



## EFFECTS OF GARLIC (*ALLIUM SATIVUM*) POWDER ON GROWTH PERFORMANCE, FEED UTILIZATION AND DISEASE RESISTANCE AGAINST *AEROMONAS HYDROPHILA* IN *LABEO CATLA*

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The present study investigated the effects of garlic (*Allium sativum*) powder on growth performance, feed utilization and disease resistance against *Aeromonas hydrophila* in *Labeo catla* over a 60 day experimental period at the College of Fisheries Science, Kamdhenu University, Veraval. Four experimental diets were formulated with a uniform protein level of 30%. A total of 120 *L. catla* fry (mean initial weight: 0.37 g) were randomly assigned to 12 plastic tanks in a completely randomized design comprising four treatments with three replicates each. The treatments included: T0 (control, without garlic powder), T1 (0.5% garlic powder), T2 (1% garlic powder) and T3 (1.5% garlic powder). Growth parameters such as mean weight, mean weight gain and percentage weight gain were recorded fortnightly. After 60 days, feed utilization parameters including Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Feed Conversion Efficiency (FCE) and Protein Efficiency Ratio (PER) were calculated. Survival and mortality rates were also recorded. Following the feeding experiment, fish were challenged with an injection of *A. hydrophila* (0.1 ml of  $1 \times 10^6$  CFU/ml suspension) and post-infection mortality was monitored for seven days. Statistical analysis revealed significant differences ( $p < 0.05$ ) among treatments. Overall, T2 demonstrated the highest performance across all measured parameters, recording the greatest mean weight ( $2.69 \pm 0.081$  g), mean weight gain ( $2.33 \pm 0.067$  g), percentage weight gain ( $648.08 \pm 11.16\%$ ), SGR ( $3.354 \pm 0.025\%$ ), survival rate ( $96.67 \pm 3.33\%$ ), FCE (0.5  $\pm$  0.006) and PER ( $1.668 \pm 0.019$ ), along with the lowest FCR ( $1.999 \pm 0.023$ ). In terms of disease resistance, both T2 and T3 achieved the highest post-challenge survival rate (90.00%). These results suggest that dietary supplementation with 1% garlic powder significantly enhances growth performance, feed utilization, survival and resistance to *Aeromonas hydrophila* in *Labeo catla*, making garlic powder a promising additive in aquaculture nutrition.

### ABSTRACT

**Keywords :** Garlic powder, *Labeo catla*, growth performance, *Aeromonas hydrophila*.

### Introduction

Aquaculture involves the controlled cultivation of aquatic animals and plants, including fish, mollusks and shellfish. It relies on active management practices like regular stocking, artificial feeding and disease prevention to enhance production and sustainability (FAO, 2022). Aquaculture contributes 47% to global aquatic animal production, with fish farming alone accounting for 54.3 million tonnes annually (FAO, 2020). As a fast-growing food sector, it addresses the

rising demand for nutritious, high-quality food (FAO, 2024).

Fish is a key source of affordable nutrition and supports livelihoods through employment and income (Narsale *et al.*, 2024). Its high protein and omega-3 content make it valuable to diets worldwide (Prakash *et al.*, 2023; Lynch *et al.*, 2016). In India, freshwater aquaculture plays a significant role in meeting fish demand, with a focus on high-value species to enhance farmer returns (Prakash *et al.*, 2023).

*Labeo catla* (Catla) is a commercially important freshwater fish species belonging to the Cyprinidae family and is widely cultured in India and South Asia. It is the second-most significant species in Indian major carp (IMC) polyculture systems and contributes substantially to freshwater aquaculture production. Catla accounts for 5.6% of global aquaculture production and 3.4% of worldwide freshwater aquaculture production (FAO, 2019). Known for its rapid growth, high protein content and consumer demand, Catla plays a vital role in food security, rural livelihoods and the economic sustainability of India's aquaculture sector.

A balanced diet is crucial in aquaculture to ensure healthy and cost-effective fish production (Craig and Helfrich, 2002). However, the increased use of antibiotics and chemicals has raised concerns over environmental safety and food quality. As a result, there is growing interest in herbal alternatives such as medicinal plants or phytobiotics, which enhance growth, feed intake and immunity in fish while being eco-friendly and cost-effective (Kaur and Ansal, 2020; Xu *et al.*, 2020). Garlic, in particular, is widely used for its natural availability and beneficial effects, including anti-stress, immunostimulatory and growth-promoting properties (Nyadjeu *et al.*, 2021).

Garlic (*Allium sativum*), a member of the Liliaceae family, has long been used as a spice and in traditional medicine. It is known as an "all-healing" herb and can be used as a flavoring and functional food. Garlic can be extracted in a number of ways, such as ethanol, aqueous and dry powder (Shin and Kim, 2004). Garlic is rich in nutrients, containing at least 33 sulfur compounds, 17 amino acids, several enzymes, minerals, vitamins and a high carbohydrate content. Its phenols provide antioxidant activity. On average, garlic's composition includes 65% water, 27.5% carbohydrates, 4.7% fiber, 2–3% organosulfur compounds and 2% protein (Gambogou *et al.*, 2018). It also contains vitamins A, B and C (Dragan *et al.*, 2008).

Garlic contains bioactive compounds such as allicin, which improve the immunity of fish. Furthermore, active compounds in garlic, such as allicin and diallyl sulfide, have antibacterial properties that protect against a variety of fish pathogens, including bacteria, viruses and parasites. This provides a significant advantage for disease management in aquaculture. Thus, using immunostimulants derived from natural sources, such as garlic, is an effective approach for boosting growth performance, improving antioxidant status and controlling immunological, hematological and serum biochemical parameters

while successfully controlling aquaculture diseases (Muahiddah and Diamahesa, 2023; Yilmaz and Ergun, 2012).

Disease outbreaks are a major constraint in aquaculture, significantly affecting production and economic growth (Yunxia *et al.*, 2001). Motile Aeromonas Septicemia (MAS), primarily caused by *Aeromonas hydrophila*, is a widespread bacterial disease leading to high mortality in both cultured and wild fish (Noga, 2010; McDaniel, 1979). This opportunistic pathogen becomes more virulent under stress and often resists antibiotics, making management difficult (Kalyankar *et al.*, 2013). With rising antibiotic resistance, natural alternatives like garlic have shown effectiveness against various pathogens, including *A. hydrophila*, offering a sustainable and eco-friendly solution for disease control in aquaculture (Agrawal *et al.*, 2001).

## Materials and Methods

### Experimental setup

The research was conducted for 60 days at the Hands-on Training Centre, College of Fisheries Science, Kamdhenu University, Veraval, followed by a 7 day disease challenge test. The experiment was conducted in a Completely Randomized Design (CRD), where the 12 tanks were distributed into 4 treatment groups and replicated 3 times.

The experimental animal used was *Labeo catla*. The fry of *L. catla* were procured from a commercial fish hatchery and transported to the experimental site in oxygenated polyethylene bags. Upon arrival, the fish were acclimated for 7 days in a 500 liter plastic tank, which was equipped with continuous aeration and provided with regular feedings. A total of 12 rectangular plastic tanks, each measuring 2 x 1 x 1 feet with a capacity of 40–50 liters, were used for the experiment. A total of 120 fish were used in the experiment. Following the acclimatization period, the fish were put into plastic tanks (10 fish per tank), where they were kept for 60 days. They were fed at the rate of 5–7% of their body weight and provided with sufficient aeration throughout the experiment.

### Experimental diet

Ingredients like Garlic, wheat flour and tapioca flour were sourced from the local market in Veraval. Garlic slices were dried using an artificial dryer at the College of Fisheries Science, Veraval and then ground into a fine powder. The garlic powder was sealed in a plastic container and refrigerated for later use in experiments. Other ingredients such as fish meal, groundnut oil cake (GNOC), fish oil and a vitamin-

mineral mix were obtained from a nearby fish processing plant in Veraval. All ingredients were finely ground, sieved for uniformity and stored in polythene bags at room temperature.

Four experimental diets were prepared, each formulated to contain 30% protein using Pearson's square method. The control diet, labelled T0, did not include garlic powder. The other diets, labelled T1, T2 and T3, included garlic powder at concentrations of

0.5, 1 and 1.5%, respectively. The weighed ingredients were ground, sieved and thoroughly mixed with water in a plastic tray to form dough. This dough was steam-cooked in an autoclave at 121°C and 15 lbs pressure for 15 minutes. After cooling, vitamins and minerals and garlic powder (as per treatment) were added and mixed. The mixture was then pelletized, sun-dried to below 10% moisture and stored in labelled, sealed containers.

**Table 1:** Composition of experimental diets

Ingredients (%)	Diet (30% protein)			
	T0	T1	T2	T3
Garlic powder	0	0.5	1	1.5
Fish meal	30	30	30	30
Groundnut oil cake	40	40	40	40
Wheat flour	14	13.7	13.6	13.4
Tapioca flour	12	11.8	11.4	11.1
Fish oil	2	2	2	2
Vitamins and Minerals	2	2	2	2
Total	100	100	100	100

### Experimental Procedure

**Growth Study:** Fish were weighed every 15 days to calculate growth and survival.

- **Mean Weight Gain (MWG)** = Final average body weight (g) - Initial average body weight (g)
- **% Weight Gain** = (Final weight (g) - Initial weight (g) / Initial weight) × 100
- **SGR (%/day)** = [ln (Final weight (g)) - ln (Initial weight (g)) / Experimental days] × 100

**Feed Utilization:** FCR, FCE, SGR and PER were calculated at the end of the experiment.

- **FCR** = Feed intake (g) / Weight gain (g)
- **FCE** = Weight gain (g) / Feed intake (g)
- **PER** = Weight gain (g) / Protein intake (g)

### Disease Resistance:

#### Preparation of *Aeromonas hydrophila* Stock Culture

*Aeromonas hydrophila* was cultured in nutrient broth at 37°C for 24 h following Das *et al.* (2015). The culture was centrifuged at 3000 rpm for 10 min, and the pellet was resuspended in PBS (pH 7.4). The suspension was adjusted to 0.5 McFarland standard ( $1 \times 10^6$  CFU/ml) and serially diluted with PBS as per Sahu *et al.* (2007).

### Challenge Test

After the 60 day feeding experiment, 10 fish per treatment were intramuscularly injected with 0.1 ml of

*A. hydrophila* suspension ( $1 \times 10^6$  CFU/ml) using a 26-gauge needle. Mortality and clinical signs were recorded for 7 days.

### Water quality management

Water quality parameters, such as temperature, pH, dissolved oxygen (DO) and alkalinity, were checked periodically. pH was recorded by a digital pH meter, temperature by the thermometer, dissolved oxygen by Winkler's method and alkalinity by the titration method.

### Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) and SPSS, with results expressed as mean  $\pm$  standard error. Mean differences were evaluated using Duncan's Multiple Range Test (DMRT) at a significance level of  $p < 0.05$ .

## Results and Discussion

#### Effects of garlic powder on the growth and survival of *Labeo catla*

The initial mean weights of *L. catla* in treatments T0, T1, T2 and T3 were  $0.36 \pm 0.032$ ,  $0.36 \pm 0.023$ ,  $0.36 \pm 0.015$  and  $0.37 \pm 0.015$  grams, respectively. The final weights for these treatments at the end of the experiment were  $1.41 \pm 0.112$ ,  $1.67 \pm 0.127$ ,  $2.69 \pm 0.081$  and  $2.35 \pm 0.118$  grams, respectively.

At the end of the experiment, the mean weight gain of *Labeo catla* fry in treatments T0, T1, T2 and T3 was recorded as  $1.05 \pm 0.085$ ,  $1.31 \pm 0.105$ ,  $2.33 \pm$

0.067 and  $1.98 \pm 0.103$  grams, respectively. Statistical analysis showed a significant difference in weight gain across the treatments ( $p < 0.05$ ). Among them, T2 demonstrated the highest mean weight gain, significantly outperforming all other groups. This suggests that the inclusion of 1% garlic powder (T2) was the most effective in promoting growth in *L. catla* throughout the study period. Comparable findings were reported by Nyadjeu *et al.* (2021), who observed the highest growth rates in *Clarias gariepinus* fry fed diets supplemented with 10 g/kg of garlic powder. Similarly, Harika and Yusufzai (2021) found improved weight gain in *Cirrhinus mrigala* when 1% garlic powder was added to their diet, supporting the present study's results. The current findings also align with those of Zare *et al.* (2021), Xu *et al.* (2020), Saghaei *et al.* (2015) and Mehrim *et al.* (2014). Furthermore, Abbas *et al.* (2023) reported that a 1.5% garlic powder supplemented diet significantly improved final weight and weight gain in *Labeo rohita* over 90 days, similar to the results of Chesti and Chauhan (2018), who observed enhanced growth performance in Amur carp (*Cyprinus carpio*) fed a 1.5% garlic-based diet.

Among all the treatments, T2 showed the highest percentage weight gain ( $648.078 \pm 11.164$ ), followed by T3 ( $534.654 \pm 5.893$ ), T1 ( $363.38 \pm 13.358$ ) and T0 ( $292.719 \pm 15.491$ ). These findings are consistent with those reported by Samson (2019), Maniat *et al.* (2014) and Mehrim *et al.* (2014).

**Table 2:** Mean weight (g), Mean weight gain (g), percentage weight gain (%) and Survival (%) of *Labeo catla* recorded in different treatments during the culture period (Mean  $\pm$  SE)

Treatment	Mean Weight (g)	Mean Weight gain (g)	Percentage Weight gain (%)	Survival (%)
<b>T0</b>	$1.41 \pm 0.112^b$	$1.05 \pm 0.085^c$	$292.719 \pm 15.491^d$	$80.00 \pm 5.774^b$
<b>T1</b>	$1.67 \pm 0.127^b$	$1.31 \pm 0.105^c$	$363.380 \pm 13.358^c$	$83.33 \pm 3.333^{ab}$
<b>T2</b>	$2.69 \pm 0.081^a$	$2.33 \pm 0.067^a$	$648.078 \pm 11.164^a$	$96.67 \pm 3.333^a$
<b>T3</b>	$2.35 \pm 0.118^a$	$1.98 \pm 0.103^b$	$534.654 \pm 5.893^b$	$93.33 \pm 3.333^{ab}$

#### Effects of garlic powder on SGR, FCR, FCE and PER of *Labeo catla*

The highest specific growth rate (SGR) was observed in treatment T2 (1% garlic) with a value of  $3.354 \pm 0.025$ , followed by T3 and T1, while the control group (T0) recorded the lowest SGR. Statistical analysis revealed that T2 had a significantly higher SGR compared to the other treatments ( $p < 0.05$ ), highlighting its strong growth-promoting potential. These findings align with those of Nyadjeu *et al.* (2021), who studied the effects of garlic and ginger powder as natural feed additives on *Clarias gariepinus* fry and found that fish fed a 1% garlic-supplemented diet showed the highest SGR. Similar results were also

In terms of survival rate, T2 (1% garlic supplementation) achieved the highest mean value of  $96.67 \pm 3.33$ , which was significantly higher than the control group. This suggests that incorporating 1% garlic powder had a positive effect on fish survival. Similar results were reported by Mehrim *et al.* (2014), who observed the highest survival rate in *Oreochromis niloticus* fed a diet containing 1% garlic powder. Harika and Yusufzai (2021) also reported comparable outcomes in *Cirrhinus mrigala*.

Garlic is known to contain several bioactive compounds that contribute to its growth-promoting properties in aquaculture species. Shalaby *et al.* (2006) reported that compounds such as allicin, alliin and organosulfur constituents especially thiosulfinate play a significant role in enhancing growth by improving digestion, nutrient assimilation and overall metabolic efficiency. Similarly, Metwally (2009) observed that dietary garlic supplementation positively influenced fish growth performance. However, the study also noted that excessive inclusion levels could negatively impact feed intake, likely due to the strong odor of garlic. This finding aligns with the observations of Mesalhy *et al.* (2008) and Platel and Srinivasan (2004), who reported that higher concentrations of garlic powder in fish diets led to reduced weight gain, potentially attributed to decreased palatability and feed consumption resulting from garlic's pungency.

reported by Harika and Yusufzai (2021), Xu *et al.* (2020), Samson (2019) and Mehrim *et al.* (2014).

The lowest feed conversion ratio (FCR) was recorded in T2 at  $1.999 \pm 0.023$ , followed by T3 at  $2.117 \pm 0.044$ , indicating better feed utilization in these groups. In contrast, the highest FCR was observed in the control group (T0) at  $2.664 \pm 0.079$ , reflecting poorer feed efficiency. The highest feed conversion efficiency (FCE) was also noted in T2 at  $0.50 \pm 0.006$ , followed by T3 and T1, with the control group showing the lowest FCE. These significant differences ( $p < 0.05$ ) suggest that garlic supplementation had a positive effect on feed utilization. These outcomes are consistent with Nyadjeu *et al.* (2021), who found that

*Clarias gariepinus* fry fed 1% garlic powder had improved FCR compared to the control. Similarly, Samson (2019) reported significantly reduced FCR in red tilapia fed diets containing 10 g/kg of garlic powder.

The highest protein efficiency ratio (PER) was achieved in the T2 group, with a mean value of  $1.668 \pm 0.019$ , indicating the most effective conversion of protein into fish biomass. This was followed by T3, which had a PER of  $1.576 \pm 0.033$ . T2 showed a significantly higher PER than the other treatments, confirming the beneficial impact of 1% garlic supplementation. These results are in line with previous studies, including Harika and Yusufzai (2021), who reported that *Cirrhinus mrigala* fed a diet with 1% garlic powder exhibited a higher PER than the control.

**Table 3:** Specific Growth Rate, Food Conversion Ratio, Feed Conversion Efficiency and Protein Efficiency Ratio of *Labeo catla* recorded in different treatments during the culture period (Mean  $\pm$  SE)

Treatment	Specific Growth Rate (SGR)	Feed Conversion Ratio (FCR)	Feed Conversion Efficiency (FCE)	Protein Efficiency Ratio (PER)
T0	$2.277 \pm 0.065^d$	$2.664 \pm 0.079^a$	$0.376 \pm 0.011^b$	$1.254 \pm 0.038^b$
T1	$2.554 \pm 0.048^c$	$2.471 \pm 0.053^b$	$0.405 \pm 0.009^b$	$1.350 \pm 0.029^b$
T2	$3.354 \pm 0.025^a$	$1.999 \pm 0.023^c$	$0.500 \pm 0.006^a$	$1.668 \pm 0.019^a$
T3	$3.080 \pm 0.016^b$	$2.117 \pm 0.044^c$	$0.473 \pm 0.010^a$	$1.576 \pm 0.033^a$

#### Effects of garlic powder on disease resistance in *Labeo catla* against *Aeromonas hydrophila*

At the end of the 60 day experimental period, a challenge test was conducted to assess disease resistance. Mortality in all groups was monitored over 7 days. Garlic supplementation had a significant positive effect on survival rates following exposure to *Aeromonas hydrophila* compared to the control group. After 7 days, treatments T2 and T3 demonstrated similar and higher survival rates, while the control group (T0) showed a lower survival rate of 50%. These findings suggest that incorporating 1% garlic powder into the diet enhances resistance to *A. hydrophila*, proving more effective than the control. Similar results were reported by Harika and Yusufzai (2018), who found that feeding *Cirrhinus mrigala* with 1% garlic powder improved resistance against *A. hydrophila*.

Khalil *et al.* (2001) proposed that the improved feed utilization observed in fish fed diets containing 1% garlic powder could be attributed to allicin, a key active compound in garlic. Allicin is known to enhance the activity of beneficial intestinal microflora, thereby improving digestion and energy efficiency, which contributes to better growth performance. However, Samson (2019) reported an increase in Feed Conversion Ratio (FCR) with higher levels of garlic supplementation. This trend may be linked to reduced feed intake, likely due to the strong odor and pungency of garlic, which can negatively affect feed palatability, particularly at higher inclusion rates. These findings highlight the importance of optimizing garlic dosage to balance its beneficial effects on feed efficiency without compromising feed acceptability.

Likewise, Chesti *et al.* (2018) observed that supplementing *Cyprinus carpio haematopterus* with 1.5% garlic powder boosted immunity against aeromonad infections.

Khodadadi *et al.* (2013) reported that garlic supplementation can strengthen the immune system and enhance disease resistance in fish, largely due to the presence of bioactive compounds such as allicin and essential vitamins like A and C. These components are known to support immune function and overall health. In agreement with this, Aly *et al.* (2008) observed higher survival rates in fish treated with garlic powder, attributing this effect to garlic's immunostimulatory properties, which help to activate and reinforce the fish's immune response. These findings suggest that garlic can play a significant role in improving fish health and resistance to infections.

**Table 4:** Disease resistance of *L. catla* in each treatment was observed throughout the experimental period after 7 days of exposure to *Aeromonas hydrophila*

Treatments	T0	T1	T2	T3
Survival (%)	$50 \pm 5.77^c$	$70 \pm 5.77^b$	$90 \pm 0.00^a$	$90 \pm 5.77^a$

### Physico-Chemical Water Parameters

During the 60 day study period, water quality

parameters including temperature, pH, dissolved oxygen (DO) and alkalinity were monitored weekly.

**Table 5:** Temperature (°C), pH, Dissolved oxygen (ppm) and Alkalinity (mg/l) of *L. catla* in each treatment was observed throughout the experimental period

Treatments	Temperature (°C)		pH		Dissolved oxygen (ppm)		Alkalinity (mg/l)	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
T0	25.67	27.33	7.23	7.70	5.60	6.33	163	197
T1	26.33	27.00	7.23	7.77	5.53	6.10	165	200
T2	25.67	27.00	7.27	7.67	5.53	6.30	164	205
T3	25.67	27.33	7.47	7.77	5.50	6.30	166	206

### Conclusion

The research assessed the effects of garlic powder on the growth performance, feed utilization and disease resistance of *Labeo catla* for 60 days. Out of all the treatments, the treatment fed with 1% garlic powder (T2) had the highest improvements, registering the highest weight gain, Specific Growth Rate (SGR), Feed Conversion Efficiency (FCE) and Protein Efficiency Ratio (PER). T2 also had the best Feed Conversion Ratio (FCR) and survival rate, indicating better feed utilization and enhanced fish health. After being challenged with *Aeromonas hydrophila*, the T2 (1% garlic) and T3 (1.5% garlic) groups of fish exhibited the strongest resistance, demonstrating garlic's immunostimulatory role. The results of this experiment indicate that supplementing the diet of *Labeo catla* with garlic powder, particularly at 1%, significantly enhances growth rate, feed efficiency and disease resistance. Thus, garlic powder can be considered as a good dietary supplement in the diet of *Labeo catla* to enhance growth and resistance to diseases.

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