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Original Article

Effect of dietary L-ascorbic acid and L-leucine and their combination on growth performance, survival and feed utilisation in *Labeo catla* fingerlings

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Abstract
The present investigation was carried out for a period of 75 days to evaluate the effects of dietary supplementation of L-ascorbic acid and L-leucine on growth performance and feed utilisation in *Labeo catla* fingerlings. A total of nine experimental diets were formulated with different levels of L-leucine and L-ascorbic acid, including a control (T_0) without supplementation. The treatments consisted of T_1 (10 g L-leucine), T_2 (15 g L-leucine), T_3 (0.3 g L-ascorbic acid), T_4 (0.4 g L-ascorbic acid), T_5 (0.3 g L-ascorbic acid + 10 g L-leucine), T_6 (0.4 g L-ascorbic acid + 10 g L-leucine), T_7 (0.3 g L-ascorbic acid + 15 g L-leucine) and T_8 (0.4 g L ascorbic acid + 15 g L-leucine). The results indicated significant improvement in the highest final weight (3.86±0.38 g), specific growth rate (1.95±0.10% day^{-1}), and protein efficiency ratio (1.74±0.08), along with the lowest feed conversion ratio (1.93±0.09) in T_6. The combination of 0.4 g L-ascorbic acid and 10 g L-leucine (T_6) was found to be the most effective and is recommended for enhancing growth and feed utilisation in *L. catla* fingerlings.

Keywords: feed utilization; growth performance; *Labeo catla*; L-ascorbic acid; L-leucine

1 | INTRODUCTION

World production of fisheries and aquaculture reached a new high of 235 million tonnes including 195 million tonnes of aquatic animals and 40 million tonnes of algae (FAO 2026). Aquaculture is one of the most promising food-producing sectors, holding significant potential for future growth. However, production must increase by 50% by 2050 to meet the rising global demand for food (Schubel and Thompson 2019; Bhadarka *et al.* 2026). In the last few years, Indian fisheries have undergone a major transformation, shifting from being primarily marine based to placing greater emphasis on inland fisheries. Inland fisheries now account for a far larger portion of the nation's total fish production. India has experienced a significant expansion in commercial carp culture in recent years. Native cyprinid carps (e.g. catla, *Labeo catla*; rohu, *Labeo rohita* and mrigal, *Cirrhinus cirrhosus*) are the important candidate species for freshwater aquaculture in India, as they contribute over 60–70% of the total freshwater aquaculture production. *Labeo catla* is an important species in India's freshwater aquaculture, contributing significantly to the country's fish production and food security, particularly in states like Bengal and Odisha (National Fisheries Development Board 2020). It is one of the Indian major carp alongside rohu and mrigal, forming the backbone of the aquaculture sector (Singh and Venkateshwarlu 2015).

Labeo catla is a significant species in aquaculture, particularly in freshwater fisheries in South Asia. It plays a key role in food security, livelihood, and economic growth in the region. However, despite its high economic potential, the farming of *L. catla* is confronted with several challenges, including poor growth rates, susceptibility to diseases, and compromised immune responses, which can lead to production losses and increased reliance on antibiotics and other treatments. Enhancing the growth performance and feed utilisation of *L. catla* fingerlings could improve the sustainability and profitability of aquaculture operations. Ascorbic acid (vitamin C), a water-soluble vitamin, is essential for all vertebrates. It plays a critical role in various physiological functions, including growth, collagen synthesis, wound healing, haematology, reproduction, iron metabolism, stress response, and immune function (Jobling 2011). Water soluble ascorbic acid is probably the most important among all vitamins, as it is a vital antioxidant that supports the immune system in fish (Omoniyi and Ovie 2018). Furthermore, vitamin C enhances iron absorption and protects against oxidative stress, which is essential for maintaining optimal health and growth performance in fish (Buettner and Jurkiewicz 1996). A deficiency in vitamin C can lead to deformities in bone or other hard tissues (Wang and Huang 2015). The recommended level for dietary ascorbic acid is 200 mg kg^{-1}, which is optimal for enhancing immune responses, while 500 mg kg^{-1} promotes better growth and survival in *L. rohita* fingerlings and helps increasing disease resistance against naturally occurring opportunistic bacteria such as *Aeromonas hydrophila* (Misra *et al*. 2007).

Essential amino acids are crucial for optimal growth, feed conversion ratio (FCR), and desirable carcass quality in animals. By supplementing amino acids, the diet's amino acid profile can be adjusted to better align with the animal's ideal balance. The amount and proportion of necessary amino acids in a protein determine its biological usefulness in the diet. If any required amino acid is either deficient or in excess relative to others, protein synthesis will be hindered. In such cases, the body will break down labile proteins to provide the necessary amino acids, ensuring protein synthesis continues. Most aquatic animals require 10 of the 20 amino acids that make up proteins because their carbon skeletons cannot be produced by the body. The animal can synthesise the other ten amino acids by transferring amino groups to carbon molecules produced during glucose and lipid metabolism, which are derived from both gluconeogenic and ketogenic amino acids (Abidi and Khan 2007). From the branched chain amino acids, only one ketogenic amino acid has the special ability to induce the release of insulin from the pancreas islet cells (Panten *et al*. 1974). This amino acid is critical for maintaining nitrogen balance and plays a key role in the regulation of muscle protein synthesis, a function first identified in the 1970s (Buse and Reid 1975; Fulks *et al*. 1975; Li and Jefferson 1978). Leucine, in collaboration with valine and isoleucine, plays a vital role in supporting and fuelling muscle function. It is crucial for haemoglobin production, helps regulate blood sugar levels, boosts growth hormone production, and is involved in stress response, energy metabolism, and muscle health. A lack of leucine in the diet can lead to significant biochemical disruptions, including stunted growth.

This research is important for filling the knowledge gap on the effects of L-ascorbic acid and L-leucine supplementation on *L. catla* fingerlings. By evaluating their impact on growth, and feed utilisation, the study could contribute to the development of more efficient and sustainable aquaculture practices. Therefore, this study is undertaken to find out the recommended level of L-ascorbic acid, L-leucine and its combination in *L. catla* fingerlings.

2 | METHODOLOGY

2.1 Study period and site

The present research work was conducted for 75 days, from 1 November 2025 to 14 January 2026. The experiment took place in the wet lab at the Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat (20°56′21.6″N 70°21′09.2″E).

2.2 Experimental animal

Healthy *L. catla* fingerlings, belonging to the Family Cyprinidae and Order Cypriniformes, were procured from the Aqua fish farm located in Ranagadh, Limbdi. The fish had a mean weight of 0.85±0.04 g. Prior to being placed in an acclimatisation tank, the fish underwent a prophylactic treatment in compliance with accepted protocol, which included a bath in a 0.05% potassium permanganate ($KMnO_4$) solution.

2.3 Acclimatisation of fish

Before stocking the fingerlings into the tanks, the bags were allowed to float on the surface of the water to adjust the temperature, after they were released and water was properly mixed. The fish were reared for one month in 250-liter rectangular plastic tanks under laboratory conditions to ensure proper acclimatisation to optimal parameters. During this period, the fish were fed with formulated floating feed at a rate of 8% of their body weight.

2.4 Experimental set-up

The investigation took place in pre-treated (2 ppm $KMnO_4$, washed 3–4 times thoroughly) 27 rectangular tanks (2×1×1 feet), which were filled with pre-quality-tested water. The experimental setup followed a CRD design. The tanks were arranged in triplicate for treatments and controls (Table 1). The experimental fish *L. catla* was stocked at the rate 1 g L^{-1} of water.

TABLE 1 Experimental groups for testing the effects of dietary L-ascorbic acid and L-leucine and their combination on growth performance, survival and feed utilisation in *Labeo catla* fingerlings.

Treatment	Feed additive (g kg^{-1})
T_0	Diet prepared without the addition of L-ascorbic acid and L-leucine (Control)
T_1	Diet prepared with 10 g L-leucine
T_2	Diet prepared with 15 g L-leucine
T_3	Diet prepared with 0.3 g L-ascorbic acid
T_4	Diet prepared with 0.4 g L-ascorbic acid
T_5	Diet prepared with 0.3 g L-ascorbic acid and 10 g L-leucine
T_6	Diet prepared with 0.4 g L-ascorbic acid and 10 g L-leucine
T_7	Diet prepared with 0.3 g L-ascorbic acid and 15 g L-leucine
T_8	Diet prepared with 0.4 g L-ascorbic acid and 15 g L-leucine

Siphoning was performed daily, and water exchange was maintained at 10–15% to compensate for losses due to siphoning and evaporation. The fish were fed at a rate of 8% of average body weight, split into two daily doses for the first 30 days, and 6% for the subsequent period. Aeration in each tank was achieved using aerators, air stones, and regulators to ensure consistent airflow. Growth parameters were monitored fortnightly. Water quality parameters, including temperature, pH, total alkalinity and dissolved oxygen were analysed at appropriate intervals. The water temperature was measured using a digital centigrade thermometer. The pH levels were determined using a digital pH meter. Dissolved oxygen was quantified through the standard Winkler's method. Finally, total alkalinity was analysed via the titration method using methyl orange and phenolphthalein indicators.

2.5 Preparation of experimental diets

Fish feed was experimental diets were formulated using fish meal, groundnut cake, wheat flour, tapioca powder, and a vitamin-mineral mixture, all ingredients procured from a local market in Veraval. Nine different isonitrogenous diets were prepared, each diet containing 30% crude protein. L-ascorbic acid and L-leucine were incorporated at varying concentrations, either individually or in combination, as detailed in Table 2. Proximate analysis of the feed ingredients was conducted, and the diets were formulated using the Pearson Square method to maintain a 30% protein level.

All ingredients were mixed with water to form a dough and cooked in an autoclave at 121°C. After 20 minutes, the dough was cooled to room temperature. A vitamin-mineral premix, L-ascorbic acid, and L-leucine was then added to the dough and blended thoroughly. The mixture was extruded through a 2 mm diameter hand pelletizer, sun dried and stored in airtight plastic jars. A control diet was prepared using the same ingredients, excluding L-ascorbic acid and L-leucine. Table 2 Details of percentage composition of the diets used in the experiment.

TABLE 2 Details of percentage composition of the diets used in the experiment.

Ingredients	Grams of ingredients per 100 grams of feed								
	T0	T1	T2	T3	T4	T5	T6	T7	T8
L-Leucine	0	1	1.5	0	0	1	1	1.5	1.5
L-Ascorbic acid	0	0	0	0.03	0.04	0.03	0.04	0.03	0.04
Fish meal	37	36	36	37	37	36	36	36	36
Groundnut oil cake	23	23	23	23	23	23	23	23	23
Wheat flour	17	17	17	17	17	17	17	17	17
Tapioca powder	17	17	16.5	16.97	16.96	16.97	16.96	16.47	16.46
Fish oil	2	2	2	2	2	2	2	2	2
Plant oil	2	2	2	2	2	2	2	2	2
Vitamins and minerals	2	2	2	2	2	2	2	2	2

2.6 Growth parameters

Growth parameters of experimental fish like mean weight gain (g), feed conversion ratio (FCR), specific growth rate (SGR), body weight gain (%), feed conversion efficiency (FCE), and protein efficiency ratio (PER), were analysed during the study. The following formulas were used to calculate the growth parameters of fish.

Mean weight gain = Final weight – Initial weight

Survival (%) = (Total No. of fish at the end of the experiment / Total No. of fish at the beginning of the experiment) × 100

SGR (%) = (Ln Final weight − Ln Initial weight × 100) / number of days

FCR = Feed given (dry weight) / Body weight gain (wet weight)

FCE = Weight gain / Feed given

PER (%) = Weight gain / Protein intake

2.7 Data analysis

Prior to analysis, the data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. Result confirmed that the data satisfied the assumptions for parametric analysis ($p > 0.05$). Consequently, a one-way ANOVA was utilized to compare treatment means at each 15-day interval, with differences considered significant at $p < 0.05$ using Duncan multiple range test (DMRT) (Snedecor and Cochran 1989).

3 | RESULTS

3.1 Growth performance

This experiment was conducted to determine the effect of L-ascorbic acid and L-leucine on growth performance of *L. catla* fingerlings over a 75-days experimental period. The results of growth parameters include final weight, body weight gain (%), and specific growth rate (SGR) illustrated in Figure 1. During the experiment the initial and final weights were recorded from 0.71±0.14 g to 0.88±0.05 g and 1.83±0.04 g to 3.86±0.38 g respectively. The final weight showed an increasing trend with the dietary supplementation of L-ascorbic acid and L-leucine compared to control. The highest final weight was observed in T6 (3.86±0.38 g) which showed a significant difference ($p < 0.05$) compared to all other treatments.

The lowest final weight was recorded in the control (1.83±0.04 g). Treatments T5 (2.79±0.16 g) and T7 (2.77±0.47 g) also showed significantly higher growth than the control but significantly lower growth than T6. In the current study, the BWG (%) exhibited a progressive increase across all the treatment groups from day 15 to day 75. However, the rate of increase varied significantly among the treatments. The highest growth performance was consistently observed in T6, reaching a maximum of 335.33±30.56% by the end of the experiment. This was followed by T7 (301.48±55.48%) and T5 (244.97±51.86%).

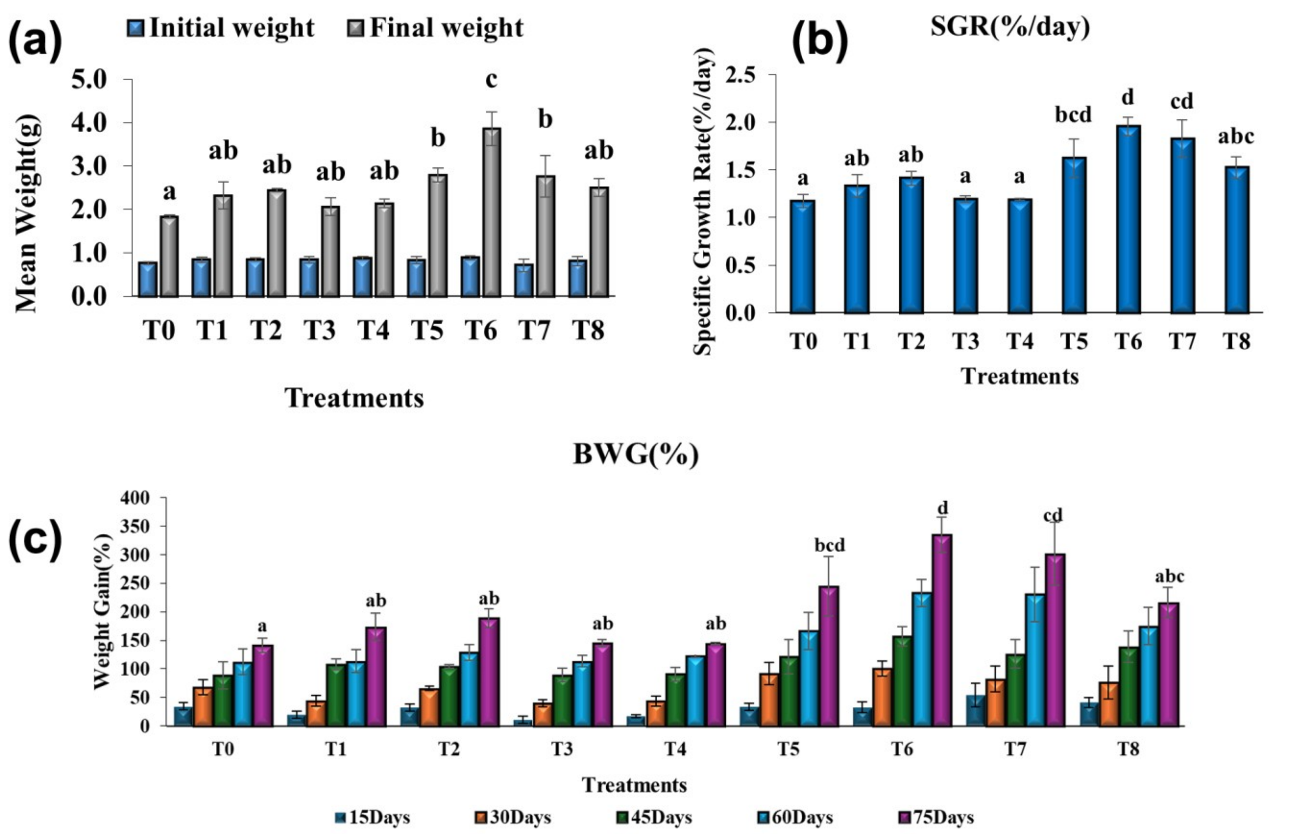

FIGURE 1 Growth performance of *Labeo catla* (a) Initial and final weight; (b) Specific growth rate (SGR); (c) Body weight gain (BWG).

Statistical analysis revealed significant differences ($p < 0.05$) between the treatment groups as the experiment progressed. Specifically, by 75th day, T6 and T7 showed a significantly higher body weight gain compared to control and treatment T3 (144.83±6.23%) and T4 (144.37±2.33%) did not show any statistically significant improvement over control. In the present study, dietary supplementation significantly improved the SGR across the different treatment groups. The highest SGR was recorded in T6 (1.95±0.10% day^{-1}), followed by T7 (1.83±0.20 % day^{-1}) and T5 (1.62±0.20 % day^{-1}). These values represent a substantial improvement in daily growth performance compared to control, which exhibited an SGR of 1.17±0.07% day^{-1}. Statistical analysis revealed that treatments T6 was significantly different ($p < 0.05$) from the control and treatments T3 (1.19±0.04 % day^{-1}) and T4 (1.19±0.01 % day^{-1}).

3.2 Feed utilisation

This experiment was conducted to determine the effect of L-ascorbic acid and L-leucine on feed utilisation of *L. catla* fingerlings over a 75-days of experimental period. The results for the feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER) obtained in the present experiment are illustrated in Figure 2. The statistical analysis showed significant differences ($p < 0.05$) in FCR across the different treatments. The control recorded the highest FCR value (3.75±0.09) indicating the least efficient conversion of feed into body mass. The lowest and most favourable FCR was observed in T6 (1.93±0.09), followed by T7 (2.20±0.30) and T5 (2.48±0.32). These three treatments exhibited a significantly better FCR compared to T0 (3.75±0.09), T3 (3.31±0.10) and T4 (3.41±0.09). Compared to the control and other treatments, T6 had the highest and most effective FER (0.52±0.02). This was followed by T7 (0.48±0.08) and T5 (0.42±0.05), they both showed significantly greater feed efficiency than the control. Moderate feed efficiency values were observed in T2 (0.36±0.02), T8 (0.36±0.03), and T1 (0.34±0.05). Lower FER values, which remained statistically similar to the control group, were recorded in T3 (0.30 ± 0.01) and T4 (0.29 ± 0.01). The control (0.27 ± 0.01) exhibited the lowest FER, reflecting the least efficient conversion of feed into biomass during the 75-day feeding trial. The PER, which measures how well dietary protein is converted to body weight, varied significantly across treatments ($p < 0.05$). The mean PER value ranged from a minimum of 0.89±0.02 in T0 to a maximum of 1.74±0.08 in T6. Treatment T6 (1.74±0.08) exhibited a significantly higher PER than T0, T3 and T7. However, no significant difference was observed between T6 and T1. L-ascorbic acid supplementation has been shown to improve feed utilization efficiency and growth performance in fish.

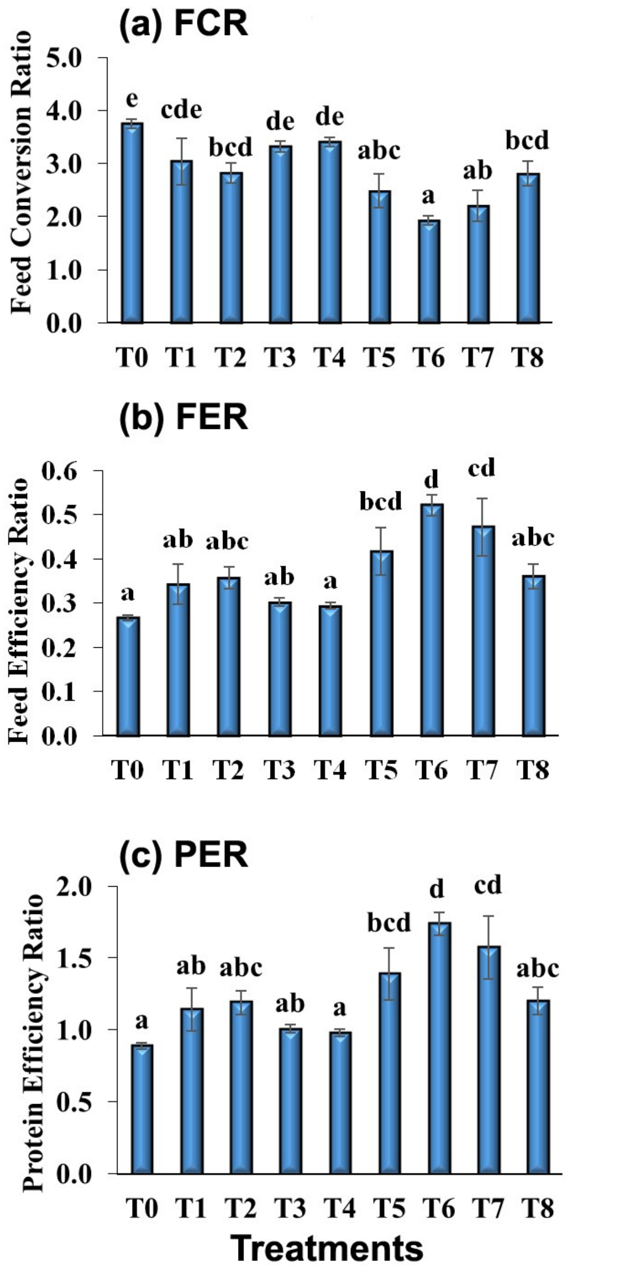

FIGURE 2 Feed utilisation of *Labeo catla* (a) Feed conversion ratio (FCR); (b) Feed efficiency ratio (FER); (c) Protein efficiency ratio (PER).

3.3 Survival rate

The survival rate of *L. catla* after the completion of the 75 day feeding trials are illustrated in Figure 3. The result indicates that the treatments T2, T3, T4, T5, T6 and T8 achieved a maximum survival rate of 100%, which was statistically comparable to T1 (97.78±2.22%) and T7 (97.78±2.22%). The T0 has the lowest survival rate (95.56±2.22%), though it still represented a high overall survival. The survival was fulfilled, demonstrating the high level of health management and the favourable culture conditions.

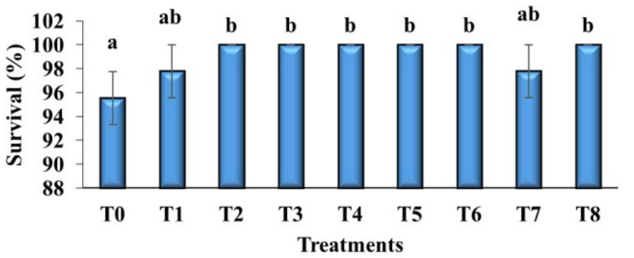

FIGURE 3 Survival rate of *Labeo catla* in different treatment groups.

3.4 Physico-chemical water parameters

Water quality parameters were monitored over a duration of 75 days at 15-day intervals. The results are summarized in Table 3. Temperature ranged from a maximum of 26.8°C–27.2°C to a minimum of 22.0°C to 23.4°C. The pH remained relatively stable and slightly alkaline, fluctuating between 7.4 and 7.9. This range is considered ideal for most freshwater fish. Optimal availability of oxygen for aquatic species was ensured by maintaining dissolved oxygen (DO) levels between 5.6 and 6.9 mg L^{-1}. Total Alkalinity ranged from 125 to 148 mg L^{-1} as $CaCO_3$, exceeding the minimum threshold of 120 mg L^{-1}.

4 | DISCUSSION

4.1 Growth performance

In the present study, higher growth was observed in T6, where experimental fish were fed with 10 g kg^{-1} of L-leucine and 0.4 g kg^{-1} of L-ascorbic acid when compared to the other treatments and the control group ($p < 0.05$). These results agree with the findings of Sultana *et al*. (2023), which revealed that dietary vitamin C at 400 mg kg^{-1} feed enhanced growth in *Labeo bata*. Similarly, Ibrahim *et al*. (2020) showed that vitamin C supplementation significantly improved the final body weight. The highest supplementation level (400 mg kg^{-1}) led to increased serum levels of vitamin C and growth hormone. Samuel *et al*. (2024) reported that in *Clarias gariepinus* supplementation of at optimum level resulted in significantly increased weight gain. It was also reported that dietary vitamin c also had positive effect on growth in *Labeo rohita* (Misra *et al*. 2007; Tewary and Patra 2007). Feng *et al*. (2023) reported that in juvenile yellow catfish, supplementation of L-leucine at an optimum level resulted in significantly higher growth. Leucine, being an indispensable amino acid for protein synthesis, plays an essential role in the growth and well-being of fish (Kim and Lee 2013; Zehra and Khan 2014). In the present study, higher level of dietary L-leucine did not improve the growth performance in *L. catla*. Reduced growth performance may be associated with an imbalance in the amino acid profile (Peres and Oliva-Teles 2007). It may also result from poor absorption or inefficient utilisation of certain amino acids, which can lead to toxic effects and physiological stress in fish (Zhou *et al*. 2020). Additionally, growth depression could be linked to antagonistic interactions among branched-chain amino acids (BCAAs). Such antagonism, well documented in mammals, typically occurs when leucine is present in excess relative to isoleucine and valine (NRC, 1993). However, evidence regarding BCAA antagonism in fish is limited and remains inconsistent across different species (Ahmed and Khan 2006). A low growth rate was observed in the control groups where experimental fish were fed with a leucine deficient diet. Previous research has shown that a deficiency of leucine in the diet disrupts the balance of branched-chain amino acids, leading to impaired intestinal digestive and absorptive enzyme activities, ultimately reducing growth performance (Wang *et al*. 2017). Therefore, the findings of the present study suggest that a combination dose of 10 g kg^{-1} of L-leucine and 0.4 g kg^{-1} of L-ascorbic acid may be helpful for increasing the growth rate in *L. catla*.

TABLE 3 Physico-chemical water parameters during the experiment.

Day	Temperature (°C)	pH	Dissolved oxygen (mg/L)	Alkalinity (mg/L as $CaCO_3$)
Day 0	26.0–26.5	7.4–7.6	5.8–6.1	125–135
Day 15	26.8–27.2	7.5–7.7	5.6–5.9	128–137
Day 30	25.4–26.3	7.6–7.8	5.9–6.2	130–140
Day 45	23.5–24.8	7.4–7.7	6.2–6.5	135–145
Day 60	22.0–23.4	7.7–7.9	6.4–6.8	138–148
Day 75	22.5–23.0	7.6–7.8	6.5–6.9	140–147

4.2 Feed utilisation

In the present study, improved feed utilisation was observed in T6, where experimental fish were fed with 10 g kg^{-1} of L-leucine and 0.4 g kg^{-1} of L-ascorbic acid when compared to the other treatments and the control group ($p < 0.05$). The results agree with the findings of Tewary and Patra (2007), who reported that dietary vitamin C supplementation improved growth performance and nutritional efficiency in *Labeo rohita*. Likewise, Misra *et al*. (2007) observed improved growth and physiological conditions in *Labeo rohita* fingerlings when diets were supplemented with vitamin C. Similar improvements in growth performance and utilisation of feed due to vitamin C supplementation have also been reported in other fish species such as Nile tilapia (Ibrahim *et al*. 2020) and common carp (Aboseif *et al*. 2022). L-Leucine is a branched-chain amino acid that plays a crucial role in regulating protein metabolism and improving nutrient utilization in fish. Dietary leucine has been reported to improve the feed utilisation in several fish species. Ahmed and Khan (2006) reported that dietary branched-chain amino acids significantly influenced growth performance and nutrient utilisation in *Cirrhinus mrigala*. Similarly, Wu *et al*. (2017) observed improved feed utilization and growth performance in black carp when diets were enriched with L-leucine. Furthermore, Feng *et al*. (2023) reported improved metabolic efficiency and growth performance in juvenile yellow catfish fed diets containing optimal levels of L-leucine. Therefore, the lowest FCR observed in treatment T6 in the current investigation may be related to the combined effect of L-ascorbic acid and L leucine, which likely enhanced protein metabolism, nutrient assimilation, and overall feed utilisation efficiency in *L. catla* fingerlings (Ahmed and Khan 2006; Misra *et al*. 2007; Tewary and Patra 2007; Wu *et al*. 2017; Feng *et al*. 2023).

4.3 Survival rate

After the completion of the 75-day feeding trials, survival rates remained high across all treatments ranging from 95.56 % to 100%. Statistical analysis revealed no significant differences in the survival rate between most of the treatment groups, indicating that the dietary supplementation of L-ascorbic acid and L-leucine did not have any adverse effect on the health of *L. catla*. However, given the consistently high survival observed across all groups, it is possible that the experimental conditions were highly optimal and did not provide a sufficiently challenging environment to detect potential differences in survival.

4.4 Physico-chemical water parameters

Throughout the experimental period, all monitored physico-chemical parameters (temperature, pH, dissolved oxygen, and total alkalinity were maintained within the optimal ranges required for *L. catla* culture. While temperature exhibited a slight gradual decrease consistent with seasonal variation, it remained within suitable threshold. The stability of pH (7.4–7.9) was supported by an alkalinity level exceeding the 120 mg L^{-1} threshold, indicating a robust buffering capacity within the system (Boyd *et al*. 2016). Furthermore, dissolved oxygen levels remained above the critical minimum of 5 mg L^{-1} (Ali *et al*. 2022). Maintaining these stable and optimal environmental conditions is important for the validity of the feeding trial. By ensuring that water quality remained consistent and favourable, we minimised the influence of external environmental stressors that could otherwise cofound fish growth and physiological responses. Consequently, the observed growth performance and feed utilisation in *L. catla* can be confidently attributed to the dietary supplementation rather than fluctuations in rearing environment.

5 | CONCLUSIONS

The finding of the present study indicates that dietary supplementation of L-ascorbic acid and L-leucine acid significantly enhance the growth performance, survival and feed utilisation in *L. catla* fingerlings. Fish fed with combined supplementation exhibited enhanced gain in weight and feed efficiency, indicating better nutrient utilisation. Among all treatments, the combination of 0.4 g L-ascorbic acid and 10 g L-leucine (T6) showed the most pronounced effects across all parameters, highlighting it as the optimal supplementation level. While this study demonstrates clear biological benefits, future research should incorporate an economic analysis to evaluate the cost-effectiveness of this supplementation, which is a critical consideration for practical development and commercial adoption of fish feed.

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ETHICAL APPROVAL

All applicable international, national and/or institutional guidelines for the care and use of animals were followed in this study.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Conceptualisation: AIB, SIY; Methodology & Experimental Design: AIB, SIY, NVR; Data analysis: AIB, AM; Manuscript preparation: AIB,HSR; Review & Editing: SIY, AIB.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available within the article.

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