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Nutritional Benefits of Crustacean Meal in the Culture of Pearl Spot (*Etroplus suratensis* Bloch 1790)

Aanand S.[1], Ramji B[2]., Somu Sunder Lingam R.[3], and Velmurugan P.[4]

[1]Assistant Professor and Head, Erode Bhavanisagar Centre for Sustainable Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Bhavanisagar 638 451, Erode, Tamilnadu, India.

[2]Technical Consultant Coastal Aquaculture Authority,5th floor, Integrated Office Complex for Animal Husbandry and Fisheries Department, Fanepet, Nandanam, Chennai-

[3]Assistant professor, Krishnagiri Barur Centre for Sustainable Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Barur, Krishnagiri dt. – 625 201, Tamilnadu, India.

[4]Assistant professor & Head, Kanyakumari Parakkai Centre for Sustainable Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Parakai., Nagercoil, Kanyakumari, Tamilnadu, India.

SUMMARY

Animal-derived protein demand is expected to double by 2050 globally, with the needs for both food and feed are expected to grow by 70%. Several animal protein sources from insects, land by-products and fisheries by-products have been evaluated as possible feed ingredients in fish production. However, no documented studies comparing animal protein sources in diet and control diet. Therefore, this review aimed to evaluate the effects of major animal-based meals such shrimp head waste, crab shell waste, and Silkworm pupae meal on the growth, nutrient utilisation, and digestive and metabolic enzyme activities of Etroplus suratensis. The discussions and summing up brings about the limitations of incorporating these in the diet of pearl spot, around 30% of that of fish meal in the diet. Crustacean meal seems to show better influence on the growth performance of pearl spot when replacing Fish meal by 30%.

INTRODUCTION

In India, aquaculture is a rapidly growing sector that contributes to the food supply, nutritional security, export earnings, employment generation, and national GDP (Krishnan et al., 2000). The fishery sector in India has recorded higher growth than the crop and livestock sectors (Kumar et al., 2006), with the aqua feed industry being vital in the development of the aquaculture sector. It contributes to 60 percent of the total production cost of aquaculture. Aqua feed depends heavily on the constant supply of fishmeal (FM), a major component of commercial fish feed. Cultured fish require a high-quality and nutritionally balanced diet for better growth (Gabriel et al., 2007). Proteins are critical nutrients needed by any fish for tissue growth, maintenance, reproduction, catabolic pathways, and energy sources. Furthermore, proteins are the most expensive component of fish feed (Philips, 2008).

Culture of Pearl Spot *Etroplus suratensis*, Bloch (1790)

E. suratensis, commonly referred to as the green chromide or Pearlspot, is one of only three cichlid species native to southern Asia, including peninsular India and Sri Lanka. As an endemic cichlid fish of commercial importance, it is prevalent in the brackish water habitats of Kerala. Widely distributed throughout India, it is traditionally cultured in the backwaters and freshwaters along the coastal regions from South Canara to Thiruvananthapuram on the west coast and later introduced to the east coast regions, such as Chilka Lake, Orissa, and Kakdwip, West Bengal. The fish is highly tolerant of a wide range of salinities, breeds in confined areas, and exhibits flexible feeding habits. It is also known by various other names, including Pearl spot cichlid, banded pearl spot, and striped chromide. In Kerala, it is referred to as Karimeen, while in Goa, it is known as Kalundar. In Odisha, it is called Kundal, and in Sri Lanka, it is known as Koraliya.

Fish meal in aquaculture

Fish meal and fish oil are considered the most nutritious and digestible ingredients for farmed fish, as well as a significant source of omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In addition, fishmeal in animal diets improves feed efficiency and growth through better food palatability and enhances nutrient uptake, digestion, and absorption. Fish meal is the primary protein source in aquafeeds because of its high protein content, excellent amino acid profile, lack of anti-nutritional factors, better nutrient digestibility, lower cost, and easy availability (Daniel, 2018). However, the supply of fishmeal and fish oil to the aquafeed industry has recently declined due to the decreased catches from the marine fishery, resulting in increased costs of both in the market to over 1600 US $ and 900-1800 US $ per metric ton (Mt). Thus, the marine sector's supply of fishmeal and fish oil cannot sustain the aquaculture industry. Owing to the high cost of FM, alternative protein sources that provide nutritional benefits similar to FM have been widely investigated.

Plant-based materials, such as soybeans, oil seeds, and cereal gluten, are increasingly used in animal feed (Daniel, 2018). However, replacing FM with large amounts of plant-based materials is not immediately viable in the aquaculture industry. This is primarily because plant-based feeds contain anti-nutritional components, non-starch polysaccharides, and fatty acid and amino acid profiles that are less suitable for fish (Daniel, 2018). The reduction in fish meal and oil supply has driven the search for alternative protein sources to sustain aquaculture (Hodar et al., 2020). Recent research has continuously involved the identification and use of alternative locally available feed sources in feed formulations. Fish meal can be produced from any type of seafood but is generally manufactured from wild-caught small marine fish possessing a high percentage of bones and oil. In addition, nutritionists recognize fish meal as a high-quality, highly digestible feed component that is preferred for the diet of most farm animals, mainly fish and shrimp.

Insect meal

Insects represent a good source of protein and are one of the best alternatives for partial or complete replacement of fish meal, mainly due to insects' versatility and ability to change their amino acid and fatty acid profiles. Insect meals are rich in high-quality proteins and contain a good percentage of lipids, carbohydrates, and specific vitamins (DeFoliart et al., 2009). Technically it is possible to use insects as an alternative protein ingredient in animal diets (Sanchez-Muros et al., 2014). Insects could be identified based on their protein content, amino acid profile, fat, mineral, and raw material availability, and produced commercially (Gasco et al., 2018). Insects are not only the most diverse group of animals but also a natural food for carnivorous and omnivorous fish, as these fish species need relatively high amounts of proteins in their diets (Nogales- M'erida et al., 2019; Tran et al., 2015; Van Huis, 2020). Among the insects, the Black soldier fly (*Hermetia illucens*), Silkworm pupae *(Bombyx mori)*, Housefly (*Musca domestica*), and Yellow mealworm (*Tenebrio molitor*) are already popular as alternative protein sources.

Studies on replacing fishmeal with insect meal over the last decade have yielded promising results. Generally, most experiments have shown good results in partially replacing FM with insect meals, depending on fish and insect species**.** This has attracted investors to invest in various start-up companies to produce insect meals (Rumbos et al., 2021). Hence, using insect meals to replace FM can revolutionize aquaculture (Yue and Shen, 2021).

Silkworm pupae meal

Unlike other insect meals, SPM, whether defatted or not, produced decent outcomes in fish feeding trials, and the fat of silkworm pupa was considered beneficial (Makkar et al., 2014; Sun et al., 2014). Silkworm pupae, a waste of the silk reeling industry, are known for their high nutritional value. The SWPs are comparatively cheaper than fish meals and can be one of the most suitable alternatives to fish meals without much effect on farmed fish species' growth.

Nutritional profile of Silkworm pupae

According to the Bureau of Indian Standards, 1971, the silkworm pupae contain 50% crude protein, 25% crude fat, 50% moisture, 3% crude fiber, and 5% ash. SWP composition, however, is dependent on the treatment process; the nutritional value of the Silkworm pupae may vary. Oven-dried Silkworm pupae contain 47.9% protein, 27% fat, 3.4% fibre, and 5.6% total ash (Fagoonee, 1983). On the contrary, pressed Silkworm pupae contain 60.77% protein, 15.30% fat, 2.73% ash, 6.37% water, and 4.63% chitin (Roychoudhury and Joshi, 1995).

Silkworm pupae as a feed for carps

Grass carp *(Ctenopharyngodon idella)* showed better growth and yield when fed with a silkworm pupae-based diet (Hora, 1962). Fingerlings of Indian major carp fed with Silkworm pupae increased more than mustard oil cake and rice bran. Common carp *Cyprinus carpio* provided with Silkworm pupae meal showed good growth performance and a better feed conversion ratio (Erencin, 1976). Pellet feed in a combination of Silkworm pupae, prawn waste or fish meal, and tapioca flour showed higher growth performance without affecting the flesh quality of common carp (*C. carpio*) (Jeyachandran and Paulraj (1977).

SWP up to 30% pupa inclusion in the feed of catla and rohu has shown the best growth (Jayaram and Shetty, 1980). Studies on the effects of various protein sources in mrigal (*Cirrhinus mrigala*) show that Silkworm pupae incorporated pellet feed yielded better growth (Borthakur 1983). Up to 100% fish meal can be replaced with silkworm pupae in the diet of Catla fingerlings with better growth and survival (Hasan, 1991). On the contrary, rohu (*L. rohita*) (Begum et al., 1994) and common carp showed better feed utilization and growth performance, only up to 50% replacement for the fish meal by silkworm pupae (Nandeesha et al., 2000).

According to Rangacharyulu et al. (2003), feeding fermented Silkworm pupae silage can obtain higher body weight, feed conversion ratio, and specific growth rate in Indian major carp. FM substitution level @ 60% or more in the diet of Jian carp significantly affected its growth; hence, a replacement level of up to 50% was recommended (Ji et al., 2015). In the fringe-lipped carp *(Labeo fimbriatus)*, protein digestibility increased and decreased at 20% and 40% inclusion levels of Silkworm pupae (Gangadhar et al., 2017). On the contrary, in mirror carp (Cyprinus carpio), an eleven-week feeding trial found that SPM was an appealing, sustainable, functional feed component in carp diets, with benefits in terms of improving growth performance and particular physiological parameters (Wan et al., 2017). SWP, due to the higher protein and fat digestibility, can be profitably used up to 30% in Catla diets (Umalatha et al., 2018).

Silkworm pupae as a feed for other freshwater fishes

Akiyama et al. (1984) observed Chum salmon (*Oncorhynchus keta*) fry obtained a better feed efficiency ratio when fed with Silkworm pupae powder. Similarly, Silkworm pupae in the diet of walking catfish (*Clarias batrachus*) showed higher specific growth rates and feed efficiency ratio (Venkatesh et al., 1986). The growth performance improved when fed with 80% non-deoiled Silkworm pupae (Habib et al., 2001). Raw silkworm pupae at 60% and deoiled Silkworm pupae at 50% in the Deccan mahseer (*Tor khudree*) diet improved their growth performance (Shyama and Keshavanath 1993). The fingerlings of ornamental silver barb (*Barbonymus gonionotus*) exhibited excellent response when fed with 38% Silkworm pupae (Mahata et al., 1994).

Dheke et al. (2013) silkworm pupae meal can be an alternative to replace shrimp meal in trout feed. A diet consisting of 100% silkworm meal could be used in the formulation of *Clarias gariepinus* fingerlings (Olaniyi et al., 2013). Similarly, Oso et al. (2014) reported that although 25% gave the best results, 100%, 75%, and 50% levels of replacement of fish meal protein with SWP in the diet of *Clarias gariepinus* juvenile are acceptable without affecting the growth and feed utilization. Silkworm pupae can also be used as a protein source in the diet of red zebra *(Maylandia estherae)* fingerlings up to 60% inclusion level (Nisha et al., 2014). Shakoori et al. (2016) reported that Silkworm pupae incorporation of up to 10% of FM did not adversely affect the meat quality, growth, or survival of rainbow trout. Besides the fish meal, Silkworm pupae can be an adequate substitute for the shrimp meal in the diet of rainbow trout, *O. mykiss* (Walbaum), with better growth and survival (Bhagat, 2017). Raja et al. (2020) concluded that SWP could be used to replace FM in the diets of rainbow sharks at a replacement level of 30% was found to be more efficient.

Silkworm pupae as a feed for marine fishes

Silkworm pupae in the diet of Japanese seabass *(Lateolabrax japonicas)* improved its energy production and crude protein digestibility by 73% and 85%, respectively (Ji et al., 2010). Lee et al. (2012) found that replacing fish meal with 10% Silkworm pupae and 10% Silkworm pupae + 20% promoted meal revealed good growth without any adverse effects on the feed utilization in olive flounder (*Paralichthys olivaceus*).

Silkworm pupae as a feed for shellfishes

A combination of soya meal (29%) and Silkworm pupae meal (16.9%) on DM basis) could replace 100% fish meal in the diet of Abalone juveniles (*Haliotis discus*) with better survival and growth (Cho, 2010). In giant freshwater prawn (*Macrobrachium rosenbergii*), Silkworm pupae at an inclusion level of 8.6% in the diet, affected the growth performance (Jintasataporn et al., 2011).

Food and feeding habits of *Etroplus suratensis*

Bhaskaran (1946) has observed the food habits of *E. suratensis* young from freshwater habitats and reported that they feed mainly on filamentous algae, marginal plants, and detritus. Chacko (1949) has dealt with the food and feeding of the fry and fingerlings and concluded that they rarely feed on higher marginal plants, insect larvae, and filamentous algae. Alikunhi (1957), while discussing the feeding habits during different stages, has reported that the larvae feed mainly on zooplankton and switch over to filamentous algae (Spirogyra) and vegetable matter from 19 mm onwards. The study of food and feeding habits of *Etroplus suratensis* has been reported by numerous authors (Keshava et al., 1988; Keenleyside et al., 1991; Ushakumari and Aravindan, 1992; Sultana et al., 1995; Bindu and Padmakumar, 2008 and Vidhya and Nair, 2012). Menon and Chacko (1956) considered *E. suratensis* a bottom feeder, as the gut contents contained a fair proportion of substratum materials. According to Hora and Pillai (1962), the pearl spot favours blue-green algae as food. Fish accept various food items, such as copepods, cladocerans, insects, and worms (Jhingran and Natarajan, 1969). Gopalakrishnan (1972) reported that the fish's juvenile stages are an omnivore that transforms into an herbivore as it grows. He also noted that the species is a vegetable feeder, depending mainly on aquatic plants, filamentous algae, and phytoplankton for food.

The young feed almost exclusively on zooplankton, the advanced fry on aquatic insect larvae, filamentous algae, and other vegetable matter. At the same time, adult fish subsist mainly on filamentous algae, aquatic macro vegetation, and planktonic organisms (Sundararaj and Krishnamurthy, 1975). Varghese (1975) reported that this species is omnivorous, showing a certain degree of specificity about the food items it consumes and displaying substantial variations in the diet in some months. Worms, shrimps, and insect larvae also form part of its food. Adult pearl spots can be fed with pelleted fish feed. Devaraj et al. (1975) reported that filamentous algae and detritus form the dominant food items in the stomach of pearl spots collected from estuarine waters, while in those samples collected from the freshwater tanks, insect larvae, and detritus were found to dominate. Fish's food and feeding habits depend upon the available food materials in the water body (Bruton, 1979), along with the stages of life and ecological conditions. Jayaprakash (1980) studied the species' food and feeding habits of different size groups. He found a gradual change in food preference as growth progressed from diatoms and zooplankton to filamentous algae and then to higher aquatic plants. Ward and Samarakoon (1981) reported that *E. suratensis* is a complete herbivore.

On the contrary, De Silva et al. (1984) observed it as a predominant macrophyte feeder and not a complete herbivore. Earlier studies also indicated the preponderance of aquatic weeds followed by detritus and algae in the diet of the adult *E. suratensis* from Pulikat Lake and Nethravati-Gurpur estuary (Prasadam, 1971; Keshava et al., 1988). Bindu and Padmakumar (2008) reported that the filamentous algae *Spirogyra*, *Oscillatoria*, *Lyngbia*, and *Fragillaria* formed the primary food of *E. suratensis* in Vembanadu Lake. Vidhya and Nair (2012) noticed a diet change with an increased fish size. Kannan et al. (2015) observed that the fish is a herbivore that sometimes switches to omnivorous feeding habits in the Thamirabarani River, Western Ghats of India. Emmanuel et al. (2019) suggested that *E. suratensis* is indicative of the predominant herbivore food preference of the species in Vellayani Lake. They were dominated by diatoms (78%), macrophytes (77%), filamentous algae (76%), and detritus (72%) followed by miscellaneous items (58%), zooplanktons (36%) and molluscs (11%). The primary food items consumed by *E. suratensis* in sarvepalli reservoir consisted of diatoms, filamentous algae, higher aquatic plants, rotifers, insect larvae, cladocerans, copepods, and other crustaceans, gastropods, and detritus (Ganesh et al., 2020).

Growth studies in *E. suratensis*

Diets containing 60- 87% protein provide a higher food conversion efficiency of *E. suratensis* in the brackish water culture ponds. Royan et al. (1982) suggested high specific growth rate (8.33%) could be obtained at a 53.62% protein level. They concluded that a feeding level of 10% live body weight per day could increase the conversion efficiency in *Etroplus suratensis*. Jayasuriya et al., (1987) Preliminary trials carried out showed that *E. suratensis* fingerlings (5 cm) cultured in cages and fed with 30% CP in the Kelani River estuary at a stocking density of 27/m^3 gained an average weight of 50 g and an average length of 14.0cm for six months. Aneykutty et al. (1994) recommended an Azolla feed of 36.93% protein for the optimum growth of *E. suratensis*. A diet containing 40% CP and testosterone propionate at a 4 ppm level exhibited additional growth increment in *Etroplus suratensis* juveniles (Sambhu and Jyaprakash, 1997). Pillai and Ali (1997) reported that 31.5% protein was optimum for *E. suratensis*. Jayaprakash and Kumar, (1999) said that a diet with 35% protein is optimum for maximum growth and high feed utilization in *E. suratensis*.

According to Debasis et al. (2006), feed supplementation was required for better growth of *E. suratensis*, and pellet feed was utilized more efficiently than mash feed. Datta and Dasgupta (2007) recorded a final weight of 73.1 g in 11 months when stocked with 7.5 g Etroplus in the freshwater culture system and recorded a survival rate of 82.6%. Palavesam et al. (2008) recommended a 25% protein diet for a higher growth rate than a 30% protein diet or other higher protein concentrations. The survival rates varied between 45% and 100% (Padmakumar et al., 2004a, 2009b). Etroplus, with an average body weight of 0.28 g, at stocking, when reared in inland saline groundwater ponds stocked @ 10,000 fish/ha, attained 150 g in 90 days with a 40% crude protein diet (Kumar et al., 2009). Biswas et al. (2013) suggest stocking a density of 150 fish per m^3 without soil base in tanks would be appropriate for raising pearl spot fingerlings in a brackishwater indoor seed-rearing system. Lekshmi et al. (2014) reported that 30% of dietary protein was estimated to be optimum for the economic rearing of *E. suratensis* fingerlings.

Bindu et al. (2016) said fry in the nursing tank attained about 20.01+0.62mm in one month and attained 30- 35mm, in two months. Yadav et al. (2021) suggested that *E. surartensis* advanced fry can be stocked at 150 m^{-3} to obtain higher biomass with good survival numbers in the biofilm-based rearing system. Parakkandi et al. (2021) reported that fish with an average weight of 5.98 ± 0.52 g at stocking grew to 90.31 ± 2.48 g in 7 months of culture with a mean weight gain of 84.33 g. Mean weight gain was low during the first two months of culture and showed a steady increase during the rest of the culture period, which could be attributed to the stress associated with the change of habitat or water quality at the initial stage of culture. Sial et al. (2021) recorded the growth performance of pearl spots (*E. suratensis*) to the extent of 0.593 g/day at stocking densities of 15 per cage, and it attained 137g in 150 days in the Gopalpur Creek shallow water system. Lekshmi et al. (2022) observed that a formulated diet had a relatively lower growth rate of 0.013g/day and attributed this to young fishes preferring a natural diet like vegetable matter and periphyton over pelleted feeds.

Enzyme assays

The studies on several species' digestive proteases helped develop cost-effective diets for their intensive farming (Alarcon et al., 1998) and the matching of an artificial diet to their nutritional needs (Furne et al., 2005). Digestive enzyme activities in fish respond to the quality and quantity of nutrient intake (Coway et al., 1981). Sambhu and Jayaprakash (2000) reported that digestive enzyme activity was maximum (590.34 units/g tissue) in the intestines of fishes fed on a diet containing TP+HCG. A substrate in culture ponds induces higher growth through increased digestive enzyme activity (Kumar et al., 2009). In fishes, the activities of major digestive enzymes are present along the whole length of the digestive tract (Sklan et al., 2004).

A comparative study on digestive enzymes such as acid and alkaline protease, amylase, and lipase activity in *E. suratensis* and *O. mossambicus*, showed that *O. mossambicus* digests the dietary proteins and fats better than *E. suratensis* (Sankar et al., 2014). Eshaghzadeh et al. (2014) suggested that the dietary inclusion of inulin had no significant effects on the digestive enzyme activities of common carp fry. Long et al. (2014) reported that BFT improved fish's digestive enzyme activity, enhancing feed utilization and growth performance. Tok et al., 2016 studied the metabolic and digestive enzyme activity of *Pangasianodon hypophthalmus* fingerlings in response to alternate feeding of different protein levels, i.e., 35P (35P/35P), alternate feeding of 1-day diet, 35P next-day diet 30P (35P/30P), alternate feeding of 1-day diet 35P next day diet 25P (35P/25P) and alternate feeding of 1-day diet 35P next day diet 20P (35P/20P) in the diet. He found that MDH and LDH activities are significantly higher in the 35P/20P group. The lowest activities were in the 35P/35P group, and digestive enzymes (protease, lipase) activities were higher in the 35P/35P group, followed by 35P/25P and 35P/30P groups, and the lowest activity was found in the 35P/20P group. And also he reported that the highest amylase activity was found in 35P/20P, followed by the 35/25P, 35/30, 35/35 groups. Jiang et al. (2016) confirm that dietary curcumin supplementation significantly improved crucian carp's growth performance and digestive enzyme activities than basal group feed. Zhang et al. (2018) replacement of FM with SM (Soya bean meal) resulted in significantly reduced protease, amylase, and lipase activity in the foregut of Japanese seabass.

Gora et al. (2018) found that MDH activity was lower in the fucoidan groups than in the control and seaweed-fed groups. LDH activity was not affected significantly by seaweed and dietary fucoidan in *Labeo rohita*. Paul et al. (2021) studied the effect of dietary lipid levels on the growth and digestive enzyme activities in *Ompok bimaculatus*. He revealed that amylase activity was decreased with increasing dietary lipid levels. The maximum alkaline protease, pepsin, and lipase activities were noticed in diet D2 (8% lipid), further he recorded the lowest LDH activity in D2. Abbas et al. (2021) investigated the growth performance, and enzyme activities of Labeo rohita fed different commercial fish feeds (Oryza (T1), AMG (T2), Aqua (T3), and Supreme (T4) and concluded that oryza feed with 25% CP showed significant growth and high protease enzyme activity. Kari et al., (2022) studied the effect of fish meal substitution with fermented soy pulp on growth performance and digestive enzyme activity. He found the highest protease activity in the control diet (0% FSP), and amylase lipase activities were higher in the diet with 50% FSP. Ezhilmathi et al. (2022) studied the effect of stocking density on the growth performance and digestive enzyme activity of Asian seabass reared in RAS. They reported that T1 (70 fish/m^3) showed a higher level of protease, amylase, and lipase than other treatments (T2-140, T3-210, T4-280, and T5-350 fish/m3).

Zafar et al. (2022) studied the growth and digestive enzyme activities of Nile tilapia reared at various stocking densities in RAS. They suggested that significantly lower ($P < 0.05$) protease, amylase, and lipase activities were observed in the 160 F group, followed by the 120 F and 80 F groups. The dietary carbohydrate sources significantly ($P < 0.05$) influenced the activity of malate dehydrogenase (MDH) and reported that MDH activity was significantly higher in the fish fed with starch feed group and the lowest activity was found in glucose and sucrose feed-based groups in *Etroplus suratensis* (Ramachandramoorthi et al., 2022).

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