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Exploring the potential of *Averrhoa carambola* extract for enhancing growth and immunity in *Labeo rohita* in hill aquaculture of North-east India

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Abstract

The growth, immunity and overall condition of fish in hill ecosystems remain suppressed due to prolonged cold climates. To address this issue, we assessed the effect of feeding fish (*Labeo rohita*; 6.41±0.34 g 6.17±0.34 cm) with a diet containing *Averrhoa carambola* var. sweet large extract as feed additive. The study was undertaken in 1 m^3 cemented cisterns, with 30 fish per tank. They were fed daily with diets containing different concentrations of carambola extract (CE): 0% (control group), 2 and 4%, equivalent to 3% of their biomass. Water quality parameters were assessed fortnightly, while monthly evaluations measured fish growth attributes. Fish were sampled on 15th, 30th and 60th day of the feeding trial for analysis of growth and blood parameters. Results showed a significant increase in specific growth rate (SGR), with a 49.5% boost in the group fed with CE-incorporated feed. Survival rates ranged from 86.7 to 88.4% for fish fed with CE, compared to 78.4% for the control group. Fish fed with 4% CE displayed positive allometric growth. Blood analysis revealed increased RBC and WBC counts, higher haemoglobin and total protein levels, and decreased glucose and cholesterol content. Serum glutamic oxaloacetic transaminase (GOT) and glutamic puruvic transaminase (GPT) activity remained normal. The optimal results were observed with a 4% CE inclusion in the diet. In conclusion, including carambola extract in fish feed effectively improved fish growth, condition and immunity. These findings are valuable for enhancing aquaculture strategies in hill ecosystems.

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Introduction

Aquaculture plays a significant role in the economic and livelihood development in hilly regions of North-east India (Kakati, 2019). However, hill aquaculture is relatively less productive compared to aquaculture that is practiced in plain lands (Das *et al*., 2018). The harsh cold climate in hill ecosystems adversely affect fish growth, immunity and disease resistance. In the hilly regions of North-eastern region (NER) of India, including Meghalaya, Sikkim, Arunachal Pradesh, Nagaland and Manipur, the annual mean water temperature rarely exceeds 20°C (Das *et al*., 2012) and during the winter season (late November to February), the water temperature drops rapidly from 22 to 6°C, causing significant stress to fish and other aquatic organisms. This unfavourable thermal condition results in poor fish growth and survival in hills.

Carambola (*Averrhoa carambola*) fruit possesses a plethora of health benefits, owing to its remarkable phytochemical composition (Lakmal *et al*., 2021). In the realm of Indian Ayurvedic science, carambola has been revered for its multifaceted therapeutic properties, such as anticancer, anti-inflammatory, antioxidant, hypoglycemic, hypolipidemic, hepatoprotective, and cardioprotective effects (Lakmal *et al*., 2021). While a wealth of research has explored the efficacy of various botanical products in fish health management (Sahu *et al*., 2007; Sahu *et al*., 2008; Sharma *et al*., 2010; Jiang *et al*., 2012; Talpur and Ikhwanuddin, 2013; Yogeshwari *et al*., 2015; Kumar *et al*., 2018; Kumar *et al*., 2021; Debnath *et al*., 2024), investigations specifically centered on carambola remain scanty. Nonetheless, based on the formidable phytochemical profile of carambola, it is reasonable to postulate its potential as a promoter of fish growth and immunity (Dwinanti *et al*., 2021). Prior fragmented studies have alluded to the immunomodulatory effects of carambola in mice and fish (Roy and Rv, 2011; Wahab *et al*., 2014; Dwinanti *et al*., 2021). Moreover, it has exhibited antibacterial properties, causing disruption to bacterial cell walls, and exhibited promising efficacy in combating motile Aeromonas Septicemia, a bacterial disease afflicting fish (Agustina *et al*., 2017). Nevertheless, these isolated investigations fall short of providing conclusive evidence regarding the efficacy of carambola in bolstering fish growth and immunity, particularly in the challenging terrain of hill ecosystems.

Given the paucity of information regarding the role of carambola in augmenting fish growth and immunity, the present study was conducted within the agro-climatic confines of Meghalaya, North-east India, employing the Indian major carp *Labeo rohita* (rohu) as the model fish species. The primary objective of this study was to ascertain the impact of carambola extract on various parameters of fish health.

Materials and methods

Experimental set-up

The research was conducted in Meghalaya, a state located in the North-eastern Region (NER) of India, within the geographical coordinates of 21°05' to 29°50'N and 85°05' to 97°05'E. The study site was situated at an elevation of approximately 950 m above the mean sea level, characterised by a typical cold to warm pre-humid climate with an annual rainfall exceeding 2000 mm. The air temperature varied between 11 and 26°C, while the water temperature ranged from 10 to 24°C. The research was carried out from February to March 2022 in cemented tanks, with each tank measuring 1 cubic meter (1.0 x 1.0 x 1.0 m) in size. Prior to commencing the study, a meticulous cleaning and sanitisation process was carried out in the tanks. This involved employing a solution of potassium permanganate ($KMnO_4$) at a concentration of 5 mg l^{-1}. Subsequently, the tanks were filled with tap water and left undisturbed for a period of two days to attain water stability. Afterward, fingerlings of *L. rohita,* collected from the farm of ICAR Research Complex for NEH Region (ICAR-RC NEHR) were introduced at 30 nos. of fish per tank, after acclimation over the course of a week. During the stocking process, the average size of the fingerlings was determined to be approximately 6.41±0.34 g, with a length of 6.17±0.34 cm. Within 24 h of stocking, the fishes were provided with experimental feeds.

Carambola (*Averrhoa carambola* var. sweet large)

Carambola was obtained from the Horticulture section of the ICAR-RC NEHR, Meghalaya. The average weight of the fruit was 58.25±3.52 g. The moisture, protein, lipid, crude fibre, calcium, phosphorus, iron, magnesium and vitamin C content of the fruit were determined using standard protocols and found to be 90.8, 1.1, 0.26, 1.02, 5.5, 16.2, 1.2, 13.2 and 22.6 mg%, respectively. For the experiment, fresh carambola extract (CE) was prepared by squeezing the fruit and straining it through a sieve. The extract was then evaluated at predetermined concentrations for the experiment.

Preparation of experimental feeds and feeding

Three experimental feeds were formulated incorporating carambola extract (CE) at different concentrations: 0% (control), 2 and 4% (Table 1). Rice bran (RB), mustard oil cake (MOC) and fish meal (FM) that were obtained from the local market served as the basic feed ingredients. Initially, the basal ingredients were thoroughly mixed with water and cooked in a pressure cooker. After cooling the mixture to approximately 40°C, vitamin-mineral mixture and cod liver oil were added. Fresh carambola extract was then incorporated into the feed mixture. The quantity of CE listed in Table 1 represents the weight of the liquid extract (20 and 40 g for the 2 and 4% treatments, respectively). The liquid extract was thoroughly mixed with other ingredients after cooking, to preserve its nutritional properties. An equivalent amount of water (40 g) was added to the control diet to maintain similar moisture content across all treatments.

The mixtures were subsequently passed through a hand pelletiser to form pellets measuring 1 mm in diameter. These pellets were then dried in a hot air oven at 40°C for two days and stored in airtight containers for future use. After preparation, the feeds were analysed for proximate composition using the methods outlined in AOAC (1980). The feeds exhibited a crude protein content ranging from 24.3 to 24.7% and a crude lipid content ranging from 6.5 to 6.8% (Table 1). Fish received the experimental feeds daily at a feeding rate of 3% of their biomass, with tanks being aerated at regular intervals throughout the study period. Additionally, a 1/3rd to 2/3rd exchange of tank water was carried out on a weekly basis.

Weekly water exchange proved sufficient to maintain low ammonia levels (0.37-0.75 ppm). Daily management included removal of excess feed and faecal matter using a fine mesh net, which, combined with moderate stocking density, ensured optimal water quality throughout the experimental period.

The experimental design followed a completely randomised design (CRD), with a total of nine tanks being utilised to evaluate the three

Table 1. Composition of experimental fish feeds prepared with the inclusion of CE

Ingredients	Control	2% CE	4% CE
RB (g)	600	580	560
MOC (g)	200	200	200
FM (g)	140	140	140
Wheat flour (g)	40	40	40
Vitamin-mineral mix (g)	15	15	15
Cod liver oil (g)	5	5	5
CE (g)	0	20	40
Total (g)	1000	1000	1000
Proximate analysis (%)			
Moisture	12.2±0.4	12.5±0.5	12.5±0.3
Crude protein	24.7±0.8	24.2±0.6	24.3±0.7
Crude lipid	6.8±0.3	6.5±0.4	6.5±0.2
Total ash	7.5±0.2	7.4±0.3	7.5±0.2
Crude fiber	48.8±1.2	49.4±1.5	49.2±1.3

Vitamin-mineral mix composition (per kg): Vitamin A 2,500,000 IU; Vitamin D_3 500,000 IU; Vitamin E 25,000 IU; Vitamin K_3 1,000 mg; Vitamin B_1 5,000 mg; Vitamin B_2 5,000 mg; Vitamin B_6 2,000 mg; Vitamin B_{12} 6 mcg; Calcium pantothenate 10,000 mg; Nicotinamide 25,000 mg; Folic acid 1,000 mg; Calcium 750 mg; Phosphorus 500 mg; Manganese 27,000 mg; Iodine 1,000 mg; Iron 7,500 mg; Zinc 15,000 mg; Copper 2,000 mg; Cobalt 450 mg.

feeding treatments in triplicates. The duration of the experiment spanned 60 days.

Analysis of water quality parameters

Water quality parameters, including temperature, dissolved oxygen (DO), total dissolved solids (TDS), pH and total ammonia, were regularly tested from each tank using the standard methods as described by Clescer *et al*. (1998). The testing was conducted between 08:00 and 09:00 hrs. Water temperature, DO, pH and TDS were measured using digital thermometer, DO meter (Make: Lutron PDO-519), pH meter (Make: Eutech Instruments PCSTestr 35) and TDS meter (Make: TDS-3) respectively. Total ammonia nitrogen (TAN) levels were analysed using ammonia testing kit (Make: API, USA). These instruments and kits were used in accordance with their respective manufacturers' instructions.

Assessment of fish growth and survival

On the 15th, 30th and 60th day post-feeding, ten fish (n=10) were randomly selected from each treatment tank for measurement and evaluation. The total length (TL) of each fish was measured to the nearest centimeter (cm) using a calibrated measuring scale and their weight was determined to the nearest 0.1 gram (g) using a precision analytical balance (Shimadzu, Model ATX224). Upon completion of the 60-day experimental period, all fish were harvested by completely draining the tanks. The total number of surviving fish was counted to calculate the survival rate. Growth performance and survival were assessed using the following standard formulae:

Weight gain (g) = Final weight (g) - Initial weight (g)

Specific growth rate (SGR, % per day) = [(Ln (Final weight) - Ln (Initial weight)) / Days of experiment] × 100

Survival rate (%) = (Number of fish harvested / Number of fish stocked) × 100

Assessment of condition of fish

At the end of the 60-days experiment, all fish were harvested, and their total length (TL) in millimeters and weight (W) in grams were measured. The statistical relationship between TL and BW was determined using the equation proposed by Le Cren (1951): $W = aL^b$, where 'a' represents the intercept and 'b' represents the slope. To calculate the values of 'a' and 'b', a linear regression analysis was performed on the natural logarithmic transformed equation: $Ln(W) = Ln(a) + b\ Ln(L)$. The coefficient of determination (r^2) was estimated from the linear regression analysis and indicates the degree of association between the length and weight of the fish. The coefficient 'R' is equal to the square root of r^2. The condition of the fish was determined based on the regression parameter 'b'.

In addition to the length-weight relationship, Fulton's Condition Factor (K) was calculated to further assess the well-being of the fish. The condition factor was determined using the formula: $K = (W \times 100)/L^3$, where W represents the weight of fish in grams and L represents the total length in centimeters (Nash *et al*., 2006). This index provides a standardised measure of fish health and nutritional status, with higher values indicating better condition.

Collection of fish blood and serum separation

On the 15th, 30th and 60th day of the feeding trial, ten fish (n=10) were randomly selected from each tank using a triangular net and then anesthetised using MS-222 solution at 0.1 ppm concentration. Five fish were utilised for blood collection, while the remaining five were used for serum collection. Blood was collected from the caudal vein employing a syringe. The collected blood was then transferred into heparinised Eppendorf tubes and the tubes were gently shaken. These blood samples were subsequently utilised for the analysis of red blood cell (RBC) and white blood cell (WBC) counts as well as haemoglobin (Hb) levels. For serum separation, the sampled blood was transferred into non-heparinised Eppendorf tubes (1.5 ml each) . The blood samples were allowed to clot for 3-4 h. Serum was separated from the clotted blood by subjecting the tubes to centrifugation at 3000 *g* for five minutes. The separated serum was then stored at a temperature of -20°C for subsequent analysis. The serum samples obtained were used for the analysis of various parameters, including glucose, total protein, total cholesterol levels, as well as for the activity of enzymes such as glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT).

Analysis of blood/serum parameters

To estimate the RBC and WBC counts, RBC diluting fluid and WBC diluting fluid from Qualigens Fine Chemicals, India, were utilised following the method described by Gupta *et al*. (2008). Hb level was determined using the cyanmethemoglobin method, as outlined in Gupta *et al*. (2008). For the estimation of glucose levels, a glucose diagnostic kit (Crest Biosystems, India) based on glucose-oxidase peroxidase (GOD/POD) method, developed by Trinder (1969) was employed. The serum protein level was estimated using a total protein kit utilising the Biuret method, as described by Gornall *et al*. (1949). The kit used for this analysis was manufactured by Crest Biosystems, India. The activity of GOT and GPT was estimated using diagnostic kits (Crest Biosystems, India) specifically designed for GOT and GPT. These kits followed the method developed by Reitman and Frankel (1957).

Statistical analysis

The data were analysed through statistical package of social science (SPSS; Version 21.0) for Windows and presented as Mean±Standard deviation (SD). The statistical analysis involved one-way analysis of variance (ANOVA) and comparison of means following Least Square Design (LSD) at 5% level of significance ($p \leq 0.05$).

Results and discussion

Water quality parameters

The water quality parameters and their respective mean values are presented in Table 2. The water temperature ranged from 21.15 to 22.05°C, DO varied between 7.25 and 8.15 ppm, pH ranged from 7.5 to 8.0, TDS measured between 78 and 88 ppm and TAN levels ranged from 0.37 to 0.75 ppm. These findings align with previous studies conducted on fish culture in Meghalaya (Majhi and Das, 2014; Das *et al*., 2018; Das *et al*., 2021). The water temperature reflects the typical hill climate in the region (Majhi *et al*., 2006). The low ammonia content in water can be attributed to the regular practice of water exchange in the tanks (Debnath *et al*., 2015).

Table 2. Water quality parameters in different treatment tanks

Feed	Temperature (^{0}C)	DO (ppm)	pH	TDS (ppm)	Total Ammonia-N (ppm)
Control	21.25±0.25	7.25±1.25	8±0	86±22	0.75±0.25
2% CE	21.25±0.05	7.85±0.85	8±0	85.5±29.5	0.375±0.13
4% CE	21.15±0.05	7.85±1.55	7.5±0.5	78±6	0.375±0.125

Fish growth and survival

Water temperature constitutes a pivotal abiotic determinant that profoundly impacts the growth trajectory and longevity of fish (Majhi *et al*., 2013). A preceding investigation carried out in the identical locale of India's NER revealed that suboptimal water temperature evoked stress responses and exerted discernible influences on the somatic growth of *Channa stewartii* (Das and Majhi, 2015). Paradoxically, in the current study, despite the perpetuation of relatively low water temperature within the range of 21.1 to 21.2^{0}C, a notable enhancement in fish performance was observed upon integrating CE into their dietary regimen. Plausible causative factors for this positive outcome encompass the presence of essential vitamins, vital minerals, and potent antioxidants within CE, which can potentially engender favourable repercussions on fish growth and overall health. These vital nutrients, when assimilated into the diet, possess the potential to exert beneficial effects on fish growth and bolster immune competency.

The findings pertaining to fish growth in the present study are depicted in Figs. 1-3. Significantly enhanced fish growth was observed among individuals nourished with feeds containing CE in comparison to those fed with non-CE-incorporated feeds. The average weight of fish after a rearing period of 60 days was estimated to be 11.4 g for fish fed with non-CE-incorporated feeds, whereas it ranged from 15.2 to 15.9 g for fish fed with CE-incorporated feeds, representing a remarkable 36.4% increase compared to the former (Fig. 1). The mean SGR was estimated to be 1.03% per day for fish fed with non-CE-incorporated feeds, while it ranged from 1.5 to 1.53% per day for fish fed with CE-incorporated feeds, exhibiting a substantial 49.5% elevation in comparison to the former (Fig. 2). In comparison to our study, Majhi *et al*. (2006) reported a specific growth rate of 1.27% per day for *Ctenopharyngodon idella* fed a plankton diet, which was 18.1 to 20.4% lower than the growth rate observed in our study. Similarly, Das *et al*. (2020) reported a specific growth rate of 0.037 to 0.08% per day for common carp fed a combination of rice polish and mustard oil cake (1:1) along with plankton produced through the application of poultry manure in Meghalaya. Additionally, Das *et al*. (2021) reported a specific growth rate of 1.15 to 1.15% per day for *Labeo gonius* and *Cyprinus carpio* co-cultured and fed diets containing rice polish and mustard oil cake (1:1). These comparative figures underscore the potential of CE usage in augmenting fish growth rates.

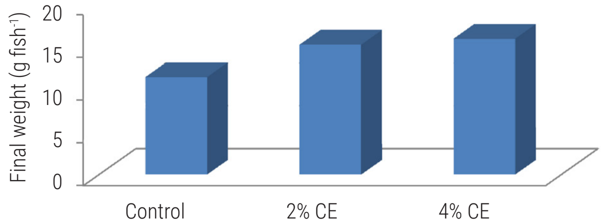

Fig. 1. Final weight in carambola extract (CE)-fed *L. rohita*. Values represent mean final weight (g) ± SD after 60 days of feeding with different concentrations of CE (0, 2 and 4%)

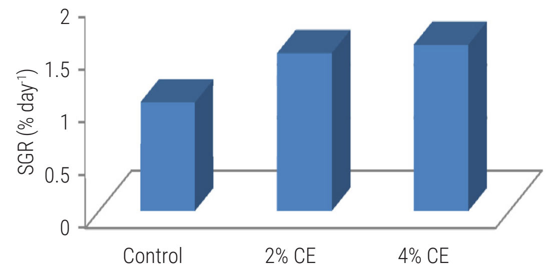

Fig. 2. SGR in carambola extract (CE)-fed fish. Values represent mean SGR (%/day) ± SD after 60 days of feeding with different concentrations of CE (0, 2 and 4%)

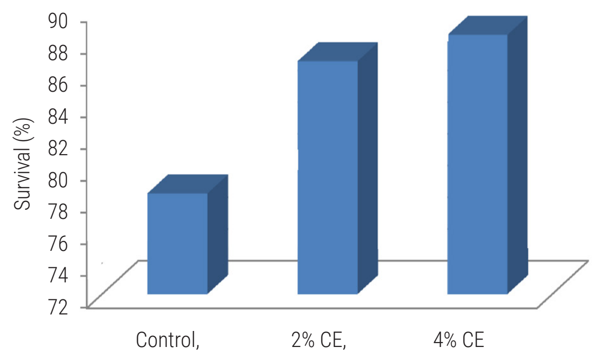

Fig. 3. Survival in Carambola extract (CE)-fed fish. Values represent mean survival rate (%) ± SD after 60 days of feeding with different concentrations of CE (0 2, and 4%)

Feeding diets incorporating CE also exhibited a beneficial effect on the survival rate of the fish under study. The survival rate was significantly higher among fish fed with CE-incorporated feeds compared to those fed with non-CE-incorporated feeds. The survival rate was estimated to be 78.4% for fish fed with non-CE-incorporated feeds, while it ranged from 86.7 to 88.4% for fish fed with CE-incorporated feeds (Fig. 3). Similar improvements in survival rates were reported by Debnath *et al*. (2024), who found that turmeric extract supplementation increased survival of *L. gonius* to 88.4-90% compared to 78.4% in control groups under the hill conditions of Meghalaya. In comparison, Das *et al*. (2021) reported a fish survival rate of 78 to 83% for *L. gonius* and *Cyprinus carpio* co-cultured and fed a diet containing rice polish and mustard oil cake (1:1).

Similarly, Das *et al*. (2020) reported a survival rate of 29.5 to 76.8% for *L. gonius* seed rearing, also fed a diet containing rice polish and mustard oil cake (1:1). These comparative results indicate that feeding CE positively influenced the survival of *L. rohita*. As no prior study in this area exists, it can be considered as the first study reporting the benefits of carambola in improving fish growth and immunity. However, based on literature reviews, the advantageous effects of feeding carambola can be attributed to the presence of L-ascorbic acid in the fruit. Sahoo *et al*. (2016) also reported enhanced growth and survival in *L. bata* fingerlings fed a diet containing ascorbic acid. In this study, the incorporation of fresh CE at a rate of 4% of feed yielded superior results compared to a 2% level of incorporation. In contrast to the present findings, Dwinanti *et al*. (2021) reported that feeding carambola juice (*Averrhoa bilimbi*) at a rate of 300 ml kg^{-1} of feed did not exhibit beneficial effects on fish, while Agustina *et al*. (2017) reported that feeding carambola at the same rate successfully cured motile Aeromonas septicemia in catfish. These discrepancies highlight the necessity for further studies in this field to uncover the underlying causes and gain a clear understanding of the effects of different feeding strategies on fish production. Debnath *et al*. (2024) reported similar immunostimulatory effects when supplementing *L. gonius* with turmeric extract in the same geographical region, suggesting that botanicals with antioxidant properties may be particularly effective in enhancing fish health under hill conditions. The specific mechanism of carambola's immunomodulatory effects remain unknown; however, based on earlier literature, it could be attributed to the action of bioactive compounds such as carambolaflavone and carambolaside M on the immunopoietic cells of organisms (Duarah *et al*., 2022).

Fish condition

The length-weight relationship is a vital aspect of fish biology as it offers insights into growth patterns, gonad development and the overall condition of the fish. The regression parameter 'b' in length-weight relationship equations provide an indirect assessment of the fish health status and general well-being. A higher 'b' value is generally associated with better fish condition (Froese *et al*., 2006). When b=3, it indicates isometric growth and ideal condition in fish. A value less than 3 suggests negative allometric growth, while a value greater than 3 indicates positive allometric growth (Pauly, 1983). In this study, fish fed without CE had a 'b' value of 1.86, while CE-fed fish showed a range of 'b' values between 2.6 and 3.5. Specifically, fish fed diets containing 4% CE exhibited a 'b' value exceeding 3.0. These results indicate that the condition of CE-fed fish is superior to that of fish fed without CE.

The Fulton's condition factor analysis confirmed our findings, with significantly higher K values in CE-fed groups ($p<0.05$) (Table 3). The K value increased progressively with higher CE concentrations, reaching 1.67±0.09 in the 4% CE group, 38% higher than the control (1.21±0.08). This improvement indicates enhanced nutritional status and physiological well-being in CE-supplemented fish. Previous research suggests that incorporating CE enhances nutrient availability and assimilation, improving fish growth and condition (Limbu *et al*., 2020). Increased condition factor also indicates elevated energy reserves, contributing to better stress tolerance during infections (Chellappa *et al*., 1995). Further studies are needed to fully validate these findings.

Effect on haematological parameters

CE-fed fish showed significant improvements in haematological parameters across all sampling periods. RBC counts increased from 0.82 x 10^6 mm^{-3} in control fish to 1.1-1.2 x 10^6 mm^{-3} in CE-fed fish (Fig. 4). Similarly, WBC counts rose from 25.4 x 10^3 mm^{-3} in control to 27.2-32.0 x 10^3 mm^{-3} in CE-fed fish (Fig. 5). Haemoglobin levels also improved from 7.8 g dl^{-1} in control to 8.1-9.3 g dl^{-1} in CE-fed fish (Fig. 6). The 4% CE inclusion level demonstrated superior efficacy compared to 2% in enhancing these parameters. These results indicate CE's immune-boosting properties, likely attributable to its essential vitamins, minerals and antioxidants (Lakmal *et al*., 2021). Comparable improvements have been documented in fish fed with other plant-based supplements including turmeric (Sahu *et al*., 2008), glycyrrhizin (Kumar *et al*., 2021) and *Withania somnifera* (Sharma *et al*., 2010). In a related study, Debnath and Sahoo (2025) demonstrated that dietary supplementation with *W. somnifera* root powder significantly enhanced immunological parameters and disease resistance in *L. rohita* challenged with *Aphanomyces invadans*, with optimal results at 0.2% inclusion level, further supporting the potential of botanical supplements in enhancing fish immunity in North-east Indian conditions.

Effect on enzymatic parameters

In the present study, there was a significant reduction in the activity of GOT and GPT in fish fed diets containing CE across all sampling

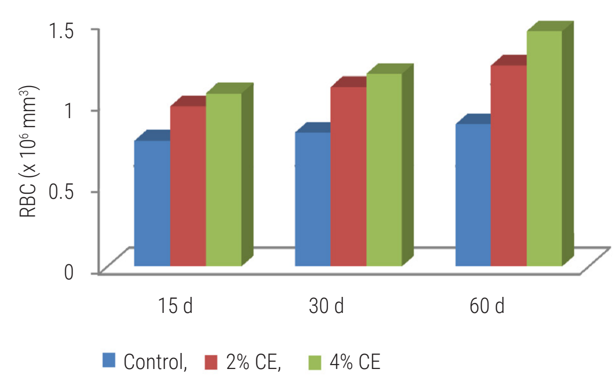

Fig. 4. RBC counts in CE-fed fish. Values represent mean RBC count ($\times 10^6$ mm^{-3}) ± SD in *L. rohita* fed with different concentrations of CE (0, 2 and 4%) on 15 th, 30 th and 60 th day of feeding trial

Table 3. Length-weight relationship parameters and Fulton's Condition Factor of *L. rohita* fed with different concentrations of carambola extract (CE)

Treatment	Length-weight relationship	r^2	b value	Growth pattern	Fulton's condition factor (K)
Control	$W = 0.0462L^{1.86}$	0.92	1.86	Negative allometric	1.21 ± 0.08^a
2% CE	$W = 0.0194L^{2.60}$	0.95	2.60	Near isometric	1.48 ± 0.11^b
4% CE	$W = 0.0096L^{3.50}$	0.97	3.50	Positive allometric	1.67 ± 0.09^c

Values (mean±SD) with different superscripts are significantly different ($p<0.05$)

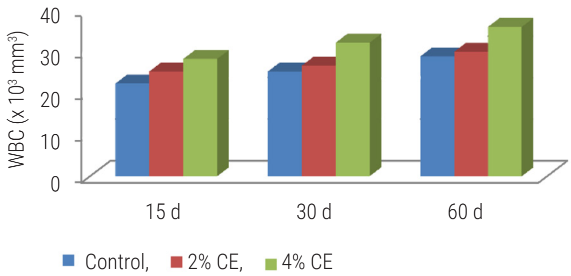

Fig. 5. WBC counts in CE-fed fish. Values represent mean WBC count ($\times10^3$ mm^{-3}) ± SD in *L. rohita* fed with different concentrations of CE (0, 2 and 4%) on 15th, 30th and 60th day of feeding trial

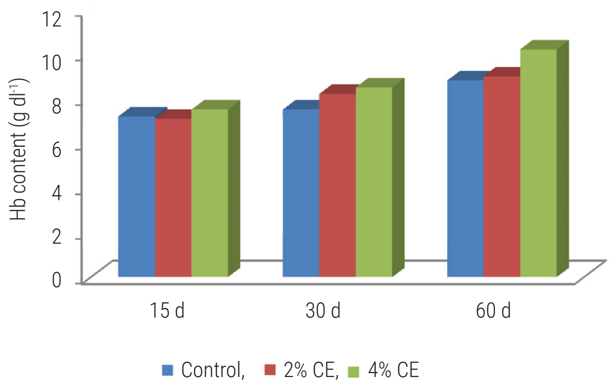

Fig. 6. Hb levels in CE-fed fish. Values represent mean Hb concentration (g dl^{-1}) ± SD in *L. rohita* fed with different concentrations of CE (0, 2 and 4%) on 15th, 30th and 60th day of feeding trial

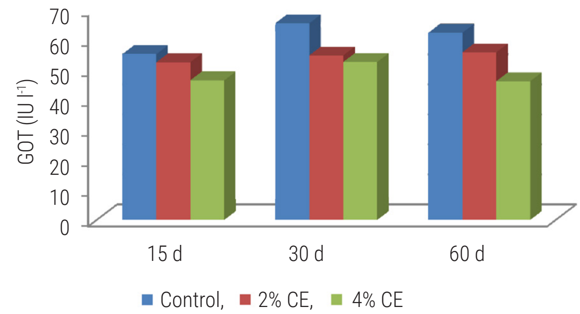

Fig. 7. GOT activity in CE-fed fish. Values represent mean GOT enzyme activity (IU l^{-1}) ±SD in *L. rohita* fed with different concentrations of CE (0, 2 and 4%) on 15th, 30th and 60th day of feeding trial

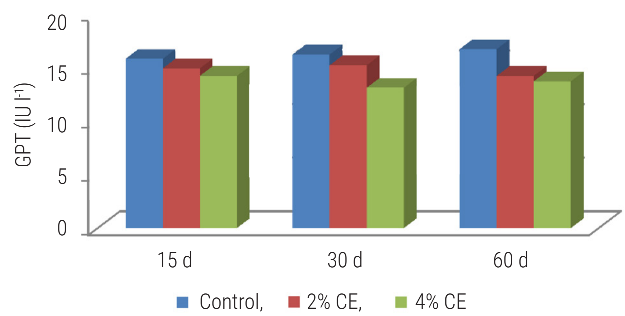

Fig. 8. GPT activity in CE-fed fish. Values represent mean GPT enzyme activity (IU l^{-1}) ±SD in *L. rohita* fed with different concentrations of CE (0, 2 and 4%) on 15th, 30th and 60th day of feeding trial

days (15th, 30th and 60th day of feeding trial). The mean activity of GOT was found to be 61 IU l^{-1} in fish fed without CE, whereas it ranged from 48.4 to 54.3 IU l^{-1} in CE-fed fish (Fig. 7). Similarly, the mean activity of GPT was estimated to be 16.3 IU l^{-1} in fish fed without CE, while it was 13.7-14.8 IU l^{-1} in CE-fed fish (Fig. 8). A higher level of inclusion (4%) of CE in the diet was more effective in improving the enzymatic parameters of the fish. The activity of GOT and GPT is commonly associated with physiological stress in animals, and a decrease in their activity indicates a normal health condition. Elevated levels of GOT and GPT in the blood often suggest liver injury or disease (Udayakumar *et al*., 2009). Based on the present study, it can be concluded that feeding CE helped the fish maintain a normal health condition, which is crucial for their growth and survival. Although a direct comparison with other studies was not possible, the observed benefits could be attributed to the presence of bioactive compounds in CE (Duarah *et al*., 2022).

Effect on biochemical parameters

CE-fed fish demonstrated superior biochemical profiles compared to the control group across all sampling periods. Blood glucose levels were significantly lower in CE-fed fish (73.7-78.8 mg dl^{-1}) than in control fish (91.2 mg dl^{-1}) (Fig. 9), while remaining within the normal range for fish (40-90 mg dl^{-1}) (Malini *et al*., 2018). Total protein levels were elevated in CE-fed fish (8.4-8.9 g dl^{-1}) compared to control fish (7.4 g dl^{-1}) (Fig. 10), indicating enhanced non-specific immune function and improved resistance to pathogenic infections (Yogeshwari *et al*., 2015). Additionally, serum cholesterol levels were markedly reduced in CE-fed fish (131.4-132.4 mg dl^{-1}) *vs* control fish (174.9 mg dl^{-1}) (Fig. 11).

These biochemical improvements correlate with enhanced physiological parameters in CE-fed fish, including increased growth rates, elevated blood cell counts and normalised enzyme activity. Higher protein levels indicate enhanced immunity and improved distribution of body fluids (Asadi *et al*., 2012). Despite carambola's oxalic acid content potentially affecting glucose metabolism (Muthu *et al*., 2016), the 2 and 4% CE concentrations proved safe based on observed physiological improvements. The *Averrhoa*

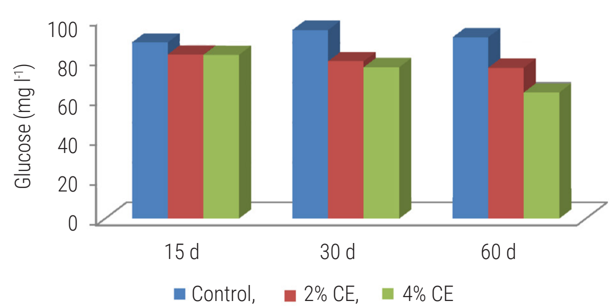

Fig. 9. Glucose level in CE-fed fish. Values represent mean blood glucose concentration (mg dl^{-1}) ± SD in *L. rohita* fed with different concentrations of CE (0, 2 and 4%) on 15th, 30th and 60th day of feeding trial

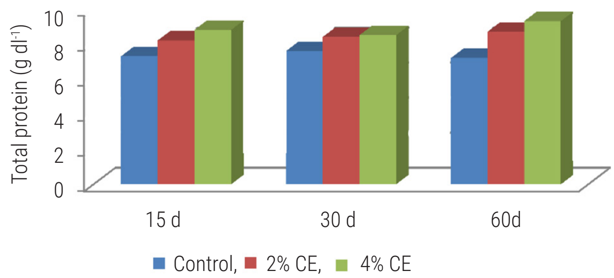

Fig. 10. Total protein level in CE-fed fish. Values represent mean serum protein concentration (g dl^{-1}) ± SD in *L. rohita* fed with different concentrations of CE (0, 2 and 4%) on 15th, 30th and 60th day of feeding trial

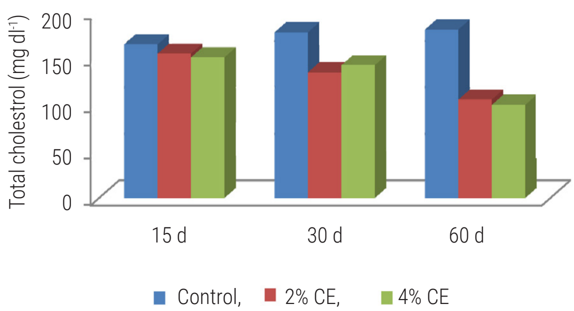

Fig. 11. Total cholesterol level in CE-fed fish. Values represent mean serum cholesterol concentration (mg dl^{-1}) ± SD in *L. rohita* fed with different concentrations of CE (0, 2 and 4%) on 15th, 30th and 60th day of feeding trial

carambola var. sweet large used might contain lower oxalic acid levels below fish toxicity thresholds, explaining the health benefits at these concentrations. Reduced blood lipid levels (Qin *et al*., 2017) and enhanced antioxidant status (Leelarungrayub *et al*., 2016) further validate CE supplementation benefits. Further research should quantify and correlate oxalic acid content across carambola varieties with fish health parameters.

In conclusion, this study demonstrates that incorporating *Averrhoa carambola* var. sweet large extract into fish diets significantly enhances aquaculture outcomes in cold hill climates. The 4% CE supplementation yielded superior results, increasing specific growth rate by 49.5% (1.53% per day *vs.* 1.03% per day in control), improving survival rates (88.4% *vs.* 78.4%), and promoting positive allometric growth (b=3.50). Haematological parameters showed marked improvements with CE supplementation: RBC counts increased by 46.3% (1.2 *vs.* 0.82 x 10^6 mm^{-3}), WBC counts rose by 26% (32.0 *vs.* 25.4 x 10^3 mm^{-3}) and haemoglobin levels improved by 19.2% (9.3 *vs.* 7.8 g dl^{-1}). CE supplementation also normalised enzyme activity (GPT/GOT) and improved biochemical profiles. We recommend incorporating 4% carambola extract in fish diets for hill aquaculture to maximise growth and enhance immunity, particularly in regions with prolonged cold climates.

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