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# BROWN SEAWEED (*Sargassum ilicifolium*) AS A NUTRITIONAL SUPPLEMENT: EFFECTS ON GROWTH AND HEMATOLOGY IN *Labeo rohita*

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**ABSTRACT:**

This experimental trial aimed to evaluate the impact of dietary supplementation with *Sargassum ilicifolium* on the growth performance and hematology of *Labeo rohita*. Fingerlings weighing an average of 2.36g were allocated into the control and three treatment groups (3%, 5%, and 7% BSW), each with triplicate (25 fingerlings/aquarium) for 90 days after acclimatized for two weeks. The results indicated that the highest growth rate ($P<0.05$) occurred in the T3 treatment group, with a final weight of 5.31±0.00a, followed by T2 (4.84±0.01b), and T1 (4.63±0.00c), while the lowest growth was observed in the control group (4.20±0.02d). Maximum weight gain was recorded in T3, which contained the highest level of Sargassum supplementation. Other treatments also showed higher weight gain than the control group. Specific growth rate and protein efficiency were also observed to be higher in treatment groups, particularly in T3, than in the control group. Hematological profiles, i.e., red blood cells (RBCs), white blood cells (WBCs), Hemoglobin (Hb), Hematocrit (HCT), blood indices and leukocytes count also showed better results in T3 and other treatments as compared to the control group. These findings suggest a high inclusion of sargassum is most effective for promoting the growth and enhancement of the hematology of *Labeo rohita*, and it is an environmentally sustainable way, as it is economical, environmentally friendly, and readily available alternative for aquaculture.

**KEYWORDS**: *Labeo Rohita*, *Sargassum Ilicifolium*, Growth Performance, Protein Efficiency Ratio, And Hematology.

## 1. INTRODUCTION

The economic value of algae has gained attention due to their diverse applications in various industries, including aquaculture. In fish feed, algae offer numerous benefits: they provide high-quality protein and essential amino acids, improve feed conversion rates, and contain bioactive compounds with immunomodulatory and anti-inflammatory properties (Veluchamy and Palaniswamy, 2020). Algae are an excellent source of omega-3 fatty acids, which improve nutrition and flesh quality in fish. By adding algae to fish diets, sustainable aquaculture practices can be supported, as this reduces the need for fishmeal and helps lessen the pressure on wild fish populations. In summary, including algae in aquaculture is beneficial as it enhances fish health and promotes sustainability (Liu *et al.,* 2023; Rathod *et al*., 2025). Algae, as primary producers, are essential to global aquatic ecosystems; they sustain marine food webs and significantly contribute to carbon dioxide fixation and oxygen production (Pereira, L. 2021; Vijayaram *et al*., 2024). They are utilized for sustenance, pharmaceuticals, cosmetics, and biofuels, among other applications. The seashore of Pakistan is home to around 70 genera and 27 distinct kinds of algae, predominantly seaweeds (Siddiqui *et al*., 2019).

Based on color, algae fall into three main groups: brown algae (Phaeophyta), green algae (Chlorophyta), and red algae (Rhodophyta) (Shahzad et al., 2023). Among the most important economic algae in Phaeophyta is sargassum. It provides a wide range of development and application prospects in industry, agriculture, food, medicine, and ecological restoration (Zou *et al*., 2022), so helping greatly to preserve the marine ecology. There are more than 400 different species of brown seaweed in the genus Sargassum, and each has special ecological use. Common in tropical locations, it produces steroids, polysaccharides, and polyphenols. Bioactive polysaccharides derived from brown seaweeds have gained significant interest among pharmacology and biochemistry researchers. Brown seaweed-derived sulfated polysaccharides have shown anti-inflammatory, antiviral, antioxidant, anti-cancer, and anticoagulant properties (Jyotsna *et al*., 2021).

Aquaculture, which has been utilized for centuries to produce food, is currently expanding rapidly (Eroldogan *et al*., 2022). Fisheries provide food for humans and industrial raw materials for national construction, contributing significantly to the national economy and contributing to the country's economy (Hu *et al*., 2021). Indian major Carp (*Labeo rohita*), commonly known as rohu, is profitable from an economic standpoint, other Labeo species could also be cultivated because of their excellent nutritional content, mouthwatering flavor, low economic worth, and high market value. *L. rohita* is one of the fish species that is most often raised in Asia (Khizar *et al*., 2024).

Sargassum is a desirable addition to the fish diet since it is a bioactive component that is rich in vitamins, minerals, and amino acids (Morais *et al*., 2020). It stimulates immune responses, fortifies antioxidant defences, and improves growth performance. Sargassum improves fish health and resilience to environmental stressors by increasing nutritional absorption, disease resistance, stress tolerance, and digestive enzyme activity. It also improves meat quality and lipid metabolism,

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rendering fish suitable for human consumption (Saade *et al*., 2020; Sabzi *et al*., 2023; Gazali *et al*., 2024).

This study aims to examine the impact of *S. ilicifolium* on the hematological parameters, growth performance, and immune response of *L. rohita*. Standard fish feed ingredients, which often rely on expensive and scarce resources such as fishmeal and soy protein, may be partially replaced with the sustainable and cost-effective feed additive Sargassum. Given the abundance of sargassum in tropical and subtropical regions, its application in aquaculture reduces dependence on imported feed sources, thus decreasing production costs for fish farmers.

## 2. MATERIALS AND METHODS

This experimental study evaluated the effect of *S. ilicifolium* supplementation on the growth performance and hematology of *L. rohita.* It was carried out at the Fisheries Laboratory, Department of Zoology, Government College University Faisalabad, for 90 days.

**Collection of *S. Ilicifolium:***

Brown seaweed (*S. ilicifolium*) was collected from Manora Beach, Karachi. The seaweed was thoroughly washed under tap water to remove any debris or impurities, followed by another wash with distilled water to ensure complete cleanliness. The cleaned seaweed was dried and ground into a fine powder. This powdered seaweed material was stored at room temperature until it was ready for further analysis.

**Experiment Design:**

One control and three experimental diets were prepared with varying amounts of sargassum powder in them. Three hundred fingerlings (2.36g) of *L. rohita* were purchased from Punjab Fish Hatchery, Satyana Road, Faisalabad. Fish were acclimatized for two weeks in the Fish Lab. During acclimatization, fish were fed the control diet of 4% of their body weight once a day. After 15 days, fish were randomly stocked in four aquariums: Control (0%BSW), T1(3%BSW), T2(5%BSW), and T3 (7%BSW), each with triplicates (25 fingerlings per aquarium), arranged using interspersion. Fish were fed with 4 % of their live body weights once a day. Feeding behaviour was also observed in the trial.

**Study of Growth Parameters:**

Total body length and weight were measured fortnightly over 90 days to track growth performance metrics, according to the approach of Al Sulivany et al. (2024). After data collection, fish were stocked back into respective aquariums. The ethics committee of the Government College University Faisalabad, Pakistan, approved the animal study protocol for scientific purposes. Approval no. is GCUF/ERC/505.

The following formulas were used to calculate the growth parameters;

$$\mathbf{K} = \frac{\boldsymbol{W \times 100}}{\boldsymbol{L}^3}$$

$$\mathbf{WG\ (g)} = \boldsymbol{Final\ body\ weight - Intial\ body\ weight}$$

$$\mathbf{SGR}(\%) = \frac{\boldsymbol{Weight\ Gain}}{\boldsymbol{Experimental\ Days}} \times \mathbf{100}$$

$$\boldsymbol{PER}\ (\%) = \frac{\mathbf{Weight\ gain}}{\mathbf{Amount\ of\ protein\ fed}}$$

**Analysis of Hematology :**

Blood samples were collected from the caudal vein of fish to assess the blood indices. The total number of red blood cells (RBCs) and white blood cells (WBCs) were assessed using a hemocytometer with a validated Neubauer counting chamber (Blaxhall & Daisley, 1973). Hemoglobin concentration estimates were obtained using the cyanomethemoglobin method, as outlined by Wedemeyer & Yastuke (1977). The microhematocrit technique (Brown *et al*., 1980) was employed to ascertain hematocrit using capillary tubes. The percentage composition of leukocytes was determined based on the identification characters listed (Ivanova, 1983). The subsequent factors were employed for calculation: MCHC (mean corpuscular hemoglobin concentration), MCH (mean corpuscular hemoglobin), and MCV (mean cell volume) utilizing the specified formulas.

$$\boldsymbol{MCHC}\ (\%) = \frac{\boldsymbol{Hb}}{\boldsymbol{PCV}} \times \mathbf{100}$$

$$\boldsymbol{MCV}\ (\mathbf{fl}) = \frac{\boldsymbol{PCV}}{\boldsymbol{RBC}} \times \mathbf{100}$$

$$\boldsymbol{MCH}\ (\mathbf{pg}) = \frac{\boldsymbol{Hb}}{\boldsymbol{RBC}} \times \mathbf{100}$$

**Statistical Analysis :**

After the possible outcomes were known, data on growth and hematology were subjected to statistical analysis. ANOVA and ± SE values were computed by using GraphPad ver. 6.0, and the difference between means was calculated by Tukey's HSD test.

## 3. RESULTS

**Growth Parameters :**

The effects of *S. ilicifolium* on the growth performance of *L. rohita* are shown in Table 1. After the 90-day trial, Weight gain (WG) and Specific growth rated (SGR) were significantly higher (P<0.05) in groups fed with 5%(T2) and 7% (T3) of *S. ilicifolium* compared to the control, with the highest WG of 2.96 g and SGR of 0.90 observed in the T3. In contrast, the control group exhibited a minimum WG of 1.57 g. The highest Protein Efficiency Ratio (PER) value of 0.092 was recorded in the 7% fed group, significantly higher compared to the control group's PER of 0.49. However, the highest condition factor was observed in the control group, and the lowest was observed in the T3 treatment. Overall, these results suggest the beneficial effects of *S. ilicifolium* on the growth performance of *L. rohita* at higher dietary levels.

**Table 1:** Effects of dietary inclusion of *Sargassum ilicifolium* on growth performance of *L. rohita*.

| Parameters | Control | T1 (BSW3%) | T2 (BSW5%) | T3 (BSW7%) | P-value |
|---|---|---|---|---|---|
| Initial Weight(g) | 2.360±0000a | 2.360±0.000a | 2.350±0.000a | 2.350±0.000a | N. S |
| Final Weight (g) | 4.200±0.030d | 4.630±0.030bc | 4.840±0.040b | 5.310±0.020a | 0.0298* |
| WG (%) | 1.570±0.029d | 2.270±0.009c | 2.490±0.019b | 2.960±0.000 a | 0.0023** |
| CF (K) | 1.820±0.020d | 1.75±0.024c | 1.70±0.019b | 1.64 ±0.159a | 0.0001** |
| SGR (%) | 0.560±0.019d | 0.750± 0.009c | 0.800± 0.030b | 0.900± 0.030a | 0.0043** |
| PER (%) | 0.049±0.001d | 0.0705±0.002c | 0.0779±0.002b | 0.092± 0.003a | 0.0038** |

Values are presented as mean± SE (n = 12 fish from each treatment), WG weight gain, CF condition factor, SGR specific growth rate, and PER protein efficiency ratio.

**Hematological Assay:**

Hematological parameters in *L. rohita* improved significantly in fish-fed enriched diets (Table 2). The RBC count was notably higher in fish fed the highest level of 7% (T3) compared to the control group ($P < 0.01$). Hemoglobin values also increased significantly across all treatment groups, with the highest level observed in the T3 (16.5), contrasting with the control group (12.3), where significant differences were noted ($P < 0.01$). WBC counts showed substantial elevation in the T3 (11.4) compared to the control (4.8) ($P < 0.01$). Hematocrit levels demonstrated a marked increase in the T3 (54.5) versus the control (35.5), with significant differences across all treatments ($P < 0.01$). While MCV, MCH and MCHC values were elevated in the enriched groups, significant differences for MCV were found compared to control ($P < 0.01$). Still, no significant differences in MCH were found between groups ($P > 0.01$). Additionally, the number of blood leukocytes, i.e., Neutrophils, Lymphocyte**s,** Monocytes**,** Eosinophils and Platelets, was higher in fish fed the enriched diets versus controls, with significant differences particularly noted in the highest inclusion level ($P < 0.01$). Overall, these results indicate that dietary supplementation with *S. ilicifolium* enhances the hematological profile of *L. rohita*, especially at higher inclusion rates

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**Table 02:** Hematological changes of *L. rohita* fed diets enriched with different levels of *Sargassum ilicifolium* (0, 3, 5 and 7%) for 12 weeks

| Parameters | Control | T1 (BSW3%) | T2 (BSW5%) | T3 (BSW7%) | P-value |
|---|---|---|---|---|---|
| Hemoglobin(g/dl) | 12.300±0.200d | 12.900±0.100bc | 13.00± 0.300b | 16.500±0.500a | 0.022* |
| RBC’s ($\times10^{-6}/\mu l$) | 3.750± 0.250d | 4.000± 0.300c | 4.200±0.400b | 5.250± 0.250a | 0.0088** |
| HCT (%) | 35.500±0.500d | 45.750± 0.750c | 48.650±0.350b | 54.500±0.500a | 0.0001** |
| MCV (fl) | 77.500±0.200d | 79.900± 0.100c | 88.700± 0.300b | 98.500±0.500a | 0.0004** |
| MCH (pg) | 25.500±0.500d | 29.350± 0.050c | 31.100±0.400b | 34.500±0.500a | 0.0004** |
| MCHC (%) | 30.800±0.200d | 33.100±0.400c | 35.050±0.750b | 37.500±0.500a | 0.0029** |
| WBC’s($\times10^{3}/\mu L$) | 4.850± 0.150d | 8.450± 0.450c | 9.850± 0.450b | 11.450±0.450a | 0.0012** |
| Neutrophils (%) | 40.300± 0.300d | 57.900± 0.200c | 63.900± 0.100b | 73.900± 0.100a | 0.0001** |
| Lymphocytes (%) | 24.550± 0.550d | 29.500± 0.500c | 32.850± 0.150b | 39.750± 0.250a | 0.0001** |
| Monocytes (%) | 2.300± 0.300d | 3.700± 0.300c | 4.600± 0.400b | 7.250± 0.550a | 0.0041** |
| Eosinophils (%) | 1.000± 0.000d | 2.000± 0.000bc | 2.500± 0.500b | 3.600± 0.600a | 0.0391* |
| Platelets($\times10^{3}/\mu L$) | 338.250±0.350d | 360.000±0.250c | 370.000±0.300b | 401.900±0.200a | 0.0001** |

Data are presented as mean ± SE (n=12 fish from each treatment). Hb: hemoglobin concentration, RBC: red blood cells, WBC: white blood cells, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

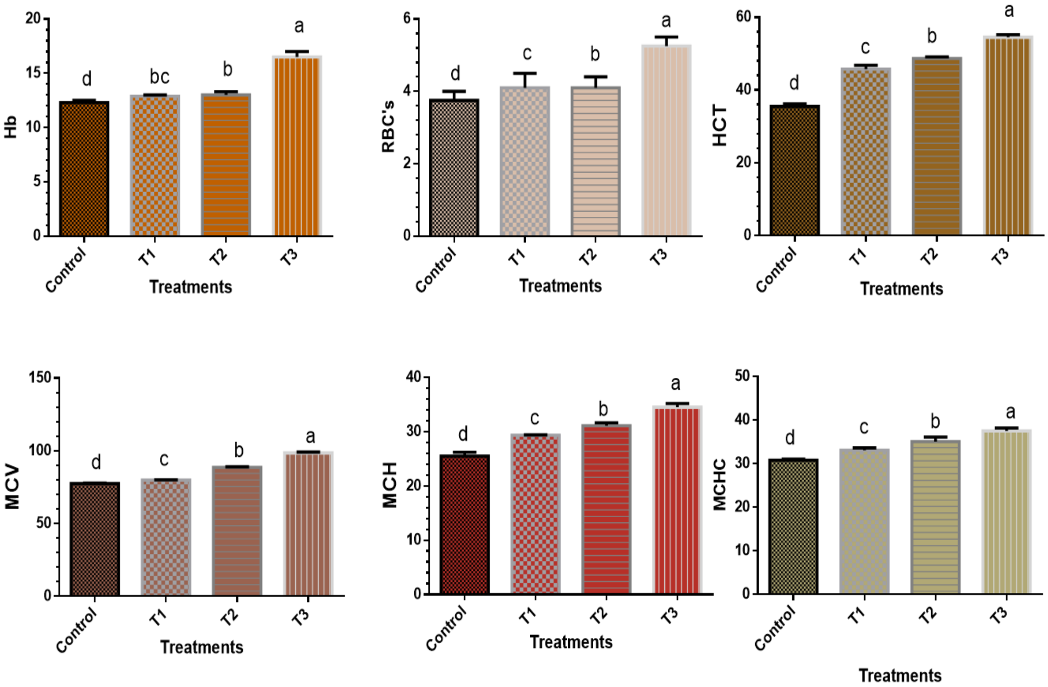


**Figure 1:** Graph illustration of hemoglobin, red blood cells, hematocrit, MCV, MCH, and MCHC of *L. rohita* reared under control and Treatment groups.

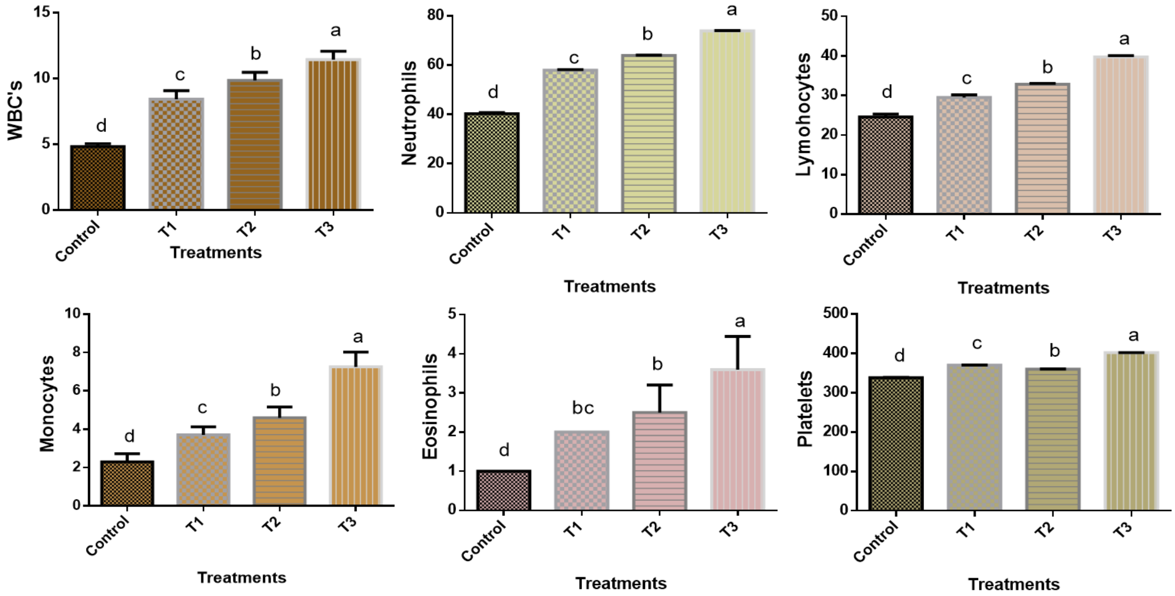


**Figure 2:** Graph illustration of white blood cells and leukocyte count of *L. rohita* reared under control and Treatment groups

## 4. DISCUSSION

In the present growth study, the inclusion of Sargassum spp. in the feed presented a significant improvement in growth rates associated with the control set. Among the different inclusion levels, the group with the highest inclusion (7%) showed the best growth performance, with the maximum final body weight (FBW), weight gain (WG), and specific growth rate (SGR). The present findings are similar to the studies in which tilapia showed better growth, WG and SGR when fed with 20 g/kg and 30 g/kg sargassum than control group (Abdelrhman *et al*., 2022), similarly Shi *et al*. (2021) stated that yellow catfish when fed with *Sargassum hornei* extract in feed enhanced growth and significant improvement in specific growth rate (SGR) and weight gain (WG) was observed. Yeganeh and Adel (2019) observed that average body weight, total body length and SGR enhance when supplemented with *S. ilicifolium* extract particularly at 7.5% inclusion. These results show a favorable association between increased seaweed inclusion and enhanced fish development, with the former showing much superior growth than the latter.

The positive effects observed in the current study align with the findings of Zeynali *et al*., (2020) and Seyed alhosseini (2023), who found that diets supplemented with *S. ilicifolium* and other seaweed species resulted in improved development performance and feed application. Based on the findings, Sargassum spp. Enhances growth in fish as it contains polysaccharides, carbohydrates and bioactive compounds that improve nutritional absorption in fish. Akbary (2019) explored that the highest body weight, condition factor and PER were observed in *Rastrelliger kanagurta* at a 15% inclusion level of *S. ilicifolium*.

In contrast, Shapawi and Zamry (2016) stated that *Sargassum polycystum* in Asian seabass did not improve growth. Similarly, *Palmaria palmata* has no significant effect on the development of Atlantic salmon Wan *et al*. (2016). The inclusion of *A. platensis* in diets of *Oncorhynchus mykiss* did not affect growth performance Teimouri *et al*. (2013). These studies showed that the growth-increasing effects of seaweeds may be wary due to different species nutritional requirements, environment, seaweed types and dosage. Plant-based protein source for fish feed has functional benefits due to its nutritional quality, sustainability, and cost-effectiveness. Asad et al., (2025).

Hematological indices, notably red blood cell count, hemoglobin, and hematocrit values, significantly improved in the current study when Sargassum spp. was added to fish diets at different levels (0, 3%, 5%, and 7%). These results are consistent with earlier research by Sabzi *et al*. (2023), which found that feeding fish species like *L. calcarifer*, *Cirrhinus mrigala*, and *Oreochromis niloticus* diets supplemented with seaweed significantly increased their RBC, Hb, and Hct levels.

With increasing dietary inclusion of Sargassum spp., a clear positive trend in RBC values was shown. This was especially true for the 7% inclusion group, which showed the highest significant rise in RBCs, Hb, and Hct when compared to the control. The developments imply that Sargassum spp. might have bioactive elements like polysaccharides and antioxidants, which, by enhancing oxygen circulation and the immune system, could help fish's blood parameters and general health.

Furthermore, the notable increase in neutrophils and WBC in diets supplemented with Sargassum spp. in fish-eating diets is a crucial indicator of immunological activation, which is consistent with the results of Yeganeh and Adel (2019), who also observed elevated WBC levels. Given that the body uses these cells to fight infections, the noted rise in neutrophils in our study points to a better immunological response. The 7% Sargassum spp. diet shown a notable increase in neutrophils, thus substantiating the hypothesis that Sargassum spp. can enhance fish immune systems.

A number of investigations have found the opposite, in contrast to these encouraging findings. A study conducted by Yang *et al*. (2014) found that fucoidan, a chemical derived from brown seaweed, had no significant effect on the red and white blood cell counts or hematocrit levels in yellow catfish.

In conclusion, the present study confirms that dietary inclusion of Sargassum spp. enhances hematological indices in fish, particularly RBC, Hb, Hct, and WBC levels. These improvements in blood demonstrate that Sargassum spp. contributes to better overall physiological status and immune function in fish. However, some studies also showed neutral and negative effects of seaweeds.

## CONCLUSION

The findings of this study demonstrated that the growth performance and immunological response of *Labeo rohita* significantly improved in the treatment groups with the dietary inclusion of *Sargassum ilicifolium* than in the control group. By comparing the treatments, the best results were observed in the treatment with 7% sargassum inclusion (T3) followed by 5%

inclusion (T2) and the least but better than control results were observed in the treatment with 3% sargassum inclusion (T1).

**Statements and Declarations**

**Conflict of interest:** Not applicable
**Consent to Participate:** All authors are agreed to submit the article in this journal
**Consent to Publish:** All authors are agreed to publish in this journal
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