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Effects of Fermented Rice Bran-based Copefloc Technology Supplemented with Mixed Microalgae (*Chlorella vulgaris* and *Aphanizomenon flos-aquae*) on Growth Performance, Digestive Enzyme Activity and Water Quality of Indian Spiny Loach (*Lepidocephalichthys thermalis*)

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ABSTRACT

Background: Indian spiny loach, *Lepidocephalichthys thermalis*, is an economically important freshwater ornamental and food fish with high nutritional value and market demand. However, limited information is available regarding its captive culture. Hence the present investigation was undertaken to assess the impact of groundnut oil cake (GNOC) with mixed algae (*Chlorella vulgaris* and *Aphanizomenon flos-aquae*) + fermented rice-bran (FRB) (1:5, 1:6, 1:7, 1:8, 1:9 and 1:10) on growth ratio, digestive enzyme activity and water quality of Indian spiny loach under captive culture conditions.

Methods: The study followed a completely randomized design (CRD) with six treatments and a control each in triplicate tanks. Juvenile *Lepidocephalichthys thermalis* were stocked at a rate of 50 numbers in all 21 experimental tanks. Groundnut oil cake (GNOC) at 8% body weight and mixed algae of 250 ml was fed to all experimental tanks throughout the rearing period. Once in a week, the fermented carbon source at various ratios (1:5, 1:6, 1:7, 1:8, 1:9 and 1:10) was added to the respective treatment tanks at a rate of 3 ml L^{-1}.

Result: The study displayed significant variations ($p<0.05$) in growth, digestive enzyme responses and water quality among the experimental groups of *Lepidocephalichthys thermalis* reared indoor. From the outcome of the study, groundnut oil cake (GNOC) with mixed algae (*Chlorella vulgaris* and *Aphanizomenon flos-aquae*) + fermented rice-bran (FRB) (1:5) produced the best overall culture performance and digestive enzyme activity of *Lepidocephalichthys thermalis* and maintain better water quality.

Key words: Copefloc, Digestive enzymes, Fermented rice bran, *Lepidocephalichthys thermalis*, Water quality.

INTRODUCTION

The Indian spiny loach (*Lepidocephalichthys thermalis*) is a small freshwater fish species which is distributed globally. The species is considered suitable for aquaculture because of its adaptability to diverse environmental conditions (Velmurugan *et al*., 2025). The distribution of this species is extensive throughout Sri Lanka and India (Pethiyagoda, 1991; Ekaratne, 2000). Burrowing behaviour exhibited by this fish helps it tolerate environmental stress and avoid predators (Natuhara, 2013). The species exhibits omnivorous feeding behaviour with preference towards small aquatic organisms (Keskar *et al*., 2014). This bottom feeding fish shows high preference towards feeding zooplanktons such as copepods (Robotmam, 1977). It is also an algal feeder which makes use of benthic algae as an excellent source of food (Abe *et al*., 2009). In Indian domestic markets Indian spiny loach has a high demand as it is rich in multiple nutrients (Manoharan *et al*., 2019). This species also possess rich medicinal value (Qin *et al*., 2002). Microalgal species such as *Chlorella vulgaris* and *Aphanizomenon flos-aquae* are rich in antioxidant compounds beneficial for aquaculture applications (Tomassi *et al*., 2024). Aquamimicry-based culture systems

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improve microbial productivity and natural live-feed availability under sustainable farming conditions (Romano, 2017).

Lepidocephalichthys thermalis naturally consumes benthic algae and zooplankton, particularly copepods, making it highly suitable for culture systems that promote natural microbial and planktonic productivity. Fermented rice bran serves as an organic carbon source supporting microbial proliferation, while *Chlorella vulgaris* and *Aphanizomenon flos-aquae* enhance the nutritional quality of the naturally developed copefloc biomass. Therefore, integrating fermented rice bran with mixed microalgae can closely mimic the natural feeding ecology of *L. thermalis*.

Although biofloc and aquamimicry-based culture systems have been investigated in several commercially important aquaculture species, information regarding their application in Indian spiny loach (*Lepidocephalichthys thermalis*) remains unavailable. Furthermore, no previous study has been done on the effects of copefloc biomass made from fermented rice bran and mixed microalgae (*Chlorella vulgaris* and *Aphanizomenon flos-aquae*) on growth performance, digestive enzyme activity and water quality in this species. Therefore, the present study aimed to determine the optimum fermented rice bran ratio for maximizing growth performance, digestive enzyme activity and improving water quality under captive culture of *Lepidocephalichthys thermalis*.

MATERIALS AND METHODS

The experiment was conducted for 60 days on growth evaluation of *Lepidocephalichthys thermalis* under captive culture conditions at Kanyakumari Parakkai Centre for Sustainable Aquaculture. The Juvenile loaches (*L. thermalis*) were collected from the wild at Parakkai, Kanyakumari district of Tamil Nadu India. Prior to experimentation, fishes were acclimated to indoor tank conditions for 15 days and the initial length and weight (4.40±0.03 cm length and 0.50±0.02 g weight) were immediately recorded. The culture tanks for rearing juvenile *L. thermalis* were fiberglass reinforced plastic (FRP) tanks ($1 \times 1 \times 0.5$ m). Total of 21 tanks were maintained indoor with a total capacity of 500 liters each filled with 250 liters of freshwater. The experimental feed used was groundnut oil cake (GNOC) and mixed algae. Mixed algae contains *C. vulgaris* and *Aphanizomenon flos-aquae* (*Afs*). Mixed algae were harvested from the wild and maintained in plastic tanks of 500 L capacity. The plastic tanks were kept in direct exposure to sunlight which act as an energy source for mixed algal cells to grow. Prior to feeding, cell densities were estimated microscopically using a Neubauer haemocytometer and averaged 5×10^6 cells mL^{-1} for *C. vulgaris* and 2.5×10^5 cells mL^{-1} for *A. flos-aquae*.

For copefloc preparation, husk-less deoiled rice bran purchased from a local rice mill was used as a carbon source. Rice bran was mixed with freshwater at six different ratios of 1:5, 1:6, 1:7, 1:8, 1:9 and 1:10 (w/v) in separate 50-L plastic containers. *Saccharomyces cerevisiae* obtained from commercial baker's yeast served as the fermentation inoculum and added at 5, 6, 7, 8, 9 and 10 g to the respective fermentation mixtures corresponding to the above dilution ratios. Aerobic conditions during fermentation were maintained through constant aeration using diffusers connected to an air blower. The mixtures were fermented for 24 h at room temperature (27-30°C). During fermentation, rice bran particles gradually disintegrated and formed a fine suspended biomass rich in microbial aggregates. After completion of fermentation, the floating and suspended microbial biomass (copefloc) was collected from the upper layer of the fermentation medium and immediately applied to the respective experimental tanks. Fresh fermentation batches were prepared every week immediately before application to maintain consistent microbial activity.

Fifty juveniles were randomly stocked into each of the 21 experimental tanks (1 control and 6 treatments, each with triplicates) : groundnut oil cake (GNOC) with mixed algae (c), GNOC with mixed algae + fermented rice-bran (FRB) biomass (1:5) (T1), GNOC with mixed algae + FRB biomass (1:6) (T2), GNOC with mixed algae + FRB biomass (1:7) (T3), GNOC with mixed algae + FRB biomass (1:8) (T4), GNOC with mixed algae + FRB biomass (1:9) (T5) and GNOC with mixed algae + FRB biomass (1:10) (T6). GNOC was fed daily at a rate of 8% body weight. Mixed algae of 250 ml (*C. vulgaris* of 12.5×10^8 cells in 250 ml (5×10^6 cells mL^{-1}) and *A. fs* of 6.2×10^7 cells in 250 ml (2.5×10^5 cells mL^{-1}) was fed to animals in control (c) and treatments T1 to T6 throughout the experiment. The fermented carbon source of 1:5 (T1), 1:6 (T2), 1:7 (T3), 1:8 (T4), 1:9 (T5) and 1:10 (T6) was supplied to the respective experimental tanks at a rate of 3 ml per litre once in a week. In each tank, 20 fishes were sampled every fortnight for growth evaluation. The proximate composition of GNOC and microalgae (*Chlorella vulgaris* and *Aphanizomenon flos-aquae*) were analysed (n = 3 samples) before initializing the experiment (Table 1). In digestive enzyme analysis, protease was estimated using modified casein hydrolysis assay (Walter 1984), p-nitrophenylpalmitate (p-NPP) hydrolysis assay was used to assess the Lipase activity (Markweg-Hanke *et al*., 1995) and the 3,5-dinitrosalicylic acid (DNS) method with starch as the substrate was used to quantify the amylase activity (Areekijseree *et al*., 2004).

Using the following standard formulas for various bio-growth indicators, growth performance was evaluated:

$$\text{Length gain (LG)} = \text{Final length} - \text{Initial length}$$

$$\text{Weight gain (WG)} = \text{Final weight} - \text{Initial weight}$$

$$\text{Weight gain percentage (WGP)} = \frac{\text{Weight gain}}{\text{Initial weight}} \times 100$$

$$\text{Specific growth rate (SGR)} = \frac{\text{Ln final weight} - \text{Ln initial weight}}{\text{Days of culture}} \times 100$$

$$\text{Survival rate (SR)} = \frac{\text{Number of fish survived}}{\text{Number of fish stocked}} \times 100$$

Water quality parameters such as temperature, pH and dissolved oxygen were recorded on a daily basis. Ammonia, nitrite and nitrate were recorded on weekly basis. Uneaten feed and accumulated debris were siphoned periodically to avoid deterioration of water quality. The standard procedures suggested by APHA (2005) were strictly followed to estimate the water quality of the experimental tanks. The fermented biomass supplementation was expected to enhance microbial utilization of nitrogenous waste compounds and stabilize water quality parameters during the culture period.

Data entry was carried out in Microsoft Excel and was analyzed in SPSS (Statistical Package for Social Sciences) software version 26.0. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test (p<0.05).

RESULTS AND DISCUSSION

Growth performance of experimental animals

One-Way ANOVA showed significant differences in final length and weight among control and six copefloc treatments (p-value <0.05) (Table 2). The weight gain percentage (%) of *L. thermalis* fed with different diet combination among the various treatments clearly indicates that groundnut oil cake (GNOC) with mixed algae (*Chlorella vulgaris* and *Aphanizomenon flos-aquae*) + fermented rice-bran (FRB) (1:5) (T1) recorded highest weight gain (0.08± 0.003 g) among all other feeds (Table 2). Similarly, T1 displayed significantly higher length gain (0.13±0.01 cm) on comparison with all other feed groups (p-value <0.05). Similar to other results, specific growth rate was higher in T1 group (p-value <0.05). T1 also recorded a survival rate of 97±0.28% which was the highest among all the other feeds (Table 2). The superior performance recorded in T1 could be associated with improved nutrient utilization and the presence of bioactive compounds from fermented rice bran and the bioactive compounds present in mixed algae (*Chlorella vulgaris* and *Aphanizomenon flos-aquae*). Comparable enhancement in growth has also been documented in common carp (Abiri *et al*., 2022), *Nile tilapia* (El-Dein *et al*., 2025) and Pacific white shrimp (Jelshina *et al*., 2026). Similar improvements in growth performance and nutrient utilization using carbon source have also been reported in *Etroplus suratensis* reared under indoor conditions (Jackqulinwino *et al*., 2024).

An experiment conducted by Tomassi *et al*. (2024) concludes that *C. vulgaris* and *A. flos-aquae* had the potential to enhance polyphenol content after 24 h fermentation. Polyphenols are growth enhancers, which play a vital role in providing better growth performance of species such as *Cyprinus carpio* (Jahazi *et al*., 2019), *Lates calcarifer* (Ahmadi *et al*., 2022), *Oncorhynchus mykiss* (Mansoori *et al*., 2024). The present findings indicate that although the control diet (GNOC) supported acceptable growth, supplementation with fermented rice bran at the 1:5 ratio further enhanced growth performance. However, higher dilution ratios did not consistently outperform the control, indicating that the beneficial effect was dependent upon the fermentation ratio.

Digestive enzyme activity

In the present study the mean protease, lipase and amylase levels (U mg^{-1} protein) were significantly different among the treatments and higher values were recorded in T1 (1:5) of 89.6±0.12, 8.6±0.11 and 3.1±0.06 (p-value <0.05)

Table 1: Proximate composition (% of dry weight) of experimental diets.

Exp diets	Protein	Fat	Carbohydrate	Ash
Groundnut oil cake	46±0.54	7.1±0.18	23±0.45	5.6±0.17
Mixed algae (*Chlorella vulgaris+Aphanizomenon flos-aquae*)	62.5±0.76	9.8±0.26	15.4±0.63	9.1±0.34

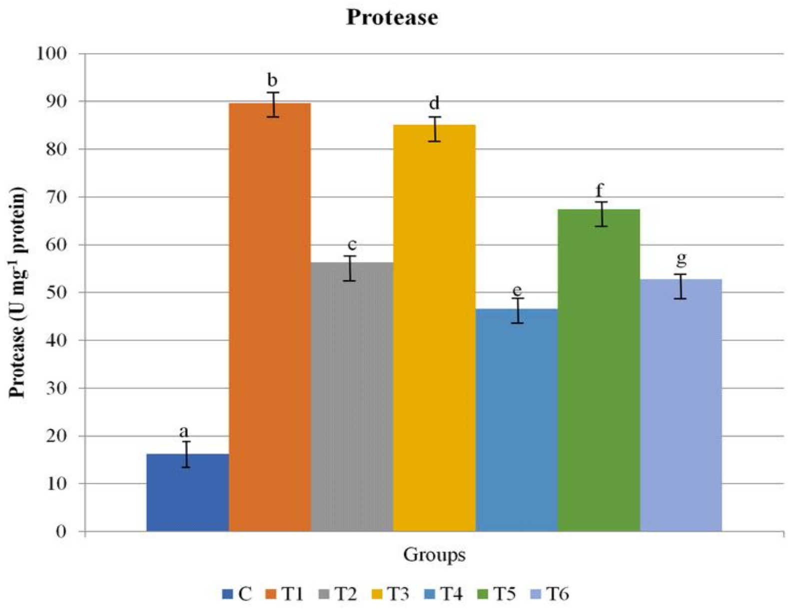
Values expressed as mean±S.E (n = 3). Post-hoc Tukey test was carried out to determine significant difference (p<0.05).

Table 2: Effects of groundnut oil cake (GNOC) with mixed algae (*Chlorella vulgaris* and *Aphanizomenon flos-aquae*) + fermented rice-bran (FRB) (at different ratios) on the growth performance of loach.

Index	Control	T1	T2	T3	T4	T5	T6	p-value
Initial length (cm)	4.40±0.03	4.40±0.03	4.40±0.03	4.40±0.03	4.40±0.03	4.40±0.03	4.40±0.03	NS
Final length (cm)	4.50±0.02^{c}	4.54±0.04^{a}	4.52±0.02^{b}	4.53±0.03^{ab}	4.53±0.02^{ab}	4.52±0.02^{b}	4.52±0.03^{b}	<0.05
Initial weight (g)	0.50±0.02	0.50±0.02	0.50±0.02	0.50±0.02	0.50±0.02	0.50±0.02	0.50±0.02	NS
Final weight (g)	0.58±0.007^{ab}	0.59±0.008^{a}	0.57±0.015^{b}	0.56±0.008^{c}	0.58±0.01^{ab}	0.57±0.009^{b}	0.56±0.019^{c}	<0.05
Length gain (cm)	0.09±0.01^{c}	0.13±0.01^{a}	0.12±0.009^{ab}	0.12±0.01^{ab}	0.12±0.007^{ab}	0.12±0.01^{ab}	0.11±0.03^{bc}	<0.05
Weight gain (g)	0.07±0.008^{b}	0.08±0.003^{a}	0.07±0.01^{b}	0.06±0.01^{c}	0.07±0.007^{b}	0.06±0.01^{c}	0.06±0.02^{c}	<0.05
Weight gain (%)	14.79±0.26^{b}	16.31±0.48^{a}	14.35±0.08^{b}	13.10±0.31^{c}	14.46±0.07^{b}	12.48±0.02^{d}	12.09±0.45^{d}	<0.05
SGR (%/day)	0.12±0.01^{b}	0.13±0.005^{a}	0.12±0.02^{b}	0.11±0.02^{c}	0.12±0.01^{b}	0.10±0.02^{d}	0.10±0.04^{d}	<0.05
Survival rate (%)	92±0.23^{d}	97±0.28^{a}	96±0.18^{ab}	95±0.21^{b}	96±0.17^{b}	93±0.26^{c}	94±0.15^{c}	<0.05

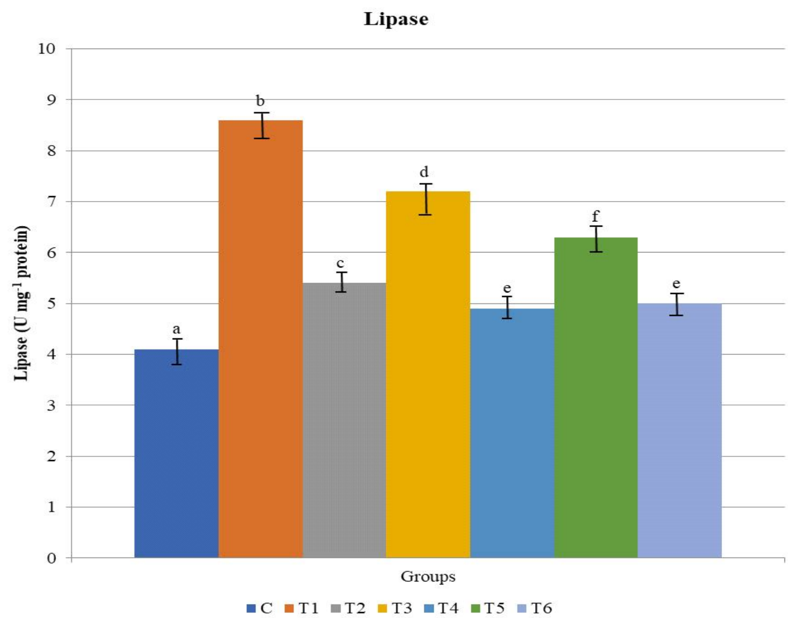
Values expressed as mean±S.E (n = 20); p-value<0.05-statistically significant, NS- No significant difference.

(Fig 1,2,3). Jelshina *et al.* (2026) recorded superior digestive enzyme activity of *Penaeus vennamei* using copefloc treated diet, which is agreed with the results obtained in the research. Susilo *et al*. (2022) analysed the digestive enzyme activity of barred Loach *Nemacheilus fasciatus* and found that at pH 7.0 protease was within the range of 80 to



Values are expressed as mean±SE (n = 10). Different lowercase letters above the bars indicate significant differences among treatments (Tukey's test, P<0.05).

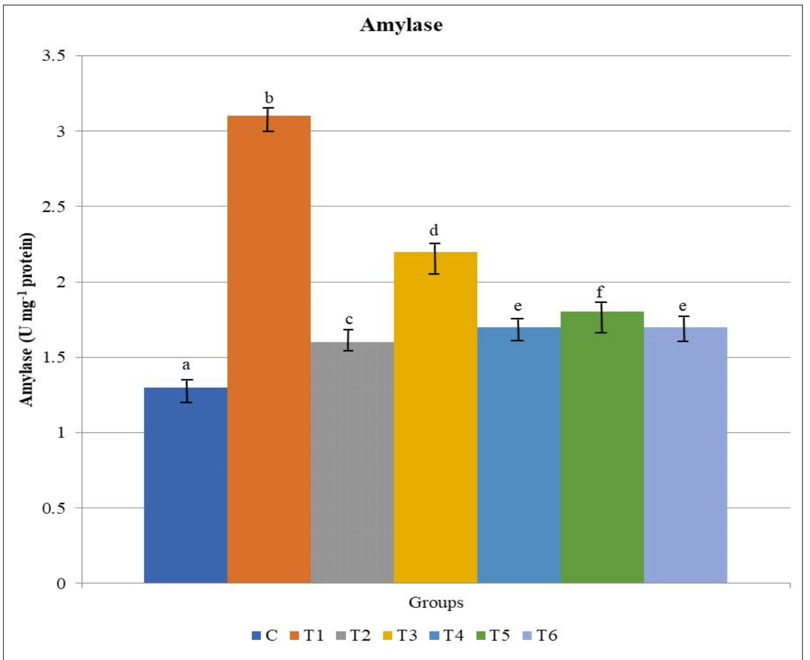
Fig 1: Protease activity (U mg^{-1} protein) of *Lepidocephalichthys thermalis* juveniles reared using groundnut oil cake (GNOC), mixed microalgae (*Chlorella vulgaris* and *Aphanizomenon flos-aquae*) and fermented rice bran (FRB) at different dilution ratios.



Values are expressed as mean±SE (n = 10). Different lowercase letters above the bars indicate significant differences among treatments (Tukey's test, P<0.05).

Fig 2: Lipase activity (U mg^{-1} protein) of *Lepidocephalichthys thermalis* juveniles reared using groundnut oil cake (GNOC), mixed microalgae (*Chlorella vulgaris* and *Aphanizomenon flos-aquae*) and fermented rice bran (FRB) at different dilution ratios.

100 U mg^{-1} protein where protease in T1 fell within this range with almost neutral pH (7.2±0.08). Since *Nemacheilus fasciatus* belongs to the same family of benthic loaches and shares comparable feeding ecology with *L. thermalis*, it provides an appropriate physiological comparison. Elevated protease activity contribute to improved protein digestion and nutrient assimilation and facilitate better growth performance of *L. thermalis* in T1 than in other treatments. *Chlorella vulgaris* shows positive effect towards promoting better protease, lipase and amylase activity in grey mullet *Mugil cephalus* (Akbary and Raeisi, 2020). Similar enhancement of digestive enzyme activities have been demonstrated in *Etroplus suratensis*, where increased protease, lipase and amylase activities were associated with improved nutrient utilization and growth (Jackqulinwino *et al*., 2025). The microalgae *Aphanozomenon-flos aquae (Afa)* contains an important bioactive compound known as phycocyanin (Scoglio *et al*., 2024). According to Hassaan *et al*. (2020), this bioactive compound enhanced digestive enzyme activities such as lipase and amylase in *Oreochromis niloticus,* which may have enhanced lipid and carbohydrate digestion in the experimental fish. Susilo and Rachmawati (2020) reported that lipase activity on barred loach was found to be better between 7.0 to 8.1 which corresponds with observations from the current experiment. A study by Yuan *et al*. (2021) found the amylase activity of loach *Paramisgurnus dabryanus* at 1.50±0.04 U mg^{-1} protein which was much closer to the results obtained in the present study.



Values are expressed as mean±SE (n = 10). Different lowercase letters above the bars indicate significant differences among treatments (Tukey's test, *P*<0.05).

Fig 3: Amylase activity (U mg^{-1} protein) of *Lepidocephalichthys thermalis* juveniles reared using groundnut oil cake (GNOC), mixed microalgae (*Chlorella vulgaris* and *Aphanizomenon flos-aquae*) and fermented rice bran (FRB) at different dilution ratios.

Table 3: Water quality parameters.

Parameters	Control	T1	T2	T3	T4	T5	T6	p-value
Temperature (°C)	26±0.08	26±0.06	26±0.07	26±0.06	26±0.08	26±0.07	26±0.07	NS
pH	8.2±0.05^{a}	7.2±0.07^{e}	7.7±0.04^{d}	7.8±0.05^{c}	7.8±0.06^{c}	7.7±0.08^{d}	8.0±0.07^{b}	<0.05
Dissolved oxygen (ppm)	3.6±0.05^{e}	5.8 ±0.06^{a}	4.4±007^{cd}	4.8±0.11^{b}	4.5±0.08^{c}	4.7±0.07^{bc}	4.4±0.12^{d}	<0.05
Ammonia (ppm)	0.14±0.04^{a}	0.02±0.01^{d}	0.06±0.04^{c}	0.05±0.02^{c}	0.08±0.04^{b}	0.08±0.06^{b}	0.07±0.04^{bc}	<0.05
Nitrite (ppm)	0.09±0.07^{a}	0.03±0.01^{d}	0.05±0.02^{c}	0.05±0.02^{c}	0.06±0.03^{bc}	0.05±0.01^{c}	0.07±0.03^{b}	<0.05
Nitrate (ppm)	0.18±0.06^{a}	0.11±0.02^{d}	0.14±0.06^{c}	0.15±0.07^{b}	0.14±0.08^{c}	0.15±0.07^{b}	0.17±0.09^{ab}	<0.05

Values expressed as mean±standard error (n = 3 water samples per treatment); p-value<0.05- statistically significant, NS- No significant difference.

Water quality parameters

Temperature in all experimental tanks recorded was 26±0.06 to 26±0.08 which remained relatively stable during the culture period (Table 3). According to Santhosh and Singh (2007) ideal temperature for the betterment of fish culture was 24 to 30°C which was within the range maintained in the present study. Ibrahim *et al*. (2023) found superior growth performance in *Clarias gariepinus* within temperature range of 20 and 27°C where *L. thermalis* was maintained within this range, that contributed to improved culture performance of the fish reared in FRP tanks. T1 recorded lower pH and higher Dissolved oxygen (ppm) of 7.2±0.07 and 5.8±0.06 than other treatment groups (Table 3). Research by Li *et al*. (2020) displayed better growth performance of juvenile GIFT at DO of 5 mg/L than 3, 4 mg/L which is consistent with the present findings. Francis-Floyd (2020) highlighted that DO at 5 ppm help better growth and health of fishes which is almost same as the findings of the present experiment. The improved dissolved oxygen observed in T1 may be associated with enhanced microbial balance, efficient organic matter degradation and sustained photosynthetic activity of the mixed microalgae, thereby reducing oxygen depletion from decomposing organic wastes. Ivoke *et al*. (2008) found better growth performance of hybrid juvenile *Heterobranchus Bidorsalis* (B and) × *Clarias gariepinus* (@ and) at pH range of 7.0 to 7.5 which is within the range recorded in the present research that helped *L. thermalis* better growth performance in T1 (7.2±0.07) compared to other treatment groups. Ademola *et al*. (2025) found better growth result of African sharptooth catfish using fermented rice bran at pH 7.45±0.45 which was agreed with the findings of the present study. Lower ammonia (ppm), nitrite (ppm) and nitrate (ppm) was recorded in T1 (1:5) of 0.02±0.01, 0.03±0.01 and 0.11±0.02 (Table 3). Shin *et al*. (2016) reported that increase in exposure to ammonia affects the growth performance in rockfish *Sebastes schlegelii* which can be a possible reason for better growth of *L. thermalis* in T1 group. Research experiments by Martínez-Córdova *et al*. (2017); Abiri *et al*. (2022) and Ajamhasani *et al*. (2023) highlighted that rice brans in the culture system serves as a source of carbon that lowers the ammonia concentration, which supports the outcome displayed. Research by Zhang *et al*. (2023) expressed that nitrite at 0.1 ppm did not cause any negative effect on growth performance of *Ctenopharyngodon idella.* Nitrite level of all treatment was below 0.1 ppm among which T1 had lower nitrite level favoured enhanced growth responses of *L. thermalis*. Similar improvements in ammonia, nitrite and nitrate reduction were recorded in previous research where carbon-source-driven microbial communities significantly improved water quality and fish performance (Ezhilmathi *et al*., 2024). Davidson *et al*. (2014) reported that high nitrate level downregulates the growth and survival of *Oncorhynchus mykiss* which is consistent with the research on *L. thermalis*. Although these comparisons involve taxonomically different fish species, they provide useful reference ranges for interpreting general physiological responses to water quality parameters.

CONCLUSION

The study highlighted that supplementation of fermented rice bran-based copefloc biomass together with mixed microalgae positively influenced culture performance of juvenile Indian spiny loach. Among the tested treatments, the 1:5 fermented rice bran ratio produced the most favourable growth, digestive enzyme activity and water quality responses under indoor rearing conditions.

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Disclaimers

The authors of this article hold responsibility for the plenitude and precision of the information furnished in the manuscript, but are in no way answerable for any type of losses arising from the use of its content. The stances and the inferences expressed in the article are exclusively of the authors and there are no views of the associated institutions.

Informed consent

The guidelines and recommendations of the University of Animal Care Committee were followed properly while handling the experimental species altering to the approval of the committee of experimental animal care.

Conflict of interest

The authors hereby affirm that there has been no difference of opinion or conflicts of interest with regard to the publication of this article. We declare that the preparation of this manuscript was not influenced at any stage by any kind of sponsorship or funding.

REFERENCES

Abe, S.I., Kobayashi, S., Kitano, S. and Nagumo, T. (2009). Differential vulnerability of benthic algae to grazing by the loach, *Niwaella delicata* (Niwa). *Diatom.* **25:** 73-78.

Abiri, S.A., Chitsaz, H., Najdegerami, E.H., Akrami, R. and Jalali, A.S. (2022). Influence of wheat and rice bran fermentation on water quality, growth performance and health status of common carp (*Cyprinus carpio* L.) juveniles in a biofloc-based system. *Aquaculture*. **555(30):** 738168.

Ademola, B.T., Samaila, M.B., Badamasi, M., Lawal, U. and Usman, S.A. (2025). Evaluating fermented and hydrolyzed rice bran for improved biofloc performance in African catfish (*Clarias gariepinus*) rearing. *Asian Journal of Fisheries and Aquatic Research*. **27(3):** 42-49.

Ahmadi, A., Bagheri, D., Hoseinifar, S.H., Morshedi, V. and Paolucci, M. (2022). Beneficial role of polyphenols as feed additives on growth performances, immune response and antioxidant status of *Lates calcarifer* (Bloch, 1790) juveniles. *Aquaculture.* **552(15):** 737955.

Ajamhasani, E., Akrami, R., Najdegerami, E.H., Chitsaz, H. and Shamloofar, M. (2023). Different carbon sources and probiotics in biofloc-based common carp (*Cyprinus carpio*) culture: Effects on water quality, growth performance, fish welfare and liver histopathology. *Journal of the World Aquaculture Society.* **54(6):** 1546-1562.

Akbary, P. and Raeisi, E. (2020). Optimum dietary level of *Chlorella vulgaris* powder as a feed additive for some blood parameters and digestive enzymatic activities of grey mullet, *Mugil cephalus*. *Iranian Journal of Fisheries Sciences.* **19(3):** 1130-1139.

APHA. (2005). Standard Methods for the Examination of Water and Wastewater. 21st Edn., American Public Health Association/ American Water Works Association/Water Environment Federation, Washington, DC.

Areekijseree, M., Engkagul, A. and Kovitvadhi, U. (2004). Temperature and pH characteristics of amylase and proteinase of adult freshwater pearl mussel, *Hyriopsis* (*Hyriopsis*) *bialatus* Simpson 1900. *Aquaculture*. **234(3):** 575-587.

Davidson, J., Good, C., Welsh, C. and Summerfelt, S.T. (2014). Comparing the effects of high vs. low nitrate on the health, performance and welfare of juvenile rainbow trout (*Oncorhynchus mykiss*) within water recirculating aquaculture systems. *Aquacultural Engineering*. **59(11):** 30-40.

Ekaratne, S.U.K. (2000). A Review of the Status and Trends of Exported Ornamental Fish Resources and their Habitats in Sri Lanka. Bay of Bengal Programme, Chennai, India. BOBP/REP/88.

El-Dein, A.N., Ezzat, A., Aboseif, A.M. and Flefil, N.S. (2025). Enhancing micronutrients bioavailability for tilapia reared in green water system using rice bran fermented with newly isolated phytase-producer, *Pediococcus pentosacious*, maintained both economic and environmental sustainability. *Discover Food.* **5:** 155. https://doi.org/10.1007/s44187-025-00434-2.

Ezhilmathi, S., Felix, S., Antony, C., Gopalakannan, A., Ahilan, B. and Hemamalini, N. (2024). Effect of distillery spent wash at different C/N ratio on biofloc based nursery culture of *Penaeus vannamei*. *Indian Journal of Animal Research*. **58(12):** 2214-2218. doi: 10.18805/IJAR.B-4843.

Francis-Floyd, R. (2020). Dissolved Oxygen for Fish Production. FA27/FA002, Rev.5/1997.EDIS.2020(5).

Hassaan, M.S., Mohammady, E.Y., Soaudy, M.R., Sabae, S.A., Mahmoud, A.M. and El-Haroun, E.R. (2020). Comparative study on the effect of dietary β-carotene and phycocyanin extracted from *Spirulina platensis* on immune-oxidative stress biomarkers, genes expression and intestinal enzymes, serum biochemical in *Nile tilapia*, *Oreochromis niloticus*. *Fish and Shellfish Immunology*. **108:** 63-72.

Ibrahim, A., Umar, A.M. and Burak, L.N. (2023). Effects of temperature variation on behaviour and growth performance of African catfish (*Clarias gariepinus*). *Journal of Environmental Microbiology and Toxicology*. **11(1):** 14-17.

Ivoke, N., Mgbenka, B.O. and Okeke, O. (2008). Effect of pH on the growth performance of *Heterobranchus bidorsalis* (B and)♂ *Clarias gariepinus* (♀) hybrid juveniles. *Animal Research International*. **4(1).** doi: 10.4314/ari.v4 i1.40808.

Jackqulinwino, A., Ahilan, B., Antony, C., Chidambaram, P., Uma, A. and Ruby, P. (2024). Influence of *in situ* biofloc production on bio growth performance, physiological immune response, digestive enzyme activity, nutrient composition and disease resistance of *Etroplus suratensis*. *Indian Journal of Animal Research.* **58(10):** 1810-1816. doi: 10. 18805/IJAR.B-5266.

Jackqulinwino, A., Ahilan, B., Antony, C., Chidambaram, P., Uma, A. and Ruby, P. (2025). Efficacy of *in situ* and *ex situ* biofloc on growth, immune-physiological responses, nutrient composition and muscle growth-related gene expression of *Etroplus suratensis*. *Indian Journal of Animal Research.* **59(1):** 39-47. doi: 10.18805/IJAR.B-5306.

Jahazi, M.A., Hoseinifar, S.H., Jafari, V., Hajimoradloo, A., Van Doan, H. and Paolucci, M. (2019). Dietary supplementation of polyphenols positively affects the innate immune response, oxidative status and growth performance of common carp, *Cyprinus carpio* L. *Aquaculture*. **517(25):** 734709.

Jelshina, G.B., Chidambaram, P., Anand, C., Dinesh, R. and Anand, T. (2026). Effects of copefloc technology using fermented rice bran on growth, digestive enzyme activity and growth gene expression in *Penaeus vannamei*. *Thalassas: An International Journal of Marine Sciences*. **42(2):** doi: 10.1007/s41208-026-01108-1.

Keskar, A., Padhye, A. and Dahanukar, N. (2014). Fighting Against All Odds: The Struggle for Existence Among Hill Stream Loaches of Northern Western Ghats. Min Newsletter FFSG. **2:** 25-29.

Li, J., Huang, K., Huang, L., Hua, Y., Yu, K. and Liu, T. (2020). Effects of dissolved oxygen on the growth performance, haematological parameters, antioxidant responses and apoptosis of juvenile GIFT (*Oreochromis niloticus*). *Aquaculture Research.* **51(1):** 3079-3090.

Manoharan, S., Kuppu, R. and Uthandakalaipandian, R. (2019). Comparative assessment of nutritional composition in raw and cooked Indian freshwater fish *Lepidocephalus thermalis* (V.). *Proceedings of the Zoological Society*. **72(2):** 154-159.

Mansoori, A., Noveirian, H.A., Hoseinifar, S.H., Sajjadi, M., Ashouri, G., Imperatore, R. and Paolucci, M. (2024). Polyphenol-rich extracts enhance growth, immune function and antioxidant defense in juvenile rainbow trout (*Oncorhynchus mykiss*). *Frontiers in Nutrition.* **5(11):** 1487209.

Markweg-Hanke, M., Lang, S. and Wagner, F. (1995). Dodecanoic acid inhibition of a lipase from *Acinetobacter* sp. OPA 55. *Enzyme and Microbial Technology*. **17(6):** 512-516.

Martínez-Córdova, L.R., Martínez-Porchas, M., Emerenciano, M.G.C., Miranda-Baeza, A. and Gollas-Galván, T. (2017). From microbes to fish: The next revolution in food production. *Critical Reviews in Biotechnology.* **37(3):** 287-295.

Natuhara, Y. (2013). Ecosystem services by paddy fields as substitutes of natural wetlands in Japan. *Ecological Engineering.* **56:** 97-106.

Pethiyagoda, R. (1991). Freshwater Fishes of Sri Lanka. Wildlife Heritage Trust of Sri Lanka, Colombo, Sri Lanka. pp. 353.

Qin, C.G., Huang, K.X. and Xu, H.B. (2002). Protective effect of polysaccharides from the loach on the *in vitro* and *in vivo* peroxidative damage of hepatocyte*. The Journal of Nutritional Biochemistry.* **13(10):** 592-597.

Robotmam, P.W.J. (1977). Feeding habits and diet in two populations of spined loach, *Cobitis taenia* (L.). *Freshwater Biology*. **7(5):** 469-477.

Romano, N. (2017). Aquamimicry: A revolutionary concept for shrimp farming-responsible seafood advocate. *Global Seafood Alliance.* pp. 1-10.

Santhosh, B. and Singh, N.P. (2007). Guidelines for Water Quality Management for Fish Culture in Tripura. ICAR Research Complex for NEH Region, Tripura Centre, Publication. pp. 29.

Scoglio, G.D., Jackson, H.O. and Purton, S. (2024). The commercial potential of *Aphanizomenon flos-aquae*, a nitrogen-fixing edible cyanobacterium. *Journal of Applied Phycology.* **36(4):** 1-26.

Shin, K.W., Kim, S., Kim, J., Hwang, S.D. and Kang, J. (2016). Toxic effects of ammonia exposure on growth performance, hematological parameters and plasma components in rockfish, *Sebastes schlegelii*, during thermal stress. *Fisheries and Aquatic Sciences*. **19(1):** 44. https://doi.org/10.1186/s41240-016-0044-6.

Susilo, U. and Rachmawati, F.N. (2020). Protease, lipase and amylase activities in barred loach, *Nemacheilus fasciatus* CV. *Jurnal Biodjati*. **5(1):** 115-124.

Susilo, U., Rachmawati, F.N., Wibowo, E.S., Pradhyaningrum, R.R., Okthalina, K. and Mulyani, M.N.A. (2022). Digestive enzyme activities in barred loach (*Nemacheilus fasciatus*, Val., 1846): Effect of pH and temperature. *Molekul.* **17(2):** 219.

Tomassi, E., Arouna, N., Caruso, M.G., Girgenti, A., Picone, P., Nuzzo, D. and Pucci, L. (2024). Fermentation of *Chlorella vulgaris* and *Aphanizomenon flos-aquae* biomass improves the antioxidant profile. *LWT.* **215(7):** 117183.

Velmurugan, A., Chidambaram, P., Velmurugan, P., Gokul, S. and Lingam, R.S.S. (2025). Influence of water pH and temperature on breeding and embryo development of Indian spiny loach (*Lepidocephalichthys thermalis*). *Animal Reproduction Science.* pp. 107859.

Walter, H.E. (1984). Proteinases. In: Methods of Enzymatic Analysis [Bergmeyer, H.U. and Grassl, M. (eds)]. Verlag Chemie, Weinheim, Deerfield Beach, Florida. pp. 271-276.

Yuan, X., Wang, C., Song, C. and Shu, G. (2021). Effect of farming patterns on growth, antioxidant activity and nonspecific immunity of loach, *Paramisgurnus dabryanus*. *Aquaculture Research*. **53(4):** 161-167.

Zhang, J.M., Fu, B., Li, Y.C., Sun, J.H., Xie, J., Wang, G.J. and Yu, E.M. (2023). The effect of nitrite and nitrate treatment on growth performance, nutritional composition and flavor-associated metabolites of grass carp (*Ctenopharyngodon idella*). *Aquaculture*. **562(15):** 738784.