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Research Article

Spirulina effect on Oreochromis Mossambicus for Better Human Health

Effect of Arthrospira Platensis (Spirulina) and Azolla Pinnata on Growth Performance and Hematology of the Freshwater Fish Oreochromis Mossambicus (Tilapia)

M.Sakthivel[1*] and J.Prakash Sahaya Leon[2]

[1,2]Department of Zoology, Government Arts College for Men, Krishnagiri, Tamilnadu, India,
Affiliated to Periyar University, Salem

Abstract: Aquaculture is identified as the viable alternative source, for increasing fish production to meet out ever growing demand for fish consumption. Due to their high nutritive value, toxic-free, environment friendly and high productivity Azolla and Spirulina were considered as promising feed. The knowledge of hematological and growth characteristics is an important tool that can be used as a sensitive and effective index to monitor pathological and physiological changes in fish. Consumption of Azolla and Spirulina by fish can have direct or indirect effects on human health. In the present study, a survey was conducted in humans to analyze the effect of Azolla, Spirulina through fish tilapia consumption and also to analyze the hematological and growth changes the fish *Oreochromis mossambicus* were exposed to different concentrations of spirulina and Azolla with commercial fish feed. Fishes were selected for the weight of (21gram) and length of (11cm) for the present experiment. In the control group, 6 fishes were exposed to 20 grams of commercial fish feed. In the first group T1 (experiment 1) 6 fishes were exposed to 8 grams of Azolla with 12 grams of commercial fish feed, in the second group T2 (experiment 2) 6 fishes were exposed 8 grams of Spirulina with 12 gram of commercial fish feed and in the third group T3 (experiment3) 6 fishes were exposed 4 grams of Azolla and 4 grams of Spirulina with 12 gram of commercial fish feed for 60 days. In the present investigation, growth level was increased when fish *Oreochromis mossambicus* was exposed to different concentrations of Spirulina and Azolla with commercial fish feed, and also significant differences were observed in the blood parameters like white blood cells (WBC), Red blood cells (RBC), Hemoglobin (HB), MCV and MCH. A questionnaire-based dietary survey of tilapia consumers were collected in detail through face-to-face interviews at fish shops located nearby KRP Dam, Krishnagiri district. Statistical data were analyzed using SPSS software and all tests were considered significant at p<0:05. Main objective of the present study is to analyze the growth and hematological effect of Azolla, Spirulina in fish tilapia and also to identify the fish (*Oreochromis mossambicus*) consuming people through questionnaire-based survey. It is concluded that more people consumed fish tilapia as a common food and fish fed with azola and spirulina showed rich nutritive value. So, the present study recommended that, if the fish tilapia is fed with azolla and spirulina, this may increase the nutritive states of human populations via fish Tilapia.

Keywords: Tilapia (*Oreochromis mossambicus*), Spirulina, Azolla, Hematology, Growth, RBC, WBC, Hb, MCV, MCH, Human, questionnaire, Survey.

***Corresponding Author**

M.Sakthivel , Department of Zoology, Government Arts College for Men, Krishnagiri, Tamilnadu, India, Affiliated to Periyar University, Salem.

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1. INTRODUCTION

Aquaculture has the greatest potential to meet the increasing demand for aquatic food and it is one of the fastest growing food producing sectors.[1] Supplemental feeding is an important measure for the management in intensive aquaculture for enhancing production of fish. Utilization of aquatic plants produces high food value and gives more animal protein at low cost. Fish tilapia is considered the most intensive cultivated freshwater fish in India. In culturing of various fish, the use of algae as a feed additive might help in effective usage of its diet.[2]*Spirulina* is one of the most widely used micro algal species in aqua feeds, due to its high contents of proteins, minerals, essential amino acids and pigments of antioxidant.[3] Spirulina is the most nourishing food source in the world.[4] *Spirulina* can be considered a feed supplement for animals having economic profit and nutritional supplement that has various health benefits for humans. It has been found that the alga can be used as another source of protein and can also be used to improve the taste and quality of meat. For initiating immune-potentiating function in carp *Spirulina* can be used.[5] Because of its high productivity, high nutritive value and ease of cultivation *Azolla pinnata* is considered to be a promising feed.[6,7] The recognition of growth and hematological characteristics (RBC, WBC, Hb, MCV, MCH) are the important tools that can be used as effective monitoring physiological and pathological changes in fishes.[8] Study of hematology in fish have diagnostic economic significance. For fisheries biologists, in recent year's fish hematology has become an important tool.[9,10] According to a recent study, omega-3s which are present in fish can help reduce high blood pressure, strokes, and atherosclerosis and prevent heart attacks[11,12]. Fish tilapia contains antioxidants and selenium that treat heart-related diseases and fight against cancer[13]. Consuming tilapia increases neurological function and also boosts brain function[14]. Tilapia contain selenium improves the hormonal functions and plays an important role in the regulation of the thyroid gland[15]. Fish tilapia is good for bones. It contains minerals such as phosphorus calcium that are used for bone maintenance and growth. The main objective of the present study is to analyze the growth and hematological effect of Azolla, Spirulina in fish tilapia and also to identify the fish (*Oreochromis mossambicus*) consuming people through questionnaire-based surveys.

2. MATERIALS AND METHOD

Fish *O. mossambicus* (Tilapia) *were* collected from the Barur Tilapia Research Center, located at Krishnagiri, Tamilnadu, India. The collected fish were transported in air filled polythene bags with water. The fish brought to the laboratory were disinfected with 0.1% potassium permanganate solution and were maintained for three weeks in a well-aerated tap water cemented tank for the acclimatization. For the sign of disease, stress, physical damage and mortality the test fish were critically screened. To maintain the optimum dissolved oxygen level, the experimental water was renewed every 24 hr. While conducting the experiments, to prevent any deviation from the modified main principle of bioassay techniques outlined by Sprague, care was taken[16] and was recommended by APHA.[17] The data variance and significance were analyzed by the test of DMRT and ANOVA.[18]

2.1 Criteria of Water

In all the experiments De chlorinated well water was used as test water. The following were the conditions of experimental water (Committee on Water Quality Criteria)[19]

Table. 1.Experimental water parameters

Parameters	Unit	Value
pH	-	7.3 to 7.5
Temperature	°C	27 ± 1
Alkalinity as $CaCO_3$	mg/L	255 to 260
Dissolved Oxygen	mg/L	7.2 to 7.4
Total hardness as $CaCO_3$	mg/L	300 to 320
Salinity	mg/L	0.42 to 0.49

(mg/L– milligram per liter)

2.2 Experimental Procedure

2.2.1 Dietary Survey

A survey was taken (weekly basis) for 2000 healthy human beings from the common population at different fish shops located nearby KRP Dam at Krishnagiri district, Tamilnadu. Dietary data were collected only on tilapia consuming people. Details of fish tilapia intake were collected from the human population and computed.

2.2.2 Experiments

Fishes were selected for the weight of 21gram and length of 11cm for the present experiment. In the control group 6 fishes were exposed to 20 gram of commercial fish feed. In the first experimental group (Exp-1) 6 fishes were exposed 8 grams of Azolla with 12 gram of commercial fish feed, in the second experimental group (Exp-2) 6 fishes were exposed 8 grams of Spirulina with 12 gram of commercial fish feed and in the third experimental group (Exp-3) 6 fishes were exposed 4 grams of Azolla, 4 gram of Spirulina and 12gram of commercial fish feed for the period of 60 days. After the experimental period of 60 days, growth (length and weight) and hematological (RBC, WBC, Hb, MCV and MCH) changes were analyzed in the fish *Oreochromis mossambicus* treated to various concentration of Azolla and spirulina with formulated fish feed and compared with control group.

2.2.3 *Experimental Setup*

Experimental work in Aquaculture Laboratory

2.2.4 *Feed Percentage*

Experiment 1 is 60 % commercial fish feed and 40% Azolla.
Experiment 2 is 60% commercial fish feed 40% Spirulina.
Experiment 3 is 60% commercial fish feed, 20% Azolla and 20% Spirulina.

2.2.5 *Growth Parameters*

Performance of growth of fish in all replicates was weighed independently and body weights of them were measured. According to Goodwin et al., specific growth rate (SGR) is calculated.[20]

$$SGR = \frac{\text{Ln [final mean body weight (g)] - Ln [initial mean body weight (g)]}}{\text{Time interval (days)} \times 100}$$

$$CF = \frac{\text{Weight (g)}}{\text{Length (cm)}^3}$$

2.3 *Analysis of Hematology*

Five fish per control and the entire experimental group (Exp-1, Exp-2, and Exp-3) were randomly chosen and anesthetized with tricaine methanesulfonate (20 mg/L). The blood sample was extracted from the caudal vein and collected in an Eppendorf tube along with EDTA anticoagulant. Neubauer's hemocytometer is used for the total count of WBC, RBC, and for Hb estimation.[21]

2.4 *Assessment of Hemoglobin Concentration (HB %)*

Sahli's Acid Hematin Method was used for estimation of hemoglobin.[22] A drop of (0.1 ml) blood and 0.1 N Hydrochloric acid which was taken up to the mark of 20 in the graduated tube was added and allowed to stand for 5 minutes until it changes to dark brown in color. The solution was diluted until it matches with standard color by adding distilled water drop by drop (mixing the solution with a stirring rod). Then reading was obtained from the scale on the graduated tube and the hemoglobin concentration was expressed as gram percent.

2.5 *Estimation of Red Blood Cells (RBC)*

After blood dilution, the RBC counting can be done manually as per the method given by Mukherjee.[23] The ruled areas of the hemocytometer at 5 centers are counted for counting total RBC and total number of RBC is measured as number/cubic millimeter. Corner small squares of hemocytometer i.e. 16 x 4 = 64 sq.mm are counted for counting white blood cells (WBC) and number of cells were

measured as number/cubic millimeter.0.5 mark of blood was drawn into the RBC pipette and immediately101 mark of diluting fluid containing, formal Citrate Solution was drawn. The solution is added and for 2 to 3 minutes, the solution is allowed to be settled and the cover glass is deposited over the ruled area. The solution was mixed gently again and the stem full of solution was drawn out and by holding the pipette at an angle of 45° , a drop of fluid was allowed to flow under the cover slip. It was allowed to settle for 2 to 3 minutes, the RBCs after settling under the cover slip were counted. Under the microscope the ruled counting area was focused and the number of RBC's were counted in fine small squares of the counting area under high power lens and the following formula is used to calculate the number of RBC per sq.mm:

$$Total\ RBC = \frac{No.0f\ Cells \times Dilution\ factor \times Depth\ factor}{Total\ NO.of\ small\ Squares} = cubic\ mm$$

2.6 Estimation of White Blood Cell (WBC)

Blood collection and processing procedure was the same as described in the above except for the dilution factor which is 1: 20. As far as the WBC counting chamber, each of these 4 squares millimeter areas was subdivided into 16 squares, by using low power objective and a maximum ocular care was taken for counting cells of the Neubauer's chamber. For the enumeration of WBC, the following formula was taken.

$$Total\ WBC = \frac{No.0f\ Cells\ Counted \times Volume\ of\ the\ Square \times Depth\ factor}{NO.of\ Squares(4)} = cubic\ mm$$

2.7 Estimation of MCV and MCH

Mean corpuscular volume is an auxiliary indicator and it is a measurement of red blood cell size. MCV value is calculated by dividing measured hematocrit value. MCV expressed in femtoliter (fL or 10^{-15} L). Diagnosis of the hematological parameter was carried out in a nearby medical diagnostic center, Krishnagiri. The following formula was used for the calculating MCV, MCH in a given sample of blood.

$$MCV = \frac{\text{hematocrit (\%)}}{\text{RBC count (millions/}mm^3\text{ blood)}} \times 10$$

$$MCH = \frac{\text{Hb (in g/d }L)}{\text{RBC (in millions/}\mu L)} \times 10$$

3. RESULT

3.1. Dietary Survey

A survey was taken frequently (weekly basis) for 2000 healthy human beings from the common population at different fish shops located nearby KRP Dam at Krishnagiri district, Tamilnadu. Dietary data were collected only on tilapia consuming people. Details of fish tilapia intake were collected from the human population and computed.

Table. 2. Survey on fish (Tilapia) consume people in various shops at KRP Dam area at Krishnagiri, Tamilnadu. (Average of four weak observations)

Average consumption of fish Oreochromis mossambicus by the human population (Number of people) at KRP Dam area at Krishnagiri (Nos.)		
Morning	Evening	Total
112	98	210
109	95	204
102	97	199
105	92	197
111	89	200
108	88	196
104	86	190
110	94	204
103	98	201
106	93	199
	Total	2000

(Average of four weeks' Observations)

3.2 Growth performance

The present results showed significant growth rate (weight and length) in *O. mossambicus* exposed to various concentrations of spirulina and azolla with commercial fish feed while compared with the controlled group.

3.2.1 Length of fish

In the first group of experiment, 6 fishes were experimented with 8 grams of Azolla and 12 gram of commercial fish feed for the period of 20, 40 and 60 days, the result shows increased level of length rate (11.9 cm, 12.6 cm and 13.3 cm) in fishes at the end of all the days of exposure period (20, 40 and 60 days) while compare with control group. In the second group of experiment, 6 fishes were experimented with 8 gram of spirulina and 12 gram of commercial fish feed for the period of 20, 40 and 60 days, the result shows increased level of length (11.7 cm, 12.8 cm and 13.6 cm) in fishes at the end of all the days of exposure period (20, 40 and 60 days) while compare with control group. In the third group of experiment, 6 fishes were experimented with 4 gram of Azolla, 4 gram of spirulina and 12 gram of commercial fish feed for the period of 20, 40 and 60 days, the result shows increased level of length rate (11.6 cm, 12.9 cm and 14.1cm) in fishes at the end of all the days of exposure period (20, 40 and 60 days) while compare with control group.

Table.3. Comparative analysis of average length (cm) of Tilapia *O. mossambicus* in different days of Exposure (20, 40 and 60 days)

Types Experiment	Initial average Length(cm)	Average Length(cm) 20 days	Average Length(cm) 40 days	Average Length(cm) 60 days
Control	11.1 ± 0.12	11.6 ±0.16	12.5 ±0.20	13.1 ±0.23
Experiment-1	11.2 ±0.15	11.9 ±0.21	12.6 ±0.25	13.3 ±0.27
Experiment-2	11.0 ±0.5	11.7 ±0.12	12.8 ±0.18	13.6 ±0.22
Experiment-3	11.3 ±0.8	11.6 ±0.15	12.9 ±0.19	14.1 ±0.28

All the values mean ± SD of six observations
Values which are not sharing common superscript differ significantly at 5% (p < 0.05)
Duncan multiple range test (DMRT)

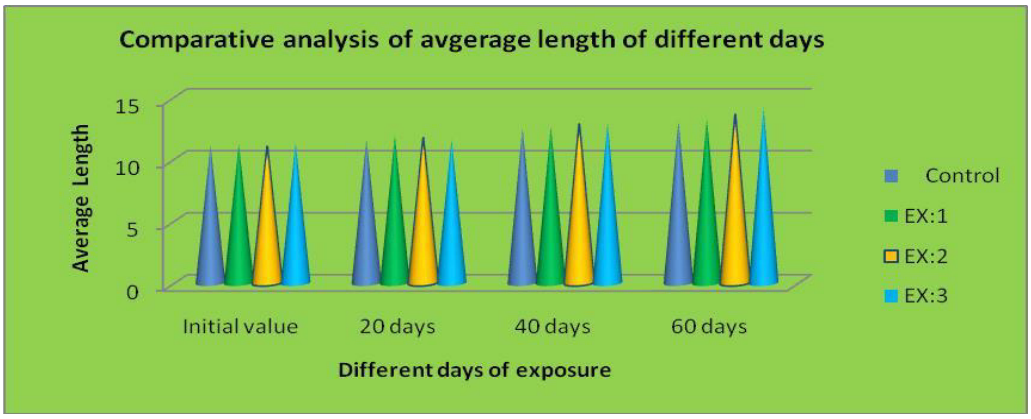

Fig 1. Comparative analysis of average length (cm) of Tilapia *O. mossambicus* in different days of Exposure (20, 40 and 60 days)

3.3 Growth performance

The present results showed significant weight increases in *O. mossambicus,* exposed to various concentrations of spirulina and azolla with commercial fish feed while compared with the controlled group.

3.3.1 Weight of fish

In the first group of experiment, 6 fishes were experimented with 8 grams of Azolla and 12 gram of commercial fish feed for the period of 20, 40 and 60 days, the result shows increased weight (23.4 gm., 26.5 gm. and 29.9 gm.) in fishes at the end of all the days of exposure period (20, 40 and 60 days) while compare with control group. In the second group of experiment, 6 fishes were experimented with 8 gram of spirulina and 12 gram of commercial fish feed for the period of 20, 40 and 60 days, the result shows increased level of length (23.9 gm., 26.9 gm. and 28.7 gm.) in fishes at the end of all the days of exposure period (20, 40 and 60 days) while compare with control group. In the third group of experiment, 6 fishes were experimented with 4 grams of Azolla, 4 gram of spirulina and 12 gram of commercial fish feed for the period of 20, 40 and 60 days, the result shows increased level of length rate (24.0 gm., 27.2 gm and 30.0 gm.) in fishes at the end of all the days of exposure period (20, 40 and 60 days) while compare with control group.

Table.4. Comparative analysis of average weight (g) of Tilapia *O. mossambicus* different days of Exposure (20, 40 and 60 days)

Types Experiment	Initial Average weight(g)	Average weight(g) 20 days	Average Weight(g) 40 days	Average weight(g) 60 days
Control	21.4 ±0.05	23.0 ±0.02	25.4 ±0.07	27.0 ±0.09
Experiment-1	21.7 ±0.02	23.4 ±0.06	26.5 ±0.08	29.9 ±0.05
Experiment-2	21.9 ±0.03	23.9 ±0.05	26.9 ±0.06	28.7 ±0.08
Experiment-3	22.1 ±0.08	24.0 ±0.07	27.2 ±0.09	30.0 ±0.03

All the values mean ± SD of six observations
Values which are not sharing common superscript differ significantly at 5% (p < 0.05)
Duncan multiple range test (DMRT)

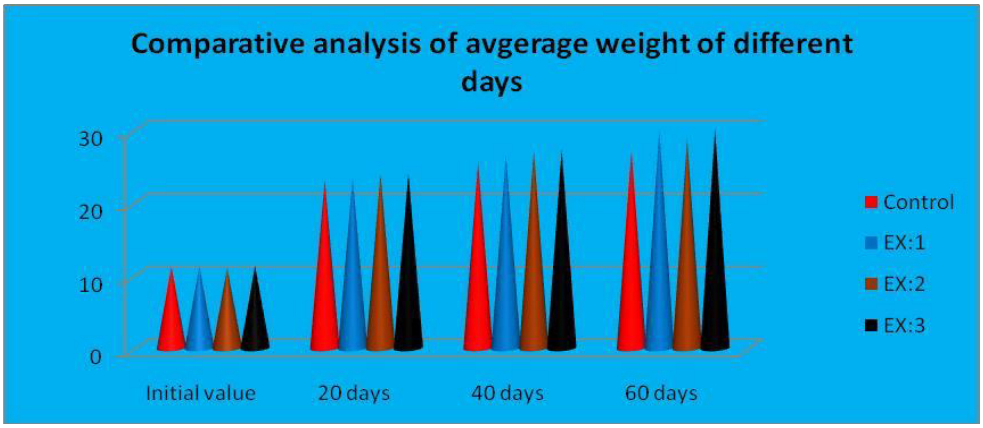

Fig 2. Comparative analysis of average weight (g) of Tilapia *O.mossambicus* in Different days of exposure (20, 40 and 60 day

The present study revealed that the growth performance demonstrated as body length and weight gain, were significantly increased in all the experimental groups while compared with control. Maximum length (14.1 cm) and body weight (30 g) were observed in group 3, the fish experimented with 4 grams of Azolla and 4 grams of Spirulina with 12gram of commercial fish feed for the period of 60 days. (Table-3, Table-4 and Figure-1and Figure-2).

3.4 Hematological Parameters

3.4.1. RBC, WBC, HB, MCV and MCH

The present results showed increased levels of RBC, WBC, Hb, MCV, MCH and in *O. mossambicus* exposed to various concentrations of spirulina and azolla with commercial fish feed while compared with the controlled group. In the first group of experiment, 6 fishes were experimented with 8 grams of Azolla and 12 gram of commercial fish feed for the period of 60 days, the result shows increased level of RBC, WBC, Hb, MCV, MCH (1.38 cu.mm, 172.3 cu.mm and 16.7 %, 147.5 fL and 40.9 pg.,) in fishes at the end of 60 days of exposure while compare with control group. In the second group of experiment, 6 fishes were experimented with 8 gram of spirulina and 12 gram of commercial fish feed for the period of 60 days, the result shows increased level of RBC, WBC, Hb, MCV, MCH (2.0 cu.mm, 184.9 cu.mm and 7.2 %, 154.5 fL and 41.3 pg.,) while compared with control group. In the third group of experiment, 6 fishes were experimented with 4 grams of Azolla, 4 gram of spirulina and 12 gram of commercial fish feed for the period of 60 days, the result shows increased level of RBC, WBC, Hb, MCV, MCH (2.8 cu.mm, 216.8 cu.mm and 8.5 %, 156.6 fL and 42.5 pg.,) while compare with control group. Maximum levels of RBC, WBC, Hb, MCV and MCH were observed in group 3 for the period of 60 days. (Table-5and Figure-3).

Table.5. Changes in Hematological parameters of O. *mossambicus* exposed to different concentrations of spirulina and azolla with formulated feed for 60 days of exposure.

	Control	Experiment 1	Experiment 2	Experiment 3
RBC (cu.Mm)	1.29 ±0.01	1.38±0.04	2.0±0.06	2.8±0.09
WBC(cu.Mm)	152.7 ± 0.08	172.3±0.03	184.9±0.02	216.8±0.06
Hb (%)	5.2± 0.02	6.7±0.05	7.2±0.04	8.5±0.08
MCV (fL)	140.6±0.01	147.5±0.4	154.7±0.08	156.6±0.06
MCH (pg)	40.3±0.07	40.9±0.03	41.3±0.02	42.5±0.05

All the values mean ± SD of six observations
Values which are not sharing common superscript differ significantly at 5% (p < 0.05)
Duncan multiple range test (DMRT)

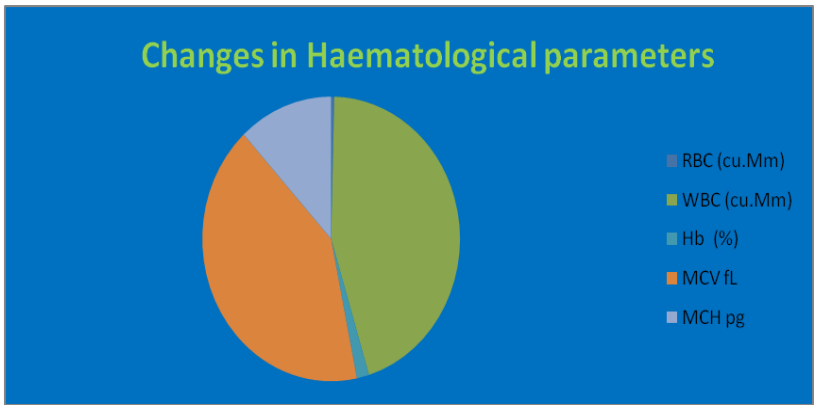

Fig 3. Changes in Hematological parameters of O. *mossambicus* exposed to different concentration of spirulina and azolla with formulated feed for 60 days of exposure.

4. DISCUSSION

A survey was admitted (weekly basis) for 2000 healthy human beings from the common population at different fish shops located nearby KRP Dam at Krishnagiri district, Tamilnadu. Dietary data were collected only on tilapia consume people. Details of fish tilapia intake was collected from human population and computed. In the present study, the data survey clearly showed that, consumption of fish tilapia by the public are increased. All the experimental groups (Exp-1, Exp-2 and Ex-3) in the present study produced the best length in growth and best weight gain for the period of 60 days. The experimental group 3 of fish fed with commercial fish feed (4 grams of Azolla, 4 gram of Spirulina and 12gram of commercial fish feed) exhibited the highest body weight and length in growth. These results cleared that the optimum dietary level of Azolla and Spirulina with commercial fish feed for *O. mossambicus* for 60 days enhanced the growth and length performance. Due to the positive effect on weight gain, the use of algae feed in aquaculture has a lot of attention.[24] Several studies showed that the physiological activity and growth performance were induced in the presence of small amount of algae in aqua feeds.[25,26] The best growth level observed with *Spirulina* supplemented diets suggested that, the *Spirulina* improved feed utilization and decreased the amount of feed necessary for animal growth, similar result have been reported by Fadl.*et al*.,.[27]The results obtained from the current study were in accordance with Ibrahim *et al*.,.[28] who found that growth rates in *O. niloticus* was improved by feed supplemented with *S. platensis* powder. Hematological parameters of tilapia with different level of spirulina, azolla feed in the present study showed significant enhancement when compared with control feed. The present results showed that, feeding *Spirulina platensis* and azolla at exposure group T1, T2 and T3 had significantly higher RBC and WBCs, MCV and MCH. Concerning the percentage of hemoglobin in fish blood of all the experimental groups fed on Spirulina and Azolla had higher than control, reflecting good nutritional values. Similar results obtained by Abdel-Tawab *etal*.,.[29] who stated that Spirulina diet increase RBCs and WBCs counts. The results showed that, the highest value of RBC, WBC and Hemoglobin were recorded in experimental (group T3) that fed on diet with 4 grams of Azolla and 4 grams of Spirulina with 12 grams of formulated fish feed.[30] The spirulina and Azolla diets were most effective to improve the hematological parameters in fish as compared with other control diet the high nutritional content of Azolla and spirulina might be the reason of it. Therefore, spirulina and Azolla supplementation could be used as a dietary supplement for the fishes. More than 80% of the global tilapia production comes from Asia [31, 32]. From the present result, it was observed that the fish *Oreochromis mossambicus* tilapia fed with azolla and spirulina showed improved level of morphological structure and hematological parameters. This may boost the immune system in human. The improvement of morphological structure is similar to the finding of Basak *et al*., [33]. Naghshi *et al.,* reported Azolla supplementation powder significantly improved daily weight gain in chickens[34.] Several benefits of tilapia consumption observed in human such as increasing metabolism, weight reduction, reducing the possibility of chronic diseases and strengthening the bones are contributed by rich nutritional content of tilapia flesh. Protein content of the tilapia is high.[35] Spirulina and Azolla was considered as an excellent source of useful nutrients as well as an excellent energy source.[36] Spirulina reduced hepatic damage.[37] From the present result, it was observed that the fish *Oreochromis mossambicus* tilapia fed with azolla and spirulina showed increased level of growth, weight and hematological parameters (RBC, WBC, Hb, MCV, MCH) and also it was observed that tilapia consuming population were increased overall. From the present result we assure and postulate that the people who consume fish tilapia fed with azolla and spirulina may get good nutrition in their food and it may increase their immune system.

5. CONCLUSION

Intensive aquaculture is highly dependent on fish meal. Spirulina and Azolla are promising new feed sources to support future animal production needs and human health. From the present result, it was concluded that the fish *Oreochromis mossambicus* tilapia fed with azolla and spirulina showed increased level of growth, weight, protein and hematological parameters and also it was observed that number of tilapia consuming people also have increased. The present result clearly showed that people who consume fish tilapia fed with azolla and spirulina may get good nutrition in their food and it may increase their immune system. Use of feed ingredients like Spirulina and Azolla would reduce the cost of commercial fish feed. Azolla and Spirulina enhances immunity in stressful conditions organisms like fish and humans. Thus, more attention is needed to increase the use of spirulina and azolla in aquaculture. The results provide awareness on usage of spirulina and azolla in aquaculture and human health. From the present result, it was observed that the fish *Oreochromis mossambicus* tilapia fed with azolla and spirulina showed increased level of growth, weight and

hematological parameters (RBC, WBC, Hb, MCV, and MCH) and also it was observed that number of Tilapia consumed people also have increased.

6. ACKNOWLEDGEMENT

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7. AUTHOR CONTRIBUTION STATEMENT

Mr.Sakthivel.M conceptualized and gathered the data with regard to this work. Dr.Prakash Sahaya Leon.J analyzed these data and necessary data were given to designing of the manuscript. All authors discussed the results and contributed to the final manuscript.

8. ABBREVIATIONS

RBC Red Blood Cell, WBC White Blood Cell, Hb Hemoglobin, MCV Mean corpuscular volume, MCH Mean corpuscular hemoglobin.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

10. REFERENCES

1. Kumari R, Ojha ML, Saini VP, Sharma SK. Effect of Azolla supplementation on growth of rogue (Labeorohita) fingerlings. J Entomol Zool Stud. 2017; 5:1116-9.
2. Mustafa MG, Nakagawa H. A review: dietary benefits of algae as an additive in fish feed. Isr J Aquacult Bamidgeh. 1995; 47:155-62.
3. Nakagawa H, Montgomery WL. Algae. In: Dietary supplements for the health and quality of cultured fish. Cambridge: CABI North American Office; 2007.
4. Ringø E, Olsen RE, Gifstad TØ, Dalmo RA, Amlund H, Hemre G-I et al. Prebiotics in aquaculture: a review. Aquacult Nutr. 2010;16(2):117-36. doi: 10.1111/j.1365-2095.2009.00731. x.
5. Watanuki H, Ota K, Tassakka ACMAR, Kato T, Sakai M. Immunostimulant effects of dietary Spirulina platensis on carp, Cyprinus carpio. Aquaculture. 2006;258(1-4):157-63. doi: 10.1016/j.aquaculture.2006.05.003.
6. M a i t y J, [P a t r a B.C.]. Can J Pure Appl Sci. 2008. Effect of replacement of fishmeal by Azolla leaf meal on growth, food utilization, pancreatic protease activity and RNA/DNA ratio in the fingerlings of Labeorohita (Ham.);323.
7. Prabina B.J., K u m a r K. Asian J Anim Sci. 2010. Dried Azolla as a nutritionally rich cost effective and immunomodulatory feed supplement for broilers; 5:20-2.
8. Kori-Siakpere O, Ake JEG, Idoge E. Haematological characteristics of the African snakehead, Parachacnnaobscura. Afr J Biotechnol. 2005;4(6):527-30.
9. Mekkawy IA, Mahmoud UM, Sayed Ael-D. Effects of 4-nonylphenol on blood cells of the African catfish Clariasgariepinus (Burchell, 1822). Tissue Cell. 2011;43(4):223-9. doi: 10.1016/j.tice.2011.03.006, PMID 21501852.
10. Sayed AH, Abdel-Tawab HS, Abdel Hakeem SS, Mekkawy IA. The protective role of quince leaf extract against the adverse impacts of ultraviolet-A radiation on some tissues of Clariasgariepinus (Burchell, 1822). J Photochem Photobiol B Biol. 2013; 119:9-14. doi: 10.1016/j.jphotobiol.2012.11.006.
11. Jain AP, Aggarwal KK, Zhang PY. Omega-3 fatty acids and cardiovascular disease. Eur Rev Med Pharmacol Sci. 2015;19(3):441-5. PMID 25720716.
12. Freeman LM. Beneficial effects of omega-3 fatty acids in cardiovascular disease. J Small Anim Pract. 2010 Sep;51(9):462-70. doi: 10.1111/j.1748-5827.2010. 00968.x, PMID 20673293.
13. Chen YC, Prabhu KS, Mastro AM. Mastrols selenium a potential treatment for cancer metastasis? Nutrients. 2013 Apr;5(4):1149-68. doi: 10.3390/nu5041149, PMID 23567478.
14. Dyall SC. Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. Front Aging Neurosci. 2015; 7:52. doi: 10.3389/fnagi.2015.00052, PMID 25954194.
15. Ventura M, Melo M, Carrilho F. Selenium and thyroid disease: from pathophysiology to treatment. Int J Endocrinol. 2017; 2017:1297658. doi: 10.1155/2017/1297658, PMID 28255299.
16. Sprague JB. 528.American soc. Test. ABC ' s of pollutant bioassay using fish biological method for assessment of water quality. ASTM STP. Materials; 1973:6-30.
17. APHA, 1989. Standard method for the examination of water and waste –water. 17th ed. American pupil. DC: Health Communications Association Washington.
18. Duncan DB. Multiple range tests for correlated and heteroscedastic means. Biometrics. 1957;13(2):359-64. doi: 10.2307/2527799.
19. Water quality criteria, 1972. A report of the Committee on Water Quality Criteria. Washington, DC: Environmental Studies Board, National Academy of Sciences, National Academy of Engineering.
20. Goodwin TW, M, Mercer EI. Fructosans. In: Goodwin TW, Mercer EI, editors, Introduction to Plant biochemistry. Oxford: Pergamon Press; 1983. p. 261-4.
21. Mishra N, Pandey PK, Datta Munshi JS, Singh BR. Haematological parameters of an air-breathing mud eel, Amphipnous cuchia (Ham.) (Amphipnoidae; Pisces). J Fish Biology. 1977;10(6):567-73. doi: 10.1111/j.1095-8649. 1977.tb04089. x.
22. Shali T. Text book of clinical pathology. Mukherjee Scward, Eimiller, editors. Vol. 1988; 1962.
23. Med Lab Technol. Tata McGraw hill publishing company Ltd., I: 242-243.
24. Ophelia S. Raman jam, P. Effic Green Algae Cyanobacteria Feed Juv Labeo Gonius. – Journal of Algal Biomass Utilization 8(3). 2017:13-22.

25. Valente LMP, Gouveia A, Rema P, Matos J, Gomes EF, Pinto IS. Evaluation of three seaweeds Gracilaria bursa-pastoris, Ulva rigida and Gracilaria cornea as dietary ingredients in European sea bass (Dicentrarchus labrax) juveniles. Aquaculture. 2006;252(1):85-91. doi: 10.1016/j.aquaculture.2005.11.052.
26. Batista S, Ozório ROA, Kollias S, Dhanasiri AK, Lokesh J, Kiron V et al. Changes in intestinal microbiota, immune- and stress-related transcript levels in Senegalese sole (Solea senegalensis) fed plant ingredient diets intercropped with probiotics or immunostimulants. Aquaculture. 2016;458:149-57. doi: 10.1016/j.aquaculture.2016.03.002.
27. Fadl SE, ElGohary MS, Elsadany AY, Gad DM, Hanaa FF, El-Habashi NM. Contribution of microalgae-enriched fodder for the Nile tilapia to growth and resistance to infection with Aeromonas hydrophila. Algal Res. 2017;27:82-8. doi: 10.1016/j.algal.2017.08.022.
28. Ibrahem MD, Mohamed MF, Ibrahim MA. The role of Spirulina platensis (Arthrospira platensis) in growth and immunity of Nile tilapia (Oreochromis niloticus) and its resistance to bacterial infection. J Agric Sci. 2013;5(6):109-17. doi: 10.5539/jas.v5n6p109.
29. Abdel-Taw wad, M,M. Ahmed, Y.AbdelHadi and M. 8th International Symposium on Tilapia in Aquaculture. Sedan; 2008. Use of Spirulina (Arthrospira Platensis) as a growth and immunity promoter for Nile Tilapia, Oreochromis niloticus (L.) fry Challenged with pathogenic Aeromonas hydrophila.
30. Adedeji OB, Adeyemo OK, Agbede SA. Effects of diazinon on blood parameters in the African catfish (Clariasgariepinus). Afr J Biotechnol. 2009;8:3940-6.
31. Eknath AE, Hulata G. Use and exchange of genetic resources of Nile tilapia (Oreochromisa niloticus). Rev Aquacult. 2009;1(3-4):197-213. doi: 10.1111/j.1753-5131.2009.01017.x.
32. Shovon BC, Majumdar Z. Rahman Heavy metals (lead, cadmium and nickel) concentration in different organs of three commonly consumed fishes in Bangladesh Fish. Aqua Cult J. 2017;8(3):1-5.
33. Basak B, Pramanik AH, Rahmnan MS, Taradar SU, Roy BC. Azolla (Azolla pinnata) as a feed ingredient in broiler ration. Int J Poult Sci. 2002;1:29-32.
34. Naghshi H, Khojasteh S, Jafari M. Investigation the effect of different levels of Azolla (Azolla pinnata) on performance and carcass characteristics of cobb broiler chicks. Int J Farm Alli Sci. 2014;3:45-9.
35. El-Sayed AM. Alternative dietary protein sources for farmed tilapia, Oreochromis spp. Aquaculture. 1999;179(1-4):149-68. doi: 10.1016/S0044-8486(99)00159-3.
36. Glombitza KW, Koh M. Secondary metabolites of pharmaceutical potentials. In: Cresswell RC, Rees TAV, Shah N, editors, Algal and cyanobacterial biotechnology. Harlow, UK: Logman; 1989. p. 161-238.
37. Romay C, Ledón N, González R. Phycocyanin extract reduces leukotriene B4 levels in arachidonic acid-induced mouse-ear inflammation test. J Pharm Pharmacol. 1999;51(5):641-2. doi: 10.1211/0022357991772646, PMID 10411225.