

Optimization of dietary carbohydrate requirement of rohu fingerlings cultured in recirculatory aquaculture system

S. Swamy¹, A.D. Deo^{1*}, M. Jayant¹, N. Shamna¹, N.P. Sahu¹, K.D. Rasal², M. Dhanalakshmi² and B.R. Prashanth¹

¹Fish Nutrition, Biochemistry and Physiology Division, ICAR-Central Institute of Fisheries Education, Mumbai-400 061, India

²Fisheries Resource Harvest and Post-Harvest Management Division, ICAR-Central Institute of Fisheries Education, Mumbai-400 061, India

³Fish Genetics and Biotechnology Division, ICAR-Central Institute of Fisheries Education, Mumbai-400 061, India

Received: 13 May 2025

Revised: 07 October 2025

Accepted: 05 January 2026

*Corresponding Author Email: ashutosh.cife19@rediffmail.com

*ORCID: <https://orcid.org/0000-0002-9341-2084>

Abstract

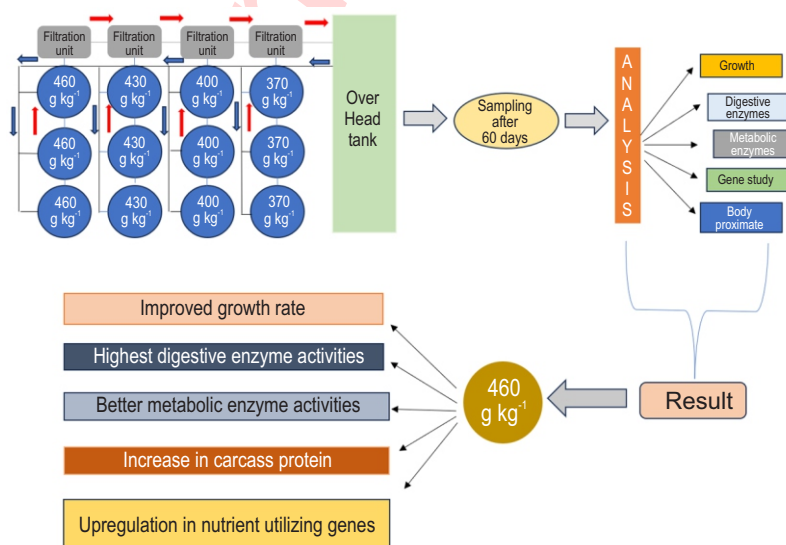
Aim: To optimize the dietary carbohydrate requirement of rohu fingerlings cultured in recirculatory aquaculture system.

Methodology: Rohu fingerlings (n=120; weighing 9.23 ± 0.10 g) were distributed across four treatment groups, each fed with varying levels of dietary carbohydrates: 370g (C37), 400g (C40), 430g (C43), and 460g (C46) per kg diet. At the end of the sampling period, the total fish biomass from each unit was measured to determine the growth performance and feed consumption. Additionally, fish were dissected to collect gill, liver and intestinal tissues to analyze digestive and metabolic enzymes and gene expression.

Results: Fish fed a diet containing 460 g of carbohydrate per kg (C46) showed significant improvement in the growth performance. Additionally, the C46 group demonstrated elevated digestive and metabolic enzyme activities, and a gene expression study, indicating more efficient nutrient digestion and uptake.

Interpretation: The dietary carbohydrate requirement for rohu fingerling in RAS has been optimized at 460g kg^{-1} for better nutrient utility and growth.

Key words: Carbohydrate utilization, Gene expression, Growth performance, *Labeo rohita*, Recirculatory aquaculture system



Introduction

Global fish production has reached peak of approximately 185.4 million tonnes in 2022, with aquaculture accounting 51% of this total (FAO, 2024). A 54% increase in the fish production is projected by 2032 (FAO, 2024) to meet the growing demand for food and nutrition (FAO, 2024). Since capture fisheries are stagnating, they cannot fully address this need; therefore, boosting aquaculture production is essential. To achieve this, various new methods are being introduced in the aquaculture sector to enhance fish production. Land and water are the primary resources required for fish farming however, water and available space for fish culture are often limited, making intensive culture systems more suitable in such cases (Crab *et al.*, 2012). However, overcrowding of fish can lead to poor water quality (Pulkkinen *et al.*, 2010). Poor pond water quality is often caused by overfeeding and improper pond management. While feed provides essential nutrition, it can also generate waste if not properly managed, contributing to many environmental issues in aquaculture (Roque d'Orbcastel *et al.*, 2009). In intensive aquaculture practices, effective nutritional management relies on two main feeding strategies: optimal feeding rate and frequency. These strategies are crucial for regulating feed intake, nutrient metabolism, and waste removal (Sun *et al.*, 2016).

Moreover, the optimal feeding rate and frequency depend on two factors: endogenous factors (such as body weight, physiological state, and species) and exogenous factors (such as environment, feed, and rearing conditions) (Bregnballe, 2010). While endogenous factors cannot be changed, it is possible to modify and optimize exogenous factors to support fish growth. Among intensive culture methods, the Recirculating Aquaculture System (RAS) is particularly well-suited for managing exogenous factors for optimal fish growth. RAS is an effective technique for reusing pond water while maintaining high water quality. Typically, these indoor, tank-based systems use mechanical, chemical, and biological filtration, along with other treatment processes, to achieve high water reuse rates. These systems help maintain water quality (such as TSS, TS, ammonia, and nitrate levels) and consistent water temperatures. Carbohydrates are a key nutrient that primarily meets the energy requirements of animals. They are generally regarded as the most abundant and cost-effective energy source, making them an essential component of fish feed to fulfill fish energy needs and support growth through their protein-sparing role (Kamalam *et al.*, 2017).

In other words, the dietary inclusion of an optimal amount of carbohydrates helps retain lipids and proteins in the fish body. Moreover, both directly and indirectly, carbohydrate metabolism influences glycolysis, lipogenesis, gluconeogenesis, and glycogen metabolism (Kamalam *et al.*, 2017). Herbivorous fish typically utilize carbohydrates more efficiently than omnivorous and carnivorous fish. Rohu, a herbivorous fish, is highly preferred among the freshwater species due to its delicate taste, consumer preference, rapid growth rate, and availability. It accounts for 40% of India's total freshwater fish production. Additionally, rohu possess the biological machinery of carbohydrate-digesting

enzymes, metabolic enzymes, and glucose transporters (GLUTs), which enable it to utilize carbohydrates efficiently. Generally, the optimal carbohydrate requirement for herbivorous fish ranges from 30 to 50% of their diet (Wilson, 1994; Hemre *et al.*, 2002; Kumar *et al.*, 2005). This optimal carbohydrate requirement for specific fish species has been experimentally determined using a dose-response curve at optimum growth. Therefore, the carbohydrates needed for each fish species should be balanced to promote growth without compromising the overall health and immunity of fish. This ensures that a specific diet can be recommended for fish in a recirculating aquaculture system. In view of the above, this study was designed to evaluate the optimum dietary carbohydrate utilization of rohu fingerlings reared in the RAS system.

Materials and Methods

Experimental animal: Rohu fingerlings were obtained from Kanamia Aqua Pvt. Ltd. hatchery, Chikli, Navsari, Gujarat, India. In oxygen-filled polythene bags, the fingerlings were transported to the ICAR-Central Institute of Fisheries Education (CIFE), Mumbai, India. The experiment is conducted in wet lab of ICAR-CIFE Mumbai during year 2024. Upon arrival, they were transferred to 1000 l cement tanks and acclimated for 15 days on a control diet consisting of crude protein (300 g kg⁻¹) and lipid (60 g kg⁻¹), administered twice daily. Adequate aeration was maintained in the tanks to ensure optimal levels of dissolved oxygen.

Experimental diet preparation: Four isonitrogenous (300 g crude protein kg⁻¹ diet), isolipidic (60 g lipid kg⁻¹ diet) diets having gradient carbohydrate levels of 370, 400, 430 and 460 g kg⁻¹ were formulated (Table 1) and designated as C37, C40, C43, and C46, respectively. Ingredients were mixed in specific ratios to prepare experimental diets with varying levels of carbohydrates. All the pre-weighed ingredients were mixed thoroughly with an accurate amount of water to prepare the dough and autoclaved for 20 min (121°C). The feed mixture was cooled and mixed uniformly with oils and heat-sensitive feed additives. This feed mixture was pelletized to prepare uniform-size pellets (2.0 mm diameter), oven-dried at 45°C, packaged in labelled containers, and stored at room temperature.

Experimental design and management: A 60-day experiment was carried out in a Recirculating Aquaculture System (RAS). The RAS had 12 circular drums having proper inlets and outlets, outfitted with mechanical cum biological filters. All drums were properly closed with lids to avoid fish escape. The RAS flow rate was maintained at 1.6 l min⁻¹. Rohu, *Labeo rohita* fingerlings (n=120; mean weight 9.23 ± 0.10 g) were distributed randomly in four treatment groups in triplicate following a complete randomized design (CRD). Four different treatment group fishes were fed with four different feeds having gradient carbohydrate levels of 370, 400, 430, and 460g kg⁻¹ diet, as shown in Table 1. Fish were fed twice daily (09:00 am and 05:00 pm) to satiety level. Uneaten feed was collected from the tank outlet daily to measure daily feed intake accurately.

Table 1: Ingredients and proximate composition of the different experimental diets

Ingredients (g kg ⁻¹)	Experimental diets			
	C37	C40	C43	C46
Fish meal ¹	60.0	60.0	60.0	60.0
Soybean meal ¹	230.0	230.0	230.0	230.0
Groundnut oil cake ¹	248.0	248.0	248.0	248.0
Starch ²	20.0	12.0	42.0	92.0
Dextrin ²	10.0	43.0	43.0	43.0
De-oiled rice bran ¹	225.0	225.0	225.0	225.0
Wheat flour ¹	75.0	80.0	80.0	40.0
Cellulose ²	60.0	34.8	0.48	0
Sardine oil ¹	20.4	18.0	18.0	15.5
Sunflower oil ¹	20.4	18.0	18.0	15.3
Gum Arabica ²	10.0	10.0	10.0	10.0
Vit- min mix#	20.0	20.0	20.0	20.0
Choline chloride ²	1.0	1.0	1.0	1.0
Butylated hydroxytoluene ²	0.2	0.2	0.2	0.2
Total	1000.0	1000.0	1000.0	1000.0
Proximate composition (g kg⁻¹) on dry weight basis				
Moisture	93.1	92.6	92.3	91.5
Crude protein	304.1	305.6	304.3	303.2
Ether extract	64.7	64.9	62.4	61.1
Crude fibre	112.3	95.0	82.2	56.2
Nitrogen free extract	370.9	405.4	432.5	463.3
Total ash	98.1	99.1	88.6	86.2
Digestible energy (MJ kg ⁻¹)	13.9	14.3	14.6	15.2

¹Source: Procured from the local market, Mumbai, India; ²Source: Hi Media Pvt. Ltd., Mumbai, India; #Source: Agrimin Forte, Vibrac, India. Composition (quantity kg⁻¹): vitamin A-700000 IU, vitamin D3- 70000 IU, vitamin E- 250 mg, nicotinamide-1000 mg, cobalt- 150 mg, copper-1200 mg, iodine-325mg,iron-1500 mg, magnesium-6000 mg, manganese-1500 mg, potassium-100mg,sodium-5.9 mg, sulphur-0.72%, zinc-9600 mg, calcium-25%, and phosphorus- 12.75%.

Water quality parameters: Water quality parameters were monitored on alternate days and kept within optimal limits during the experimental period. The temperature, pH, and dissolved oxygen were tested using a portable pH meter (Eutech,China), digital thermometer cum dissolved oxygen recorder (MERCK, Germany), respectively. The nitrite, ammonia, and carbon dioxide levels were measured following the standard methods (APHA, 2007).

Sampling: The total biomass of individual drums was recorded every 15 days to calculate the growth parameters and feed intake. Before weighing, fish were not fed for 12 hr to ensure gut emptiness. Three fish were selected randomly from each treatment for the whole-body composition study. Additionally, the intestine, gill, and liver tissues were collected from three fish per group solution. The tissues were homogenized in sucrose solution (0.25M) and the 0.5% tissue extract was obtained by centrifuging the homogenates, thereafter, it was stored at -20°C for subsequent enzyme analysis.

Proximate composition study: The proximate composition of the diets and the whole fish body was analyzed using the

standard AOAC (2005) protocol. The moisture was measured using a hot air oven, crude protein was estimated by the Kjeldahl method (LABQUEST), ether extract was assessed through solvent extraction (Soxtron), and crude fiber was analyzed by the acid-alkali digestion method (Fibrotec, FT122 FOSS). Ash content was measured with a muffle furnace. The nitrogen-free extract (g kg⁻¹) in the diet was calculated as 1000 – {ether extract + crude protein + crude fiber + total ash}, while total carbohydrate (g kg⁻¹) for fish whole body composition was calculated as 1000 – {moisture + crude protein + total ash + ether extract}. The digestible energy (MJ kg⁻¹) of the diets was estimated by the method described by Halver (1976).

Growth and nutrient utilization parameters: Weight gain (WG), weight gain percentage (WGP), feed conversion ratio (FCR), specific growth rate (SGR), and survival were calculated using the formula provided (Halver, 1976).

Analysis of digestive and metabolic enzyme activities: Total protein contents in the gill, intestine, and liver tissues were quantified by Lowry's method (Lowry *et al.*, 1951). Intestinal amylase and protease were assayed following the standard

Table 2: Forward and reverse primers of different genes for real-time PCR

Gene	Forward primer	Reverse primer	Amplicon size	Accession No.
GLY	TGAACCTCCAAACCAAGCCATC	CTCTGTCGTGATAGGGCAGTCC	200	XM_051105893.1
PFK	AAGCCATCGCGGTTCTGACGAGC	TGGTGACGCTCTGCCAGTTGGCGA	167	XM_051122636.1
GRH	TGCGTTGTCGTGCCAGCCACTTG	CTGGGATCTCTGGCTCAGCAA	162	LC_271259
β -actin	CACTGCTGCTTCTCCTCTCTCC	GATACCGCAAGACTCCATACCCA	141	EU184877.1

GLY- Glycogen synthase; PFK-Phosphofructokinase; GRH- Ghrelin; β -actin

protocol of Rick and Stegbauer (1974) and Drapeau (1976), intestinal lipase (LPS) was assayed by the modified method of Moreno-Arias *et al.* (2017). Hexokinase (HXK), iso-citrate dehydrogenase (IDH), glucose-6-phosphate dehydrogenase (G6PD), and Aminotransferases activities were estimated following the methods of Wroblewski and Laude (1955), Ochoa (1955), Bergmeyer *et al.* (1974), and Wooten (1964), respectively. The catalase and superoxide dismutase activities were evaluated according to the methods described by Takahara *et al.* (1962) and Misra and Fridovich (1972), respectively.

Total RNA extraction and cDNA synthesis: Liver tissues from the fish were collected for gene expression analysis. Liver total RNA was isolated using TRIZOL reagent (Invitrogen, USA) following the protocol recommended by the manufacturer (Simms *et al.*, 1993). The purity (260/280 ratio) and concentration of RNA was measured with a Nanodrop spectrophotometer (Thermo Scientific, USA). To eliminate any residual DNA, the RNA was treated with D Nase as per the manufacturer's guidelines (Fermentas, USA). The RNA was then used for cDNA synthesis using a cDNA synthesis kit (Thermo Scientific, USA), and either utilized right away or preserved at -20°C for further use.

Expression study by quantitative real-time PCR (q-PCR): In this experiment, three genes were selected, namely, glycogen synthase (GLY), phosphofructokinase (PFK), and ghrelin (GRH) (Table 2). The selected genes were optimized for perfect annealing condition. PCR conditions for all genes were: 94°C for 5min, 94 °C for 15 sec, 60 °C for 20 sec, 72 °C 20 sec and 72 °C 5 min. The real-time PCR was conducted using SYBR Green q-PCR master mix without ROX (Agilent, USA). A 10 μ l reaction mixture comprising 5 μ l of 2X Itaqtm universal SYBR Green q-PCR Master mix, 0.5 μ l forward primer, 0.5 μ l reverse primer, 1 μ l of cDNA, and the remaining nuclease-free water was used for quantification. The CT (threshold cycle) value represented the quantified mRNA value. After each PCR reaction, a melting curve analysis was conducted to assess the amplified products. mRNA expression levels were determined following the comparative CT method. Each PCR reaction was performed in duplicate, and the gene expression levels were normalized by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), with β -actin serving as the reference gene (Table 2). The quantified result was analyzed by the following formulae:

$$\Delta Ct = \text{Target gene ct value} - \text{Reference gene ct value}$$

$$\Delta\Delta Ct \text{ value} = \Delta Ct \text{ value of treatment group} - \Delta Ct \text{ value of control group}$$

Statistical analyses: The impact of varying dietary carbohydrate levels on growth, nutrient utilization, and the activities of digestive, oxidative stress, and metabolic enzymes was significantly assessed by One-way ANOVA and polynomial contrast analysis in SPSS 22.0. Duncan's multiple range test under post hoc was applied to determine the significant variations between and among the treatment groups at a 5% level of significance ($p < 0.05$). The relationship between different response parameters and dietary carbohydrate was established using Pearson correlation analysis using R version 4.2.3. The results in the tables were presented as means \pm standard error (SE).

Results and Discussion

In this study, the physico-chemical water parameters (Table 3), including water temperature, varied from 27.81 to 28.52°C, which was preferable ambient temperature for rohu culture (Sarkar and Rahman, 2025). The pH range 7.9 to 8.31 was ideal for carp culture (Meena *et al.*, 2025). Dissolved oxygen ranged from 6.32 to 7.04 mg l⁻¹, well above the lowest requirement of 5 mg l⁻¹ for fish culture (Patel and Singh, 2025). Throughout the experimental period, the dissolved carbon dioxide was undetectable. The levels of ammonia-N and nitrite-N remained within the acceptable limits, ranging from 0.04 to 0.08 mg l⁻¹ and 0.03 to 0.07 mg l⁻¹, respectively, staying below the tolerance threshold for carp (Kumar *et al.*, 2025). Diets in the present experiment were iso-lipidic (60 g kg⁻¹), iso-nitrogenous (300g kg⁻¹), and hetero caloric with increasing carbohydrate levels, viz., 370 g kg⁻¹ (C37), 400 g kg⁻¹ (C40), 430 g kg⁻¹ (C43), and 460 g kg⁻¹ (C46), respectively. The ether extract, crude protein, and digestible energy ranged from 61.1 to 64.9 g kg⁻¹, 303.2 to 305.6 g kg⁻¹ and 13.9 to 15.2 MJ kg⁻¹, respectively (Table 1).

The whole body proximate of fish composition of fish is given in Table 5. The crude protein, total carbohydrate, moisture, and crude lipid were considerably affected by dietary carbohydrate ($p < 0.05$). In this study, the body crude fat and crude protein significantly increased ($p < 0.05$) with increasing dietary carbohydrate, with higher values noticed in the C46 group, which correlates with the protease and transaminase enzymes' activities. Additionally, the lipid levels in the carcass were significantly higher in the C46 group, which further aligns with the elevated G6PD activity observed in the same group. These results are consistent with Lei *et al.* (2016), who reported that G6PD and fatty acid synthase are directly linked to fatty acid synthesis, suggesting that the C46 group utilized nutrients more

Table 3: Water quality parameters of different experimental groups during the experimental period of 60 days

Parameters	Diets			
	C37	C40	C43	C46
Temperature (°C)	27.81±2.10	28.34±1.82	28.14±1.92	28.52±1.58
Dissolved oxygen (mg l ⁻¹)	7.04±0.46	6.58±0.68	7.17±0.41	6.32±0.81
pH	8.24±0.38	8.31±0.29	7.90±0.62	8.15±0.46
Ammonia (mg l ⁻¹)	0.07±0.004	0.04±0.003	0.08±0.007	0.05±0.006
Nitrite (mg l ⁻¹)	0.03±0.006	0.07±0.005	0.05±0.002	0.03±0.004
Nitrate (mg l ⁻¹)	0.07±0.003	0.06±0.001	0.04±0.002	0.06±0.005
Dissolved carbon dioxide	Nil	Nil	Nil	Nil

Data is presented as Mean ± SE (n = 3); ¹Different experimental diet with graded carbohydrate levels (C37- 370g carbohydrate kg⁻¹ diet; C40- 400g carbohydrate kg⁻¹ diet; C43- 430g carbohydrate kg⁻¹ diet; C46- 460g carbohydrate kg⁻¹ diet)

Table 4: Growth, feed and nutrient utilization and survival of rohu fingerlings cultured in recirculatory aquaculture system with graded dietary carbohydrate levels for period of 60 days

Parameters	Treatments ¹				SEM ²	p-value		
	C37	C40	C43	C46		Overall	Linear	Quadratic
Initial Weight (g)	9.22±0.17	9.43±0.15	9.37±0.16	8.90±0.45	0.128	0.521	0.411	0.231
Final Weight (g)	12.15±0.16 ^a	12.63±0.50 ^{ab}	13.70±0.63 ^b	14.08±0.32 ^b	0.301	0.046	0.008	0.906
Weight gain (g)	2.93±0.07 ^a	3.20±0.36 ^a	4.33±0.53 ^b	5.18±0.16 ^b	0.306	0.005	0.001	0.412
Weight gain percentage	31.76±1.15 ^a	33.83±3.38 ^{ab}	46.16±5.27 ^{bc}	58.60±4.40 ^c	3.631	0.004	0.002	0.217
Condition factor	0.87±0.04 ^a	0.98±0.03 ^{ab}	1.10±0.02 ^{ab}	1.3±0.04 ^b	0.03	0.038	0.010	0.329
Specific growth day (% day ⁻¹)	0.46±0.01 ^a	0.48±0.04 ^a	0.63±0.06 ^b	0.77±0.05 ^b	0.041	0.004	0.001	0.242
Feed conversion ratio	3.79±0.14 ^b	3.63±0.40 ^b	2.67±0.22 ^{ab}	2.07±0.10 ^a	0.242	0.007	0.001	0.452
Survival (%)	95.55±2.22 ^a	100.00±0.00 ^b	100.00±0.00 ^b	100.00±0.00 ^b	0.749	0.052	0.028	0.081

Data is presented as Mean ± SE (n = 3), values with different superscripts in the same column differ significantly (p<0.05). ¹Different experimental diet with graded carbohydrate (C37- 370g carbohydrate kg⁻¹ diet; C40- 400g carbohydrate kg⁻¹ diet; C43- 430g carbohydrate kg⁻¹ diet; C46- 460g carbohydrate kg⁻¹ diet) ²SEM, average standard error of means

efficiently than other groups. Similar findings were reported in *Barbodes carnaticus* by Gangadhar et al. (2019), further supporting this trend.

All the parameters related to growth and nutrient utilization, i.e., final body weight (FBW), WG, WG%, SGR, and FCR, were found to be significantly (p <0.05) affected by the dietary carbohydrate levels (Table 4). There was an increasing trend of FBW, WG, WG%, and SGR with dietary carbohydrate levels. The highest FBW, WG, WG%, and SGR were observed in the C43 and C46 groups (p<0.05). Additionally, the Condition factor was significantly higher in the C46 group compared to other groups. The cultured environment of rohu fingerlings in the present study was conducive to growth and survival. The domesticated fish species condition factor value was near one or more than 1 in better culture conditions (Masram et al., 2022). The FCR value trailed just the reverse trend of these parameters. These findings corroborate the findings of Anand et al. (2018), who described that the ideal dietary carbohydrate requirement for rohu fingerlings was 450 g kg⁻¹ when the diet consisted 300 g kg⁻¹ protein. Among digestive enzymes, including amylase, lipase and protease activity, were significantly (p <0.05) affected due to

increasing levels of dietary carbohydrates (Table 6). The amylase activity was maximum (p<0.05) in the higher dietary carbohydrate-fed group (C46). The amount of carbohydrate substrate and amylase activity were direct by co-related, which is consistent with the results observed in Silver pompano (*Trachinotus ovatus*) fed varying dietary carbohydrates (Zhou et al., 2015). Lipase activity was found to be maximum at higher levels of dietary carbohydrate fed group (C43 and C46). Similarly, the lipase enzyme activity aligns with the findings on *Lateolabrax maculatus* (Zheng et al., 2023). However, there is a disagreement on how dietary carbohydrates affect lipase, activity and no reliable results exist, therefore, this topic requires further research.

Metabolic enzyme activity, including hexokinase (HXK), isocitrate dehydrogenase (IDH), glutamate pyruvate transaminase (GPT), and glutamate oxaloacetate transaminase (GOT) activities in muscle and liver, varied significantly (p<0.05) among different dietary groups (Table 7). HXK activity was significantly highest in the group receiving dietary 460 g kg⁻¹ (C46), whereas the lowest carbohydrate-fed group (C37) demonstrated reduced HXK activity. The hexokinase, a prime enzyme of glycolysis, enhances the activity with substrate

Table 5: Whole body proximate (g kg⁻¹; wet weight basis) of rohu fingerlings cultured in a recirculatory aquaculture system with graded dietary carbohydrate levels for a period of 60 days

Parameters	Treatments ¹				SEM ²	p-value		
	C37	C40	C43	C46		Overall	Linear	Quadratic
Moisture	767.11±1.63 ^c	763.92±0.62 ^{bc}	762.35±2.43 ^{ab}	758.50±0.55 ^a	1.102	0.01	0.514	0.883
Crude protein	144.24±0.57 ^a	142.86±0.97 ^a	151.45±1.01 ^{ab}	159.14±0.45 ^b	0.592	0.044	0.014	0.333
Ether extract	26.81±1.23 ^a	28.77±1.02 ^a	31.90±1.12 ^b	35.13±1.23 ^c	1.623	0.009	0.034	0.806
Ash	36.43±1.17 ^b	32.13±1.20 ^a	34.40±1.24 ^{ab}	42.73±0.55 ^c	1.048	0.001	0.004	0.008
Total carbohydrate	21.23±1.00 ^a	25.38±0.11 ^b	27.91±0.33 ^c	31.25±0.46 ^d	1.116	0.001	0.001	0.906

Data is presented as Mean ± SE (n = 3), values with different superscripts in the same column differ significantly (p < 0.05). ¹Different experimental diet with graded carbohydrate levels (C37- 370g carbohydrate kg⁻¹ diet; C40- 400g carbohydrate kg⁻¹ diet; C43- 430g carbohydrate kg⁻¹ diet; C46- 460g carbohydrate kg⁻¹ diet); ²SEM, average standard error of means.

Table 6: Digestive enzyme activities of rohu fingerlings cultured in a recirculatory aquaculture system with graded dietary carbohydrate levels for a period of 60 days

Parameters	Treatments ¹				SEM ⁵	p-value		
	C37	C40	C43	C46		Overall	Linear	Quadratic
Amylase ²	0.87±0.14 ^a	1.00±0.06 ^a	1.07±0.02 ^a	1.45±0.01 ^b	0.072	0.004	0.001	0.147
Lipase ³	113.86±4.13 ^{ab}	82.21±9.81 ^a	138.01±6.71 ^b	146.95±17.04 ^b	8.807	0.010	0.011	0.092
Protease ⁴	0.14±0.06	0.16±0.03	0.18±0.05	0.15±0.07	0.011	0.211	0.348	0.127

Data is presented as Mean ± SE (n = 3), values with different superscripts in the same column differ significantly (p < 0.05). ¹Different experimental diet with graded carbohydrate levels (C37- 370g carbohydrate kg⁻¹ diet; C40- 400g carbohydrate kg⁻¹ diet; C43- 430g carbohydrate kg⁻¹ diet; C46- 460g carbohydrate kg⁻¹ diet); ²Amylase activity is expressed as micromole of maltose released/mg protein min⁻¹; ³Lipase activity is expressed as mole of paranitrophenyl released/mg protein min⁻¹; ⁴Protease activity is expressed as millimole of tyrosine released/mg protein min⁻¹; ⁵SEM, average standard error of means.

Table 7: Metabolic and stress enzyme activities of rohu fingerlings cultured in a recirculatory aquaculture system with graded dietary carbohydrate levels for period of 60 days

Parameters	Treatments ¹				SEM ⁹	p-value		
	C37	C40	C43	C46		Overall	Linear	Quadratic
HXK ²	5.70±0.64 ^a	12.36±0.90 ^b	13.42±0.13 ^b	14.46±0.94 ^b	1.112	0.001	0.001	0.020
IDH ³	0.07±0.00 ^a	0.06±0.00 ^a	0.12±0.02 ^b	0.74±0.01 ^c	0.086	0.001	0.001	0.001
G6PD ⁴	2.91±0.20 ^a	5.02±0.34 ^{ab}	10.31±1.10 ^{ab}	12.82±0.84 ^b	0.743	0.021	0.003	0.326
GOT ⁵ (Liver)	136.75±2.18	123.48±2.43	130.95±4.12	135.02±3.07	9.766	0.101	0.293	0.201
GOT ⁵ (Muscle)	15.72±1.26	22.83±5.63	15.02±5.07	14.18±1.89	1.980	0.436	0.502	0.344
GPT ⁶ (Liver)	0.41±0.03 ^a	1.99±0.21 ^b	1.96±0.06 ^b	2.84±0.04 ^c	0.268	0.001	0.003	0.003
GPT ⁶ (Muscle)	1.32±0.09	1.43±0.17	1.29±0.30	1.19±0.07	0.081	0.827	0.528	0.581
SOD ⁷ (Liver)	14.38±0.04 ^b	13.70±0.09 ^b	10.38±0.53 ^a	10.59±0.13 ^a	0.553	0.001	0.002	0.147
SOD ⁷ (Gill)	50.81±2.06 ^b	22.57±0.46 ^a	23.92±0.82 ^a	21.63±1.17 ^a	3.716	0.001	0.004	0.001
CAT ⁸ (Liver)	34.03±0.08 ^c	34.79±0.11 ^c	24.94±1.39 ^a	27.84±0.47 ^b	1.289	0.001	0.001	0.182
CAT ⁸ (Gill)	32.79±1.20 ^c	30.79±0.39 ^c	16.37±0.48 ^b	10.44±0.20 ^a	2.865	0.001	0.001	0.021

Data is presented as Mean ± SE (n = 3), values with different superscripts in the same column differ significantly (p < 0.05). ¹Different experimental diet with graded carbohydrate levels (C37- 370g carbohydrate kg⁻¹ diet; C40- 400g carbohydrate kg⁻¹ diet; C43- 430g carbohydrate kg⁻¹ diet; C46- 460g carbohydrate kg⁻¹ diet) ²Hexokinase activity is expressed as nanomole mg⁻¹ protein min⁻¹; ³Isocitrate dehydrogenase activity is expressed as micromole mg⁻¹ protein min⁻¹; ⁴G6PD, Glucose-6-phosphate dehydrogenase, the activity is expressed as nanomole mg⁻¹ protein min⁻¹; ⁵GOT, glutamic-oxaloacetic transaminase, the activity is expressed as nanomole mg⁻¹ protein min⁻¹; ⁶GPT, glutamic-pyruvic transaminase, the activity is expressed as nanomole mg⁻¹ protein min⁻¹; ⁷SOD (Superoxide dismutase) activity is expressed as mmole of epinephrine decomposed mg⁻¹ protein min⁻¹; ⁸Catalase activity is expressed as mmole of hydrogen peroxide decomposed mg⁻¹ protein min⁻¹; ⁹SEM, average standard error of means

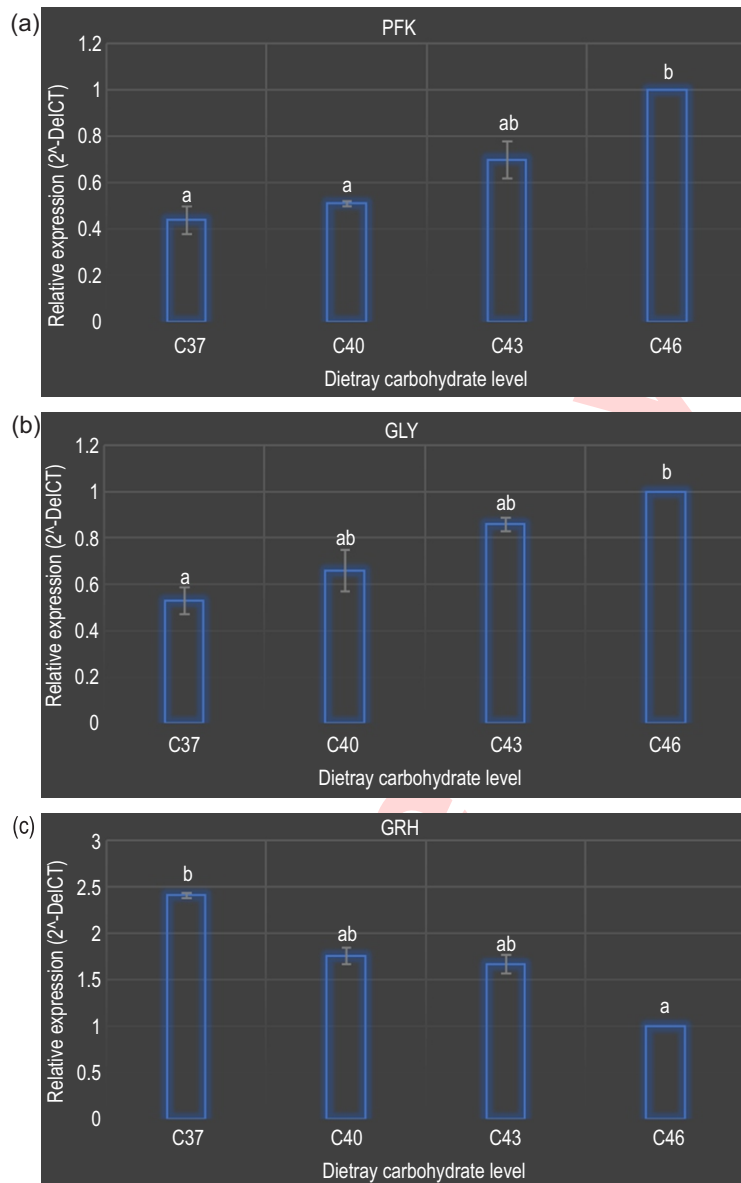


Fig. 1: The relative gene expression of Phosphofructokinase (a), Glycogen synthase (b), and Ghrelin (c) in the liver of rohu fingerlings cultured in a recirculatory aquaculture system with graded dietary carbohydrate levels for a period of 60days. C37- 370g carbohydrate/kgdiet; C40- 400g carbohydrate/kgdiet; C43- 430g carbohydrate/kgdiet; C46- 460g carbohydrate/kgdiet. Data is presented as Mean \pm SE (n = 3), values with different superscripts in the same column differ significantly ($p < 0.05$).

glucose in the animal body. Hexokinase activity increased in parallel with dietary carbohydrate levels, consistent with Bai *et al.* (2023). The produced product from the glycolysis cycle further enters TCA cycle for energy production. Isocitrate dehydrogenase (IDH) is an important enzyme in that pathway. Hence, IDH activity showed a significant upward trend, with the highest activity recorded in the C46 group. These findings are in agreement with Gopika *et al.* (2019), who observed that isocitrate dehydrogenase activity was highest in fish that efficiently utilized nutrients. Liver GPT activities varied significantly, with the highest

liver GPT activity recorded in the C46 group, indicating enhanced protein metabolism through the process of transamination. A similar observation was reported in hybrid fish (*Megalobrama amblycephala* ♀ \times *Culter alburnus* ♂) by Bai *et al.* (2023). Furthermore, stress enzymes, catalase and superoxide dismutase activities in the gill and liver was significantly affected by dietary carbohydrate levels ($p < 0.05$) (Table 7). The lowest SOD level in both the gill and liver was detected in the high carbohydrate fed groups (C43 and C46). Similarly, the catalase activity in the both tissues was lowest in the C46 group,

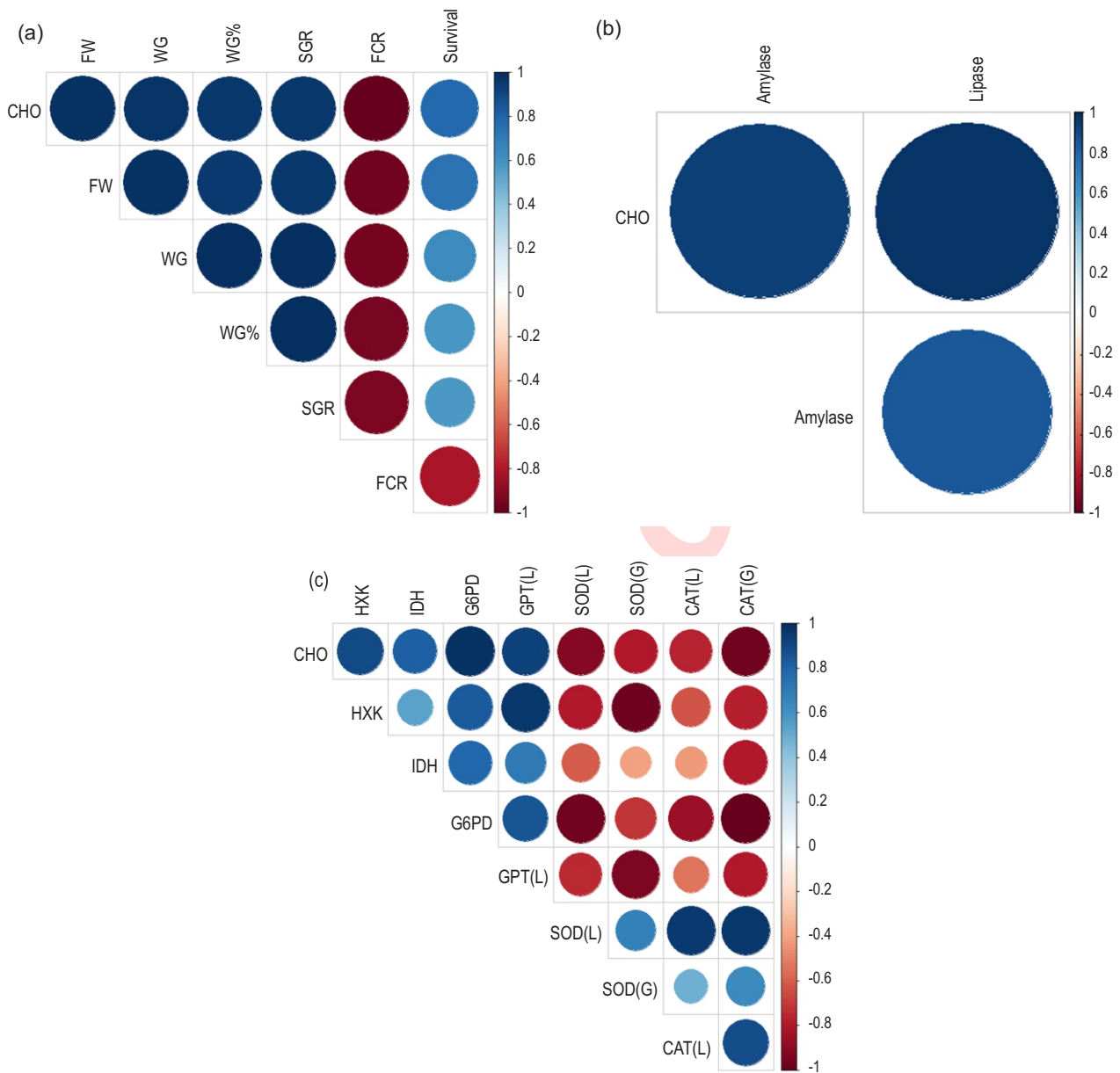


Fig. 2: Karl- Pearson correlation plot between dietary carbohydrate and growth parameters (a), digestive enzymes (b), and metabolic enzymes (c) of rohu fingerlings reared under RAS system fed with graded dietary carbohydrate levels for a period of 60days.

suggesting a negative correlation between increasing dietary carbohydrate levels, and SOD and catalase activities. Reduced stress in the animal, improved the nutrient utilization and growth. This result is consistent with the findings in the top mouth culter (*Culter alburnus*) reported by Bai *et al.* (2023), fed with different carbohydrate levels. Pearson correlation analysis was conducted between the dietary carbohydrate level and growth parameters, digestive enzymes, metabolic enzymes, and serum parameters, as shown in Fig. 2. The growth rates (WG, WGP & SGR; $r=0.93$ to 0.96) indices were positively correlated with dietary carbohydrate (CHO). At the same time, FCR was negatively correlated with

dietary carbohydrate ($r=-0.89$). Mean while, the digestive enzyme activities like lipase and amylase were positively correlated ($r=0.91$ to 0.97). HXK, IDH and G6PD exhibited a linear correlation with dietary carbohydrates ($r=0.87$, $r=0.80$, and $r=0.92$) following a growth trend. Meanwhile, the hepatic GPT activity was moderately positively correlated with dietary carbohydrate ($r=0.79$). Additionally, the SOD and catalase activities were negatively correlated ($r= -0.88$ to -0.95). The dietary carbohydrate level significantly affected the selected three genes, PFK, GLY and GRH ($p<0.05$), as shown in Fig. 1. The PFK expression was considerably higher in the C46 group

compared to the other ($p < 0.05$), which strongly supports the trend found in hexokinase enzyme activity during the metabolic enzyme assay. However, this result contrasts with the findings of Bai *et al.* (2023) in the top mouth culter. Similarly, the GLY expression was highly upregulated by the fish fed with 460 g carbohydrate kg^{-1} diet (C46), and the lowest was observed in the C37 group significantly ($p < 0.05$). Kamalam (2017) reported that excess glucose in the fish's body leads to glycogen synthesis.

This study found the highest upregulation of the GLY gene in the group fed with the optimum dietary carbohydrate (460g carbohydrate kg^{-1} diet), further supporting this metabolic pathway. Ghrelin hormone is considered a hunger hormone; its expression was considerably down-regulated in the C46 group compared to all other treatments ($p < 0.05$). This suggests that fish efficiently metabolizing carbohydrates for energy display reduced ghrelin expression, this is in line with the findings of Santos *et al.* (2020), where fish with improved nutrient utilization showed lower ghrelin expression.

Dietary carbohydrate levels significantly impacted growth performance, feed conversion, and the activities of digestive and metabolic enzymes in rohu fingerlings cultured in a recirculatory aquaculture system. The comprehensive analysis of digestive, metabolic, and gene expression responses revealed that a dietary carbohydrate level of 460 g kg^{-1} was the optimal requirement for maximizing the growth and health of rohu fingerlings in this system. This finding highlights the importance of tailored nutrition in enhancing the overall efficiency and productivity of aquaculture practices. Fixing the dietary carbohydrate level in the diet, further protein, and lipid levels can also be studied for rohu fingerlings reared in the RAS system.

Acknowledgments

The authors would like to express their gratitude to the Director of ICAR-Central Institute of Fisheries Education, Mumbai, for providing the necessary infrastructure for the experiment.

Authors' contribution: **S. Swamy:** Contributed to conceptualization, methodology, sampling, laboratory analysis, acquisition of data, validation, data curation, and drafting of the manuscript; **A.D. Deo:** Contributed to the conception and design of the study, supervision, analysis, interpretation of data, critical revision, validation and data curation; **M. Jayant:** Contributed to the analysis and interpretation of data, critical revision, validation, data curation and visualization; **N. Shamna:** Contributed to the interpretation of data and critical revision; **N.P. Sahu:** Contributed to the interpretation of data, critical revision, validation, and data curation; **K.D. Rasal:** Performed interpretation of data, validation; **M. Dhanalakshmi:** Performed sampling, analysis and software; **B.R. Prashanth:** Contributed sampling, analysis.

Funding: Not applicable.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: The animals used in the present study were handled as per the standard procedure approved by the Committee- Ethics and Animal Care of ICAR- Central Institute of Fisheries Education, Mumbai, India, and The CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests (Animal Welfare Division), India.

Conflict of interest: The authors declare that there is no conflict of interest.

Data availability: All the data analyzed for this study are included. Any extra data of interest, if needed, are available from the Corresponding Author upon reasonable request.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology*.

References

- Anand, G., I.A. Bhat, T. Varghese, S.A. Dar, N.P. Sahu, M.D. Aklakur, S. Kumar and S. Sahoo: Alterations in non-specific immune responses, antioxidant capacities and expression levels of immunity genes in *Labeo rohita* fed with graded level of carbohydrates. *Aquaculture*, **483**, 76–83 (2018).
- AOAC. Official Methods of Analysis of AOAC International. 16th ed. Washington, D.C., USA, pp. 25–28 (2005).
- APHA-American Public Health Association. Standard Methods for Examination of Water and Wastewater. 12th Edn., Washington, D.C., (2005).
- Bai, J., C. Li, Z. Tang, C. Wu and Z. Wei: Comparative study of carbohydrate levels on growth, oxidative stress and glucolipid metabolism of hybrid fish between *Megalobrama amblycephala* (♀) × *Culter alburnus* (♂) and *Culter alburnus*. *Reprod. Breed.*, **3**, 131–142 (2023).
- Bergmeyer, H.U. and E. Bernt: UV-Assay. In: Methods of Enzymatic Analysis. Academic Press pp. 613–617 (1974).
- Bregnballe, J.: A guide to recirculation aquaculture: an introduction to the new environmentally friendly and highly productive closed fish farming systems. FAO publisher, 100 pages (2015).
- Crab, R., T. Defoirdt, P. Bossier and W. Verstraete: Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture*, **356**, 351–356 (2012).
- d'Orbcassel, E.R., J.P. Blancheton and J. Aubin: Towards environmentally sustainable aquaculture: Comparison between two trout farming systems using life cycle assessment. *Aquac. Eng.*, **40**, 113–119 (2009).
- Drapeau, G.: Protease from *Staphylococcus aureus*. *Meth. Enzymol.*, **45**, 469–475 (1976).
- FAO: The State of World Fisheries and Aquaculture 2024 – Blue Transformation in Action. Rome, 232 pages (2024).
- Gangadhar, B., N. Sridhar, H. Umalatha and S.S. Giri: Effect of dietary carbohydrate levels on the growth of fingerlings of Carnatic carp *Barbodes carnaticus* (Jerdon 1849). *Indian J. Fish.*, **66**, 146–150 (2019).
- Halver, J.E.: Fish Nutrition. 2nd Edn. Academic Press, New York (1976).
- Hemre, G.I., T.P. Mommsen and A. Kroghdahl: Carbohydrates in fish

- nutrition: effects on growth, glucose metabolism, and hepatic enzymes. *Aquac. Nutr.*, **8**, 175–194 (2002).
- Kamalam, B.S., F. Medale and S. Panserat: Utilization of dietary carbohydrates in farmed fishes: new insights on influencing factors, biological limitations, and future strategies. *Aquaculture*, **467**, 3–27 (2017).
- Kumar, S., N.P. Sahu, A.K. Pal, D. Choudhury, S. Yengkokpam and S.C. Mukherjee: Effect of dietary carbohydrate on haematology, respiratory burst activity and histological changes in *L. rohita* juveniles. *Fish Shellfish Immunol.*, **19**, 331–344 (2005).
- Kumar, S., R. Sharma and A. Singh: Ammonia stress-induced physiological and histological changes in tropical carps under varying carbohydrate loads. *Int. J. Fisher.*, **24**, 102–115 (2025).
- Lei, C., H. Ji, J. Zhang and J. Li: Effects of dietary DHA/EPA ratios on fatty acid composition, lipid metabolism-related enzyme activity, and gene expression of juvenile grass carp, *Ctenopharyngodon idellus*. *J. World Aquacult. Soc.*, **47**, 287–296 (2016).
- Livak, K.J. and T.D. Schmittgen: Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻ΔΔCT method. *Methods*, **25**, 402–408 (2001).
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275 (1951).
- Masram, V., P. Singh, S.N. Datta and G. Tewari: Length weight relationship and condition factor of *Labeo rohita* (ham.) collected from domesticated and riverine habitats. *Indian J. Ecol.*, **49**, 869–872 (2022).
- Meena, D.K., P. Das and S. Sahoo: *Terminalia arjuna* bark powder as a nutritive additive for enhanced growth and immunity in *Labeo rohita* aquaculture. *Aquacul. Int.*, **33**, 45–60 (2025).
- Misra, H.P. and I. Fridovich: The purification and properties of superoxide dismutase from *Neurospora crassa*. *J. Biol. Chem.*, **247**, 3410–3414 (1972).
- Moreno-Arias, A., J.A. López-Elías, A. Miranda-Baeza, M.E. Rivas-Vega, L.R. Martínez-Córdova and J.C. Ramírez-Suárez: Replacement of fishmeal by vegetable meal mix in the diets of *Litopenaeus vannamei* reared in low-salinity biofloc system: effect on digestive enzymatic activity. *Aquacult. Nutr.*, **23**, 236–245 (2017).
- Ochoa, S.: Malic enzyme: malic enzymes from pigeon and wheat germ. *Meth. Enzymol.*, **1**, 323–326 (1955).
- Patel, V. and K. Singh: Sustainable development and engineering solutions for oxygen management in intensive tropical cyprinid culture. Sustainable Development and Engineering Solutions for Tourism, Agriculture and Food Sectors II, pp. 88–102 (2025).
- Pulkkinen, K., L.R. Suomalainen, A.F. Read, D. Ebert, P. Rintamäki and E.T. Valtonen: Intensive fish farming and the evolution of pathogen virulence: the case of columnaris disease in Finland. *Proc. R. Soc. B. or Proc. R. Soc. B. Biol. Sci.*, **277**, 593–600 (2010).
- Rick, W. and H.P. Stegbauer: α-Amylase: Measurement of reducing groups. In: *Methods of Enzymatic Analysis* (ED.: H.U. Bergmeyer). Academic Press, San Diego, CA, pp. 885–890 (1974).
- Santos, W.M., L.S. Costa, J.F. López-Olmeda, N.C.S. Costa, F.A.C. Santos, C.G. Oliveira, H.O. Guilherme, R.N. Bahiense, R.K. Luz and P.A.P. Ribeiro: Dietary protein modulates digestive enzyme activities and gene expression in red tilapia juveniles. *Animal*, **14**, 1802–1810 (2020).
- Sarkar, M. and T. Rahman: Modelling urban future aquaculture integrating CA-ANN models for temperature-growth dynamics in Indian Major Carps. *J. Mar. Sci. Engin.*, **13**, 250–268 (2025).
- Simms, D., P.E. Cizdziel and P. Chomczynski: TRIzol: A new reagent for optimal single-step isolation of RNA. *Focus*, **15**, 532–535 (1993).
- Sun, G., Y. Liu, D. Qiu, M. Yi, X. Li and Y. Li: Effects of feeding rate and frequency on growth performance, digestion and nutrients balances of Atlantic salmon (*Salmo salar*) in recirculating aquaculture systems (RAS). *Aquac. Res.*, **47**, 176–188 (2016).
- Takahara, S. and M. Ogata: Spectrometric studies on the catalase in acatalasemic red blood cell extracts, Stages 2 and 3 by Herbert-Pinsent. *Proc. Japan Acad.*, **38**, 779–782 (1962).
- Wilson, R.P.: Utilization of dietary carbohydrate by fish. *Aquaculture*, **124**, 67–80 (1994).
- Wooten, I.D.P.: Microanalysis. In: *Medical Biochemistry* (Ed. J.A. Churchill). 4th Edn., Churchill Ltd., London, UK, pp. 101–107 (1964).
- Wroblewski, F. and J.S. Laude: LDH activity in blood. *Proc. Soc. Exp. Biol. Med.*, **90**, 210–213 (1955).
- Zheng, L., Z. Wang, B. Zhang, L. Yan, P. Wang, C. Zhao, H. Lin, L. Qiu and C. Zhou: Effects of high dietary carbohydrate levels on growth performance, enzyme activities, expression of genes related to liver glucose metabolism, and the intestinal microbiota of *Lateolabrax maculatus* juveniles. *Fishes*, **8**, 431 (2023).
- Zhou, C., X. Ge, J. Niu, H. Lin, Z. Huang and X. Tan: Effect of dietary carbohydrate levels on growth performance, body composition, intestinal and hepatic enzyme activities, and growth hormone gene expression of juvenile golden pompano, *Trachinotus ovatus*. *Aquaculture*, **437**, 390–397 (2015).