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# POTENTIAL OF *PADINA GYMNOSPORA*, AN ALGAL BASED FORMULATED FEED ON GROWTH PERFORMANCE, NUTRITIONAL AND PROTEIN INDICES OF CULTIVABLE BRACKISHWATER FISH *ETROPLUS SURATENSIS*- AN INITIATIVE IN REPLACING FISH MEAL FOR SUSTAINABLE FISH FARMING

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## ABSTRACT

This analysis is to assess the potential of replacing conventional fish meal used in fish diet with *Padina gymnospora* supplementation under laboratory feeding conditions for 30 days on growth performance parameters, feeding indices, feed conversion efficiency and protein indices in fingerlings of *Etroplus suratensis*. Proximate composition, β- carotene, total carotenoids, vitamin E and C content of commercial control feed and *Padina gymnospora* formulation was analysed in control and experimental feed. The analysis determined better growth performance index and significant increase in nutritional indices in fishes fed on a formulated diet.



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# INTRODUCTION

In order to provide a sustainable supply of fish, the aquaculture industry has grown quickly in the last several years. Since fish meal is the most expensive ingredient, demand for it will only rise as the aquaculture industry is expected to grow. This will have a detrimental effect on pelagic fish, which are the primary source of fish meal. A top research priority worldwide is the utilization of less expensive protein sources to replace fish meal entirely or in part. In certain fish species, the development of diets free of fish meal (FM) has been partially successful. Many studies are currently being conducted in an effort to identify FM's sustainable replacement. In order to achieve a cheap and sustainable aquaculture practice, it has been suggested that increasing the amount of plant protein in fish diets can reduce the price of FM in fish feeds (Hafeziah*et al*., 2014; Gora *et al*., 2018). Biologically active chemicals can be found in seaweeds, which are particularly rich in vital nutrients for animal and human nutrition. When it comes to raising aquatic species like fish, mollusca and shrimp, microalgae are crucial. The type of seaweed utilized, how much of it is incorporated, in the aquafeed all seem to have an impact on the outcome for fish culture aspects. The expenses associated with production, harvesting, and processing macroalgae before adding them to fish diets will determine whether or not they are used in fish feeds and the cost incurred in this algal feed processing can be compared with fish meal incorporation in diet and based on this a suitable diet can be formulated. *Etroplus suratensis* (Pearl spot) is a brackish water euryhaline fish included in the family Cichlidae inhabits in both brackish and freshwater ecosystem (Padma kumar*et al*., 2012). It can withstand wide range of temperature and salinity conditions as it has highly efficient cellular stress response mechanism and osmoregulatory mechanism (Chandrasekar *et al*., 2014). Ability to adapt wide salinity regimes increase the profitability of *Etroplus suratensis* in aquaculture (Arun Kumar *et al*., 2020). The purpose of the study is to ascertain how supplementing *Etroplus suratensis* with seaweed *Padina gymnospora* through diet will affect the species in place of fishmeal in the diet, without impairing growth.

# MATERIALS AND METHODS

***Collection of marine algae*****:** *Padina gymnospora* (*P.gymnospora*) were collected from the Gulf of Mannar Region of Mandapam Coast Tamilnadu. The samples were washed thoroughly with tap water then shade dried for about four weeks and ground into a fine powder and stored in air-tight containers till further use.

***Feed Preparation from feed ingredients:*** The diets were prepared by the method of Jayaram and Shetty (1981). The fine powdered algae was mixed other ingredients green gram, soyabean meal, Tapioca, rice bran, cod liver oil, and egg albumin. The other ingredients were procured from local market. Dry fish meal was used only in control diet along with ground nut oil cake. Weighed amounts were mixed thoroughly using a common blender and mixer. The powered ingredients were mixed with vitamin-mineral premix. Tapioca was used as the binder in preparation of control and experimental feeds. Finely ground ingredients were mixed thoroughly with required amount of water and corn flour was added to make a thick smooth dough. The dough was mixed thoroughly to ensure uniform dispersal of ingredients before pelletization. The dough was then transferred to an aluminum container and steam cooked in pressure cooker for 15 min. The dough pellets (2 mm diameter size) were prepared by a hand pelletizer and were air dried in an hot air oven at $40^0$C. After sun drying, they were stored in air- tight containers and kept in refrigerators for use during feeding trial. The control and experimental diets were formulated as per the composition given in Table 1.

**Table 1. Composition of formulated feed (in percentage) for control and experimental fishes (Ingredients refer to g/100 gm of feed prepared)**

| S.No | Feed Ingredients | Control feed | Experimental feed |
|---|---|---|---|
| 1 | Dry fish meal | 15 | 0 |
| 2 | *Padinagymnospora* | 0 | 50 |
| 3 | Ground nut oil cake | 30 | 0 |
| 4 | Green gram | 10 | 10 |
| 5 | Soybean meal | 12 | 12 |
| 6 | Rice bran | 15 | 10 |
| 7 | Tapioca | 10 | 10 |
| 8 | Cod liver oil | 3 | 3 |
| 9 | Egg albumin | 2 | 2 |
| 10 | Vitamin-Mineral mix | 3 | 3 |
| | Total | 100 | 100 |

***Collection and Maintenance of experimental animals*:** Active and healthy fingerlings of *E. suratensis* were procured from a private hatchery located at Puducherry, and packed in aerated polythene bags and transported to the laboratory. After transportation, fishes were acclimatized to saline water for two weeks, following which they were transferred to bore water for the conduct of experiment. The current study was performed infour uniformly sized circular fiber-reinforced plastic tanks of 500 L. The tanks were washed with clean water and disinfected with potassium permanganate solution. The tanks were filled with 300 L of borewaterand were constantly aerated. To prevent undesirable materials from entering the tanks, a fine-mesh filter bag (60 um) was utilised for water filling. They were fed normal fish feed twice a day in the morning and evening. Left over feed if any was removed by siphoning, two hours post feeding. The water from the plastic tanks was changed twice in a week and replenished with fresh borewater. The accumulated faeces from the bottom were siphoned out daily. Continuous oxygen supply was provided through aerator and the fishes were maintained at 12 hrs light / dark regime.

***Experimental Design:*** Acclimated fingerlings of *E. suratensis* weighing 5.0 to 5.6 g and 4.3 to 4.4 cms in length were divided into four groups consisting of 10 fishes in each group. Feeding initially 2% body weight for two week followed by 5% of the body weight twice daily for 30 days.

***Control Feed*:** *E. suratensis* fingerlings fed with groundnut oil cake and dry fish meal mix.

***Experimental Feed:*** *E. suratensis* fingerlings fed 50% *Padina gymnospora* formulated feed. Fishes were handled with a clean hand net. Fishes were equally distributed in the plastic tanks with two replication for each diet treatment. Aeration was continuously provided by aerators. Feed was given for a period of 30 days, twice daily at 09.30 am and 4.30 pm. Feeding period was two hours. After the feeding time, the unconsumed food remaining in the tub was collected by siphoning out with a tube, causing least disturbance to the fish. About 75% of the water in the plastic tanks was changed three times a week. On the subsequent day before feeding, faecal matter accumulated in the plastic tanks was siphoned out. The unconsumed feed and faecal matter was collected, dried and weighed to calculate the feeding rate. All weighing were made in digital balance to an accuracy of 1 mg. The amount of food given, the amount food unconsumed were recorded daily before and after feeding. The body weight of the fishes were recorded once in 10 days. Sampling was done and the quantity of feed given was re-adjusted, after each sampling, based on the weight recorded.

***Feed Content Analysis:*** Proximate composition was determined using AOAC methods (2003). β- carotene, total carotenoids, vitamin E and C was determined following the standard procedures from British Pharmacopoeia, 2018 in both control feed and *Padina gymnospora* formulated experimental feed.

***Calculation of growth,nutritional and protein indices*:** Growth indices (Hopkins, 1992), Feeding and protein indices (Castell and Tiews, 1980) were calculated according to the respective standard formula.

***Statistical Analysis:*** Data expressed as Mean ± SD. Software package SPSS 16.0 version was used to carry out the statistical analysis. Student 't ' test was used to compare various indices between control and experimental groups and the statistical significance was tested at 1% and 5% levels.

# RESULTS

***Proximate composition and Vitamin content of the feed*:** The experimental feed composition show an increase in carbohydrate, protein, fat, fibre, ash and moisture content when compared to the control feed (Table 2). β- carotene, total carotenoids, vitamin E and C show an increase in the formulated feed when compared to control feed (Table 3).

**Table 2. Proximate composition of control feed and *Padina gymnospora* formulated experimental feed (Values are expressed as %)**

| Proximate analysis | Control feed | Experimental feed |
|---|---|---|
| Carbohydrates | 10.89 | 4.01 |
| Protein | 29.02 | 37.08 |
| Fat | 6.05 | 7.56 |
| Fibre | 1.39 | 2.91 |
| Ash | 9.64 | 12.53 |
| Moisture | 5.28 | 9.39 |

**Table 3. Content of β- carotene, total carotenoids, vitamin E and C in control feed and *Padina gymnospora* formulated experimental feed**

| Vitamin | Control feed | Experimental feed |
|---|---|---|
| β- carotene | 13.95 | 15.1 |
| Total carotenoids | 16.45 | 30.54 |
| Vitamin E (Tocopherol) | 19.6 | 35.3 |
| Vitamin C (Ascorbic acid) | 16.89 | 29.58 |

Values of β- carotene and Total carotenoids expressed as μg / 100 gm dry weight
Values of Vitamin E and Vitamin C expressed as mg / 100 gm dry weight

***Growth performance:*** The various growth parameters viz Specific weight gain, length gain, growth rate, specific growth rate and relative weight gain were significantly ($p < 0.01$) increased in the experimental group of fishes fed on *Padina gymnospora* based formulated feed compared to the fishes fed on a basal control diet (Table 4).

***Feeding indices:*** Feed conversion efficiency, feeding rate, feed absorbed, absorption rate and absorption efficiency, was significantly increased ($p < 0.01$) in the experimental groups when compared to control diet fed fishes. A significant decline ($p < 0.05$) was seen in feed conversion ratio of experimental fed fishes when compared to control diet fishes (Table 5).

***Protein indices*:** Protein conversion ratio, Protein conversion efficiency and its utilization noted significant changes ($p < 0.01$) in formulated diet fed group when compared to control diet fed fishes (Table 6).

quality of the muscle in cultured fish. Antioxidant health benefits are provided by these vitamins (Agregan *et al*., 2016). When compared to control feed, fish fed formulated feed based on *Padina gymnospora* exhibited a significant improvement in nearly all growth indices. Fish fed on millet supplemented diet showed improved growth performance in *Labeo rohita* (Reka *et al*., 2022). Similar findings have also been observed by earlier research on feed substitutes in cultivable fishes (Saravanan *et al*., 2015; Reka *et al*., 2021). One possible explanation for the higher survival rate of *Etroplus suratensis* fingerlings fed an experimental diet could be the bioactive compounds present in the sea weeds.

**Table 4. Potential of *Padina gymnospora* based formulated feed on growth performance in fingerlings of *Etroplus suratensis***

| Growth performance | Control feed | Experimental feed | t- value | P- value |
|---|---|---|---|---|
| Specific weight gain (gm) | 2.22± 0.02 | 4.26± 0.01 | 248.07 | < 0.01** |
| Length gain (cm) | 2.12± 0.01 | 3.24± 0.27 | 12.70 | < 0.01** |
| Growth rate (gm/day) | 0.049± 0.001 | 0.094± 0.002 | 56.33 | < 0.01** |
| Specific growth rate (%) | 4.93± 0.15 | 9.46± 0.01 | 332.629 | < 0.01** |
| Relative weight gain (%) | 0.55± 0.08 | 0.99± 0.01 | 72.372 | < 0.01** |
| Condition factor (%) | 83.42± 0.06 | 97.49±0.16 | 139.235 | < 0.01** |
| Survival Rate (%) | 83.38±0.04 | 91.66±0.20 | 79.119 | < 0.01** |

Values are Mean ± SD (n=10) observations; ** denotes significance at 1% level

**Table 5. Potential of *Padina gymnospora* based formulated feed on feeding indices in fingerlings of *Etroplus suratensis***

| Feeding indices | Control feed | Experimental feed | t- value | P- value |
|---|---|---|---|---|
| Feed conversion ratio (gm) | 0.90± 0.01 | 0.56± 0.03 | 34.33 | < 0.01** |
| Feed conversion efficiency (%) | 104.3± 0.02 | 200.16± 1.05 | 201.73 | < 0.01** |
| Feeding rate (gm/day) | 0.190± 0.008 | 0.20± 0.001 | 13.41 | < 0.01** |
| Food absorbed (mg /day) | 1.45± 0.02 | 1.67± 0.05 | 9.6 | < 0.01** |
| Absorption rate (mg /gm /day) | 0.12 ± 0.001 | 0.15± 0.001 | 74.0 | < 0.01** |
| Absorption efficiency (%) | 67.32 ± 0.26 | 76.42± 0.16 | 58.52 | < 0.01** |

Values are Mean ± SD (n=10) observations :**p < 0.01 denotes significance at 1% level

**Table 6. Potential of *Padina gymnospora* based formulated feed on protein indices in fingerlings of *Etroplus suratensis***

| Protein indices | Control feed | Experimental feed | t- value | P- value |
|---|---|---|---|---|
| Protein efficiency (or) protein conversion ratio (gm) | 0.07± 0.001 | 0,09± 0.008 | 27.48 | < 0.01** |
| Protein conversion efficiency (%) | 15.17± 0.03 | 21.35± 0.17 | 86.79 | < 0.01** |
| Protein utilization (gm) | 0.15± 0.003 | 0.21± 0.001 | 47.70 | < 0.01** |

Values are Mean ± SD (n=10) observations;** denotes significance at 1% level

# DISCUSSION

The majority of studies have recently looked into many facets of feeding fish with effective feed additives that impact their health and growth performance (Maghawri *et al*., 2023). The current study examines the appropriateness of a commercial diet with addition of a value based algae in place of fish meal, using ingredients that are complete algal proteins. The effect was assessed in brackishwater fish *Etroplus suratensis* against a standard baseline control diet. Entrepreneurs in the aquafarming industry are now forced to look for less expensive alternative plant-based protein sources. Algae is put directly to feed as a supplement to provide value. A recently developed algal diet ought to meet the fishes' nutritional needs while also having a high level of feed acceptance (Lekang *et al*., 2017). Polysaccharides, carbohydrates, proteins, lipids, fatty acids, ashes, and dietary fibers are abundant in macroalga *Padina* sp. (Subramanian *et al*., 2015). Its significance as a natural source of immunostimulant, antibacterial, antioxidant, antimicrobial, and hepatoprotective properties has lately drawn the attention of researchers (Ansari *et al*., 2019). Due to their high concentrations of macronutrients, micronutrients, and bioactive substances, marine seaweeds are a great source of nutrition (Ravi and Subramanian, 2017). Additionally, they are a good source of dietary fibers, which are crucial for enhancing the functional qualities of food (Elleuch *et al*., 2010). Vitamins are bountiful in algal diets (Martin *et al*., 2011). The formulated feed utilized in this study has higher levels of β-carotene, total carotenoids, vitamin E, and vitamin C than the commercial regular feed. Therefore, it is evident that algal meals can be a great supply of micronutrients needed for fish growth and the protein and fat production. African sea bass, *Lates calcarifer*, fed *Saragassum wightii* included formulated feed showed higher survival rates (Tuller *et al*., 2014). The *Padina gymnospora*-based formulated feed's higher protein content suggests that it has the ability to promote growth and improve feed conversion efficiency. When compared to fish fed a control diet, the FCR values were lower for the group fed a formulated feed based on *Padina gymnospora*. The above results are consistent with research done on *Lates calcarifer* fed a high-protein meal prepared by *Saragassum wightii* (Kodithuwakku*et al*., 2017). Regarding the feeding parameters of blue gourami fed on *Spirulina plantensis*, similar results were observed (Rinna Hamlin *et al*., 2013). As seen in many freshwater fish, where the feed conversion ratio is known to drop with increasing dietary protein content, it is proven that lower the ratio, higher the quality of the feed (Saravanan *et al*., 2017; EdTehal and Sayed Hussain, 2017). Fish nutritional requirements are influenced by feeding rates. The quality of the feed has a major impact on the efficiency of feed usage (Khan *et al*., 2012; Gnanavel *et al*., 2019). When compared to fish on a regular basal diet, the current study shows that juvenile fish fed an experimental diet had higher food absorption, absorption rate, and efficiency. Due to an acceleration of dietary algae's nutrient absorption, macroalgae *Ulva fasciata* and *Enteromorpha flaxusa* enhanced the palatability and digestibility of rabbitfish fry while also having an impact on growth (Abdel Aziz *et al*., 2017). Peixoto *et al*. (2016) observed that European sea bass fed with *Gracilaria*, *Fucus*, and *Ulva* showed improvements in their ability to digest food and in how much they ingested. The study's findings provides an information on how possible components derived from seaweeds are quickly and readily absorbed into the diets. An increase in the activity of digestive enzymes or an increased rate of assimilation may be the cause of the rise in absorption activities and conversion efficiency (Poongodi *et al*., 2012). The usage and conversion of protein in fingerlings of *Etroplus suratensis* fed on formulated meal based on *Padina gymnospora* was significantly improved growth performance. *Spirulina pacifica* partially substituted for fish meal in the diets of parrotfish *Oplegnathus fasciatus* resulted in decreased FCR and better PER (Kim *et al*., 2013). *Pangasianodon hypophthlamus* fed with fucoidan rich extract from *Saragassum wightii*, the fishes were found to exhibit growth-promoting properties (Prabhu *et al*., 2016). Comparable findings on PER were found in *Labeo rohita* fed on *Saragassum wightii* and its fucoidan-rich extract (Gora *et al*., 2018) and in *Marsupenaeus japonicus* supplemented with dietary fucoidan (Traifalgar *et al*., 2010). According to Peixoto *et al*. (2016), adding seaweed to aqua feeds may be a useful strategy for improving the immune system and general health of farmed fish species. It has been proposed that replacing fish meal in the diet with a combination of other dietary protein sources worked better.

# CONCLUSION

Fisheries resources now provide a large portion of the protein used in commercial aquafeeds, thus finding new and sustainable sources of protein is essential to the aquaculture industry's continued growth. Alternative protein sources are being looked for in order to replace fish meal in aquaculture feeds. In the current work, algal sources have been substituted for animal lipid sources in order to maximize energy. The results of this study demonstrated that formulated feed based on *Padina gymnospora* can be fed to *pearl spot* in processed pellet form as it fits their nutritional needs and promotes improved growth.

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