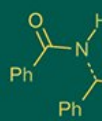


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Effect of mulberry (*Morus alba*) leaf extract on growth and survival of *Labeo catla*

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Abstract

The present study was conducted to evaluate the effect of dietary supplementation of mulberry (*Morus alba*) leaf extract on the growth performance and survival of *Labeo catla*. A 60-day feeding trial was carried out at the Hands-on Training Centre, Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval. Five experimental diets containing different levels of mulberry leaf extract 0% (control), 0.25%, 0.5%, 0.75% and 1% were formulated with a uniform crude protein level of 30%. A total of 200 *L. catla* were randomly distributed into 20 experimental tanks following a Completely Randomized Design (CRD) with five treatments and four replications. The results revealed that dietary supplementation of mulberry leaf extract significantly improved the growth performance and survival of *L. catla*. Among all the treatments, fish fed with 0.75% mulberry leaf extract (T3) exhibited the highest final body weight (2.23 ± 0.021 g), mean weight gain (1.73 ± 0.021 g) and specific growth rate (2.492 ± 0.009), along with the lowest feed conversion ratio (1.968 ± 0.033) and highest survival rate (90%). The control group showed the poorest performance for all growth and survival parameters. The improved performance in T3 may be attributed to the presence of bioactive compounds in mulberry leaves, which enhance nutrient utilization and support better physiological condition of the fish. The findings of the present study suggest that dietary incorporation of 0.75% *Morus alba* leaf extract can effectively enhance growth performance, feed utilization efficiency and survival of *Labeo catla*. Therefore, mulberry leaf extract can be considered a promising natural feed additive and a sustainable alternative to synthetic growth promoters in freshwater aquaculture.

Keywords: Mulberry (*Morus alba*) leaf extract, *Labeo catla*, growth performance

Introduction

Aquaculture has become one of the fastest-growing food-producing sectors and plays a significant role in ensuring global food security, improving human nutrition and supporting economic development (Li *et al.*, 2019; Perschbacher, 2015)^[16]. It represents a major sector of the global food production industry, with an average annual growth rate of 2.3% and a total fish production of approximately 96.4 million tonnes recorded in 2020 (FAO, 2020). In recent decades, aquaculture has contributed more than half of the total fish consumed worldwide (Naderi *et al.*, 2017)^[14]. India is the second-largest producer of fish and aquaculture products globally, with inland fisheries contributing a major proportion of total fish production.

Fish provide an excellent source of high-quality protein, essential fatty acids, vitamins and minerals that are important for human health and development. Among freshwater fish species, *Labeo catla* (Catla), belonging to the family Cyprinidae, is one of the most commercially important Indian major carps due to its rapid growth, high consumer preference and significant market value. It is commonly cultured in polyculture systems along with *Labeo rohita* and *Cirrhinus mrigala*, where it contributes substantially to increased fish production and farm profitability. Its high market demand and consumer acceptance make it an important species in freshwater aquaculture in India (Mohanty *et al.*, 2013)^[13].

In aquaculture, disease outbreaks and environmental stress remain major constraints affecting fish health, growth performance, survival and overall aquaculture productivity. Antibiotics and synthetic chemicals have been widely applied in aquaculture for the prevention and control of bacterial infections. The excessive use of antibiotics and synthetic chemicals for health management has raised concerns regarding antimicrobial resistance,

chemical residues, environmental pollution and potential risks to human health. Therefore, there is an increasing need to develop safe, sustainable and eco-friendly alternatives that can improve fish health and performance.

In recent years, the use of medicinal plants and their bioactive compounds has gained considerable attention as natural feed additives in aquaculture. Herbal supplements have been reported to enhance growth performance, survival and promote the overall health of cultured fish. Among these medicinal plants, *Morus alba* (white mulberry) has received significant attention because of its rich nutritional composition and diverse biological activities. The leaves of *Morus alba*, a plant native to Asian regions including India, China and Japan, have been extensively employed in traditional medicine, animal nutrition and sericultural practices. Mulberry is widely cultivated in tropical and subtropical areas due to its high nutritional value (Al-Kirshi *et al.*, 2013) [1].

The leaves of *M. alba* are rich in crude protein, essential fatty acids, vitamins, minerals and various bioactive compounds such as flavonoids, alkaloids, polyphenols, polysaccharides, saponins, tannins, phytosterols and triterpenes (Nomura *et al.*, 1983; Kusano *et al.*, 2002; Chen *et al.*, 2005; Chan *et al.*, 2016; Chen *et al.*, 2021) [2, 3, 5, 11, 15]. These compounds exhibit antioxidant, antibacterial, anti-inflammatory, immunomodulatory, hypolipidemic and antidiabetic activities, which may positively influence growth performance, digestive enzyme activity, antioxidant status and gut microbial balance in fish (Feng *et al.*, 2021; El-Sayyad, 2015; Hao *et al.*, 2018; He *et al.*, 2018; Wang *et al.*, 2012) [6, 8, 9, 17]. However, the direct application of mulberry leaves may be limited due to the presence of certain anti-nutritional factors. Therefore, extraction methods such as ethanol extraction are employed to reduce undesirable compounds while preserving and concentrating valuable bioactive constituents. Considering the nutritional and pharmacological potential of *M. alba*, its application as a natural dietary supplement may provide an effective strategy to enhance growth performance and survival of cultured fish while reducing dependence on synthetic additives.

Therefore, the present study was designed to evaluate the effects of mulberry leaf extract supplementation on the growth performance and survival of *Labeo catla*. This approach may contribute to the development of sustainable and environmentally friendly practices in freshwater aquaculture.

2. Materials and Methods

2.1 Location of experiment

The experiment was conducted at the Hands-on Training Centre, Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval, for a period of 60 days from October to December 2025. A total of 20 plastic aquarium tanks (50 L capacity; 2 × 1 × 1 ft) were arranged in a Completely Randomized Design (CRD) consisting of five treatments with four replicates each. The tanks were cleaned and disinfected with potassium permanganate (4 mg L⁻¹) before filling with clean water.

Labeo catla were procured from a local hatchery, transported in oxygen-filled polythene bags and acclimatized for seven days in a 500 L FRP tank under proper aeration and feeding. After acclimatization, 200 fish were randomly stocked in the experimental tanks at a

density of 10 fish per tank, and adequate aeration was maintained throughout the experimental period.

2.2 Collection of feed ingredients to prepare diet

The experimental diets were formulated using various feed ingredients, including fish meal, groundnut oil cake, wheat flour, vegetable oil, fish oil, tapioca flour and vitamin-mineral premix. All feed ingredients were procured from local markets, while fresh mulberry leaves were collected from a nearby area. The collected leaves were sun-dried for 3-4 days to reduce moisture content before processing. Mulberry leaf extract was prepared using ethanol as a solvent through a conventional solvent extraction technique. The proximate composition of all dietary ingredients was determined according to established standard analytical methods. All ingredients were finely ground using a grinder and sieved to obtain a uniform particle size. The processed powdered ingredients were then packed in high-density polyethylene (HDPE) bags and stored at room temperature until further use in diet preparation.

2.2.1 Preparation of mulberry leaf extract

Fresh leaves of *Morus alba* were collected and air-dried under natural conditions. The dried leaves were subsequently pulverized into a fine powder using an electric blender. Approximately 180 g of the powdered material was immersed in 70% ethanol in a conical flask and maintained at room temperature for seven days with intermittent shaking to facilitate efficient extraction. Following the extraction period, the supernatant was separated carefully, and the ethanol was removed using a rotary evaporator. The obtained crude extract was then freeze-dried, transferred into sterile containers and preserved at -20°C until further experimental use (De Oliveira *et al.*, 2015) [5].

2.3 Experimental diet preparation

Five experimental diets were formulated with a uniform crude protein level of 30%. The control diet (T0) was prepared without *Morus alba* leaf extract, while T1, T2, T3, and T4 diets were supplemented with 0.25%, 0.5%, 0.75% and 1% mulberry leaf extract, respectively. The feed ingredients were weighed according to the formulation, finely ground, sieved and thoroughly mixed. Water was added to obtain a homogeneous dough, which was steam-cooked by autoclaving at 121°C and 15 lbs pressure for 15 min. After cooling, the required amounts of vitamin-mineral premix and mulberry leaf extract were incorporated and the mixture was pelletized using a mechanical pelletizer. The prepared pellets were sun-dried until the moisture content was reduced below 10% and stored in labelled airtight plastic containers until further use.

Table 1: Composition of experimental diets

Ingredients (%)	Diet (30% protein)				
	T0	T1	T2	T3	T4
Mulberry leaf extract	0	0.25	0.5	0.75	1
Fish meal	35	35	35	35	35
GNOC	30	30	30	30	30
Wheat flour	17	17	17	17	17
Tapioca flour	12	11.75	11.50	11.25	11
Plant oil	2	2	2	2	2
Fish oil	2	2	2	2	2
Vitamin & mineral	2	2	2	2	2
Total	100	100	100	100	100

2.4 Measurement of growth and survival

At the completion of the 60-day experiment, the final body weight and survival rate of fish were recorded. Growth performance parameters including mean weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) were calculated to evaluate the effect of the experimental diets.

- 1) Body weight gain (g) = (Final body weight - Initial body weight)
- 2) FCR (Feed conversion ratio) = Amount of feed given/Body Weight gain
- 3) SGR (Specific growth rate) = $\ln[(\text{Final body weight}) - \ln(\text{Initial body weight})] \times 100 / \text{days of the experiment}$
- 4) Survival rate% = (No. of fish survived / No. of fish stocked) $\times 100$

2.5 Analysis of water quality parameters

During the experiment period, water quality parameters such as temperature, pH, dissolved oxygen (DO) and total hardness were checked every week. The water temperature was recorded with a thermometer and pH was measured using a digital pH meter and pH indicator solution. The dissolved oxygen and hardness in the water was determined by titration method.

2.6 Statistical analysis

Statistical analysis was performed using one-way Analysis of Variance (ANOVA) in SPSS software. The obtained results were presented as mean $\pm$ standard error (SE) and significant differences among treatment means were determined using Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$.

3. Results and Discussion

3.1 Effects of mulberry leaf extract (MLE) on the growth and survival of *Labeo catla*

The initial mean weights of catla in the five experimental treatments were 0.48 ± 0.010 g (T0), 0.47 ± 0.008 g (T1), 0.47 ± 0.017 g (T2), 0.50 ± 0.007 g (T3) and 0.48 ± 0.013 g (T4). After 60 days of the experimental period, the final mean weights increased to 1.53 ± 0.024 g in T0, 1.66 ± 0.015 g in T1, 1.85 ± 0.024 g in T2, 2.23 ± 0.021 g in T3 and 2.09 ± 0.012 g in T4, with the highest final weight observed in T3 and the lowest in T0.

The mean weight gain of *Labeo catla* was evaluated for all five treatments using the mean weight data. At the end of the 60-day experimental period, the highest mean weight gain was recorded in treatment T3 (1.73 ± 0.021 g), followed by T4 (1.59 ± 0.012 g), T2 (1.35 ± 0.024 g), T1 (1.16 ± 0.015 g) and the lowest value was observed in the control treatment T0 (1.03 ± 0.024 g).

Statistical analysis indicated a significant difference ($p < 0.05$) in mean weight gain among all the experimental treatments. The maximum mean weight gain was observed in treatment T3 (0.75% mulberry leaf extract), which was significantly higher than the other treatments, suggesting its better performance in promoting the growth of *L. catla*. Similar findings were reported by Ma *et al.* (2024) in *Siniperca chuatsi* (mandarin fish), where supplementation of 0.75% mulberry leaf extract in the diet resulted in the highest mean weight gain, specific growth rate and feed efficiency among the experimental groups. Similarly, Shekarabi *et al.* (2020) observed that the inclusion of 0.75% black mulberry juice powder in the diet significantly

($P < 0.05$) enhanced weight gain, specific growth rate and survival rate, indicating the growth-enhancing effects of black mulberry juice powder in aquafeeds.

Table 1: Effect of different treatments on growth performance, feed conversion ratio, specific growth rate, and survival rate

Treatment	Mean weight (g)	Mean weight gain (g)	Feed conversion ratio (FCR)	Specific growth rate (SGR)	Survival Rate (%)
T0	1.53 ± 0.02 4 ^e	1.03 ± 0.02 4 ^e	2.533 ± 0.04 5 ^e	1.935 ± 0.04 6 ^d	77.50 ± 2.50 0 ^b
T1	1.66 ± 0.01 5 ^d	1.16 ± 0.01 5 ^d	2.380 ± 0.01 8 ^d	2.094 ± 0.04 3 ^c	82.50 ± 2.50 0 ^{ab}
T2	1.85 ± 0.02 4 ^c	1.35 ± 0.02 4 ^c	2.203 ± 0.03 6 ^c	2.286 ± 0.04 7 ^b	85.00 ± 2.88 6 ^{ab}
T3	2.23 ± 0.02 1 ^a	1.73 ± 0.02 1 ^a	1.968 ± 0.03 3 ^a	2.492 ± 0.00 9 ^a	90.00 ± 0.00 0 ^a
T4	2.09 ± 0.01 2 ^b	1.59 ± 0.01 2 ^b	2.065 ± 0.01 5 ^b	2.442 ± 0.05 5 ^a	85.00 ± 2.88 6 ^{ab}

After 60 days of the experimental period, the feed conversion ratio (FCR) values recorded for treatments T0, T1, T2, T3 and T4 were 2.533 ± 0.045 , 2.380 ± 0.018 , 2.203 ± 0.036 , 1.968 ± 0.033 and 2.065 ± 0.015 , respectively. The lowest FCR was observed in treatment T3 (1.968 ± 0.033), followed by T4 (2.065 ± 0.015), T2 (2.203 ± 0.036) and T1 (2.380 ± 0.018), while the highest FCR was recorded in the control treatment T0 (2.533 ± 0.045). Statistical analysis revealed that the FCR values differed significantly ($p < 0.05$) among all the experimental treatment groups. Similar findings were reported by Ma *et al.* (2024) in mandarin fish, where dietary supplementation with 0.75% mulberry leaf extract significantly reduced the FCR compared to other treatment groups. Likewise, Shekarabi *et al.* (2020) observed that rainbow trout (*Oncorhynchus mykiss*) fed a diet containing 0.75% black mulberry juice powder exhibited the lowest and most efficient FCR, demonstrating superior feed utilization compared to the other dietary treatments.

The specific growth rate (SGR) values varied among the different treatment groups after the 60-day experimental period. The highest SGR was recorded in treatment T3 (2.492 ± 0.009), followed by T4 (2.442 ± 0.055), T2 (2.286 ± 0.047), T1 (2.094 ± 0.043) and the lowest value was observed in T0 (1.935 ± 0.046). Statistical analysis indicated that treatment T3, containing 0.75% MLE, showed a significantly higher SGR compared to the other treatment groups. Although T3 and T4 exhibited the highest SGR values and did not differ significantly from each other, both treatments were significantly superior to T0, T1, and T2. The T2 treatment showed a moderate improvement in SGR compared to T0 and T1. Shekarabi *et al.* (2020) investigated the impact of black mulberry juice powder supplementation on the growth performance of rainbow trout (*Oncorhynchus mykiss*). The study revealed that the diet containing 7.5 g/kg black mulberry juice powder significantly enhanced growth parameters, including weight gain, survival rate, feed conversion efficiency and specific growth rate (SGR) compared to other dietary treatments. Similarly, Zhou *et al.* (2023) reported that supplementation of mulberry leaf extract in the diet improved the specific growth rate (SGR) of spotted sea bass (*Lateolabrax maculatus*).

The survival rate of *Labeo catla* varied among different treatments. The highest survival rate (90%) was recorded in

T3, while the lowest survival rate (77.50%) was observed in T0. The survival rates in treatments T3, T4, T2, T1 and T0 were 90%, 85%, 85%, 82.50% and 77.50%, respectively. The results indicated that T3 showed the best survival performance among all treatments, whereas T0 showed the lowest survival. Among all the treatments, T3 containing 0.75% mulberry leaf extract exhibited the highest survival rate. The survival rates in T2 and T4 were statistically similar, while T1 showed a moderate survival rate, which was lower than T2 and T4 but higher than the control treatment. Therefore, T3 was found to be the most effective treatment for improving survival. Similar results were reported by Shekarabi *et al.* (2020), who observed that dietary supplementation of 0.75% black mulberry powder significantly ($P<0.05$) enhanced survival, feed efficiency, weight gain, and specific growth rate in Rainbow trout (*Oncorhynchus mykiss*), with the 0.75% supplementation level showing the best overall performance. Likewise, Zhou *et al.* (2023) reported that the inclusion of mulberry in the diet significantly improved the survival rate of Spotted seabass.

Conclusion

A 60-day feeding experiment was carried out to assess the influence of dietary supplementation of Mulberry (*Morus alba*) leaf extract on the growth performance and survival of *Labeo catla*. Five experimental diets containing different levels of mulberry leaf extract (0-1%) were prepared with a uniform crude protein content of 30%. Among the tested diets, the treatment supplemented with 0.75% mulberry leaf extract exhibited the best growth performance, showing maximum mean weight gain and specific growth rate (SGR), along with the lowest feed conversion ratio (FCR) and highest survival rate. The findings suggest that the inclusion of 0.75% mulberry leaf extract in the diet can effectively improve the growth and survival of *L. catla*, indicating its potential as a natural feed additive for promoting sustainable aquaculture.

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