



Effect of Different Levels of *Spirogyra* Incorporated Diet on Bio Growth Performance, Digestive Enzyme Activity and Haematology of *Etroplus suratensis*

Salkapuram Sandeep Kumar¹, S. Selvaraj¹, Cheryl Antony¹, S. Aruna¹, P. Ruby¹

10.18805/IJAR.B-5504

ABSTRACT

Background: *Etroplus suratensis* is omnivorous feeder, but mainly feeds on filamentous algae especially *Spirogyra*. The preference of pearlspot towards *Spirogyra* as an important food item, made us utilize the *Spirogyra* as an ingredient in the feed of pearlspot at different levels to evaluate its impact on growth and other parameters.

Methods: The experiment was conducted to evaluate the effect of *Spirogyra* on bio growth performance, digestive enzyme activity and haematology of *E. suratensis*. The experiment was performed with different levels of *Spirogyra* incorporated diet - S0, 10, 12.5, 15, 17.5, 20, 22.5 and 25%. The pearlspot fingerlings of initial weight of 14.91 ± 0.11 was stocked in the 300 litre FRP tank for 60 days.

Result: The highest growth performance of pearlspot was found in 22.5% *Spirogyra* incorporated diet. The average weight gain was highest in 22.5% SP (26.55 ± 0.22 g), followed by 20% SP (26.11 ± 0.25 g), control (25.83 ± 0.47 g), 25% SP (24.78 ± 0.15 g), 17.5% SP (24.46 ± 0.16 g), 15% SP (23.03 ± 0.16 g), 12.5% SP (22.21 ± 0.34 g), 10% SP (22.16 ± 0.80 g). The digestive enzyme activity of amylase (0.026 ± 0.001 U mg protein⁻¹ min⁻¹), protease (0.164 ± 0.02 U mg protein⁻¹ min⁻¹) and lipase (0.486 ± 0.01 U mg protein⁻¹ min⁻¹) were found to be highest in 22.5%SP treatment, followed by other treatments. The haematology parameters RBC and WBC count increased with increase in the incorporation of *Spirogyra* in diet. The study concluded that the incorporation of *Spirogyra* at 22.5% improved the growth performance and feed utilization of pearlspot.

Key words: Digestive enzymes, *Etroplus suratensis*, Growth performance, Haematology, *Spirogyra*.

INTRODUCTION

The global fisheries and aquaculture production has reached upto 232.2 million tons, in which aquaculture has contributed about 130.9 million tons in 2022 (FAO, 2024). Aquaculture, the fastest growing food producing sector, meets the demand for aquatic food products all over the world. It also plays role in global food security, reduction of poverty by providing employment by intensifying and expanding systems through sustainable development (Subasinghe, 2009). Aquaculture is one the ideal sources for compensating the protein requirement in less time, which provides employment and economic growth for the nation (Ruby, 2022). Brackishwater aquaculture through farming contributed 4 billion USD annually to national income (CIBA, 2022). Algae are rich source of proteins, fatty acids, vitamins and minerals which can be extensively used in aquaculture for promoting the growth and is economically viable (Sivakumar, 2018). Many brackishwater fish species are available for potential aquaculture, among them *E. suratensis* is one of the species that adapts to both freshwater as well as brackish water and suitable for the traditional and advanced aquaculture practices (Lakra, 2011). *E. suratensis* is well known as pearlspot or green chromide, also locally called as Karimeen in Kerala, belonging to family Cichlidae, endemic to peninsular India and Sri Lanka (Padmakumar, 2012). The relationship

¹Dr. M.G.R. Fisheries College and Research Institute, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Ponneri-601 204, Tamil Nadu, India.

Corresponding Author: Salkapuram Sandeep Kumar, Dr. M.G.R. Fisheries College and Research Institute, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Ponneri-601 204, Tamil Nadu, India. Email: sandeepsalkapuram@gmail.com

How to cite this article: Kumar, S.S., Selvaraj, S., Antony, C., Aruna, S. and Ruby, P. (2025). Effect of Different Levels of *Spirogyra* Incorporated Diet on Bio Growth Performance, Digestive Enzyme Activity and Haematology of *Etroplus suratensis*. Indian Journal of Animal Research. 1-6. doi: 10.18805/IJAR.B-5504.

Submitted: 10-10-2024 **Accepted:** 05-12-2024 **Online:** 30-01-2025

between the fish and its feeding majorly depends on abundance and availability of food items (Jhingran, 1969). Pearlspot is an omnivorous fish, majorly feeds on filamentous algae, aquatic plants, copepods and detritus. Among all the food items, pearlspot majorly feeds on the filamentous algae *Spirogyra* (Joseph, 1988). Keeping this point in view and very few studies were studied by *Spirogyra* incorporation in feed, the present study is carried out using *Spirogyra* at different levels in feed to find the impact on growth performance, digestive enzymes and haematology of *E. suratensis*.

MATERIALS AND METHODS

Experimental design

The experiment was conducted in PRFF centre, Pazhaverkadu under Tamil Nadu Dr. J. Jayalalithaa Fisheries University in triplicate manner utilizing 24 tanks, capacity of 300 litres with completely randomized design (CRD). The pearlspot fishes collected from Pulicat lake, Pazhaverkadu are disinfected with KMnO_4 , stocked in FRP tank and fed with commercial feed for about 2 weeks for acclimatizing. Prior to collection of fish, tanks were cleaned thoroughly and disinfected and filled with 70% water and adequate aeration was provided. The pearlspot fish of initial body weight of 14.91 ± 0.11 g at the rate of 25 fish per each tank were stocked and fed with 2% body weight, fed twice a day. Daily 10% water is exchanged by removing the uneaten feed particles and faecal matter present at the bottom of the tank to maintain good water quality. The sampling was performed fortnightly by taking the weights of fish individually to observe the increase in growth and also to adjust the feeding.

Experimental diets

Spirogyra collected was shade dried and prepared into powder and incorporated in different levels in feed. The proximate composition of the *Spirogyra* consists of 16.10% of crude protein, 2.50% of fat, 3.40% of ash and 12.30% fibre. The eight feeding trial diets with various levels of *Spirogyra* were prepared including control diet. The feed formulation and proximate composition of *Spirogyra* incorporated diets are presented in the Table 1.

Water quality parameters

Water quality parameters were maintained within optimum range. pH, Dissolved oxygen (DO), Temperature and Salinity were measured on daily basis with the help of pH meter, DO meter, digital thermometer and refractometer. Alkalinity, total hardness, calcium hardness, magnesium hardness, ammonium and nitrite were analysed weekly once using standard procedure and presented in the Table 2 (APHA, 2005).

Growth parameters

The bio-growth parameters in terms of average weight gain (AWG), feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER), specific growth rate (SGR), average daily gain (ADG) and survival rate were calculated every two weeks during sampling using formulae.

Digestive enzyme activity

Digestive enzyme analysis was performed at the end of the feeding trial. Initially sample homogenate (5%) was prepared by homogenizing the tissue in 0.25M sucrose solution using pestle and mortar, the centrifuged at 4°C for 20 minutes to obtain a supernatant and is stored at -20°C in a deep freezer, till the enzyme analysis is done. The protease, amylase and lipase activity were performed using Casein digestion method (Drapeau, 1976 and Sarath, 1989); Dinitro salicylic acid (Rick and Stagbauer, 1974) and Titrimetric method (Cherry and Crandall, 1932).

Table 1: Feed formulation and proximate composition of experimental diets (g/kg of diet).

Ingredients	Different levels of incorporation of <i>Spirogyra</i>							
	0%	10%	12.5%	15%	17.5%	20%	22.5%	25%
Fish meal	240	240	240	240	240	240	240	240
Soya bean meal	240	240	240	240	240	240	240	240
Wheat bran	165	140	125	110	95	80	65	50
Corn flour	165	90	80	70	60	50	40	30
<i>Spirogyra</i>	-	100	125	150	175	200	225	250
Wheat gluten	150	150	150	150	150	150	150	150
Fish oil	10	10	10	10	10	10	10	10
Di calcium phosphate	10	10	10	10	10	10	10	10
Vit mineral premix	10	10	10	10	10	10	10	10
Salt	7	7	7	7	7	7	7	7
Arginine	2	2	2	2	2	2	2	2
Vit E	1	1	1	1	1	1	1	1
Proximate composition								
Moisture	9.31	9.18	9.21	9.26	9.27	9.31	9.31	9.32
Crude protein	44.81	44.69	44.65	44.60	44.54	44.51	44.47	44.43
Crude lipid	5.70	5.75	5.79	5.84	5.89	5.92	5.95	5.98
Ash	4.71	4.76	4.81	4.84	4.89	4.93	4.97	5.18
Fibre	3.50	3.56	3.62	3.69	3.74	3.80	3.83	3.87
Carbohydrate	31.97	32.06	31.92	31.77	31.67	31.53	31.47	31.22

Haematological analysis

The blood samples from each treatment were collected at the end of the feeding trial. Using 1 mL sterile syringe the blood samples are collected from the caudal vein of fishes. Prior to the collection of blood, the syringes were coated with EDTA (2.7%) which acts as an anticoagulant. The fishes were anesthetized using clove oil and blood samples are collected and stored in EDTA coated vials (1 mL). The Haemoglobin level in blood was determined by Cyanmethaemoglobin method (Drabkin, 1946). The haematocrit level in blood was determined by Microhematocrit method (Nelson and Morris, 1989). The white blood cells (WBC) and red blood cells (RBC) counts were determined by Haemocytometer. The MCV, MCH and MCHC values were calculated using Wintrobe (1934).

MCH (fL) = (haemoglobin × 10) / Erythrocytes

MCV (per µl) = (haematocrit × 10) / Erythrocytes

MCHC (g dl⁻¹) = (haemoglobin × 100) / Haematocrit

Data analysis

The statistical analysis of the data was analysed using SPSS, 20.0 by ONE WAY ANOVA along with Duncan's multiple range test followed by Tukey's test for post hoc comparison of means with significance level $P < 0.05$ among the treatment groups.

RESULTS AND DISCUSSION

Effect of *Spirogyra* on growth performance of *E. suratensis*

The results calculated for growth parameters and feed utilization at the end of the feeding trial of 60 days are presented in the Table 3. The final body weight among the treatments showed significant difference with highest body weight in 22.5% SP treatment (26.55±0.63 g), followed by 20%SP treatment (25.98±0.25 g), control (25.82±0.24 g) and least weight gain in 10%SP (22.16±0.34 g). The better FCR (1.87±0.05), FER (0.54±0.02) and PER (1.21±0.03) and ADG (0.19±0.005) were recorded in 22.5% SP treatment. Previous study on *Spirogyra* incorporated diet in Catla fingerlings concluded average weight gain was

Table 2: Water quality parameters maintained during the trial period.

Parameter	Optimum range
pH	7.6 – 7.9
Temperature (°C)	26 – 28
Dissolved oxygen (mg/L)	5 – 6.5
Salinity (ppt)	14.5 – 16
Alkalinity (mg/L)	144 – 156
Calcium (mg/L)	103 – 110
Magnesium (mg/L)	562 – 594
Total hardness (mg/L)	2200 – 2450
Ammonium (mg/L)	0.02 – 0.07
Nitrite (mg/L)	0.2 – 0.3
Nitrate (mg/L)	0.01 – 0.2

Table 3: Bio-growth parameters of pearlspot fed with different levels of *Spirogyra* incorporated diet.

<i>Spirogyra</i> diet	Initial weight (g)	Final weight (g)	Average weight gain (g)	FCR	FER	PER	SGR (%/day)	ADG (g/fish/day)	SR (%)
0% SP (C)	14.90±0.11	25.82±0.24 ^b	10.92±0.14 ^b	1.99±0.26 ^e	0.50±0.06 ^b	1.14±0.01 ^b	3.98±0.02 ^b	0.18±0.002 ^b	93.33±6.65 ^a
10% SP	14.90±0.11	22.16±0.34 ^e	7.26±0.41 ^e	3.00±0.17 ^a	0.33±0.01 ^e	0.76±0.04 ^e	3.30±0.09 ^f	0.12±0.006 ^e	88.88±7.69 ^c
12.5% SP	14.90±0.11	22.54±0.27 ^e	7.64±0.25 ^e	2.85±0.09 ^b	0.35±0.01 ^e	0.80±0.02 ^e	3.39±0.05 ^e	0.13±0.004 ^{de}	92.59±6.81 ^b
15% SP	14.90±0.11	23.03±0.16 ^d	8.13±0.23 ^d	2.68±0.07 ^c	0.37±0.01 ^d	0.85±0.02 ^d	3.49±0.04 ^d	0.13±0.003 ^d	90.00±4.69 ^c
17.5% SP	14.90±0.11	24.46±0.16 ^c	9.56±0.14 ^c	2.27±0.03 ^d	0.44±0.00 ^d	1.00±0.01 ^c	3.76±0.02 ^c	0.16±0.002 ^c	95.59±6.81 ^a
20% SP	14.90±0.11	25.98±0.25 ^b	11.08±0.26 ^b	1.93±0.04 ^e	0.51±0.01 ^{ab}	1.17±0.02 ^{ab}	4.02±0.03 ^{ab}	0.18±0.004 ^{ab}	92.59±6.81 ^b
22.5% SP	14.90±0.11	26.55±0.63 ^a	11.65±0.33 ^a	1.87±0.05 ^e	0.54±0.01 ^a	1.21±0.03 ^a	4.10±0.04 ^a	0.19±0.005 ^a	92.59±6.81 ^b
25% SP	14.90±0.11	24.78±0.15 ^c	9.88±0.14 ^c	2.20±0.03 ^d	0.45±0.00 ^c	1.03±0.01 ^c	3.82±0.02 ^c	0.16±0.002 ^c	85.22±1.07 ^d

Values in the same row with different manuscripts differ significantly ($p < 0.05$) among the treatments.

Table 4: Digestive enzyme activity of pearlspot fed with different levels of *Spirogyra*.

Digestive enzymes	Spirogyra incorporated treatments							
	0%	10%	12.5%	15%	17.5%	20%	22.5%	25%
Amylase	0.025±0.001 ^a	0.015±0.000 ^d	0.016±0.000 ^d	0.018±0.01 ^c	0.023±0.01 ^b	0.025±0.000 ^a	0.026±0.001 ^a	0.023±0.04 ^b
Protease	0.157±0.08 ^{ab}	0.122±0.09 ^d	0.129±0.10 ^{cd}	0.136±0.17 ^c	0.144±0.009 ^{bc}	0.161±0.18 ^{ab}	0.164±0.02 ^a	0.153±0.07 ^{ab}
Lipase	0.454±0.02 ^{ab}	0.386±0.11 ^e	0.409±0.06 ^{de}	0.427±0.02 ^{cd}	0.449±0.01 ^c	0.461±0.01 ^{ab}	0.486±0.02 ^a	0.463±0.03 ^{ab}

Values in the same row with different manuscripts differ significantly ($p < 0.05$) among the treatments.

Units: Amylase: U mg protein⁻¹ min⁻¹; Protease: U mg protein⁻¹ min⁻¹; Lipase: U mg protein⁻¹ min⁻¹

Table 5: Haematological parameters of pearlspot fed with different levels of *Spirogyra* incorporated diet.

Parameters	0%SP	10%SP	12.5%SP	15%SP	17.5%SP	20%SP	22.5%SP	25%SP
Hb	6.71±0.12 ^f	7.04±0.04 ^e	7.26±0.11 ^d	7.41±0.16 ^c	7.55±0.12 ^{bc}	7.69±0.09 ^b	7.84±0.21 ^a	7.49±0.23 ^{bc}
Ht	21.42±0.28 ^e	25.96±0.13 ^d	26.34±0.08 ^d	26.92±0.12 ^c	27.43±0.07 ^b	27.84±0.21 ^{ab}	28.31±0.11 ^a	27.12±0.09 ^c
RBC	1.38±0.06 ^e	1.42±0.05 ^e	1.49±0.03 ^{de}	1.57±0.04 ^d	1.68±0.01 ^{bc}	1.77±0.02 ^b	1.92±0.05 ^a	1.65±0.03 ^{bc}
WBC	10.23±0.14 ^e	10.45±0.09 ^{de}	10.63±0.11 ^d	10.79±0.23 ^{cd}	10.93±0.05 ^c	11.62±0.21 ^{ab}	11.76±0.24 ^a	11.53±0.08 ^b
MCV	155.21±0.17 ^d	182.81±0.34 ^a	176.77±0.13 ^{ab}	171.46±0.21 ^b	163.27±0.09 ^c	157.28±0.14 ^d	147.44±0.09 ^e	164.36±0.42 ^c
MCH	48.62±0.07 ^b	49.57±0.11 ^a	48.72±0.06 ^b	47.19±0.01 ^c	44.94±0.15 ^d	43.45±0.04 ^e	40.83±0.06 ^f	45.39±0.02 ^d
MCHC	31.32±0.04 ^a	27.11±0.20 ^d	27.56±0.03 ^{bc}	27.52±0.01 ^c	27.62±0.05 ^b	27.52±0.08 ^c	27.69±0.03 ^b	27.61±0.04 ^b

Values in the same row with different manuscripts differ significantly ($p < 0.05$) among the treatments.

Hb - gm dl⁻¹; Ht - %; RBC - 10⁶ cells/mm³; WBC - 10⁶ cells/mm³; MCV - per µl; MCH - fl; MCHC - g dl⁻¹.

lower in experimental diet treatments than control, but observed increase in weight of fish with increase in incorporation level of *Spirogyra*, FCR values also followed the same and best weight gain and FCR among the *Spirogyra* treatment was observed at 37% and 40% incorporation level (Kumar *et al.*, 2004). The similar results were observed with the striped mullet fed with Ulva meal and reported incorporation of 20% meal in diet enhanced the growth performance of the fish (Waseef, 2001). Several early studies reported that algal diets improved the growth performance of fish (Olvera- Novoa, 1998; Khanzadeh, 2016; Younis, 2018; Priyatharshni, 2024).

Digestive enzyme activity

The digestive enzyme activity of protease, amylase, lipase exhibited the significant difference among the treatments ($p < 0.05$). The results of the digestive enzyme activity were presented in the Table 4. The highest enzyme activity of amylase (0.026 ± 0.001), protease (0.164 ± 0.02) and lipase (0.486 ± 0.01) were recorded in 22.5% SP treatment. The activity of digestive enzymes significantly increased with increase in the level of *Spirogyra* incorporation in diet may be due to the dietary fibre digestion by the fish. Similarly, results were observed in increased digestive enzyme activity of Nile tilapia in amylase and lipase activity in 7.5 and 10 g kg^{-1} dried periphyton incorporated diets (Saleh, 2022). The similar results for the increase in the digestive enzyme activity of the amylase, lipase and protease was observed (Akbar, 2018; Anle, 2018).

Haematological analysis

The haematological analysis was performed at the end of the trial, values are presented in Table 5. The significant increase in the levels of RBC and WBC count was observed, with increase in the *Spirogyra* incorporation level. The highest RBC (1.92 ± 0.04), WBC (11.76 ± 0.13) count and Haemoglobin value (7.84 ± 0.05) was found in 22.5% SP treatment. The haematology is one of the indicators of the fish health in relation to the supplementary feed and good efficiency of transport of oxygen from gills to tissues indicate the Hb, RBC and Ht levels in blood (Adhikari, 2004; Cook, 2013). Previous studies have also concluded that incorporation of algae in feed helped in the improvement of the haematological parameters (Nur, 2020; Rahman, 2023).

CONCLUSION

From the above study, it is concluded, the *Spirogyra* has effect on the growth performance, digestive enzyme activity and haematological parameters of *Etroplus suratensis*. *Spirogyra* can also be utilized as alternative source for few ingredients such as corn flour, wheat bran, rice flour.

ACKNOWLEDGEMENT

The authors are thankful to the Dean, Dr. M.G.R. Fisheries College and Research Institute, Tamil Nadu Dr.J. Jayalalithaa Fisheries University, Ponneri 601 204, Tamil Nadu, India for rendering the facilities to conduct the present study.

Conflict of interest

Every step taken was compliant with the responsible parties' ethical standards, the authors declare that they have no conflict of interest.

REFERENCES

- Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C.T. and Ayyappan, S. (2004). Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). *Ecotoxicology and Environmental Safety*. 58(2): 220-226.
- Akbary, P. and Jahanbakhshi, A. (2018). Growth yield, survival, carcass quality, haematological, biochemical parameters and innate immune responses in the grey mullet (*Mugil cephalus* Linnaeus, 1758) fingerling induced by Immunogen® prebiotic. *Journal of Applied Animal Research*. 46(1): 10-16.
- Anle, X., Zhongbao, L., Jingbo, S., Xiaowei, H. and Yongchun, H. (2018). Effects of fermented *Enteromorpha prolifera* on growth performance, non-specific immunity and digestive enzyme activity of pearl gentian grouper. *wmmfjzb*. 40(4): 96-105.
- APHA. (2005) Standard methods for the examination of water and wastewater. American Water Works Association, Environment Federation, Washington, DC
- Cherry, L.S., Crandal, L.A. (1932). The specificity of pancreatic lipase; its appearance in the blood after pancreatic injury. *Am. J. Physiol. Leg. Cont.* 100(2): 266-273.
- CIBA [Central Institute of Brackishwater Aquaculture]. Annual report (2022). Central Institute of Brackishwater Aquaculture. Chennai.
- Cook, D.G., Iftikar, F.I., Baker, D.W., Hickey, A.J. and Herbert, N.A., (2013). Low-O₂ acclimation shifts the hypoxia avoidance behaviour of snapper (*Pagrus auratus*) with only subtle changes in aerobic and anaerobic function. *Journal of Experimental Biology*. 216(3): 369-378.
- Drabkin, D.L. (1946). Spectrophotometric studies XIV. The crystallographic and optical properties of the hemoglobin of man in comparison with those of other species. *Journal of Biological Chemistry*. 164(2): 703-723.
- Drapeau, G.R. (1976). Protease from *Staphylococcus aureus*. In: *Methods in Enzymology*. 45: 469-475.
- FAO. (2024). The State of World Fisheries and Aquaculture (2022). Blue Transformation in Action. Rome, FAO.
- Jhingran, V.G. and Natarajan, A.V. (1969). A study of the fisheries and fish populations of the Chilka Lake during the period 1957-65. *J. Inland Fish. Soc. India*. 1(1): 49- 126.
- Joseph, P.S. and Mohan Joseph, M., 1988. Feeding habits of the Pearl-Spot *Etroplus suratensis* (Bloch) in the Nethravati-Gurpur estuary. In *The First Indian Fisheries Forum. Proceedings. Asian Fisheries Society*. (pp. 203-206)
- Khanzadeh, M., Esmaeili Fereidouni, A. and Seifi Berenjestanaki, S. (2016). Effects of partial replacement of fish meal with *Spirulina platensis* meal in practical diets on growth, survival, body composition and reproductive performance of three-spot gourami (*Trichopodus trichopterus*)(Pallas, 1770). *Aquaculture international*. 24: 69-84.

- Kumar, M.H., Gajaria, S.C. and Radha, K.S. (2004). Growth and development of catla (*Catla catla*) fed with different levels of diet containing *Spirogyra* sp. Bioresource Technology. 95(1): 73-76.
- Nelson, D.A., Morris, M.W. (1989). Basic Methodology, Haematology and Coagulation. In: Clinical diagnosis and Management by Laboratory Methods. [Nelson, D.A., Henry, J.B. (Eds)]. 17th Edn. Philadelphia, USA: WB Saunders. Part IV. p 578-625.
- Nur, A., Hossain, M.F., Hasan, M.N., Zannat, S., Chakraborty, K. and Rafiquzzaman, S.M. (2020). Effect of selected seaweed powder as a fish feed on growth and immune system of tilapia (*Oreochromis niloticus*). International Journal of Fish Aquaculture Studies. 8(4): 24-30.
- Olvera Novoa, M.A., Domínguez Cen, L.J., Olivera Castillo, L. and Martínez Palacios, C.A. (1998). Effect of the use of the microalga *Spirulina maxima* as fish meal replacement in diets for tilapia, *Oreochromis mossambicus* (Peters), fry. Aquaculture research. 29(10): 709-715.
- Padmakumar, K.G., Bindu, L. and Manu, P.S. (2012). *Etioplos suratensis* (Bloch), the State Fish of Kerala. J. Biosci. 37(6): 925-931, Ind. Acad. Sci.
- Priyatharshni, A., Antony, C., Uma, A., Ahilan, B., Chidambaram, P., Ruby, P. and Prabu, E. (2024). Effect of dietary seaweed supplementation on physiological responses of genetically improved farmed Tilapia. Indian Journal of Animal Research. 58(4). doi: 10.18805/IJAR.B-5295.
- Rahman, M., Al Mamun, M.A., Rathore, S.S., Nandi, S.K., Kari, Z.A., Wei, L.S., Tahiluddin, A.B., Rahman, M.M., Manjappa, N.K., Hossain, A. and Nasren, S. (2023). Effects of dietary supplementation of natural *Spirulina* on growth performance, hemato-biochemical indices, gut health and disease resistance to *Aeromonas hydrophila* of Stinging catfish (*Heteropneustes fossilis*) fingerling Aquaculture Reports. 32: 101-727.
- Rick, W. and Stegbauer, H.P. (1974). α -Amylase Measurement of Reducing Groups. In: Methods of Enzymatic Analysis, Academic Press. pp. 885-890.
- Ruby, P., Ahilan, B., Antony, C., Manikandavelu, D., Selvaraj, S., and Moses, T.L.S. (2022). Evaluation of effect of the different stocking densities on growth performance, survival, water quality and body indices of pearlspot (*Etioplos suratensis*) fingerlings in biofloc technology. Indian Journal of Animal Research. 56(8). doi:10.18805/IJAR.B-4922.
- Saleh, R.S., Mohammady, E.Y., El Haroun, E. and Hassaan, M.S. (2022). Dietary dried periphyton can improve growth, digestive enzyme, serum biochemical, antioxidant response and intestinal morphometric of Nile tilapia. Aquaculture Research. 53(18): 6463-6477.
- Sarath G, De la Motte RS, Wagner FW (1989). Protease Assay Methods. In: Proteolytic Enzymes: A Practical Approach. [Beynon, R., Bond, J. (eds)]. IRL, Oxford. pp 25-56.
- Singh, A.K. and Lakra, W.S. (2011). Risk and benefit assessment of alien fish species of the aquaculture and aquarium trade into India. Reviews in Aquaculture. 3(1): 3-18.
- Sivakumar, N., Sundararaman, M. and Selvakumar, G. (2018). Evaluation of growth performance of *Penaeus monodon* (Fabricius) fed diet with partial replacement of fishmeal by *Spirulina platensis* (Sp) meal. Indian Journal of Animal Research. 52(12): 1721-1726. doi: 10.18805/ijar.B-3438.
- Subasinghe, R., Soto, D. and Jia, J. (2009). Global aquaculture and its role in sustainable development. Reviews in aquaculture. 1(1): 2-9.
- Wassef, E.A., El Masry, M.H. and Mikhail, F.R. (2001). Growth enhancement and muscle structure of striped mullet, *Mugil cephalus* L., fingerlings by feeding algal meal based diets. Aquaculture research. 32: 315-322.
- Wintrobe, M.M. (1934). Anemia: classification and treatment on the basis of differences in the average volume and hemoglobin content of the red corpuscles. Archives of Internal Medicine. 54(2): pp.256-280.
- Younis, E.S.M., Al-Quffail, A.S., Al-Asgah, N.A., Abdel-Warith, A.W.A. and Al-Hafedh, Y.S. (2018). Effect of dietary fish meal replacement by red algae, *Gracilaria arcuata*, on growth performance and body composition of Nile tilapia *Oreochromis niloticus*. Saudi Journal of Biological Sciences. 25(2): 198-203.