



L-amino acids and their derivatives supplementation enhances growth and metabolic responses in *Labeo catla* (Hamilton, 1822)

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Manuscript history

Received 1 March 2026 | Accepted 14 August 2026 | Published online 16 October 2026

Citation

Prashanth BR, Aklakur M, Varghese T, Jayant M, Pailan GH, Deo AD, Roy U, Venkat CSS (2027) L-amino acids and their derivatives supplementation enhances growth and metabolic responses in *Labeo catla* (Hamilton, 1822). Journal of Fisheries 15(1): 151210. DOI: 10.17017/j.fish.1233

Abstract

Improving feed intake is a key strategy for enhancing the production efficiency of the culture system. The present work was conducted to evaluate the efficiency of selected amino acids and their derivatives as feeding stimulants and attractants on growth and physio-biochemical parameters of *Labeo catla*. Four hundred and eighty (30.95±1.86 g) fish were distributed in a completely randomised design for a 60-day feeding trial. Eight iso-nitrogenous (30%) and iso-caloric (375 Kcal 100g⁻¹) practical diets were formulated: control (no supplementation), Ala (1.5% supplemental alanine), Arg (1.25% supplemental arginine), Bet (0.5% supplemental betaine), Glu (1% supplemental glutamic acid), Gly (1% supplemental glycine), Hyp (1% supplemental hydroxyproline) and Lys (1.5% supplemental lysine). Dietary supplementation significantly ($p < 0.05$) improved feeding response, weight gain (WG), weight gain percentage (WG%), protein efficiency ratio (PER), average daily gain (ADG), and specific growth rate (SGR), with the highest growth performance observed in the Hyp group followed by Arg, Ala and Bet. The activities of digestive enzymes (amylase, protease and lipase) and metabolic enzymes (glucose-6-phosphate dehydrogenase, G6PDH, alanine aminotransferase, ALT; aspartate aminotransferase, AST) were significantly ($p < 0.05$) upregulated. Furthermore, supplemented diets elevated antioxidant enzyme profiles while reducing malate dehydrogenase and lactate dehydrogenase activity, indicating lower physiological stress. These findings demonstrate that functional amino acid addition in feed, particularly 1% hydroxyproline, 1.25% arginine, and 1.5% alanine, acts as an effective feeding stimulant. This offers a practical approach to formulate better feeds for *L. catla*.

Keywords: amino acids; antioxidant enzymes; digestion; feeding stimulants; *Labeo catla*; metabolism

1 | INTRODUCTION

The food from aquaculture is well recognised for its key

role in promoting food security and nutritional needs (Pradeepkiran 2019). With the stationary growth in cap-

ture fisheries (inland and marine), the production has remained around 90 million tons since 1990 (FAO 2022). However, the aquaculture sector continued to grow, and for the first time, it surpassed capture fisheries production. Furthermore, it is expected that fish and shellfish production using culture-based fisheries will reach 111 million tons by 2032 (FAO 2024). Aquaculture production can be further enhanced by implementing intensive culture technologies and utilizing higher-quality feed to increase fish growth (Ngoc *et al.* 2021). The growth of aquaculture, coupled with the rising cost of feed, has necessitated interventions in the feed manufacturing process and formulation. To produce cost-effective and nutritionally complete feed, the exploration of alternative feed ingredients, including various feed additives offers a promising approach.

Amino acids are added to the feed as additives, as they act as feed stimulants, enhancing growth by increasing feed intake of fish (Li *et al.* 2009). Previous research has demonstrated a significant improvement in digestive enzymatic activities such as protease, amylase and lipase upon addition of fish diets with specific amino acids (Li *et al.* 2009; Dar *et al.* 2019; Wang *et al.* 2022). These improvements in digestive efficiency led to better nutrient absorption and utilisation, ultimately promoting faster and healthier fish growth (Zhao *et al.* 2015; Jiang *et al.* 2016). According to Nguyen *et al.* (2020), the amino acid inclusion in aquafeeds reduces the reliance on traditional, high-cost protein sources, thus addressing economic and sustainability concerns. Thus, using amino acids as feed stimulants and growth enhancers presents a viable strategy for advancing aquaculture feed technology. This approach not only promotes the optimal health and growth of fish but also helps the aquaculture industry remain sustainable economically and environmentally (Hancz 2020).

Fish live in aquatic systems, where detecting food and early intake without much wastage are major concerns (Li *et al.* 2020). There is always a certain amount of feed that gets wasted because of problem in the detection of feed by the fish and hence contributes to water quality deterioration (Hancz 2020). Therefore, utilization of feed at the earliest is necessary to avoid the problem of leaching. Use of binders as feed additives will help to ensure that the feed is intact after placing in water body (Tacon 1987), but instead improving feeding response and optimising feeding frequency can be the most efficient way to reduce loss and improve growth of the animal cultured (Craig *et al.* 2017). The intervention in feed manufacturing by the addition of feeding stimulants in feed has opened a big niche of feed additives in the category of feed stimulants and attractants. Feed stimulants enhance the palatability and feed acceptance (Alemayehu *et al.* 2018).

Amino acids are mostly used and studied as feed

stimulants. These also play a prominent role in fish nutrition. Li *et al.* (2009) have reported crucial roles of certain AAs and their metabolites in regulating essential metabolic pathways related to feed intake, nutrient utilization, growth, immune response, behavior, larval development, reproduction, and the ability to cope with environmental changes and pathogens among different fish species. Although according to Adams and Johnsen (1986), the stimulation effect of different compounds in herbivorous fish and carnivorous fish generally vary but, according to Carr *et al.* (1996), in both groups, a common role of food sensing is performed by the AAs. There is a scarcity of research for species specific response of amino acids as an attractant in feed.

Labeo catla was selected for the experiment due to its easy availability, market demand and higher growth rate. *L. catla* production ranked fourth among cultivated finfish in 2022, contributing more than 4 million tons to global fisheries production (FAO 2024). Similarly, the Indian major carp's production in India has reached 6.736 MMT in the year 2022–23 (Handbook on fisheries statistics 2023). This study is aimed at understanding the effect of amino acids fortified feed in terms of feed intake, growth, metabolic and antioxidant responses of *L. catla*.

2 | METHODOLOGY

2.1 Experimental fish

Four hundred and eighty farm-reared advanced *L. catla* fingerlings (30.95 ± 1.86 g) of the Indian Council of Agricultural Research-Central Institute of Fisheries Education (ICAR-CIFE), Motipur, were procured and stocked in an FRP tank of 2000 L capacity. Fish were properly conditioned and acclimatised for 15 days after stocking. Water was exchanged daily at the rate of 20% using groundwater. During the acclimatization, the fish were fed commercial feed (30% CP) twice a day until satiation.

2.2 Experimental design

The experiment was conducted in p-lined rectangular ponds (14×14 square feet each) located in ICAR-CIFE, Motipur, India (GPS coordinates: latitude: 26.258489, longitude: 85.173947). The experiment consisted of eight treatments, viz., control, without any AA supplementation in the diet and experimental diets using Ala (1.5% supplemental L-alanine), Arg (1.25% supplemental L-arginine), Bet (0.5% supplemental betaine hydrochloride), Glu (1% supplemental L-glutamic acid), Gly (1% supplemental L-glycine), Hyp (1% supplemental L-hydroxyproline), Lys (1.5% supplemental L-lysine) in triplicates following the completely randomised design (CRD). The experimental trial was conducted for 60 days. Randomly, four hundred and eighty healthy fish were selected and distributed into eight groups with twenty-four ponds (20 fish per pond). The water quality parameters,

including temperature, pH, ammonia, nitrite, and nitrate were examined every week at the water and soil quality laboratory of the ICAR-CIFE Motipur Centre, according to APHA, (2005) standard techniques.

2.3 Experimental diets

The locally available ingredients (Table 1) were selected for fish feed preparation and proximate analysis (crude protein and ether extract) was performed following standard techniques (AOAC 2005). Eight experimental diets (around 30% crude protein) containing different amino acids were formulated and prepared (Table 2). Soybean meal, fish meal, and mustard oil cake were used as sources of protein. Fish oil and vegetable oils were the lipid sources, and rice bran, maize flour, and wheat flour were the carbohydrate sources. Choline chloride was also used in addition to the vitamin-mineral premix. The micronutrients, like vitamins and minerals were also added to the prepared feeds. Other miscellaneous ingredients, such as vitamin C, butylated hydroxytoluene (BHT) and carboxymethyl cellulose (CMC) were added to the diets.

TABLE 1 Proximate composition of locally available ingredients used to prepare feed.

Ingredients	CP ¹ %	EE ² %
Fish meal	52	5
Soybean meal	44	2
GNOC	45	9
Mustard oil cake	34	7
Wheat flour	12.1	2
Rice bran	12.5	2
Corn flour	10.3	2
Sunflower oil	0	100
Fish oil	0	100

¹CP, crude protein; ²EE, ether extract

2.4 Feeding trial

A feeding trial was carried out for 60 days from 15 May 2023 to 14 July 2023 at the Motipur centre of ICAR-CIFE, Bihar. During the experiment, the fish were given feed at 4% of body weight twice a day at 9:00 and 17:00 hours. The following parameters were calculated to understand feed utilization.

$$WG (\%) = (FW (g) - IW (g)) \times 100 / IW (g)$$

$$SGR = (\ln FW (g) - \ln IW (g)) \times 100 / \text{Feeding trial duration}$$

$$FCR = \text{Feed given (dry weight in g)} / WG (\text{wet weight in g})$$

$$PER = WG (g) / \text{Protein intake through feed (g)}.$$

Where WG is weight gain, FW is final weight of fish, IW is initial weight of fish, FCR is feed conversion ratio, and PER

is protein efficiency ratio.

2.5 Feeding response

The following equation was used to calculate the feeding response:

$$\text{Feeding response} = ((\text{Total feed given} - \text{Left over feed collected})) / (\text{Total feed given}) \times 100$$

Fifteen fish (mean weight of 30 g) per treatment in triplicate were stocked in plastic crates (100 L). Fish were acclimatized to the system for a week. Continuous aeration was provided and the optimum water quality was maintained. Commercial feed (30% CP) was provided twice a day during the period of acclimatisation.

The feeding response was studied by providing feed and collecting the remaining feed after 20 minutes of feeding. The leftover feed was dried and weighed. The study was conducted two times a day. The efficiency of each feed was analysed individually using this method and expressed in percentage. The aim was to understand the stimulation effect of amino acids in feed.

2.6 Proximate composition

To perform whole body composition analysis, sampling was done at the end of the feeding trial by using randomized sampling method in triplicate from each treatment. A similar procedure was used for experimental diets. The proximate analysis, which includes moisture, crude protein, crude lipid, crude fibre, total ash and free extract was estimated using the standard method of AOAC (2005).

2.7 Enzyme analysis

Following the feeding trial, fish were left unfed for a day before sampling. For enzymatic analysis, different tissues (liver, muscle and intestine) were collected and homogenised in sucrose solution (chilled condition) of 0.25 M using a homogeniser. Following homogenisation, centrifugation was done at 5000 rpm at 4°C for 10 minutes. Later, the supernatant was collected for analysis purposes. Lowry's method was used for estimating the total protein content of the above-mentioned tissue samples (Lowry *et al.* 1951). The activities of digestive enzymes *viz.* protease, amylase and lipase were estimated by standard methods of Drapeau (1976), Cherry and Crandell (1932) and Rick and Stegbauer (1974), respectively.

Protein metabolic enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities of the liver sample were assayed using the standard technique of Wooten (1964). The Lohr and Waller (1965) technique was used to measure the amount of G6PDH in the hepatic tissue. Assay of lactate dehydrogenase was done by following the Wroblewski and Ladue (1955) method. Malate dehydrogenase was estimated using a similar reaction combination, with the exception

of the substrate (1 milligram oxaloacetate millilitre⁻¹ of chilled triple distilled water) (Ochoa 1955).

Using the Misra and Fridovich (1972) technique, the SOD (superoxide dismutase) activity was calculated and represented as 50% suppression of epinephrine auto-

oxidation mg⁻¹ protein min⁻¹. Catalase activity was analysed according to Takahara *et al.* (1960). The nanomoles of H₂O₂ decomposed min⁻¹ mg⁻¹ protein was used to express the enzyme activity.

TABLE 2 Formulation and proximate composition of experimental diets.

Ingredients (g/kg)	Diets (treatments ¹)							
	C	Ala	Arg	Bet	Glu	Gly	Hyp	Lys
Fish Meal	50	50	50	50	50	50	50	50
Soybean meal	305	272	280	297	285	285	285	272
GNOC	195	190	188.5	190	190	190	190	190
Mustard oil cake	140	140	140	140	140	140	140	140
Wheat flour	90	100	100	100	100	100	100	100
Rice bran	110	110	110	110	110	110	110	110
Maize flour	39.1	52.1	48.1	37.1	44.1	44.1	44.1	52.1
Groundnut oil	10	10	10	10	10	10	10	10
Soyabean oil	20	20	20	20	20	20	20	20
Fish oil	10	10	10	10	10	10	10	10
Vit-Min mix ²	20	20	20	20	20	20	20	20
CMC	10	10	10	10	10	10	10	10
Choline	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
BHT	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin C	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Alanine	0	15	0	0	0	0	0	0
Arginine	0	0	12.5	0	0	0	0	0
Betaine	0	0	0	5	0	0	0	0
Glutamic acid	0	0	0	0	10	0	0	0
Glycine	0	0	0	0	0	10	0	0
Hydroxyproline	0	0	0	0	0	0	10	0
Lysine	0	0	0	0	0	0	0	15
Total	1000	1000	1000	1000	1000	1000	1000	1000
Proximate composition of the diet (on a % dry matter basis)								
Dry matter	91.65	91.76	91.46	91.1	91.76	91.59	91.65	91.34
Crude protein	29.78	30.83	30.58	30.1	30.17	30.01	29.9	29.47
Ether extract	7.23	7.19	7.09	7.01	7.34	7.21	7.34	7.11
Crude fibre	7.81	7.89	7.76	7.65	7.69	7.72	7.98	7.72
Nitrogen free extract	47.47	46.61	47.42	47.66	47.32	47.82	47.4	48.56
Total ash	7.71	7.48	7.15	7.58	7.48	7.24	7.38	7.14
Digestible energy	374.07	374.47	375.81	374.13	376.02	376.21	375.26	376.11

¹Treatments- C, 0% amino acid; T1, 1.5% Alanine; T2, 1.25% Arginine; T3, 0.5% betaine; T4, 1% glutamic acid; T5, 1% Glycine; T6, 1% Hydroxyproline; T7, 1.5% Lysine

²Composition of the Vitamin-mineral mixture (quantity/kg): Vitamin A, 7,00,000 IU; Vitamin D3, 70,000 IU; Vitamin E, 250 mg; Co, 150 mg; Cu, 1200 mg; I, 1500 mg; Mg, 6000 mg; K, 100 mg; Na, 5.9 mg; Mn, 1500 mg; S, 0.72%; Zn, 9600 mg; DL-Methionine, 1000 mg; Ca, 25.5%; P, 12.75%

2.8 Feed cost analysis

Feed cost analysis was used to summarise the economic value of adding amino acids in the diet of *L. catla*. The cost of experimental feed was calculated from the ingredient and amino acid prices based on their inclusion level. Finally, FCR was used to estimate the cost of feed involved in producing one kg of fish (Agbayani 2002).

Feed cost (Rs / kg) fish = FCR × Estimated cost (Rs / kg) feed [100 Rs = 1.06 USD]

2.9 Data analysis

For the analysis of the data, ANOVA (one-way analysis of variance) in SPSS 25.0 for Windows was used. To test the significant differences between the mean values of differ-

ent treatment groups, Duncan's multiple range test was used at a probability level of 5% ($p < 0.05$). The obtained data were expressed as $\mu \pm SE$, where μ is the mean and SE is standard error.

3 | RESULTS

3.1 Whole body composition of *Labeo catla*

No significant differences were observed in the carcass composition of *L. catla* among the nutrients, with crude protein level as an exception. Among the treatments, significant variation was demonstrated by crude protein levels ($p < 0.05$). Increased crude protein percentage in the body of *L. catla* was observed in Hyp, Ala and Arg-supplemented groups ($p < 0.001$), as shown in Table 3.

3.2 Feeding response, growth performance and nutrient utilisation

The significantly higher feeding response was observed

upon supplementation of amino acids in the diet when compared to the control diet. A significant increase in intake was observed in Hyp, Glu, Ala and Bet groups ($p < 0.05$). The Arg group also showed improved intake when compared to the control. The moderate increase was also observed in the Gly and Lys groups (Figure 1).

The effect of amino acids on growth and nutrient utilisation of *L. catla* during the experimental period is displayed in Table 4. In the Hyp group, a higher percentage in weight gain was observed, followed by the Arg and Ala groups. The control group, which received no additional amino acids, exhibited the lowest weight gain. A comparable pattern was observed in the case of SGR and PER. The group fed with hydroxyproline (Hyp) showed significantly the lowest FCR in comparison with other treatments ($p < 0.05$). Arg group also exhibited significantly lower FCR in comparison to the control group ($p < 0.05$).

TABLE 3 The whole-body proximate composition of *Labeo catla* of different experimental groups (on a % wet weight basis) reared for 60 days ($n = 3$).

Treatments ¹	Moisture	CP ²	TL ³	TA ⁴	TC ⁵
Control	74.28±0.31	14.99 ^a ±0.12	3.88±0.05	3.41±0.13	3.44±0.33
Ala	73.38±0.26	16.23 ^d ±0.15	4.07±0.04	3.52±0.15	2.80±0.27
Arg	73.46±0.19	16.12 ^{cd} ±0.11	4.06±0.12	3.37±0.14	2.92±0.16
Bet	74.03±0.32	15.78 ^{bc} ±0.10	3.95±0.04	3.52±0.13	2.71±0.14
Glu	74.16±0.29	15.62 ^b ±0.10	3.89±0.05	3.38±0.13	2.95±0.13
Gly	74.16±0.21	15.74 ^{bc} ±0.19	3.91±0.10	3.52±0.15	2.68±0.39
Hyp	73.83±0.23	16.08 ^{cd} ±0.11	3.92±0.05	3.63±0.14	2.54±0.31
Lys	74.02±0.31	15.38 ^b ±0.12	3.89±0.15	3.43±0.13	3.35±0.28
p-value	0.233	<0.001	0.623	0.871	0.264

¹Control, without any supplementation of amino acids in the diet; Ala, supplementation of 1.5% alanine; Arg, supplementation of 1.25% arginine; Bet, supplementation of 0.5% betaine; Glu, supplementation of 1% glutamic acid; Gly, supplementation of 1% glycine; Hyp, supplementation of 1% hydroxyproline; Lys, supplementation of 1.5% lysine. ²CP, Crude protein; ³TL, Total lipid; ⁴TA, Total ash; ⁵Total carbohydrate. Data are expressed as mean $\pm$ S.E

TABLE 4 Growth parameters and nutrient utilization of *L. catla* supplemented with dietary L-amino acids ($n = 3$).

Treatments	WG ¹ (%)	SGR ²	FCR ³	PER ⁴
Control	113.06 ^a ±2.77	1.26 ^a ±0.02	2.13 ^d ±0.05	1.57 ^a ±0.04
Ala	163.55 ^c ±2.01	1.62 ^{cd} ±0.01	1.47 ^{ab} ±0.02	2.27 ^c ±0.03
Arg	171.09 ^c ±2.24	1.66 ^d ±0.01	1.40 ^{ab} ±0.02	2.38 ^c ±0.03
Bet	151.85 ^b ±4.76	1.54 ^{bc} ±0.03	1.58 ^{bc} ±0.05	2.11 ^b ±0.07
Glu	141.20 ^b ±3.19	1.47 ^{bc} ±0.02	1.70 ^c ±0.04	1.96 ^b ±0.04
Gly	120.54 ^a ±6.67	1.32 ^a ±0.05	2.00 ^d ±0.11	1.67 ^a ±0.09
Hyp	174.77 ^c ±0.89	1.68 ^d ±0.01	1.37 ^a ±0.01	2.43 ^c ±0.01
Lys	112.60 ^a ±4.11	1.26 ^a ±0.03	2.14 ^d ±0.08	1.56 ^a ±0.06
p-value	<0.001	<0.001	<0.001	<0.001

¹WG%, Weight gain percentage; ²SGR, Specific growth rate (%/day); ³FCR, Feed conversion ratio; ⁴PER, Protein efficiency ratio. Data are expressed as mean $\pm$ S.E.

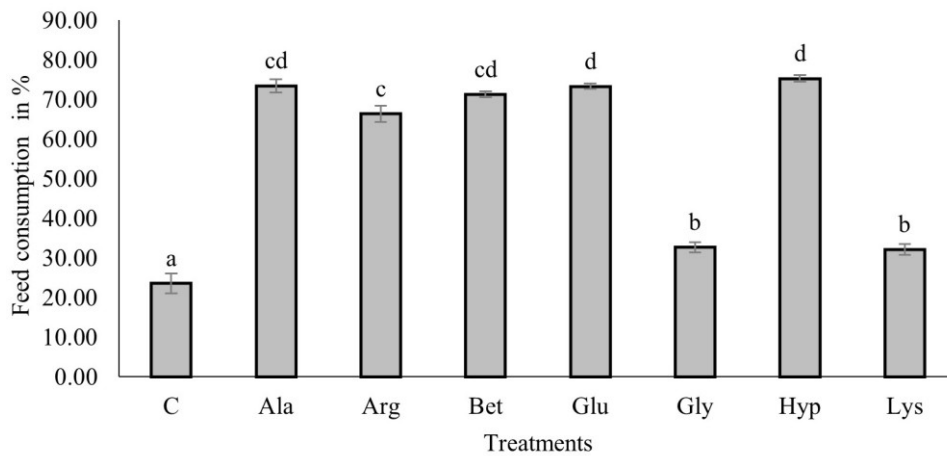


FIGURE 1 Feed consumption (%) of *Labeo catla* fed with different experimental diets. Bars with different superscripts on the top differ significantly ($p < 0.05$). Data are expressed as mean $\pm$ S.E, $n = 6$ (One-way ANOVA).

3.3 Digestive enzyme activities

Figure 2, 3 and 4 illustrate the impact of different amino acid supplementation on the digestive enzyme activities of *L. catla*. The results showed comparable ($p < 0.05$) variations in enzyme activities due to amino acid supplementation. The protein-digesting enzyme activity, *viz.*, protease in the intestine of fish, was higher in the amino acid-supplemented groups. The highest amylase and lipase

activities were noticed in the Ala group. The highest protease activity was reflected in the Arg group, which had been supplemented with 1.25% arginine. The control group exhibited the lowest protease and lipase activities, while the Lys group (1.5% lysine supplemented) demonstrated the lowest amylase activity. These results show the influence ($p < 0.05$) of amino acids on the enzymatic activities in *L. catla*.

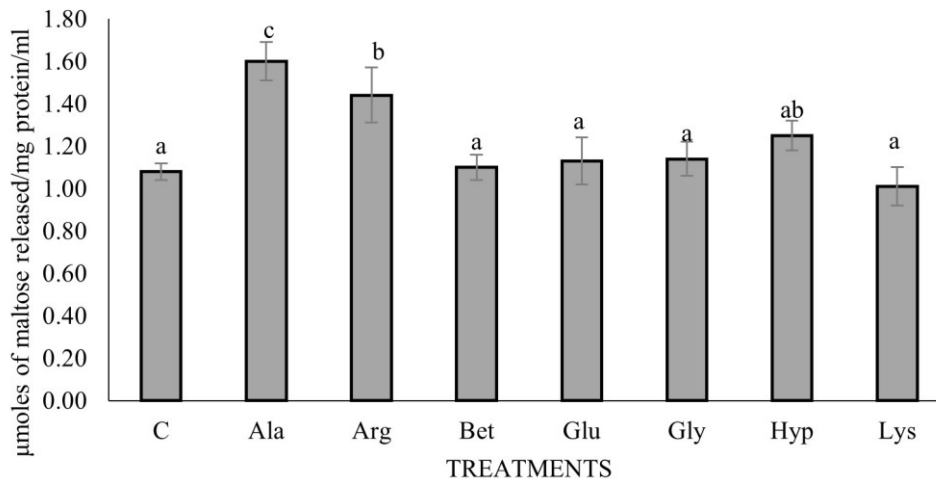


FIGURE 2 Amylase activity in *Labeo catla* fed with different experimental diets for 60 days. Bars with different superscripts on the top differ significantly ($p < 0.05$). Data are expressed as mean $\pm$ S.E, $n = 6$ (One-way ANOVA).

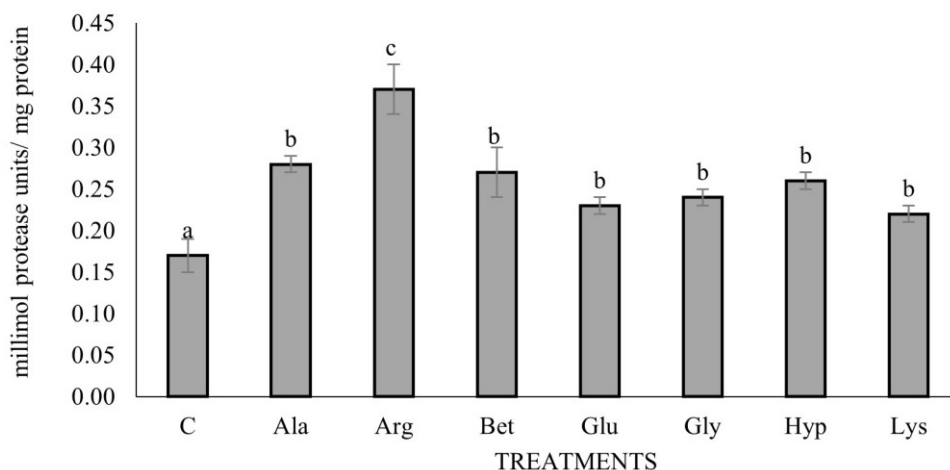


FIGURE 3 Protease activity in *Labeo catla* fed with different experimental diets for 60 days. Bars with different superscripts on the top differ significantly ($p < 0.05$). Data are expressed as mean $\pm$ S.E, $n = 6$ (One-way ANOVA).

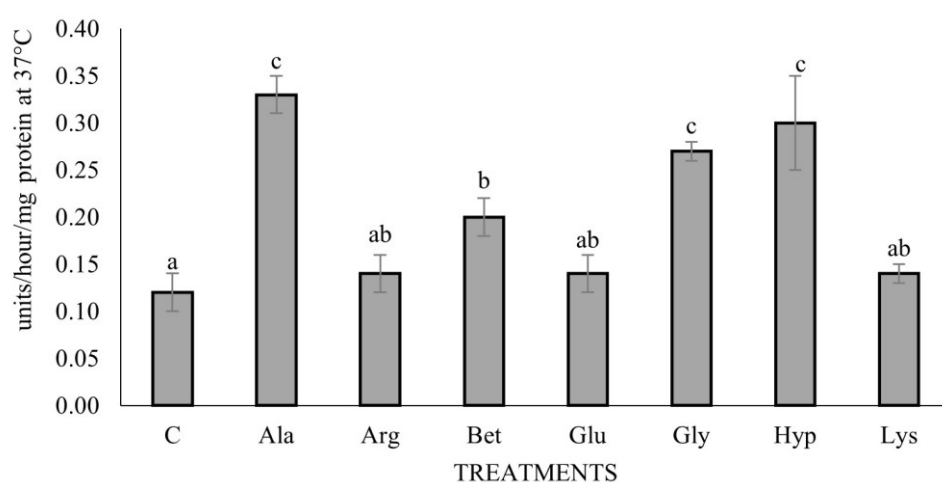


FIGURE 4 Lipase activity in *Labeo catla* fed with different experimental diets for 60 days. Bars with different superscripts on the top differ significantly ($p < 0.05$). Data are expressed as mean $\pm$ S.E, $n = 6$ (One-way ANOVA).

3.4 Metabolic enzyme activities

3.4.1 Protein metabolic enzymes: The data presented in Table 5 show the remarkable variation ($p < 0.05$) in the activity of AST in the liver of *L. catla*. The minimal activity was found in the control group. The supplementation with betaine (Bet) and Hydroxyproline (Hyp) significantly increased the activity of AST ($p < 0.05$). Arg, Glu, Gly and Lys groups showed elevated ($p < 0.05$) AST activity. Whereas the Ala group showed a moderate increase.

Among the different experimental groups, a significant variation was shown in the activity of ALT ($p < 0.05$). The control group recorded the lowest activity. AA supplemented groups showed a significant ($p < 0.05$) increase. In comparison to all the treatments, the Hyp group exhibited the highest activity, and in the groups of Arg, Bet and Glu a moderate increase was observed. Ala, Gly and Lys groups also showed elevated ($p < 0.05$) ALT

activity.

3.4.2 Carbohydrate metabolic enzymes: The activity of LDH upon inclusion of different amino acids in the diet of *L. catla* is shown in Table 5. In the control group, the highest ($p < 0.05$) activity was recorded. Glu, Gly, and Lys groups showed similar activity as the control group. Supplementation with alanine (Ala) and betaine (Bet) significantly ($p < 0.05$) reduced the activity of LDH. Moderate decrease in the activity was found in Arg and Hyp groups ($p < 0.05$).

Hepatic MDH activity of *L. catla* is presented in Table 5. Higher activity of MDH was noticed in the control group. Ala and Hyp groups exhibited similar activity to that of control. Gly, Arg, Bet, Glu and Lys groups showed significantly ($p < 0.05$) decreased MDH activity.

TABLE 5 Metabolic enzyme activities in the liver of *Labeo catla* supplemented with dietary L-amino acids.

Treatments ¹	AST ¹	ALT ²	LDH ³	MDH ⁴	G6PDH ⁵
Control	7.56 ^a ±0.20	6.89 ^a ±0.57	11.20 ^c ±0.2	0.66 ^c ±0.03	0.11 ^a ±0.006
Ala	11.92 ^b ±1.53	12.51 ^{bc} ±0.51	9.37 ^a ±0.37	0.70 ^c ±0.03	0.26 ^c ±0.027
Arg	13.33 ^{bc} ±1.16	14.38 ^{cd} ±0.23	9.90 ^{ab} ±0.05	0.53 ^b ±0.03	0.19 ^b ±0.015
Bet	14.92 ^c ±0.57	15.06 ^{cd} ±0.21	9.53 ^a ±0.17	0.48 ^b ±0.03	0.12 ^a ±0.002
Glu	12.78 ^{bc} ±0.92	14.34 ^{cd} ±1.17	11.25 ^c ±0.46	0.48 ^b ±0.03	0.11 ^a ±0.009
Gly	13.12 ^{bc} ±0.42	13.13 ^{bc} ±0.55	11.45 ^c ±0.11	0.35 ^a ±0.01	0.17 ^b ±0.016
Hyp	14.83 ^c ±0.63	16.23 ^d ±1.55	10.59 ^{bc} ±0.45	0.69 ^c ±0.01	0.17 ^b ±0.003
Lys	14.20 ^{bc} ±0.43	10.90 ^b ±0.09	10.88 ^c ±0.08	0.46 ^b ±0.02	0.13 ^a ±0.003
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

Data are expressed as mean $\pm$ S.E, $n = 6$ (One-way ANOVA); mean values in the same column with different superscripts differ significantly ($p < 0.05$). ¹AST, Aspartate aminotransferase activity expressed in nanomoles of oxaloacetate released/min/mg protein; ²ALT, Alanine aminotransferase activity expressed in nanomoles of sodium pyruvate released/min/mg protein; ³LDH, Lactate dehydrogenase activity expressed in Units/mg protein/min; ⁴MDH, Malate dehydrogenase activity expressed in Units/mg protein/min; ⁵G6PDH, Glucose-6-phosphate dehydrogenase activity expressed in units/min/mg protein at 37°C.

3.4.3 Lipogenic enzyme activity: G6PDH activity in the liver of *L. catla* exhibited significant ($p < 0.05$) differences across treatments. The group without amino acid supplementation had the lowest ($p < 0.05$) activity. Supplementation with alanine (Ala) increased ($p < 0.05$) G6PDH activity. Addition of Arginine (Arg) showed a significant increase. Betaine (Bet) supplementation also showed increased activity ($p < 0.05$). Glutamine (Glu) supplementation showed a moderate increase. Supplementation with glycine (Gly) did not show any significant variation. Hydroxyproline (Hyp) supplementation showed a slight decrease in activity. Lysine (Lys) supplementation significantly ($p < 0.05$) increased G6PDH activity.

3.5 Antioxidant enzymes

The SOD activity in the hepatic tissue sample of *L. catla* of different treatments is shown in Figure 5 and 6. The activity of the SOD enzyme was augmented ($p < 0.05$) in amino acid-supplemented groups, with the elevated activity ob-

served in Ala and Arg groups. The lowest ($p < 0.05$) SOD activity was observed in the control group.

A statistical variation and increased activity in amino acid-added groups was observed in the catalase enzyme activity in comparison with other treatments and the control group ($p < 0.05$). Among all the groups, the Ala group showed the highest activity, followed by the Arg group and Hyp group. Reduced catalase activity was observed in non-amino acids supplemented group ($p < 0.05$).

3.6 Feed cost analysis

The economic evaluation of experimental diets for *L. catla* is presented in Table 6. The feed cost required to produce 1 kg of fish was lower in most amino acid supplemented groups than in the control group. The lysine fed group is an exception. Among the supplemented diets, Ala, Arg, Hyp and Bet showed the lowest feed cost per kilogram of fish produced.

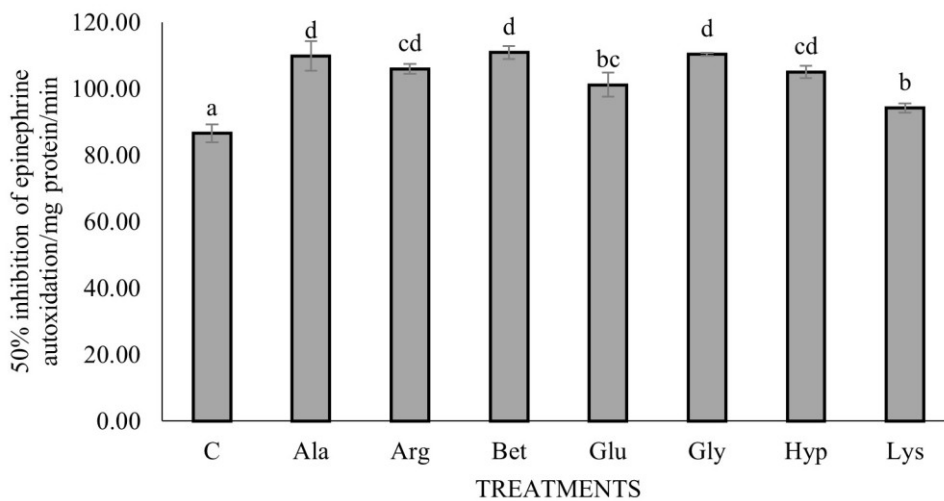


FIGURE 5 SOD activity in the liver of *Labeo catla* fed with different experimental diets for 60 days. Bars with different superscripts on the top differ significantly ($p < 0.05$). Data are expressed as mean $\pm$ S.E, $n = 6$ (One-way ANOVA)

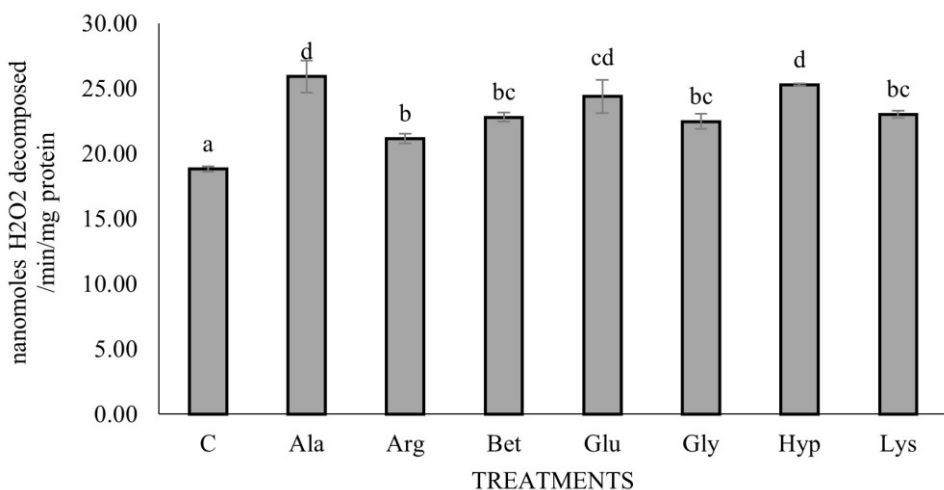


FIGURE 6 Catalase activity in the liver of *Labeo catla* fed with different experimental diets for 60 days. Bars with different superscripts on the top differ significantly ($p < 0.05$). Data are expressed as mean $\pm$ S.E, $n = 6$ (One-way ANOVA)

TABLE 6 Economic analysis of experimental diets.

Feeds	Base formula cost (Rs/Kg) feed	Cost of additive (Rs/Kg)	Added cost (Rs/Kg) Feed	Estimated cost (Rs/Kg) Feed	FCR	Feed cost (Rs/Kg) Fish
Control	49.25	0	0	49.25	2.13	104.9025
Ala	49.25	300	4.5	53.75	1.47	79.0125
Arg	49.25	610	7.625	56.875	1.4	79.625
Bet	49.25	400	2	51.25	1.58	80.975
Glu	49.25	215	2.15	51.4	1.7	87.38
Gly	49.25	175	1.75	51	2	102
Hyp	49.25	950	9.5	58.75	1.37	80.4875
Lys	49.25	140	2.1	51.35	2.14	109.889

100 Rs = 1.06 USD.

4 | DISCUSSION

Feeding stimulants and attractants can play an important role in reducing the environmental impact caused by uneaten feed. At the same time, feeding stimulants can improve the growth performance of the animal through improvement of the palatability of the feed (Papatryphon and Soares 2000). In the present experiment, the amino acid supplementation resulted in both enhancement of feeding and growth as per the above hypothesis.

4.1 Feeding response, growth performance and nutrient utilisation

The supplementation of amino acids in the diet of *L. catla* improved the feeding response and growth performance of the fish. A similar effect was observed by Li *et al.* (2022) upon addition of amino acids as attractant in the diet of *Cyprinus carpio* var. Further, an improvement in the growth of salmon due to increased feed intake was observed when the diet was supplemented with AA (Kousoulaki *et al.* 2013). However, different amino acid supplementation had varying effects on feeding. The highest effect was observed in Hyp, Glu, Ala and Bet groups ($p < 0.05$). A similar result was observed with hydroxyproline supplemented to *Larimichthys crocea* (Wei *et al.* 2016), glutamic acid to *Micropterus salmoides* (Jiang *et al.* 2024), betaine to grouper (Lim *et al.* 2016) and alanine along with other amino acids to *C. carpio* var (Li *et al.* 2022). Increased feeding could make more nutrients available for fish and can promote fish growth.

It was observed that growth performance was generally enhanced due to dietary amino acids. However, different amino acids had varying growth performances, as observed in the present study. The hydroxyproline-supplemented group had better growth, followed by arginine, alanine and betaine. Similarly, when hydroxyproline was supplemented in the diet of *Siniperca chuatsi*, *Carassius auratus*, and *Nibea coibor*, an improvement in growth performance and nutrient utilisation was observed (Rong *et al.* 2020; Cao *et al.* 2022; Feng *et al.* 2022). The improved growth was also observed with the supplementation of arginine in *Oncorhynchus mykiss*,

betaine in *Labeo rohita*, alanine in *O. mykiss*, glutamic acid in *Pagrus major* and glycine in *C. carpio* (Ozyurt and Polak 2001; Dar *et al.* 2019; Tola *et al.* 2019; Wang *et al.* 2022; Abbasi 2023). Hence, it can be interpreted that amino acids serve a dual role as both feeding stimulants and nutrients in *L. catla*, thereby enhancing growth performance.

4.2 Whole body composition of *L. catla*

The chemical composition of *L. catla* of different treatments did not show any variation ($p > 0.05$) except for the protein content ($p < 0.05$). However, a similar result to the present research has been observed by Li *et al.* (2022). Li *et al.* (2022) observed a significant change in the crude protein content of the whole fish body by the addition of complex amino acids 1.6% (alanine, arginine and glycine). In *C. carpio*, an improved protein synthesis efficiency was observed when the feed was supplemented with a coating of lysine (de la Higuera *et al.* 1998). The increased crude protein level was observed mainly in alanine, arginine and hydroxyproline-supplemented diets. Similarly, in a previous study, Rong *et al.* (2020) revealed that the crude protein content of the whole body was increased with dietary hydroxyproline, while other parameters remained unaffected. The improvement in crude protein with supplementation of dietary arginine in *O. mykiss* (Wang *et al.* 2022) and betaine in *Carassius auratus gibelio* (Dong *et al.* 2018) coincides with the present study.

4.3 Digestive enzyme activities

The key enzymes of digestion, like amylase, protease and lipase, are good parameters to understand the digestive capacity in fish (Gisbert *et al.* 2009). The result showed a swift increase in digestive enzyme activity of *L. catla* when fed with an amino acid-supplemented experimental diet. The improved activities of these enzymes provide a valid explanation for the better growth performance of the experimental fish. Similarly, according to Dar *et al.* (2019), elevated amylase and protease activities were observed in *L. rohita* when supplemented with betaine.

Similarly, Wang *et al.* (2022) reported significant improvement in amylase and lipase activity in *O. mykiss* when supplemented with arginine in the diet.

4.4 Metabolic enzyme activities

The aspartate and alanine aminotransferases play a significant role in the interconversion of amino acids, and they indicate either gluconeogenesis activation or body protein synthesis (Knox and Greengard 1965; De Silva and Anderson 1994). Enhanced aminotransferase activities can indicate both the breakdown of amino acids for fulfilling energy requirements or the anabolic synthesis of non-essential amino acids, depending upon the availability of non-protein energy sources (Chuphal *et al.* 2024). In the present study, both ALT and AST were studied in the muscle and liver. An increase in the aminotransferase activity was observed with the amino acid supplementation in the diets. Parallel results were observed when lysine and betaine were supplemented in *L. rohita* (Dar *et al.* 2019). This study explains the improvement in dietary protein utilisation, as well as improved protein synthesis and hence growth.

Lactate dehydrogenase is an NADH-dependent enzyme and is responsible for the reduction of pyruvate to lactate and back. It can act as an indicator of anaerobic respiration (Murrey *et al.* 2000). The increase in LDH activity may be due to the production of substrate (Lactate) required for gluconeogenesis (Chatterjee *et al.* 2006). In the following experiment, with the addition of some of the AAs, a reduction in LDH activity was observed, whereas some AAs showed parallel activities with the control. This may be due to active feeding and the reduction of stress in the animal. Malate dehydrogenase (MDH) converts malate to oxaloacetate in the TCA cycle, and its activity is elevated during glycogenesis from amino acids (Dey 1984). MDH activity of some amino acid-supplemented groups is in parallel with the control group, whereas certain groups showed reduced activity. Similar results were observed in LDH and MDH activities upon supplementation of betaine in *L. rohita* (Kumar *et al.* 2012). There isn't much research on how LDH and MDH behave when amino acids are added as feeding stimulants.

G6PDH is considered to be the essential enzyme in the pentose phosphate pathway (PPP). This pathway helps in producing NADPH. Then NADPH helps in the synthesis of FA and further in converting GSSG (glutathione disulfide) to GSH (glutathione) in GPx (glutathione peroxidase) through GPx (glutathione peroxidase) activity (Lehninger 2002). Thus, it is depicted as a lipogenic enzyme. Here in this study, the G6PDH significantly increased with the dietary supplementation of amino acids in the diet of *L. catla*. This result may be attributed to the role of these additives in fatty acid synthesis (Liang *et al.* 2017; Liao *et al.* 2024) or their potential involvement in

the induction of lipogenesis-related hormones.

4.5 Antioxidant enzymes

The antioxidant enzymes have a direct correlation with the health status of fish (Zhou *et al.* 2014). One of the main enzymes in a biological system's defense mechanism against lipid peroxidation is SOD, which transforms active oxygen molecules into non-toxic substances (Zimmermann *et al.* 1973). Superoxide dismutase and catalase are present in fish and are responsible for scavenging reactive oxygen free radicals (Pastor *et al.* 1988). The study indicated increased SOD (superoxide dismutase) activity and CAT (catalase) activity in the liver, with amino acid supplementation in the diet. A parallel study on *O. mykiss* also observed increased antioxidant properties when the diet included arginine supplementation (Wang *et al.* 2022).

Further, Li *et al.* (2022) observed improved SOD and CAT activity when using an AA mixture as an attractant. Hu *et al.* (2010) also reported that addition of glycine in the diet of *Litopenaeus vannamei* can significantly improve the antioxidant enzyme activities. The increased growth along with enhanced SOD and CAT activity is observed in the present study; hence, amino acid addition improved the antioxidant capacity of fish along with optimum growth in the fish.

4.6 Feed cost analysis

Feed cost is the highest operational expense in aquaculture and hence it determines the economic feasibility of dietary interventions (Mohamad *et al.* 2026). In the present study, the lower feed cost required to produce 1 kilogram of fish in most amino acid supplemented groups indicates improved feed utilization efficiency (Stone *et al.* 2024). The economic evaluation was based on FCR, indicating diets with lower FCR required less feed to produce same quantity of fish, thereby reducing the production cost (Onomu and Okuthe 2024). Among the dietary treatments, supplementation with alanine, arginine, hydroxyproline and betaine showed the lowest feed cost per kilogram of fish produced. Overall, the analysis clearly demonstrates the superior cost-effectiveness of production with stimulants. However, the additional economic benefit in the field may arise from reduced costs associated with water quality maintenance.

5 | CONCLUSIONS

The rising demand for aquaculture products has made it vital to boost fish production by utilizing intensive culture technologies and enhancing fish development by using higher quality diets. The present research evaluated the stimulative effect of selected amino acids and their derivatives on the performance of *L. catla*. The results of growth parameters, digestive, metabolic and antioxidant

enzyme activities have proved the stimulative effect of selected amino acids and their derivatives. From the study, it can be observed that supplementation of amino acids improves feeding response and growth performance in *L. catla*. The best effect was observed with hydroxyproline (1%), arginine (1.25%) and alanine (1.5%) supplementation in feed. It is a beneficial study because studies on individual amino acids as attractants on carp are very scarce. However, the assessment of the combination of these compounds and their impact has to be explored. Similarly, this study can be done over a long time in a large water body where feed detection is a major problem. The findings from this study on the attraction and stimulation of feed intake in *L. catla* suggest that similar experiments could be conducted with different compounds with similar properties and extended to other carp species as well.

ACKNOWLEDGEMENTS

The authors of this study acknowledge the Director, ICAR-CIFE, Mumbai, for the support in conducting the research.

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 authors affirm that they have no known financial or interpersonal conflicts that would have seemed to have an impact on the research presented in this study.

AUTHORS' CONTRIBUTION

B.R. Prashanth: Data curation, Investigation, Software, Writing – original draft; Md Aklakur: Conceptualization, Supervision, Writing – review & editing; Tincy Varghese: Writing – review & editing, Formal analysis; Manish Jayant: Supervision, Investigation, Writing-review & editing; G.H. Pailan Writing – review & editing; Ashutosh D. Deo: Writing – review & editing; Udipta Roy: Formal analysis, Writing – review & editing; Chundru Sri Sai Venkat: Formal analysis, Writing – review & editing.

AI-USE STATEMENT

The authors did not use any AI to generate, analyse or interpret data in the following study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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