A SEMINAR PAPER ON

EFFECT OF SEAWEED AS A SUPPLEMENT OF FISH FEED ON GROWTH AND IMMUNE SYSTEM OF FISH

Course Title: Seminar

Course Code: FBE 598

Term: Summer, 2018

SUBMITTED TO:

Course Instructors	Major Professor
1. Dr. Md. Mizanur Rahman Professor BSMRAU 2. Dr. A. K. M. Aminul Islam Professor BSMRAU 3. Dr. Md. Rafiqul Islam Professor BSMRAU 4. Dr. Dinesh Chandra Shaha Assistant Professor BSMRAU	Dr. S. M. Rafiquzzaman Associate Professor Department of Fisheries Biology & Aquatic Environment

SUBMITTED BY:

Sultana Razia

MS Student

Reg. No.: 13-05-3031

Department of Fisheries Biology & Aquatic Environment

BANGABANDHU SHEIKH MUJIBUR RAHMAN AGRICULTURAL UNIVERSITY

SALNA, GAZIPUR 1706

ABSTRACT

Formulating of new innovative fish feed is the major challenges for profitable aquaculture. This study was conducted to review the growth performance and immune response of different fishes by supplementing seaweed in fish feed. As this is a review paper, so all the data were collected from secondary sources. Results found that, average weight gain (WG) of Red Tilapia (*Orechromis sp*) and *O. niloticus* increased up to 15% and 30% supplement of *Ulva* and *Hypnea musiformis*. In case of *Catla catla*, seaweeds *Chlorodesmis fastigiata*, *Padina tetrastomatica* and *Stoechospermum marginatum* showed WG 13.38g/month, 11.56 g/month, 9.05g/month respectively that are higher than the control (6.48g/month). All others parameters regarding growth such as specific growth rate (SGR), feed conversion ratio (FCR) etc. showed highest results in seaweed treated fish than the commercial feed. Regarding the immune response, highest RBC count were 3.64×10^{6} cells mm^{-3} and 3.67×10^{6} cells mm^{-3} for *O. niloticus* and *Cyprinus carpio* for 30% *H. muciformis* and 35% *G. corticata* supplementation. Highest WBC count was 15.12×10^{3} cells mm^{-3} in *O. niloticus* for 30% *H. muciformis*. In case of *Mugil cephalus* highest WBC was found for 1% *Sargassum whitti* supplement. All others parameters of blood for immune response such as mean corpuscle volume (MCV), hemoglobin, hematocrit etc. showed highest results in seaweed treated feed compared. From the above results, seaweed supplemented feeds showed best results compared to control feed which increased the total aquaculture production and immune system of fish.

Key Words: SGR, FCR, average weight gain, MCV, hematocrit, hemoglobin

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CHAPTER 1

INTRODUCTION

Bangladesh is a low-lying, riverine country located in South Asia (24°34' to 26°38' North, 88°01' to 92°41' East). The total area of the country is 147,570 sq. km (56,977 sq. miles). Bangladesh is in the transitional zone for the flora and fauna of the Indian subcontinent and Southeast Asia, and is part of the Indo-Burma biodiversity hotspot. The country features a great diversity of natural ecosystems. The natural ecosystems of Bangladesh include several types of forests, freshwater wetlands and coastal and marine habited where many important floral and faunal biodiversity are found. The marine waters of Bangladesh are having 442 species of fish, 453 species of birds, 36 species of marine shrimps, about 336 species of mollusks, in which 151 genera has been identified. In addition, 3 lobsters and 7 species of turtles and tortoises, 3 sponges, 16 crabs, 3 lobsters, 10 frogs, 3 crocodiles, 24 snakes, 3 otters, 1 porcupine, 9 dolphins and 3 species of whale, 336 molluscs and 3 starfish/echinoderms also found in the Bangladesh territory (Hossain *et al.*, 2014). According to Sarkar *et al*., (2016), about 193 seaweed species of 94 genera belonging to only three major divisions i.e. Chlorophyta-green algae, Phaeophyta-brown algae and Rhodophyta-red algae are available in Bangladesh. Among the available seaweed species, 19 species of 14 genera are considered as economically important. Around 60 seaweed species are found from Sundarbans and among them about 155 seaweed species are found in Cox's Bazar. We have a huge resource in our country but it is not used so much. This is a potential sector to focus on the utilization of marine resources like seaweed.

Seaweeds are available in the coastal climatic zones throughout the world i.e. from the warm tropics to the icy Polar Regions. Seaweeds have been reported to be used for various purposes by the Romans, Egyptians, Japanese, and Chinese in the ancient time (Drugs.com, 2015). The extent and scope of seaweeds utilization in this modern world is increasing (McHugh, 2003) day by day. There are about 10,000 kinds of seaweeds in the world.

Fisheries is the second most valuable productive and dynamic sectors in Bangladesh which plays a significant role providing food, nutrition, incomes, livelihoods, employment and earning foreign exchange. The total annual fish production in Bangladesh was estimated at 3.68 million tons in 2014–2015, of which 2.06 million tons (55.93%) were obtained from inland aquaculture, 1.02 million tons (28%) from inland capture fisheries and 0.59 million tons (16.07%) from marine fisheries (FRSS,2016). Fish culture in ponds is the main freshwater aquaculture practice in Bangladesh. Fish contributes 60% of national animal protein consumption. The country's export earnings from this sector are 1.92% in 2014-15. Fisheries sector earns second highest foreign exchange of the country. The contribution of fisheries sector in Bangladesh is 3.69% of GDP and 23.12% in agriculture during 2014-2015. Now Bangladesh is 5th in position in freshwater aquaculture (FRSS, 2016). For higher production aquaculture intensification is occurring but there is a problem of sustainability. Feed, seed and disease infestation are major threats of aquaculture intensification. Quality seed is the prerequisite for successful fish culture. So, seed production in hatchery is very significant in this stage. But in hatchery of Bangladesh, survivability rate of fingerlings are very low. Even the fingerlings which go through the hatchery period mortality occurs in grow out pond and their growth rate is not satisfactory as well. They are easily affected by different diseases because their immune system is very poor. For solving this problem it is important to use such foodstuffs having various nutritional factors and immune enhance capability. Again feed cost is one of the largest operational costs in the aquaculture industry. According to Pereira *et al.*, (2012) fish feeding constitutes over 50% of operating cost in intensive aquaculture. An important approach for reducing feed costs in commercial aquaculture need to develop proper feed management, husbandry strategies and efficient broadcasting of the predetermined ration to the culture system.

Seaweed can enhance growth and immune system of fishes reducing the feed cost as it contains many nutrients and bioactive compounds. Seaweeds have higher nutrients than any land vegetables. It is a tremendous source of micronutrients including calcium, magnesium, zinc, iron and selenium. More importantly, seaweed is a great source of iodine and also contains omega-3 fatty acids, DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid). Seaweeds have different types of carbohydrates that fishes lack digestive enzymes to digest, like: carrageenan, fucan, galactan, and many more. Gut micro-biota use these carbohydrates as their food. Seaweeds has a great quantity of vitamins, including beta-carotene, which is the precursor of vitamin A, vitamin B group, including B12, vitamin C, D, E and K. Calcium and iron tend to accrue at much higher levels in seaweed than in terrestrial plants. Seaweeds promote the production of white blood cells, the cells that are responsible for fighting off invasive bacterial and viral infections which can increase the immune system of fish. Many types of seaweed also contain anti-inflammatory and anti-microbial agents. It is also rich in a polysaccharide of alginic acid which has been revealed to suppress cholesterol absorption and discharge heavy metals from the body as well as act to prevent artery hardening (Yoon *et al.*, 2008). Acid water-soluble polysaccharide of fucoidan in the seaweed has showed the effects of anti-coagulation of blood, anti-tumor, anti-cancer and anti-oxidation (Allen *et al.*, 2001) and these benefits further increase the thrombin, Factor Xa, urokinase-type plasminogen activator and tissue plasminogen activator. In addition, alginate oligomer, which is a degradation product of alginates by an enzyme of seaweed, in a large quantity, can directly and indirectly influence the activation of immune system in the body by increasing cytokine secretion and improving the bioavailability of zinc. Therefore it is necessary to apply such technology for better growth performance and immune response of fish.

OBJECTIVES:

Based on above facts the objectives of this reviewed paper are-

1) To review the effects of seaweed on growth performance of fish
2) To highlight the effects of seaweed on immune response of fish

CHAPTER 2

MATERIALS AND METHODS

This seminar paper is exclusively a review paper. All 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. Finding related to my topic have been reviewed with the help of the library facilities of Bangabandhu Sheikh Mujibur Rahman Agricultural University. I got suggestions and valuable information from my major professor and my course instructors. After collecting all the available information, I myself complied that collected information and prepared this seminar paper.

CHAPTER 3

REVIEW OF FINDINGS

3.1 Definition of seaweed:

Seaweed is a colloquial term for the common name of mostly macroscopic and multicellular marine algae, which do not have root systems or flowers, leaves, stems, fruits and seeds and generally grow and live attached to rock or other hard substrata below the high-water mark or remain drifted in the oceans (Chapman, 1973).

Seaweeds appearance somewhat resembles non-arboreal terrestrial plants which has Thallus: the algal body, Lamina or blade: a flattened structure that is somewhat leaf-like, Sorus: a spore cluster, Air bladder: a flotation-assisting organ on the blade on kelp, Float: a flotation-assisting organ between the lamina and stipe, Stipe: a stem-like structure, may be present or absent, Holdfast: a specialized basal like structure providing attachment to a surface, often a rock or another algae. Haptera: a finger like extension of the holdfast anchoring to a benthic substrate and Frond: the stipe and blade are collectively known as the Frond (figure 1).

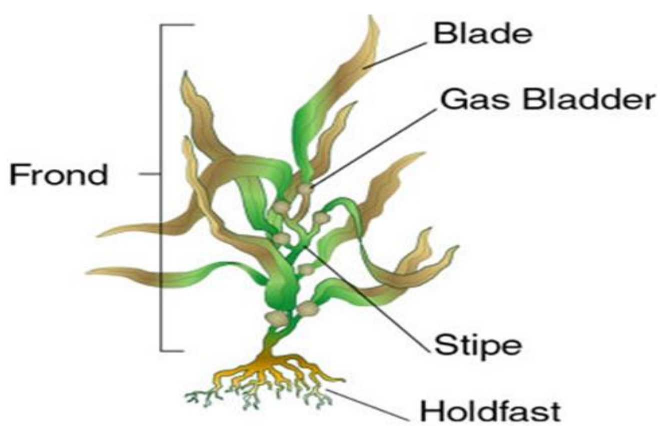

Figure1: Structure of Seaweed (Sarkar *et al*., 2016)

3.1.1 Types of Seaweeds

According to Sarkar *et al*., (2016), about 193 seaweed species of 94 genera are available in Bangladesh. Among the available seaweed species, 19 species of 14 genera are considered as economically important. There are three types of seaweed in our country.

Chlorophyta-green seaweed/algae:

Green algae or seaweeds have chloroplasts that contain chlorophyll *a* and *b*, giving them a bright green color that's why it is called chlorophyta algae. It also contains the accessory pigments beta-carotene and xanthophyll. The cell walls of green seaweed or algae usually contain cellulose and store carbohydrate in the form of starch. All green algae have mitochondria with flat cristae and paired flagella that are used to move the cell, when it is present in the seaweed. They are anchored by a cross-shaped system of microtubules and fibrous strands. Flagella are only present in the motile male gametes and are absent from the gametes of Pinophyta. Green Seaweed such as *Enteromorpha clathrata, E. intestinalis, Ulva lactuca, U. intestinalis* etc.

Phaeophyta-brown seaweed/algae:

The Brown algae or seaweeds are belonging to the group Heterokontophyta having chloroplasts surrounded by four membranes, suggesting an origin from a symbiotic relationship between a basal eukaryote and another eukaryotic organism. Most brown algae contain the pigment fucoxanthin, which is responsible for the distinctive greenish-brown color that gives them their name. Brown algae are unique among heterokonts in developing into multicellular forms with differentiated tissues, but they reproduce by means of flagellated spores and gametes that closely resemble cells of other heterokonts. Examples: *Padina australis, Sargassum caryophyllum, S. flavicans, S.vulgare, Ectocarpus breviarticulatus, Dectyota bratayresii* etc.

Rhodophyta-red seaweed/algae:

The red algae or seaweeds form a distinct group characterized by having eukaryotic cells without flagella and centrioles, chloroplasts that lack of external endoplasmic reticulum and which contain unstacked (stoma) thylakoids, and use phyco-bili-proteins as accessory pigments, which give them their red color. Red algae store sugars as floridean starch, which is a type of starch that consists of highly branched amylopectin without amylose, as food reserves outside their plastids. Most red algae are also multicellular, macroscopic, marine, and reproduce sexually. The red algal life history is typically an alternation of generations that may have three generations rather than two. Examples: *Hypnea musciformis, H. pannosa, H. boergesenii, Gracilaria textorii, Halymenia floresia, Halymenia discoidea, H. floridana* etc.

3.2 Supplementary feed

Supplementary feed is one of the most prime exogenous requisite for proper fish culture. The supplementary fish feeds are either be vegetable foods (e.g. pulse, cereals, grains, yeast, plant parts etc.) or animal origin (e.g. fish meal, meat meal, blood meal, meat and bone meal, internal parts of animal body etc.). Whatever the feeds type, the criteria for a successful fish feeds are, (i) readily acceptable, (ii) having high conversion rate (iii) availability (iv) highly keeping quality and (v) low cost.

Several studies are directed to an efficient and cost-effective seaweed supplementary fish feed because of the importance of fish as a protein source for human and animal's diet. Seaweed plays an important role in efficient aquaculture production as it gives high influences not only to the production costs, but also to the fish health, growth and immunity. Aqua feed is the major determinant that influences the successful growth and intensification of aquaculture production.

3.3 Seaweed Supplementary feed for growth performance of fish

The effect of seaweeds as feed supplements in fish diet which show significant results on growth performance, feed efficiency and body composition of fish. Different studies indicate that the seaweed Supplementary feed for growth performance of fish was higher and effective than commercial feed.

3.3.1Average weight gain (WG) and Specific growth rate (SGR)

Weight gain (g) = Mean final weight (W2) - Mean initial weight (W1)

Specific growth rate (SGR % per day) = $\frac{\text{LogW2 – LogW1}}{\text{Time}} \times 100$

Where,

W_1 = the initial live body weight (g) at time T_1 (day)

W_2 = the final live body weight (g) at time T_2 (day)

Nader *et al.*, (2010) was carried out the study about the effect of green seaweeds (*Ulva* sp.) as feed supplements in fish diet on growth performance, feed efficiency and body composition of Red Tilapia (*Oreochromis sp.*). Six isocaloric diets (236 kcal metabolic energy/100g diet) containing 26% crude protein, with different levels of green seaweed *Ulva sp.* (0, 5, 10, 15, 20 and 25% of fish diet) were used for 9 weeks**.** Results showed that weight gain and specific growth rate (SGR) increased significantly with increasing *Ulva* level in fish diet up to 15%

(Table 1). Increasing the level of *Ulva* in the diets from 15% suggested additional advantage in growth of fish.

Table 1: Weight gain (WG) and specific growth rate (SGR%/day) of red tilapia (*Oreochromis sp*.) fed at different dietary levels of seaweeds (*Ulva sp.)*

Treatments	WG(g)	SGR%/day
Control	7.98	3.32
5%	8.12	3.33
10%	9.54	3.53
15%	9.75	3.56
20%	8.57	3.42
25%	7.42	3.22

Source: (Nader *et al.*, 2010)

Nur, 2017 was carried out the study about the effect of seaweed powder as a supplement of feed on growth and immune system of Tilapia *(Oreochromis niloticus).* This experiment was carried out in earthen pit with four treatments (T_1: Control and T_2: 10%, T_3: 20% and T_4: 30% seaweed as fish meal supplement, respectively) with three replications. Fishes were reared in earthen pits from 28th April to 17th June, 2017. Sampling was done 7 days of interval to collect the different parameter of growth. Results found that, the average weight gain of fish was 9.95g, 11.69g, 11.72g and 12.49g for T_1, T_2, T_3 and T_4, respectively and specific growth rate (SGR % day^{-1}) of T_1, T_2, T_3 and T_4 was 4.79, 5.13, 5.29 and 5.67, respectively. So it can be said that growth was significantly increased with the increased supplement rate of fish meal by seaweed powder.

Table 2: Average weight gain and Specific growth rate (SGR) of *O. niloticus* fed at different dietary levels of seaweeds (*H. musciformis*)

Treatment	Average weight gain(g)	Specific growth rate (%/day)
T_1	9.95	4.79
T_2	11.69	5.13
T_3	11.72	5.29
T_4	12.49	5.67

Source: (Nur, 2017)

Savita *et al.*, (2010) was evaluated the growth performance of Indian major carp (*Catla catla*), was assessed over a period of six months through formulated feeds consisting of three seaweeds, namely *Chlorodesmis fastigiata*, *Padina tetrastomatica* and *Stoechospermum marginatum*. A relatively slow average growth rate (6.48g/month) in fishes was observed in the control group.

Meanwhile, the maximum and rapid growth rate (13.38g $month^{-1}$) was observed with Feed-A supplemented with *C. fastigiata*. Similarly, a comparable growth rate was also observed with Feed-B (11.56g $month^{-1}$) with *P. tetrastomatica*. However, the growth rate in fishes was relatively lower (9.05g $month^{-1}$) with Feed-C containing seaweed *S. marginatum* (Figure 2). The data of the study clearly demonstrated that seaweeds, such as *C. fastigiata* and *P. tetrastomatica* could be used in commercial formulated feed for the better growth of fingerlings of major carps.

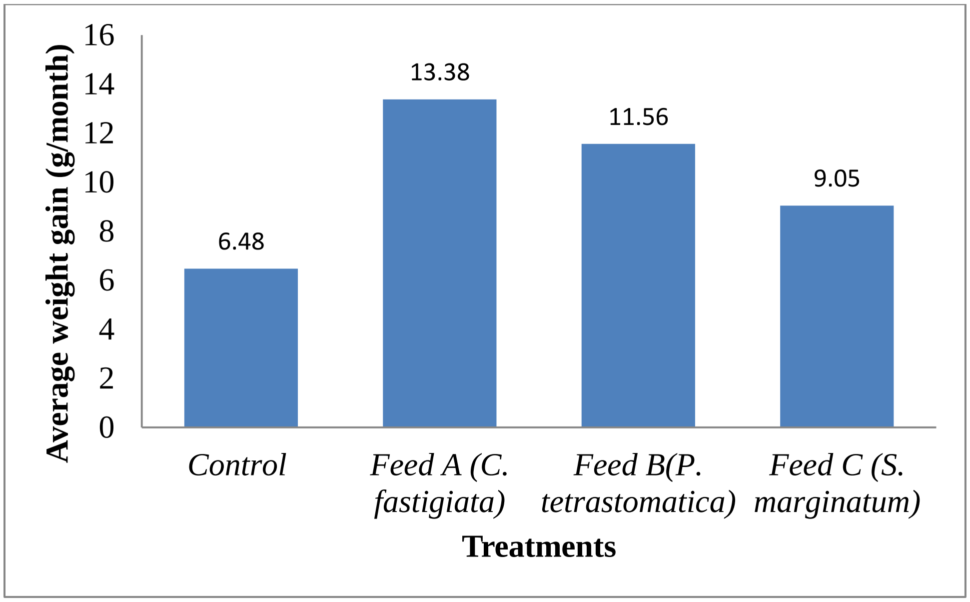

Figure 2: Growth performance in *Catla catla* when fed with different seaweed supplemented feeds

(Source: Savita *et al.*, 2010)

3.3.2 Relative percent (%) increment of weight gain

Relative percent (%) increment of weight gain compare between the percent (%) of weight gain of treatments and control.

$$\text{Percent (\%) of weight gain} = \frac{\text{Mean final weight} - \text{Mean initial fish weight}}{\text{Mean initial fish weight}} \times 100$$

Relative percent increment of weight gain of T_2, T_3 and T_4 was 84.26%, 87.17% and 133.18%, respectively, which was significantly different at each treatment (Figure 3) (Nur, 2017). So it can be concluded that growth was significantly increased with the increased supplement rate of fish meal by seaweed powder.

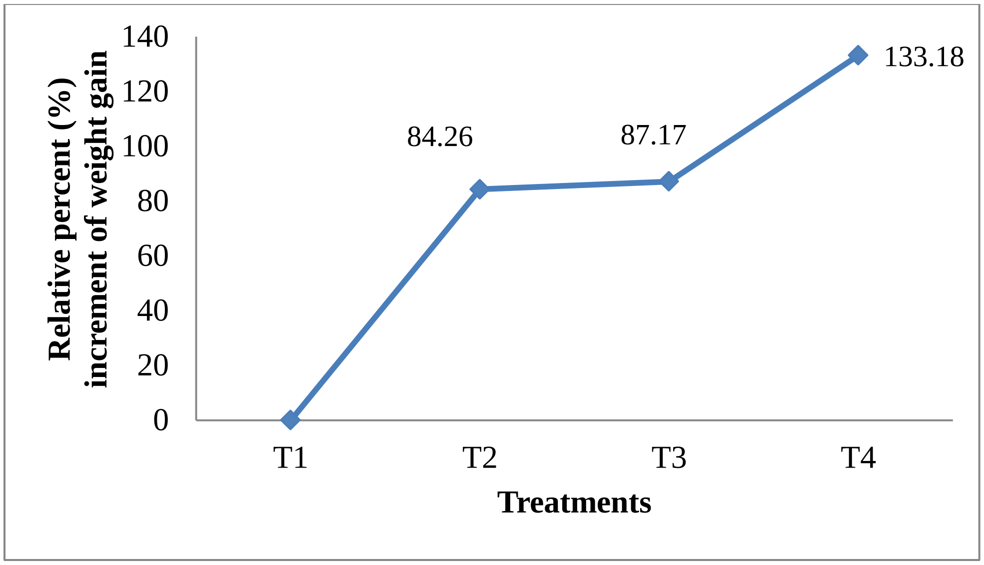

Figure 3: Relative percent (%) increment of weight gain of *O. niloticus* fed at different dietary levels of seaweeds (*H. musciformis*)

(Source: Nur, 2017)

According to Nader *et al.*, (2010), relative percent increment of weight gain of control, 5%, 10%, 15%, 20% and 25% *Ulva sp.* supplemented diet was 70.6%, 71.2%, 82.2%, 84.7%, 74.5% and 65.0%, respectively, which was significantly different at each treatment (Figure 4). So it can be concluded that increasing the level of *Ulva* in the diets from 15% suggested additional advantage in growth of fish.

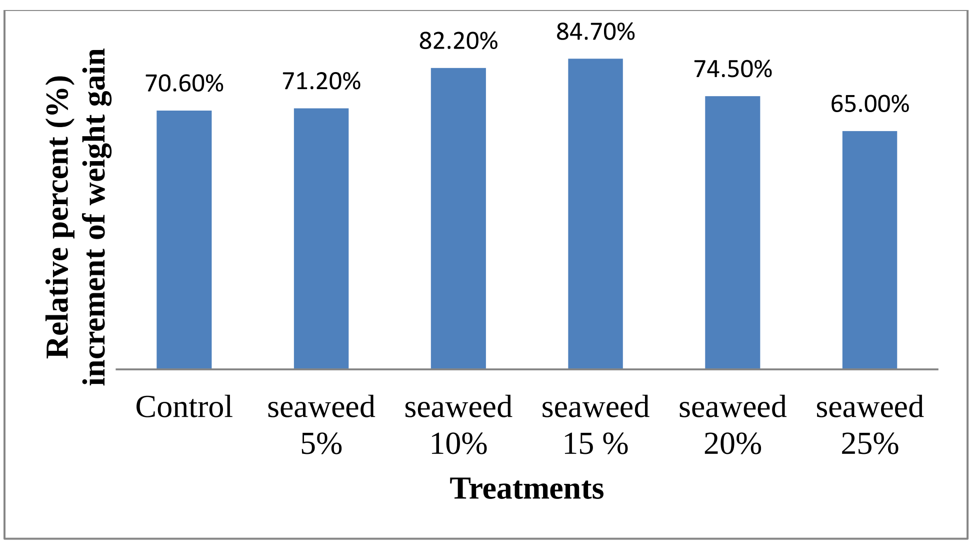

Figure 4: Relative percent (%) increment of weight gain of red tilapia (*Oreochromis sp.*) fed at different dietary levels of seaweeds (*Ulva sp.)*

(Source: Nader *et al.*, 2010)

3.3.3 Feed conversion ratio (FCR)

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total Feed consumption}}{\text{Total body weight gain of fish}}$$

FCR value of T_1, T_2, T_3 and T_4 was 1.27, 1.15, 1.12 and 1.02, respectively (Figure 5). FCR of *O. niloticus* fed with seaweed supplement feed differ significantly than control. In this study, the FCR value for Tilapia was better than control and in suitable range (Nur, 2017). So it can be concluded that the growth performance of seaweed treated treatments show better result than the control.

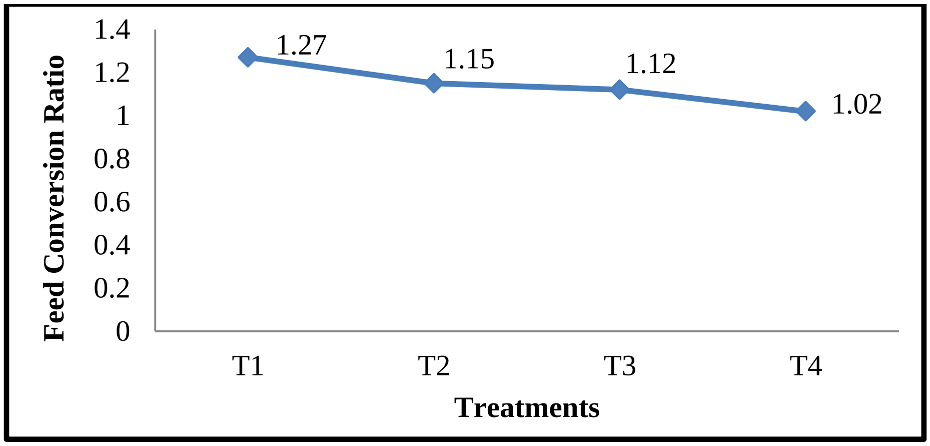

Figure 5: Feed conversion ratio (FCR) of *O. niloticus* fed at different dietary levels of seaweeds (*H. musciformis*)

Source: (Nur, 2017)

The average Food Conversion Ratio (FCR) calculated for all the different feeds and showed the minimum value by Feed-C (1.77), Feed-A (0.57) and Feed-B (0.67) with compare to control (2.54)(Table 3). The data of the study clearly demonstrated that seaweeds, such as *C. fastigiata* and *P. tetrastomatica* could be used in commercial formulated feed for the better growth of fingerlings of major carps. (Source: Savita *et al.*, 2010)

Table 3: Growth performance in *Catla catla* when fed with different seaweed supplemented feeds

Treatments	Control	Feed A (*C. fastigiata*)	Feed B(*P. tetrastomatica*)	Feed C (*St. marginatum*)
FCR	2.54	0.57	0.67	1.77

(Source: Savita *et al.*, 2010)

Felix and Alan, (2014) studied about the substitution seaweed (*Kappaphycus alvarezii*) by replacing fish meal in giant freshwater prawn (*Macrobrachium rosenbergii*). This was carried out by incorporating fermented *K. alvarezii* (FK) at three levels, 10%, 20% and 30% in diets.

The best FCR value of 1.14 was observed in prawn fed with FK at 10% incorporation followed by FK at 20% incorporation (1.15) (Table 4). The results of the study suggest that fermented *K. alvarezii* could be incorporated up to 30% level without compromising growth.

Table 4: FCR value of prawns fed with fermented *Kappaphycus alvarezii* incorporated at three different concentrations

Treatments	Control	FK 10%	FK 20%	FK 30%
FCR	1.78	1.14	1.15	1.16

(Source: Felix and Alan, 2014)

3.3.4 Hepatosomatic Index (HSI)

Hepatosomatic Index (HSI) is defined as the ratio of liver and body weight. It provides an indication on status of energy reserve of an animal.

Hepatosomatic index (HSI) = $\frac{\text{Liver weight (g)}}{\text{Body weight(g)}} \times 100$

HSI values of *O. niloticus* were 2.67, 3.05, 3.06 and 3.6 in treatments T_1, T_2, T_3 and T_4, respectively where T_1: Control and T_2: 10%, T_3: 20% and T_4: 30% seaweed as fish meal supplement. HSI of *O. niloticus* fed with seaweed supplemented feed differ significantly compared with control (Figure 6). So it can be said that growth of seaweed treated fishes are higher than the control. (Nur, 2017)

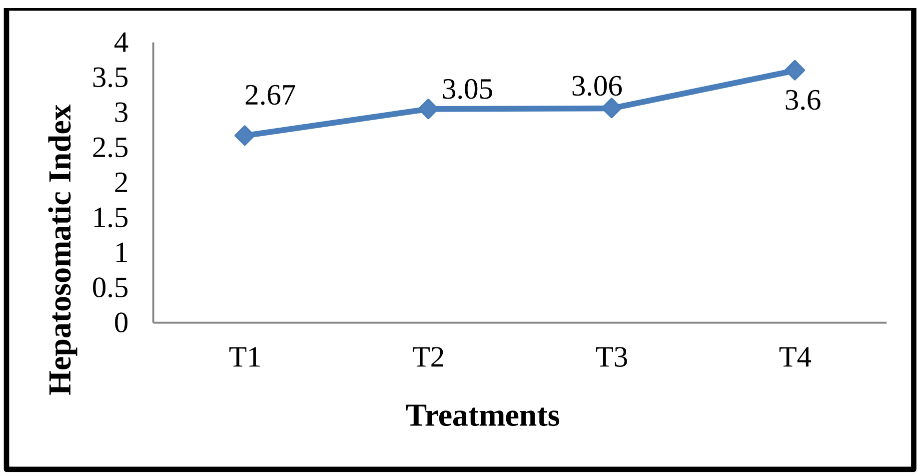

Figure 6: Hepatosomatic Index (HSI) of *O. niloticus* fed at different dietary levels of seaweeds (*H. musciformis*)

Source: (Nur, 2017)

According to Maria *et al*., (2016), a control diet (without supplementation) was tested against 6 practical diets supplemented either with *Gracilaria sp*, *Ulva sp*, or *Fucus sp*, at 2.5 or 7.5% levels. Sea bass juveniles (24.0g) were fed the experimental diets for 84 days (Table 5). Overall, the results indicate that the use of dietary seaweed supplementation improves the growth performance of European Sea bass.

Table 5: HSI of European Sea bass fed with *Gracilaria sp*, *Ulva sp*, or *Fucus sp*, at 2.5 or 7.5% levels

Treatments	Control	Gr 2.5	Ul 2.5	Fu 2.5	Gr 7.5	Ul 7.5	Fu 7.5
HSI	1.20	1.50	1.20	1.30	1.30	1.40	1.40

Source: (Maria *et al*., 2016)

3.4 Seaweed Supplementary feed for immune response of fish

Complete Blood Count (CBC), which evaluates the composition of the blood that based on RBC, WBC, Hemoglobin, Hematocrit values, MCV, MCH, and MCHC. These parameters provided the valuable information for fishery biologists in the assessment of fish health.

3.4.1Red Blood Cell (RBC) count

In this study, RBC was increased in the T_2, T_3 and T_4 than T_1 where T_1: Control and T_2: 10%, T_3: 20% and T_4: 30% seaweed as fish meal supplement, respectively. The highest RBC was counted in T_4 (3.64×10^6 cells mm^{-3}) at 4th week and lowest in T_1 (1.00×10^6 cells mm^{-3}) at 1st week (Table 6). It is a good immunological sign of *O. niloticus* because gradually increasing the RBC indicated that hemoglobin increased and transported more oxygen in the blood and also prevented from anemia. (Nur, 2017)

Table 6: RBC count in different treatments fed at different dietary levels of seaweeds (*H. musciformis*)

Treatment	RBC (cells mm^{-3})			
	1st Week	2nd Week	3rd Week	4th Week
T_1	1.00×10^6	1.21×10^6	1.42×10^6	1.64×10^6
T_2	1.15×10^6	1.47×10^6	1.95×10^6	2.32×10^6
T_3	1.31×10^6	1.68×10^6	2.13×10^6	2.98×10^6
T_4	1.54×10^6	1.85×10^6	2.57×10^6	3.64×10^6

Source: (Nur, 2017)

Radhika and Mohaideen (2016) chosen three types of seaweed for the study namely *Gracilaria corticata*, *Ulva lactuca* and *Stocheospermum marginatum*. The fish feed was formulated using 35% of seaweed. The fishes [Common carp (*Cyprinus carpio)* (weight 10±5g)] were divided into five groups where four group were infected with *Aromonas hydrophila* (1.6×10^4 CFU $fish^{-1}$) and one groups remained uninfected and this group along with negative control group was fed with control feed only and the infected groups were fed with seaweed mixed experimental diet. The experiment was carried out for 28 day. Every 7th day the blood parameter RBC was recorded. The blood parameter has increased much in seaweed treated treatments rather than the control from first day to last day (Figure 7). From this study we can understand that the fish fed with seaweed incorporated diet had good immune stimulants properties.

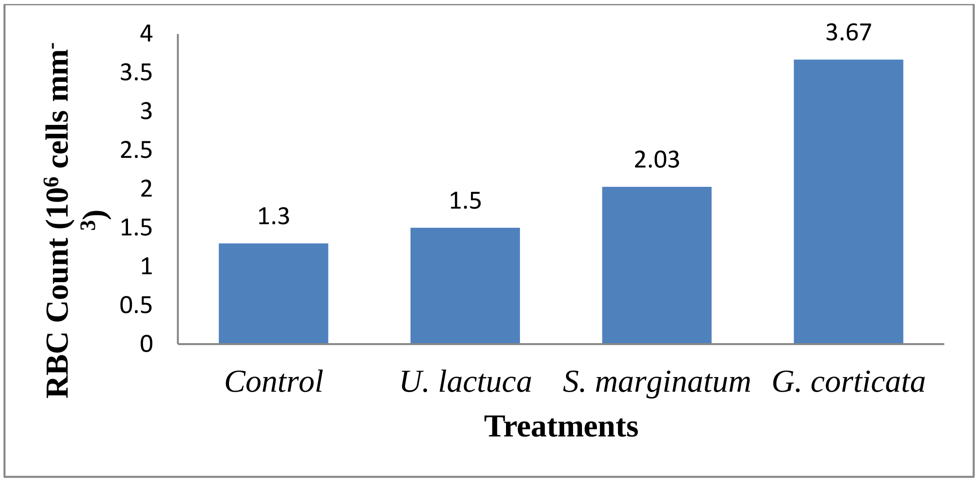

Figure 7: RBC count in different treatments fed with different seaweeds

Source: (Radhika and Mohaideen , 2016)

3.4.2 White Blood Cell (WBC) count

Kanimozhi *et al.*, (2013) was determined the effect of water extract of seaweeds *Sargassum whitti* on the immune mechanisms and disease resistance in *Mugil cephalus*. The Seaweed *(S. whitti)* was extracted by using hot water extract method. After extraction it was mixed with feed at different concentration. Fishes were feed with different doses of 0.5, 1.0 and 1.5% (kg diet^{-1}) body weight of water extract of seaweed. The immune mechanisms were assessed in terms of counting of White Blood Cells (WBC). The functional immunity was assessed by a challenge with live *Pseudomonas fluorescence.* In this study, 1% seaweed showed a significantly increasing WBC when compare with other diet 0.5 and 1.5% seaweed diet and control feed (Figure 8).

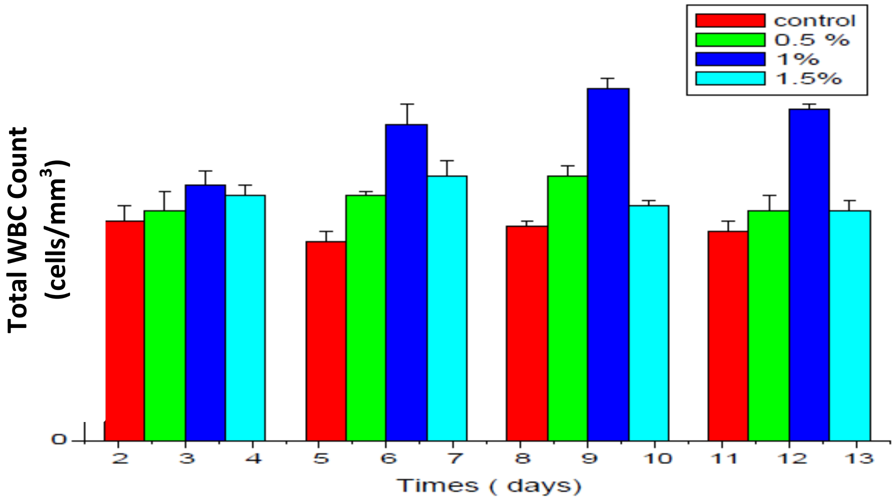

Figure 8: Total white blood cell count of *Mugil cephalus* infected with *Pseudomonas fluorescence,* fed at different dietary levels of seaweed (*Sargassum whitti)*

Source: (Kanimozhi *et al.*, 2013)

In this study, WBC was increased in the T_2, T_3 and T_4 than T_1. The highest WBC was counted in T_4 (15.12×10^3 cells mm^{-3}) at 4th week and lowest in T_1 (10.06×10^3 cells mm^{-3}) at 1st week (Table 7). WBC was increased gradually which was indicated the good sign of immunity and prevent the fish from invasive bacteria and virus. (Nur, 2017)

Table 7: WBC count of *O. niloticus* blood in different treatments fed at different dietary levels of seaweeds (*H. musciformis*)

Treatment	WBC (cells mm^{-3})			
	1st Week	2nd Week	3rd Week	4th Week
T_1	10.06×10^3	10.66×10^3	11.46×10^3	12.36×10^3
T_2	10.56×10^3	11.34×10^3	12.26×10^3	13.35×10^3
T_3	10.82×10^3	11.88×10^3	13.06×10^3	14.43×10^3
T_4	11.12×10^3	12.34×10^3	13.58×10^3	15.12×10^3

Source: (Nur, 2017)

3.4.3 Hemoglobin (Hgb/Hb) measure

Radhika and Mohaideen (2016) chosen three types of seaweed for the study namely *Gracilaria corticata*, *Ulva lactuca* and *Stocheospermum marginatum*. The fish feed was formulated using 35% of seaweed. The fishes [Common carp (*Cyprinus carpio)* (weight 10±5g)] were divided into five groups where four group were infected with *Aromonas hydrophila* (1.6×10^4 CFU $fish^{-1}$) and one groups remained uninfected and this group along with negative control group was fed with control feed only and the infected groups were fed with seaweed mixed experimental diet. The experiment was carried out for 28 day. Hemoglobin was measured more at 28th day in *G. corticata* (5.67 g dL^{-1}). There was a steady rise in the value from the 1^{st} to 28^{th} day. The Red seaweed *(G. corticata)*, which showed the highest level of hemoglobin; while the level was lower in control group at the 28^{th} day (4.33 g dL^{-1}) was found (Table 8).

Table 8: Hemoglobin (Hgb/Hb) measure in different seaweed treated treatment

Treatments	**Control**	***Stocheospermum marginatum***	***Ulva lactuca***	***Gracilaria corticata***
Hgb/ Hb (g dL^{-1})	4.33	4.99	5.20	5.67

Source: (Radhika and Mohaideen , 2016)

The blood parameter has increased much in seaweed treated treatments rather than the control from first day to last day. From this study we can understand that the fish fed with seaweed incorporated diet had good immune stimulants properties.

According to Nur, 2017, hemoglobin was increased in the T_2, T_3 and T_4 than T_1 where T_1: Control and T_2: 10%, T_3: 20% and T_4: 30% seaweed as fish meal supplement, respectively. After 4^{th} week the highest hemoglobin was measured in T_4 (12.4 g dL^{-1}) and lowest in T_1 (7.8 g dL^{-1}) (Figure 9). Increasing hemoglobin rate is a good indicator for the oxygen transportation capacity of fish, thus making it possible to establish relationships with the oxygen concentration available in the habitat and the health status of these fish. This study indicated a better immunity in seaweed treated treatments than control.

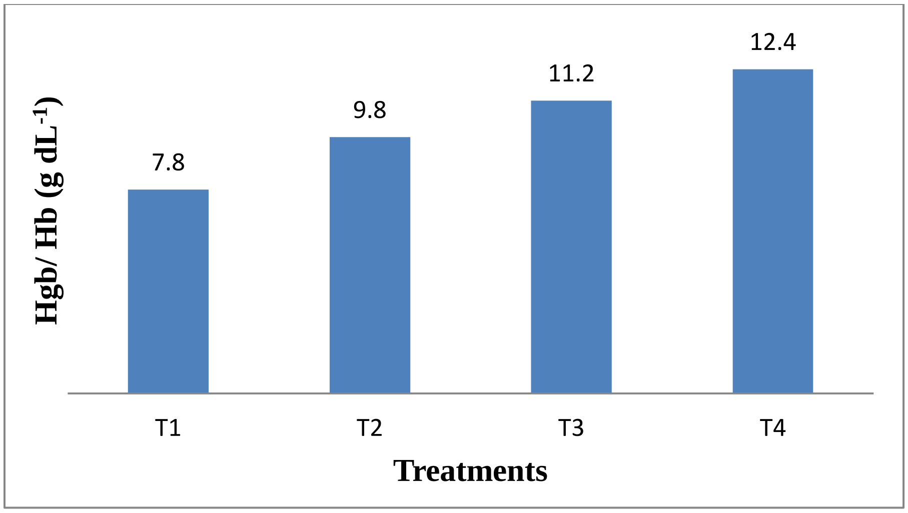

Figure 9: Hemoglobin (Hgb/Hb) measure *O. niloticus* blood in different treatments fed at different dietary levels of seaweeds (*H. musciformis*)

Source: (Nur, 2017)

3.4.4 Hematocrit (HCT) measure

In this study, hematocrit was increased in the T_2, T_3 and T_4 than Control. The highest hematocrit was measured in T_4 (36.8 %) at 4^{th} week and lowest in T_1 20.5 % at 1^{st} week (Table 9). Like hemoglobin, the hematocrit percentage is good indicator for the oxygen transportation capacity and the health status of the fish. Result of this study indicated a better immunity in seaweed treatments than control. (Nur, 2017)

Table 9: Hematocrit (HCT) measure from *O. niloticus* blood in different treatments fed at different dietary levels of seaweeds (*H. musciformis*)

Treatments	HCT (%)			
	1^{st} Week	2^{nd} Week	3^{rd} Week	4^{th} Week
T_1	20.5	22.3	24.4	27.2
T_2	22.4	24.5	26.9	30.2
T_3	24.7	26.4	28.6	32.9
T_4	26.2	28.1	31.5	36.8

Source: (Nur, 2017)

3.4.5 Cumulative Survival Rate

Kanimozhi *et al.,* (2013) was determined the effect of water extract of seaweeds *Sargassum whitti* on the immune mechanisms and disease resistance in *Mugil cephalus*. The Seaweed *(S. whitti)* was extracted by using hot water extract method. After extraction it was mixed with feed at different concentration. Fishes were feed with different doses of 0.5, 1.0 and 1.5% (kg $diet^{-1}$) body weight of water extract of seaweed. Relative Percent Survival (RPS) was assessed by a challenge with live *Pseudomonas fluorescence.* Cumulative survival rate after 24 days of challenge test, experiment group showed 80% survival and control showed 70% survival. After 120 days of experimental studies 70%, 80%, 65 %, and 30% survival were observed in 0.5%, 1%, 1.5% seaweed diet and Control group respectively (Figure 10).

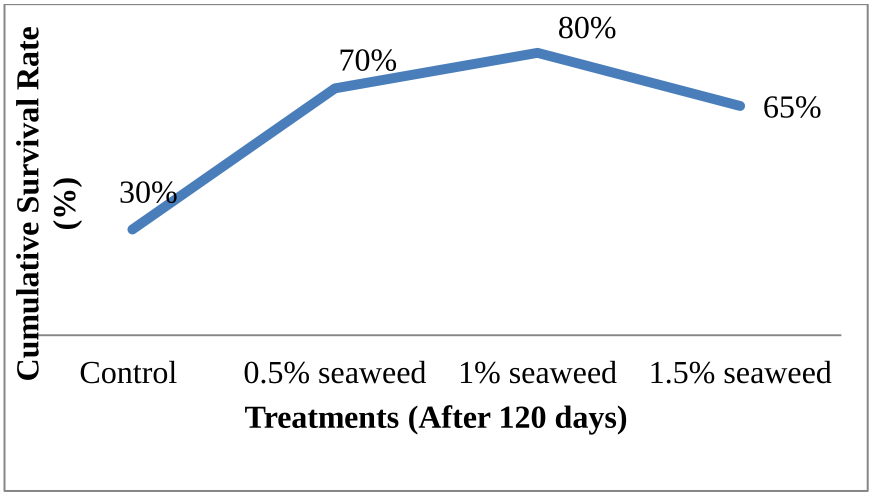

Figure 10: Cumulative survival rate of *Mugil cephalus* in different treatments fed at different seaweed diet

Source: (Kanimozhi *et al.*, 2013)

3.4.5 Lysozyme activity

Lysozyme is a fish defense element, which causes lysis of bacteria and activation of the complement system and phagocytes by acting as opsonin (Magnadottir, 2006).

This result indicated a better immunity in seaweed treatments than control. Fish fed with seaweed-containing diets feed at 1 and 1.5% lysozyme activities increased than control group (Kanimozhi *et al.*, 2013) (Figure 10).

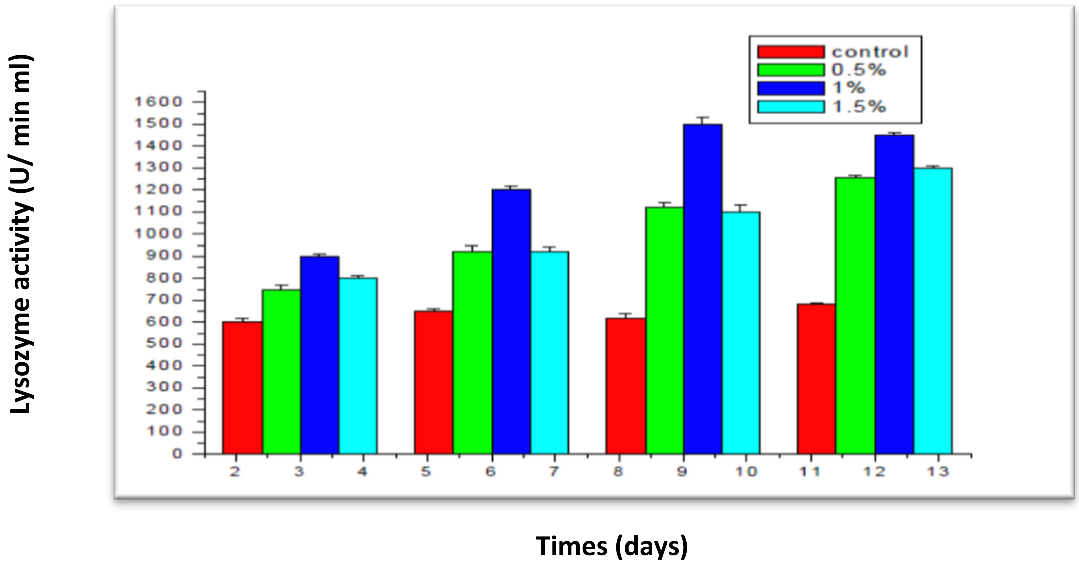

Figure 11: Lysozyme activity of *Mugil cephalus* in different treatments fed at different seaweed diet

Source: (Kanimozhi *et al.*, 2013)

3.5.6 Mean Corpuscle Volume (MCV) measure

Mean Corpuscle Volume of this study was measured where highest and lowest value was recorded in T_4 (152.6 fl) and T_1 (170.2 fl), respectively (Figure: 12). (Nur, 2017)

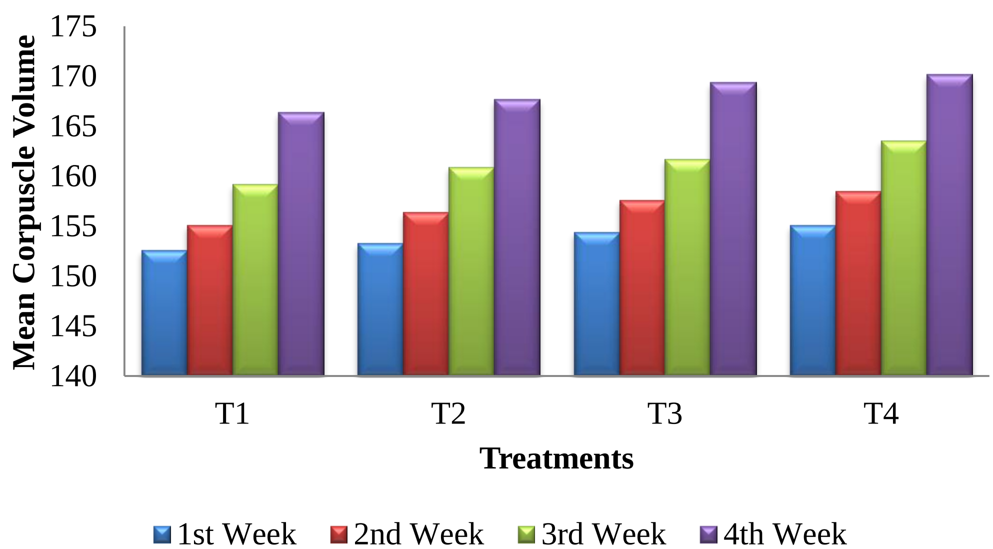

Figure 12: Mean Corpuscle Volume (MCV) of *O. niloticus* blood in different treatments fed at different dietary levels of seaweeds (*H. musciformis*)

Source: (Nur, 2017)

Here T_1: Control and T_2: 10%, T_3: 20% and T_4: 30% seaweed as fish meal supplement, respectively. This study concluded that seaweed enhances immune system of fish better than the commercial feed only.

3.4.7 Mean Corpuscle Hemoglobin Concentration (MCHC) measure

Radhika and Mohaideen (2016) chosen three types of seaweed for the study namely *Gracilaria corticata*, *Ulva lactuca* and *Stocheospermum marginatum*. The fish feed was formulated using 35% of seaweed. The fishes [Common carp (*Cyprinus carpio)* (weight 10±5g)] were divided into five groups where four group were infected with *A. hydrophila* (1.6×10^4 CFU $fish^{-1}$) and one groups remained uninfected and this group along with negative control group was fed with control feed only and the infected groups were fed with seaweed mixed experimental diet. After 28 days the highest MCHC value was found in *Gracilaria corticata* (30.54 g/L) and the lowest value was found in control (23.59 g/L) (Table 10).From this study we can understand that the fish fed with seaweed incorporated diet had good immune stimulants properties.

Table 10: MCHC value of *Cyprinus carpio* fed at different seaweed

Treatments	Control	*S. marginatum*	*U. lactuca*	*G. corticata*
MCHC (g/L)	23.59	27.67	29.03	30.54

Source: (Radhika and Mohaideen , 2016)

MCHC showed highest result in seaweed supplemented feed with compare to control. MCHC values are not measured directly, but calculated based on the hemoglobin value (Hgb). Abnormally high or low levels of MCHC determined by blood testing, results can be an indication of a number of problems within the body which are ranging from nutrient deficiencies to chronic diseases. In this study, the highest and lowest value of MCHC was measured in T_4 (30.3 g L^{-1}) and T_1 (22.4 g L^{-1}), respectively (Figure: 12). Erythrocytes are containing the normal value of hemoglobin (normal MCHC) are called normochromic. When the MCHC is abnormally low they are called hypochromic, and when the MCHC is abnormally high called hyper chromic. This study MCHC range indicated that it is in normochromic range. (Nur, 2017)

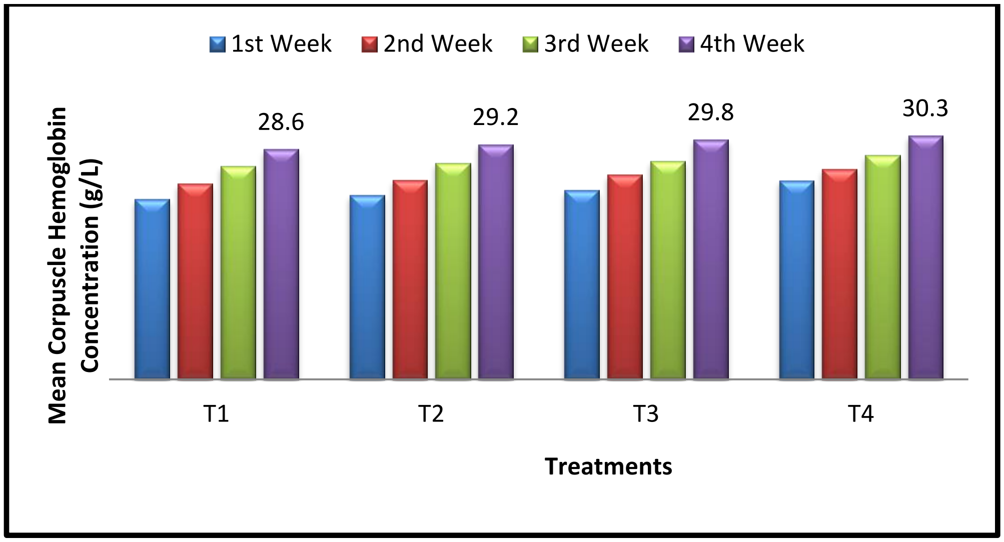

Figure 13: Mean Corpuscle Hemoglobin Concentration (MCHC) of *O. niloticus* blood in different treatments fed at different dietary levels of seaweeds (*H. musciformis*)

Source: (Nur, 2017)

From the above result, seaweed supplemented feeds are showed best result compared to control feed which increased the total aquaculture production and immune system of fish.

CHAPTER 4

CONCLUSION

In conclusion, this study establishes a strong base for seaweed with the aim of better growth and immune system of fish.

- The effect of seaweed as a feed ingredient in fish diet showed significant results on growth performance of fish. This study indicates that seaweed supplemented feed for growth performance of fish was higher and effective than commercial feed.
- The fish immune system is responsible for destroying microorganisms through acquired and innate components, with humeral and cellular process that perform together in an attempt to prevent the outbreak of diseases. Complete Blood Count (CBC), which evaluates the composition of the blood that based on RBC, WBC, HB, hematocrit values, MCV, MCH, and MCHC, has provided valuable information for fishery biologists in the assessment of fish health. All the hematological parameters were showed highest result in seaweed supplemented feed with compare to control which indicated the increasing of immune mechanism.

From this study, it can be suggested that seaweed is a good food ingredient for aquaculture and also it can enhance the growth as well as the immune system of fishes.

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