_1)}{T-t}$ × 100

Where,

$\ln W_t$ = Natural log of weight at time T

$\ln W_1$ = Natural log of initial weight

T= Final time

t= Initial time.

Feed Conversion Ratio (FCR)

The FCR is simply the amount of feed it takes to grow a kilogram of fish. Feed conversion ratio (FCR) was determined by the following formula as suggested by Payne (1987).

FCR = $\frac{\text{Feed (g) consumed by the fish}}{\text{Weight (g) gain of the fish } (W_2 - W_1)}$

Where,

W_2= Final weight

W_1=Initial weight

Against each respective value all statistical analyses were carried out by using the computer package SPSS (version 11.5) and Microsoft Excel.

Estimation of survival rate

Survivality of Nile tilapia (*Oreochromis niloticus*) fish for each treatment was estimated on the basis of number of fish gathered at the end of the 6weeks, 12 weeks, 18 weeks and 24 weeks of rearing of the fishes in the experimental ponds. Survivality of tilapia was calculated by counting the actual number of fish survived, divided by the initial number stocked and multiplying by 100, and thus

Survival rate (%) = $\frac{\text{No. of actual fish survived}}{\text{No. of actualfish stocked}}$ × 100

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2.9 Fatty Acids Estimation

Principle

Fat and fatty acids are extracted from fish by hydroelectric method. Fat into ether, and then methylated to fatty acids methyl esters (FAMEs). FAMEs are quantitatively measured by gas Chromatography.The profile of fatty acids was done followinggas Chromatographic (GC) method (Nicholus, *et. al.,* 1995). Fatty acids were obtained from lipids by saponification using NaOH dissolved in methanol H_2O mixture (hydrolysis with alkali).

Flow chart represents typical procedure for analysis of fatty acid.

Fat

⇩

Hydolysis

⇩

Methylated to FAMES

⇩

GC Quantificatificaton
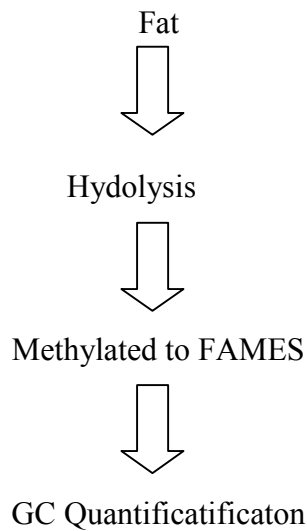
Procedure

Sample was methylated into fatty acid methyl ester using HCL and methanol mixture, which can be easily identified by gas Chromatography

⇩

Fatty acid methyl e easter was separated using mixture of hexane and anhydrous diethayle ether

⇩

For the organic phase aqueous NaOH was used as base wash and the upper organic layer was separated

⇩

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(2-3) µl of sample was injected and analyzed using Gas Chromatogrphy, with capillary column and flame ionization detector

⇩

The chromatogram was used for calculation

⇩

Standard fatty acids were analyzed simultaneously

⇩

Based on the retention time and peak area of the standard fatty acids, each fatty acid in the unknown sample identified

A. Fish flesh are dried in Oven

B. Sample preperation for fat estimation

C. Sample preparation for fatty acid estimation

Plate 5: Sample ready for fatty acid determination.

2.10 Fecundity

Fecundity is the mean or average number of eggs per brood and the intensity of animal reproduction is determined by both the average number of eggs per brood and how often the eggs are laid by the female per unit time (Winberg, 1971). Female *O. niloticus* produces only a few hundreds of offspring in a single spawn but under favorable conditions they spawn frequently every 4 to 6 weeks (Pullin, 1991). Tilapia is a good fish for warm-water aquaculture. They are easily spawned, use a wide variety of natural foods as well as formulated feeds, tolerate poor water quality, and grow rapidly at warm temperatures. These attributes, along with relatively low input costs, have made tilapia the most widely cultured freshwater fish in tropical and subtropical countries (Biswas et al., 2005; Fasakin et al., 2005, El-Saidy and Gaber 2005; Peña-Mendoza *et al.* 2005 Borgeson *et al.*, 2006; Tsadik and Bar, 2007 and Tahoun, 2007). Fecundity is one of the most important biological aspects of fish which plays a significant role to evaluate the commercial potentialities of fish stock. This must be known to assess the abundance and reproductive potential of a fish stock (Das *et al.*, 1989). It is important to know the fecundity of a fish species for efficient fish culture and effective management practices (Miah and Dewan, 1984). There are many possible reasons for the low production of tilapia fry. These include too low density of broodstock, inappropriate sex ratios, inadequate spawning techniques, broodstock nutrition and high fry mortality (Salama, 1996). Poor broodstock productivity, owing to low fecundity and asynchronous spawning cycles, remains one of the most significant outstanding constraints upon commercial tilapia production and its future expansion. Broodstock productivity clearly represents the most significant constraint on commercial tilapia production. Increased knowledge of the factors regulating brood stock productivity is therefore of great importance to the further development of tilapia culture (Coward and Bromage, 2000).

Procedure

Matured Nile tilapia are collected from four experimental ponds.Then the ovaries of fish were taken out very carefully and preserved in well labeled vials containing 5% buffered formalin for subsequent studies. Gravimetric (Islam and Talbot, 1968; Evans, 1969; Doha and Hai, 1970) method was followed to determine the fecundity of fish. In this case, the external connective tissues were removed from the surface of the ovaries. Moisture of the ovaries was removed with the help of blotting paper. Weight of ovaries

was recorded in gram with the help of a fine electric weighing balance. Then 0.01 g of each ovary was taken separately from anterior, middle and posterior regions of each lobe. The number of matured and immature eggs for each portion were sorted out and counted with the help of a needle and magnifying glass. The mean number of eggs in 0.01 g was determined and then multiplied by the total weight of the ovary, which gave the total number of eggs *i.e.*, the fecundity of the respective fish.

2.11 Statistical analysis

Statistical analysis was performed with the statistical package for the social sciences (SPSS) v.20.0 software package (SPSS, SAS Institute Inc. Gary, USA) and Microsoft office excels 2007. The data were analyzed to determine the descriptive statistics such as Mean, Standard Error of Mean, Standard Deviation, Minimum and Maximum value. Multiple Comparisons were done with Turkey HSD test with one way ANOVA (Analysis of Variance) at 5% level of significance.

A. Female tilapia containing ovary

B. Removed Ovary from female tilapia

C. Treatment T1 released fry

Plate 6: Counting egg for fecundity analysis.

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Chapter 3
Results

3.2 Effects of different phytoplankton on growth performance of Nile tilapia

Physio–chemical parameters are important factor to enhance the growth of fish in aquaculture. In this investigation the average temperature of water is (28.43±0.50) degree Celsius, the average of dissolved oxygen is (7.53±1.995) mg/l, the range of $p^h$is (7.85±0.18), the average conductivity is (441.55±5.55) µs/cm, the average light intensity is (40.62±5.67) Klux, and the average total dissolved solid is (239.23±9.69) mg/l (Appendix C).

3.1.1 Condition Factor (K, %)

At the 24 weeks culture, the highest value of condition factor (K) of Tilapia was observed in treatment T3 (K= 1.73) and the lowest value was found in all treatments (K = 1.10) at o week. Condition factor found in treatment T3 found significantly higher than that of T1 at 24 weeksculture period.
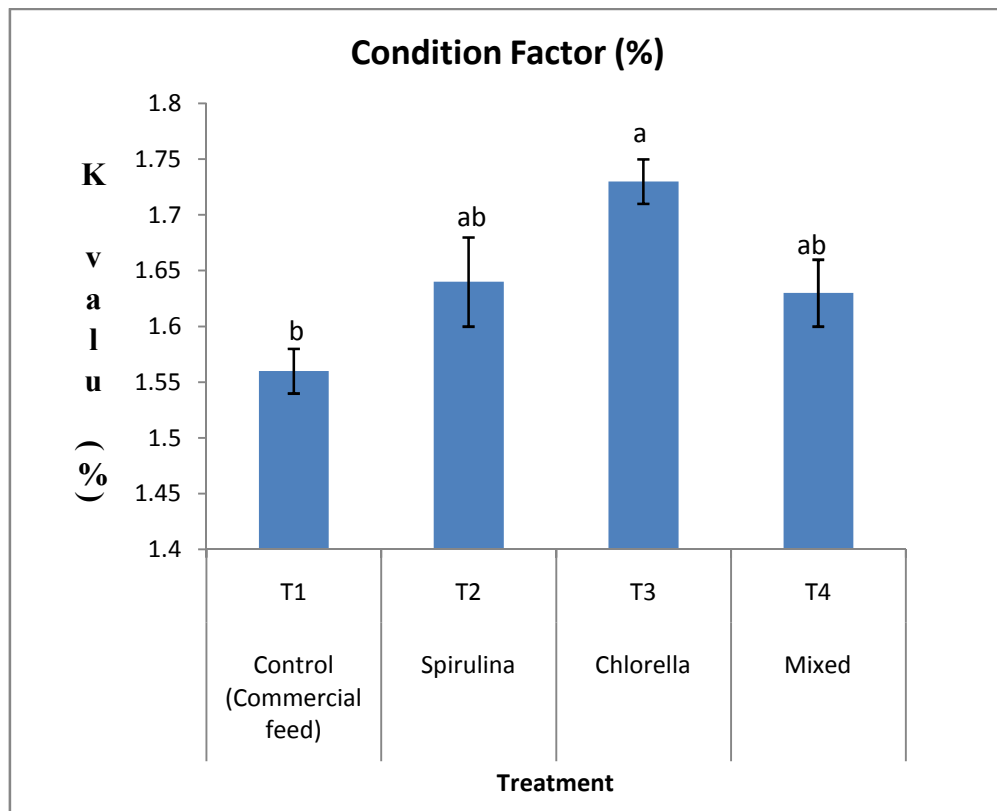
Fig.1: Condition Factor, K (%), (Mean ± SEM) of tilapia fish cultured for24 weeks with four treatments.

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Table 5 Condition Factor, K (Mean ± SEM) at 24weeks rearing period.

Treatment	Condition Factor (%)				
	O week	6 weeks	12 weeks	18 weeks	24 weeks
T1	1.10±0.06	1.51±0.08	1.51±0.07	1.48±0.03	1.56±0.02[b]
T2	1.10±0.06	1.49±0.02	1.59±0.50	1.59±0.05	1.64±.04[ab]
T3	1.10±0.06	1.60±0.04	1.61±0.04	1.65±0.05	1.73±0.02[a]
T4	1.10±0.06	1.57±0.04	1.51±0.03	1.55±0.03	1.63±0.03[ab]

Values are mean ± SEM of duplicate groups of 10 fish. Means in the same column with different superscripts are significantly different at P<0.05.

3.1.2 Average daily gain (ADG)

At the end of 24 weeks rearing, the average daily gain was observed highest value in treatment T2 (0.45±0.09) and lowest was observed in treatment T1 (0.02±0.01). And then the ADG value (ADG = 0.0312) of T3 was higher than T4 and lower than T1 while the ADG value (ADG = 0.) of T4 greater than T1 and lesser than T2 and T3 respectively. There is no significant difference among the treatments at 0.05 levels.
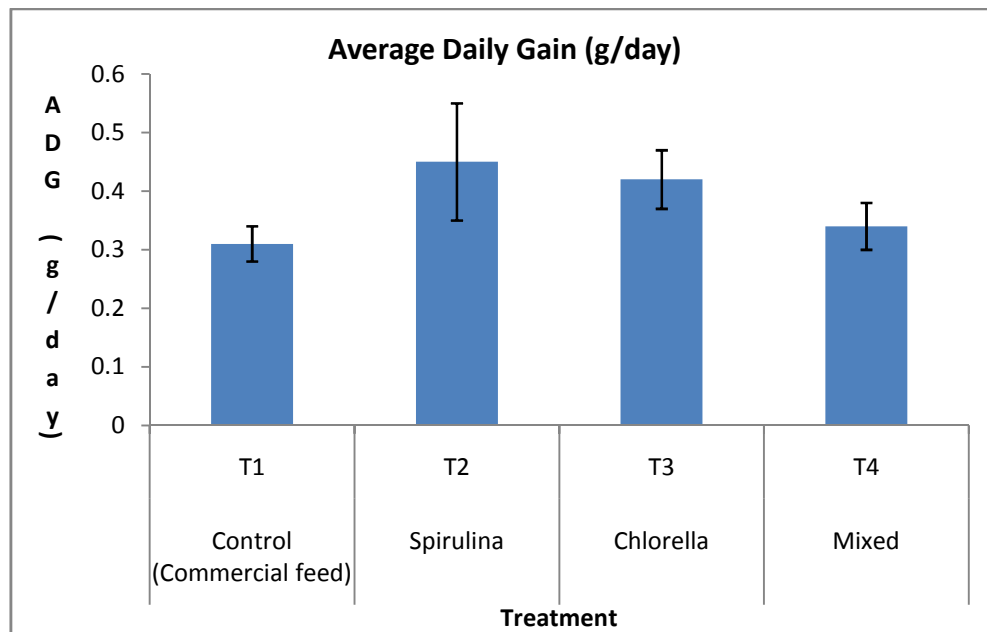
Fig.2:Average Daily Gain, ADG (g/day), (Mean ± SEM) of tilapia fishcultured for 24 with four different treatments.

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Table 6: Average Daily Gain, ADG (Mean ± SEM) at 24 weeks rearing period.

Treatment	ADG (%0			
	6 weeks	12 weeks	18 weeks	24 weeks
T1	0.02 ± 0.01^c	$0.08\pm0.02b$	0.16 ± 0.02^{bc}	0.31 ± 0.03
T2	0.12 ± 0.02^{ab}	$0.26\pm0.02a$	$0.37\pm0.09a$	0.45 ± 0.09
T3	0.17 ± 0.03^a	$0.25\pm0.04a$	0.29 ± 0.05^{ab}	0.42 ± 0.05
T4	0.09 ± 0.01^b	$0.09\pm0.02b$	0.08 ± 0.03^c	0.34 ± 0.04

Values are mean ± SEM of duplicate groups of 10 fish. Means in the same column with different superscripts are significantly different at $P<0.05$.

3.1.3 Specific Growth Rate (SGR %)

The highest specific growth rate (11.93±0.28) was achieved by treatment T3 and the lowest value (0.77±0.14) was obtained in (Table 5). Specific growth rate of treatment T3 (11.93±0.28) significant difference than treatment T4 (0.77±0.14) at 24 weeks of rearing.
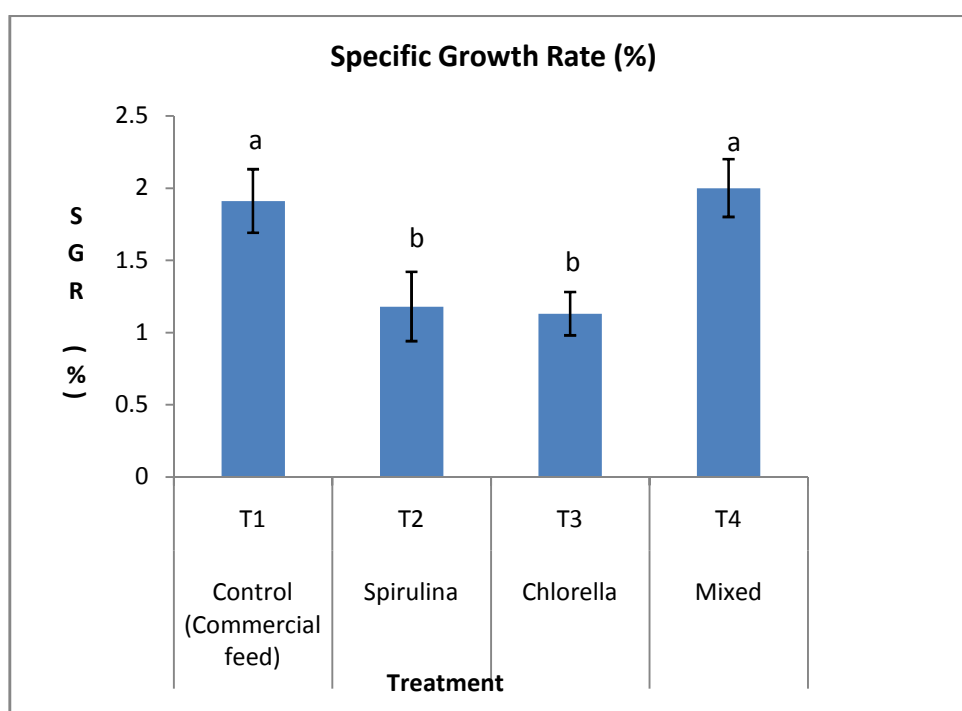
Fig.3: Specific Growth Rate, SGR (%), (Mean ± SEM) of tilapia fish cultured for 24 weeks with   four different live feed.

Table 7. Specific Growth Rate, SGR (Mean ± SEM) at 24 weeks rearing period.

Treatment	SGR (%)			
	6 weeks	12 weeks	18 weeks	24 weeks
T1	6.58 ± 0.46^{b}	4.05 ± 0.58^{a}	2.55 ± 0.37^{ab}	1.91 ± 0.22^{a}
T2	11.13 ± 0.31^{a}	2.89 ± 0.53^{ab}	5.04 ± 0.01^{a}	$1.18 \pm .24^{b}$
T3	11.93 ± 0.28^{a}	$2.35 \pm 0.16 ab$	1.33 ± 0.10^{ab}	1.13 ± 0.05^{b}
T4	10.71 ± 0.31^{a}	1.65 ± 0.26^{b}	0.77 ± 0.14^{b}	2.00 ± 0.10^{a}

Values are mean ± SEM of duplicate groups of 10 fish. Means in the same column with different superscripts are significantly different at $P < 0.05$.

3.1.4 Feed Conversion Ratio (FCR)

The feed conversion ratio of *Oreochromis niloticus* fish kept in four different small ponds and fed on four different type of feed have been calculated 24 weeks study period.The highest food conversion ratio (7.31±1.30) in treatment T4 and lowest Food conversion Ratio (0.08±0.01) in treatment T3. There is no significance difference among the all treatment at 24 weeks culture period.
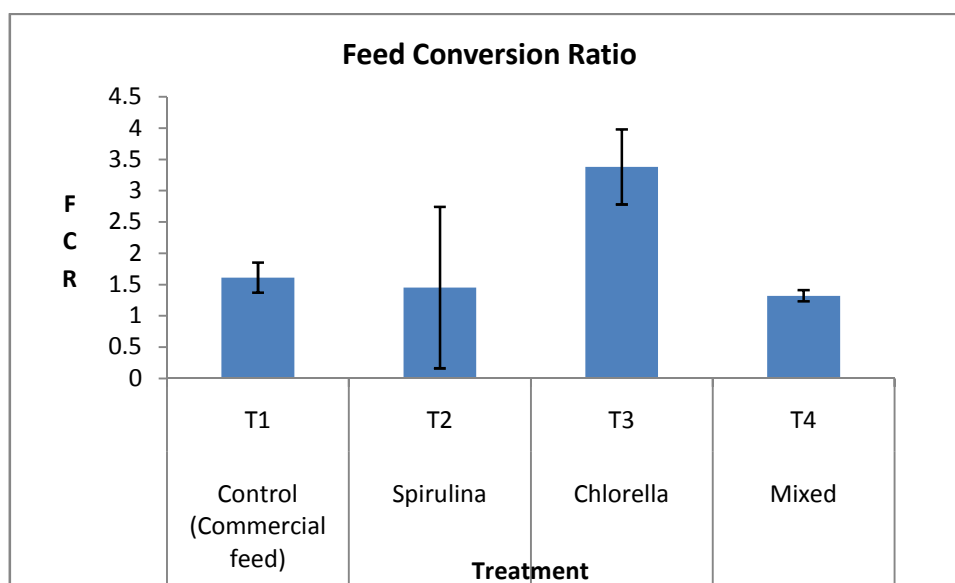
Fig. 6: Feed Conversion Ratio, FCR, (Mean ± SEM) of tilapia fish cultured for 24 weeks with four different treatments.

Table 8 Feed Conversion Ratio, FCR (Mean ± SEM) at 24 weeks rearing period.

Treatment	FCR			
	6 weeks	12 weeks	18 weeks	24 weeks
T1	0.84 ± 0.14^a	1.57±0.85	1.62 ± 0.42^b	1.61±0.24
T2	$.11\pm0.01^b$	2.47±0.90	3.84 ± 1.05^{ab}	1.45±1.29
T3	0.08 ± 0.01^b	3.47±1.23	5.34±0.10ab	3.38±0.60
T4	0.13 ± 0.02^b	4.64±1.26	7.31 ± 1.30^a	1.32±0.09

Values are mean ± SEM of duplicate groups of 10 fish. Means in the same column with different superscripts are significantly different at P<0.05

3.1.5.Survival Rate (%)

The values of survival rate of the experimental fish nile tilapia (*Orechromis niloticus)* rearing in four small ponds fed on four different types of feed have been represented in table number at the end of 6, 12, 18 , and 24 weeks culture period. The values of survival rate of fish are expressed in percentage showing in figure and table. The values of survival rate highest in treatment T3 (92.8 %) and lowest in treatment T2 (88.4 %) after 24 weeks culture period.
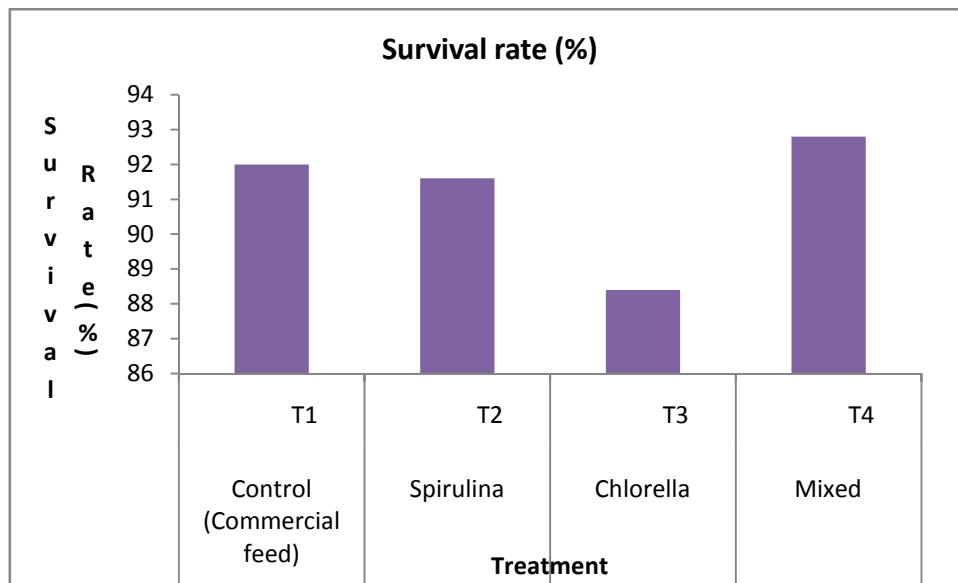
Fig. 5 Survival rate of tilapia fish cultured for 24 weeks with four different treatments.

Table 9: Survival rate of tilapia fish at 24 weeks rearing period.

Treatment	Survival Rate (%)			
	6 weeks	12 weeks	18 weeks	24 weeks
T1	98.4	96.4	94	91.6
T2	92.4	90.4	90	88.4
T3	98	95.6	94	92.8
T4	98	96	94.4	92

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3.3 Fatty acids analysis

Fatty acid is a carboxylic acid with a long aliphatic chain.Fatty acids are the building blocks of the fat in our bodies and in the food we eat. During digestion, the body breaks down fats into fatty acids, which can then be absorbed into the blood. Fatty acid molecules are usually joined together in groups of three, forming a molecule called a triglyceride. Fatty acids are mainly two types. They are Saturated and Unsaturated fatty acids.

3.2.1 Adrenic Acid

Docosatetraenoic acid is a ω-6 fatty acid with the trivial name adrenic acid. This is a naturally occurring polyunsaturated fatty acid formed through a 2-carbon chain elongation of Arachidonic acid. The highest amount Adrenic acid found in treatment T3 (2.30±0.03) and lowest Adrenic acid found in treatment T4 (1.02 ± 0.05). There is no significant difference among all the treatments at 0.05 levels.

Fig.6 : Variations of Adrenic Acid with different types of Treatment during 24 weeks culture.

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Table 10: Adrenic acid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	2.08 ± 0.45
T_2 (*Spirulina*)	1.81 ±0 .18
T_3 (*Chlorella*)	2.30 ±0.03
T_4 (Mixed)	1.02 ± 0.05

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at P<0.05

3.2.2. Eicosapentanoic Acid

Eicosapentaenoic acid (EPA or also Eicosapentaenoic acid) is an omega-3 fatty acid. In physiological literature, it is given the name 20:5(n-3). It also has the trivial name timnodonic acid. In chemical structure, EPA is a carboxylic acid with a 20-carbon chain and five *cis* double bonds; the first double bond is located at the third carbon from the omega end. EPA is a polyunsaturated fatty acid (PUFA) that acts as a precursor for prostaglandin-3 (which inhibits platelet aggregation), thromboxane-3, and leukotriene-5 eicosanoids. Studies of fish oil supplements, which contain EPA, have failed to support claims of preventing heart attacks or strokes. The highest amount of eicosapentanoic acid in treatment T3 (1.83±0.22) and lowest eicosapentaenoic is found in treatment T4 (0.22±0.03). The amount of Eicosapentaenoic acid in treatment T3 is highly significant different than T1, T2 and T4 respectively.

Fig 7: Variations of Eicosapentanoic Acid with different types of treatment during 24 weeks culture period

Table 11: Eicosapentanoic Acid (%) at 24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	$0.56\pm.02^b$
T_2 (*Spirulina*)	$0.52\pm.22^b$
T_3 (*Chlorella*)	1.83 ± 0.22^a
T_4 (Mixed)	0.22 ± 0.24^b

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at $P<0.05$

3.2.3. Docosahexaenoic Acid

Docosahexaenoic acid (DHA) is an omega-3 fatty acid that is a primary structural component of the human brain, cerebral cortex, skin, sperm, testicles and retina. It can be synthesized from alpha-linolenic acid or obtained directly from maternal milk or fish oil. The highest amount Docosahexaenoic acid is found in treatment T1 (2.70±0.53) and lowest in treatment T2 (0.07±0.02). The amount of Docosahexaenoic acid is highly significant difference in treatment T1 than Treatment T2 and T3.

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Fig. 8: Variations of Docosahexaenoic Acidwith different types of treatment during 24 weeks culture period.

Table 12: Docosahexaenoic Acid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	2.70 ± 0.53^a
T_2 (*Spirulina*)	0.07 ± 0.02^b
T_3 (*Chlorella*)	0.16 ± 0.02^b
T_4 (Mixed)	1.98 ± 0.11^a

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at $P<0.05$

3.2.4 Arachidic Acid

Arachidic acid, also called eicosanoic acid is the saturated fatty acid with a 20-carbon chain. The highest amount Arachidic acid is found in treatment T3 (2.33±0.53) and lowest in treatment T4 (0.36±0.12). There is no significance among all the treatments at 0.05 levels.

Fig 9: variations Arachidic Acidwith different types of treatment during 24 weeks culture period.

Table 13: Arachidic Acid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	1.16±1.11
T_2 (*Spirulina*)	1.09±0.25
T_3 (*Chlorella*)	2.33±0.53
T_4 (Mixed)	0.36±0.12

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at $P<0.05$

3.2.5.Docosapentaenoic Acid

Docosapentaenoic acid (DPA) is a dietary omega-3 fatty acid mainly found in fish, fish oil, seal oil and red meat. The highest amount of Docosapentaenoic acid is found in treatment T3 (2.30±0.07) and lowest intreatment T1 (0.88±0.46). There are significant difference among T1, T2 and T3.

35
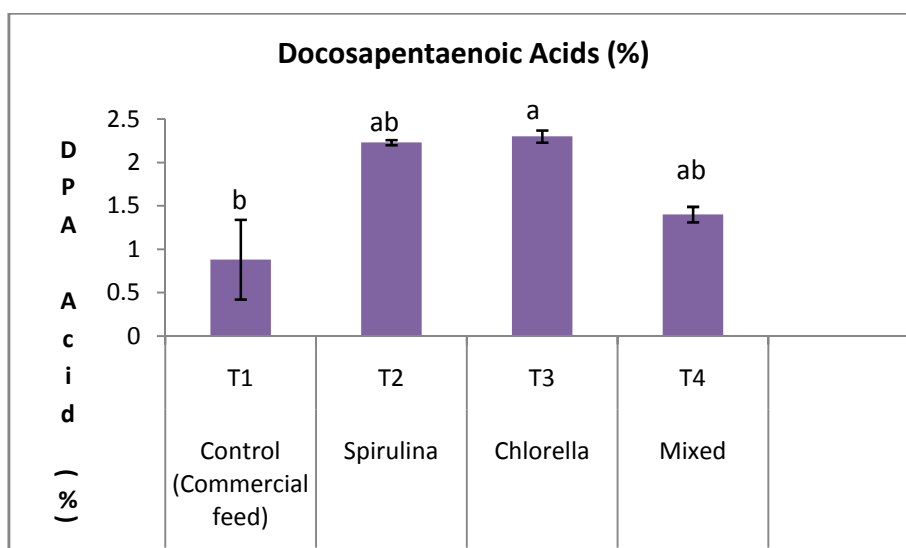
Figure10: Docosapentaenoic Acidwith different types of treatment during 24 weeks culture period.

Table 14:DocosapentaenoicAcid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	0.88 ± 0.46^b
T_2 (*Spirulina*)	2.23 ± 0.03^{ab}
T_3 (*Chlorella*)	2.30 ± 0.07^a
T_4 (Mixed)	1.40 ± 0.09^{ab}

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at P<0.05

3.2.6 Docosatrienoic Acid

Docosatrienoic acid is a rare ω-3 fatty acid not readily detected in normal phospholipids PUFA pools. The highest amount of Docosatrienoic acid is found in treatment T3 (2.14±0.03) and lowest intreatment T4 (0.88±0.46).There is no significance among all the treatments at 0.05 levels.

Fig. 11: variations of Docosatrienoic Acidwith different types of treatment during 24 weeks culture period.

Table 15: DocosatrienoicAcid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	1.45±0.45
T_2 (*Spirulina*)	2.12±0.17
T_3 (*Chlorella*)	2.14±0.03
T_4 (Mixed)	1.40±0.11

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at $P<0.05$

3.2.7. Eicosadienoic Acid

Eicosadienoic acid is a rare, naturally occurring n-6 polyunsaturated fatty acid (PUFA) found mainly in animal tissues. The highest amount of Eicosadieenoic acid is found in treatment T4 (2.81±1.17) and lowest intreatment T1 (2.81±1.17).
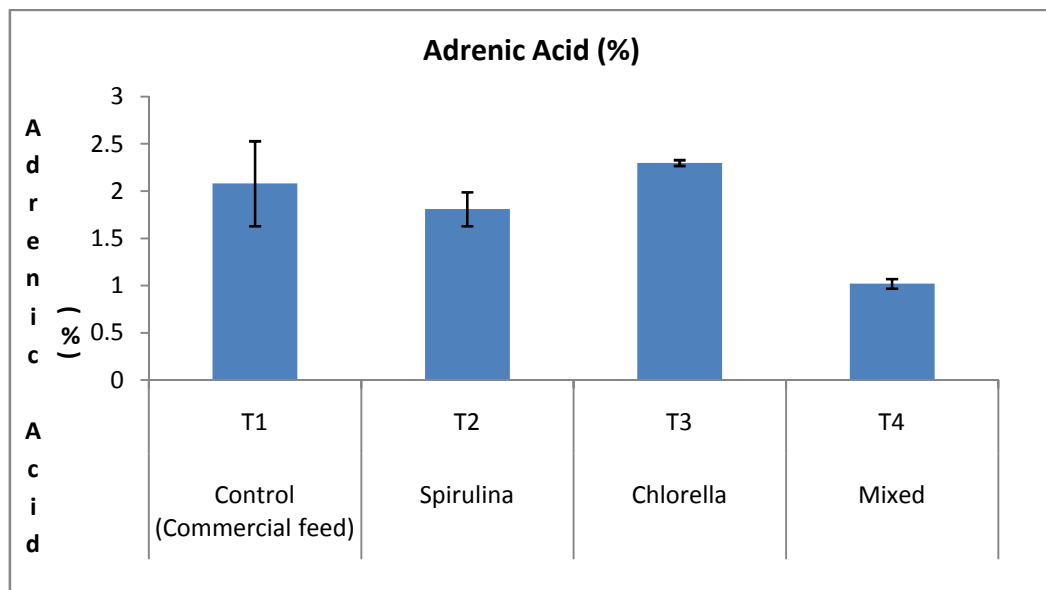
Eicosadienoic Acid (%)

Fig. 12: variations of Eicosadienoic Acidwith different types of treatment during 24 weeks culture period.

Table 16:Eicosadienoic Acid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	0.52±0.24
T_2 (*Spirulina*)	1.49±0.13
T_3 (*Chlorella*)	1.21±0.13
T_4 (Mixed)	2.81±1.17

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at P<0.05.

3.2.8. Eicosatrienoic Acid

Eicosatrienoic Acid (20:3ω-3) is a rare polyunsaturated fatty acid of the ω-3 series. The highest amount of Eicosatrienoic acid is found in treatment T3 (2.81±1.17) and lowest intreatment T4 (1.50±0.02).There is no significance among all the treatment at 0.05 levels.
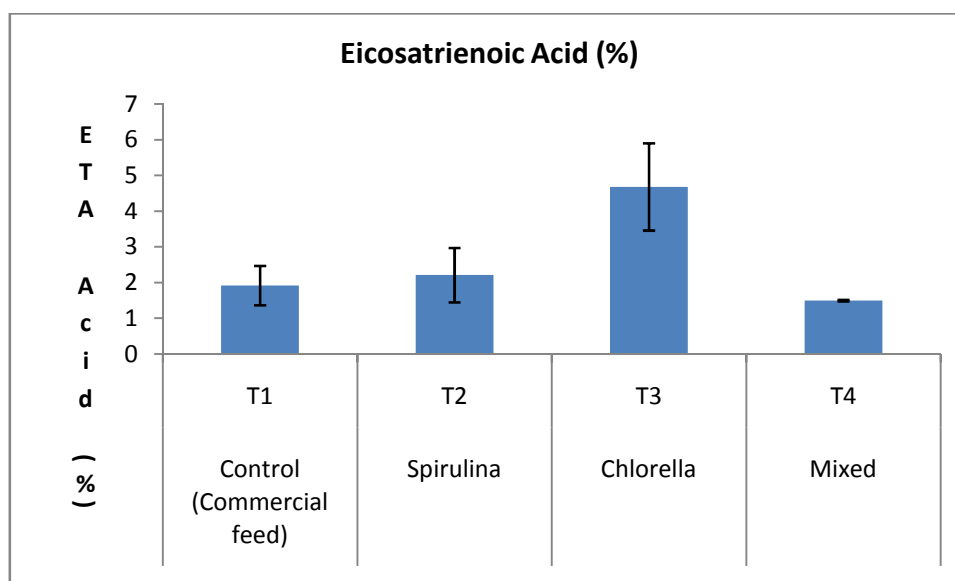
Fig. 13: variations of Eicosatrienoic Acidwith different types of treatment during 24 weeks culture period.

Table 17:EicosatrienicAcid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	1.92±0.55
T_2 (*Spirulina*)	2.21±0.76
T_3 (*Chlorella*)	4.68±1.22
T_4 (Mixed)	1.50±0.02

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at P<0.05

3.2.9. Hexadecadienoic Acid

Hexadecadienoic acid is a conjugated dienoic fatty acid metabolite of conjugated linoleic acid (CLA). The highest amount of Eicosatrienic acid is found in treatment T2 (0.98±0.36) and lowest intreatment T1 (0.52±0.04).There is no significance among all the treatment at 0.05 levels.
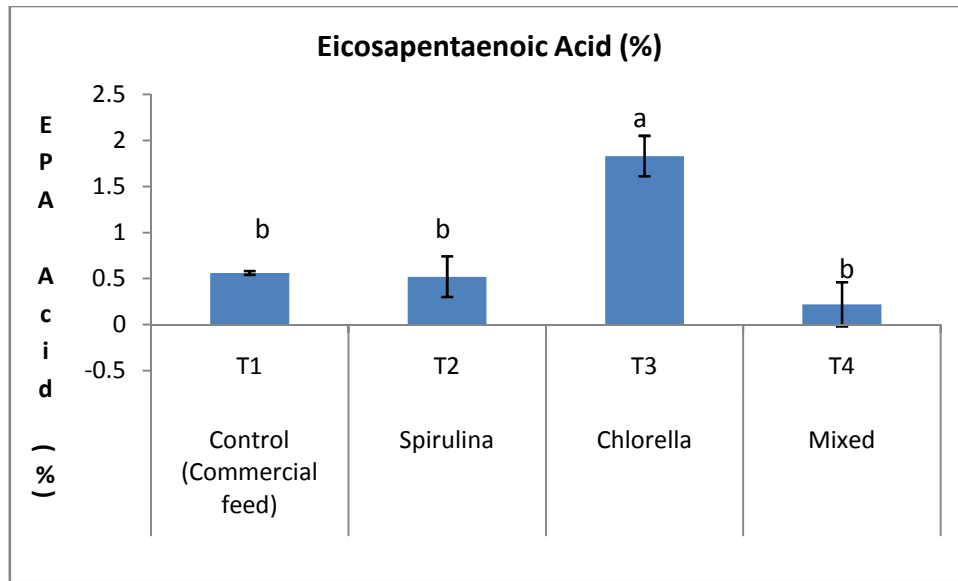
Hexadecadienoic Acid (%)
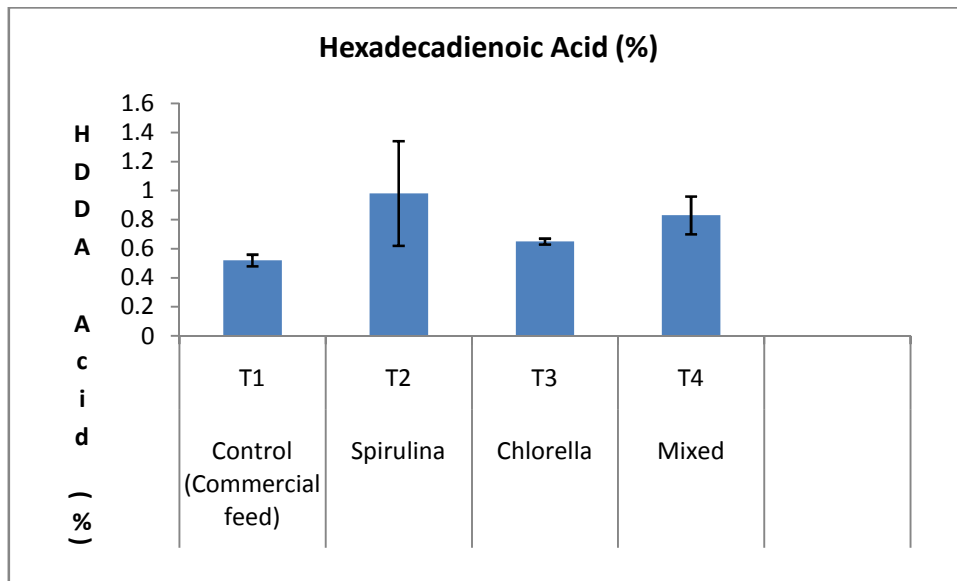
Fig. 14: Variations of Hexadecadienoic Acidwith different types of treatment during 24 weeks culture period.

Table 18:EicosatrienicAcid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	0.52±0.04
T_2 (*Spirulina*)	0.98±0.36
T_3 (*Chlorella*)	0.65±0.02
T_4 (Mixed)	0.83±0.13

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at P<0.05

3.2.10.Hexadecatrienoic Acid

Hexadecatrienoic Acid is a polyunsaturated fatty acid having 16 carbon atoms and three double bonds.The highest amount of Hexadecatrienoic acid is found in treatment T4 (1.18±0.03) and lowest intreatment T2 (0.83±0.02).There is no significance among all the treatments at 0.05 levels.
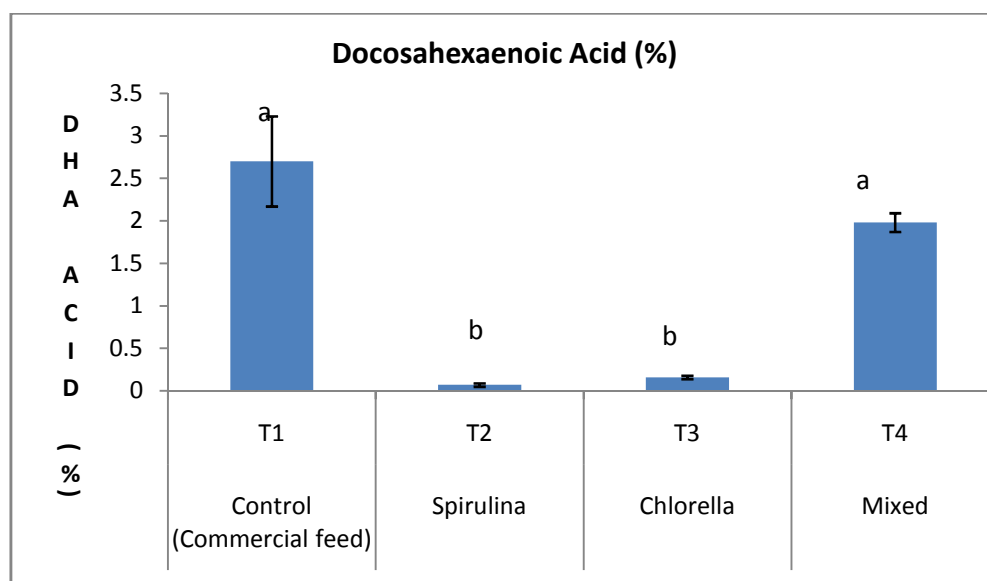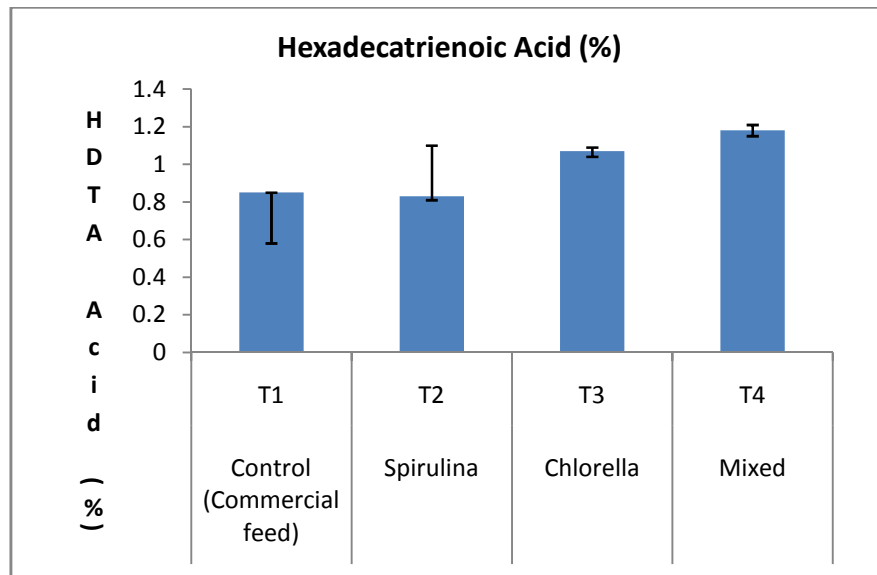
Fig. 15: Variations of Hexadecatrienoic Acid with different types of treatment during 24 weeks culture period.

Table 19:Hexadecatrienoic Acid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	0.85±0.27
T_2 (*Spirulina*)	0.83±0.02
T_3 (*Chlorella*)	1.07±0.03
T_4 (Mixed)	1.18±0.03

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at P<0.05

3. 2.11.Linoleic Acid

Linoleic acid (LA) is a polyunsaturated omega-6 fatty acid. It is a colorless liquid at room temperature. From the chemistry perspective, Linoleic acid is a carboxylic acid with an 18-carbon chain and two *cis* double bonds; with the first double bond located at the sixth carbon from the methyl end. The highest amount of Linoleic acid is found in treatment T4 (21.77±0.93) and lowest intreatment T1 (16.95±0.99). The Treatment T4 is Significantly different than T1 and T3 Treatment.

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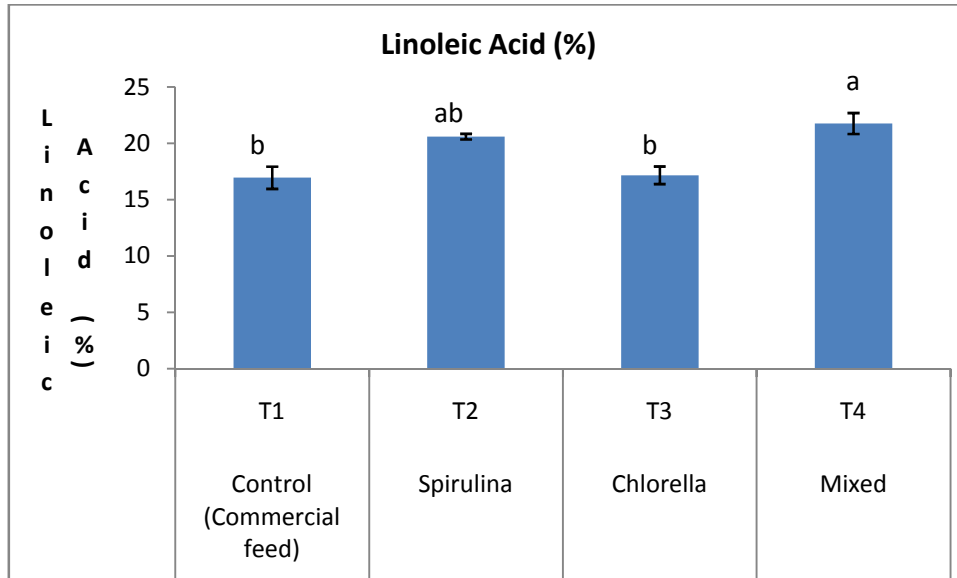
Fig16: Variations of Linoleic Acid with different types of treatment during 24 weeks culture period.

Table 20: Linoleic Acid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	16.95 ± 0.99^b
T_2 (*Spirulina*)	20.60 ± 0.25^{ab}
T_3 (*Chlorella*)	17.17 ± 0.78^b
T_4 (Mixed)	21.77 ± 0.93^a

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at P<0.05

3.2.12.Linolenic Acid

Linolenic acid is a type of fatty acid. It can refer to either of two octadecatrienoic acids with an 18-carbon chain and three double bonds. The highest amount of Linolnic acid is found in treatment T3 (3.99±0.13) and lowest intreatment T1 (2.17±0.1).There is no significance among all the treatment at 0.05 levels.
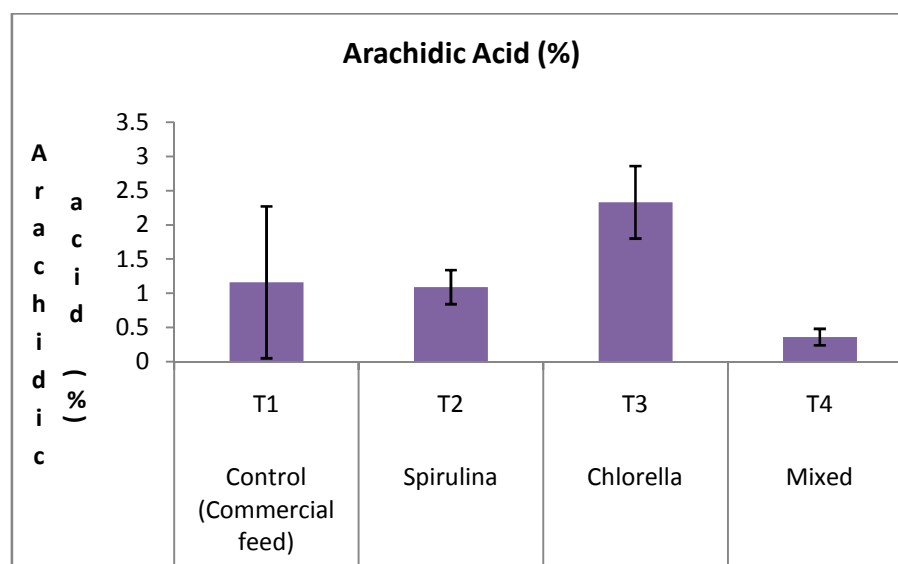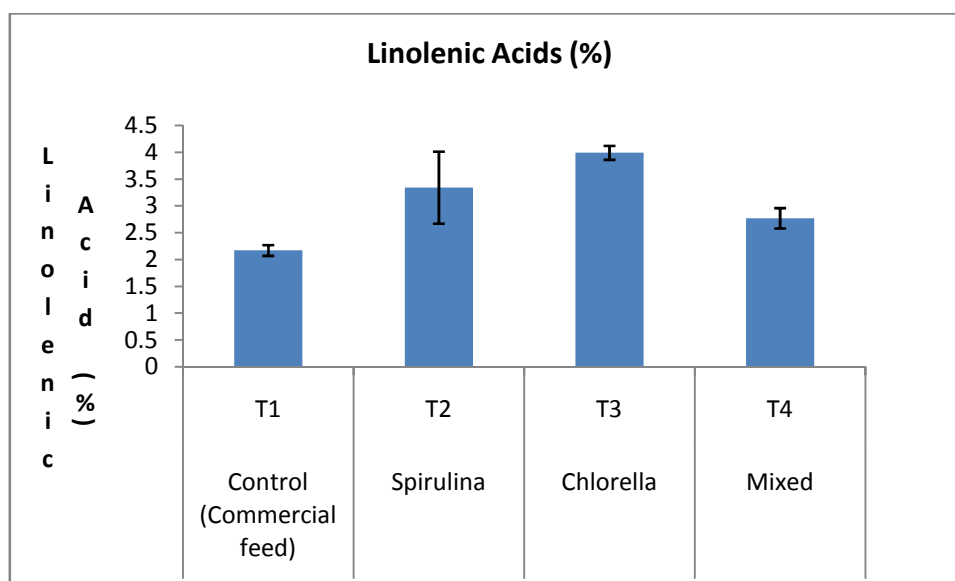
Fig17: Variations of Linolenic Acid with different types of treatment during 24 weeks culture period.

Table 21: Linolenic Acid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	2.17±0.1
T_2 (*Spirulina*)	3.34±0.67
T_3 (*Chlorella*)	3.99±0.13
T_4 (Mixed)	2.77±0.19

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at P<0.05

3.2.13.Myristic Acid

Myristic acid, also called tetradecanoic acid, is a common saturated fatty acid with the molecular formula $CH_3(CH_2)_{12}COOH$. A myristate is a salt or ester of myristic acid. The highest amount of myristic acid is found in treatment T2 with *Spirulina* (1.52±0.32) and lowest intreatment T1 withContol (1.19±0.55).There is no significance among all the treatments at 0.05 levels.
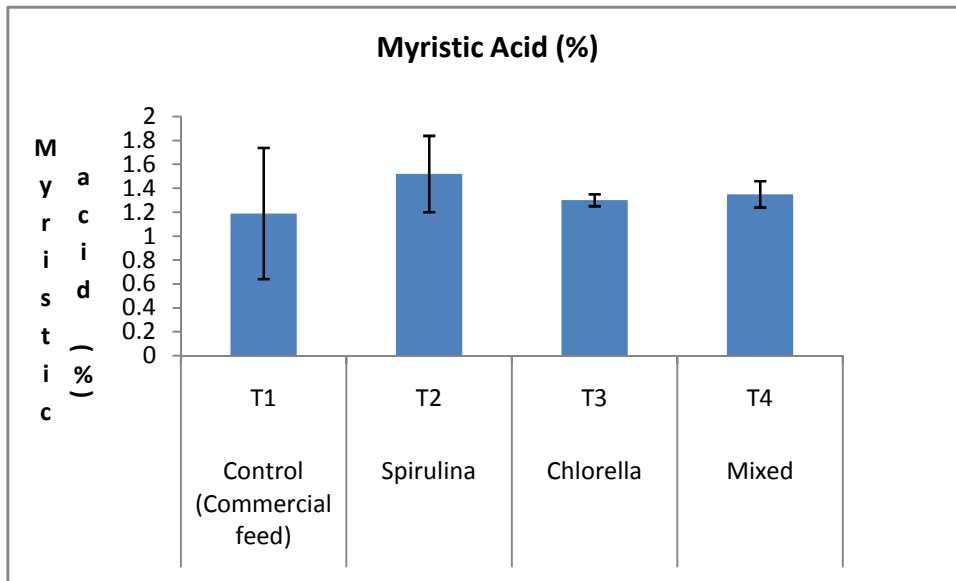
Fig. 18:   Variations of Myristic Acid with different types of treatment during 24 weeks culture period.

Table 22: Myristic Acid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	1.19±0.55
T_2 (*Spirulina*)	1.52±0.32
T_3 (*Chlorella*)	1.3±0.05
T_4 (Mixed)	1.35±0.11

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at P<0.05

3.2.14.Palmitic Acid

Palmitic acid, or hexadecanoic acid in IUPAC nomenclature, is the most common fatty acid (saturated) found in animals, plants and microorganisms. Its chemical formula is $CH_3 (CH_2)_{14}COOH$. The highest amount of Palmitic Acid acid is found in treatment T4 (24.17±0.32) and lowest intreatment T1 (20.63±0.0.43).There is no significance among all the treatment at 0.05 levels.
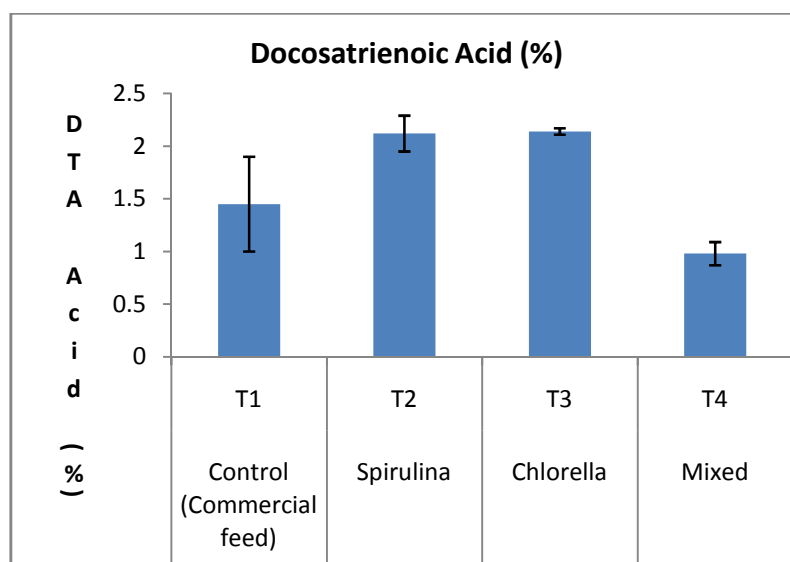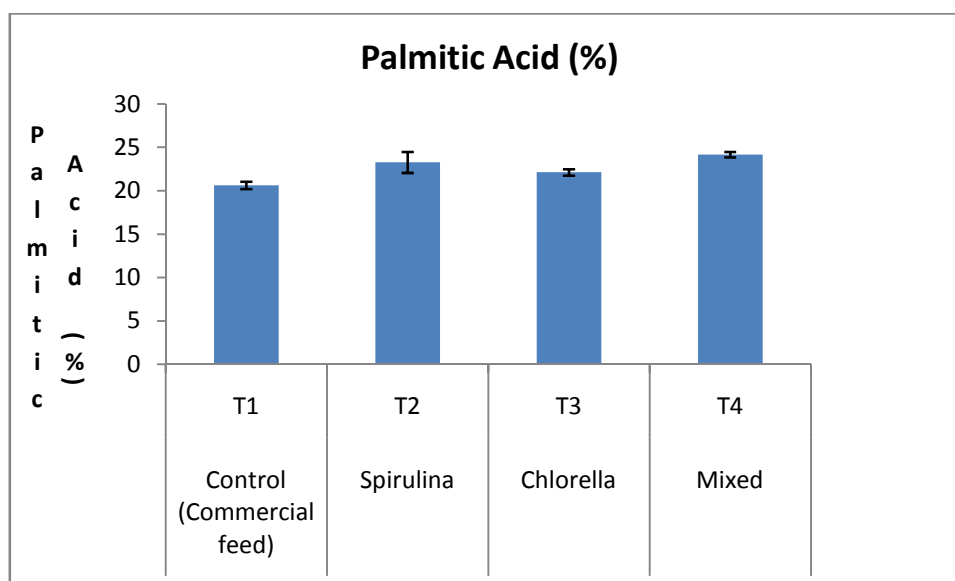
Fig. 19: Variations of Palmitic Acid with different types of treatment during 24 weeks culture period.

Table 23: Palmitic Acid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	20.63±0.0.43
T_2 (*Spirulina*)	23.27±1.21
T_3 (*Chlorella*)	22.13±0.37
T_4 (Mixed)	24.17±0.32

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at P<0.05

3.2.15.Stearic Acid

Stearic acid is a saturated fatty acid with an 18-carbon chain and has the IUPAC name octadecanoic acid. It is a waxy solid and its chemical formula is $C_{17}H_{35}CO_2H$. The highest amount of Stearic Acid is found in treatment with mixed T4 (24.17±0.32) and lowest intreatment T1 with control (20.63±0.0.43).There is no significance among all the treatments at 0.05 levels.
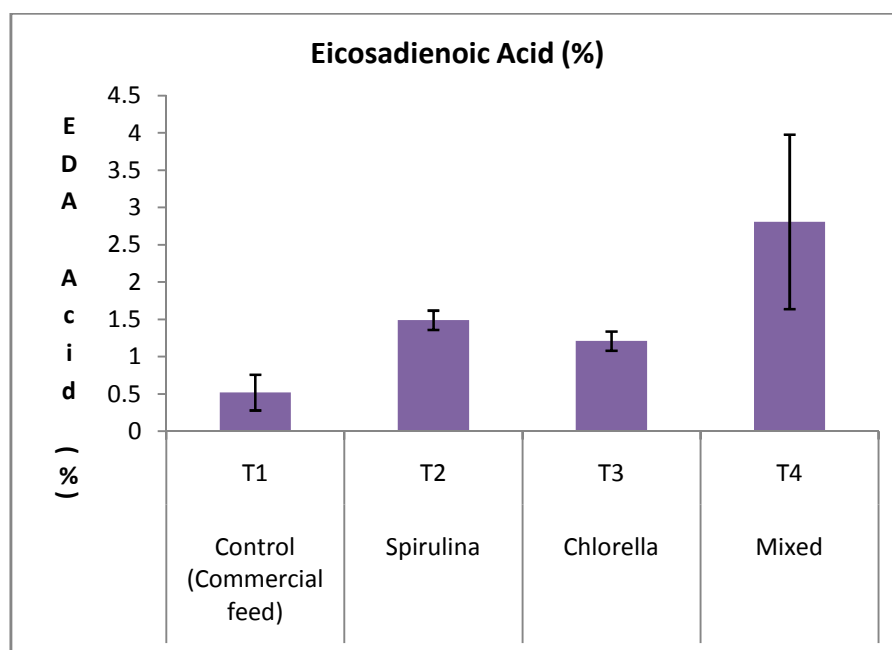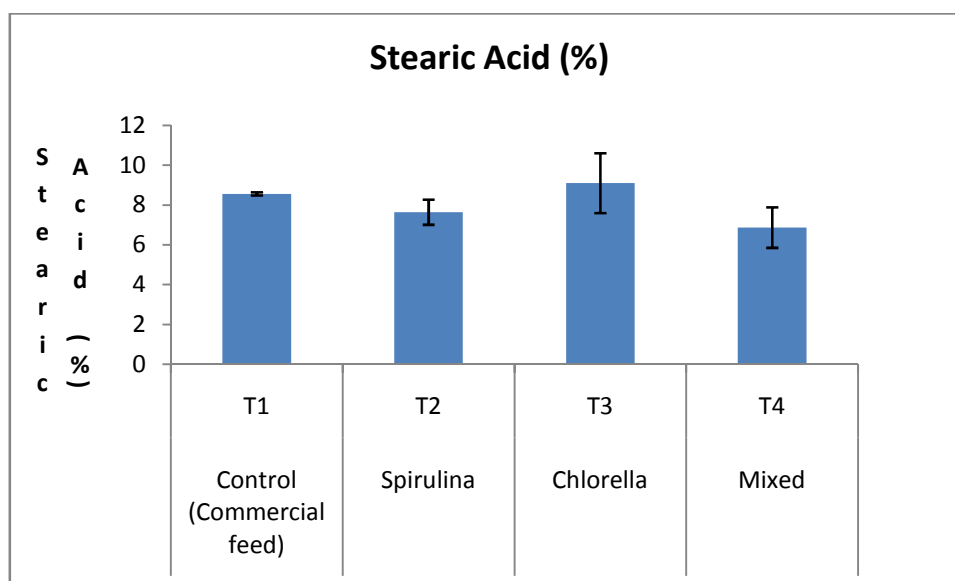
Fig. 20: Variations of Stearic Acid with different types of treatment during 24 weeks culture period.

Table 24: Stearic Acid (%) at24 weeks rearing period.

Treatment	Weight (Mean±SEM)
T_1 (Control, Commercial feed)	8.56±0.08
T_2 (*Spirulina*)	7.64±0.63
T_3 (*Chlorella*)	9.1±01.05
T_4 (Mixed)	6.87±01.02

Values are mean ± SEM of duplicate groups of 2 samples. Means in the same column with different superscripts are significantly different at $P<0.05$

3.4 Fecundity analysis

3.3.1 Fecudity of nile tilapia

The highest ficandity value is achieved by treatment T3 (2212±14) and lowest fecundity value is found in treatment T4 (968±176). There are significant difference among all treatments.
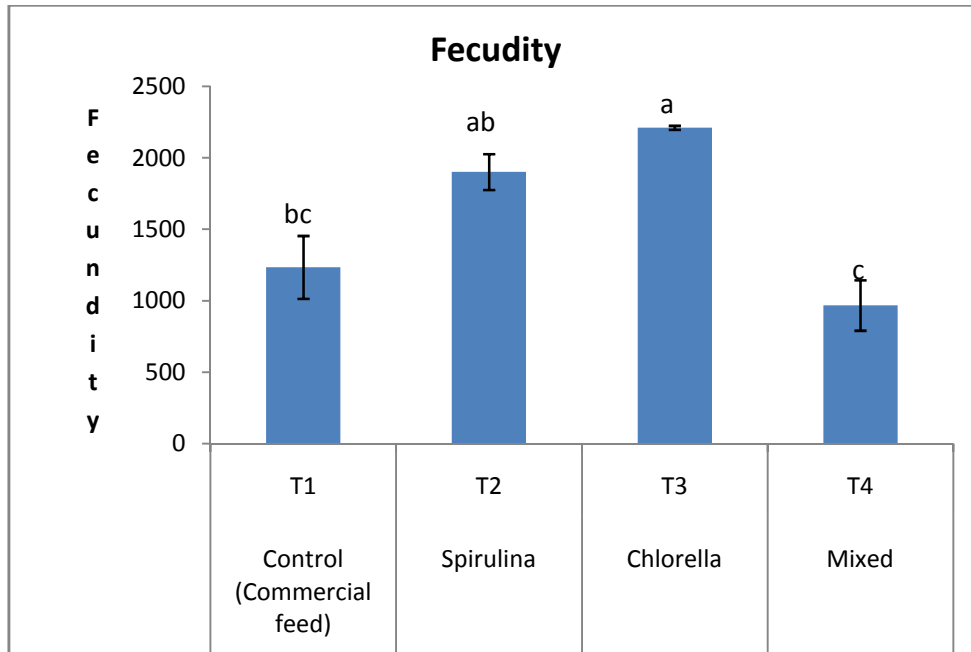
Fig. 21: Fecundity variations of Nile tilapia with four different treatment after 24 weeks rearing period

Table 25: Fecundity estimation at 24weeks rearing period.

Treatment	Fecundity (Mean±SEM)
T_1 (Control, Commercial feed)	1235±220^{bc}
T_2 (*Spirulina*)	1902±126^{ab}
T_3 (*Chlorella*)	2212±14^{a}
T_4 (Mixed)	968±176^{c}

Values are mean ± SEM of duplicate groups of 2 fish samples. Means in the same column with different superscripts are significantly different at P<0.05

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3.3.2 Ova Diameter of Nile tilapia

The highest ova diameter value is achieved by treatment T3 (5.05±0.05) and lowest ova diameter value is found in treatment T4 (3.53±0.15). There are highly significant differences of ova diameter value among all treatments.
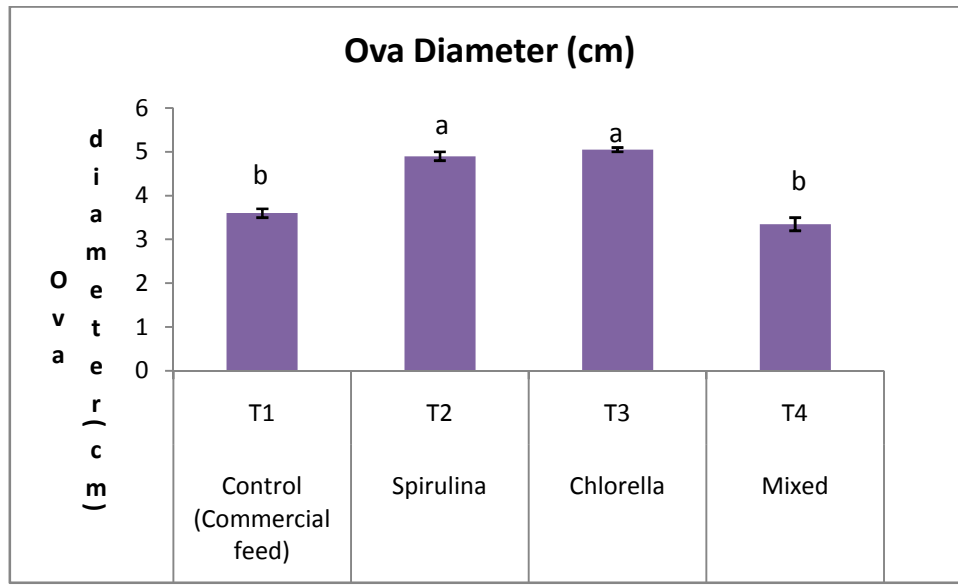
Fig.22: Ova diameter variations of nile tilapia with four different treatment after 24 weeks rearing period.

Table 26: Ova diameter in cm estimation at 24weeks rearing period.

Treatment	Ova diameter in cm (Mean±SEM)
T_1 (Control, Commercial feed)	3.60 ± 0.10^b
T_2 (*Spirulina*)	4.90 ± 0.10^a
T_3 (*Chlorella*)	5.05 ± 0.05^a
T_4 (Mixed)	3.53 ± 0.15^b

Values are mean ± SEM of duplicate groups of 2 fish samples. Means in the same column with different superscripts are significantly different at P<0.05

Chapter 4

Discussion

This investigation described the growth performance, fatty acids profiles, survival rate andfecundity of Nile tilapia (*Oreochromis niloticus)* through different Phytoplankton and Plant Derivativesduring 24 weeks culture period.In present study tilapia was selected for analysis because of its availability and easy culture and also because of its great acceptance to the poor people of Bangladesh to meet their nutritional requirements. As tilapia is omnivorous fish, different kinds of feeds are supplied to determine the effect of feeding regime on the growth performance of fish and also the fatty acids composition.

4.1 Growth performances

Growth performances in terms of total length (TL), body weight (BW), specific growth rate (SGR), feed conversion ratio (FCR), average daily gain (ADG) and condition factor (K) were recorded among four types of treatment.

4.1.1 Condition factor (K)

Condition factor of this study showed the highly variation and better performance in treatment T3. At the 24 weeks culture, the hightest value of condition factor (K) of Tilapia was observed in treatment T3 (K= 1.73) and the lowest value was found in all treatment (K = 1.10) at o weeks.Condition factor found in treatment T3 is found significantly higher than that of T1 at 24 weeksculture period.Rahman *et al*. (1997) in a study on the survival and growth of cat fish giving selected supplemental feeds got the values of condition factor between 0.51 to 0.87. Besra (1997) observed K value nearly 1.0 in *Anabas testudineus*. So the Condition factor of treatment T3 ((K= 1.73) is higher than other treatment.

4.1.2 Average Daily Gain (ADG)

This study Nile tilapia represents similar or less variation Average Daily Gain (ADG). The average daily gain was observed highest value in treatment T2 (0.45±0.09) and lowest was observed in treatment T1 (0.02±0.01).There is no significance difference among the treatments at 0.05 levels.Increased ADG of the fish suggested that the fish were able to regulate osmotic pressure of the body fluid; this was in agreement with suggestions of Nilkolsky (1963), the more the osmo-regulatory adaptation, lesser the

difference between the compositions and pressures of the internal fluid of the organism and its external environment.

4.1.3 Specific Growth Rate (SGR, %)

From this study Specific Growth Rate was found in significantly different variation.Highest specific growth rate (11.93±0.28) was achieved by treatment T3 and the lowest value (0.77±0.14).Specific growth rate of treatment T3 (11.93±0.28) significant difference than treatment T4 (0.77±0.14) at 24 weeks of sampling. This finding resembles the Medawar's (1945) fifth law "the specific growth rate declines more and more slowly as the organism increases in age" at the various conditions. Minot (1990) was the person to recognize that for most animals the specific growth rate is highest early in life and that it typically decreases with increasing age, becoming zero in some animals. Fishes usually have a rage of requirements (i.e., temperature, DO, PH, Light intensity, turbidity and so on.) for a growth and successful spawn.

4.1.4 Feed Conversion Ratio (FCR)

From This investigation, Feed Conversion Ratio of tilapia was similar at different treartments. The highest feed conversion ratio (7.31±1.30) in treatment T4 and lowest Feed Conversion Ratio (0.08±0.01) in treatment T3. There is no significant difference among the all treatments at 0.05 levels during 24 weeks culture period.Doolgindachabaporn (1994) found that the FCR value of *Crypinus carpio* rages from 1.8 to 3.0 and Akand et.al, 1989 found FCR value 2.0 to 2.7 in case of *Heteroneustes fossilis*.So FCR of tilapia in treatment T4 is more than other treatments.

4.1.5 Survival Rate (%)

The values of survival rate highest in treatment T3 (92.8 %) and lowest in treatment T2 (88.4 %) after 24 weeks culture period. Patricio (2004) who reported high survival 90% at with rapid changes in temperature used for Egyptian (Lake Manzala) strain of blue tilapia. These results are in contrast with Dan and Little (2000) who revealed that tilapia showed similarly high survival rates in deep and shallow ponds (97-100%). So the survival rate of tilapia are highly and better performance.

4.2 Fatty Acids Analysis

The high level of oleic, Palmitoleic and Arachidonic acids had been reported as a characteristics property of fresh water oils (Andrade *et al.* 1995; Osman *et al.*2001). The analysis of the fatty acid content indicates that fish are superior in terms of the nutritional value. Most abundant fatty acids are found in tilapia fish such as palmitic,myristic and stearic acids for saturated fatty acids, palmitoleic and oleic for monounsaturated fatty acids, arachidic, eicosapentaenoic, docosahexaenoic acid.

The highest amount of eicosapentanoic acid in treatment T3 (1.83±0.22) and lowest eicosapentaenoic is found in treatment T4 (0.22±0.03). The amount of Eicosapentaenoic acid in treatment T3 (*Chlorella*) is highly significant different than T1, T2 and T4 respectively. The highest amount Docosahexaenoic acid is found in treatment T1 (2.70±0.53) and lowest in treatment T2 (0.07±0.02). The amount of Docosahexaenoic acid is highly significant difference in treatment T1 (Commercial feed) than Treatment T2 *(spirulina)* And T3(*Chlorella*).(Ng *et al*. 2001), was found 10.6% eicosapentaenoic acid and 12.7% docosahexaenoic acid using oil based diets.

Stanceby (1982) also reported that 20-25% of total fatty acids in some fish lipids were palmitic acid. In this investigation, the highest amount of Palmitic Acid acid is found in treatment T4 (24.17±0.32) and lowest in treatment T1 (20.63±0.0.43). There is no significance among all the treatment at 0.05 levels. Above result supports our findings.

Molnar *et al*. (2012) found the amount of Linolenic acid is (1.48±0.17) and Eicosatrienic acid is (0.81±0.09) using soybean meal in tilapia fish. The highest amount of Linolenic acid is found in treatment T3 (3.99±0.13) and lowest intreatment T1 (2.17±0.1).There is no significance among all the treatment at 0.05 levels.The highest amount of Eicosatrienoic acid is found in treatment T3 (2.81±1.17) and lowest intreatment T4 (1.50±0.02).There is no significance among all the treatment at 0.05 levels.

In this study, the highest amount of Myristic acid is found in treatment T2 with *Spirulina* (1.52±0.32) % and lowest intreatment T1 with Control (1.19±0.55) %.The highest amount of Stearic Acid is found in treatment with mixed T4 (24.17±0.32) % and lowest intreatment T1 with control (20.63±0.0.43) %.The highest amount of Linoleic acid is found in treatment with mixed T4 (21.77±0.93) % and lowest intreatment T1 with control (16.95±0.99) %. The Treatment T4 is Significantly difference than T1 and T3 Treatment. Petinuci, *et. Al*., 2008 observed that the amount of Myristic, Stearic, and

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Linoleic acids using fishbone flour are (23.8±3.5), (54.4±0.60) and (109.60±11.60) mg/g, respectively.

4.3 Fecundity

The highest fecundity value is achieved by treatment T3 (2212±14) and lowest fecundity value is found in treatment T4 (968±176). There are significant differences among all treatments. Nyakuni, (2009) observed number of eggs per female ranged from 412 to 2380 eggs with an overall mean fecundity of 854. These findings confirm that fecundity in *O. niloticus* is variable.

Chapter 5

Conclusion and Recommendations

5.1 Conclusion

In this experiment, the significant effects of different phytoplankton and plant derivatives on growth performance, fatty acids profiles, survival rate and fecundity of Nile tilapia (*Oreochromis niloticus*) were investigated. Growth performance of Nile tilapia can cope with the different types of physiochemical parameters such as temperature, pH, DO, conductivity, total dissolved solids light intensity and so on. From the experiment conducted it was observed that the length and weight of tilapia fish varied with different phytoplankton and plant derivatives. The best growth performance was observed in *Chlorella* and *Spirulina*.*Spirulina* has the best proximate composition for human nutritional interest and thus it was found to be the better option to produce healthy fish for human use. Culture of Nile tilapia with different compound feed utilization in small ponds to improve growth and to enhance fatty acid composition of producing fish, for the well-being of consumers.

5.2 Recommendations

❖ Present study is laboratory based, so field level studies are required to identify the actual effect of *Spirulina* and *Chlorella*on tilapia.

❖ To enhance the production of Nile tilapia further research should be undertake to identify the mechanisms which are associated with the regulation of response to different phytoplankton.

❖ Research should be carried out to check the potentiality of other phytoplankton species as alternative food source rather than commercial feeds.

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Appendices

Appendix A

1. Condition factor

Zero_Week

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T1	10	1.0950173
T2	10	1.0950173
T3	10	1.0950173
T4	10	1.0950173
Sig.		1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Six_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T2	10	1.4858025
T1	10	1.5089269
T4	10	1.5731360
T3	10	1.5999271
Sig.		.431

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Twelve_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T4	10	1.5055348
T1	10	1.5075131
T2	10	1.5937080
T3	10	1.6079471

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Sig.			.499

Means for groups in homogeneous subsets
are displayed.

a. Uses Harmonic Mean Sample Size =
10.000.

Eighteen_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T1	10	1.4849127
T4	10	1.5471329
T2	10	1.5918637
T3	10	1.6456405
Sig.		.051

Means for groups in homogeneous subsets
are displayed.

a. Uses Harmonic Mean Sample Size =
10.000.

Twenty_four_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T1	10	1.5597602	
T4	10	1.6323727	1.6323727
T2	10	1.6398414	1.6398414
T3	10		1.7312279
Sig.		.174	.064

Means for groups in homogeneous subsets are
displayed.

a.   Uses Harmonic Mean Sample Size = 10.000.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	.000	3	.000	.000	1.000
Zero_Week	Within Groups	1.163	36	.032		
	Total	1.163	39			
	Between Groups	.086	3	.029	1.026	.393
Six_Weeks	Within Groups	1.003	36	.028		
	Total	1.089	39			
Twelve_Weeks	Between Groups	.090	3	.030	1.145	.344

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	Within Groups	.943	36	.026		
	Total	1.033	39			
	Between Groups	.139	3	.046	2.590	.068
Eighteen_Weeks	Within Groups	.646	36	.018		
	Total	.785	39			
	Between Groups	.148	3	.049	6.763	.001
Twenty_four_Weeks	Within Groups	.263	36	.007		
	Total	.411	39			

2.ADG

Six_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05		
		1	2	3
T1	10	.0175714		
T4	10		.0919048	
T2	10		.1153333	.1153333
T3	10			.1667857
Sig.		1.000	.757	.150

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Twelve_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T1	10	.0764762	
T4	10	.0884762	
T3	10		.2480476
T2	10		.2567619
Sig.		.994	.998

Means for groups in homogeneous subsets are
displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Eighteen_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05

		1	2	3
T4	10	.0787619		
T1	10	.1623095	.1623095	
T3	10		.2912143	.2912143
T2	10			.3681905
Sig.		.653	.292	.708

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Twenty_four_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T1	10	.3106190
T4	10	.3366667
T3	10	.4213571
T2	10	.4525476
Sig.		.345

Means for groups in homogeneous subsets

are displayed.

a. Uses Harmonic Mean Sample Size =

10.000.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	.115	3	.038	13.709	.000
Six_Weeks	Within Groups	.101	36	.003		
	Total	.216	39			
	Between Groups	.290	3	.097	8.495	.000
Twelve_Weeks	Within Groups	.409	36	.011		
	Total	.699	39			
	Between Groups	.502	3	.167	6.496	.001
Eighteen_Weeks	Within Groups	.927	36	.026		
	Total	1.429	39			
	Between Groups	.137	3	.046	1.288	.293
Twenty_four_Weeks	Within Groups	1.273	36	.035		
	Total	1.409	39			

2. SGR (%)

Six_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T1	10	6.5764255	
T4	10		10.7092638
T2	10		11.1268060
T3	10		11.9343356
Sig.		1.000	.079

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Twelve_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T4	10	1.6526448	
T3	10	2.3484911	2.3484911
T2	10	2.8927366	2.8927366
T1	10		4.0474471
Sig.		.259	.067

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Eighteen_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T4	10	.7697386	
T3	10	1.3260271	1.3260271
T1	10	2.5458474	2.5458474
T2	10		5.0370640
Sig.		.679	.107

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

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Twenty_four_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T3	10	1.1309367	
T2	10	1.1791639	
T1	10		1.9126829
T4	10		1.9980524
Sig.		.998	.988

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Six_Weeks	Between Groups	172.052	3	57.351	47.188	.000
	Within Groups	43.753	36	1.215		
	Total	215.805	39			
Twelve_Weeks	Between Groups	30.683	3	10.228	4.647	.008
	Within Groups	79.241	36	2.201		
	Total	109.924	39			
Eighteen_Weeks	Between Groups	107.850	3	35.950	2.867	.050
	Within Groups	451.409	36	12.539		
	Total	559.259	39			
Twenty_four_Weeks	Between Groups	6.453	3	2.151	6.351	.001
	Within Groups	12.193	36	.339		
	Total	18.646	39			

3. FCR

Six_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T3	10	.0747910	
T2	10	.1066546	
T4	10	.1278907	
T1	10		.8429539
Sig.		.954	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Twelve_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T1	10	1.5650460
T2	10	2.4744761
T3	10	3.4690209
T4	10	4.6431052
Sig.		.199

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Eighteen_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T1	10	1.6203617	
T2	10	3.8357039	3.8357039
T3	10	5.3397294	5.3397294
T4	10		7.3135848
Sig.		.175	.224

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Twenty_four_Weeks

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T4	10	1.3219459
T2	10	1.4452859
T1	10	1.6061180
T3	10	3.3844532
Sig.		.200

Means for groups in homogeneous subsets are displayed.

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a. Uses Harmonic Mean Sample Size = 10.000.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Six_Weeks	Between Groups	4.120	3	1.373	26.165	.000
	Within Groups	1.889	36	.052		
	Total	6.009	39			
Twelve_Weeks	Between Groups	52.493	3	17.498	1.511	.228
	Within Groups	416.760	36	11.577		
	Total	469.253	39			
Eighteen_Weeks	Between Groups	173.520	3	57.840	3.657	.021
	Within Groups	569.448	36	15.818		
	Total	742.968	39			
Twenty_four_Weeks	Between Groups	28.247	3	9.416	1.803	.164
	Within Groups	187.945	36	5.221		
	Total	216.192	39			

5. Physico-chemical parameters

Case Summaries[a]

			Temperature	Ph	DO	Conductivity	Light_Intensity	TDS
Treatment	T1	1	30.10	9.50	9.10	489	11.80	202
		2	29.40	7.10	7.00	494	16.50	200
		3	27.80	7.33	6.10	413	20.50	265
		4	30.70	8.73	3.75	410	63.50	266
		5	24.50	7.59	4.10	461	75.70	278
		Total Mean	28.5000	8.0500	6.0100	453.40	37.6000	242.20
		Total Std. Error of Mean	1.11131	.45812	.98214	18.013	13.27720	16.978
		Total N	5	5	5	5	5	5
	T2	1	29.70	7.50	6.10	448	31.80	184
		2	28.70	7.53	5.20	440	14.50	198
		3	27.70	7.60	4.50	405	22.40	260
		4	30.70	7.46	4.60	426	79.50	271
		5	25.20	7.48	45.00	472	78.30	305
		Total Mean	28.4000	7.5140	13.0800	438.20	45.3000	243.50
		Total Std. Error of Mean	.94340	.02441	7.98507	11.164	13.98917	22.892
		Total N	5	5	5	5	5	5
	T3	1	30.00	9.80	7.80	417	21.80	170
		2	28.80	7.58	6.70	412	17.50	180
		3	28.00	7.58	5.10	432	27.10	274

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		4		30.70	7.43	4.15	427	78.10	273
		5		24.10	7.44	3.98	459	53.50	293
			Mean	28.3200	7.9660	5.5460	429.40	39.6000	238.00
	Total	Std. Error of Mean	1.15386	.45965	.74204	8.201	11.48251	26.013
		N	5	5	5	5	5	5
		1		30.10	9.35	6.50	487	31.50	197
		2		29.30	7.39	5.30	472	16.00	188
		3		28.10	7.52	4.80	408	25.58	261
T4		4		30.70	7.53	5.46	412	65.60	263
		5		24.30	7.56	5.40	447	61.30	257
			Mean	28.5000	7.8700	5.4920	445.20	39.9960	233.20
	Total	Std. Error of Mean	1.13666	.37115	.27760	15.740	9.91271	16.704
		N	5	5	5	5	5	5
		Mean		28.4300	7.8500	7.5320	441.55	40.6240	239.23
Total	Std. Error of Mean			.50022	.17788	1.99545	6.658	5.66710	9.691
	N			20	20	20	20	20	20

a. Limited to first 200 cases.

Appendix B

1. Adrenic acid (%)

ANOVA

Adrenic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.881	3	.627	5.345	.070
Within Groups	.469	4	.117		
Total	2.350	7			

Adrenic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T4	2	1.021600
T2	2	1.812200
T1	2	2.083700
T3	2	2.304100
Sig.		.065

Means for groups in homogeneous subsets
are displayed.

a. Uses Harmonic Mean Sample Size =
2.000.

ANOVA

Eicosapentaenoic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.051	3	1.017	21.350	.006
Within Groups	.191	4	.048		
Total	3.241	7			

Eicosapentaenoic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T4	2	.219250	
T2	2	.515750	
T1	2	.559300	

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T3	2		1.825200
Sig.		.487	1.000

Means for groups in homogeneous subsets are
displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

3. Docosahexaenoic acid

ANOVA

Docosahexaenoic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.497	3	3.499	23.633	.005
Within Groups	.592	4	.148		
Total	11.089	7			

Docosahexaenoic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T2	2	.064950	
T3	2	.159150	
T4	2		1.984350
T1	2		2.704850
Sig.		.994	.364

Means for groups in homogeneous subsets are
displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

3. Arachidic acid

ANOVA

Arachidic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.963	3	1.321	1.658	.311
Within Groups	3.187	4	.797		
Total	7.150	7			

Arachidic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1

T4	2	.360950
T2	2	1.087050
T1	2	1.162950
T3	2	2.326100
Sig.		.265

Means for groups in homogeneous subsets
are displayed.

a. Uses Harmonic Mean Sample Size =
2.000.

4. Arachidonic acid

ANOVA

Arachidonic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.300	3	1.433	1.150	.431
Within Groups	4.983	4	1.246		
Total	9.283	7			

Arachidonic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T2	2	2.651550
T3	2	3.391500
T4	2	3.431900
T1	2	4.691900
Sig.		.379

Means for groups in homogeneous subsets
are displayed.

a. Uses Harmonic Mean Sample Size =
2.000.

5.Docosapentaenoic Acid

ANOVA

Docosapentaenoic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.811	3	.937	8.364	.034
Within Groups	.448	4	.112		
Total	3.259	7			

Docosapentaenoic_Acid

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Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T1	2	.881550	
T4	2	1.399350	1.399350
T2	2	2.229200	2.229200
T3	2		2.304200
Sig.		.052	.164

Means for groups in homogeneous subsets are
displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

6. Docosahexaenoic acid

ANOVA

Docosahexaenoic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.497	3	3.499	23.633	.005
Within Groups	.592	4	.148		
Total	11.089	7			

Docosahexaenoic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T2	2	.064950	
T3	2	.159150	
T4	2		1.984350
T1	2		2.704850
Sig.		.994	.364

Means for groups in homogeneous subsets are
displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

7. Eicosadienoic acid

ANOVA

Eicosadienoic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.525	3	1.842	2.531	.196
Within Groups	2.911	4	.728		
Total	8.437	7			

Eicosadienoic_Acid

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Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T1	2	.515900
T3	2	1.216250
T2	2	1.490250
T4	2	2.809150
Sig.		.166

Means for groups in homogeneous subsets
are displayed.

a. Uses Harmonic Mean Sample Size =
2.000.

8. Eicosatrienoic acid

ANOVA

Eicosatrienoic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.352	3	4.117	3.476	.130
Within Groups	4.739	4	1.185		
Total	17.091	7			

Eicosatrienoic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T4	2	1.495050
T1	2	1.920350
T2	2	2.207200
T3	2	4.683550
Sig.		.133

Means for groups in homogeneous subsets
are displayed.

a. Uses Harmonic Mean Sample Size =
2.000.

9. Hexadecadienoic acid

ANOVA

Hexadecadieonic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.244	3	.081	1.088	.450
Within Groups	.299	4	.075		
Total	.543	7			

Hexadecadieonic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T1	2	.520450
T3	2	.647900
T4	2	.825050
T2	2	.980900
Sig.		.434

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

10. Hexadecaditrienoic acid

ANOVA

Hexadecaditrieonic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.175	3	.058	1.586	.325
Within Groups	.147	4	.037		
Total	.321	7			

Hexadecaditrieonic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T2	2	.834850
T1	2	.852150
T3	2	1.071700
T4	2	1.183950
Sig.		.381

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

11. Linoleic acid

ANOVA

Linoleic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35.496	3	11.832	9.390	.028
Within Groups	5.040	4	1.260		
Total	40.537	7			

Linoleic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T1	2	16.949950	
T3	2	17.171750	
T2	2	20.604400	20.604400
T4	2		21.773300
Sig.		.099	.738

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

12. Linolenic acid

ANOVA

Linolenic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.647	3	1.216	4.764	.083
Within Groups	1.021	4	.255		
Total	4.668	7			

Linolenic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T1	2	2.170450
T4	2	2.765050
T2	2	3.335100
T3	2	3.992650
Sig.		.073

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

13. Myristic acid

ANOVA

Myristic_Acid

	Sum of Squares	df	Mean Square	F	Sig.

Between Groups	.114	3	.038	.184	.902
Within Groups	.829	4	.207		
Total	.943	7			

Myristic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T1	2	1.190150
T3	2	1.300500
T4	2	1.352500
T2	2	1.521700
Sig.		.881

Means for groups in homogeneous subsets
are displayed.

a. Uses Harmonic Mean Sample Size =
2.000.

14. Palmitic acid

ANOVA

Palmitic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.997	3	4.666	4.947	.078
Within Groups	3.773	4	.943		
Total	17.769	7			

Palmitic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T1	2	20.632900
T3	2	22.130600
T2	2	23.269400
T4	2	24.171650
Sig.		.071

Means for groups in homogeneous subsets
are displayed.

a. Uses Harmonic Mean Sample Size =
2.000.

15. Stearic acid

ANOVA

Stearic_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.838	3	1.946	1.056	.460
Within Groups	7.374	4	1.843		
Total	13.211	7			

Stearic_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T4	2	6.868850
T2	2	7.641450
T1	2	8.563450
T3	2	9.095650
Sig.		.452

Means for groups in homogeneous subsets
are displayed.

a. Uses Harmonic Mean Sample Size =
2.000.

16. Margaric Acid

ANOVA

Margaric_Acid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	3	.000	.087	.964
Within Groups	.023	4	.006		
Total	.024	7			

Margaric_Acid

Tukey HSD

Treatment	N	Subset for alpha = 0.05
		1
T1	2	.185850
T4	2	.205900
T2	2	.219100
T3	2	.219400
Sig.		.968

Means for groups in homogeneous subsets
are displayed.

a. Uses Harmonic Mean Sample Size =
2.000.

Appendix C

Fecundity Analysis

Body_weight

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T4 Mixed	2	19.3550	
T1 (Control /Commercial Feed)	2	28.2100	
T2 Spirulina	2		49.3900
T3 Chorella	2		51.4600
Sig.		.065	.819

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Body_length

Tukey HSD

Treatment	N	Subset for alpha = 0.05		
		1	2	3
T4 Mixed	2	10.300		
T1 (Control /Commercial Feed)	2		11.650	
T2 Spirulina	2			13.850
T3 Chorella	2			14.200
Sig.		1.000	1.000	.429

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Fecundity

Tukey HSD

Treatment	N	Subset for alpha = 0.05		
		1	2	3
T4 Mixed	2	968.00		
T1 (Control /Commercial Feed)	2	1234.50	1234.50	
T2 Spirulina	2		1902.00	1902.00
T3 Chorella	2			2212.00
Sig.		.649	.119	.551

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Ova_diameter

Tukey HSD

Treatment	N	Subset for alpha = 0.05	
		1	2
T4 Mixed	2	3.350	
T1 (Control /Commercial Feed)	2	3.500	
T2 Spirulina	2		4.900
T3 Chorella	2		5.050
Sig.		.759	.759

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Body_weight	Between Groups	1502.342	3	500.781	89.245	.000
	Within Groups	22.445	4	5.611		
	Total	1524.787	7			
Body_length	Between Groups	20.550	3	6.850	161.176	.000
	Within Groups	.170	4	.043		
	Total	20.720	7			
Fecundity	Between Groups	1994038.375	3	664679.458	13.896	.014
	Within Groups	191336.500	4	47834.125		
	Total	2185374.875	7			
Ova_diameter	Between Groups	4.850	3	1.617	71.852	.001
	Within Groups	.090	4	.022		
	Total	4.940	7			