



Impact of Dietary Lipid Levels on Growth Performance, Metabolic Enzymes and Carcass Composition in *Labeo rohita* Fingerlings Reared at Low Temperature in a Recirculating Aquaculture System (RAS)

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ABSTRACT

Background: The present experimental study investigated the optimal dietary lipid levels to improve the growth rate and metabolic efficiency in *Labeo rohita* fingerlings raised at 18°C±1°C in a recirculating aquaculture system (RAS) simulating the winter conditions.

Methods: Six experimental diets, each with similar crude protein content (300 g kg⁻¹) but varying lipid levels (40-140 g kg⁻¹) and digestible energy (15-17.95 MJ kg⁻¹) were prepared. Over a two-month feeding trial, fingerlings (8±0.09 g) were fed until they were satiated twice daily.

Result: Polynomial regression and broken-line analysis determined that 90 g kg⁻¹ lipid was optimal for growth and feed efficiency, with peak values for average final weight (AFW), protein efficiency ratio (PER) and feed conversion ratio (FCR). Important metabolic enzymes such as glucose-6-phosphatase (G6Pase), Glucose-6-phosphate dehydrogenase (G6PDH) and carnitine palmitoyltransferase1 (CPT1) similarly peaked at this lipid level, whereas higher lipid intake significantly (p<0.05) impaired enzyme activity. The ideal lipid level for AFW, as estimated by the quadratic equation ($y = -0.001x^2 + 0.1774x + 5.2264$, $r^2 = 0.98$), was 90.33 g kg⁻¹ and 90.03 g kg⁻¹ by broken-line analysis ($r^2 = 0.99$), with an averaging 90.18 g kg⁻¹. For feed conversion ratio (FCR), the quadratic equation ($y = 0.0005x^2 - 0.09x + 6.35$, $r^2 = 0.96$) predicted 91.82 g kg⁻¹, with the broken-line analysis indicating 89.34 g kg⁻¹, averaging 90.58 g kg⁻¹. For protein efficiency ratio (PER), the quadratic equation ($y = -0.0003x^2 + 0.05x - 0.734$, $r^2 = 0.95$) estimated 90.14 g kg⁻¹, whereas the broken-line analysis suggested 89.34 g kg⁻¹, with an average of 89.74 g kg⁻¹. All things considered, the optimal dietary lipid requirement for optimizing growth rate and nutrient efficiency was approximately 90 g kg⁻¹. These findings underscore the critical role of dietary lipid optimization in improving growth, nutrient utilization and metabolic health of *Labeo rohita* under winter culture conditions. To confirm these findings under different production systems and temperatures, more investigation is required.

Key words: Average final weight (AFW), Feed conversion ratio (FCR), *Labeo rohita*, Protein efficiency ratio (PER), Recirculating aquaculture system (RAS), Specific growth rate (SGR).

INTRODUCTION

Dietary lipids are essential for fish growth, development and overall health, particularly under suboptimal environmental conditions such as low temperatures (Glencross *et al.*, 2024). Low-temperature environments pose significant challenges to fish, such as reduced metabolic rates, impaired feeding efficiency and compromised growth (Correa *et al.*, 2023). Fish have developed adaptive behavioral and physiological mechanisms, collectively known as stress responses, to mitigate the adverse effects of temperature fluctuations (Currie *et al.*, 2014). These responses include enhanced antioxidant capacity, shifts in glucose metabolism and adjustments in fatty acid metabolism, which are essential for maintaining homeostasis and survival under thermal stress (Deng *et al.*, 2020). Important metabolic pathways, that are involving glucose-6-phosphatase (G6Pase) and peroxisome proliferator-activated receptors (PPARs), play crucial roles in glucose and lipid homeostasis during thermal stress (Wang *et al.*, 2022). Furthermore, the

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mechanistic target of rapamycin (mTOR) signaling pathway has been implicated in cold resistance, signifying its importance in physiological adaptation to low temperatures. Lipids are essential for cellular architecture, immunological response and metabolic regulation in species such as the commonly cultivated freshwater fish *Labeo rohita*. They also provide a concentrated energy source (Rasal *et al.*, 2023; Turchini *et al.*, 2024).

In recirculating aquaculture systems (RAS), low temperatures can still challenge fish physiology (Colombo *et al.*, 2024). Imbalances in dietary lipids, whether excessive or deficient, can lead to metabolic disorders, affecting growth and survival (Meng *et al.*, 2019; Das *et al.*, 2021). Optimizing lipid levels, particularly essential fatty acids (EFAs) like EPA and DHA, which enhance membrane fluidity, energy metabolism and immunological function, improve growth under thermal stress (Egessa *et al.*, 2024). *Labeo rohita* often known as rohu is an important freshwater species in Indian aquaculture, renowned for its rapid growth and high feed conversion efficiency but facing challenges at low temperatures (Murmu *et al.*, 2020; Surasani *et al.*, 2022). This study evaluates optimal dietary lipid levels for *L. rohita* fingerlings at low temperatures, assessing growth, feed intake, nutrient utilization, lipid metabolism and body composition to improve aquaculture sustainability in winter conditions.

MATERIALS AND METHODS

Experimental design

The feeding trial was conducted using a Recirculating Aquaculture System (RAS) with a combined filtration and a flow rate of 1.8 L min⁻¹. Two hundred seventy *L. rohita* fingerlings (8±0.09 g) were divided into six experimental groups (L4, L6, L8, L10, L12 and L14) in triplicate (15 fish/tank), fed twice daily to satiation, with biweekly weight measurements to adjust feeding rates. Mortality was monitored daily and water quality parameters (DO, pH, temperature, hardness, alkalinity, CO₂, ammonia and nitrites) were regularly measured by APHA (2005) method.

Experimental diet preparation

Six isonitrogenous practical diets (300 g/kg crude protein) were prepared with varying lipid levels viz., 40 g/kg (L4), 60 g/kg (L6), 80 g/kg (L8), 100 g/kg (L10), 120 g/kg (L12) and 140 g/kg (L14), corresponding to energy values of 1514-1795 MJ/kg (Table 1). The proportion of unsaturated to saturated fatty acids in the diets remained at 1:1. The ratio of cod liver oil to coconut oil was changed to alter energy levels. After blending, steaming, cooling and pelletizing the ingredients (apart from oils and additives), they were dried at 40°C to less than 10% moisture content and kept in a freezer at -40°C.

Collection of data

To evaluate growth and survival, the total biomass and fish count were noted both prior to stocking and at the end of the trial. After that, fish were chosen at random and given

50 µl/l of clove oil to make them unconscious. The entire body proximate composition of three fish was analyzed. In order to get tissue samples, fish were dissected at 4°C. Liver weight was measured and estimated following dissection.

Growth, feed conversion, nutrient utilization and body indices

The following formulae were used for the experiment.

$$WG (g) = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$SGR (\%/day) = \frac{\ln \text{ final weight (g)} - \ln \text{ initial weight (g)}}{\text{Duration (days)}} \times 100$$

$$FCR = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

$$PER = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

$$LER = \frac{\text{Weight gain (g)}}{\text{Lipid intake (g)}}$$

$$\text{Survival (\%)} = \frac{\text{Total alive animal}}{\text{Total stocked fish}} \times 100$$

Analysing fatty acids and proximate composition

A proximate analysis of the diets and the entire body of the fish was conducted using the established procedures of the AOAC (2015). A temperature of 105°C was maintained in a hot air oven until a constant weight was reached in order to assess the moisture content. Soxhlet apparatus (Soxtron, India) was used to estimate EE content, while Kjeldahl assembly (Kjeltron, India) was used to estimate CP content. In order to estimate the crude fiber (CF) content, fat-free samples were first digested using acid and alkali in a Fibretec (Fibrotron, India) and then burned for six hours at 550°C in a muffle furnace. By burning the samples for six hours at 550°C in a muffle furnace, the total ash (TA) content was calculated. The following formulas were used in the subtraction approach to get the NFE of diets and TC of shrimp samples.

$$NFE (\%) = \{100 - (CP\% + EE\% + CF\% + TA\%)\}$$

$$TC (\%) = \{100 - (CP\% + \text{Lipid}\% + TA\%)\}$$

Finally, the proximate components except water were expressed on dry weight basis.

Enzyme assays

Preparation of tissue homogenate

A 5% intestinal tissue homogenate was prepared using a Teflon-coated homogenizer (REMI Equipment, Mumbai, India) and cold 0.25 M sucrose solution at 4°C. The homogenate was centrifuged (Heraeus Megafuge 8R centrifuge, Thermo Fisher Scientific, Germany) at 5000 rpm for 10 min and the supernatant was stored at -20°C for the enzyme assay.

Metabolic enzymes

The Marjorie technique (1964) utilizes grams of phosphorus released per minute per milligram of protein at 37°C to

assess glucose-6-phosphatase activity. By measuring the amount of D-glucose that was phosphorylated per minute at 30°C, the Easterby and O'Brien method (1973) was used to measure hexokinase activity. With glucose-6-phosphate di-sodium salt as a substrate, glucose-6-phosphate dehydrogenase activity was measured (DeMoss, 1955). The amount of enzyme activity was measured in units per minute per milligram of protein at 37°C.

Analytical statistics

Statistical analysis was conducted using SPSS Version 22.0 with one-way ANOVA after ensuring for homogeneity of variance. Data are presented as mean $\pm$ SE and significant differences between means at the 5% probability level were determined using DMRT with post hoc analysis.

RESULTS AND DISCUSSION

Water quality parameters

Throughout the experiment, the water temperature varied between $18 \pm 0.5^\circ\text{C}$, pH between 7.2 and 8.6, dissolved oxygen between 9.02 and 9.23 mg/L and hardness between 272 and 288 mg L⁻¹. The amount of ammonical nitrogen, nitrite and nitrate fell between 0.05 and 0.11, 0.05 and 0.08 and 0.068 and 0.97, respectively.

Fatty acid composition of the experimental diets

Table 2 depicts the fatty acid content of the experimental diets, including the ratio of saturated to unsaturated fatty acids (USFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA). While MUFA

Table 1: Feed formulation and proximate composition of the experimental diets.

Ingredient's composition (g kg ⁻¹)	Experimental diets ¹					
	L40	L60	L80	L100	L120	L140
Fish meal	60.00	60.00	60.00	60.00	60.00	60.00
Defatted soybean meal	300.00	300.00	300.00	300.00	300.00	300.00
Groundnut oil cake	60.00	60.00	80.00	80.00	80.00	80.00
De-oiled rice bran	300.00	283.80	266.80	242.80	222.80	200.80
Corn flour	100.00	100.00	100.00	100.00	100.00	100.00
Wheat flour	100.00	100.00	100.00	100.00	100.00	100.00
Coconut oil	16	23.00	31.00	40.00	50.00	59.00
Fish oil	2.00	11.00	20.00	35.00	45.00	57.00
BHT ²	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride	2.00	2.00	2.00	2.00	2.00	2.00
CMC ³	20.00	20.00	20.00	20.00	20.00	20.00
*Vitamin-mineral premix	20.00	20.00	20.00	20.00	20.00	20.00
Cellulose	19.80	0.00	0.00	0.00	0.00	0.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Proximate composition⁴ (g kg⁻¹; on dry weight basis)						
Dry matter	908.00	910.70	918.50	920.20	928.90	935.10
Crude protein	301.00	302.50	300.61	300.82	299.60	299.49
Ether extract	41.30	60.61	79.69	98.71	120.49	140.11
Crude fiber	72.70	64.20	64.10	41.40	45.10	45.20
Nitrogen-free extract ⁵	510.90	495.90	475.80	480.30	465.00	458.20
Total ash	74.12	76.39	79.59	78.98	69.92	57.15
DE ⁶ (MJ kg ⁻¹)	15.14	15.64	15.99	16.78	17.32	17.95
P: E ⁷ (g protein MJ ⁻¹ DE)	19.88	19.34	18.70	17.92	17.29	16.68

*Composition of vitamin-mineral premix (Agrimin 3503; quantity kg⁻¹) Vitamin A, 7,00,000 IU; Vitamin D₃, 7,00,000 IU; Vitamin E, 250 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B₆, 1,000 mg; Vitamin B₁₂, 6 mcg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 1 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 325 mg; Fe, 1,500 mg; Zn, 6,000 mg; Cu, 1,000 mg; Co, 150 L- lysine, 10 g; DDL-Methionine 10 g; Selenium 125 mg; Vitamin C, 2500 mg.

¹Experimental diets viz. L40, L60, L80, L100, L120 and L140 contain 40, 60, 80, 100, 120 and 140 g lipid kg⁻¹ diet with equal proportion of saturated and unsaturated fatty acids.

²BHT Butylated hydroxytoluene.

³CMC Carboxymethyl cellulose.

⁴Proximate composition of experimental diet- Data were presented as mean (n = 3).

⁵NFE% nitrogen-free extract = $[100 - (\text{CP}\% + \text{EE}\% + \text{CF}\% + \text{TA}\%)]$; $\text{TC}(\%) = 100 - (\text{CP}\% + \text{LIPID}\% + \text{TA}\%)$.

⁶DE (MJ kg⁻¹) digestible energy = $\{16.74 \times \text{CP} (\text{g/kg})\} + \{37.66 \times \text{EE} (\text{g/kg})\} + \{16.74 \times \text{NFE} (\text{g/kg})\}$ (Halver, 1976).

⁷P:E- Protein-to-energy ratio.

and PUFA levels significantly increased from 6.82 g kg⁻¹ to 38.82 g kg⁻¹ (p<0.05) and 13.82 g kg⁻¹ to 31.23 g kg⁻¹ (p<0.05), respectively, SFA content increased from 20.68 g kg⁻¹ in L4 to 70.11 g kg⁻¹ in L14 (p<0.05). The SFA/USFA ratio was constant at approximately 1.00 across diets, but the n-3/n-6 ratio increased from 0.27 in L4 to 1.12 in L14 (p<0.05).

Growth rate and nutrient utilization

Table 3 demonstrates that dietary lipid levels had a significant (p<0.05) impact on fish growth and utilization of nutrients efficiency. The lipid between 80 and 100 g kg⁻¹ produced the

maximum AFW, PER and FCR. Using polynomial regression analysis, the exact dietary lipid requirement was determined. The ideal lipid level for AFW (Fig 1), FCR (Fig 2) and PER (Fig 3) was determined by the quadratic equation to be around 90 g kg⁻¹, whereas the broken-line analysis (r²=0.99) showed 90.03 g kg⁻¹, averaging 90.18 g kg⁻¹ (for AFW).

Metabolic enzyme activities

Dietary lipid levels had a significant (p<0.05) impact on *Labeo rohita*'s important metabolic enzymes (G6Pase, G6PDH and CPT1), with optimal activity noted at lipid levels

Table 2: Fatty acid composition of the experimental diets (g kg⁻¹ diet).

Fatty acids	L4	L6	L8	L10	L12	L14
Saturated fatty acids (SFA)						
8:00	1.92	3.3	4.42	5.63	6.76	7.71
10:00	1.51	2.07	2.85	3.67	4.69	5.49
12:00	6.62	10.4	13.59	17.65	21.53	25.5
14:00	2.71	4.43	6.11	8	10	11.71
15:00	0.12	0.07	0.05	0.06	0.04	0.04
16:00	6.36	8.33	10.42	11.56	14.05	15.75
17:00	0.18	0.25	0.43	0.66	0.42	0.9
18:00	0.94	1.21	1.53	1.57	1.87	2.45
20:00	0.01	0.04	0.12	0.2	0.19	0.11
22:00	0.11	0.07	0.13	0.15	0.39	0.18
23:00	0.17	0.02	0.07	0.13	0.13	0.11
24:00.00	0.03	0.16	0.18	0.08	0.19	0.15
ΣSFA	20.68±0.34 ^a	30.33±0.39 ^b	39.88±0.42 ^c	49.35±0.44 ^d	60.26±0.59 ^e	70.11±0.61 ^f
Monounsaturated fatty acids (MUFA)						
14:1n-5	0	0.06	0.11	0.33	0.37	0.29
16:1n-7	0.35	0.82	1.64	2.77	4	4.64
17:1n-7	0.07	0.2	0.26	0.43	0.31	0.41
18:1n-7	3.17	4.24	5.1	4.08	6.18	6.77
18:1n-9	3.01	4.85	7.02	9.72	12.69	15
20:1n-9	0.19	1.16	2.26	3.97	5.49	6.47
22:1n-9	0.03	1.04	1.72	2.92	3.87	5.24
Total MUFA	6.82±0.13 ^a	12.37±0.18 ^b	18.12±0.21 ^c	24.22±0.34 ^d	32.92±0.44 ^e	38.82±0.51 ^f
Polyunsaturated fatty acids (PUFA)						
18:2n-6	10.57	12.1	13.41	12.53	11.52	12.82
18:3n-3α	1.19	1.37	1.71	1.89	1.86	1.85
18:3n-3γ	0.09	0.27	0.43	0.92	1.05	0.52
20:2n-6	0.16	0.32	0.49	0.54	0.84	1.46
20:3n-3	0.02	0.04	0.15	0.35	0.28	0.42
20:4n-6	0.14	0.16	0.29	0.45	0.37	0.45
20:5n-3	0.85	1.71	2.65	3.98	5.56	6.51
22:6n-3	0.81	1.93	2.61	4.48	5.84	7.2
Σn-3	2.96	5.33	7.55	11.61	14.57	16.5
Σn-6	10.86	12.58	14.18	13.52	12.72	14.72
Σn-3/Σn-6	0.27±0.01 ^a	0.42±0.01 ^b	0.53±0.01 ^c	0.86±0.01 ^d	1.14±0.01 ^e	1.12±0.01 ^e
ΣPUFA	13.82±0.11 ^a	17.91±0.14 ^b	21.73±0.17 ^c	25.13±0.16 ^d	27.32±0.19 ^e	31.23±0.22 ^f
ΣUSFA	20.65±0.30 ^a	30.28±0.34 ^b	39.84±0.39 ^c	49.35±0.44 ^d	60.24±0.47 ^e	70.05±0.51 ^f
ΣSFA/ΣUSFA	1.001	1.002	1.001	1.000	1.000	1.001

Values are expressed as Mean ± SE, n=3; Mean values in the same column with different superscripts differ significantly (P<0.05).

¹Experimental diets viz. L40, L60, L80, L100, L120 and L140 contain 40, 60, 80, 100, 120 and 140 g lipid kg⁻¹ diet with equal proportion of saturated and unsaturated fatty acids.

of 80-100 g kg⁻¹ (Table 4). To determine the ideal dietary lipid content for the maximum enzyme activity in *Labeo rohita*, polynomial regression and broken-line analysis were used. Hexokinase ($y = -0.0001x^2 + 0.0174x - 0.2344$, $r^2 = 0.96$) (Fig 4); G6Pase ($y = -0.0004x^2 + 0.0707x - 0.9441$, $r^2 = 0.96$) (Fig 5); G6PDH ($y = -0.0054x^2 + 0.8986x - 11.973$, $r^2 = 0.96$) (Fig 6); CPT1 ($y = -0.0054x^2 + 0.8986x - 11.973$, $r^2 = 0.96$) (Fig 7).

The health of fish depends on dietary lipids, such as saturated and unsaturated fatty acids, which enable energy

storage, membrane fluidity and cold adaption. In cold-water aquaculture, optimal lipid levels balance protein-to-energy ratios, improving development and immunity while lowering nitrogenous waste and environmental effect (Luc *et al.*, 2024). An excessive intake of fat can reduce fish output, increase body lipid accumulation, impair nutrient digestion and cause conditions like fatty liver syndrome. The effects of the ideal dietary lipid require on development, nutrition utilization and body composition are investigated in this study for *L. rohita* fingerlings in a low-temperature RAS.

Table 3: Growth, nutrient utilization and survival of *Labeo rohita* fingerlings fed with different experimental diets for 60 days.

Treatments	AIW (g)	AFW (g)	PER	FCR	LER
L40	8.05±0.01	10.88±0.14 ^a	0.98±0.05 ^a	3.43±0.16 ^d	4.88±0.36 ^c
L60	8.03±0.01	12.11±0.2 ^b	1.22±0.06 ^{bc}	2.74±0.12 ^{bc}	4.91±0.28 ^c
L80	8.04±0.02	13.30±0.26 ^d	1.68±0.08 ^d	1.99±0.09 ^a	6.31±0.3 ^e
L100	8.06±0.02	13.19±0.23 ^{cd}	1.63±0.09 ^d	2.06±0.11 ^a	6.01±0.27 ^d
L120	8.03±0.01	12.73±0.24 ^{bc}	1.30±0.06 ^c	2.57±0.12 ^b	3.26±0.16 ^b
L140	8.06±0.01	10.88±0.12 ^a	1.08±0.05 ^{ab}	3.09±0.14 ^{cd}	2.32±0.11 ^a
<i>p-value</i>	0.087	0.001	0.001	0.001	0.001

Data are expressed as mean ± SE, n = 3; Mean values with different superscripts in the same column differed significantly (P<0.05). AIW: Average initial weight; AFW: Average final weight; WG%: Weight gain percentage; SGR: Specific growth rate; FCR: Feed conversion ratio; PER: Protein efficiency ratio.

¹Experimental diets viz. L40, L60, L80, L100, L120 and L140 contain 40, 60, 80, 100, 120 and 140 g lipid kg⁻¹ diet with equal proportion of saturated and unsaturated fatty acids.

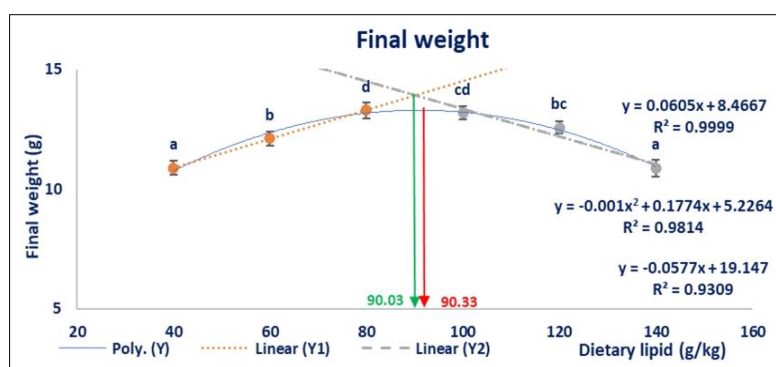


Fig 1: Quadratic (second-order polynomial) regression and broken-line linear analysis of final weight against increasing dietary lipid.

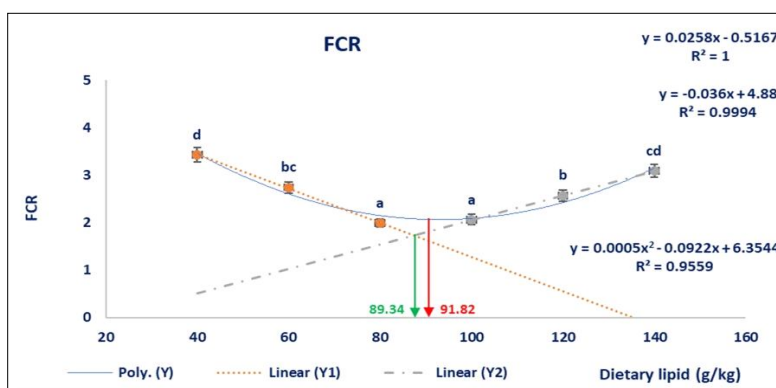


Fig 2: Quadratic (second-order polynomial) regression and broken-line linear analysis of feed conversion ratio (FCR) against increasing dietary lipid.

In order to avoid carp from consuming less feed when the temperature drops below 18°C, the fish were raised in water with an ideal quality of 18.0±0.5°C (Mohapatra *et al.*, 2011).

Aquaculture relies significantly on water quality since it affects fish survival, growth and health (Das *et al.*, 2004). A pH of 7.9-8.3 (Ayyappan *et al.*, 2006), dissolved oxygen levels of 8.32-9.1 mg L⁻¹ (above the 5 mg L⁻¹ minimum) and ammonia levels of 0.04-0.08 mg L⁻¹ (within the carp tolerance range) were all part of the *Labeo rohita* culture conditions used in this investigation (Das *et al.*, 2004). To evaluate the effects of low temperatures, the temperature was kept at 18±0.5°C throughout the period of the experiment. In order to investigate lipid requirements, six isonitrogenous, heterolipidic (L40 to L140) and hetero-energetic (15.14-17.95 MJ kg⁻¹) diets were prepared with 300.67 g kg⁻¹ protein and P:E ratios of 16.68-19.88 g protein MJ⁻¹DE in accordance with accepted standards (Halver, 2002). The crude fiber (6.99-7.52%), total ash (6.46-7.95%)

and crude protein (30.02-30.4%) in the diet meet the nutritional requirements of Indian Major Carp (Baruah *et al.*, 2005). Fish growth at low temperatures depends on dietary lipid since it gives them energy and facilitates their absorption of nutrients (Luc *et al.*, 2024). Within this investigation, *Labeo rohita* fingerlings raised in a RAS at 18±0.5°C demonstrated excellent development and feed efficiency at 90 g kg⁻¹ of dietary lipid level. This amount was consistently found to be optimal by polynomial regression and broken-line analyses, with AFW, PER and FCR peaking at 90.18 g kg⁻¹, 89.74 g kg⁻¹ and 90.58 g kg⁻¹, respectively. This balance is necessary since excessive fat deposition, early satiation, decreased feed intake, inadequate protein intake and metabolic stress were all brought on by greater lipid levels (e.g., 120-140 g kg⁻¹), whereas lower levels (e.g., 40-60 g kg⁻¹) resulted in energy deficits. These results are consistent with those of Abdel-Ghany *et al.* (2021), who reported that Nile tilapia growth was optimal at 70-85 g kg⁻¹

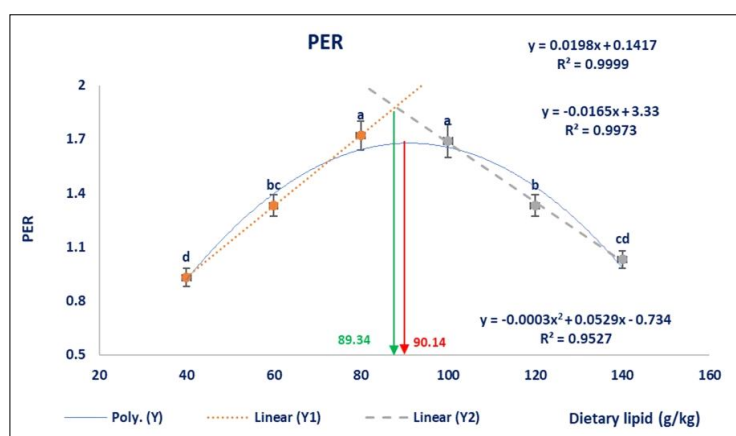


Fig 3: Quadratic (second-order polynomial) regression and broken-line linear analysis of protein efficiency ratio (PER) against increasing dietary lipid.

Table 4: Liver metabolic enzymes activities of liver and muscle tissue of *Labeo rohita* fingerlings fed with different experimental diets for 60 days.

Treatments ¹	L40	L60	L80	L100	L120	L140	p-value
Hexokinase ²	0.3±0.01 ^b	0.4±0.01 ^c	0.52±0.02 ^{de}	0.45±0.02 ^d	0.32±0.01 ^b	0.15±0.01 ^a	0.001
G6Pase ³	1.23±0.05 ^b	1.64±0.07 ^c	2.12±0.09 ^d	1.85±0.11 ^c	1.3±0.09 ^b	0.62±0.03 ^a	0.001
G6PDH ⁴	15.65±0.69 ^b	20.87±0.92 ^c	26.95±1.19 ^d	23.48±1.03 ^c	16.52±0.79 ^b	7.83±0.34 ^b	0.004
CPT 1 ⁵	55.67±2.45 ^b	74.23±3.27 ^c	95.88±4.22 ^d	83.51±3.67 ^c	58.76±2.82 ^b	27.84±1.22 ^a	0.003
Malic enzyme ⁶	0.11±0.01 ^b	0.15±0.01 ^c	0.19±0.01 ^d	0.17±0.01 ^{cd}	0.12±0.01 ^b	0.07±0.01 ^a	0.001

All values are expressed as Mean ± SE, n=3; Mean values in the same column with different superscripts differ significantly (P<0.05).

¹Experimental diets viz. L40, L60, L80, L100, L120 and L140 contain 40, 60, 80, 100, 120 and 140 g lipid kg⁻¹ diet with equal proportion of saturated and unsaturated fatty acids.

²Hexokinase expressed in milliunits min⁻¹ mg⁻¹ protein.

³Glucose 6 phosphatases (G6Pase) expressed as µg phosphorus released min⁻¹ mg⁻¹ protein at 37°C.

⁴G6PDH, glucose 6 phosphate dehydrogenase expressed as unit min⁻¹ mg⁻¹ protein at 37°C.

⁵CPT1, carnitine palmitoyltransferase¹ expressed as units min⁻¹ mg⁻¹ protein at 37°C;

⁶Malic enzyme, expressed in milliunits min⁻¹ mg⁻¹ protein.

⁷LDH, lactate dehydrogenase, expressed as micromoles of NAD⁺ released min⁻¹ mg⁻¹ protein.

⁸MDH, malate dehydrogenase, expressed as NADH oxidized min⁻¹ mg⁻¹ protein.

lipid and Mishra and Samantaray (2004), who noticed better PER and SGR in rohu at 8% lipid in the food reared in 21°C. The significance of balancing lipid content and fatty acid composition was confirmed by similar findings in young cobia, striped bass and darkbarbel catfish (Lutfi *et al.*, 2023; Siciliani *et al.*, 2023).

Hexokinase phosphorylates glucose to start glycolysis and the endoplasmic reticulum's glucose-6-phosphatase (G6Pase) completes glycogenolysis and gluconeogenesis

(Xia *et al.*, 2024). The study found that G6Pase activity peaked at 90 g kg⁻¹ lipid level and then sharply declined beyond 100 g kg⁻¹, indicating that this level is ideal in order to encourage gluconeogenesis. According to Paul *et al.* (2021) in butter catfish and Atasever *et al.* (2014) in brown trout, excess lipids above this level may result in feedback inhibition or impair enzyme function, so this lipid level of 90 g kg⁻¹ provides adequate energy and metabolic precursors to support gluconeogenesis without causing metabolic

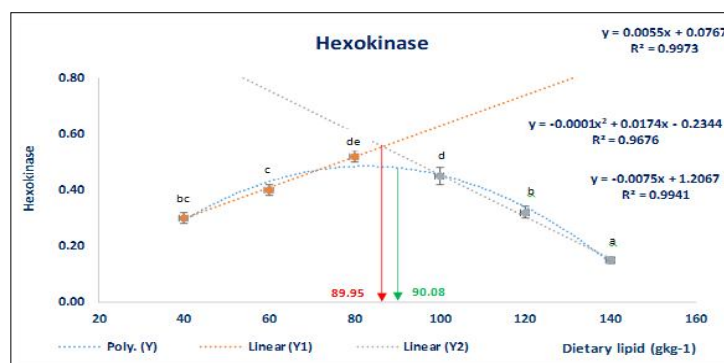


Fig 4: Quadratic (second-order polynomial) regression and broken-line linear analysis of hexokinase activity against increasing dietary lipid.

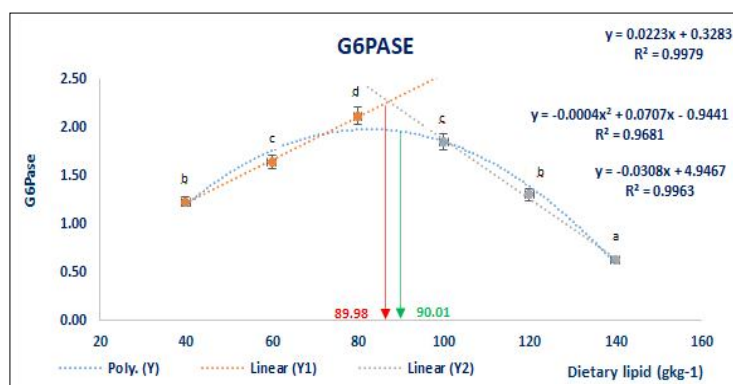


Fig 5: Quadratic (second-order polynomial) regression and broken-line linear analysis of G6Pase activity against increasing dietary lipid.

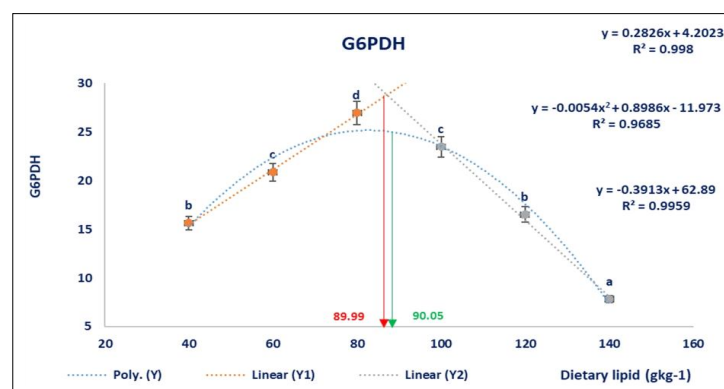


Fig 6: Quadratic (second-order polynomial) regression and broken-line linear analysis of G6PDH activity against increasing dietary lipid.

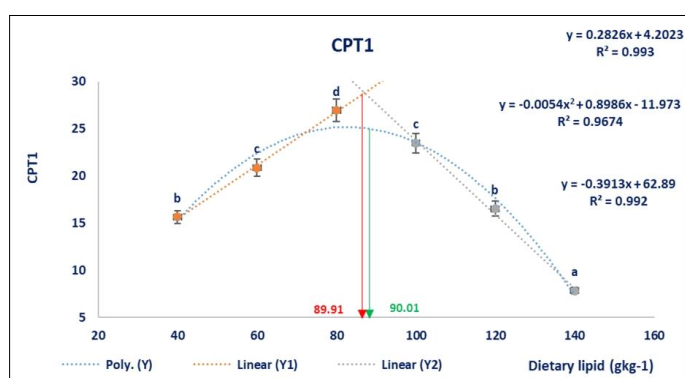


Fig 7: Quadratic (second-order polynomial) regression and broken-line linear analysis of CPT1 activity against increasing dietary lipid.

overload. The pentose phosphate pathway's NADPH synthesis depends on glucose-6-phosphate dehydrogenase (G6PDH), which likewise exhibited peak activity at 90 g kg⁻¹ lipid and decreased after 100 g kg⁻¹. Since excess lipids may interfere with glucose metabolism and lower NADPH production-which is essential for antioxidant defense and lipid biosynthesis-this reduction is most likely the result of either poor insulin sensitivity or cellular stress at higher lipid levels. In addition, this tendency has been observed in rainbow trout (Hemre and Sandnes, 1999) and butter catfish (Paul *et al.*, 2021). An important enzyme in β -oxidation, carnitine palmitoyltransferase-1 (CPT-1), was most active at 90 g kg⁻¹ lipid and less effective above this range. Since excess lipids may affect mitochondrial function, this suggests a shift in metabolic pathways caused by substrate saturation or oxidative stress at higher lipid levels. This interpretation is comparable with observations reported from rainbow trout (Turchini *et al.*, 2013) and juvenile turbot (Peng *et al.*, 2014).

Supplying a nutritionally appropriate diet for rohu in RAS at freezing temperatures is essential for their development, immunity and ability to adapt physiologically. Arachidonic acid and other polyunsaturated fatty acids, in particular, assist reduce winter stress and promote homeoviscous adaption when consumed in adequate amounts. Understanding fatty acid dynamics in feed formulation is made easier by this work, which also emphasizes the necessity of PUFA-enriched diets for rohu in cold-water RAS and suggests more research in this area of study.

CONCLUSION

In conclusion, based on the findings of the present study highlights the significance of dietary lipids, with an ideal level of 90 g kg⁻¹, for promoting growth, nutrient utilization and metabolic efficiency of *Labeo rohita* fingerlings at low temperatures (18±0.5°C). Excessive lipid intake (>100 g kg⁻¹) caused metabolic stress and reduced growth rate, emphasizing the need for accurate lipid management in cold-water aquaculture to ensure optimal physiological fish health and performance.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors proclaim no competing interest.

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