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Effect of selected seaweed powder as a fish feed on growth and immune system of tilapia (*Oreochromis niloticus*)

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Abstract
Formulating new fish feed is one of the major challenges for profitable aquaculture. With this view, this study was designed to find out the effect of seaweed as replacement of fish meal on growth and immune response of Tilapia. This experiment was carried out in earthen pit with four treatments (T_1: Control, T_2: 10%, T_3: 20% and T_4: 30% seaweed as fish meal replacement) with three replications of each. Fishes were reared in very small earthen ponds called pits for 60 days. Results found that, the average weight gain, the relative percent (%) increment of weight gain and specific growth rate (SGR % day^{-1}) was highest in T_4 followed by T_3, T_2 and T_1 (P<0.05). Furthermore, feed conversion ratio (FCR) of experimental Tilapia in T_1, T_2, T_3 and T_4 was 1.27±0.35, 1.15±0.18, 1.12±0.07 and 1.02±0.28, respectively which was significantly different (P>0.05). Regarding the immune response of Tilapia, the blood parameters such as red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH) and mean corpuscle hemoglobin concentration (MCHC) were measured. Results found that the highest number of RBC (3.23×10^6 cells mm^{-3}) and WBC (12.86×10^3 cells mm^{-3}) were found in T_4 along with other blood parameters compared to T_3, T_2 and T_1. So, it can be inferred that seaweed has positive impact on growth and immune system of fish.

Keywords: Seaweed, fish meal, tilapia, growth, immune system

1. Introduction
In the beginning, Bangladesh used to rely on capture fisheries rather than culture fisheries in regard of fish production. But gradually culture fisheries got popular and took the place of capture fisheries. In recent years aquaculture fisheries contribute almost 28% higher than that of capture fisheries to total fish production. Now Bangladesh is 5th in position in freshwater aquaculture [1]. For higher production aquaculture intensification is being done but there is a problem of sustainability. Feed, seed and disease infestation are major threats of aquaculture intensification. Quality feed and seed is the prerequisite for successful fish culture. So, seed production in hatchery and functional feed are very significant in this context. Another perspective is that different commercial feeds that are usually used by the farmers and hatchery owners focused more on growth rather than immunity of fishes. As a result, in the hatcheries of Bangladesh, survivability rate of fingerlings is very low because of immune compromised broodfish. Even the fingerlings which pass through the hatchery period mortality occurs in grow out stage and their growth rate then 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 feedstuffs having various nutritional factors and immune enhancing capability that could be used as a growth promoter alongside immune enhancer For this aspect seaweed can be one of the best options as it is nutritionally enriched and available in our country. Seaweeds are considered as rich sources of bioactive compounds as they are able to produce a great variety of biological activities not only against human pathogens but also against fish pathogens [2, 3]. Now a days seaweed is an alternative plant feed stuff that is increasingly being used in aqua feeds because of it's nutritional quality, lower cost and availability. Some scientists have already worked on it and got positive results [4, 5, 6]. There are 193 seaweed species including 19 commercially important species, belonging to 94 genera, found in

Bangladeshi coast, mostly in St. Martin Island, Cox's Bazar and Sundarbans Mangrove forest [7]. In spite of having variety of seaweeds in Bangladesh it is yet to be used as fish feed. So, it will be a milestone in fisheries sector of Bangladesh if seaweed is incorporated in aqua feedstuffs that not only boost up the production but also ensure the long overdue sustainability. By regarding all, this study was carried out to determine the effect of seaweed as feed on the growth and the immune response of Tilapia *(O. niloticus)*.

2. Materials and Methods

2.1 Experimental site and design of experiment

The experiment was conducted in the backyard of Faculty of Fisheries, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur. It was conducted for a period of 60 days to determine the effect of selected seaweed powder as a supplement of fish feed on growth and immune system of Tilapia (*O. niloticus*) in a randomized completely block design (RCBD) into four different treatments having three replications each containing 75 fish (Table 1).

Table 1: Experimental design of Tilapia (*Oreochromis niloticus*) fry rearing

Treatment	Pit size (ft^3)	No. of fish/pit	Feed replaced by seaweed (%)
T_1	75	75	0
T_2	75	75	10
T_3	75	75	20
T_4	75	75	30

2.2 Experimental fish

Tilapia fish was selected because of its ability to tolerate adverse situation in accordance with its availability. Uniform sized fry of tilapia was selected for each pit.

2.3 Selection and collection of Seaweed (*H. musciformis*)

Among 194 seaweed species found in Bangladesh red seaweed *H. musciformis* was selected for the experiment due to their availability and low costing. It has plenty of essential nutrients, especially trace elements and several other bioactive substances. It contains very high-quality protein and has all the essential and non-essential amino acids and that's why we selected the red seaweed species *(H. musciformis)* for this research. Seaweed was collected from the St. martins island of Cox's bazar district (Figure 1)

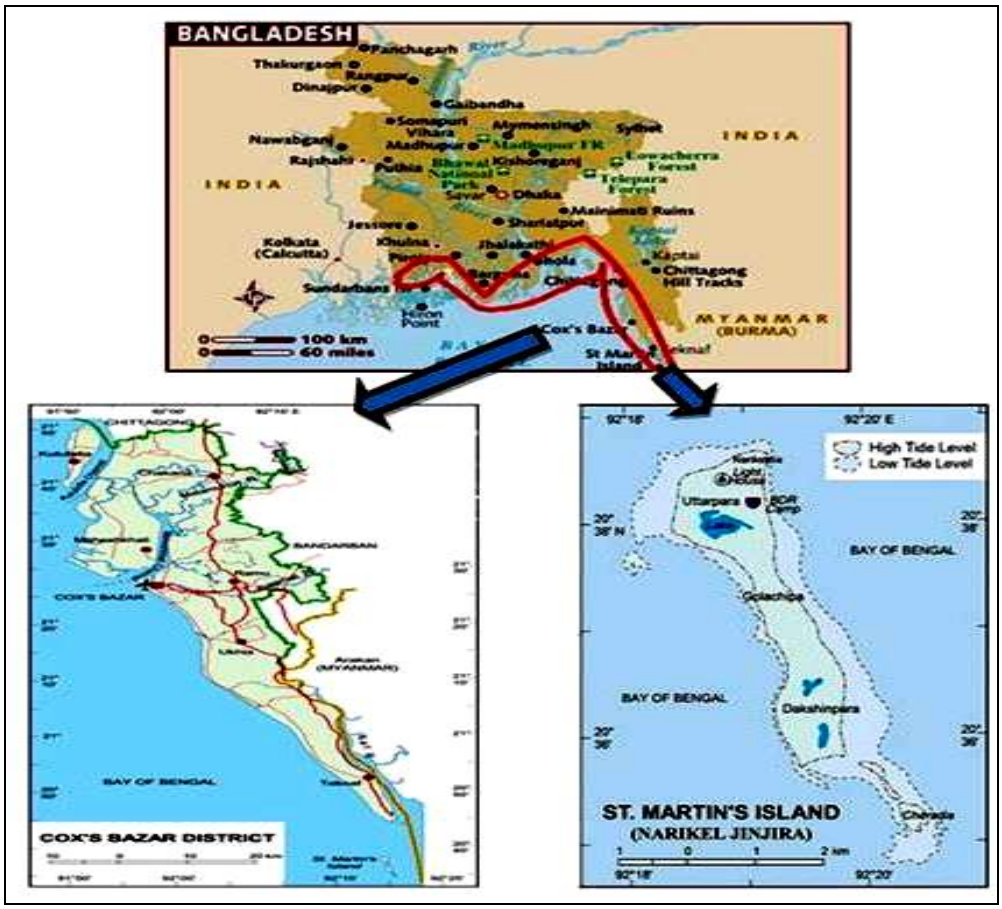

Fig 1: Seaweed collection area

2.4 Experimental feed formulation and proximate composition analysis

Experimental feed was formulated in the laboratory of the department of Fisheries Biology and Aquatic Environment (FBE) at Fisheries Faculty of BSMRAU. At first, all ingredients were collected before feed preparation such as dried fish meal, mustard oil cake, rice bran, wheat bran and seaweed. Feeds were prepared with seaweed ingredients at three different levels of replacement like 10%, 20% and 30% respectively, where control was prepared by commercial feed solely (Table 2). The feed was formulated by the Pearson square method. Then, the ingredients were mixed together.

Table 2: Percentage of feed ingredients used in Experimental diets

Ingredients	Control Feed	10% Seaweed replacement feed	20% Seaweed replacement feed	30% Seaweed replacement feed
Fish Meal	36.37	32.73	29.10	25.46
Seaweed	0.0	3.64	7.27	10.91
Rice Bran	27.78	27.78	27.78	27.78
Wheat Bran	14.15	14.15	14.15	14.15
Mustard Oil Cake	19.19	19.19	19.19	19.19
Ata (Binder)	2.5	2.5	2.5	2.5

The mixture was slowly mixed with hot-water (80 °C) in a proportion of 70:30 (v/w) to accomplish agglutination. The dough was passed through a meat chopper (Brand-Filizola) to obtain pellets of 2 mm diameter and dried for 3 days under the sunlight and then stored at room temperature. Then, nutritional properties of formulated feed were analyzed in lab of Department of Aquaculture; Bangladesh Agricultural University, Mymensingh (Table 3).

Table 3: Results of Proximate Composition analysis of feed (% Moisture basis)

Name of item	Moisture	Crude lipid	Crude protein	Ash	Crude fiber	Carbohydrate
Control	11.60	9.55	41.50	10.82	4.20	22.33
10% Supplemented	11.83	8.20	38.63	13.89	4.88	22.57
20% Supplemented	13.10	6.83	31.10	15.57	6.20	27.20
30% Supplemented	15.02	4.32	27.21	17.03	7.45	30.37

2.5 Setting up of pit and rearing of fish

The experiment site was cleanedup first and the plants and weeds were manually removed from the site. To set up the pit (small earthen pond), the soil was excavated in which length and width were 5 ft each and depth was 3 ft. Tilapia (*O. niloticus*) fry were collected from a commercial fish hatchery. Fish were reared in Pit for acclimatization. In the beginning of the experiment, fishes were weighted individually and uniform sized fishes were selected. There were 61 fishes per pit. Triplicate groups of fish were fed with control and seaweed supplemented feed during experimental period. The fish was fed twice a day near satiation.

2.6 Fish sampling and weighing

Sampling was done fortnightly for monitoring growth parameters. Fish were harvested by scoop net and randomly 25-30 fish were weighted by digital electric balance to measure different growth parameters.

2.7 Monitoring water quality

pH, Ammonia (mg l^{-1}), Dissolved oxygen (mg l^{-1}) and Temperature (°C) of each pit was measured daily at 8 am by using digital device.

2.8 Growth parameter

Following formulas were used to evaluate the growth parameters:

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

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

$$\frac{\text{Mean final fish weight} - \text{Mean initial fish weight}}{\text{Mean initial fish weight}}$$

$$\text{Percent (\%) of weight gain} = \frac{\text{Ln } W_2 - \text{Ln } W_1}{\text{Time}} \times 100$$

$$\text{Specific growth rate (SGR \% per day)} = \frac{\text{Log W2} - \text{Log W1}}{\text{Time}} \times 100$$

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)

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

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

2.9 Collection and preservation of blood

Blood was collected after the experimental period was over. Before blood collection, the needle was soaked into 2% heparin solution as an anticoagulant. Blood was collected by syringe from the caudal peduncle of fish. After that the blood was transferred into the EDTA (Ethylene Diamine Tetra Acetic Acid) tube and stored at 4 °C for determining the haematological parameters.

Haematological parameters like Leukocyte (WBC), erythrocyte (RBC), hematocrit (Hct), hemoglobin (Hb), MCV, MCH and MCHC were determined in the FBE laboratory of BSMRAU.

2.9.1 Determination process of haematological parameters

Red blood cell (RBC) and white blood count (WBC): Red blood cell (RBC) and white blood cell (WBC) were counted using a haemocytometer with Neubauer counting chamber as described by Blaxhall 1972 [8]. The following formula was used to calculate the number of RBC and WBC per milliliter of the blood sample: Number of cells = (Number of cells counted × dilution) / (Area counted × depth of fluid).

Hemoglobin (Hb): Blood haemoglobin concentration (Hb) can be measured on microliter blood samples and has been used widely and arguably. The most accurate method of determining Hb Drabkin's method [9] was used to determine Hb.

Hematocrit (Hct): Hematocrit (Hct) was determined by means of centrifugation. The pipettes containing samples were centrifuged for 3 min with a speed of 3000 rpm and placed on the reading device and read-off.
MCV, MCH and MCHC was determined using a method originally devised by Yokoyama 1947 and later on modified by Christensen *et al.*, (1978) [10].

3. Results and Discussion

3.1 Water quality parameters

The average temperature, pH, ammonia and dissolve oxygen level during the experimental period was 28 ^{0}C, 7.58, 0.4 mg/l and 5.54 mg/l, respectively. However, during experimental period, the water quality parameters of experimental sites did not differ significantly (P>0.05).

3.2 Growth performance of *O. niloticus*

3.2.1 Average weight gain and Specific growth rate (SGR)

In this study, the average final weight gain of *O. niloticus* was ranged from 9.95±0.05g to 12.49±0.09g (Table 4). Highest result was recorded in T_4 (12.49±0.09g) and the lowest in T_1 (9.95±0.05g) which had significant difference (P<0.05). With other treatments, Seaweed supplemented feed showed the best result in terms of growth performance of *O. niloticus*. Specific growth rate (% day^{-1}) of *O. niloticus* was higher in T_4 (5.67±0.06g) than control (4.79±0.15g), which differed significantly (P>0.05). Resley *et al.*, (2009) [11] reported that average weight gain and SGR value as 8.9 to 13.5g and 4.7 (% day^{-1}) respectively in the growth and survival of juvenile cobia, in a recirculating aquaculture system. Rakocy *et al.* [12], (2006) found that SGR value was 4.4 (% day^{-1}) in an intensive Nile Tilapia in basil aquaponic production system. So, the result of this study indicated that the overall growth performance of seaweed supplemented feed was highly satisfactory.

Table 4: Average weight gain and Specific growth rate (SGR) of *O. niloticus*

Treatment	Average weight gain(g)	Specific growth rate (%)
T_1	9.95 ± 0.05	4.79 ± 0.15
T_2	11.69 ± 0.21	5.13 ± 0.08
T_3	11.72 ± 0.32	5.29 ± 0.12
T_4	12.49 ± 0.09	5.67 ± 0.06

3.2.2 Relative percent (%) increment of weight gain

Relative percent increment of weight gain of T_1, T_2, T_3 and T_4 were 78.2±0.32 84.26±0.31, 91.17±0.38 and 103.2±0.11%, respectively, which was significantly different (P<0.05) at each treatment (Figure 2).

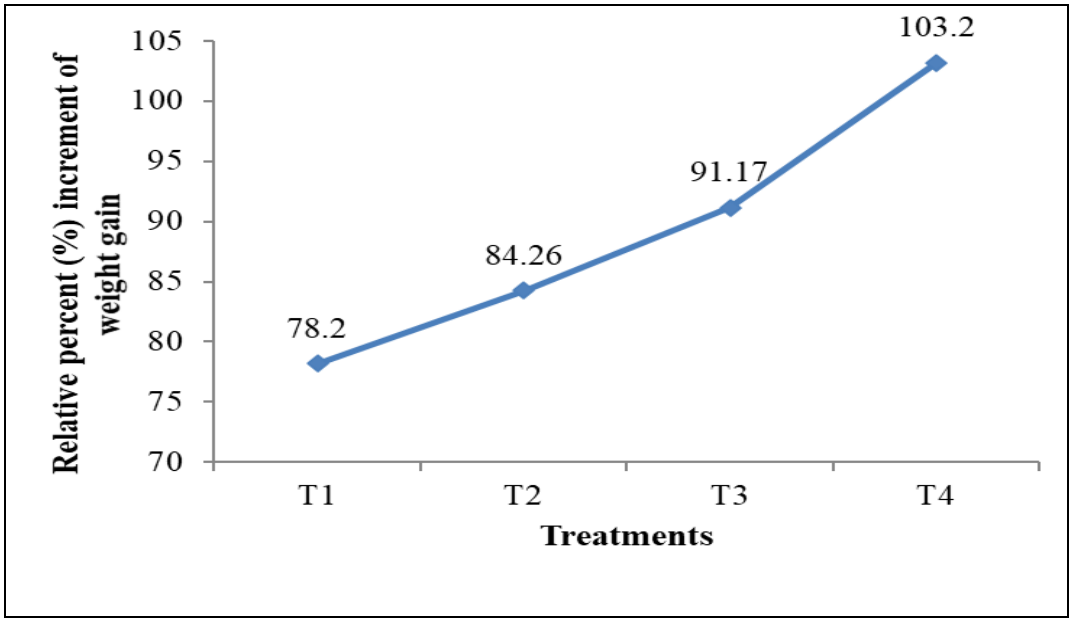

Fig 2: Relative percent (%) increment of weight gain of *O. niloticus*

Salam *et al.*, (2014) [13] conducted an experiment on nutrient recovery from fish farming wastewater in aquaponics system for plant and fish integration and found that mean weight gain (%) of Tilapia was 926.18% using spinach (*Ipomoea aquatica*) for 115 days. This indicated that the total aquaculture production of fish was increased than control.

3.2.3 Feed conversion ratio (FCR)

FCR value of T_1, T_2, T_3 and T_4 was 1.27±0.35, 1.15±0.18, 1.12±0.07 and 1.02±0.28, respectively. FCR of *O. niloticus* fed with seaweed supplement feed differed significantly (P<0.05) from control (Figure 3).

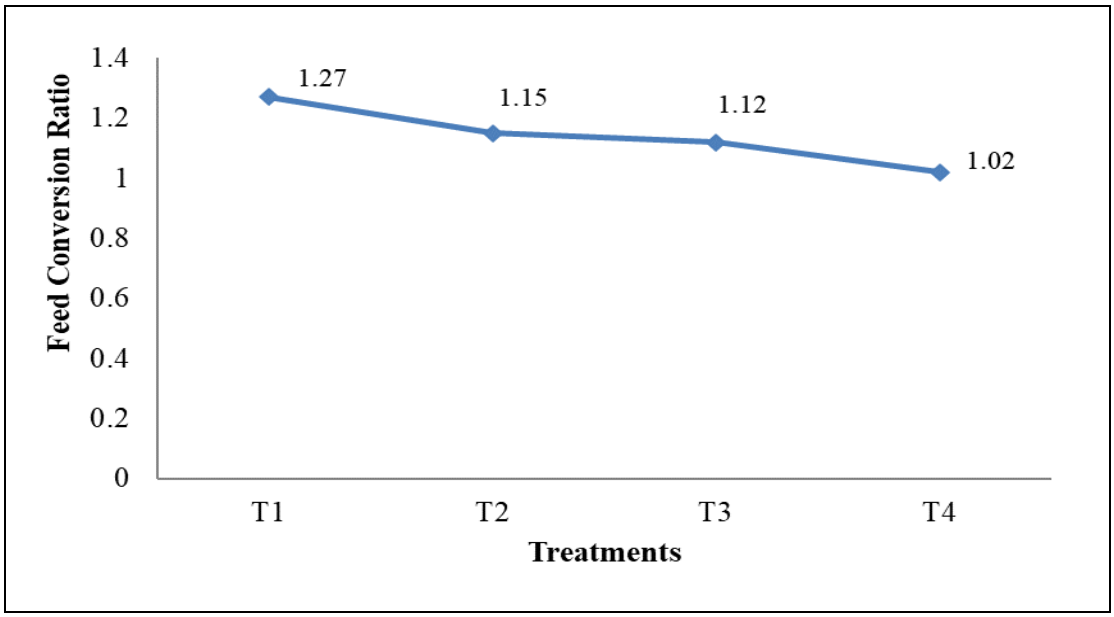

Fig 3: Feed conversion ratio (FCR) of *O. niloticus*

Cruz and Ridha (2001) [14] conducted an experiment on growth and survival rates of Nile Tilapia (*O. niloticus*) juveniles reared in a recirculating system fed with floating and sinking pellets and found that the FCR value of the sinking pellets (2.03) was significantly lower than the floating pellets (2.48) because the protein percentage of sinking pellets was significantly higher than floating pellets. Watanabe *et al*., (2002) [15] studied that the FCR value of Tilapia was 1.5-2.0. In this study, the FCR value for Tilapia was better than control and in suitable range.

3.2.4 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. 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. HSI of *O. niloticus* fed with seaweed supplemented feed differed significantly (P<0.05) compared with control (Figure 4). Ogunji *et al*., (2008) [16] evaluated the growth performance and nutrient utilization of Nile Tilapia (*O. niloticus*) where found that the hepatosomatic index of fishes were 3.08 and 3.64 fed with fish meal and mageal. Resley *et al*., (2009) [11] obtained HSI value of 2.4 to 3.5 in a recirculating aquaponic system of Cobia juveniles. These previous results support the hepatosomatic index value of this study.

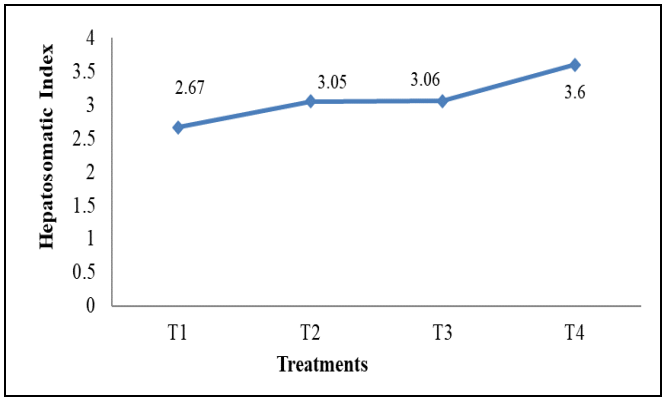

Fig 4: Hepatosomatic Index (HSI) of *O. niloticus*

3.3 Immune response of *O. niloticus* in pit culture system

Complete Blood Count (CBC), is the evaluation of the composition of the blood based on RBC, WBC, HB, Haematocrit values, MCV, MCH, and MCHC. These parameters provides the valuable information for fishery biologists in the assessment of fish health.

3.3.1 Red blood cell (RBC) and white blood cell (WBC) count

The highest RBC was counted in T_4 (3.23×10^6) and lowest in T_1 (2.22×10^6) (Table 5). According to Radhika and Mohaideen (2016) [17], there was a gradual increase in the RBC count from the 1st day to the 28th day where the maximum RBC value observed in the fish fed with *G. corticata* diet on the 28th day (3.67×10^6 cells mm^{-3}) than *S. marginatum* (2.03×10^6 cells mm^{-3}) and *U. lactuca* (1.50×10^6 cells mm^{-3}). 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.

Table 5: RBC and WBC count in different treatments

Treatment	RBC (cells mm^{-3})	WBC (cells mm^{-3})
T_1	2.22×10^6	12.36×10^3
T_2	2.47×10^6	12.42×10^3
T_3	2.98×10^6	12.73×10^3
T_4	3.23×10^6	12.86×10^3

The highest WBC was counted in T_4 (12.86×10^3 cells mm^{-3}) and lowest in T_1 (12.36×10^3 cells mm^{-3}) (Table 4). According to Radhika and Mohaideen (2016) [17] WBC level also gradually increased in 1st day to 28th day. The values being high for the Red seaweed *G. corticata* (21.67×10^3 cells mm^{-3}) was noted and lowest level was found in control feed (18×10^3 cells mm^{-3}). Francesco F *et al.*, (2012) [18] counted the WBC level that gradually increased in that study where highest and lowest value of WBC was found 20.00×10^3 and 16.30×10^3 cells mm^{-3}, respectively. WBC was increased gradually which indicated the good sign of immunity and prevented the fish from invasive bacteria and virus.

3.3.2 Hemoglobin (Hgb/Hb) measure and Hematocrit (HCT) measure

In this study, hemoglobin was increased in the T_2, T_3 and T_4 than T_1. The highest hemoglobin was measured in T_4 (12.4 g dL^{-1}) and lowest in T_1 (7.8 g dL^{-1}) (Figure 5, A). Hemoglobin was measured highest in seaweed fed fish [17.18]. 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.

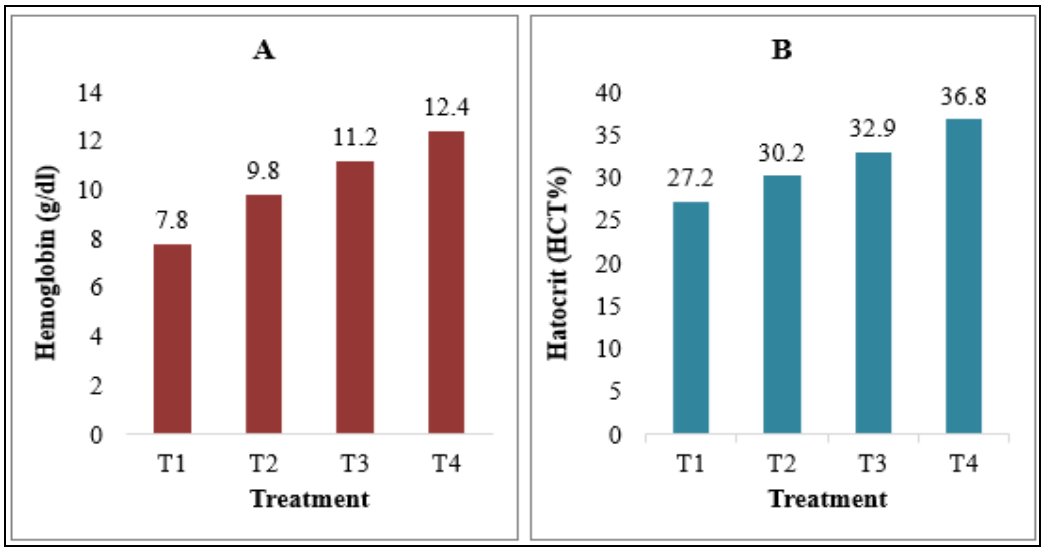

Fig 5: (A) Hemoglobin and (B) Hematocrit measurement of *O. niloticus*

In this study, the highest hematocrit was measured in T_4 (36.8 %) and lowest in T_1 20.5 % (Fig 5, B) that is supported by Francesco F *et al.*, (2012). [18] Like hemoglobin, the hematocrit percentage is good indicator for the oxygen transportation capacity and the health status of the fish. From this above discussion, result of this study was in suitable range and indicated a better immunity than control.

3.3.3 Mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH) and mean corpuscle hemoglobin concentration (MCHC) measurement

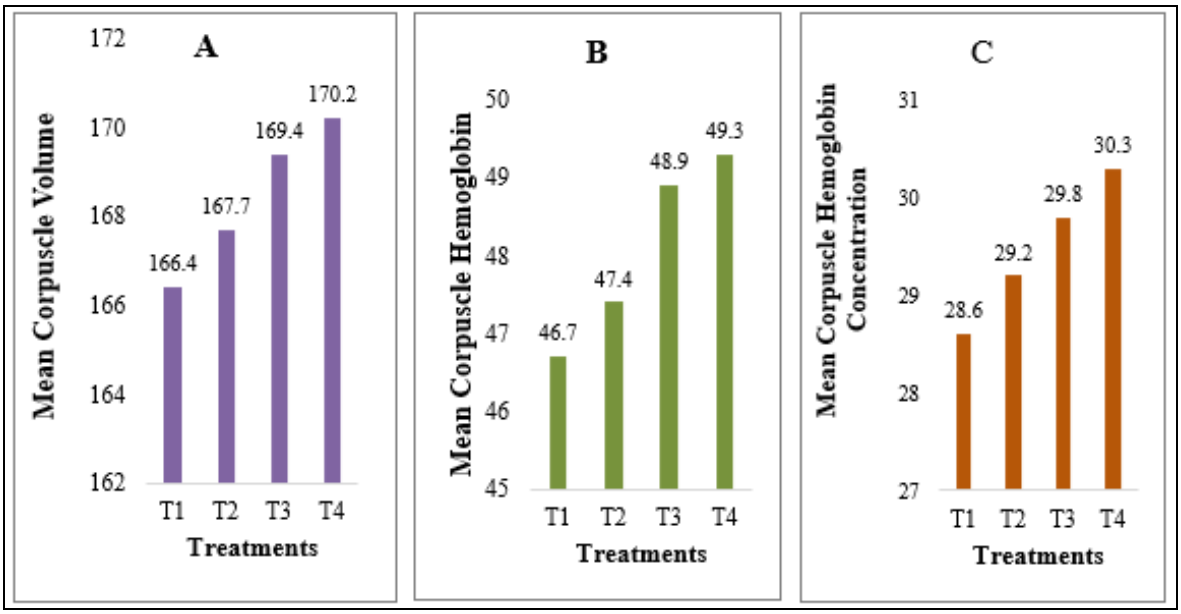

Fig 6: (A) Mean corpuscle volume, (B) Mean corpuscle hemoglobin (MCH) and (C) Mean corpuscle hemoglobin concentration of *O. niloticus*

The highest and the lowest mean corpuscle volume (MCV) value was recorded in T_4 (170.2fl) and T_1 (166.4 fl), respectively (Figure: 6, A). According to Radhika and Mohaideen (2016) [17], mean corpuscular volume (MCV) of the fishes treated with seaweeds *G. corticata* (72.78 fl), *S. marginatum* (92.81 fl) and control (81.67 fl) were lower when compared to the positive control (127.78 fl) and *Ulva lactuca* (128.22 fl). Similar type of result was also found by Francesco, F. *et al.*, (2012). [18] From above discussion, this study was in suitable range and indicated a better immunity than control.

The highest MCH was measured in T_4 (49.3 pg) and lowest in T_1 (46.7 pg) (Figure: 6, B). It depends on hemoglobin (Hb) synthesis when it decreases Hb synthesis reduces and causes anemia. If it is increased, the MCH value is able to diminish in hypochromic anemia.

In this study, the highest and lowest value of MCHC was measured in T_4 (30.3 g l^{-1}) and T_1 (28.6 g l^{-1}), respectively

(Figure: 6, C). In terms of MCH and MCHC, results are similar to the result found by Radhika and Mohaideen (2016) and Francesco, F. *et al.*, (2012) [17, 18]. But in this research, the blood parameter was in good range which increased the overall immunity of the fish. Erythrocytes 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. In this study MCHC range indicated that it is in normochromic range.

4. Conclusion

This study has established a substantive base for incorporating seaweed as a fish feed with the aim of better growth and immune system of fish. This study indicates that seaweed replaced feed has a better growth performance. On top of that all the haematological parameters observed also showed highest result in seaweed replaced feed compared to control which indirectly indicates the better performance of immune mechanism. A positive correlation between percentage of seaweed increment in feed and better growth besides immune response has been observed. Further study is imperative to optimize the rate of seaweed replacement in fish feed stuffs and select the best seaweed. However, it can be recommended that seaweed could be used as an essential feed ingredient for fish feed production.

5. Acknowledgement

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