

Performance of crustacean and insect meal based diets on the growth and digestive enzyme profile of pearlspot *Etroplus suratensis* (Bloch, 1790)

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Abstract

A feeding trial of 75 days was conducted in tanks to study the performance of shrimp head meal (SM), crab shell meal (CM) and silkworm pupae meal (SWP) on the growth and digestive enzyme profile of pearlspot, *Etroplus suratensis*. Four iso-nitrogenous diets with 31.5% crude protein replacing 50% fishmeal (FM) in the diets were formulated viz., SM (T₁), CM (T₂), SWP (T₃) and a control (C). Pearlsport advanced fry, 2 cm in length and 1.1 g in weight, was stocked at 60 nos per tank. After 75 days of the experiment, feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER) were observed to be significantly better ($p < 0.05$) in all treatment groups (T₁, T₂ and T₃) than in control (C). T₂ (CM) fed fishes exhibited a maximum specific growth rate (SGR) of 3.25 ± 0.09 , and significantly higher ($p < 0.05$) weight gain (11.54 ± 0.78 g) than T₁, T₃ and control. In enzyme assays, T₂ (CM) fed fishes exhibited significantly higher ($p < 0.05$) protease activity than other treatments. Lipase activity was significantly higher ($p < 0.05$) in T₃. However, there was no significant difference with control diet-fed fishes. Significantly higher ($p < 0.05$) activities of amylase, malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) were observed in all the treatments compared with control. The outcomes of the present study indicate that crab meal (31.5% CP) could be effectively utilised as a 50% replacement for fishmeal in the diet of *E. suratensis* advanced fry for higher yields.



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Introduction

Aquatic foods have a significant role in food security and nutrition and are a substantial source of protein for human consumption. They also serve as a unique and diverse source of essential omega-3 fatty acids and micronutrients. FAO (2022) estimated that a record 214 million t of fisheries and aquaculture products were produced in 2020, including 178 million t of aquatic animals and 36 million t of algae, primarily due to the growth of aquaculture, predominantly in Asia. In 2019, the consumption *per capita* reached a record of 20.5 kg, despite a modest decrease to 20.2 kg in 2020 (FAO, 2022).

Aquaculture has substantially contributed to global food and nutritional security

by offering fish and fishery products as affordable alternatives to animal-derived proteins to feed the expanding human population. Aquaculture production highly depends on artificial feeds, which account for up to 50-60% of the production cost. Fish meal (FM) is the primary source of aqua feed for reasons such as high protein content, excellent amino acid profile, lack of anti-nutritional factors, better nutrient digestibility, less cost and easy availability (Daniel, 2018). However, the supply of fishmeal and fish oil to the aqua feed industry has recently been declining due to the decreased catches from the marine fishery, resulting in their increased costs to over US \$ 1600 and 900-1800 per t. Thus, the marine sector's supply of fishmeal and fish oil cannot sustain the aqua feed

industry. Due to the high cost of FM, alternate protein sources that provide similar nutritional benefits as FM are being searched widely.

The more expensive fishmeal has been replaced by several sources of plant protein, single-cell protein and animal protein in part or whole (Yigit *et al.*, 2016). Plant-based materials such as soybeans, oil seeds and cereal gluten are increasingly used in animal feeds (Daniel, 2018). However, replacing FM with plant-based material is not immediately worthwhile in aquaculture. This is primarily because plant-based feed contains anti-nutritional components, non-starch polysaccharides, and fatty acid and amino acid profiles, which are less suitable for fish (Daniel, 2018). Due to higher protein and lipid content, superior essential amino acids and excellent palatability, animal protein sources have commonly been considered ideal substitutes for fishmeal in formulating fish diets (Robinson *et al.*, 1998; 1999). The reduction in fish meal and fish oil supply drives the search for alternative protein sources to sustain aquaculture (Hodar *et al.*, 2020). Recent research continuously involves identifying and using alternative locally available feed sources in fish feed formulations.

According to FAO (2013), animal-derived protein demand is expected to double globally by 2050. Furthermore, future food and feed needs are expected to grow by 70%. Several animal protein sources from insects, land byproducts and fisheries byproducts have been evaluated as possible feed ingredients in fish production (Arunlertaree *et al.*, 2008; Ayadi *et al.*, 2012; Chor *et al.*, 2013; Aladetohun *et al.*, 2013; Mountino *et al.*, 2017). However, there are no documented studies comparing various animal protein sources such as crustacean and insect meals. Therefore, this study aimed to evaluate the effects of partial replacement of fishmeal by using shrimp head waste, crab shell waste and silkworm pupae meal on the growth, nutrient utilisation and digestive and metabolic enzyme activities of the pearlspot *Etroplus suratensis*.

Materials and methods

The present study evaluated the dietary inclusion of crustacean and insect protein sources such as SM, CM and SWP to replace fish meal in the diet of *E. suratensis*. The study adopted all the rules and regulations for experimental animal care and procedures by Tamil Nadu Dr. J. Jayalalithaa Fisheries University (TNJFU), Nagapattinam, Tamil Nadu, India.

Experimental setup

The study was conducted at Erode Bhavanisagar Centre for Sustainable Aquaculture (EBCeSA), Bhavanisagar, Erode District, Tamil Nadu, India. The experimental trial was conducted in triplicates R1, R2 and R3 in 3 m dia x 1m high circular tanks. Tanks were covered with bird fencing nets as a bio-security measure. The tanks were cleaned twice weekly to remove leftover feed and unwanted material to ensure proper water quality.

Experimental animal

Pearlspot advanced fry, 2 cm in length and 1.1 g in weight, was stocked in the tanks. The experimental animals were stocked @ 60 numbers per tank.

Experimental diets

The feed ingredients used for the experiment were fish meal (FM), shrimp meal (SM), crab meal (CM), silkworm pupae meal (SWP), groundnut oil cake, rice bran, wheat flour and vitamin-mineral premix. Four Iso-nitrogenous diets with 31.5% crude protein were formulated as per Pillai *et al.* (1997), replacing 50% fish meal by SM (T₁), CM (T₂) and SWP (T₃) with the control diet (C) containing only FM. The feeding trial was conducted for 75 days, with a feeding rate of 5% of the body weight. The composition and proximate analysis of the experimental diets are given in Tables 1 and 2.

Assessment of proximate feed composition such as crude protein, crude lipid, crude fibre moisture and ash content was done at EBCeSA, TNJFU, Tamil Nadu, following standard protocols (AOAC, 2005).

Analysis of water quality parameters

Water quality parameters viz., total dissolved solids, ammonia, nitrate, nitrite and inorganic phosphate were analysed and recorded every fortnight, starting from stocking to the end of the experimental trial using standard methods (APHA, 2005).

Fish sampling and harvesting

Fish were sampled by netting out a minimum of 50% of the stock every fortnight to monitor the growth performance and to determine the biomass for calculating the quantity of experimental feed. The feed quantity was adjusted based on the fish biomass in the tanks. Feeding was stopped 24 h prior to final weight and length measurements. At the end of the trial, fish were harvested by draining the tanks and all the surviving fish were counted to calculate the survival rate of each treatment, including those fed on control diet.

Table 1. Feed composition of the experimental diets

Ingredient	Experimental diets (g per 100 g)			
	C	T1	T2	T3
Fish meal	59	29.5	29.5	29.5
Shrimp meal	-	29.5	-	-
Crab meal	-	-	29.5	-
Silkworm pupae meal	-	-	-	29.5
Groundnut oil cake	10	10	10	10
Rice bran	8	8	8	8
Tapioca flour	19	19	19	19
Vitamins	1	1	1	1
Minerals	3	3	3	3

Table 2. Proximate composition of the experimental diets

Proximate composition (%)	C	T1	T2	T3
Crude protein	32	31.7	31.9	31.2
Crude lipid	5.74	4.15	3.62	5.52
Crude fibre	4.37	9.41	9.2	3.1
Moisture	8.5	7.6	7.1	7.8
Ash	8.3	11.1	12.5	4.2

Growth performance analysis

Fortnightly, the growth performance was recorded by measuring the weight of the fish using a standard weighing balance (accuracy of 0.001 g). In each sampling, 20 fish were randomly collected from each tank with minimal stress and body weight was recorded. The weight gain, specific growth rate (SGR), survival, feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated using standard formulae (Dong *et al.*, 2018; Tan *et al.*, 2018):

Weight gain (g) = Final weight (g) – Initial weight (g)

Specific growth rate (SGR, % day⁻¹) = $[(\log_e \text{ Final weight} - \log_e \text{ Initial weight}) \times 100] / \text{Number of days}$

Survival rate (%) = $[\text{Total number of fish harvested} / \text{Total number of fish stocked}] \times 100$;

Feed conversion ratio = Feed given (g) / Body weight gain (g)

Feed efficiency ratio = Body weight gain (g) / Feed given (g)

Protein efficiency ratio = Net weight gain (g) / Protein fed (g)

Analysis of enzyme activity

Fish samples (5 nos. pertank) were collected at the end of the experimental trial and a 5% tissue homogenate was prepared using a mortar and pestle. The whole procedure was conducted in ice-cold conditions. Homogenised samples were centrifuged at 5000 rpm for 10 min at 4°C. Later, the supernatant was collected in a 5 ml test tube and stored in a deep freezer at -20°C for enzyme assay.

Protein quantification of fish tissues was done as per Lowry *et al.* (1951). The casein digestion method (Drapeau, 1976) was used to estimate protease activity in the samples. Lipase activity was determined using the standard titrimetric method described by Cherry and Crandall (1932) based on the release of fatty acids due to the enzymatic hydrolysis of olive oil. Amylase activity was determined by the Di-nitro salicylic acid (DNS) method (Rick and Staggbauer, 1974) using starch as a substrate. Lactase dehydrogenase (LDH) activity was assayed in tissues by adopting the standard methodology of Wroblewski and Ladue (1955), using sodium pyruvate as a substrate. The malate dehydrogenase (MDH) activity was assayed by the method of Ochoa (1955), using oxaloacetate solution as a substrate. All enzyme activities were measured in terms of changes in the absorbance, using spectrophotometer (Systronics, PC-based double beam spectrophotometer 2202), except lipase activity, and expressed as specific activity (μ mg protein⁻¹). One unit of protease, amylase, and lipase activities were expressed as 1 μ g of tyrosine, maltose and fatty acid released per minute, respectively. MDH and LDH activity was expressed as units mg protein⁻¹ min⁻¹ at 25°C, where 1 unit was equal to Δ 0.01 OD min⁻¹ at 37°C.

Statistical analysis

The data collected were processed and analysed by one-way ANOVA using statistical software SPSS version 20.0 at a 5% significance level to test for significant differences between the mean values of various treatments, and by using the Duncan Multiple Range test (IBM SPSS Statistics for Windows, Version 20.0. IBM Corp., Armonk, NY).

Results and discussion

Water quality

Standard procedures (APHA, 2005), were adopted for water quality analysis. During the 75-day experimental period, the mean values of water temperature, pH, DO, ammonia-N, nitrite-N, nitrate-N, inorganic phosphate, free CO₂, total hardness, total alkalinity, total suspended solids and total dissolved solids were in the optimal level (Table 3) in all the treatment and control tanks throughout the experimental trial.

Assessment of growth performance of fish

At the end of the experiment, a significant difference in the growth performance (weight gain, SGR, FCR, FER, PER and survival) of pearlspot fed with different experimental and control diets was observed. Fish fed with crab meal (T₂) showed significantly higher ($p < 0.05$) body weight gain (11.54 $\pm$ 0.78 g) followed by T₁ (SM) (10.19 $\pm$ 0.91 g), T₃ (SWP) (9.56 $\pm$ 0.67 g), than the control (5.99 $\pm$ 0.18 g) diet fed fishes. SGR was observed to be higher in T₂; however, there was no significant difference ($p > 0.05$) between T₁ (SM) and T₂ (CM). FCR, FER and PER were found to be significantly higher ($p < 0.05$) and there was no significant difference between the treatment groups, T₁, T₂ and T₃, while poor performance was observed in control (C) diet-fed fishes. The survival rate was noted to be significantly higher ($p < 0.05$) in the control groups, followed by T₂, yet there was no significant difference ($p > 0.05$) between T₁ and T₂. T₃ diet-fed fishes exhibited the lowest survival rate. The growth parameters and mean weight gain of fishes fed with different treatments such as T₁ (SM), T₂ (CM), T₃ (SWP) and control diets are depicted in Table 4 and Fig. 1, respectively.

Enzyme assays

Enzyme activities of fishes fed with experimental feed significantly influenced the growth performance and survival. Enzyme activities of *E. suratensis* given different treatments such as control, T₁ (SM), T₂ (CM) and T₃ (SWP) are given in Fig. 2, 3, 4 and 5. Crab meal (T₂) diet-fed fishes exhibited higher protease activity than other treatments. Lipase activity was significantly higher ($p < 0.05$) in silkworm pupae-fed fishes (T₃). However, both protease and lipase

Table 3. Mean values of the water quality parameters recorded during the experimental trial

Parameters	Mean $\pm$ SD
Temperature (°C)	33.28 $\pm$ 0.74
pH	8.43 $\pm$ 0.3
Dissolved oxygen (ppm)	6.08 $\pm$ 0.57
Total alkalinity (ppm)	67.25 $\pm$ 10.47
Total hardness (ppm)	173.08 $\pm$ 7.94
Free CO ₂ (ppm)	5.54 $\pm$ 0.86
Total dissolved solids (ppm)	0.47 $\pm$ 0.08
Total suspended solids (ppm)	0.19 $\pm$ 0.11
Ammonia (ppm)	0.16 $\pm$ 0.1
Nitrite (ppm)	0.13 $\pm$ 0.09
Nitrate (ppm)	2.78 $\pm$ 0.44
Phosphate (ppm)	1.78 $\pm$ 0.77

Table 4. Growth performance of the fishes fed with different experimental diets

Growth parameters	Diets			
	C	T1	T2	T3
Mean initial weight (g)	1.1 g	1.1 g	1.1 g	1.1 g
Mean final weight (g)	7.09±0.18	11.29±0.91	12.64±0.78	10.66±0.67
Percentage weight gain (%)	544.54±16.08 ^a	926.66±82.53 ^b	1049.09±70.61 ^c	868.79±61.02 ^b
Specific growth rate (% per day)	2.48±0.03 ^a	3.1±0.11 ^{bc}	3.25±0.09 ^c	3.02±0.09 ^b
Feed conversion ratio	1.91±0.11 ^b	1.34±0.16 ^a	1.34±0.09 ^a	1.27±0.1 ^a
Feed efficiency ratio	0.52±0.03 ^a	0.75±0.08 ^b	0.75±0.06 ^b	0.79±0.06 ^b
Protein efficiency ratio	1.67±0.1 ^a	2.38±0.26 ^b	2.37±0.16 ^b	2.51±0.19 ^b
Survival rate (%)	80.55±2.55 ^c	65.56±3.47 ^{ab}	70.56±4.19 ^b	60.55±2.55 ^a

Values are presented as mean ± standard deviation.

Values in the same row with different superscripts differ significantly ($p < 0.05$) for each parameter.

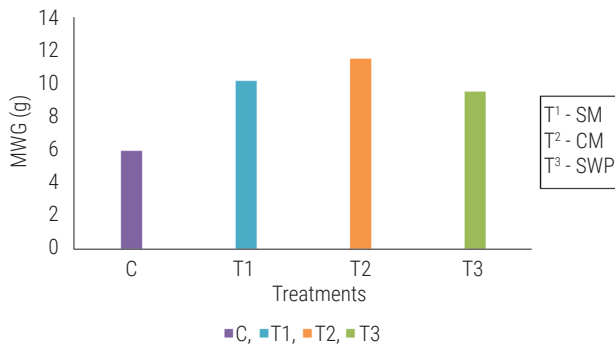


Fig. 1. Mean weight gain of fishes fed with different experimental and control feeds

activities were not significantly different ($p > 0.05$) in the control group fed fish. The highest amylase activity was not significantly different ($p > 0.05$) between the treatments but was significantly different from the control group. Fishes fed with treatment diets T₁ (SM), T₂ (CM) and T₃ (SWP) exhibited significantly higher ($p < 0.05$) MDH and LDH activities in the intestine. In contrast, the lowest activity was observed in control diet-fed fishes.

In the present study, all the treatments exhibited better growth than the control. The T₂ (crab meal) diet (31.5%) was found to be significantly better at 50% replacement of fish meal than the other treatments T₁, T₃ and the control. the findings were in line with the growth reported in African giant catfish (*Heterobranchus longifilis*) fed with crab meal (30% crude protein, CP) as a substitute for fish meal in its diet (Karemah, 2013). Further, similar performance was reported in Nile tilapia (*Oreochromis niloticus*), wherein juveniles

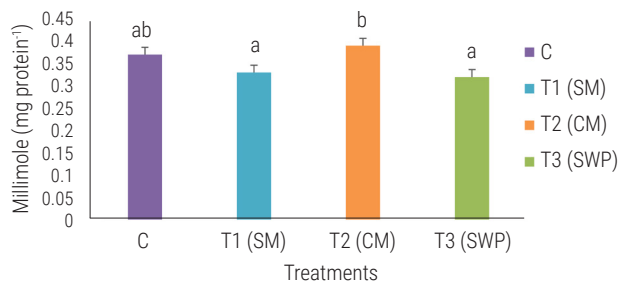


Fig. 2. Protease activity of fishes fed with different experimental and control feed

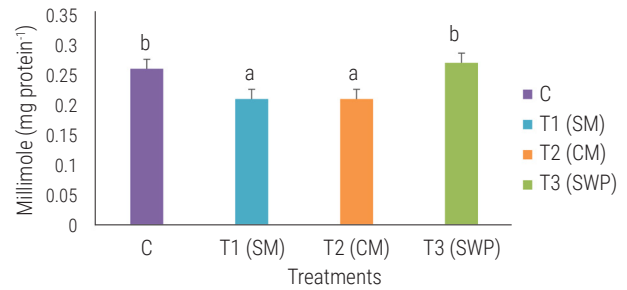


Fig. 3. Lipase activity of fishes fed with different experimental and control feeds

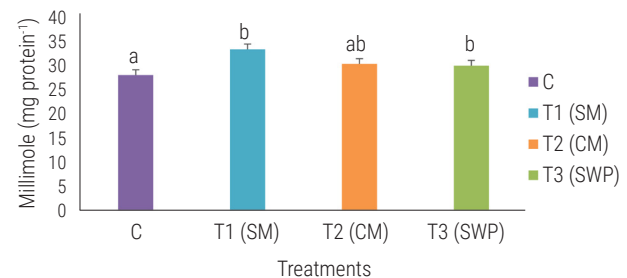


Fig. 4. Amylase activity of fishes fed with different experimental and control feeds

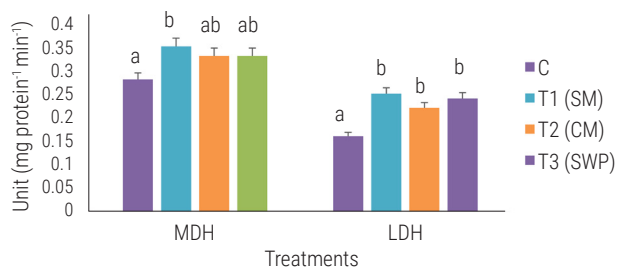


Fig. 5. Malate dehydrogenase (MDH) and Lactate dehydrogenase (LDH) activity in fishes fed with different experimental and control feeds

fed with red crab meal and chickpea meal showed better growth performance in terms of final body weight than different animal (fish silage meal, whey meal, bovine blood meal and red crab meal) and plant (extruded bean, extruded chickpea meal, coconut paste, *Jatropha curcas* meal and chickpea meal) based by-products

(Montoya-Mejia *et al.*, 2017). Similarly, red crab meal (RCM, @15%) is a suitable partial replacement for fish meal in the diet of *Penaeus vannamei* juveniles, with improved growth, feed conversion and protein efficiency (Villarreal *et al.*, 1994; Goytortua-Bores *et al.*, 2006). Significantly better growth was observed in juvenile *Penaeus californiensis* when RCM partially replaced dietary fish meal (Civera *et al.*, 1994; Villarreal *et al.*, 2004). Furthermore, the study clearly stated that crab meal could partially replace fish meal in *H. longifilis* for noticeable growth compared with the control diet (FM) (Valeta, 2020).

Feed utilisation parameters such as FCR, FER and PER were significantly higher in T₂-CM diet-fed fishes than in control. Furthermore, it was observed that there was no significant difference among the values of FCR, FER, and PER across all treatments. The highest PER values (2.37±0.16) of crab meal-fed fishes in this study compared favourably with those of *H. longifilis* fed with a crab-meal diet of 30% CP (Karemah, 2013). Shrimp meal and silkworm pupae meal also performed significantly ($p < 0.05$) higher than the control diet, which had fish meal alone. The present results are in line with the results in common carp fed a diet with partial replacement of fishmeal by shrimp meal (Al-Jader and Al-Khshali, 2021). The lowest FCR (1.34±0.16) value obtained in T₁ (SM) in this study was comparable with the results of Rajanandini *et al.* (2014) and Fall *et al.* (2020) where they recorded better FCR (1.30 and 1.42) at 30% CP for 50% replacement of fishmeal by shrimp meal in the diet of Koi carp and Nile tilapia respectively. Further, the better growth performed by silkworm pupae meal strongly correlated with the findings by Nandeesha *et al.* (2000), who suggested that SWP can be used up to 50% incorporation level without any adverse effect in common carp. Similar results were observed in Jian carp fed with SWP at 50% replacement of fish meal in diets without compromising growth performance (Ji *et al.*, 2015).

Dheke and Gubhaju (2013) found a lower FCR of 1.33 in rainbow trout when fed with shrimp meal compared to silkworm pupae meal. However, there was no significant difference between the two treatments, and these findings align with the results observed in the present study. Also, lower FCR (2.1) was reported in silkworm pupae compared to fish meal in carp (Rangacharyulu *et al.*, 2003). Pillai and Ali (1997), suggested that 31.5% protein was optimum for *E. suratensis*. This reflection supports the result of the present study. The survival rate of *E. suratensis* fluctuates between 45 to 100% (Padmakumar *et al.*, 2004a). More than 50% of survival was observed in all treatments and the highest was recorded in the control group (80.55±2.55%). The control diet had the highest survival, followed by the crab meal diet. A similar survival rate was also observed by Ayala-borboa *et al.* (2013) who found survival was 90 and 79% for the shrimps (*P. vannamei*) fed with fish meal and red crab meal, respectively. Hence, crab meal (31.5 % CP) can be effectively utilised as a partial replacement for fish meal in the diet of *E. suratensis*. Further, shrimp meal and silkworm pupae meal also can be used as a partial substitution for fish meal in the diet of *E. suratensis* with no adverse effect on growth performance, feed utilisation and survival.

The digestive enzymes play a significant role in the development and growth of fish. The capability of the fish to utilise ingested nutrients depends on the activities of various digestive and metabolic enzymes in the digestive tract. To understand the digestive physiology and metabolic activity of pearlspot, digestive

enzymes such as protease, amylase, and lipase as well as metabolic enzymes such as malate dehydrogenase (MDH) and lactate dehydrogenase (LDH), were analysed and recorded from the intestine of fishes at the end of the trial. The results showed that digestive and metabolic enzymes were not deleteriously affected by the experimental diets. Experimental evidence on the metabolic and digestive enzyme profile of *E. suratensis* is insufficient for preparing proficient compound feeds. The crab meal (T₂) diet-fed fishes exhibited significantly higher ($p < 0.05$) protease activity than other treatments; however, it was not significantly different from control. This result agreed with the previous findings, where high protease activity in tilapia fed with red crab was reported (Montoya-Mejia *et al.*, 2017). Further, similar results and observations also found high proteolytic activity in *P. vannamei* provided with red crab meal (Ayala-barboa *et al.* (2013).

Lipase activity was significantly higher ($p < 0.05$) in T₃; however, there was no significant difference with the control diet (fish meal) fed fishes. This result was in line with the report of Montoya-Mejia *et al.* (2017) observing high lipase activity in tilapia fed with fish silage-based diets. The high protease and lipase activity recorded in the control group in the present study is on par with the results recorded in African catfish fed with control (FM) diet and 50% of fish meal replacement with fermented soy pulp (FSP) (Kari *et al.*, 2022). Similarly, the highest protease and lipase activity was found in Japanese seabass fed with fishmeal-based diets than the treatment diets (Zang *et al.*, 2018). High lipase activity in silkworm pupae (T₃) diet-fed fishes in this study was comparable with the results observed in common carp, *Cyprinus carpio* fed with high levels of non-de-fatted silkworm pupae (Nandeesha *et al.*, 2000). Higher amylase activity in shrimp meal-fed fishes in this study was comparable with findings in hybrid snakeheads provided with shrimp paste (Fang *et al.*, 2019).

The MDH and LDH activities were significantly higher ($p < 0.05$) in all the treatments than in the control diet-fed fishes observed in this study. However, there was no significant difference between these treatments. It is suggested that the metabolic activity of treatment diets-fed fishes was better than control diet-fed fishes. The increased protease and lipase activity found in T₂ (crab meal) and control (FM) diet-fed fishes were coupled with enhanced protein and lipase digestion, promoting faster growth in fish than other treatments. But in the case of fish meal, the lowest growth was recorded, possibly due to the various food preferences of *E. suratensis* with multiple sizes and seasons. This suggestion coincides with the findings of Sundararaj and Krishnamurthy (1975), who reported that advanced fry of pearlspot mainly feeds on aquatic insect larvae-based feeds.

It can be concluded that crab meal performed better than shrimp meal and silkworm pupae (SWP) meal, at 50% replacement of fish meal in the diet of *E. suratensis*. However, the shrimp and SWP meals also performed better than the control (FM). Hence, these ingredients can also be used in the diet of *E. suratensis*. Better nutrient utilisation and growth performance of crab meal in the diet can be attributed to their balanced digestive and metabolic enzyme activities throughout the experimental trial. Therefore, crab meal at a 50% replacement level can be utilised successfully in the diet of pearlspot as a cost-effective supplementary feed ingredient for sustainable aquaculture development, particularly by reducing the over-dependence on fish meal. It would also mean reduced

environmental impact as excess crustacean wastes are being utilised that would otherwise be discarded into the environment.

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