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To Assess the Growth Performance of *Heteropneustes fossilis* by the Help of Plant Protein *Moringa oleifera* as a Partial Replacement

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ABSTRACT: This study assesses the growth performance of *Heteropneustes fossilis* using *Moringa oleifera* leaves as a partial replacement for fish meal in the diet. Conducted over 90 days, fingerlings were fed isonitrogenous diets with varying inclusion levels of moringa leaves. Proximate analysis revealed that increasing moringa inclusion decreased crude protein but increased fiber and carbohydrate content. Growth parameters, including weight gain, feed conversion ratio (FCR), specific growth rate (SGR), and protein efficiency ratio (PER), were monitored. The control diet showed superior growth performance and feed efficiency compared to moringa-based diets. Despite reduced growth, high survival rates indicated potential health benefits from moringa inclusion. Further research is needed to optimize moringa inclusion levels and enhance its nutrient profile for better growth outcomes in *Heteropneustes fossilis*.

Keywords: Sustainable aquaculture, *Moringa oleifera*, Fish diets, Protein efficiency, Environmental impact, Economic feasibility.

INTRODUCTION

India's fishery industry supports millions of livelihoods, food security, and nutrition, making it essential to the economy. As a significant protein source, fish helps reduce hunger and malnutrition. India ranks third globally in fish and aquaculture production, contributing 16% of the world's inland fish output and 5% of marine fish output. In 2021-2022, India produced 162.48 lakh metric tons of fish. The top five fish-producing states were Andhra Pradesh, West Bengal, Karnataka, Odisha, and Gujarat. Fish catch disposal methods include fresh, frozen, curing, canning, and more, with fresh fish marketing being the most common. From 2013 to 2021, India's fish catches steadily increased. In 2020-2021, Andhra Pradesh, West Bengal, and Odisha had the largest fish harvests. Fish, a key protein source, is cooked in various ways and preserved for flavor and texture. States with high per capita fish consumption include Uttar Pradesh, Odisha, Bihar, Kerala, and Tamil Nadu, with Kerala and Lakshadweep leading in prawn consumption. Tripura has the highest proportion of fish-consuming households. In 2022, India exported 1.36 million metric tons of seafood worth $7.76 billion, mainly to the US and China, with frozen shrimp being the top export. The fishing industry contributes about 1.1% to the Indian economy. The Fisheries and Aquaculture Infrastructure Development Fund (FIDF) has approved 105 proposals worth Rs. 4923.94 crore. Artificial feed is crucial for the aquaculture industry as it provides essential nutrients for aquatic organisms growth and development. It comes in various forms to meet different species dietary needs, including energy sources, proteins, fats, carbs, vitamins, minerals, and other nutrients. Formulated or manufactured feed is crucial for the aquaculture industry's growth due to its affordability and efficiency (Sikotariya and Yusufzai 2019). While animal protein, known for high digestibility and nutrient content, has been traditionally used (Olsen and Hasan 2012), it is expensive. Plant-based proteins can replace fish meal in freshwater fish diets (Wang *et al*., 2016). Aquaculture feed energy includes proteins, lipids, and carbohydrates (Xie *et al*., 2017). Fish require higher protein levels than terrestrial animals for growth, necessitating efficient protein utilization to reduce feed costs (NRC, 2011).Lipids in fish feed provide energy and aid in the absorption of essential nutrients. Optimal dietary lipid content is 7-9% for carp and prawn, and 4% for IMCs. Carbohydrates enhance extruded feed quality and spare protein for energy (Hemer *et al*., 2002). Fish carbohydrate utilization varies by species (Kamalam *et al*., 2017). Digestibility for carp is 55-60%, with different optimal levels for various fish. Plant-based and animal protein sources have replaced fishmeal due to their nutrient content and palatability. The global market for animal-derived protein is expected to double by 2050.Potential animal protein sources for fish production include insects, land by-products, and fisheries by-products, but no studies compare these with control diets (Raswiswi *et al*., 2021). *Heteropneustes fossilis*, known as Singhi or stinging catfish, is a highly valued freshwater air-breathing fish in the Indian subcontinent and Southeast Asia (Shukla *et al*., 2018). It adapts to fresh and brackish water with varying oxygen levels (Pillay, 2001) and is noted for its resilience (Dehadrai, 1978). Singhi commands a premium price due to its nutritional and medicinal value, high digestibility, and palatability. It can be cultivated in monoculture and polyculture systems (Haniffa, 2009). Singhi fish are omnivores with a varied diet and use aerial respiration, thriving in low-oxygen environments (Nandi *et al*., 2023). The moringa tree, indigenous to northeastern India, is cultivated throughout the tropics and subtropics (Jahn, 1986). Moringa leaves, native to Southeast Asia, have significant commercial, industrial, and medicinal applications. They are needed in aquaculture due to their affordability, despite some anti-nutritional factors (Sherif *et al*., 2014). Moringa leaves show antioxidant and hypercholesterolemic properties (Chumark *et al*., 2008), and serve as a promising protein source in fish diets (Chiseva, 2006). They have bactericidal properties (Caceres *et al*., 1991) and bioactive substances like flavonoids and polyphenols that enhance fish immune systems. Moringa leaves contain 25-32% crude protein (Makkar & Becker 1996; Soliva *et al*., 2005), making them a high-quality fish meal replacement (Khetran *et al*., 2018). They have multiple uses in agriculture, pharmaceutics, human health, and livestock (Falowo *et al*., 2018). The Pearson square method is a simple technique for balancing nutrient ratios in feed mixtures, particularly useful for crude protein (Wagner and Stanton, 2012). In Indian aquaculture, common ingredients like groundnut oil cake and rice bran are used in a 1:1 ratio, though mustard oil cake is sometimes substituted. Semi-intensive carp farming often relies on farm-made feeds due to the lack of commercial options, with many farmers enhancing traditional mixtures with locally available ingredients. Pellet-form complete diets are becoming more popular, and unconventional feed ingredients are also used (Singh *et al*., 2018).

MATERIALS AND METHODS

Experimental site. The research was completed for ninety days in 2023–2024 at the Sarma fish breeding farm, located at Gandatwisa, Dhalai Tripura, Tripura, India (Lat 23.587943°, Long 91.814617°).

Experimental sites description. The Sarma Fish Breeding Farm, established in 1980 and managed by TTAADC in Khumulung, West Tripura, is located in Gandatwisa, Dhalai, about 115 kilometers from Agartala. Under the supervision of the Superintendent of Fisheries, the farm encompasses four hatcheries for Eco-carp, Magur and Singhi, Pangasius, and Tengara species, along with a gene bank for Tengara. It maintains seventy-five ponds for various aquaculture activities including culture, rearing, nursing, and broodstock. The farm boasts advanced facilities for water quality testing, a training hall, and a hostel that collectively educate over 2000 farmers annually, significantly enhancing aquaculture practices in the region. Its breeding efforts focus on a diverse range of species, including exotic carps like Cyprinus carpio, Ctenopharyngodonidella, and Hypophthalmichthys molitrix, Indian major carps, and other species such as Anabas testudines, various Channa species, Oreochromis niloticus, Puntius sarana, Labeobata, Labeogonius, and catfish like Heteropneustes fossilis, Clariasbatrachus, Pangasius pangasius, and Mystus species. Situated close to the Saima River, which flows into Dumbur Lake and forms the Gomati River, the farm plays a pivotal role in fisheries and aquaculture research. Oversight extends to managing operations in Dumbur Lake, monitoring pond water quality, and providing comprehensive training to farmers under the auspices of the Superintendent of Fisheries' office.

Collection of ingredients. A leaf of *Moringa oleifera* was gathered from a locality area nearby and properly sun-dried. In order to prevent early spoilage, samples of feed ingredients were also gathered from the market. Fish meal, rice bran, wheat bran, wheat flour, mustard oil cakes, vitamin and mineral capsules, and soy oil were among the samples. The samples were then properly sun-dried and sealed in polyethylene bags.

Experimental design. There were nine one-meter-diameter cement tanks that could hold 200 liters of water each. Two treatments and one control unit with three replications each were used in the current investigation. Potassium permanganate (KMnO4) was used to disinfect each tank before adding singhi fish. Tap water was then added to each tank. From a private hatchery, the *H. fossilis* was obtained. Singhi weighed an average of about 1.18 g. With appropriate aeration, 15 H. fossilis were added to each tank.

Feed preparation. Using readily available fresh fish feed ingredients, three experimental diets were created using Pearson's square technique (Wagner & Stanton 2012). Two diets (T1 with T2) were created by partially substituting moringa leaf for fish meal, which was the basis of diet C1, the control diet. The necessary ingredients were ground and thoroughly combined with water to prepare the diet. Using a pellet machine, all diets were turned into pellets that were stored in polythene bags for later use after being sun-dried for a few days.

Table 1: Formulation of the control & experimental diets.

Ingredients (g/100g diet)	Diets		
	C1 (0%)	**T1 (25%)**	**T2 (50%)**
Fish meal	44.37g	33.27g	22.18g
M. oleifera	0g	11.09g	22.18g
M.O.C	44.37g	44.37g	44.37g
Wheat flour	5.62g	5.62g	5.62g
Rice bran	5.62g	5.62g	5.62g
Vitamins & minerals capsules	0.5g	0.5g	0.5g
Soyabean oil	1ml	1ml	1ml

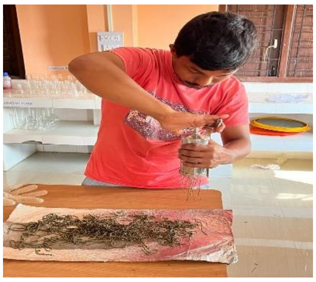

Fig. 1. Making of feed using kitchen press.

Feeding strategy. For the first month, feeding was done twice a day at 9:00 a.m. and 5:00 p.m. at a rate of 3% of body weight, and for the next few months, at 5% of body weight. Every day, uneaten food was siphoned out. Exchange of water took place once every seven days.

Proximate analysis. A series of laboratory techniques known as "proximate analysis" are used to identify the main ingredients in food items, animal feed, and other organic materials. It offers a numerical evaluation of the feed's composition and reveals information about its nutritional worth. When analyzing proximally, moisture, crude protein, crude fat (lipids), ash, after that crude fiber are the main elements examined.

Water quality parameters. For both natural and artificial water bodies to remain healthy and productive, water quality is a crucial component of aquatic ecosystems. Water quality has a direct impact on aquatic organisms' ability to grow, survive, and reproduce in aquaculture. Thus, maintaining and adjusting water quality parameters is essential for aquaculture operations to be successful. API freshwater kit has been used to check the parameters throughout the period.

Growth analysis of fish. For the growth performance, the weight of Singhi fishes were considered at regular intervals of 15 days. The final sampling was already determined by following parameters given below:

Weight gain (g) = Mean final weight − Mean initial weight

Specific growth rate (SGR) (% / Day) = 100 × (ln Wf - ln Wi) /Δt

Feed conversion ratio (FCR) = Feed fed (dry weight)/ Live weight gain (g)

Protein Efficiency Ratio (PER) = Net weight gain (g)/Protein applied in the feed (g)

Survival rate (%) = (Total amount of fish that were harvested / Total number of fish stocked) × 100

Statistical analysis. Mean value & standard deviation were calculated from the result using MS-Excel sheet. The data of growth performance of control diet & experimental diets were analyzed using MS-Excel one-way ANOVA. This included significant results, ($p<0.05$) were taken as rejection of the null hypothesis significant differences between the treatments.

RESULT AND DISCUSSION

The fingerlings of *Heteroneustes fossilis*, weighing an average of 15.86 ± 0.94 g, 18.09 ± 0.55 g, and 19.52 ± 0.86 g, were acquired from a nearby farm and placed in the rearing cemented tank. This was done for each of the three treatments (C1, T1, and T2). Fish were sampled and the growth parameters were tracked every 15 days during the course of the 90-day study period. An isonitrogenous feed containing 40% CP was fed to the animals at a bodyweight percentage of 3% during the first month, rising to 5% during the second and third. Using the methods or techniques, throughout the cultured periods, the water quality in the experimental rearing tanks was maintained and observed every fifteen days. Every weekend, a quarter or so of the waste material, including the excrement, was siphoned off and exchanged for water.

Table 2: Proximate analysis of formulated feed.

Treatments	Crude protein (%)	Crude Lipid (%)	Crude Fibre (%)	Ash (%)	Moisture (%)	NFE (%)
C1	40.8	8.9	8.2	10.7	8.9	22.5
T1	36.7	8.5	9.9	9.7	8.5	26.7
T2	33	9.1	10.7	8.2	9.4	29.6

The control diet (C1) had the highest crude protein content at 40.8%. Substitution with *Moringa oleifera* in T1 and T2 led to a decrease in crude protein levels, indicating that fish meal provided a higher protein concentration compared to moringa leaves. This aligns with findings by Makkar and Becker (1996) that *Moringa oleifera* leaves contain around 25-32% crude protein, which is lower than traditional fish meals. Lipid content slightly decreased with 25% Moringa inclusion (T1) but increased again with 50% inclusion (T2), showing that *Moringa oleifera* leaves have comparable lipid content to fish meal. This is consistent with reports by Grubben and Denton (2004) that Moringa leaves have substantial crude fat content.

Crude fibre content increased with higher inclusion levels of *Moringa oleifera*. Higher fiber in T1 and T2 could affect digestibility and nutrient absorption, as noted by Chiseva (2006). The ash content decreased with higher levels of Moringa inclusion, suggesting a lower mineral content in moringa leaves compared to fish meal. Ferreira *et al*., (2008) highlighted that although *Moringa oleifera* has beneficial minerals, it might not match the mineral richness of fish meal. Moisture content showed slight variations, with T2 having the highest. This indicates that Moringa inclusion does not significantly alter the moisture content of the diets, supporting the findings of Soliva *et al*. (2005).

NFE, representing carbohydrates, increased with higher levels of *Moringa oleifera*, suggesting that moringa leaves are higher in carbohydrates compared to fish meal. This is consistent with Kamalam *et al.* (2017), who stated that Moringa leaves can provide significant carbohydrate content.

Table 3: Growth parameters of different treatments (mean ± standard deviation).

Growth Parameters	Treatments		
	Control diets	**Treatment-1**	**Treatment-2**
Weight gain (g)	49.83 ± 1.62	41.32 ± 0.60	39.36 ± 1.21
Feed Conversion Ratio (FCR)	2.33 ± 0.08	2.59 ± 0.08	2.74 ± 0.17
Specific Growth Rate (SGR) (%)	1.58 ± 0.06	1.32 ± 0.03	1.22 ± 0.05
Protein Efficient Ratio (PER)	1.24 ± 0.04	1.03 ± 0.01	0.98 ± 0.03
Survival (%)	73.33 ± 6.67	71.10 ± 13.87	64.44 ± 10.18

The control group (C1) exhibited the highest weight gain (50.76 ± 2.3 g), significantly higher than T1 (41.94 ± 0.53 g) and T2 (40.12 ± 1.26 g). This indicates that traditional fish meal diets promote better growth compared to diets with moringa leaf inclusion. Similar results were reported by Haniffa (2009), highlighting the effectiveness of fish meal in promoting growth. The FCR was lowest in the control group (2.33 ± 0.08), indicating better feed efficiency compared to T1 (2.59 ± 0.08) and T2 (2.74 ± 0.17). Higher FCR values in treatments with moringa leaf inclusion suggest reduced feed efficiency. This aligns with findings by Shukla *et al*. (2018), where alternative plant-based proteins showed lower efficiency than fish meal. SGR was highest in the control group (1.58 ± 0.06%), followed by T1 (1.32 ± 0.03%) and T2 (1.22 ± 0.05%). The decreasing SGR with increased moringa inclusion supports the findings of Wang *et al.* (2016), who noted that plant proteins often result in lower growth rates due to differences in digestibility and nutrient composition. PER was highest in the control group (1.24 ± 0.04), followed by T1 (1.03 ± 0.01) and T2 (0.98 ± 0.03). The lower PER in moringa-based diets indicates less efficient protein utilization, corroborating studies by Olsen and Hasan (2012), where plant proteins were found to be less efficient compared to animal proteins. Survival rates were relatively high across all treatments, with C1 at 73.33 ± 6.67%, T1 at 71.10 ± 13.87%, and T2 at 64.44 ± 10.18%. Despite lower growth performance, the high survival rates in moringa treatments indicate the potential for moringa to support fish health, aligning with findings by Nandi *et al.* (2023) on the resilience of *Heteropneustes fossilis* in varied dietary conditions. While *Moringa oleifera* shows potential as a partial replacement for fish meal, its inclusion at higher levels (T1 and T2) results in reduced growth performance and feed efficiency. These findings are consistent with previous studies on alternative protein sources (Sherif *et al*., 2014; Chiseva, 2006). Further research is needed to optimize the inclusion levels of moringa leaf and enhance its nutrient profile for better growth outcomes in *Heteropneustes fossilis*.

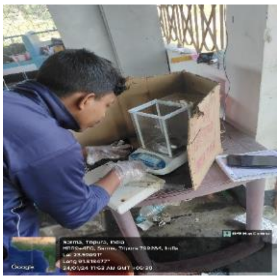

Fig. 2. Weighing of fish.

Decrease in weight gain in T1 & T2 probably due to the presence of some antinutrients in the moringa leaf such as phenol, tannin & saponin (Richter *et al.*, 2003).

All the growth parameters, including WG, FCR, SGR, and PER, have significant differences ($p<0.05$), but survival rate does not have significant differences ($p>0.05$).

CONCLUSIONS

Using *Moringa oleifera* leaf in fish diets shows promise for sustainable aquaculture by reducing reliance on expensive and unsustainable fish meal. It supports initiatives for environmentally friendly feed options and enhances profitability and productivity for fish farmers. The study's limitations include a small sample size and controlled environment, limiting broader applicability. Future research should address long-term effects, explore interactions with other nutrients, and evaluate ecological impacts and economic feasibility. Further studies should focus on broader species applicability, bioavailability of nutrients, and ecological implications of using *Moringa oleifera* leaf in aquaculture. Economic analyses comparing costs and benefits will aid in practical implementation and sustainability of these practices.

FUTURE SCOPE

Investigate *Moringa oleifera*'s effects on the entire lifecycle of *Heteropneustes fossilis*. Analyze bioactive compounds in *Moringa oleifera* to enhance fish growth and optimize feed formulations. Compare *Moringa oleifera* with other plant-based protein sources for sustainable aquaculture. Study environmental impacts of *Moringa oleifera* in fish diets, including water quality and nutrient cycling. Assess cost-effectiveness and profitability of *Moringa oleifera* in commercial fish feeds. Investigate *Moringa oleifera*'s effects on fish health, immunity, pathogen resistance, and stress tolerance. Explore *Moringa oleifera*'s influence on gene expression related to growth and metabolism for breeding. Research integration of *Moringa oleifera* with sustainable practices like polyculture and IMTA.

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Conflict of Interest. None.

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