A SEMINAR PAPER ON

USE OF MICROALGAE AS FISH FEED INGREDIENT FOR ENHANCED GROWTH PERFORMANCE AND IMMUNITY

Course Title: Seminar

Course Code: AQC 598

Term: Summer, 2020

SUBMITTED TO:

Course Instructors

Dr. A. K. M. Aminul Islam
Professor
Dept. of Genetics and Plant Breeding,
BSMRAU.

Dr. Md. Mizanur Rahman
Professor
Dept. of Soil Science,
BSMRAU.

Dr. Dinesh Chandra Shaha
Associate Professor
Dept. of Fisheries Management,
BSMRAU.

Dr. Md. Sanaullah Biswas
Associate Professor
Dept. of Horticulture,
BSMRAU.

Major Professor

Dr. Md. Amzad Hossain
Professor & Head
Dept. of Aquaculture,
BSMRAU.

SUBMITTED BY:

Joya Saha
Reg. No: 19-05-5300
MS Student
Department of Aquaculture

BANGABANDHU SHEIKH MUJIBUR RAHMAN AGRICULTURAL UNIVERSITY

SALNA, GAZIPUR-1706

Use of Microalgae as Fish Feed Ingredient for Enhanced Growth Performance and Immunity[1]

By

Joya Saha[2]

ABSTRACT

Microalgae are microscopic algae typically found in freshwater and marine water systems and considered as an important source of nutrients such as protein, lipid, carbohydrate, antioxidant, essential amino acid, fatty acid etc. The use of microalgae as a fish feed ingredient is increasing rapidly. This seminar paper is to review the effect of using microalgae in culture of different fish for better growth and immunity. Microalgal species such as *Spirulina platensis, S. maxima, Spirogyra* sp., *Chlorella vulgaris* etc. have been mostly used in aquaculture. In one finding, 10% replacement of fishmeal with dried *S. platensis* showed better growth performance of *Cyprinus carpio* compared with the control one. Another finding showed that, the growth rate of *Pungasius sutchi* fed with 5% *Spirulina* supplement showed better growth. Highest value for average weight gain of fish was observed to be 84 g and lowest value for average weight gain was observed in feed without *Spirulina* was 78.6 g. One experiment showed that, level of Catalase, Lysozyme and IgG (Immunoglobulin G) value were found to be higher in *S. Platensis* supplemented groups in case of mono-sex tilapia. The lysozyme was higher with 1.5% *Spirulina* supplementation (7.28 μ/ml) compared to control group (3.94 μ/ml) that indicates better immune response. Another experiment showed that, the specific growth rate was found best for African catfish fed with 25% *Chlorella vulgaris* diet 7.86 ± 0%day^{-1}, and worst at 6.77 ± 0.07%day^{-1} for the control group having 0% algal meal. Another study found positive result of using *Spirogyra* sp. incorporated into diet formulations (30% and 40% inclusion) on the growth and body composition of catla (*Catla catla*). And all these findings prove the efficient use of microalgae as a fish feed ingredient for enhanced growth and immunity of fish.

Keywords: Microalgae, fish feed, growth performance, immunity

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[1]A seminar paper presented at seminar course on July 23, 2020

[2]MS student, Department of Aquaculture, BSMRAU, Gazipur-1706

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Chapter I
INTRODUCTION

Aquaculture is one of the fastest growing animal food producing sectors, and in the last three decades (1980–2010), world food fish production of aquaculture has expanded, at an average annual rate of 8.8 percent by almost 12 times (Yoshimatsu *et al.*,2014). In 2016, global fish production was about 171 million tons with aquaculture representing 47 percent of the total production (FAO, 2018). The total first sale value of fisheries and aquaculture production in 2016 was calculated at USD 362 billion, of which USD 232 billion was from aquaculture production (FAO, 2018). Bangladesh is one of the world's leading fish producing countries with a total production of 42.77 lakh MT in FY 2017-18, where aquaculture production contributes 56.24 percent of the total fish production (DoF, 2018). In recent years, world population is increasing rapidly and thus food security has become a serious issue with this rapidly increasing world population. Fisheries have always played an important socio-economic role in many countries and communities. Fish is a vital resource towards poverty reduction and food security for poorer households. The main constrains in aquaculture are seed and feed. Fish feed generally constitutes 60–70% of the operational cost in intensive and semi- intensive aquaculture system (Singh *et al.*, 2006). Due to the high cost of fish feed, we have to think about the alternative of the present commercial feed. Conventional agriculture has not been able to supply enough food therefore, new alternative and unconventional food sources have to be searched to feed this much crowded world.

Algae are a diverse group of aquatic, photosynthetic organisms mainly categorized as either macroalgae (i.e. seaweed) or microalgae (unicellular). Algae can be a good alternative source of protein for cultured fish, particularly in tropical and subtropical regions where algae production is high (El-Hindawy *et al.*, 2006). Microalgae can grow in a wide range of habitats, have several fold higher biomass production than plants, can divide fast with simple nutritional requirements, can accumulate useful metabolites, and its availability is not dependent on wild fish harvesting for fishmeal (Hemaiswarya *et al.*, 2011). The use of microalgae as fish feed inputs has been studied with inspiring results. Microalgae are a wide group of photosynthetic heterotrophic organisms consisting of vital amino acids, protein, minerals, vitamins, chlorophylls and some forms of antioxidants and bioactive substances (Kwak *et al.*, 2012). They are necessary for larvae nutrition during life cycle of many commercially important fish. The nutritional value of microalgae is determined by their size, shape, digestibility, and biochemical compositions (Brown *et al.*, 1997). They are used not only as basic nutrients and immunostimulants but also as a source of pigments to color the skin on the other.

Microalgae have the potential to minimize the dependence on conventional raw materials in aquafeeds. The use of microalgae could have significant beneficial effects and could potentially replace or reduce common feed stuff because of their nutritional quality and positive effect on the rate of growth of aquatic species due to increased triglyceride and protein deposition in muscle, improved resistance to disease, decreased nitrogen output into the environment, omega-3 fatty acid content, physiological activity, and carcass quality (Becker, 2004). Microalgae with its added advantages such as a long history of its food use, easy cultivation, and high nutritional content make it an important source for immunomodulation studies (Raja *et al.*, 2010). Algal supplements have been reported to contain many compounds that serves as non-specific immunostimulants, improving the innate defense mechanisms in the animal, providing enhanced resistance to pathogens and thus improve the immunity (Shah *et al.*, 2018).

In order to gain a good understanding of how microalgae can be used in fish feeds, a large number of well-designed feeding trials are still required to evaluate the potentiality of microalgae as a fish feed ingredient (Shah *et al.*, 2018). However, it already seems that microalgae will play a significant role in the effort to move the formulation of aqua-feed to a more sustainable future. Available microalgal biomass with better composition and cheaper price will allow producing more available and reliable aqua-feed and this will free the aquaculture industry to keep growing and fulfil current and future demands.

From this review paper, it would be possible to find out the effect of using microalgae as fish feed ingredients for enhanced growth and immunity of fish.

OBJECTIVES

The study has undertaken to accomplish the following objectives:

- To evaluate the importance of using microalgae as a fish feed ingredient
- To find out the effect of using microalgae on growth performance and immunity.

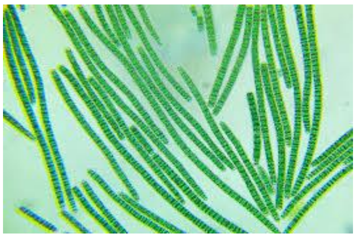
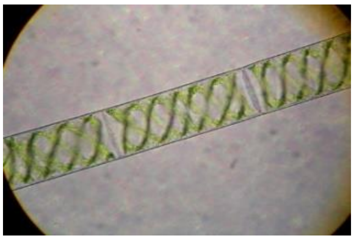
Chapter II
MATERIALS AND METHODS

All the information of this seminar paper has been collected from the secondary sources as it is just a review paper. During the preparation of this review paper, I went through various relevant books, journals, proceedings, reports, publications, internet etc. I got valuable suggestions and information from my major professor and my course instructors. After collecting all the available information, I myself complied the collected information and prepared this seminar paper.

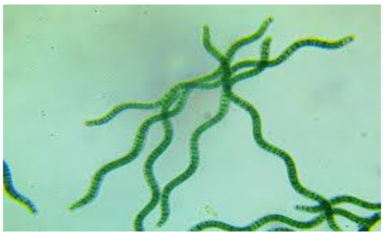
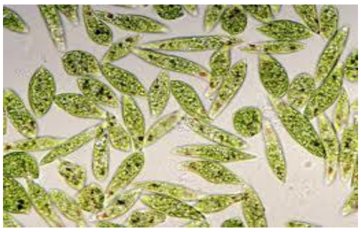
Chapter III
REVIEW OF FINDINGS

3.1 Microalgae

Microalgae are the main component of phytoplankton, and they are by far the most important primary producers of the majority of aquatic systems (Falkowski *et al.*, 2007). Microalgae are microscopic algae generally found in both freshwater and marine water systems, living in the water column and sediment. They are unicellular species and exist individually, or in chains or groups. Their sizes can range from a few micrometers (µm) to a few hundred micrometers. Unlike higher plants, they do not have roots, stems, or leaves. Microalgae are adapted to an environment dominated by viscous forces. They are capable of performing photosynthesis which is important for life on earth. According to James *et al.*, (2019), microalgae generate approximately half of the atmospheric oxygen and use simultaneously the greenhouse gas carbon dioxide to grow autotrophically. Microalgae, together with bacteria forms the base of the food web and also provide energy for all the trophic levels above them.


Spirulina platensis *Spirogyra* sp. *Chlorella vulgaris*

S. maxima *Euglena* sp.

Figure 1. Some important microalgal species under microscope. (Source: Raja *et al*., 2010, Hasan *et al.*, 2009)

Thousands of microalgae exist on the earth but a few strains are used in the aquaculture. This is due to the difference in culture techniques. Over 50,000 species of microalgae, with a rich biodiversity exist all over the world. In case of Bangladesh, updated data is not available as we do not have a national microalgae collection and culture centre.

Table 1: Classes, genera and species of major currently named microalgae grown for food in aquaculture, and their main utilization

Class	Genus	Species	Main Utilization
Cyanophyceae	*Arthrospira (Spirulina)*	*platensis, maxima*	LPF
Bacillariophyceae	*Skeletonema*	*costatum, pseudocostatum*	BM, PS
	Phaeodactylum	*tricornutum,*	BM, PS
	Chaetoceros	*calcitrans, gracilis*	BM, PS
Chlorophyceae	*Chlorella*	*minutissima, vulgaris*	LPF
	Dunaliella	*tertiolecta, salina*	LPF, PS
	Nannochloris	*atomus*	BM
Prasinophyceae	*Tetraselmis (Platymonas)*	*suecica, striata, chuii*	LPF, BM, PS
	Pyramimonas	*virginica*	BM
Cryptophyceae	*Rhodomonas*	*salina, baltica, reticulata*	BM, PS
Eustigmatophyceae	*Nannochloropsis*	*Oculata*	LPF
Prymnesiophyceae	*Isochrysis*	*Galbana*	BM, PS
	Pavlova (Monochrysis)	*lutheri, salina*	BM, PS

LPF: Food for live preys of fish larvae

BM: Food for bivalve mollusc larvae

PS: Food for peneid shrimp larvae

(Source: Shields *et al*., 2012)

3.2 Importance of microalgae as fish feed ingredient

The importance of microalgae as fish feed ingredient is increasing day by day because of their appropriate size, high nutritional value, high growth rate, antioxidant property, disease resistance power etc. The main importance of microalgae for aquaculture are linked with nutrition (as single component or as food additive). Various data on chemical composition of algae give the basic information on the nutritive potential of the algae biomass (Brown *et al*., 1997). Microalgae are mainly important for larval nutrition, either for direct consumption of molluscs and peneid shrimp or indirectly as feed for the live prey fed to small fish larvae (Muller-Feuga, 2000). Gross chemical compositions of various microalgae have been determined by various authors in order to reveal their nutritional and feeding value for using them successfully as fish feed ingredient.

3.2.1 Nutritional value of microalgae

Major Nutrient (protein, carbohydrate, lipid)

Several microalgae contains high protein, carbohydrate and lipid and therefor considered as an important fish feed ingredient. According to Roy *et al.*, (2015) there are more protein in diatoms in comparison to chlorophycean members. *Spirulina, Scenedesmus* and *Chlorella* are considered as a source of single cell protein due to 40–70% protein content. In case of, microalgae, nutritional value of protein component is considered to be high if their EAA (essential amino acid) composition is close to the requirement of the feeding animals (Webb and Chu, 1983). Carbohydrates plays a significant role in digestibility of the total algal biomass. Carbohydrates of algae are mainly found in the form of starch, cellulose, sugars and other polysaccharides. Algal lipids are mainly composed of glycerol, sugars or bases, esterified to fatty acids bearing carbon numbers in the range of C12–C22, that may be either saturated or unsaturated.

Table 2. Protein, carbohydrate and lipid contents of few microalgae

Algae	Protein (%)	Lipid (%)	Carbohydrate (%)
Spirulina platensis	50–65	8-14	4-9
Chlorella sp.	51-58	12-17	14-22
Euglena sp.	39-61	14-18	14-20
Anabaena sp.	48-50	25-30	4-7
Pavlova sp.	24-29	6-9	9-14
Spirulina maxima	60-71	13-16	6-7
Spirogyra sp.	6-20	33-64	11-21
Porphyridium cruentum	28-39	9-14	40-57
Dunaliella sp.	49-57	4-32	6-8

(Source: Roy *et al*., 2015)

Amino acid

The amino acid composition of almost all algae compares with that of other food proteins, with minor deficiencies in sulfur containing amino acids methionine and cysteine (Becker, 2004). Various literature are available on amino acid profile of different algae viz. *Spirulina maxima, S. platensis, Scenedesmus obliquus, Chlorella vulgaris, Dunaliella bardawil, D. salina*. *Spirulina platensis* showed almost similar amino acid profile as egg, even better in some cases (Roy *et al*., 2015).

Fatty acid

Among all the microalgal species more than 100 species have been analyzed for their fatty acid composition. These fatty acids are essential components of the diet of humans and animals and also becoming important feed additives in aquaculture. It has been reported that, *S. platensis* serves as a valuable source of linolenic acid and about 20–30% of its fatty acids consist of this compound (Becker, 2004). Cyanobacterial lipids have 25–60% of PUFA (Poly Unsaturated Fatty Acid) and are rich in essential fatty acids such as C: 18 linoleic (18:2) and linolenic (18:3) acids and their C-20 derivatives, eicosapentaenoic acids (20:5) and arachidonic acid (20:4). Pure cultures of green algae contain primarily C: 16, C: 18 fatty acids with a high degree of unsaturation (Roy *et al.*, 2015).

Pigments

At present there are more than 400 known carotenoids but only very few are used commercially. These carotenoids includes β-carotene, astaxanthin lutein, zeaxanthin, lycopene and bixin (Del *et al.,* 2000). Some carotenoids can act as provitamin A and can be converted in the body to vitamin A (Garcia -Gonzalez *et al.*, 2005). Carotenoids is known to have medical applications and also can be used as ingredient for nutritional supplement. *Chlorella* pigments have also been studied to be used as coloring agent and for taste and flavor adjustments in healthy food industry (Gouveia *et al*., 2003). One of the most important characteristic of the algae is their vast range of pigment content like, chlorophylls, carotenoids, phycobiliproteins, xanthophyll's etc.

Vitamins

Microalgae are a good source of different vitamins that are essential in aquaculture. A number of vitamins are present in rather higher concentrations in algae than that of other traditionally considered rich conventional sources (Roy *et al*., 2015).

3.2.2 Microalgae as immunostimulants

In addition to the nutritional benefits, algal supplements have been reported to contain numerous compounds that serve as non-specific immunostimulants, improving the innate defense mechanisms in fish and provide enhanced resistance to pathogens (shah *et al*., 2018). Many microalgal species have been reported to produce anti-microbial compounds that are effective against particular bacterial, viral, and other pathogens (Falaise *et al.*, 2016). The development of genetic engineering technologies for algal species shows the possibility of ‘functionalizing’ the algal feed by engineering the algal cell to produce a novel bioactive, such as a vaccine, growth hormone, or anti-microbial agent.

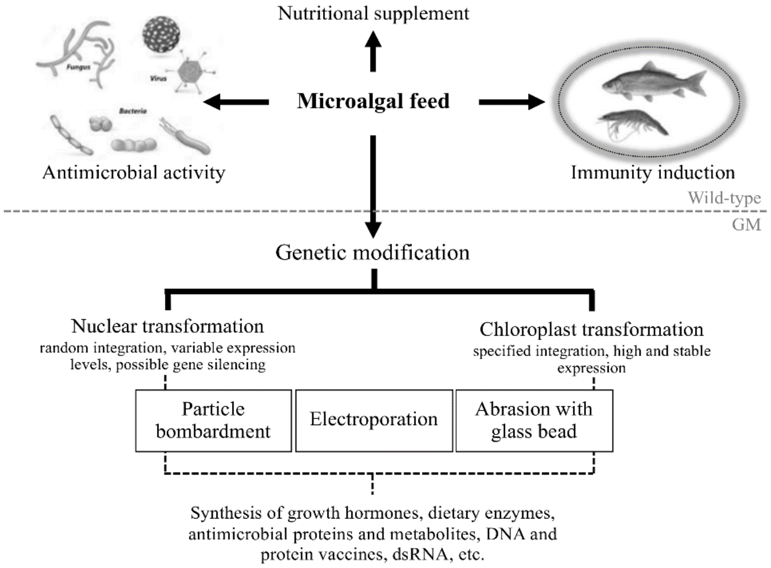


Figure 2. Overview of the current strategies for microalgal exploitation in aquaculture. (Source: Charoonnart *et al.*, 2018)

3.3 Feeding microalgal feed to fish

Microalgal feed has been successfully employed for the mass raising of commercially important fishes like tilapia (*Oreochromis sp*.), common carp (*Cyprinus carpio),* atlantic salmon *(Salmo salar*), shrimp (*Penaeus monodon*), red seabream (*Pagrus major*), gilthead seabream (*Sparus aurata*), juvenile atlantic cod (*Gadus morhua*), rainbow trout (*Oncorhynchus mykiss*) etc.

3.4 Application of microalgal feed in aquaculture

Different algae already plays a vital role in aquaculture. It is known that the addition of microalgae to larval fish culture tanks confers lots of benefits, such as preventing bumping against the walls of the tanks, enhancing predation on zooplankton, improving the nutritional value of zooplankton, as well as improving larval digestive and immune functions. It is already reported that, larvae of some fishes benefit greatly by direct ingestion of microalgae (Reitan *et al*., 1997). One study has shown that, live zooplankton could be eliminated from the larval diet of Red Drum (*Sciaenops ocellatus)* if microalgae were fed along with a formulated micro particulate diet (Lazo *et al.*, 2000). Larval feeds probably deserves the most attention in efforts to discover how algae can best be used in fish feeds.

Table 3. Some studies on applications of microalgae biomass as feed for aquaculture

Microalgal species	Aquaculture species	%Replacement of fish meal/ inclusion level	Effect on fish	Reference
Nannochloropsis sp.	Gilthead seabream	0.5 and 1% inclusion in feed	Enhanced defense activity	Cerezuela *et al.,* (2012)
Nannochloropsis sp. *Isochrysis* sp.	Juvenile Atlantic cod	15% replacement of fish meal protein	Feed intake and growth improved in the fish.	Walker *et al.,*(2011)
Arthrospira sp.	Golden barb	20% replacement of fishmeal in diet	increased growth rates of fish	Hajiahmadian *et al.,* (2012)
Spirulina platensis	Nile tilapia	0.5 to 2% inclusion in feed	improved the health conditions of fish	Ibrahem *et al.,* (2013)
Dunaliella sp.	Pacific white shrimp	1–2% inclusion of microalgal meal	Survival rate of shrimp increased	Medina-Félix (2014)
Arthrospira maxima	Red tilapia fingerling	Up to 30% replacement of fishmeal	No negative impact on growth performance	Rincón *et al.,* (2012)
Pavlova viridis	European sea bass	50–100% replacement of fish oil in diets	No negative effects on the growth	Haas *et al.,* (2016)

3.5 Effect of microalgal feed on growth performance and immune response of fish

Various studies have been conducted to find out the effect of microalgae as a fish feed ingredient for better growth rate and immunity of fish.

3.5.1 Effect of *Spirulina* on growth rate of fish

Abdulrahman *et al.,* (2013) conducted an experiment for 42 days and observed that dried *Spirulina platensis* have potential effect on growth at an optimum concentration of 5 g/kg for common carp (*Cyprinus carpio*). Diets were prepared by adding 1 gm *Spirulina*/kg diet (T2), 3 gm *Spirulina*/kg diet (T3), 5 gm *Spirulina*/kg diet (T4) and with no *Spirulina* in diet (T1). The mean value level in the group received 5 g/kg is higher in all the tested parameters with significant difference indicates the optimum dietary level of *S. platensis* for *C. carpio* is 5 g/kg. In Table 4 it has been shown that, *Spirulina* improved the Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR) to enhance growth performance. The results of the study are in accordance with (Ibrahem *et al.*, 2013) who found that feed supplemented with *S. platensis* powder improved the feed conversion ratio and growth rates in striped jack, (*Pseudocaranx dentex)* and Nile tilapia(*Oreochromis niloticus*).

Table 4. Effect of adding *Spirulina* in some growth parameters for common carp (*Cyprinus carpio)*

Treatments	Weight gain (g)	Daily growth rate (%)	Specific growth rate (% day^{-1})	Relative growth rate (%)	FCR	FER
T1	5.88	0.14	0.124	12.83	2.14	46.99
T2	3.49	0.15	0.127	10.92	1.13	49.92
T3	5.34	0.13	0.119	12.23	1.62	62.48
T4	6.89	0.17	0.147	15.31	1.07	62.47

(Source: Abdulrahman, 2014)

According to Jana *et al.*, (2014), the growth rate of *Pungasius sutchi* feeding with 5% *Spirulina* supplement shows better growth. Highest value for average weight gain in the fishes was observed in feeding with 5% Spirulina which came out to be 84 g and lowest value for average weight gain in the fish was observed in feed without Spirulina was 78.6 g. (Figure 3).

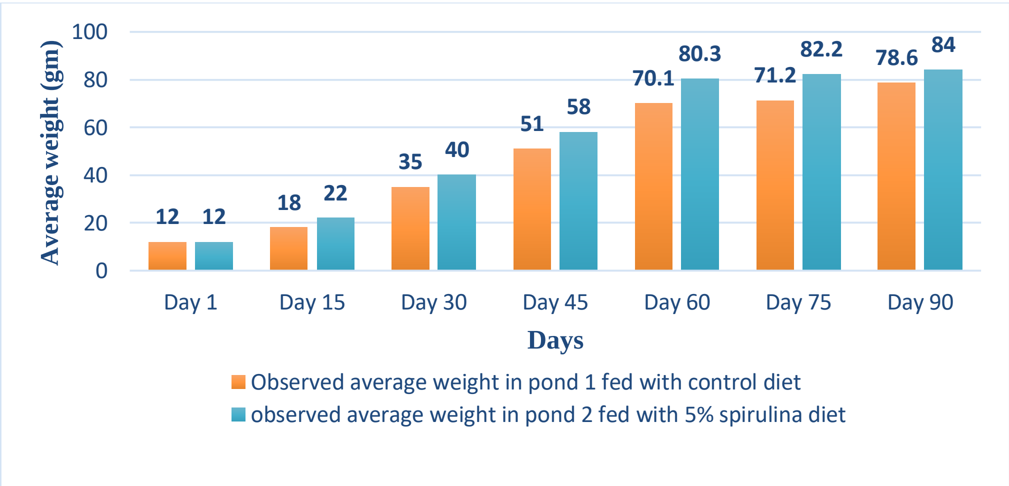


Figure 3. Observed average weight (g) of *Pungasius sutchi* using *Spirulina* supplemented feeds. (Source: Jana *et al.*, 2014)

Highest value for average length in the fishes was observed in feed with 5% Spirulina which came out to be 13.07 cm and lowest value for average length in the fish was observed in feed without *Spirulina* (control) as 12.25 cm. Hence, feed impregnated with 5% Spirulina was found best as it had the highest value for average length in the fishes (Figure 4).

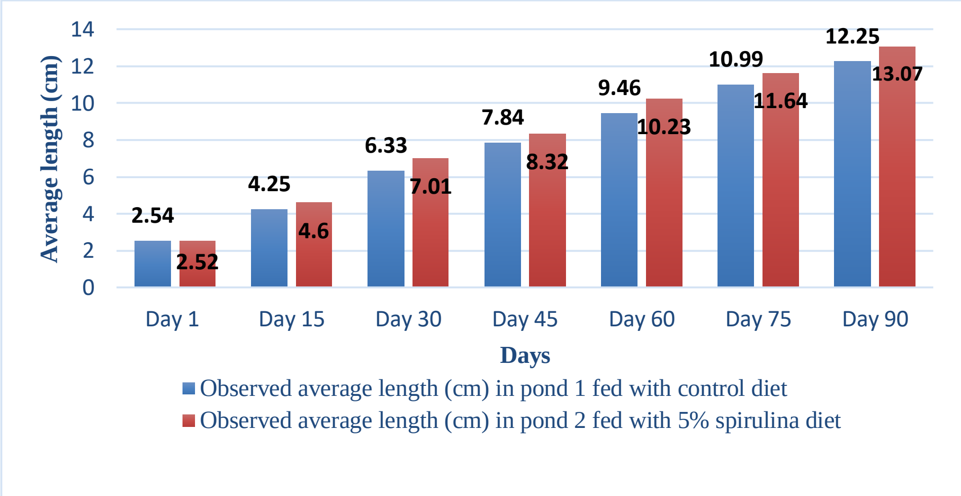


Figure 4. Observed average length (cm) of *Pungasius sutchi* using *Spirulina* supplemented feeds. (Source: Jana *et al*., 2014)

3.5.2 Effect of *Spirulina* supplementation on immune response of fish

Spirulina supplementation surely affect the immune response of fish. Amer, (2016) conducted an experiment and observed that, level of Catalase, Lysozyme and IgG value were found to be higher in *S. Platensis* supplemented groups. He found in case of mono-sex tilapia, the lysozyme is higher with 1.5% *Spirulina* supplementation (7.28 µ/ml) compared to control group (3.94 µ/ml) (Table 5) that indicates better immune response. This finding was according to Ragap *et al*., (2012) who found that *Spirulina* treated tilapia (10mg/fish) recorded highest level of lysozyme compared to control groups (1mg/fish). Lysozyme, detected in the blood, mucus and organs of various fish, plays an important bactericidal role in the nonspecific defense against pathogens primarily through lytic actions on the pathogen cell wall. High lysozyme activity is desirable in cultured fish because it acts against infection when fish are kept at high densities and exposed to high bacterial loads. Thus *Spirulina* increases immune responses by promoting phagocytic and natural killer activities.

Table 5. Effect of supplementation of *S. Platensis* on lysozyme, IgG, IgM values and catalase activity in serum of Nile Tilapia after feeding the experimental diets for 75 days

Parameters	SP0 (control)	SP0.5%	SP1%	SP1.5%
Lysozyme(µ/ml)	3.94 ±0 .04	5.10 ±0 .52	6.60±0 .34	7.28 ±0 .71
IgG(ng/ml)	46.66± 7.37	81.15 ± 15.65	108.47 ± 21.67	132.62 ± 6.84
IgM(µg/ml)	5.53 ±0 .18	5.83 ± 0.80	6.61 ±0 .86	6.77± 1.57
Catalase (µmol/ml)	40.58±11.70	65.93 ± 8.41	68.89± 6.91	48.69 ± 6.91

(Source: Amer, 2016)

Yeganeh *et al*., (2015) conducted an experiment and evaluate the effect of diets containing 0, 2.5, 5, 7.5 and 10% of *Spirulina platensis* on hematological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss).* The RBC, WBC and hemoglobin levels increased significantly in the diets supplemented with *S. platensis.* Since the first use of immunostimulants in aquaculture, so many studies have been conducted to evaluate their ability to enhance the performance and welfare of fish. Some substances such as lipopolysaccharide (LPS), chitin and glucan have been successfully used to improve the fish immune defense. The results of the study showed that hemoglobin and RBC count increased with increasing of dietary *Spirulina* (Table 6). This condition may indicate that dietary *Spirulina* facilitates oxygen transportation capacity and thus improves the wellbeing of fish (Talpur *et al.,* 2012). Higher blood hemoglobin and RBC in fish fed with SP 7.5 and SP 10 diets may be related to presence of iron element in *Spirulina*. RBC can be regarded as an indicator of oxidative status as it is one of the major production sites of free radicals (Babak *et al.,* 2012). Thus, consumption of *S. platensis* which is known as a natural antioxidant, can prevent RBC degeneration. .WBC is well known as one of the first lines of body defense against infectious agents caused by microbial and chemical factors (Talpur *et al.,* 2012).

Table 6. Hematological parameters in rainbow trout fed different levels of *S. platensis* over 10 weeks of experimental period

Parameters	**Control**	**SP 2.5%**	**SP 5%**	**SP 7.5%**	**SP 10%**
RBC ($\times 10^6$ µL)	1.81 ± 0.27	1.75 ± 0.33	1.80± 0.44	2.15 ± 0.36	2.73 ± 0.41
WBC ($\times 10^3$ µL)	7.16 ± 0.59	7.01 ± 0.62	8.63 ± 0.25	8.24 ± 0.39	10.31 ± 0.35
Hematocrit (%)	41.66 ± 2.08	40.44 ± 2.87	40.88 ± 3.97	40.66 ± 1.20	41.11 ± 3.89
Hemoglobin (g/dL)	6.76 ± 0.29	7.07 ± 0.70	6.48 ± 0.44	8.08 ± 0.29	7.96 ± 0.51

(Source: Yeganeh *et al*., 2015)

Yeganeh *et al*., 2015 also found in their study that, plasma cortisol significantly reduced in fish fed on diets containing *Spirulina* (Figure 5). Corticosteroids, principally cortisol, have been shown to increase different acute and chronic stressed conditions, e.g. handling, confinement, poor water quality, temperature shock and pathogen infection (Barton, 2000; Fridell *et al.,* 2007). So Prolonged elevation in concentrations of cortisol can have negative effects on behavior, immune response, growth performance. Plasma cortisol of *Spirulina* treatments was 1.3-2.2 folds lower than the control group and this was combined with lower glucose concentrations. This indicates that fish fed *Spirulina* supplemented diet may have a greater ability to fight against the stress conditions.

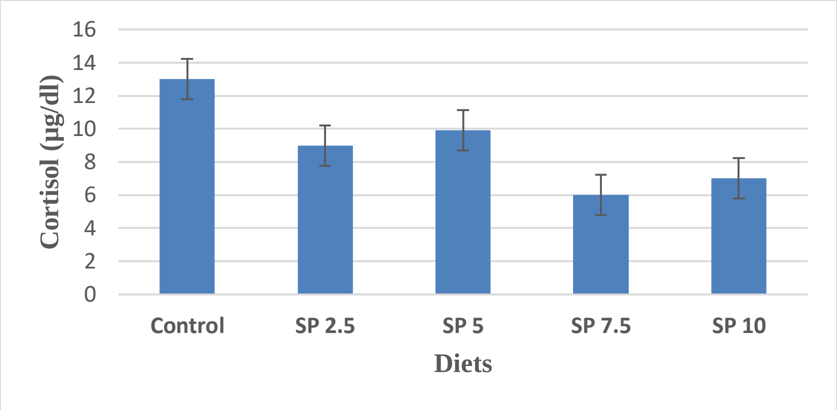


Figure 5. Cortisol concentration of red sea bream fed with *S. platensis* supplemented diets for over 10 weeks. (Source: Yeganeh *et al.*, 2015)

Yeganeh *et al.*, 2015 also found that, there were no significant differences in serum lactate between treated and the control fish (Figure 6). Catabolized amino acids and glycogen are the sources of white muscle lactic acid. When sufficient oxygen is not available for aerobic cells, glycolysis gives rise to elevated lactate production. This may be due to reduced ventilation, circulation, after exhaustive exercise and also in hypoxia. Stable lactate and increased RBC in their study may indicate that both oxygen availability and use were in optimum ranges.

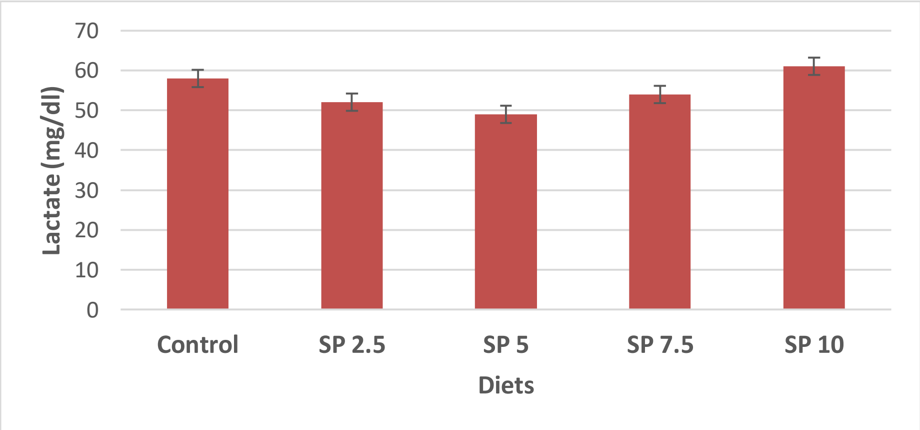


Figure 6. Lactate concentration of red sea bream fed with *S. platensis* supplemented diets for over 10 weeks. (Source: Yeganeh *et al.*, 2015)

3.5.3 Effect of *Chlorella* on growth rate of fish

According to Enyidi (2017), *Chlorella vulgaris* has good-quality protein with amino acids rich in methionine, lysine and alanine. He conducted an experiment with four diets having

C. vulgaris as the main source of protein for African catfish (*Clarias gariepinus)*.The treatment diets included 25% (F1), 15% (F2), 5% (F3) and 0% (F4) of *Chlorella* respectively. After 60 days of experimental period it was observed that, the specific growth rate was found best for the catfish fed with 25% *C. vulgaris* diet 7.86 ± 0% day^{-1} , and worst at 6.77 ± 0.07% day^{-1} for the control group F4 having 0% algal meal.

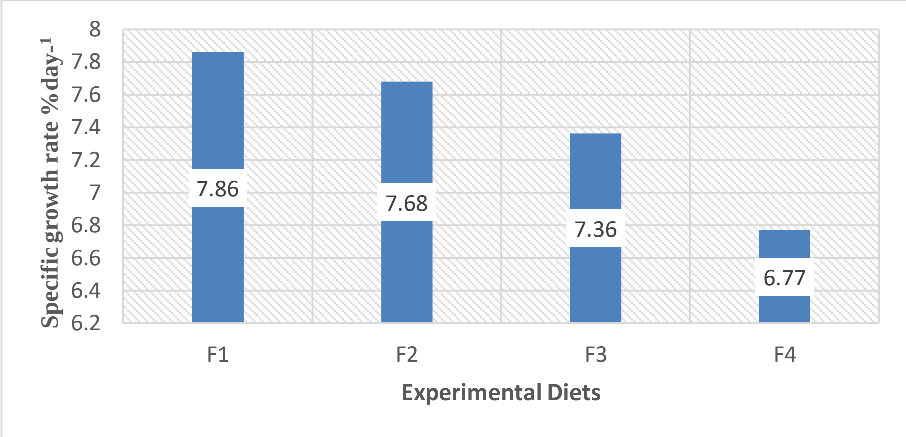


Figure 7. Specific growth rate (SGR) of African catfish *Clarias gariepinus* fed with algae *C. vulgaris* diets. (Source: Enyidi, 2017)

Zhang *et al.*, (2014) conducted an experiment to find out the effect of *Chlorella* on the growth rate and immunity of gibel carp, *Carassius auratus*. Six experimental diets were prepared that contains *Chlorella* levels of 0, 0.4, 0.8, 1.2, 1.6 and 2.0% (C, E1, E2, E3, E4, E5), respectively. After 60 days of experimental period they found the SGR (Specific growth rate) of fish in five groups fed with *Chlorella* were 1.16±0.03, 1.26±0.05, 1.27±0.01, 1.32±0.02 and 1.31±0.01, which were higher than that of control group (1.10±0.01) (Figure:8).

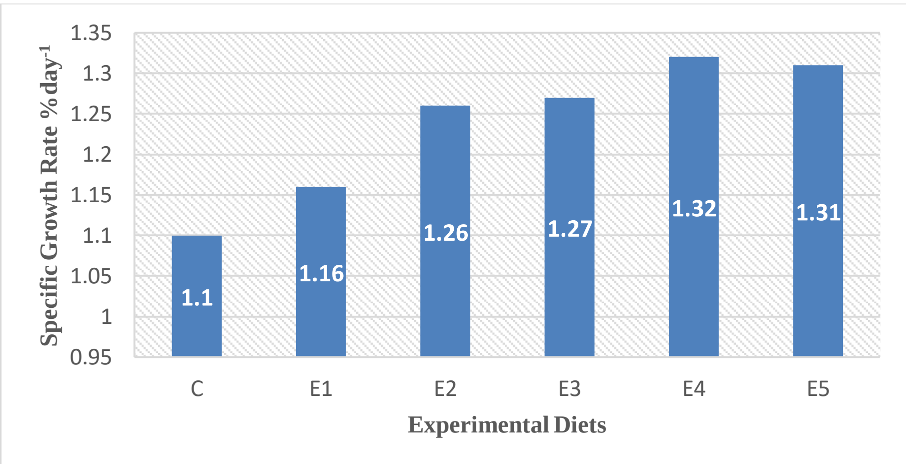


Figure 8. Specific growth rate (SGR) of gibel carp *(Carassius auratus)* fed with algae *Chlorella* diets after 60 days of experimental period (Source: Zhang *et al.*, 2014)

According to Maliwat *et al*., (2017), *Chlorella vulgaris* can act as an important ingredient in the diets of giant freshwater prawn, *Macrobrachium rosenbergii* postlarvae. They prepared test diets using a fishmeal-based-positive control diet (D0) and four basal diets with inclusion levels of 2% (D2), 4% (D4), 6% (D6) and 8% (D8) *C. vulgaris*. Postlarvae of *M. rosenbergii* were randomly stocked (mean initial body weight of 0.19 ± 0.02 g) in 30-L tanks for evaluation of growth performance. Results showed that specific growth rate, survival rate, was significantly higher in postlarvae fed D4 and D6 (Table 7).

Table 7. Growth performance of *Macrobrachium rosenbergii* postlarvae fed diets with increasing levels of *Chlorella vulgaris*

Parameters	**Control (D0)**	**2% (D2)**	**4% (D4)**	**6% (D6)**	**8% (D8)**
Initial weight (g)	0.19 ± 0.04	0.21 ± 0.01	0.19± 0.03	0.20 ± 0.02	0.19 ±0.02
Final weight (g)	0.93 ± 0.06	0.79 ±0.07	1.03 ±0.11	0.92 ± 0.08	0.73 ±0.08
Specific growth rate (%day^{-1})	1.39± 0.10	1.15 ± 0.04	1.47 ± 0.09	1.34 ±0.03	1.16± 0.00
Feed intake (g)	22.85± 4.98	14.45 ± 4.20	19.48±5.86	18.53 ± 2.18	17.29 ±1.84
FER	0.03 ± 0.00	0.04 ±0.01	0.05 ± 0.01	0.04 ± 0.00	0.03 ±0.00
Survival rate %	72.22±4.84	66.67 ± 6.67	65.56 ±11.28	78.89 ± 5.56	65.56 ±5.88

(Source: Maliwat *et al*., 2017)

3.5.4 Effect of *Chlorella* supplementation on immune response of fish

According to Zhang *et al.*, (2014) *Chlorella* could increase the levels of immunoglobulin M and D in some tissues of gibel carp (*Carassius gibelio*) which indicates that dietary *Chlorella* can be involved in regulating adaptive and innate immunity. They examined some immune proteins that reflect the fish immune status by using ELISA (Enzyme-linked immunosorbent assay) method. As shown in Figure 9, IgM level in blood was increased in the group supplemented with 0.4% *Chlorella*. Similarly to the IgM level, the IgD was only increased in the blood in the 0.8% *Chlorella* group (Figure 10). From many studies it has been found that, *Chlorella* could be involved in the regulation of animal adaptive and innate immunity. Cerezuela *et al*., (2012) observed that microalgae could increase the expression of major histocompatibility complex class I- (MHC-I) of gilthead seabream. Zhang *et al.*, (2014) found in his experiment that, the *Chlorella* could significantly increase the serum IgM and IgD levels of gibel carp. Increasing of IgD, one of the immunoglobins involved in mucosal defence Salians *et al.*, (2011) suggested that Chlorella might play some important role in the mucosal immunity.

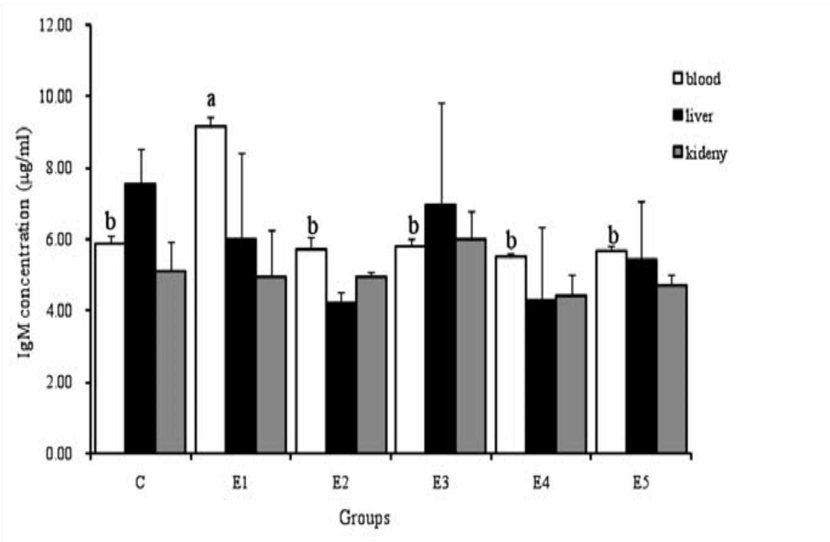


Figure 9. Effect of dietary *Chlorella* on the level of Immunoglobulin M in the blood, liver and kidney of gibel carp. (Source: Zhang *et al.*, 2014)

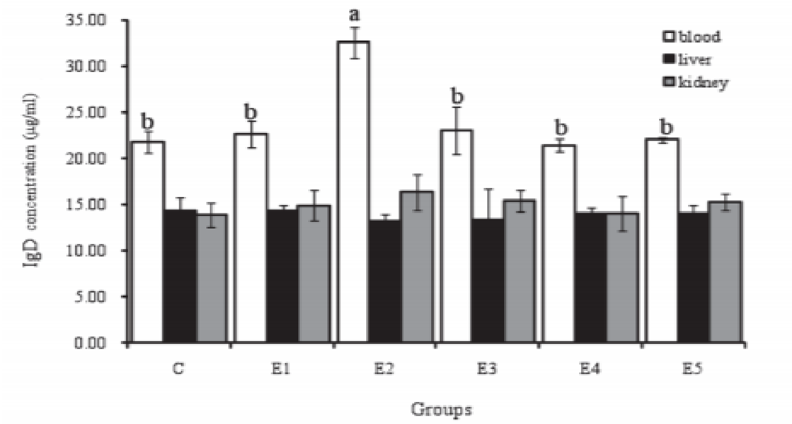


Figure 10. Effect of dietary *Chlorella* on the level of Immunoglobulin D (IgD) in the blood, liver and kidney of gibel carp. (Source: Zhang *et al.*, 2014)

3.5.3 Effect of *Spirogyra* on better growth and immunity of fish

Kumar *et al.*, (2004) conducted an experiment to know the effect of *Spirogyra* sp. incorporated into diet formulations on the growth and body composition of *Catla catla*. The diets were prepared by using spirogyra dry powder in different amounts such as 0% (control), 10%, 25%, 37% and 40%. After 45 days of feeding trial, they found that net and average weight gains are lower in the experimental diet fed groups (Table 8) but it was interesting that the linear increase in specific growth rate with increase in the algal content of the feed. The significant increase in SGR, Average Daily Growth in the experimental groups (37% and 40%) can be attributed to the presence of higher essential amino acids in *Spirogyra*.

Table 8. Feed conversion and morphological parameters in *Catla catla* after feeding with *Spirogyra* enriched feeds for 45 days

Diets	Initial body weight (g)	Final body weight(g)	Net weight gain(g)	Average daily growth(g)	Specific Growth rate %day^{-1}
HK1 (0%)	8.0±1.01	25.0± 0.01	17.0± 3.1	0.37±0.02	2.52±0.03
HK2 (10%)	10.0± 0.98	19.3± 2.01	9.3 ±0.79	0.28±0.01	1.46±0.01
HK3 (25%)	10.0± 0.63	17.0± 1.97	7.0±2.21	0.15±0.01	1.1 ±0.07
HK4 (37%)	10.0± 0.07	21.0 ±1.07	11.0 ±4.71	0.24±0.11	1.64±0.11
HK5 (40%)	8.0± 0.45	18.0 ±1.14	10.0 ±1.0	0.22±0.01	1.82±0.91

(Source: Kumar *et al*., 2004)

3.5.4 Effects of replacing fishmeal with a defatted microalgae meal (*Haematococcus pluvialis*) for better growth and survival of juvenile shrimp

Ju *et al*., (2012) conducted an experiment to evaluate the effect of partial replacement of fishmeal protein by a defatted microalgae meal (DMM) in a diet of *Litopenaeus vannamei*. The DMM was a by-product of astaxanthin produced from *Haematococcus pluvialis.* The test diets were prepared by using DMM (3, 6, 9 and 12% in a diet) to replace 12.5%, 25%, 37.5% and 50% of a fishmeal protein in a control diet. After an 8-week feeding trial, Shrimp fed the DMM-3% *Haematococcus pluvialis* showed a significantly higher growth rate (1.25 g/wk.) than that (1.11 g/wk.) of the shrimp fed the DMM-0% (Table 9).

Table 9. Effects of replacing fishmeal with a defatted microalgae meal in the control diet on growth and survival of juvenile shrimp, *Litopenaeus vannamei*

Diets	Initial weight (g)	Final weight(g)	Survival rate %	FCR	PER	PWG %	SGR %day^{-1}
DMM 0%	1.12	10.04	91.7	2.28	1.36	796.4	3.91
DMM 3%	1.06	11.06	95.8	2.13	1.40	943.4	4.11
DMM 6%	1.09	10.12	97.9	2.21	1.44	828.4	3.93
DMM 9%	1.10	10.88	87.5	2.27	1.39	889.1	4.07
DMM 12 %	1.08	10.28	95.8	2.22	1.44	851.9	3.96
Commercial feed	1.08	7.03	100	2.63	0.95	550.9	3.18

(Source: Ju *et al*., 2012)

Chapter IV
CONCLUSIONS

- Microalgal biotechnology has begun to develop in the middle of the last century but it has numerous commercial applications. Microalgae plays a vital role in aquatic food chain and are popularly used in rearing of aquatic animals like mollusks, shrimps and fishes at different growth stages. They can be used to enhance the nutritional value and immunity of fish feed owing to their chemical composition thus they play a crucial role in aquaculture. However, the collection, drying and pelletization of algae need considerable time and effort. Cultivation costs would have to be taken into consideration. Therefore, further cost-benefit on-farm trials that take these costs into consideration are needed before any definite conclusions on the future application of algae as fish feed can be drawn.

- Nevertheless, the results of many research studies shows that, algae as dietary additives contribute to an increase in growth and immunity of cultured fish due to effective assimilation of dietary protein, betterment in physiological activity, stress response, starvation tolerance, disease resistance and carcass quality. Moderate growth responses and better immune response were generally recorded when dried algal meal were used as a partial replacement of fishmeal protein. Only about 10-15% of dietary protein requirement can be met by algae in test diets without compromising growth and immunity. There is a decrease in fish performance when dietary incorporation of algal meal rises above 15-20%. Total replacement of fishmeal by algal meal commonly shows very poor growth responses. Apart from commonly observed impaired growth, the use of algae as the single source of protein in fish feed can also result in malformation. Further, this microalgal feed reduced mortality in the critical larval stages and enhances their survival. Hence microalgal feed of two microalgae *Spirulina* sp. and *chlorella* sp. are highly recommended as fish feed for better growth and immunity of fish.

REFERENCES

Abdulrahman, N. M. (2014). Evaluation of *Spirulina* spp. as food supplement and its effect on growth performance of common carp fingerlings. *International Journal of Fisheries and Aquatic Studies*, *2*(2), 89-92.

Amer, S. A. (2016). Effect of *Spirulina platensis* as feed supplement on growth performance, immune response and antioxidant status of mono-sex Nile Tilapia (*Oreochromis niloticus)*. *Benha Veterinary Medical Journal*, *30*(1), 1-10.

Babak, N., Reza, I.M., Ali, S. (2012). Effects of *Rheum rebis* Extract on the Blood Parameters and Responses of Rutilus frisii kutum under Heat Stress. *Global Veterinaria* 8(3), 222-228.

Barton, B.A. (2000). Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. *North American Journal of Aquaculture* (62), 12-18.

Becker, W. (2004). 18 microalgae in human and animal nutrition. In *Handbook of microalgal culture: biotechnology and applied phycology* (Vol. 312). Wiley Online Library.

Brown, M. R., Jeffrey, S. W., Volkman, J. K., & Dunstan, G. A. (1997). Nutritional properties of microalgae for mariculture. *Aquaculture*, *151*(1-4), 315-331.

Cerezuela, R., Guardiola, F.A., Meseguer, J., Esteban, M.Á. (2012). Enrichment of gilthead seabream (*Sparus aurata L.*) diet with microalgae: effects on the immune system. Fish Physiol. Biochem. 38:1729- 1739.

Charoonnart, P., Purton, S., & Saksmerprome, V. (2018). Applications of microalgal biotechnology for disease control in aquaculture. *Biology*, *7*(2), 24.

Del Campo, J. A., Moreno, J., Rodríguez, H., Vargas, M. A., Rivas, J., & Guerrero, M. G. (2000). Carotenoid content of chlorophycean microalgae: factors determining lutein accumulation in *Muriellopsis sp.* (Chlorophyta). *Journal of Biotechnology*, *76*(1), 51-59.

DoF. (2018). *Yearbook of Fisheries Statistics of Bangladesh*, 2017-18. Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh: Ministry of Fisheries, 2018. Volume 35: p. 129.

El-Hindawy, M. M., Abd-Razic, M. A., Gaber, H. A., & Zenhom, M. M. (2006). Effect of various level of dietary algae *Scenedesmus* sp. on physiological performance and digestibility of Nile tilapia fingerlings. In *1st Scientific Conference of the Egyptian Aquaculture Society*. *Sharm El-Sheikh–Sinai*, *Egypt* (pp. 137-149).

Enyidi, U. D. (2017). *Chlorella vulgaris* as protein source in the diets of African catfish *Clarias gariepinus*. *Fishes*, *2*(4), 17.

Falaise, C., François, C., Travers, M. A., Morga, B., Haure, J., Tremblay, R., & Leignel, V. (2016). Antimicrobial compounds from eukaryotic microalgae against human pathogens and diseases in aquaculture. *Marine drugs*, *14*(9), 159.

Falkowski, P. G., & Raven, J. A. (2007). Photosynthesis and primary production in nature. *Aquatic photosynthesis 2nd Ed Princeton University Press, Princeton*.

Food and Agriculture Organization of the United Nations. (2018). The State of World Fisheries and Aquaculture 2018–Meeting the sustainable development goals. *FAO*.

Fridell, F., Gadan, K., Sundh, H., Taranger, G., Glette, J., Olsen, R., Sundell, K., Evensen, Ø. (2007). Effect of hyper oxygenation and low water flow on the primary stress response and susceptibility of Atlantic salmon *Salmo salar L.* to experimental challenge with IPN virus. Aquaculture 270, 23-35.

García-González, M., Moreno, J., Manzano, J. C., Florencio, F. J., & Guerrero, M. G. (2005). Production of *Dunaliella salina* biomass rich in 9-cis-β-carotene and lutein in a closed tubular photo bioreactor. *Journal of biotechnology*, *115*(1), 81-90.

Gouveia, L., & Empis, J. (2003). Relative stabilities of microalgal carotenoids in microalgal extracts, Technologies, *4*(2), 227-233.

Haas, S., Bauer, J. L., Adakli, A., Meyer, S., Lippemeier, S., Schwarz, K., & Schulz, C. (2016). Marine microalgae *Pavlova viridis* and *Nannochloropsis* sp. as n-3 PUFA source in diets for juvenile European sea bass (*Dicentrarchus labrax L.). Journal of applied phycology*, *28*(2), 1011-1021.

Hajiahmadian, M., Vajargah, M. F., Farsani, H. G., & Chorchi, M. M. (2012). Effect of *Spirulina platensis* meal as feed additive on growth performance and survival rate in golden barb fish, *Punius gelius* (Hamilton, 1822). *Journal of Fisheries International*, *7*(3-6), 61-64.

Hasan, M. R., & Rina, C. (2009). *Use of algae and aquatic macrophytes as feed in small-scale aquaculture: a review* (No. 531). Food and Agriculture Organization of the United Nations (FAO).

Hemaiswarya, S., Raja, R., Kumar, R. R., Ganesan, V., & Anbazhagan, C. (2011). Microalgae: a sustainable feed source for aquaculture. *World Journal of Microbiology and Biotechnology*, *27*(8), 1737-1746.

Ibrahem, M. D., Mohamed, M. F., & Ibrahim, M. A. (2013). The role of *Spirulina platensis (Arthrospira platensis*) in growth and immunity of Nile tilapia (*Oreochromis niloticus*) and its resistance to bacterial infection.

James, I., Yoon, L. W., & Chow, Y. H. (2019, August). Effect of phosphorus-limited nutrients on growth and glucose production from microalgae. In *AIP Conference Proceedings* (Vol. 2137, No. 1, p. 020005). AIP Publishing LLC.

Jana, A., Saroch, J. D., & Borana, K. (2014). Effect of *Spirulina* as a feed supplement on survival and growth of *Pungasius sutchi*. International Journal of Fisheries and Aquatic Studies, 1(5), 77-79.

Ju, Z. Y., Deng, D. F., & Dominy, W. (2012). A defatted microalgae (*Haematococcus pluvialis*) meal as a protein ingredient to partially replace fishmeal in diets of Pacific white shrimp (*Litopenaeus vannamei*, Boone, 1931). *Aquaculture*, *354*, 50-55.

Kumar, M. H., Gajaria, S. C., & Radha, K. S. (2004). Growth and development of catla *(Catla catla*) fed with different levels of diet containing *Spirogyra* sp. *Bioresource technology*, *95*(1), 73-76.

Kwak, J. H., Baek, S. H., Woo, Y., Han, J. K., Kim, B. G., Kim, O. Y., & Lee, J. H. (2012). Beneficial immunostimulatory effect of short-term *Chlorella* supplementation: enhancement of Natural Killer cell activity and early inflammatory response (Randomized, double-blinded, placebo-controlled trial). *Nutrition journal*, *11*(1), 53.

Lazo, J. P., Dinis, M. T., Holt, G. J., Faulk, C., & Arnold, C. R. (2000). Co-feeding micro particulate diets with algae: toward eliminating the need of zooplankton at first feeding in larval red drum (*Sciaenops ocellatus*). *Aquaculture*, *188*(3-4), 339-351.

Maliwat, G. C., Velasquez, S., Robil, J. L., Chan, M., Traifalgar, R. F., Tayamen, M., & Ragaza, J. A. (2017). Growth and immune response of giant freshwater prawn *Macrobrachium rosenbergii* postlarvae fed diets containing *Chlorella vulgaris* (Beijerinck). *Aquaculture Research*, *48*(4), 1666-1676.

Medina-Félix, D., López-Elías, J. A., Martínez-Córdova, L. R., López-Torres, M. A., Hernández-López, J., Rivas-Vega, M. E., & Mendoza-Cano, F. (2014). Evaluation of the productive and physiological responses of *Litopenaeus vannamei* infected with WSSV and fed diets enriched with *Dunaliella* sp. *Journal of invertebrate pathology*, *117*, 9-12.

Muller-Feuga, A. (2000). The role of microalgae in aquaculture: situation and trends. *Journal of applied phycology*, *12*(3-5), 527-534.

Ragap, H. M., Khalil, R. H., & Mutawie, H. H. (2012). Immunostimulants effects of dietary *Spirulina platensis* on tilapia *Oreochromis niloticus*. *Journal of Applied Pharmaceutical Science*, *2*(2), 26.

Raja, R., & Hemaiswarya, S. (2010). Microalgae and immune potential. In *Dietary Components and Immune Function* (pp. 515-527). Humana Press, Totowa, NJ.

Reitan, K. I., Rainuzzo, J. R., Øie, G., & Olsen, Y. (1997). A review of the nutritional effects of algae in marine fish larvae. *Aquaculture*, *155*(1-4), 207-221.

Rincón, D. D., Velásquez, H. A., Dávila, M. J., Semprun, A. M., Morales, E. D., & Hernández, J. L. (2012). Substitution levels of fish meal by *Spirulina maxima* meal in experimental diets for red tilapia fingerlings (*Oreochromis* sp.). *Revista Colombiana de Ciencias Pecuarias*, *25*(3), 430-437.

Roy, S. S., & Pal, R. (2015, June). Microalgae in aquaculture: a review with special references to nutritional value and fish dietetics. In *Proceedings of the Zoological Society* (Vol. 68, No. 1, pp. 1-8).

Salians, I., Zhang, Y.A., Sunyer, J.O., (2011). Mucosal immunoglobulins and B cells of teleost fish. Dev. Comp. Immunol. 35:1346- 1365.

Sarker, P. K., Kapuscinski, A. R., Lanois, A. J., Livesey, E. D., Bernhard, K. P., & Coley, M. L. (2016). Towards sustainable aquafeeds: complete substitution of fish oil with marine microalga *Schizochytrium* sp. improves growth and fatty acid deposition in juvenile Nile tilapia (*Oreochromis niloticus*). *PloS one*, *11*(6), e0156684.

Singh, P. K., Gaur, S. R., & Chari, M. S. (2006). Growth performance of *Labeo rohita* (Ham.) fed on diet containing different levels of slaughter house waste. *J. Fish. Aquat. Sci*, *1*(1), 10-16.

Shah, M. R., Lutzu, G. A., Alam, A., Sarker, P., Chowdhury, M. K., Parsaeimehr, A. & Daroch, M. (2018). Microalgae in aquafeeds for a sustainable aquaculture industry. *Journal of applied phycology*, *30*(1), 197-213.

Shields, R., & Lupatsch, I. (2012). Algae for aquaculture and animal feeds. *TATuP-Zeitschrift für Technikfolgenabschätzung in Theorie und Praxis*, *21*(1), 23-37.

Talpur, A.D., Ikhwanuddin, M., 2012. Dietary effects of garlic *(Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection in Asian sea bass, *Lates calcarifer* (Bloch). Aquaculture 364, 6-12.

Walker, A. B., & Berlinsky, D. L. (2011). Effects of partial replacement of fish meal protein by microalgae on growth, feed intake, and body composition of Atlantic cod. *North American Journal of Aquaculture*, *73*(1), 76-83.

Webb, K. L. (1983). Phytoplankton as a food source for bivalve larvae. In *Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish* (Vol. 2, pp. 272-291).

Yeganeh, S., Teimouri, M., & Amirkolaie, A. K. (2015). Dietary effects of *Spirulina platensis* on hematological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). *Research in Veterinary Science*, *101*, 84-88.

Yoshimatsu, T., & Hossain, M. A. (2014). Recent advances in the high-density rotifer culture in Japan. *Aquaculture International*, *22*(5), 1587-1603.

Zhang, Q., Qiu, M., Xu, W., GAO, Z., Shao, R., & Qi, Z. (2014). Effects of dietary administration of *Chlorella* on the immune status of gibel carp, *Carassius auratus gibelio*. *Italian Journal of Animal Science*, *13*(3), 3168.