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on

Use of *Spirulina* in Fish Culture

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Use of *Spirulina* in Fish Culture[1]

By

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ABSTRACT

The use of blue green algae *Spirulina* in aquaculture has several potential advantages over the culture of fish. This seminar is to review the effect of using *Spirulina* in culture of different fishes as a replacement and nutrient supplement and its potentiality of using as an alternative source of protein in fish feed. *Spirulina* has high quality protein content (58%), which is more than other commonly used plant sources. So it can be used as an alternative protein source in fish feed. Generally *Spirulina* is used in fish feed as a replacement of fish meal and a nutritional supplement. In case of Common carp (*Cyprinus carpio*) replacing fishmeal up to 10% with *Spirulina* showed doubled weight gain up to 16.59 gm compared to the control group 8.37gm. The growth rate was found higher when *Pungasius sutchi* feeding with 5% *Spirulina* supplement. The growth performance and the mean survival rate higher (100%) than the control group (80%) when Tilapia (*Oreochromis niloticus*) was fed with 5% dietary *Spirulina platensis.* Inclusion of 10% *S. platensis* as a natural pigment source resulted in the highest carotenoid deposition (1.2mg/l) which resulted better coloration in Rainbow trout. Using *Spirulina* in fish diet improves the haematoloical parameters and immune response and makes the cultured fish healthy and disease resistant. Only 5% inclusion of *S. platenssis* in the diet of Tilapia (*O. niloticus*) improved the haematological parameters where the Erythrocyte count (RBCs), Haemoglobin (Hb), Haematocrit (PCV), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Leucocyte count (WBCs) were significantly increased. Replacement of fishmeal with 100% *Spirulina* also reduced the cost of feed by reducing the incidence cost (46.21tk) compared to the control groups (83.21 tk) with 0% *Spirulina*. And all these findings prove use of *Spirulina* as a potential nutritional supplement and a better alternative source of protein in fish feed with lots of beneficial effects.

Keywords: *Spirulina*, Growth promoter, Haematological parameters, Immune response, Carotenoid, Antioxidant properties.

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CHAPTER 1

INTRODUCTION

Spirulina is a filamentous and multicellular blue-green microalgae which belongs to two separate genera *Spirulina* and *Arthrospira* and consists of about 15 species. Of these, *Arthrospira platensis* is the most common and widely available *Spirulina* and most of the published research and public health decision refers to this specific species. *Spirulina* is an available microalgae found all over the world. It is considered one of the most concentrated natural sources for nutrition to both terrestrial and aquatic animals. *Spirulina* contains high protein contents (up to 70% in dry weight) and lipids (7–16%) (Vonshak, 1997). It is receiving increasing attention for its bioactive components such as vitamins (especially vitamin A and B12), minerals, polyunsaturated fatty acids, carotenes and other pigments that have antioxidants activity (Reddy *et al*., 2000). Therefore, *Spirulina* could be an excellent source of useful nutrients (Glombitza and Koh, 1989) as well as a good energy source that can be used as crucial component for animal feeding (Kim *et al*., 2013). According to Qureshi *et al*., 1996 *Spirulina* is suitable for animal feeding and also as supplement nutrients for humans.

Spirulina can also be cultured easily and cost effectively using low cost culture medium. It grows in water, can be harvested and processed easily and has significantly high macro- and micronutrient contents. It has been commercially cultivated for more than 10 years in different countries of the world due to its high nutritional content; e.g. protein, amino acid, vitamin, minerals, essential fatty acid and β- carotene (Vonshak, 1997)

Fish is playing an important dietary animal protein source for human nutrition. According to nutritionists, fish is an excellent substitute of protein for red meat, which contains all the essential amino acid (methionine, cystine, lysine, threonine, tryptophan, arginine, isoleucine, valine, hitidine, phenylalanine etc.) and minerals *viz*., iodine, phosphorus, potassium, iron, copper and vitamin A and D in desirable concentrations (Sandhu, 2005). It serves as valuable source of protein to a healthy diet because of its low carbohydrate and unsaturated fat, especially Omega 3 contents (Razvi, 2006). Bangladesh is one of the world's leading fish producing countries with a total production of 41.34lakh MT, where aquaculture contributes 56.44 % to total production, inland open water contributed 28%, and marine water contributed 16% (DoF, 2011). According to FAO statistics 2017, Bangladesh is ranked 4th in world aquaculture production. 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 by almost 12 times, at an average annual rate of 8.8 % (Yoshimatsu, T. and Hossain, M.A., 2013). 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). Fish meal is regarded as the best dietary protein source because it is very palatable and provides an excellent balance of essential amino acids and essential fatty acids as well as highly digestible energy (Tacon, 1993). The rapid growth of aquaculture has resulted in higher demand for FM and consequently its high price is expected to be further increased by continuous growth in its requirement (Hardy and Tacon, 2002). Furthermore, sustainability of FM production from wild fish is questionable (Naylor *et al*., 2000). To reach a sustainable aquaculture, new alternative protein sources including cheaper plant or animal origin proteins are needed to be introduced for stable aqua feed production (Higgs *et al*., 1995). And it has been found that the alga can be used as an alternative source of protein and can also be used to improve the color, flavor and quality of meat (Al-Badri, 2010). *Spirulina* is one of the most frequently used microalgae in aquatic animal feeds due to its high contents of protein, vitamins, essential amino acids, minerals, essential fatty acids and antioxidant pigments such as carotenoids (Nakagawa and Montgomery, 2007).

In recent years, food security is becoming serious issue with rapidly increasing world population. The global population has increasing and, in order to maintain current level of per capita consumption of aquatic foods, the world will require an additional 23 million tons by 2020. This additional supply will have to come from aquaculture (FAO, 2012). Conventional agriculture has not the ability to supply enough food therefore, new alternative and unconventional food sources have to be searched to feed this much crowded world. Increasing practice of aquaculture can be a solution for producing more fish meet up the protein requirement of this large population of the world. For that we need to produce more fish with less feed cost. It is suggested that the increased use of plant protein in fish diets can reduce the cost of FM and feeds (Lim and Lee, 2009). And this why use of dietary *Spirulina* in fish feed can be a good solution.

Spirulina has been used as a complementary dietary ingredient of feed for fish, shrimp and poultry, and increasingly as a protein and vitamin supplement to aqua feeds. China is using this micro-alga as a partial substitute of imported forage to promote the growth, immunity and viability of shrimp. In Bangladesh it is not used popularly in the fish farms and this is why it is not produced commercially as a fish feed ingredient. But *Spirulina* appears to have considerable potential for development, especially as a small-scale crop for nutritional enhancement, livelihood development and environmental mitigation. FAO fisheries statistics has given hint at the growing importance of this product. *Spirulina* production in China was first recorded at 19 080 tons in 2003 and rose sharply to 41 570 tonnes in 2004, worth around US$7.6 million and US$16.6 million, respectively. However, there are no apparent figures for production in the rest of the world. This suggests that despite the widespread publicity about *Spirulina* and its benefits, it has not yet received the serious consideration. So this algae should be used efficiently and commercial production of *Spirulina* should be started fish a better nutrition and an alternative protein source for fish culture. This will help Bangladesh to achieve self-sufficiency in fish production and meet up the demand of animal protein.

1.1Objective

The study has undertaken to accomplish the following objectives

- To review the utilization of *Spirulina* as an alternative source of protein for fish feed
- To review the advantageous effects of *Spirulina* in fish culture.

CHAPTER 2

MATERIALS AND METHODS

This seminar paper is exclusively a review paper so all of the information has been collected from the secondary sources. During the preparation of the review paper, I went through various relevant books, journals, proceedings, reports, publications, internet etc. Findings related to my topic have been reviewed with the help of the library facilities of Bangabandhu Sheikh Mujibur Rahman Agricultural University. I got suggestion and valuable 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 3

REVIEW OF FINDINGS

3.1 *Spirulina*: Use and effect in fish culture

3.1.1 As a replacement of fish meal

S. platensis can be used as a replacement of fishmeal in fish diets for its high protein content and no negative impacts. Abdulrahman *et al*. (2014) found better growth and survival rate by replacing fish meal with *Spirulina* in case of common carp (*Cyprinus carpio*). In that experiment 5 experimental diets were used and fishmeal protein was replaced by *Spirulina* from the standard diet at 0 (T1), 5 (T2), 10 (T3), 15 (T4) and 20% (T5) levels (Table 3.1). And 10% replacement of fishmeal with *Spirulina* showed a significant higher body weight as compared with the control ones and other replacements. Nandeesha *et al*. (2001) found that fishmeal can replace upto 25% with *Spirulina* and resulted better growth in case of *Labeo rohita*.

Table 3.1 Effect of replacing fishmeal with *Spirulina* on carp (*Cyprinus carpio*) weight gain and survival rates

Treatments	Initials weight (gm)	Weight gain (gm)	Survival (%)
T1	37	8.375^{b}	64.286^{b}
T2	37.25	12.663^{ab}	92.857^{a}
T3	34	16.593^{a}	85.714^{a}
T4	36.25	13.000^{a}	92.857^{a}
T5	37.25	15.033^{a}	78.571^{ab}

(Source: Abdulrahman *et al*., 2014)

3.1.2 As a growth promoter

Abdulrahman *et al*, (2014) observed that dried *S. platensis* found to be of potential effects on growth at an optimum concentration of 5 g/kg for common carp. 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 for studying period. *Spirulina* improved the Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR) (Table 3.2) to enhance growth performance. Duncan and Klesius (1996) reported that *Spirulina* contains high amounts of vitamins and minerals and it is a good source of protein for animal feed. The results of the current 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 (*O. niloticus*).

Table 3.2 Effect of adding *Spirulina* in some growth parameters for common carp (C. *Cyprinus carpio*) reared from 42 days

Treatments	Weight gain	Daily GR	Specific GR	Relative GR	FCR	FER
T1	5.88 b	0.14 a	0.124 b	12.83 b	2.14 a	46.99 b
T2	3.49 c	0.15 a	0.127 b	10.92 c	1.13 b	49.92 b
T3	5.34 b	0.13 a	0.119 bc	12.23 b	1.62 b	62.48 a
T4	6.89 a	0.17 a	0.147 a	15.31 a	1.07 bc	62.47 a

(Source: Abdulrahman, 2014)

Jana *et al*. (2014) observed the growth *Pungasius sutchi* feeding with 5% *Spirulina* supplement. Highest value for average weight gain in the fishes was observed in feed impregnated with 5% *Spirulina* which came out to be 60.4 g and lowest value for average weight gain in the fish was observed in feed without *Spirulina* was 57.7 g. (Fig.3.1).

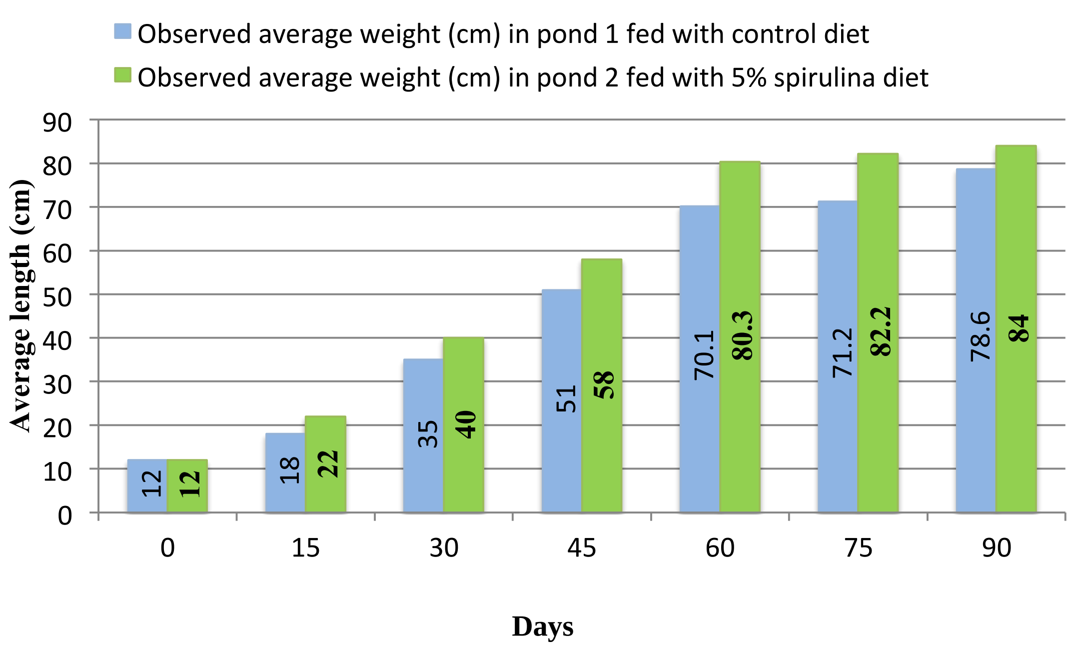

Figure 3.1: Observed average weight (g) of *Pangasius sutchi* using different feeds. (Source: Jana A. *et al*., 2014)

Highest value for average length in the fishes was observed in feed impregnated 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* as 12.26 cm. Hence, feed impregnated with 5% *Spirulina* was found best as it had the highest value for average length in the fishes (Figure 3.2).

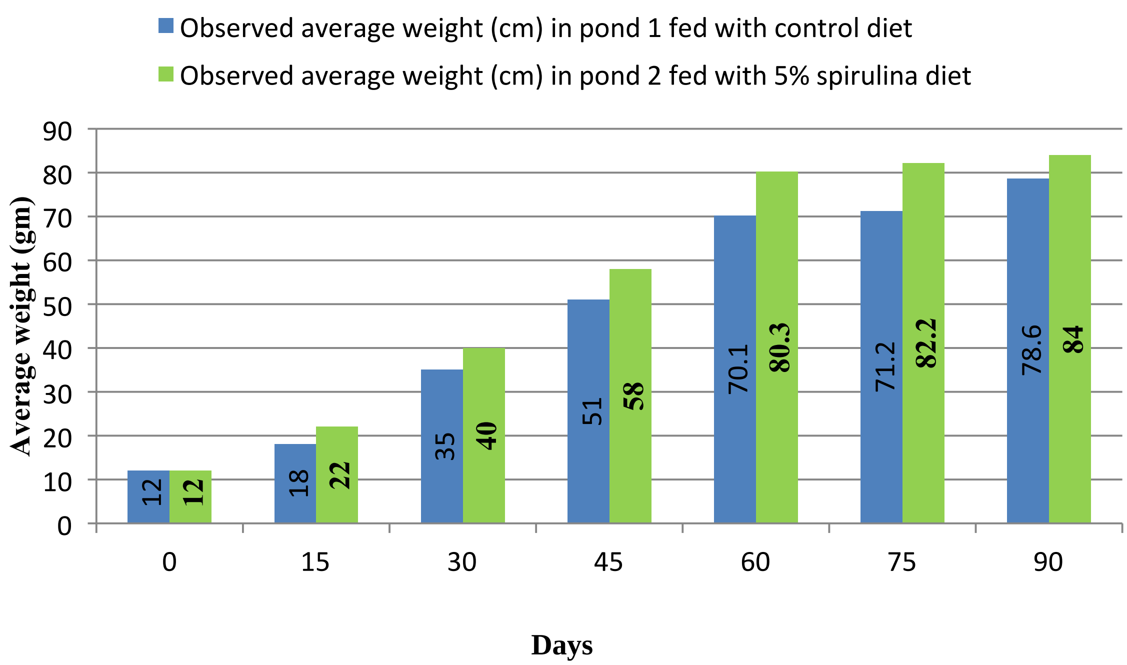

Figure 3.2: Observed average length (cm) of *Pangasius sutchi* using different feeds. (Source: Jana A. *et al.*, 2014)

The present study proved that dietary *Spirulina* supplementation has inceased fish growth. In this study, it was observed that length, weight gain, and survival of *Pangasius sutchi* was significantly best with the addition in *Spirulina* content in the feed. These results came because of the improvement in feed intake and nutrient digestibility. Moreover, *Spirulina* contains several nutrients especially vitamins and minerals that may help in fish growth promotion. These results agree with those found by several researchers (Belay *et al*., 1996 and Hirahashi *et al*., 2002) who reported that feeding *Spirulina* to fish improved survival and growth rates.

Amer (2016) found that mono-sex tilapia supplemented with 1% *S. Platensis* have higher body weight and lower FCR than the control group and other groups. On the other hand Moe (2011) also observed that of only 5% *S. platensis* inclusion of *Spirulina* in the diet of *Oreocromis niloticus* improved the growth significantly (Figure 3.3). On the other hand addition of 5% *S. platensis* in the diet also showed the best growth performance and survival rate in case of *Anabas testudineus*. The improved growth and feed utilization, on supplementation with live *Spirulina* had resulted because of the improved feed intake of fish and nutrient digestibility.

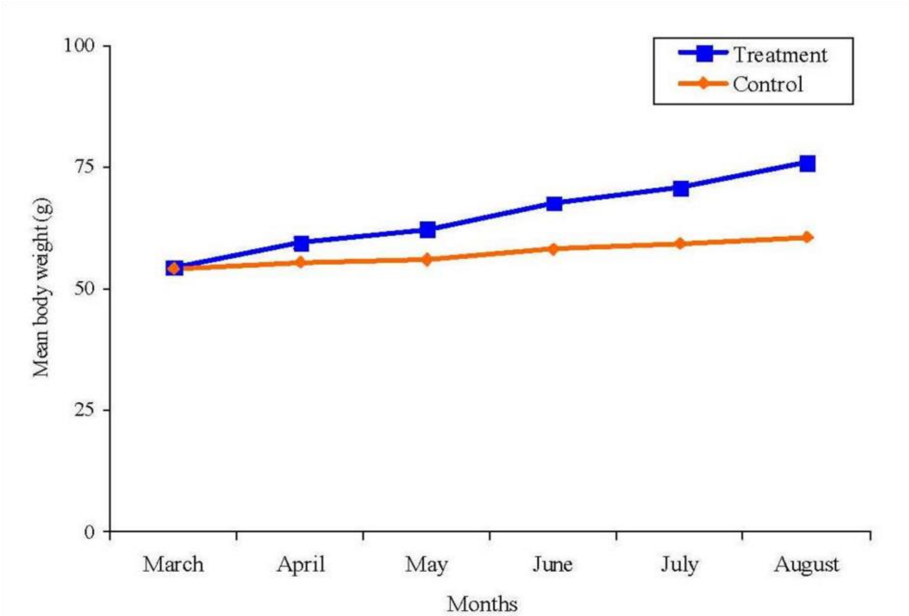

Figure. 3.3 Comparison of monthly mean body weight for treatment (*S. platensis*) and control in *O. niloticus.* (Source: Moe, 2011)

3.1.3 For increasing survival of fish

Moe (2011) found that growth performance and the mean survival rate higher (100%) than that of the control group (80%) Tilapia (*O. niloticus*) was fed with 5% dietary *S. platensis* (Table 3.3). Jana *et al*. (2014) also found 94% and 80% survival rate in the feed impregnated with *Spirulina* at all the 5% age levels and without *Spirulina*, respectively at the termination of the experiment. (Figure 3.4). So *Spirulina* improved survibility rate of fish.

Table 3.3 Growth performance of *O. niloticus* fed *S. platensis* supplementary and control diets

Month	*S. platensis* supplementary diet				Control diet			
	BW (g)	WG (g)	AGR %	Survival %	BW (g)	WG (g)	AGR %	Survival %
March	54.38			100	54.09			100
April	59.43	5.05	17.41	100	55.38	1.29	4.45	100
May	62.25	2.82	9.72	100	56.00	0.62	2.14	100
June	67.59	5.34	18.41	100	58.14	2.14	7.38	90
July	70.76	3.17	10.93	100	59.29	1.15	3.97	80
August	75.95	5.19	17.90	100	60.60	1.31	4.52	80
Mean	65.06	3.60	12.40*	100	57.25	1.09	3.74*	91.66
After the experiment	75.95	21.58	12.40	100	60.60	6.51	3.74	80

BW = Body weight, WG = weight Gain, AGR = Absolute Growth Rate

(Source: Moe, 2011)

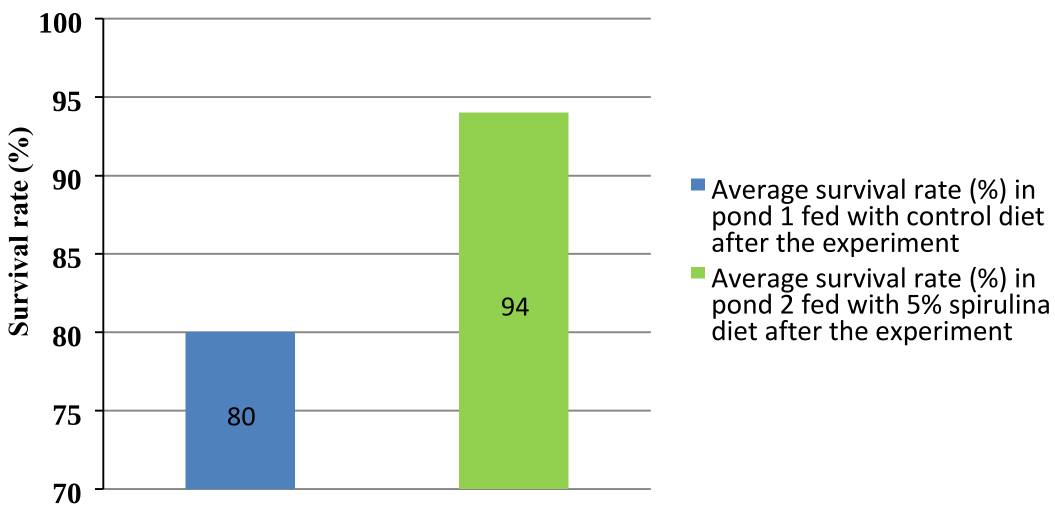

Figure 3.4 Survival Rate (%) of *Pangasius sutchi* using different feeds. (Source: Jana A. *et al.*, 2014).

3.1.4 Improving coloration as a natural source of carotenoid

Teimouri *et al.* (2013) evaluated the effects of diets containing 0, 2.5%, 5%, 7.5% and 10% *S. platensis* and synthetic astaxanthin (50 mg) on skin and fillet pigmentation as well as growth performance of rainbow trout (*Oncorhynchus mykiss*). Inclusion of 10% *S. platensis* as a natural pigment source resulted in the highest carotenoid deposition in both tissues (Figure 3.5). This result demonstrated that *S. platensis* can be used as an alternative source of natural carotenoid instead of synthetic astaxanthin in rainbow trout diets. In crustaceans carotenoids and carotenoproteins are responsible for the various colourations (Britton *et al*., 1981). Astaxanthin has been shown to be the predominant carotenoid associated with the red body colour of the black tiger prawn *Penaeus monodon* (Howell and Matthews, 1991). Although animals lack the biosynthetic pathways to synthesize carotenoids, certain crustacean and koi species are unique because they have the ability to convert dietary β-carotene and zeaxanthin directly into astaxanthin. *S. platensis* strain pacifica has the highest levels of β-carotene as well as zeaxanthin than any natural source. Both of them are converted to astaxanthin through an oxidative process resulting red pigment desired by consumers.

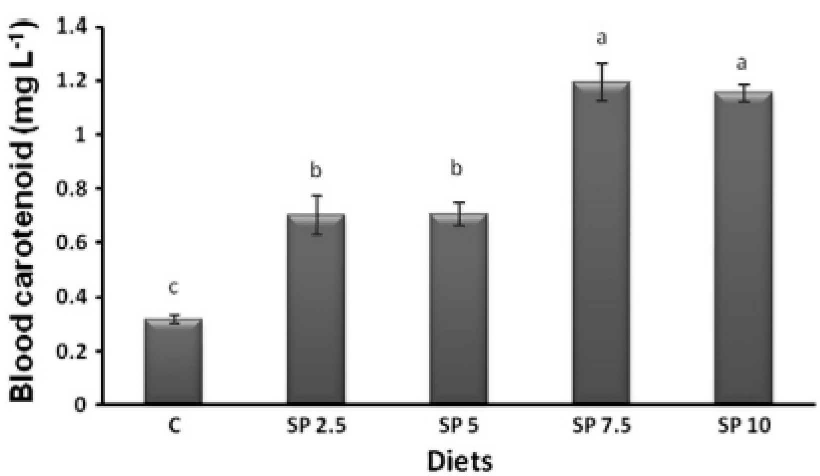

Figure 3.5: Fillet carotenoid concentration (mg/kg) in rainbow trout feeding on different levels of *S. platensis* and synthetic astaxanthin. (Source: Teimouri *et al.*, 2013)

The efficiency of *Spirulina* as a pigment for *P. monodon* is especially attributable to zeaxanthin, which can be converted into astaxanthin via 4-ketozeaxanthin. This finding suggests that dietary zeaxanthin is rapidly metabolized to astaxanthin in *P. monodon*. That may be the principal reason why *Spirulina* is such an efficient pigmentation source. A noticeable increase in carotenoid content of the carapace of black tiger shrimp (*Penaeus monodon*) resulted when *Spirulina* supplemented diets were given. The maximum effect was found when the diet was supplemented with 3 % *Spirulina* (Nur *et al*., 1993). A practical strategy for the improved pigmentation of cultured *P. monodon* is the incorporation of 3% *Spirulina* for one month before harvest. Chlorophyll in *Spirulina* acts as a cleansing and detoxifying factor against toxic substances, it is also used as a food additive to improve coloration in ornamental fish (James *et al*., 2006) and as a probiotic agent (Ramakrishnan *et al*., 2008).

3.1.5 Improving Antioxidant properties

According to Amer (2016) *Spirulina platensis* supplementation increased antioxidant protective capacities monosex tilapia. *S. Platensis* supplementation decreased significantly (P<0.05) malondialdehyde (MDH) formation compared to control group. Activity of glutathione reductase (GH) enzyme significantly higher in *S. Platensis* supplemented groups compared to control (P<0.05) (Table 3.4). A tendency toward increasing antioxidant enzymes and decreasing lipid peroxidation in S. platensis supplemented diets suggests that, among algal species, *Spirulina* has been reported to prevent oxidative damage by scavenging free radicals and active oxygen (Kok *et al.*, 1990). So *S. platensis* can also be supplemented in diet as an effective natural antioxidant source. Wang *et al*. (2007) also concluded that *Spirulina* had better carotenes and other pigments that have antioxidants activity.

Table 3.4 Effect of *S. Platensis* supplementation on antioxidant status of Nile tilapia muscle after feeding the experimental diets for 75 days.

Parameter	Control (0%)	T1 (0.5%)	T2 (1%)	T3 (1.5%)
MDA (µmol/gm)	122.55±8.74 ^{a}	57.17±12.13 ^{b}	58.03±5.76^{b}	63.60±24.31^{b}
GR(ng/gm)	1.51±0.21 ^{c}	2.69±0.34^{b}	3.20±0.23^{b}	4.19±0.09^{a}

(Source: Amer 2016.)

3.1.6 Improving hematological parameters

According to Moe, 2011, only 5% inclusion of *S. platenssis* in the diet of Tilapia (*O. niloticus*) improved the haematological parameters. He observed that *S. platenssis* significantly increased the Erythrocyte count (RBCs), Haemoglobin (Hb), Haematocrit (PCV), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Erythrocyte Sedimentation Rate (ESR) (Table 3.5) and (Figure 3.6) and Leucocyte count (WBCs) (Table 3.6) and (Figure 3.7). He concluded that *S. platensis* incorporated diet fed to *O. niloticus* improved haematological parameters reflecting weight gain due to good health.

Table 3.5 Mean values of haematological parameters in *O. niloticus* fed *S. platensis* incorporated and control diets

Parameter	Unit	Group	N	Value Mean ± SD	P value
Erythrocyte count (RBCs)	$x10^6/mm^3$	Treatment	15	1.65 ± 0.56	0.001
		Control	5	1.06 ± 0.04	
Haemoglobin (Hb)	g/dl	Treatment	15	6.20 ± 1.09	0.000
		Control	5	3.20 ± 0.52	
Haematocrit (PCV)	%	Treatment	15	19.33 ± 2.92	0.000
		Control	5	8.4 ± 1.14	
Mean Cell Volume (MCV)	fl	Treatment	15	128.56 ± 44.21	0.001
		Control	5	79.62 ± 13.49	
Mean Cell Haemoglobin (MCH)	pg	Treatment	15	41.32 ± 14.85	0.019
		Control	5	30.14 ± 4.47	
Mean Cell Haemoglobin Concentration (MCHC)	g/dl	Treatment	15	32.36 ± 2.47	0.243
		Control	5	39.25 ± 11.21	
Erythrocyte Sedimentation Rate (ESR)	mm/1st hr	Treatment	15	5.4 ± 1.12	0.003
		Control	5	8.0 ± 2.35	

(Source: Moe, 2011)

Table 3.6 Mean values of leucocyte count and differential count of *O. niloticus* fed *S. platensis* supplementary and control diets.

Parameter	Unit	Group	N	Value Mean ± SD	p value
Leucocyte count (WBCs)	$\times 10^3/mm^3$	Treatment	15	14.39 ± 6.47	0.064
		Control	5	10.84 ± 1.47	
Lymphocytes	%	Treatment	15	51.13 ± 7.13	0.001
		Control	5	35.40 ± 9.56	
Monocytes	%	Treatment	15	35.40 ± 9.56	0.384
		Control	5	7.80 ± 2.59	
Neutrophils	%	Treatment	15	38.00 ± 7.17	0.001
		Control	5	55.40 ± 10.29	
Eosinophils	%	Treatment	15	0.87 ± 0.83	0.327
		Control	5	1.40 ± 1.52	
Basophils	%	Treatment	15	0.13 ± 0.35	0.416
		Control	5	0.00 ± 0.00	

(Source: Moe, 2011)

Haematological evaluations is a routine practice for determining health status in fish and also other terrestrial animals (Tavares-Dias *et al*, 2006). Blood is a suitable means of indicating and identifying the effects of stress, environment and health status of fish in a given area. Blood cell count is a stable index and normally fish tries to maintain it between certain limits. Blood composition is usually altered during diseases or malnutrition conditions. So Fish blood is very important to accurately evaluate the health of species (Celik, 2004). Using *Spirulina* in fish diet improves these haematoloical parameters and makes the cultured fish healthy and disease resistant.

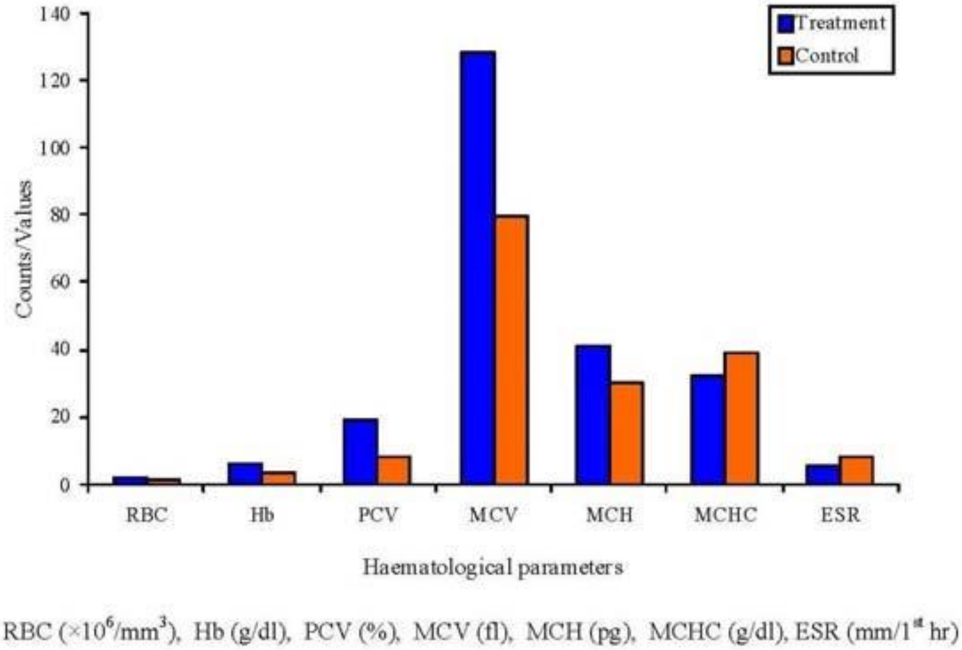

Figure 3.6: Comparison of mean haematological indices of *O. niloticus* in treatment (*S. platensis*) and control (Source: Moe, 2011)

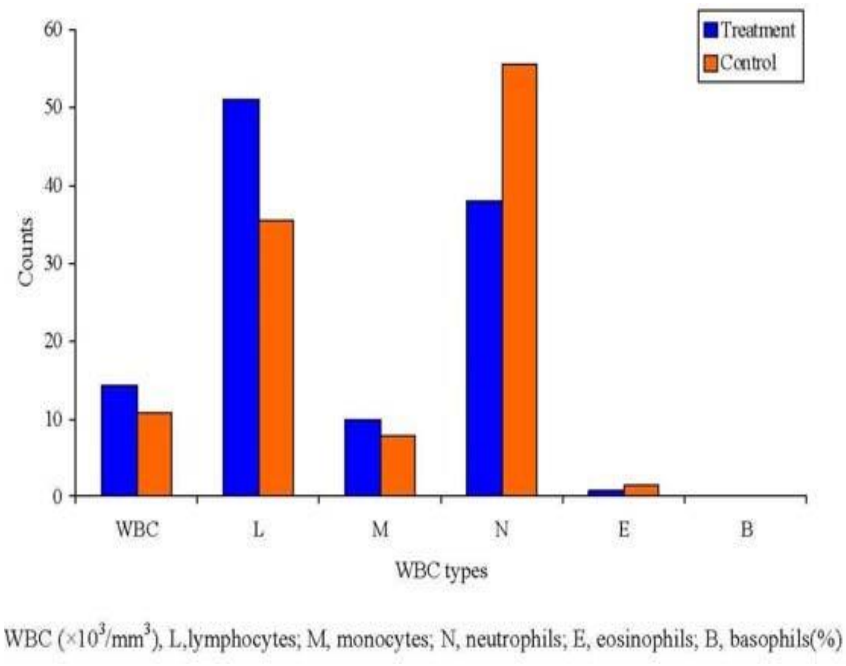

Figure 3.7: Comparison of mean total WBC and leucocyte counts of *O. niloticus* in treatment (*S. platensis*) and control (Source: Moe, 2011)

The blood reveals conditions within the body of fish long before any outward manifestation of disease. The close contact of environment to fish makes them susceptible to physicochemical changes reflected in their blood (Wepener *et al.*, 1992). Decrease in haemoglobin concentration denotes restricted ability of fish to provide sufficient oxygen to the tissues and this results in decline of physical activity (Nussey *et al.*, 1995). Prolonged reduction in haemoglobin content is harmful for oxygen transportation and any kind of blood dyscrasia and deterioration of the erythrocytes could be described as pathological condition in fishes exposed to toxicants (Shah, 2006). Structural damage to red blood cells membranes occurs when fish are exposed to toxicant which results in haemolysis and impairment in haemoglobin synthesis and stress-related release of red blood cells from the spleen and hypoxia (Wilson *et al.*, 1993). On the other hand ESR is a non-specific hematological parameter that may indicate the presence and intensity of a disease state. In case of in *Clarias gariepinus* Onusiriuka and Ufodike (2000) reported that increased ESR values under exposure to toxicants indicated polycythemia, dehydration and stress. Blood parameters have been used as guide of indicating fish health status in a number of fish species to detect physiological changes as a result of stress condition such as transportation, handling, hypoxia and acclimation. The analysis of haematological parameters helps to understand the link of blood characteristics to the habitat and adaptability of the species to the environment. Haematological parameters are closely linked to the response of the animal to the environment, an indication that the environment where fishes live could exert some influence on the haematological characteristics (Alwan *et al.*, 2009). This results indicates that using *Spirulina* will improve the health status of fish.

3.1.7 Increasing immune response

Spirulina supplementation positivily affect the immune response of fish. Amer (2016) observed that level of Catalase, Lysozyme and IgG value were found to be higher *S. Platensis* supplemented groups. He found the lysozyme higher with 1.5% *Spirulina* supplementation (7.28 μ/ml) compared to control group (3.94 μ/ml) (Table 3.7) in case of mono-sex tilapia which indicates better immune response. This finding was in accordance with (Ragap *et al*., 2012) who found that *Spirulina* -treated tilapia (10 mg/ fish) recorded the highest levels of lysozyme compared with *Spirulina* –treated tilapia (1 mg / fish), vaccinated and control groups. Lysozyme, detected in the blood, mucus and organs of various fish, plays on 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 (Grinde *et al*., 1988). Similarly, in case of Nile tilapia *S. platensis* supplement up to 10% increase lyzozyme level (Ibrahem *et al*., 2013). It also affected some innate and humoral immunity parameters. Moreover, *Spirulina* increases immune responses by promoting phagocytic and natural killer activities (Qureshi and Ali, 1996).

Table 3.7 Effect of *S. Platensis* supplementation on lysozyme, IgG, IgM values and catalase activity in serum of Nile Tilapia after feeding the experimental diets for 75 days

Parameters	Sp0	Sp0.5	Sp1	Sp1.5
Lysozyme (μ/ml)	3.94 ±0 .04[c]	5.10 ±0 .52[bc]	6.60±0 .34[ab]	7.28 ±0 .71[a]
IgG(ng/ml)	46.66± 7.37[c]	81.15 ± 15.65[b]	108.47 ± 21.67[a]	132.62 ± 6.84[a]
IgM(μg/ml)	5.53 ±0 .18[a]	5.83 ± 0.80[a]	6.61 ±0 .86[a]	6.77± 1.57[a]
Catalase (μmol/ /ml)	40.58 ± 11.70[b]	65.93 ± 8.41[a]	68.89± 6.91[a]	48.69 ± 6.91[a]

Means within the same row carrying different superscripts are significantly different at ($P \leq 0.05$)

(Source: Amer 2016)

Macias-Sancho *et al.* (2014) observed that the apoptotic index showed significant differences (p b 0.05) in Shrimp (Litopenaeus vannamei) being fed a diet with total FM replacement. A. platensis stimulates the immune systemdirectly by reducing apoptosis (table 3.8). *Spirulina* has long been recognized as a potential immunostimulant. It augments components of mucosal and systemic immune system through the activation of non-specific immune system. Its aqueous extract was reported to influence the immune system by enhancement of phagocytic activity and stimulation of NK cells (Ravi *et al*., 2010). Nakono *et al*. (2003) recorded that the lack of cellulose from the cellular structure of *Spirulina* render it easily digestible, thus, increase fish appetite, improve feed intake and nutrient digestibility and in turn enhance the health of fish, increasing the ability to fight off infections through the reduction of stress levels.

Table 3.8 Total protein concentration (TPC), granular (GH) and hyaline (Hh) hemocyte percentages and hemolymph cellular apoptosis in shrimp fed different replacement levels of *A. platensis.*

Diet	TPC (mg/mL)	GH (%)	Hh (%)	Apoptosis
0	120.13 ± 0.64^{b}	65.40 ± 0.72^{b}	34.60 ± 0.72^{a}	4.00 ± 1.20^{a}
25	119.66 ± 1.15^{b}	71.27 ± 2.20^{a}	28.73 ± 2.20^{b}	3.93 ± 1.10^{a}
50	125.13 ± 2.00^{a}	72.80 ± 2.46^{a}	27.20 ± 2.46^{b}	2.60 ± 0.35^{ab}
75	124.73 ± 1.47^{a}	74.67 ± 1.10^{a}	25.33 ± 1.10^{b}	2.47 ± 0.70 ^{ab}
100	124.73 ± 2.60^{a}	72.40 ± 2.46^{a}	27.60 ± 2.46^{b}	1.73 ± 0.41^{b}

(Source: Macias-Sancho *et al.,* 2014)

3.1.8 Reducing cost of fish feed

A new alternative protein sources including cheaper plant or animal origin proteins are needed to be introduced for stable aqua feed production inorder to make aquaculture sustainable (Higgs *et al*., 1995). It is suggested that the increased use of plant protein in fish diets can reduce the cost of FM and feeds (Lim and Lee, 2009). Sheekh *et al*. (2014) found that the use of *A. platensis* meal in red tilapia feed resulted in decrease of feed cost (cost/kg feed) and incidence cost as well as increase profit index (Table 3.9). The maximum reduction incidence cost (IC) was achieved with diet containing 75% *A. platensis* meal and caused maximum profit. So he recommended to use *A. platensis* as commercial nutrient source for large scale culture of fish in general and tilapia in particular. That means *Spirulina* is a cheaper feed ingredient than others of animal origin. Now a days China is using *Spirulina* as a partial substitute of imported feed to promote the growth, immunity and viability of prawns (example *Penaeus monodon*). *Spirulina*-containing feed was also found to reduce the cultivation time and mortality, and increase shell thickness of scallop.

Table 3.9 Cost-benefit analysis of red tilapia fed diet containing (*Spirulina*) *Arthrospira platensis*

Diet	control	50%	75%	100%
Cost per kg feed	4.01	3,43	3.19	2.84
Change %	100	85.53	79.55	70.82
Incidence cost	83.21	63.7	41.28	46.21
Change %	100	76.55	49.60	55.53
Profit index	.11	.14	.22	.19
Change %	100	127.27	200	172.73

Incidence cost = feed cost to produce 1 kg fish
Profit index = value of fish /cost of feed consumed, 1 kg fresh fish equals 6 LE

(Source: Sheekh *et al*., 2014)

The cost of fish feed can also be reduced by culturing *Spirulina* in a low cost medium. The cost of medium can be reduced by replacing the high cost chemicals with low cost chemicals or lacally available organic matters. For example the use of NPK fertilizer in *Spirulina* culture medium. Kumari *et al*. (2015) found that using NPK fertilizer were better than standard culture media, and after using newly formulated NPK-10:26:26 fertilizer medium for growth of *Spirulina* 50.0 % cost was saved compared to the standard culture media.

3.1.9 As an alternative source of protein

The rapid growth of aquaculture has resulted in higher demand for FM and consequently its high price is expected to be further increased by continuous growth in its requirement (Hardy and Tacon, 2002). Furthermore, sustainability of FM production from wild fish is questionable (Naylor *et al*., 2000). A number of algal species have been used in aquaculture mainly for nutritional applications. *Spirulina* makes it as potential FM replacer in aquafeed formulation (Hanel *et al*., 2007). It has been found it can be used as an alternative source of protein and can also be used to improve the color, flavor and quality of meat (Al-Badri, 2010). The protein content *Spirulina* is very high (58 %), which is higher than other plant sources such as dry soybeans (35 %), peanuts (25 %) or grains (8–10%). (Table 3.10). A special value of *Spirulina* is that it is easily digested due to the absence of cellulose in its cell walls and after 18 hours more than 85 % of its protein is digested and assimilated (Sasson, 1997).

Table 3.10 Typical composition of commercially available feed ingredients and algae species (per dry matter)

	% Crude Protein	% Crude Lipid	% Crude Carbohydrate*	% Ash	Gross Energy MJ/kg
Fishmeal	63.0	11.0	-	15.8	20.1
Poultry meal	58.0	11.3	-	18.9	19.1
Corn-gluten	62.0	5.0	18.5	4.8	21.3
Soybean	44.0	2.2	39.0	6.1	18.2
Wheat meal	12.2	2.9	69.0	1.6	16.8
Spirulina	58.0	11.6	10.8	13.4	20.1
Chlorella	52.0	7.5	24.3	8.2	19.3
Tetraselmis	27.2	14.0	45.4	11.5	18.0
Gracilaria sp	34.0	1.5	37.1	26.9	13.4
Gracilaria sp	10.0	0.9	50.1	34.0	11.2
Ulva lactuca	37.4	2.8	42.2	17.4	15.7
Ulva lactuca	12.5	1.0	57.0	24.5	11.2
Schizochytrium	12.5	40.2	38.9	8.4	25.6

* Carbohydrates calculated as the difference % DM – (% protein + % lipid + % ash)

(Source: Ayoola, 2010)

3.1.10 For improving fish flesh quality

Abdulrahman *et al*, (2014) found better growth and flesh quality by replacing fish meal with *Spirulina* in case of common carp. During experiment 5 experimental diets were prepared and fishmeal protein was replaced by *Spirulina* from the standard diet at 0% (T1), 5% (T2), 10% (T3), 15% (T4) and 20% (T5) levels. The data in Table 3.11 showed that fish meat composition data of protein were 24.145, 24.005, 20.935, 20.910 and 17.865% for the T5, T3, T2, T1 and T4, respectively, there were T5 had significant ($p<0.05$) differences as compared with the control ones and other treatments no significant ($p<0.05$) difference between T5 and T3. As *Spirulina* contain good amount protein and it improves performance of fishes by improving its flesh quality so it is possible to use *Spirulina* as a protein source in aquaculture industry. (Promya and Chitmanat, 2011).

Table 3.11: Effect of replacing fishmeal with *Spirulina* on fish flesh

Treatment	Protein %	Lipids %	Ash %	Moisture %
T1	20.910 b	1.623 a	1.057 a	73.503 b
T2	20.935 b	0.737 b	1.193 a	71.663 c
T3	24.005 a	1.527 a	1.110 a	74.397 b
T4	17.865 c	1.047 b	0.967 a	73.530 b
T5	25.145 a	1.483 a	1.177 a	76.400 a

(Source: Abdulrahman *et al.*, (2014)

CHAPTER 4

CONCLUSIONS

A large portion animal protein requirement of human is met up by fish protein. Total fish production in the world is largely depended on the aquaculture of fish. And the profitability in fish culture hugely dependent on fish feed because feed constitute more 65% of the production cost. This is why a cheap alternative and nutritionally balanced feed ingredient is very essential to make fish feed as well as fish production sustainable in the world. And in this case *Spirulina* is a better option and it has a good potentiality to increase the fish production of Bangladesh also.

The microalgae *Spirulina* is easily available all over the world. Its culture technique is also easy and cost effective. Different countries of the world has already started to produce it commercially and use this microalgae in their fish as a nutrient supplement or replacement of fish meal because of its quality. *Spirulina* could be an excellent source of useful nutrients for fish as well as a good energy source that can be used as crucial component for fish feeding. It is receiving increasing attention for its protein content which higher than other plant protein used in fish feed. The bioactive components such as vitamins (especially vitamin A and B12), minerals, polyunsaturated fatty acids, carotenes and other pigments that have antioxidants activity have make *Spirulina* suitable for using in fish feed. And because of these qualities *Spirulina* can be used in fish culture to increase the growth, survival, immune response, disease resistance, improving the flesh quality, coloration, haematology of fish. Now a days it is also used for improving water quality in the fish culture unit.

The most important reason behind using *Spirulina* as a plant protein source in fish culture because of is availability, nutritional quality and its cost effectiveness. So it can be concluded that *Spirulina* is a potential feed ingredient and its use in fish culture is very effective.

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