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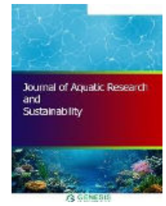

Original Research

Red seaweed, *Gracilaria* sp. a source of ingredients for the formulation of fish feed

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Abstract

Recent research on aquafeed has focused on using low-cost and nutrient-rich non-conventional feedstuffs. So, seaweeds can be considered a non-conventional feed ingredient for the aquafeed industry. This study evaluated the potentiality of selected seaweed (*Gracilaria* sp.) enriched feeds by assessing their effects on the growth performance, feed utilization, carcass composition, and palatability of *Hypophthalmichthys molitrix*. The study was conducted for 90 days under four treatments in 12 cages set in a pond at the Department of Fisheries, University of Rajshahi, Bangladesh. Four test feeds were made where one was without seaweed designed as SW0 (control) and three were enriched with seaweed (*Gracilaria* sp.) at the rates of 5, 10, and 15%, which were designed as SW1, SW2, and SW3, respectively. Sampling was conducted biweekly to evaluate weight increase. At the end of the study, the mean weight gain (MWG), specific growth rate (SGR), survival rate, and feed conversion ratio (FCR) were estimated using standard methods. In addition, carcass composition (protein, lipid, carbohydrate, moisture, and ash content) and palatability indicators (flavor, taste, and texture) of the fish were evaluated by following AOAC and organoleptic sensory methods. Significantly higher MWG and SGR were found in the fish of SW2, followed by the fish of SW1 and SW0, and lower in SW3, whereas the FCR was found better in the fish of SW2 compared to other treatments. The results of chemical analysis showed no significant difference in carcass composition but relatively higher carcass protein and lipid recorded in the fish of SW2. For the palatability test, a significantly higher organoleptic score was recorded in the fish of SW2 while lower in SW0. The outcomes of the study suggested that including 10% *Gracilaria* sp. can be effective in diets for *H. molitrix* with no negative results on the growth, carcass composition, and palatability indicators.

1. Introduction

Fish has high nutritional value because of having rich contents of protein, amino acid composition, and fatty acids (Ahmed *et al.*, 2022). The production of protein-rich foods has significant implications for national food security. By promoting aquaculture, it can be raised significantly. The most crucial element for increasing aquaculture output and profitability is feed. In fact, aquaculture production heavily depends on the external aquafeeds or nutrients supply to the aquaculture system (Tacon and Metian, 2015). Aquafeed production has been widely recognized as one of the fastest-expanding agricultural industries in the world (Ali, 2024).

Fishmeal, a commonly used feed ingredient, is currently scarce and expensive, while the plant feed ingredients (soybean meal and mustard oil cake meal) contain anti-nutritional elements that have increased interest in seeking alternative feedstuffs for aquaculture feeding. Recent research in aquaculture has focused on the use of environmentally friendly and nutrient-rich non-conventional feedstuffs that contain necessary amino acids, fatty acids, vitamins, and minerals. Therefore, the exploration and utilization of available alternative feedstuffs for aquaculture is more relevant to reduce feed costs as well as to improve the quality of feed. In this context, nutrient-rich seaweeds can be considered a non-conventional feed ingredient for aquafeeds (Mwendwa *et al.*, 2023a). They can be a sustainable and suitable alternative ingredient in aquafeeds considering their nutrient profiles (Costa *et al.*, 2021; Panteli *et al.*, 2024).

Seaweeds are typically found in large quantities in the near-shore marine habitats of all the world's oceans. The coasts of Saint Martin Island, Chittagong, and Cox's Bazar provided reports of 200 marine algal species, or seaweeds including *Hypnea*, *Gracilaria*, *Gelidium*, *Enteromorpha*, *Halimeda*, *Padina*, *Dictyota*, *Caulerpa*, *Hydroclathrus*, *Sargassum*, *Kappaphycus*, and *Porphyra* which have substantial commercial importance (Islam *et al.*, 2022). Sarkar *et al.* (2016) also reported fourteen commercially important seaweed taxa, among which *Hypnea*, *Caulerpa*, *Enteromorpha*, *Gracilaria* contain high levels of essential nutrients. Seaweeds are a good source of protein, fatty acids, vitamins, fiber, macro- and trace elements, and significant bioactive substances (Penalver *et al.*, 2020). *Gracilaria* sp. contains high crude protein, amino acid profiles, β-carotene, phosphorus, and low crude lipid and heavy metals (Aziz *et al.*, 2021). Seaweeds' diverse nutritional qualities have led to research into them in an effort to find new, natural sources of useful components for animal foods.

However, several researchers have used seaweeds as alternative ingredients in diets for different fish species and reported positive effects on growth, feed utilization, protein deposition, disease resistance, and carcass quality of fish (Hussein, 2017; Xuan *et al.*, 2019; Nur *et al.*, 2020; Mwendwa *et al.*, 2023b). Still, there are currently few studies conducted in Bangladesh on the potentiality of seaweeds as feed ingredients for carp fish. Therefore, the present study aimed to evaluate the potentiality of selected seaweed

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(*Gracilaria* sp.) enriched feeds by assessing their effects on the growth, carcass composition, and palatability of *H. molitrix* as a candidate aquaculture species in Bangladesh.

2. Materials and Methods

2.1 Ethical approval

No animals were harmed during the experiment. All procedures performed in this investigation were in accordance with the ethical guidelines provided by the International Council for Laboratory Animal Science (ICLAS) for researchers.

2.2 Study area

The study was carried out in 12 cages placed in a research pond located at the northern side of the Faculty of Fisheries, University of Rajshahi, Bangladesh (Figure 1) for 90 days (3 months) from June to August 2022. The cages (each 2.72 m^3) were made of iron rods and covered by a special synthetic nylon knotless net with a 5 mm mesh size with an opening for supplying feed and handling fish during sampling.

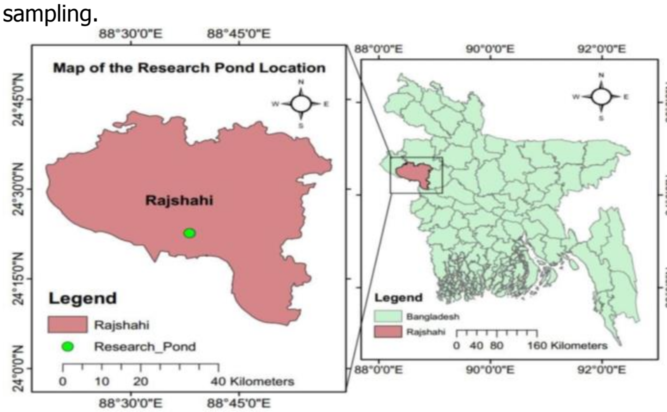

Figure 1. Map of the research pond location.

2.3 Collection and preparation of seaweeds

The selected seaweed (*Gracilaria* sp.) was collected from the coast of St. Martin Island, Cox's Bazar. At first, the collected seaweed was soaked for 24 hours in a solution containing 5 g of maize-cob ash (potassium hydroxide) diluted at a concentration of 5 g/l. Then, it was boiled in water for 5 minutes at 100 °C. Treated seaweed was also dried to a consistent weight following each treatment. Before use, the dried seaweed was crushed and sieved using a 2 mm mesh screen, and kept in a polyethylene bag.

2.4 Experimental design

Four types of feed were used in this trial as four treatments (Table 1). ACI Group's commercial carp grower feed treated the control (SW0). Three test feeds (SW1, SW2, and SW3) were formulated by using different levels of dried seaweed with the conventional feed ingredients. A fully randomized design was used to assign the treatments to the cages.

Table 1. Design of experimental cage setup.

Treatment	Cage No.	Feed
SW0	C1, C10 and C5	Feed without *Gracilaria* sp. as control
SW1	C7, C3 and C12	Feed with 5% *Gracilaria* sp. inclusion
SW2	C2, C9 and C6	Feed with 10% *Gracilaria* sp. inclusion
SW3	C8, C4 and C11	Feed with 15% *Gracilaria* sp. inclusion

2.5 Feed formulation

For the formulation of three test feeds, conventional feed ingredients (rice polish, mustard oil cake, fish meal, wheat bran, molasses, soybean oil, and vitamin premix) were used along with the selected seaweed (Table 2). The proximate compositions of these ingredients were evaluated, and the formulation was done using spreadsheet analysis. The protein content of the formulated feed was targeted to match that of the control feed (determined earlier) to obtain an iso-protein diet across all the treatments. For the preparation of feed, the required amount of each ingredient was weighed and mixed properly with the optimum amount of water to form the dough. A pelleting machine was used to extrude the dough and turn it into pellets. After being sun-dried, the pellets were sealed in polythene bags and kept at 4 °C for storage. The chemical analysis of the test feeds was done through ensuing standard methods (AOAC, 2005), and the data are shown in Table 3. The values obtained through chemical analysis of the test feeds showed no significant variation.

Table 2. Dietary inclusion level of different ingredients in three formulated feeds.

Ingredients (%)	Test feeds		
	SW1	SW2	SW3
Rice polish	25	20	18
Mustard oil cake	35	36	32
Fish meal	15	14	15
Wheat bran	15	15	15
Seaweed	5	10	15
Molasses	2.5	2.5	2.5
Soya-bean oil	2	2	2
Mineral premix	0.5	0.5	0.5

Table 3. Proximate composition of the experimental feeds.

Parameters (%)	Treatments			
	SW0	SW1	SW2	SW3
Moisture	13.92 ± 1.46^{a}	14.27 ± 1.33^{a}	13.94 ± 0.99^{a}	14.13 ± 1.34^{a}
Lipid	5.71 ± 0.63^{a}	6.29 ± 0.49^{a}	6.18 ± 0.86^{a}	5.83 ± 0.76^{a}
Protein	25.98 ± 3.69^{a}	26.11 ± 3.35^{a}	26.16 ± 2.17^{a}	26.06 ± 3.31^{a}
Ash	10.16 ± 1.27^{a}	9.57 ± 1.15^{a}	9.24 ± 1.14^{a}	10.31 ± 1.17^{a}
Carbohydrates	36.52 ± 3.67^{a}	38.22 ± 2.25^{a}	37.23 ± 3.08^{a}	37.19 ± 3.12^{a}

2.6 Experimental fish and feeding of fish

One hundred thirty fry of *H. molitrix* were collected from a nearby fish farm. Fish were transported in a van utilizing a scientifically appropriate method, equipped with an aeration system. The fish were adapted to the experimental environment for seven days before the beginning of the main study. Throughout this period, fish were given a commercial carp diet at the rate of 5% of their body weight. Following the acclimation phase, ten fish were transferred into each cage. Every day, the feeds were given twice, at 9:30 a.m. and 4:30 p.m., at a rate of 5% (2.5% + 2.5%) of the body weight each time. Throughout the study duration, fish were weighed biweekly, and the feed quantity was adjusted correspondingly.

2.7 Monitoring of water quality parameters

Throughout the study period, various physicochemical parameters of water, including temperature, dissolved oxygen (DO), pH, alkalinity, carbon dioxide (CO_2), and ammonium-nitrogen (NH_4-N) were checked biweekly. The temperature was recorded by using a Celsius thermometer. The pH was checked by a digital pH meter. DO, CO_2, total alkalinity, and NH_4-N concentrations were determined by using the HACH Kit (Model: DR/2010). Throughout the study period, the values of physicochemical parameters of water were within the appropriate range for aquaculture and didn't show any notable variations across the treatments.

2.8 Sampling for growth study

On the first day of the experiment, the first sampling was completed, and the weight of the fish in each cage was noted. Then, it was done every two weeks to keep data on the fish's weight. An electronic balance was used to weigh fish with a 0.1 g precision.

2.9 Analysis of growth performance and feed utilization

Growth performance and feed utilization by mean weight gain (MWG), specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR) were calculated by using the following formula,

$$\text{MWG} = \text{Mean Final Weight (MFW)} - \text{Mean Initial Weight (MIW)}$$

$$\text{SGR} = \frac{\ln(\text{MFW}) - \ln(\text{MIW})}{\text{Culture Duration (Days)}} \times 100$$

$$\text{SR} = \frac{\text{No.of Fish Harvested}}{\text{No.of Fish Stocked}} \times 100$$

$$\text{FCR} = \frac{\text{Feed Fed}}{\text{Live Weight Gain}}$$

2.10 Analysis of carcass composition

Following the trial, three fish were collected from each cage. After that, muscle from various body parts was taken out and refrigerated for storage. To determine the protein, lipid, carbohydrate, moisture, and ash content, the fish muscle samples were taken and analyzed according to the standard method (AOAC, 2005).

2.11 Palatability test

For the palatability test, fish flesh in the form of loins from each treatment was cooked in a traditional boiling method. The fish loins from each treatment were marked and cooked together to avoid any cooking bias. After consuming the cooked fish, selected expert panelists gave their scores blindly on the fish's flavor, taste, and texture following the specific structured scaling system (Table 4) described by Huss (1995).

Table 4. Organoleptic/sensory scoring scale for palatability test.

Palatability indicators			Score
Flavor	Taste	Texture	
Species-specific	Meaty flavor	Firm/elastic	10
Fresh fish	Sweet	Firm/springy	8
Slightly fishy or slightly sour	Slightly fishy	Less firm	6
Sour and stale	Slightly sour/some off flavor	Softer	4
Strong ammonia	Slightly rotten	Very soft	2
Rotten smell	Spoiled	Slippery	0

2.12 Statistical analysis

Using SPSS-21 software (SPSS, USA), statistical analysis was conducted through one-way analysis of variance (ANOVA) and Duncan's multiple-range test. To find the significant difference values, P=0.05 was applied.

3. Results

3.1 Growth performance and feed utilization of the fish

The result showed that the fish of SW2 had the considerably greatest MFW, MWG, and SGR, followed by the fish of SW1 and SW0, and the lowest in the fish of SW3, while there was no significant difference among the fish of SW0, SW1, and SW3. The highest value of FCR was found in the fish of SW0, while the lowest value was found in the fish of SW2, but no significant difference was found among the fish of SW0, SW1, and SW3. During the study period, no mortality was shown in the fish from different treatments (Table 5).

Table 5. The mean values of growth parameters under four treatments.

Parameters	Treatments			
	SW0	SW1	SW2	SW3
Initial weight (g)	253.16±12.56[a]	252.94±14.77[a]	253.76±13.78[a]	253.91±11.09[a]
Final weight (g)	478.56±24.33[b]	484.96±27.73[b]	509.64±23.37[a]	470.82±23.71[b]
Weight gain (g)	223.50±13.26[b]	233.1±17.86[b]	251.33±13.98[a]	216.91±21.63[b]
SGR (%)	0.77±0.05[b]	0.80±0.06[b]	0.88±0.04[a]	0.73±0.06[b]
FCR	2.49±0.11[b]	2.45±0.11[b]	2.19±0.14[a]	2.57±0.14[b]

* Fish given control feed was assigned to SW0, while fish given seaweed-enriched feed at 5, 10, and 15% were assigned to SW1, SW2, and SW3, respectively. A significant difference between values in the same row with different superscripts is indicated by P=0.05.

3.2 Carcass composition of the fish

The carcass crude protein content of fish from each treatment did not differ significantly, while the fish from SW2 were considered to have a comparatively greater value than the fish from the other treatments. The crude lipid and carbohydrate contents in the fish of SW2 and SW3 were relatively higher than the fish of other treatments.

Table 6. Carcass composition of the fish under four treatments.

Parameters (%)	Treatments			
	SW0	SW1	SW2	SW3
Protein	15.15±0.39[a]	15.13±0.42[a]	15.47±0.39[a]	15.35±0.23[a]
Lipid	2.19±0.19[a]	2.21±0.12[a]	2.45±0.11[a]	2.26±0.13[a]
Carbohydrate	3.11±0.32[a]	3.19±0.34[a]	3.46±0.57[a]	3.38±0.53[a]
Ash	3.21±0.21[a]	3.12±0.16[a]	3.35±0.15[a]	3.23±0.19[a]
Moisture	75.84±0.76[a]	75.53±0.85[a]	74.39±0.71[a]	74.80±0.91[a]

* Fish given control feed was assigned to SW0, while fish given seaweed-enriched feed at 5, 10, and 15% were assigned to SW1, SW2, and SW3, respectively. A significant difference between values in the same row with different superscripts is indicated by P=0.05.

Estimated carcass ash and moisture contents in the fish among the treatments also showed no significant variation but relatively higher ash content was recorded in the fish of SW2 and lower in the fish of SW1, whereas the higher moisture content in the fish of SW0 and the lower in the fish of SW2 (Table 6).

3.3 Palatability of cooked fish flesh

The organoleptic sensory scores were collected from a selected panel of consumers to evaluate the palatability of the cooked fish flesh. Significantly higher scores of flavor, taste, and texture were obtained in the fish of SW2 and lower in the fish of SW0, but the fish between SW1 and SW3 showed no significant difference. The sum of the scores of three organoleptic criteria (flavor, taste, texture) was also higher in the fish of SW2 and lower in the fish of SW0 (Table 7).

Table 7. Organoleptic score on cooked fish flesh under four treatments.

Organoleptic criteria	Treatments			
	SW0	SW1	SW2	SW3
Flavor	7.97±0.20[c]	8.12±0.20[b]	8.79±0.19[a]	8.19±0.17[b]
Taste	7.79±0.13[c]	7.97±0.23[b]	8.82±0.13[a]	8.03±0.16[b]
Texture	7.63±0.13[c]	8.13±0.23[b]	8.48±0.15[a]	8.22±0.17[b]
Total Score	23.47±0.18[c]	24.21±0.24[b]	26.09±0.19[a]	24.44±0.23[b]
Rank	4th	2nd	1st	3rd

* Fish given control feed was assigned to SW0, while fish given seaweed-enriched feed at 5, 10, and 15% were assigned to SW1, SW2, and SW3, respectively. A significant difference between values in the same row with different superscripts is indicated by P=0.05.

4. Discussion

4.1 Growth performance and feed utilization

To determine the potential impact on growth and feed consumption of *H. molitrix*, seaweed (*Gracilaria* sp.) was added to the feed at several doses. As per the study's findings, the fish fed 10% seaweed-enriched feed (SW2) showed the highest weight gain, specific growth rate, and lower feed conversion ratio, while the fish fed 15% seaweed-enriched feed (SW3) showed the lowest mean weight gain, specific growth rate, and higher feed conversion ratio. The growth data indicated that the addition of a certain level of seaweed in the feed enhanced the growth of the fish. The finding of the present study was more or less comparable with the report of Al-Asgah *et al.* (2016), who found that *C. gariepinus* may consume up to 10% of *G. arcuata* in their diets. They also found that the fish were fed a diet containing up to 20% and 30% of this ingredient had lower growth and feed utilization. Hussein (2017) stated that the diets of Nile tilapia can be supplemented with up to 5% seaweed without causing any negative effects or anatomical abnormalities. Xuan *et al.* (2019) found that feeding juvenile red sea bream (*Pagrosomus major*) a diet containing 3% *G. lemaneiformis* could enhance their growth and feed utilization. They also reported that feeding this fish with a diet containing 15% *G. lemaneiformis* was also feasible because it did not affect growth performance. In another study by Xuan *et al.* (2013), no reduced growth was found in juvenile black sea bream (*Acanthopagrus schlegelii*) fed diets based on *Gracilaria lemaneiformis* even at the 15% inclusion level. Additionally, they noted that growth performance was noticeably low when the addition of *Gracilaria* sp. reached 20%. Nur *et al.* (2020) observed that Nile tilapia given 30% seaweed meal showed positive growth. The findings of the previous reports are more or less supportive of the present study. It was also observed that the rate of seaweed included in fish diets may be determined by the fish's feeding behavior as well as the species of seaweed.

4.2 Carcass composition

The results of carcass analysis of the fish fed with the feed enriched with different doses of *Gracilaria* sp. and the control feed showed no significant differences in the body composition among the treatments. This finding was in accordance with the results obtained by Sotoudeh and Jafari (2017), who reported that supplementation of the experimental diets with *G. pygmaea* did not affect the carcass composition of juvenile rainbow trout. Though carcass lipid content showed no significant difference among the treatments, relatively higher lipid content was found in the fish of SW2 and SW3 (where 10 and 15% seaweed-enriched feed were used). This finding contrasted with that of Valente *et al.* (2006), who found that at the inclusion level of 5–100% *Gracilaria* diets, the lipid content of juvenile European sea bass (*Dicentrarchus labrax*) lowered which might be due to the physiological variation of the fish.

The carcass ash and moisture contents also showed no significant difference among the treatments but relatively higher moisture contents were found in SW0 (where control feed was used). The finding was also showed that increasing seaweed percentage has a relatively inverse relation to moisture contents in the carcass composition. Ahmed *et al.* (2022) reported that the moisture and lipid content of fish muscle are inversely correlated, as indicated in the current study, where increased lipid content was observed in the fish of SW2 and SW3, corresponding to substantially lower moisture content. The findings of the current study reveal that the addition of dietary *Gracilaria* sp. into fish feed has no negative effect on the carcass composition of *H. molitrix*.

4.3 Palatability

According to the overall organoleptic score, the fish flesh of SW2 had the highest score and was ranked first, followed by the fish flesh of SW3 in second place, SW1 in third place, and SW0 in fourth place. The results of the palatability test of the cooked fish flesh indicated that the inclusion of *Gracilaria* sp. in the fish feed can modify the palatability indicators (flavor, taste, and texture) and the fish fed with 10% seaweed enriched feed had the highest scores for taste, texture, and flavor. This puts the fish fed 10% seaweed enriched feed under the score "10" on the score table, indicating that they had better fresh fish flavor, taste, and texture quality, i.e., most palatable compared to the fish groups fed with other test feeds used in this study. This may be the result of the fish having relatively higher lipid content in their carcasses (Table 6). Food texture and flavor are known to be influenced by lipids, which can be found in foods as free oil or fat scattered throughout a solid matrix or as emulsions. By generating volatile oxidation products and transferring the flavor of short-chain free fatty acids, lipids enhance the flavor of food (Shahidi and Weenen, 2005). Nevertheless, there are hardly any studies on how the seaweed meal affects the palatability of fish flesh, and more investigation is needed to reach a firm conclusion.

4. Conclusions

The study concluded that the addition of a certain level (10%) of *Gracilaria* sp. in the feed enhanced growth performance, feed conversion ratio, and overall palatability of *H. molitrix* without any adverse effects.

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Data availability

Data will be made available on request.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contribution

Conceptualization: Md. Jeshan Ali and Md. Mahabubur Rahman; **Data collection:** Md. Jeshan Ali and Mst. Khadiza Khatun; **Data analysis:** Md. Risad Sarkar and Adnan Shabbir; **Figure preparation:** Md. Risad Sarkar. All authors critically reviewed the article and agreed to submit the final version of the article.

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