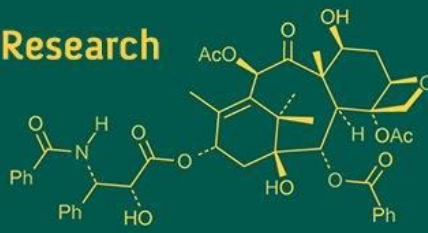


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Influence of dietary shrimp waste on pigmentation, growth and survival in the three-spot gourami, *Trichopodus trichopterus*

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Abstract

This study investigated the effects of shrimp waste-supplemented diets on growth, feed utilization, survival, and carotenoid pigmentation of *Trichopodus trichopterus* over a 45-day feeding trial. Four diets were formulated: a control (T₀) and three diets containing graded levels of shrimp waste (T₄, T₅, and T₆). Growth parameters were monitored biweekly, while specific growth rate (SGR), feed conversion ratio (FCR), survival, and total carotenoid content were evaluated at the end of the experiment. Fish fed shrimp waste-supplemented diets showed significantly improved growth performance ($p < 0.05$) compared to the control. The highest final weight, weight gain, and SGR were recorded in the T₆ group, along with the lowest FCR, indicating enhanced feed efficiency. Survival rates did not differ significantly among treatments, confirming the safety of shrimp waste inclusion. Total carotenoid levels increased markedly with shrimp waste supplementation, with maximum pigmentation observed in T₆. The findings demonstrate that shrimp waste is an effective and sustainable dietary ingredient for improving growth, feed efficiency, and coloration in *T. trichopterus*.

Keywords: *Trichopodus trichopterus*, shrimp waste, growth, feed efficiency, survival

Introduction

Ornamental fish keeping is the world's second-largest hobby, with over 2,000 species traded and Asia contributing nearly 65% of the global market value (Muyot *et al.*, 2019) [21]. The global ornamental fish trade has grown at an annual rate of about 14% since 1985. India possesses rich freshwater ornamental fish diversity, particularly in the Western Ghats, though the trade is dominated by exotic species, while marine ornamentals contribute only 2% due to limited culture technologies.

Gouramis (family Osphronemidae) are among the most popular ornamental fishes, comprising about 133 freshwater species distributed mainly in tropical Asia. The blue gourami (*Trichogaster trichopterus*), native to Southeast Asia, is widely favored in aquariums for its attractive coloration and peaceful nature (Jena *et al.*, 2019; Sinha, 2017; Degani 1993; Froese and Pauly, 2014) [14, 8, 11].

The market value of ornamental fish is largely influenced by body colour, which depends on carotenoids obtained through diet, as fish cannot synthesize these pigments (Lau *et al.*, 2023; Garcia and Lara, 2013) [18, 12]. Dietary carotenoids, particularly astaxanthin, are therefore widely used to enhance pigmentation in ornamental fishes (Matsuno *et al.*, 2001) [19].

Crustaceans are rich natural sources of carotenoids, especially astaxanthin, along with other bioactive compounds that support antioxidant activity and immunity (Babin *et al.*, 2019; Kalidoss *et al.*, 2020; Simat *et al.*, 2022) [5, 15, 28]. Shrimp waste, comprising shells and heads, contains high levels of carotenoids, proteins, chitin, and fatty acids, and has been shown to improve growth, immunity, and pigmentation in fish while offering a cost-effective alternative to synthetic pigments. The shrimp processing plant's earnings would be enhanced by retrieving these valuable components from the waste. One of the most accessible raw materials for the extraction of carotenoids is the waste shrimp produced by the Indian shrimp processing sector. (Anbazhahan *et al.*, 2014; Yi *et al.*, 2015; Oushani *et al.*, 2020; Cahu *et al.*, 2012) [1, 34, 23, 7].

Therefore, the present study aimed to evaluate the effects of dietary shrimp waste on pigmentation, growth, and survival of *Trichogaster trichopterus*.

Materials and Methods

Feed Ingredients and Diet Formulation

The ingredients and proximate composition of the experimental diets are presented in Tables 1 and 2. Proximate analysis of feed ingredients and diets was performed following AOAC (1995) [3]. Shrimp waste collected from a local processing unit was washed, dried, and ground before use. Four iso-nitrogenous diets (30% crude protein) were formulated using the Pearson square method, comprising three shrimp waste powder-based diets (T₄-T₆) and a control diet (T₀).

Table 1: Proximate composition of ingredients

Ingredients (%)	Crude Protein (%)	Crude Fat (%)	Ash (%)	Moisture (%)
Fish meal	54.63	4.8	14.9	7.83
GNOC	38.38	4.61	8.36	7.39
Wheat flour	13.5	1.1	5.28	7.67
Tapioca flour	0.23	0.54	0.18	11.29
Shrimp waste	21.24	10.85	16.05	12.82

Table 2: Composition of experimental diets

Ingredient (%)	Diets			
	T ₀ Control	T ₄ (5%)	T ₅ (10%)	T ₆ (15%)
Spinach	00.00	00.00	00.00	00.00
Shrimp waste	00.00	05.00	10.00	15.00
Sterilized fish meal	25.00	24.00	23.00	22.00
Ground nut oil cake	38.00	38.00	37.00	36.00
Wheat flour	13.00	11.00	10.00	08.00
Tapioca powder	14.00	12.00	10.00	09.00
Sunflower oil	04.00	04.00	04.00	04.00
Fish oil	04.00	04.00	04.00	04.00
Vitamin and minerals	02.00	02.00	02.00	02.00
Total	100	100	100	100

Experimental Protocol

Growth Performance Parameters

Growth performance was evaluated using the following indices:

- Weight gain (%) = [(Final weight - Initial weight)/(Initial weight)] × 100
- Specific growth rate (SGR, %) = [(ln final weight - ln initial weight)/Rearing period (days)] × 100
- Feed conversion ratio (FCR) = Total intake (g)/Total wet weight gain (g)
- Survival rate (%) = (Total no. of live animals/Total no of initial animals) × 100

Total Carotenoid Analysis

Total carotenoid concentration (TCC) in the muscle tissue of *Trichopodus trichopterus* was analyzed immediately after completion of the feeding trial following the pigment extraction method described by Olson, (1979). 1 gram of muscle tissue, excluding the head and alimentary canal, was placed in a 10 ml screw-capped clear glass vial and mixed with 2.5 g of anhydrous sodium sulphate. The tissue was gently homogenized, followed by the addition of 5 ml chloroform, and the mixture was stored overnight at 0 °C. After the formation of a clear chloroform layer (approximately 1-2 cm above the residue), absorbance was

measured at 380, 450, 470, and 500 nm using a spectrophotometer. A blank prepared using the same procedure was used for comparison. The wavelength showing maximum absorbance was selected for calculation of carotenoid concentration.

$$\text{TCC } (\mu\text{g g}^{-1}) = [(\text{Absorption at maximum wavelength} \times 10)/(0.25 \times \text{Sample weight})]$$

Where, 10 = dilution factor and 0.25 = extinction coefficient

Analysis of water quality parameters

Water quality parameters like temperature, pH, dissolved oxygen (DO) level, alkalinity, and hardness were analyzed every fortnight following standard methods given by APHA (1998) [4]. Partial water exchange was carried out in every 15 days to maintain acceptable water quality parameters.

Statistical Analysis

All experimental data were tabulated and statistically analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range test was applied for post-hoc comparison of treatment means, and differences were considered significant at $p < 0.05$.

Results

Water quality maintenance

During the feeding trial, water quality parameters were maintained within suitable and consistent ranges across all experimental tanks (Table 3). Water temperature varied between 28.9 and 30.0 °C, pH ranged from 7.8 to 8.6, and dissolved oxygen levels were maintained between 5.7 and 6.6 mg L⁻¹. Total hardness and alkalinity ranged from 160-250 mg L⁻¹ and 150-210 mg L⁻¹, respectively. These values were comparable among the control and treatment groups and remained within acceptable limits for *Trichopodus trichopterus*. Water quality was monitored fortnightly, and no signs of stress or disease were observed during the 45-day experimental period, indicating that environmental conditions were suitable and did not influence the experimental outcomes.

Table 3: Water quality parameters of experimental tanks

Parameters	T ₀		T ₄		T ₅		T ₆	
	Max	Min	Max	Min	Max	Min	Max	Min
Water temperature (°C)	29.9	28.9	29.8	29.1	29.6	28.9	30.0	29.0
pH	8.6	7.8	8.3	7.8	8.5	8.0	8.5	7.9
Dissolved oxygen (mg L ⁻¹)	6.2	5.8	6.6	5.9	6.5	5.7	6.3	5.8
Total hardness (mg L ⁻¹)	240	180	210	160	210	150	250	190
Total alkalinity (mg L ⁻¹)	180	160	170	160	210	150	170	160

Growth performance and feed utilization of *Trichopodus trichopterus*

All experimental diets were readily consumed by *Trichopodus trichopterus* throughout the feeding trial, indicating good acceptability of the shrimp waste supplemented feeds. The initial body weights of fish were similar among all treatment groups, confirming uniform experimental conditions.

After 45 days, growth performance varied noticeably among treatments, with shrimp waste-fed groups showing better growth than the control. Fish in the T₆ treatment attained the highest final body weight, weight gain (%), and specific growth rate (SGR), followed by T₅ and T₄, while the lowest

values were recorded in the control group (T_0). The improved growth observed in shrimp waste supplemented groups may be associated with enhanced feed palatability, better nutrient availability, and the presence of carotenoid-rich bioactive compounds that promote digestion and antioxidant protection.

Feed utilization efficiency was also improved, as indicated by a significantly lower feed conversion ratio (FCR) in T_6

compared to other treatments, whereas the control group showed the highest FCR.

Survival remained high and did not differ significantly among treatments, demonstrating that shrimp waste inclusion did not negatively affect fish health. Overall, dietary incorporation of shrimp waste, particularly at higher inclusion levels, effectively enhanced growth performance and feed utilization efficiency in *T. trichopterus* without compromising survival (Table 4).

Table 4: Growth performances, feed utilization and survival of fish fed with shrimp waste powder

Parameter	T_0	T_4	T_5	T_6
Initial weight (g)	2.94±0.01	3.05±0.02	3.06±0.01	3.02±0.01
Final weight (g)	4.64±0.03	5.20±0.06	5.36±0.06	5.52±0.04
Weight gain (%)	58.11±0.77 ^a	70.48±1.26 ^b	75.40±1.01 ^c	82.98±0.80 ^d
Specific growth rate (SGR, % day ⁻¹)	3.79±0.04 ^a	4.78±0.10 ^b	5.13±0.08 ^c	5.56±0.06 ^d
Feed conversion ratio (FCR)	2.32±0.02 ^a	1.92±0.04 ^b	1.79±0.02 ^c	1.63±0.01 ^d
Survival rate (%)	83.33±3.33 ^a	90.00±5.77 ^a	96.66±3.33 ^a	96.66±3.33 ^a

Note: T_0 , T_1 , T_2 , and T_3 were supplemented with 0, 5, 10, and 15 g shrimp waste powder 100 g⁻¹, respectively. The data presented in Mean ± SD

Total carotenoid concentration study in fish tissue

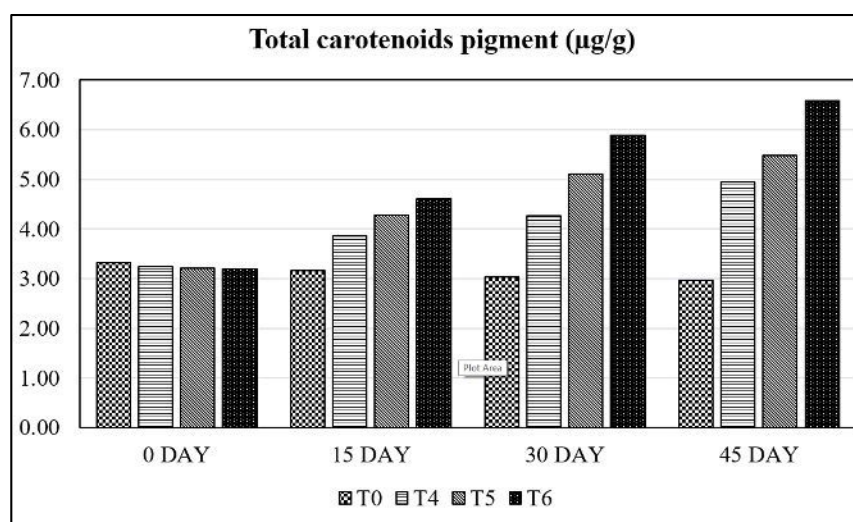
Total carotenoid content in *T. trichopterus* increased significantly with dietary shrimp waste supplementation. Initial carotenoid levels were similar among all treatments; however, a marked increase was observed in shrimp waste-fed groups over the experimental period (Figure). At the end of 45 days, fish fed the T_6 diet showed the highest carotenoid concentration, followed by T_5 and T_4 , while the control group recorded the lowest value (Table 5). Statistical analysis revealed significant differences ($p < 0.05$) in carotenoid content among all treatments, indicating a dose-dependent enhancement of pigmentation with shrimp waste inclusion. The results confirm that shrimp waste-based diets

effectively improve carotenoid deposition and coloration in *T. trichopterus*.

Table 5: Total carotenoid concentration of fish fed with shrimp waste powder

Treatments	Days			
	0	15	30	45
T_0	3.32±0.05 ^a	3.16±0.02 ^a	3.03±0.04 ^a	2.96±0.05 ^a
T_4	3.24±0.10 ^a	3.87±0.04 ^b	4.28±0.10 ^c	4.95±0.04 ^d
T_5	3.21±0.04 ^a	4.27±0.10 ^b	5.11±0.04 ^c	5.48±0.08 ^d
T_6	3.20±0.08 ^a	4.81±0.17 ^b	5.89±0.04 ^b	6.59±0.07 ^b

Note: The data presented in Mean±SD



Discussion

The significantly higher mean weight gain and specific growth rate (SGR) observed in shrimp waste-fed treatments align with earlier findings that highlight the growth-promoting potential of shrimp by-products in ornamental fish diets. Mohanta *et al.*, (2013) [20] reported improved growth in blue gourami fed prawn waste-based diets, while Kannan *et al.*, (2014) [17] demonstrated increased growth in goldfish with higher inclusion levels of shrimp head meal. Similar trends have been documented by Boonyaratpalin and Unprasert, (1989) [6], Salma *et al.*, (2013) [26], Rathnayake *et al.*, (2016), and Pattanaik *et al.*, (2021) [24],

further supporting the effectiveness of shrimp waste as a dietary supplement.

Feed conversion ratio (FCR) improved significantly with increasing shrimp waste inclusion, indicating enhanced feed utilization efficiency. The lowest FCR recorded in the highest shrimp waste treatment suggests improved digestibility and efficient conversion of feed into biomass. This improvement may be associated with the carotenoid content and other functional components present in shrimp waste, which are known to enhance metabolic efficiency and reduce physiological stress. Comparable reductions in FCR with shrimp waste-supplemented diets have been

reported by Vasanthakumaran *et al.*, (2020) ^[32], Fall *et al.*, (2011) ^[10], Kalinowski *et al.*, (2005) ^[16], and Mohanta *et al.*, (2013) ^[20].

Survival rates remained high across all treatments, with no statistically significant differences among control and shrimp waste-fed groups. This indicates that shrimp waste supplementation did not negatively affect fish health and confirms its safety as a feed ingredient. Similar survival outcomes have been reported in ornamental fish fed shrimp-based diets by Vijayagopal *et al.*, (2008) ^[33] and Anka *et al.*, (2016) ^[2].

A pronounced enhancement in total carotenoid content was observed in fish receiving shrimp waste-supplemented diets, with the highest pigmentation recorded in the maximum inclusion group. Shrimp waste is a natural source of carotenoids, particularly astaxanthin, which is readily deposited in fish tissues and contributes to improved skin coloration. The significant increase in carotenoid levels observed in this study corroborates earlier findings that demonstrate the effectiveness of shrimp waste as a natural pigment source in ornamental fish feeds. Similar improvements in coloration have been reported by Haque *et al.*, (2021) ^[13], Ramya *et al.*, (2016) ^[25], Diler and Gokoglu, (2004) ^[9], Subamia *et al.*, (2013) ^[29], Uthayasiva *et al.*, (2014) ^[31], Seidgar (2015) ^[27], and Tran *et al.*, (2022) ^[30].

In conclusion, the present study confirms that shrimp waste can be effectively utilized as a sustainable and economical feed ingredient for *Trichopodus trichopterus*, resulting in improved growth performance, feed utilization, survival, and pigmentation. The use of shrimp waste in ornamental fish diets not only enhances fish quality but also offers an environmentally responsible approach to managing seafood processing by-products.

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Ethical statement

All experimental procedures involving live fish were conducted in accordance with the guidelines approved by the Institutional Animal Ethics Committee, Department of Aquaculture, Kamdhenu University. Throughout the study, fish were handled with utmost care to minimize stress and ensure their well-being.

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