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Effect of dietary lipid levels on growth, intestinal enzyme activity, and expression of genes involved in long chain polyunsaturated fatty acid synthesis in scale carp, *Cyprinus carpio* var. *communis* fingerlings

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Abstract
Lipids play a significant role in growth and various metabolic activities in fish, inclusion of appropriate level of lipid is therefore essential in aquaculture. A 70-day feeding trial was conducted to evaluate the effect of dietary lipids levels on growth performance, hemato-biochemical parameters, serum parameters, intestinal enzyme activity, and expression of genes involved in long chain polyunsaturated fatty acids in scale carp fingerlings. *Cyprinus carpio* var. *communis* fingerlings (1.57 ± 0.02 g/fish) were fed isonitrogenous diets (428 g/kg crude protein) containing varying lipid levels (20, 40, 60, 80,100 and 120 g/kg). Fish were fed twice daily to triplicate group of 20 fish per tank at 09:00 and 16:00 hours at the rate of 4% body weight/day. The results show that growth parameters including live weight gain (LWG%), protein efficiency ratio (PER), specific growth rate (SGR%), feed conversion ratio (FCR) and proximate composition vary significantly ($P < 0.05$) with different dietary lipid levels. While, no significant ($P > 0.05$) differences in body ash content were observed among the treatments. Hematological and serum parameters also showed significant variation among the treatment. Higher enzymatic activity, with the exception of amylase, was observed at 60.0 g/kg lipid diet. Fatty acid desaturase 2 (FADS2) and elongase of very long chain fatty acids (ELOVL5) mRNA showed higher relative expression at 60.0 and 80.0 g/kg lipid fed diets, respectively. Based on the findings of quadratic regression analysis the optimal dietary requirement of linseed oil as a lipid source for maximum growth in scale carp fingerlings was established at 68.0 g/kg in presence of 20.0 g/kg of cod liver oil of dry diet.

Keywords: Scale carp, Lipid level, hemato-biochemical parameters, growth performance.

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1. Introduction

Fishes have higher dietary protein requirement than mammals and birds because fish primarily depend on protein to fulfill their energy requirement (Li et al., 2020; Li et al., 2021). However, excessive protein content in diet drives up feeding expenses and also results in sub-optimal growth performance and higher nitrogen excretion (Talukdar et al., 2020; Pang et al., 2024). Therefore, to optimize the protein utilization, diets have been designed with increased amounts of energy sources other than proteins, such as lipids and carbohydrates, that reduce the reliance on dietary protein. Apart from energy, lipids also provide vital functional components such as fatty acids, phospholipids, and cholesterol, which play a crucial role in fish growth, development, and overall well-being (Leaver et al., 2008; Sun et al., 2013). It is widely recognised that incorporating lipids into fish diets helps minimize protein usage, improve growth rates, and make aquaculture more cost-effective while reducing its environmental footprint (Wang et al., 2005; Xie et al., 2021).

The inclusion of appropriate lipid levels in the diets of fish is both quantitatively and qualitatively significant, due to its fundamental involvement in biological functions. These include acting as the main source of energy for metabolism as well as supplying vital fatty acids required for physiological processes, assisting in the absorption and distribution of fat-soluble vitamins, and preserving the structural integrity and fluidity of cell membranes, all of which are essential for the best possible development, reproduction, and growth (Wang et al., 2021; Sargent et al., 2002). However, insufficient lipid levels in fish diets often result in suboptimal growth performance (Perez-Velazquez et al., 2016). Conversely, overabundance of dietary lipids in the diet negatively affects fish health while also diminishing growth and feed efficiency (Peng et al., 2017; Wang et al., 2021). Moreover, diets with elevated lipid levels are more prone to oxidation, which can affect their quality over time (Pang et al., 2024). Optimum dietary lipid levels for various fish species are influenced by multiple factors, such as life stage, species-specific requirements, environmental conditions, and overall nutritional balance. Hence, determining the appropriate lipid levels of culturable fish species is crucial for nutritional efficiency, environmental sustainability, and economic viability (Chupal et al., 2021).

In general, humans primarily rely on marine organisms for the supply of n-3 LC-PUFAs (Pereira et al., 2003; Marrero et al., 2022). Due to this, a significant number of species are now experiencing overexploitation, which has resulted in a scarcity of these

natural lipid sources. Aquaculture has been suggested as the most promising option for satisfying the rising need for fish and other seafood on a worldwide scale (FAO, 2020). Ironically, finfish aquaculture feed production is vastly reliant on fish meal and fish oil (FAO, 2020), creating an unsustainable approach that confines its growth. Therefore, it's essential to look for an alternative to fish oil, especially the vegetable oils, as they are more cost-effective and easier to obtain. Consequently, vegetable oils (VOs) lack LC-PUFAs, which can negatively impact fish health and diminish their nutritional value for human consumption (Perez et al., 2014; Marrero et al., 2022). So, amidst this backdrop, an escalating interest has emerged in unraveling the capacities and mechanisms governing the steps in the production of LC-PUFAs in cultured fish species.

Freshwater fishes exhibit a notable capability to convert C18 PUFAs into C20-22 PUFAs, including arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Ren et al. 2012). The biosynthetic pathways of LC-PUFAs involve the synergistic action of elongases (ELOVL) and fatty acyl desaturases (FADS), such as FADS2 and ELOVL5 (Castro et al., 2016; Xie et al., 2021). FADS2 is a membrane-bound protein located in the endoplasmic reticulum, serving as the initial enzyme in the production of LC-PUFAs containing 20 or more carbon atoms. FADS operate by introducing a double bond between an existing double bond and the carboxylic group. While elongases serve as rate-limiting enzymes crucial for elongating fatty acids in the pathway (Castro et al. 2016). ELOVL5 exhibits preference for elongating C18 and C20 substrates, while ELOVL2 exhibits preference for C20 and C22 PUFA as substrates (Monroig et al., 2009). However, the ability of elongation and desaturation varies among different fish species (Monroig et al., 2011), which warrants that the exact mechanism of these genes with respect to species is imperative.

C. carpio var. *communis* is an omnivorous fish commonly known as scale carp. The common carp is a highly viable large-sized candidate species for freshwater aquaculture, offering significant potential for commercial production as well. It is a member of the largest freshwater family, Cyprinidae, renowned for its high nutritional value, delightful taste, easy digestibility, affordability, and widespread availability. Owing to its rapid growth and ease of cultivation, the fish is identified as one of the most extensively cultivated species in freshwater aquaculture worldwide (Guler et al., 2008; Yousefi et al., 2020; Hassan et al., 2022).

The objective of the present study was to establish the dietary lipid requirement and to explore the impacts of various dietary lipid levels on growth, fatty acid composition, hematology, proximate composition, serum profile and relative mRNA expression of genes involved in LC-PUFAs biosynthesis.

Materials and Methods

2.1 Formulation and Prepration of experimental diets

Six isonitrogenous diets (428 g/kg crude protein) with varying lipid levels, containing graded levels of gross energy varied from 16.48-20.25 KJ/g, dry diets were designed. The diets were formulated with casein and gelatin as source of protein, cod liver oil and linseed oil were used as lipid source. The level of linseed oil was varied at 20, 40, 60, 80, 100 and 120 g/kg, whereas cod liver oil was fixed at 20 g/kg in all the experimental diets, the diets were labelled as D_1, D_2, D_3, D_4, D_5 and D_6 (Table 1). Gelatin was dissolved in water through the process of heating and stirring in a bowl. Subsequently, casein and other ingredients were thoroughly mixed using a Hobart Corporation mixer. Once the mixture reached at 40 °C, vitamins premixes and oils were added to it with constant stirring. Lastly, carboxymethyl cellulose (CMC) was added and thoroughly mixed in order to obtain dough, later on the diet was processed using a pelletizer which was equipped with a 2mm die for the production of suitable sized pellets. However, the moisture content was reduced below 100 g/kg through the desiccation in hot air oven at 40 °C. The dried pellets were ground up, sieved and kept in storage at 4 °C until use.

Table 1. Composition of experimental diets used for estimating the lipid requirement of common carp, *Cyprinus carpio* var. *communis* fingerlings.

	Experiments diets					
Ingredients (g kg^{-1}, dry diet)	**20.0 (D_1)**	**40.0 (D_2)**	**60.0 (D_3)**	**80.0 (D_4)**	**100.0 (D_5)**	**120.0 (D_6)**
Casein[1]	414.60	414.60	414.60	414.60	414.60	414.60
Gelatin[2]	103.60	103.60	103.60	103.60	103.60	103.60
Dextrin	220.0	220.0	220.0	220.0	220.0	220.0
Cod liver oil[3]	20.0	20.0	20.0	20.0	20.0	20.0
Linseed oil[4]	20.0	40.0	60.0	80.0	100.0	120.0
Mineral mix[5]	40.0	40.0	40.0	40.0	40.0	40.0
Vitamin mix [6]	30.0	30.0	30.0	30.0	30.0	30.0
Carboxymethyl cellulose	40.0	40.0	40.0	40.0	40.0	40.0

Alpha cellulose	111.80	91.80	71.80	51.80	31.80	11.80
Total	1000	1000	1000	1000	1000	1000
Calculated crude protein (g kg^{-1})	428.0	428.0	428.0	428.0	428.0	428.0
Gross energy[7] (kJ g^{-1}, dry diet)	16.48	17.23	17.99	18.74	19.49	20.25
Proximate composition of experimental diet (g kg^{-1})						
Dry matter	912.32	913.65	914.62	916.65	917.82	918.80
Lipid content	428.4	428.9	427.7	428.8	428.2	427.6
Analyzed crude protein	41.63	61.62	80.84	101.62	121.22	139.68
Estimated Gross energy (kJ g^{-1}, dry diet)	16.65	17.41	18.08	18.81	19.61	20.33

[1]Crude protein (80%), [2]Crude protein (93%), Loba Chemie, India; [3]Sea cod pure cod liver oil, Universal Nutri science Private Limited, Mumbai, India. [4]India unrefined Linseed oil, Nashiel, Chemical Private Limited, Ahmedabad, India. [5]Halver 2002 mineral ($AlCl_3$. $6H_2O$, 150; $ZnSO_4$. $7H_2O$, 3000; CuCl,100; $MnSO_4$.4-$6H_2O$, 800; KI,150; $CoCl_2$.$6H_2O$,1000 mg kg^{-1}; plus USP # 2 Ca (H_2PO_4)2. H_2O, 135.8; $C_6H_{10}CaO_6$ 327.0; $C_6H_5O_7Fe$.$5H_2O$, 29.8; $MgSO_4$.$7H_2O$, 132.0; KH_2PO_4 (dibasic), 239.8; NaH_2PO_4.$2H_2O$, 87.2; NaCl, 43.5 (g kg^{-1}); [6]vitamin mix (choline chloride 5000: thiamin HCL 50; riboflavin 200; pyridoxine HCL 50; nicotinic acid 750; calcium pentothenate 500; inositol 2000; biotin 5.0; folic acid 15; ascorbic acid 1000; menadione 40; alpha-tocopheryl acetate 400; cyanocobalamine 0.1 (g kg^{-1}). [6]Calculated on the basis of physiological fuel values 4.5, 3.5 and 8.5 kcal g^{-1} for protein, carbohydrate and fat, respectively (Jauncey, 1982).[7]Estimated on Bomb calorimeter (Model 6400; Parr, Moline, Illinois, USA).

2.2 Feeding trial

C. carpio var. *communis* fingerlings were obtained from the Govt. Fish Seed Farm, district Manasbal for the purpose of this experiment. Which were transported in oxygen filled polythene bags to experimental station at University of Kashmir. Subsequently, the fish were prophylactically treated with a 1:3000 solution of $KMnO_4$, so as to remove any infection and stocked in circular plastic fish tank having water capacity of 600 L. Before starting the feeding trial, the fish were acclimated in laboratory conditions by feeding H_{440} diet (Halver, 2002) for a duration of fortnight. Later on, desired number of acclimated fingerlings, 1.59 ± 0.02 g (ABW± SD), were randomly distributed in 18 circular troughs with 70 L water holding capacity, supplied with continuous water flow. 20 fish were housed in tank for each treatment level 3 replicates were made. The rate of water flow for each tank was consistently kept at a range of 1.0-1.5 liters per minute. The diets were fed at the rate of 4% body weight/day into two equal amounts and fed at 09:00 and 17:00, hours daily. Fish were starved on the days designated for recording weekly weights. A digital top-loading balance was used to measure the initial and weekly weights (Sartorius CPA-224S, Goettingen, Germany). The fish excreta were removed through siphoning every morning before the daily feeding. Additionally, any unconsumed feed, if detected, was gathered, oven dried, and re-weighed to estimate the exact amount of feed consumed by the fish during the entire period feeding trial.

2.3 Proximate analysis

On the initiation of the feeding trial, 40 fish from the acclimatized lot were sacrified for determination of somatic indices and initial proximate analysis. Similarly, after the completion of the experiment, the final weight from each replicate was recorded on the last day. Subsequently, 12 fish were chosen for analysing the final proximate composition from each dietary treatment. The proximate composition of experimental diets, as well as the initial and final body constituents was analysed by using standardized methods (AOAC, 1995). All experimental samples were subjected to oven drying at a temperature of 105 ± 1°C for a duration of 24 hours to ascertain the percentage of dry matter. Subsequently, the crude protein content in each sample was measured using Kjeldahl principal with the help of Kjeltec, 8400 (FOSS, Denmark). For estimating crude lipid, the solvent extraction method was employed with petroleum ether 40-60 °C B.P and the extraction was carried out with the help of soxtec automatic analyzer, Awanti 2050 (FOSS Denmark). The content of ash of both feed and fish samples was determined after incinerated the samples in muffle furnace at 650 °C for 4-6 hours.

2.4 Water quality analysis parameters

During the entire feeding trial physico-chemical parameters such as, pH, dissolved oxygen (DO), temperature, free carbon dioxide (CO_2), and total alkalinity, adhering to standard methods (APHA, 1992). Before the daily feeding, water samples were taken for examination of above parameters. Using a mercury thermometer, the water's temperature was found to be between 24.3-25.9 °C. Winkler's iodimetric method was used to analyse, DO content which determined to be between 6.5 and 7.4 mg/L. Similarly, titrimetric

techniques were used to measure total alkalinity and free carbon dioxide, which showed values between 87 to 124 mg/L and 4.4 to 6.8 mg/L, respectively. With the application of a digital pH metre (pH ep-HI 98107, USA), the pH was tested and was found to be in between 7.2 to 7.5.

2.5 Blood collection and analysis

Five fish from each dietary treatment (n = 5× 3) were selected randomly for the assessment of the hematological parameters. For collection of blood caudal peduncle was severed, and the samples were taken for estimation using heparinized (Na-heparinized) and non-heparinized capillary tubes. All hematological analyses were conducted within a 2-hours after each extraction. Hemoglobin content (Hb), red blood cell (RBC), white blood cell (WBC) counts, and hematocrit (Hct%) content were to be determined from blood taken in heparinized tubes. The length and weight of each fish were recorded prior to blood collection to assess the condition factor (K). However, the liver was dissected from the same fish for estimation of the hepatosomatic index (HSI) specimen which were sacrificed for blood collection.

2.6Analysis of serum parameters

Blood collected in tubes without heparin underwent rapid centrifugation in a microcentrifuge (REMI-12C) at 4100 × g at 4 °C for ten minutes to extract serum. After serum separation, reagent rotors were loaded with serum samples using a 100 µL pipette to test for different parameters. Every dosage was subjected to triplicate tests to assess variables including, albumin, globulin, cholesterol, triglycerides and total protein as well as ALT and AST. Assessment of the serum analytes was carried out using an automated vet scan biochemistry analyzer VS2 (Abaxis, USA).

2.7 Intestinal enzymatic activities

The intestine samples (n = 3× 3) were gathered and homogenized within a saline solution at a proportion of 10 volumes. After, that the homogenate underwent centrifugation for a duration of 20 minutes at 4 °C at 6000 × g in order to collect the supernatant, which was then frozen at -20 °C for further examination. The protease activity was measured using the procedure outlined by Moore and Stein (1948) employing bovine serum albumin as the substrate, while the technique outlined by Furne et al. (2005) was utilized to assay the activities of lipase and amylase.

2.8 RNA isolation and Quantitative (q) PCR

TRIzol method was used to extract the RNA from fish muscles. Briefly, equal weight of fish tissues (100 mg) was homogenized in1 ml of TRIzol reagent (Invitrogen) was added and allowed to stand at room temperature for 5 minutes. Following this, 200 µl of chloroform was incorporated into the mixture, which was then allowed to incubate for 2 minutes before being subjected to centrifugation at 14,000 rpm for 15 minutes. The liquid phase was moved to a fresh, sterilized tube. Subsequently, 0.5ml of pure isopropanol were introduced, and the mixture underwent incubation for a duration of 10 minutes and underwent for centrifugation at 14,000 rpm for another 10 minutes. Carefully supernatant was discarded, pellet was washed with 75% ethanol (0.5ml) and centrifuged at 7000 rpm for 5 minutes. Then pellet was air dried for 10 minutes followed by dissolving the pellet in nuclease free water. The equal amount of RNA from all samples was then reverse transcribed into complementary DNA using the Thermo Fischer Scientific RevertAid™ first strand cDNA synthesis kit. qPCR was carried out according to the manufacturer's instructions using PowerUp™ SYBR™ Green master mix (Thermo Fischer Scientific) and the CFX 96 Real-Time System (Bio-Rad). The oligonucleotide sequences used for amplification in qPCR are provided in Table 2.

Table 2. Primers used for the real time PCR analysis.

Gene	Forward Primer (5′-3′)	Reverse primer (3′-5′)	Amplicon size (bp)	Accession no.	Primer efficiency (%)
FADS 2	AGAAATCCGGAGAAATCT GGCT	ACTGGCGGTTTAGTTGA TGTCT	122	AF309557	102.25
ELOV L5	GATTGACGACACTTCGTC CG ′	GAAAGTGTGGCTGCAGT GTG	121	KF924199	107.23
β- actin	GGACTCTGGTGATGGTGT CA	CTGTAGCCTCTCTCGGT CAG	138	M24113.1	99.89

FADS2= Fatty acid desaturase, ELOVL5= Elongase of very long chain fatty acids 5

2.9 Statistical analysis.

The results were expressed as mean ± SEM. One-way analysis of variance (ANOVA) (Snedecor and Cochran, 1967; Sokal and Rohlf, 1981) followed by Duncan's multiple range test, was used to evaluate significant difference among the treatments. A significance threshold of $P < 0.05$ was used. The data analysis was performed by using SPSS Statistics Version 22.0 (SPSS Inc., Chicago, IL, USA). Additionaly, the optimal dietary lipid level required for common carp fingerlings was determined using a second-degree polynomial regression analysis (Zeitoun et al. 1976).

3. Results

3.1Growth performance

The growth performance and feed utilization data of fingerlings of scale carp fed various levels of lipid-based testing diets for 70 days are presented in table 3. The survival of fish during the entire growth trial was recorded 100 percent. The highest values of growth attributes, like live weight gain (LWG%), protein efficiency ratio (PER), specific growth rate (SGR%) and lowest feed conversion ratio (FCR) was observed in the group that supplied a diet containing 60.0 g/kg of lipid. While as, a notable decline in growth performance ($P < 0.05$) data occurred, when the lipid content was increased from 80.0 g/kg to 120.0 g/kg, respectively. LWG and FCR data were employed in quadratic regression analysis to determine the optimal lipid requirement for the maximum growth of scale carp fingerlings. The results of the present study indicate that the optimal inclusion level of linseed oil as a dietary lipid source for maximum growth of *C. carpio* var. *communis* fingerlings was approximately 68.0 g/kg, in the presence of 20.0 g/kg cod liver oil in the diet.

Table 3. Growth, FCR, PER, protein deposition ratio and percentage survival of common carp, *C. carpio* var. *communis* fingerlings fed diets containing varying levels of dietary lipid levels for 10-weeks*.

	Varying lipid levels (g kg^{-1}, dry diet) in the experimental diets					
	20.0 (D_1)	**40.0 (D_2)**	**60.0 (D_3)**	**80.0 (D_4)**	**100.0 (D_5)**	**120.0 (D_6)**
Average initial weight (g)	1.55 ± 0.01	1.58 ± 0.01	1.59 ± 0.01	1.55 ± 0.01	1.57 ± 0.01	1.60 ± 0.013
Average final weight (g)	4.90 ± 0.02^{e}	6.23 ± 0.07^{c}	7.60 ± 0.06^{a}	7.10 ± 0.05^{b}	6.28±0.04^{c}	5.57±0.03^{d}
Live weight gain (%)[1]	216.12 ± 3.29^{e}	294.30 ± 3.77^{c}	377.98 ±4.37^{a}	358.06 ± 3.8^{b}	300.00 ± 3.56^{c}	266.24 ± 3.24^{d}
Specific growth rate (%)[2]	1.64 ± 0.01^{e}	1.96 ± 0.01^{c}	2.23 ± 0.01^{a}	2.17 ± 0.01^{b}	1.97 ± 0.01^{c}	1.78 ± 0.01^{d}
Feed conversion ratio (FCR)[3]	2.46 ± 0.02^{a}	1.75 ± 0.03^{c}	1.35 ± 0.02^{d}	1.56 ± 0.03^{c}	1.87 ± 0.02^{b}	2.04 ± 0.03^{a}
Protein efficiency ratio (PER)[4]	0.94 ± 0.01^{d}	1.33 ± 0.03^{b}	1.71 ± 0.04^{a}	1.49 ± 0.03^{ab}	1.24 ± 0.02^{c}	1.14 ± 0.01^{d}
Body protein deposition (BPD%)[5]	14.44 ± 0.35^{cd}	18.83 ± 0.37^{b}	29.94 ± 0.52^{a}	24.41 ± 0.23^{b}	19.94 ± 0.56^{c}	17.59 ± 0.47^{d}
Survival (%)	100	100	100	100	100	100

*Mean value of 3 replicates ± SEM; Mean values sharing the same superscript are not significantly different ($P > 0.05$).
[1]Live weight gain (%), Final body weight–initial body weight/initial weight×100
[2]Specific growth rate (SGR %) = 100 × (In final wet weight (g)-In initial wet weight g)/duration (days)
[3]Feed conversion ratio (FCR) = Dry weight of feed consumed / Wet weight gain
[4]Protein efficiency ratio (PER) =Wet weight gain (g) / Protein consumed (g, dry weight basis)
[5]Body protein deposition (BPD %) = 100 × (BWf × BCPf) – (BWi × BCPi) / [TF × CP]
Where BWi and BWf=mean initial and final body weight (g), BCPi and BCFf = mean initial and final percentage of muscle protein
TF=Total amount of diet consumed and CP=Percentage of crude protein of the diet.

3.2Whole body composition

The data on whole-body composition of the experimental fish, obtained at the conclusion of the 10-week feeding trial, also showed significant ($P < 0.05$) differences with respect of the inclusion of various lipid levels (Table 4). There was an upward trend observed in whole body crude protein content of each group with the increase of lipid in the diet up to 60.0 g/kg, however, beyond this level the protein content of fish significantly decreased. while, body fat content exhibited significant ($P < 0.05$) increasing pattern with each incremental level and reaching its highest peak up to the group that fed a diet containing 120.0 g/kg lipid, which was the highest lipid inclusion level in the present study. There was no notable difference ($P > 0.05$) observed in whole-body ash content across the incremental levels. The highest value of condition factor (K) was seen in fish group fed a diet containing 60.0 g/kg lipid and reduced thereafter. The hepatosomatic index (HSI) progressively increased with the addition of each lipid levels and the highest value of HSI was noticed in the group of fish that were fed with 120.0 g/kg lipid in the diet.

Table 4. Carcass composition, condition factor, hepatosomatic index of common carp, *C. carpio* var. *communis* fingerlings fed diets containing varying levels of dietary lipid levels for 10-weeks*.

	Varying lipid levels (g kg^{-1}, dry diet) in the experimental diets						
	Initial	**20.0 (D_1)**	**40.0 (D_2)**	**60.0 (D_3)**	**80.0 (D_4)**	**100.0 (D_5)**	**120.0 (D_6)**
Moisture (%)	77.32± 0.31	76.88 ± 0.25^{a}	75.71 ± 0.37^{b}	74.10 ± 0.31^{c}	73.47 ± 0.15^{d}	72.67 ± 0.19^{d}	72.22 ± 0.16^{d}
Protein (%)	13.90 ± 0.13	12.85 ± 0.13^{f}	14.33 ± 0.9 ^{e}	16.89 ± 0.6^{a}	16.05 ± 0.7^{b}	15.69 ± 0.6^{c}	15.22 ± 0.9^{d}
Fat (%)	3.67 ± 0.7	4.35 ± 0.9^{e}	4.88 ± 0.7^{d}	5.29 ± 0.5^{c}	5.42 ± 0.6^{c}	5.89 ± 0.7^{b}	6.12 ± 0.8^{a}
Ash (%)	2.45 ± 0.3	2.66 ±0.4^{a}	2.78 ±0.4^{a}	2.88 ±0.4^{a}	2.24 ± 0.3^{a}	2.30 ± 0.2^{a}	2.25 ± 0.3^{a}
Condition factor (K) [1]	1.45 ± 0.04	1.29±0.03^{e}	1.41 ± 0.04^{d}	1.79±0.03^{a}	1.68±0.04^{b}	1.67±0.03^{b}	1.56±0.04^{c}
Hepatosomatic index (HSI) [2]	1.79 ± 0.06	1.70 ± 0.04^{c}	1.75 ± 0.06^{c}	1.95 ± 0.06^{b}	2.06 ± 0.04^{c}	2.12 ± 0.07^{b}	2.65 ± 0.07^{a}

*Mean value of 3 replicates ± SEM; Mean values sharing the same superscript are not significantly different ($P > 0.05$)
[1]Condition factor = body weight (g)/body length (cm^3) × 100. [2]Hepatosomic index (%) = liver weight (g)/body weight (g) × 100.

3.3Hematological parameters and intestinal enzyme activities

In the present study, hematological parameters and intestinal enzyme activity revealed significant ($P < 0.05$) differences with respect to each incremental level of lipid in the testing diets (Table 5). The highest hemoglobin (Hb) content 8.41 g/dl was observed in fish fed a diet containing 60.0 g/kg of lipid, while the lowest Hb content was noted with fish group fed 20.0 g/kg (D_1) and 120.0 g/kg (D6) lipid diets. Hematocrit (Hct) values improved with each increasing lipid levels up to 60.0 g/kg and thereafter a significant decline in Hct content was observed. The highest red blood cell (RBC) count of 2.98×10^{6} mm^{-3} was also noted in fish fed a diet containing 60.0 g/kg of dietary lipid, whereas, the lowest value was achieved with fish fed a diet having 120.0 g/kg of lipid. Likewise, fish subjected to varying levels of lipid exhibited notable differences ($P < 0.05$) in their leukocyte (WBC) count. A significantly higher WBC count 2.58×10^{4} mm^{-3} was observed in fish fed the diet containing 120.0 g/kg lipid content. The activities of intestinal digestive enzymes of scale carp fingerlings also showed notable alteration with fish fed varying lipid levels. Significantly ($P< 0.05$) maximum activity of protease was observed in fish fed with 60.0 g/kg of lipid. Lipase also shows a similar trend, Nonetheless, no significant ($P>0.05$) difference in amylase activity was noted with the increasing lipid contents in the diet.

Table 5. Hematological indices and intestinal enzymatic activities of common carp, *C. carpio* var. *communis* fingerlings fed diets containing varying levels of dietary lipid for 10-weeks*.

		Varying levels of lipid levels (g kg^{-1}, dry diet) in the experimental diets					
	Initial	**20.0 (D_1)**	**40.0 (D_2)**	**60.0 (D_3)**	**80.0 (D_4)**	**100.0 (D_5)**	**120.0 (D_6)**
Hb (g dL^{-1}) [1]	5.03 ± 0.08	6.31 ± 0.10^{e}	7.42 ± 0.23^{b}	8.41 ± 0.42^{a}	7.74 ± 0.07^{bc}	7.04 ± 0.08^{cd}	6.69 ± 0.07^{e}
Hct (%) [2]	18.90 ± 0.29	22.92 ± 0.33^{d}	27.50 ± 0.31^{c}	33.86 ± 0.41^{a}	30.40 ± 0.51^{b}	26.75 ± 0.49^{c}	23.66 ± 0.37^{d}
RBC (x10^{6}/mm^{3})[3]	2.40 ± 0.03	2.66 ± 0.04^{c}	2.83± 0.02^{b}	2.98 ± 0.01^{a}	2.53± 0.03^{d}	2.49 ± 0.02^{d}	2.44 ± 0.02^{d}
WBC (x10^{4}/mm^{3})[4]	2.47± 0.02^{a}	2.42 ± 0.04^{ab}	2.30 ± 0.03^{c}	2.16 ± 0.03^{d}	2.26 ± 0.03^{c}	2.34 ± 0.02^{b}	2.58 ± 0.03^{a}
Protease activity (U mg/protein)	10.29 ± 0.09	12.40 ± 0.18^{e}	13.63 ± 0.14^{d}	16.56 ± 0.19^{c}	18.29 ± 0.13^{a}	17.65 ±0.23^{b}	16.59 ± 0.19^{c}
Lipase activity (U mg/ protein)	0.34 ± 0.04	0.47 ± 0.04^{e}	1.18 ± 0.02^{a}	1.72 ± 0.02^{a}	1.55 ± 0.04^{b}	1.35 ± 0.04^{c}	1.19 ± 0.03^{d}
Amylase activity (U mg/ protein)	16.33 ± 0.55	16.66 ± 0.59^{a}	16.54 ± 0.58^{a}	16.49 ± 0.58^{a}	16.44 ± 0.60^{a}	16.42 ±0.63^{a}	16.38 ± 0.60^{a}

*Mean value of 3 replicates ± SEM; Mean values sharing the same superscript are not significantly different ($P > 0.05$).
[1]Hb, Hemoglobin, [2]Hct, Hematocrit. [3]RBC, Red blood cell. [4]WBC, White blood cell

3.4 Serum indices

Fish fed various levels of lipid levels over the 10-week feeding trial displayed notable variation ($P < 0.05$) in serum parameters across each incremental level (Table 6). There was a significant increase in serum albumin, total protein, cholesterol and triglycerides levels with increasing lipid levels in the diets and reaching maximum with the fish fed 120.0 g/kg of lipid in the diet. In contrast alanine transaminase (ALT) and aspartate transaminase (AST) exhibited a decreasing pattern with the gradual increase in lipid in the diet until reaching 60.0 g/kg, afterwards an upward trend was observed.

Table 6. Effects of lipid levels on blood serum parameters of common carp, *C. carpio* var. *communis* fingerlings fed diets containing varying levels of dietary lipids for 10- weeks.*

	Varying levels of lipid levels (g kg^{-1}, dry diet) in the experimental diets					
	20.0 (D_1)	**40.0 (D_2)**	**60.0 (D_3)**	**80.0 (D_4)**	**100.0 (D_5)**	**120.0 (D_6)**
Albumin (g L^{-1})	11.13 ± 0.02^{f}	12.51 ± 0.03^{e}	13.71 ± 0.03^{d}	14.50 ± 0.06^{c}	15.44 ± 0.03^{b}	16.90 ± 0.02^{a}
Globulin (g L^{-1})	22.10 ± 0.04^{a}	21.50 ± 0.03^{a}	20.50 ± 0.03^{c}	21.52 ±0.04^{b}	21.60 ± 0.03^{b}	22.55 ± 0.02^{a}
Total protein (g L^{-1})	33.23 ± 0.04^{c}	34.0 ± 0.06^{d}	34.21 ± 0.04^{d}	36.02 ± 0.09^{e}	37.04 ± 0.03^{b}	39.44 ± 0.01^{a}
Total cholesterol (mmol L^{-1})	3.01 ± 0.0^{e}	3.08 ± 0.02^{de}	3.18 ± 0.04^{d}	3.32 ± 0.4^{c}	3.47 ± 0.04^{b}	3.60 ± 0.2^{a}

Triglycerides (mmol L^{-1})	1.80 ± 0.03^{c}	1.86 ± 0.4^{d}	1.93 ± 0.2^{d}	2.04 ± 0.2^{e}	2.19 ± 0.3^{b}	2.35 ± 0.3^{a}
ALT (U L^{-1}) [1]	22.93 ± 1.18^{c}	22.58 ± 1.10^{a}	14.88 ± 1.43^{c}	17.01 ± 1.01^{c}	18.78 ± 1.15^{b}	23.65 ± 1.14^{a}
AST (U L^{-1}) [2]	138.89 ± 2.64^{b}	134.65 ± 2.51^{bc}	125.11 ± 2.93^{d}	132.50 ± 2.12^{c}	135.76 ± 2.05^{b}	145.56 ± 3.77^{a}

*Mean value of 3 replicates ± SEM; Mean values sharing the same superscript are not significantly different ($P > 0.05$)
[1]Alanine aminotransferases.
[2]Aspartate aminotransferase.

3.5 Relative expression of FADS2 and ELOVL5 genes

In the present study, the impact of lipid levels on the relative mRNA expression levels of FADS2 and ELOVL5 genes in common carp, *C. carpio* var. *communis* fingerlings were studied (Figures 1 and 2). The highest relative expression of FADS2 and ELOVL5 mRNA ($P < 0.05$) in muscle tissue was observed in groups that were fed a diet containing 60.0 and 80.0 g/kg lipid as compared to those groups that were fed diets with other lipid levels.

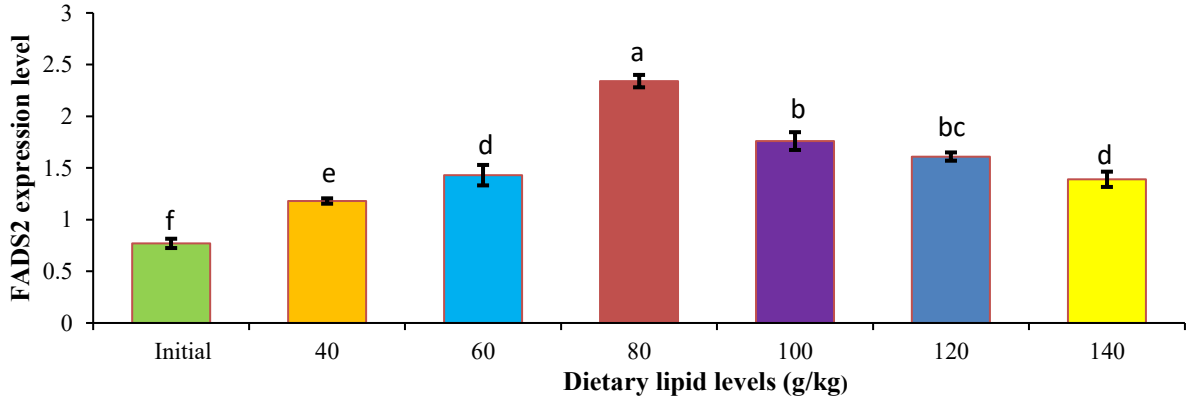

Fig.1 Relative expression levels of Fatty acid desaturase 2 (FADS2) in the muscle of *C. carpio* var. *communis* fingerlings fed diets containing varied lipid levels (g/kg) over a period of 10 weeks. Results are shown as the mean ± SEM.

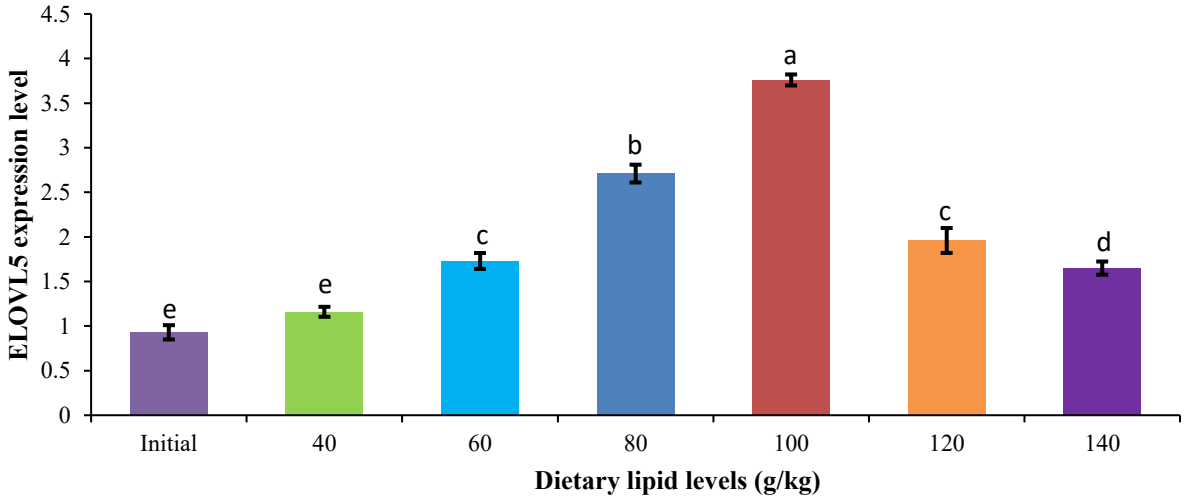

Fig.2 Relative expression levels of elongase of very long chain fatty acid 5 (ELOVL5) gene in the muscle of *C. carpio* var. *communis* fingerlings fed diets containing varied lipid levels (g/kg) over a period of 10 weeks. Results are shown as the mean ± SEM.

4. Discussion

Lipids serve as a vital energy source for both the growth and developmental processes of fish. They are efficiently metabolized by fish and are indispensable for ensuring normal growth and physiological development (Guo et al., 2019; Li et al., 2024). In the current study, various attributes, like LWG (%), PER, SGR, and BPD, increased when the scale carp fingerlings were supplied with lipid levels in the range of 20.0 – 60.0 g/kg, and afterwards, a decreasing trend in these parameters was obtained. It indicates that increasing lipid levels within an optimal range can supply adequate energy for fish activity. This allows proteins to be utilized primarily for anabolic processes, maximizing their contribution to the development and enhancing fish growth. Conversely, excessive lipid can disrupt the balance between energy and protein content in the feed, resulting in reduced growth rates and lower feed efficiency in fish. During the present study, scale carp fingerlings fed diets with lipid levels ranging from 80.0 to 120.0 g/kg were associated with a gradual decline in LWG, SGR, and BPD. The decrease in growth at high lipid-containing diets could result from the suppression of new fatty acid production and a diminished capacity of fish to digest and absorb lipids (Sargent et al., 1989; Luo et al., 2014; Han et al., 2014). Fish fed high lipid diets also exhibited an increase in FCR, accompanied by a reduction in both PER and SGR, respectively. The highest FCR value was recorded in fish fed with a diet possessing 120.0 g/kg (D6) of dietary lipid, which represents a higher value than the values observed in other treatment groups. Similarly, fish fed a diet containing 60.0 g/kg (D3) lipid displayed a significantly higher PER content than those fed other diets. These results indicate that higher lipid levels adversely affect nutrient utilization in fish, aligning with findings from earlier studies (Chatzifotis et al., 2010; Yong et al., 2015; Royuela et al., 2015; Bonvini et al., 2015). The HSI also increased progressively with rising lipid levels and showed the highest HSI was found in fish fed with a diet containing 120.0 g/kg (D6) lipid, indicating excessive lipid deposition in the liver. An increase in HSI with elevated lipid levels has also been reported in previous studies (Mohantay et al., 2008; Xu et al., 2011; Ren et al., 2012; Han et al., 2014). Additionally, fish fed a diet containing 60.0 g/kg (D3) of lipid demonstrated a higher condition factor, suggesting they were in optimal health, as an adequate lipid supply supported vital physiological functions.

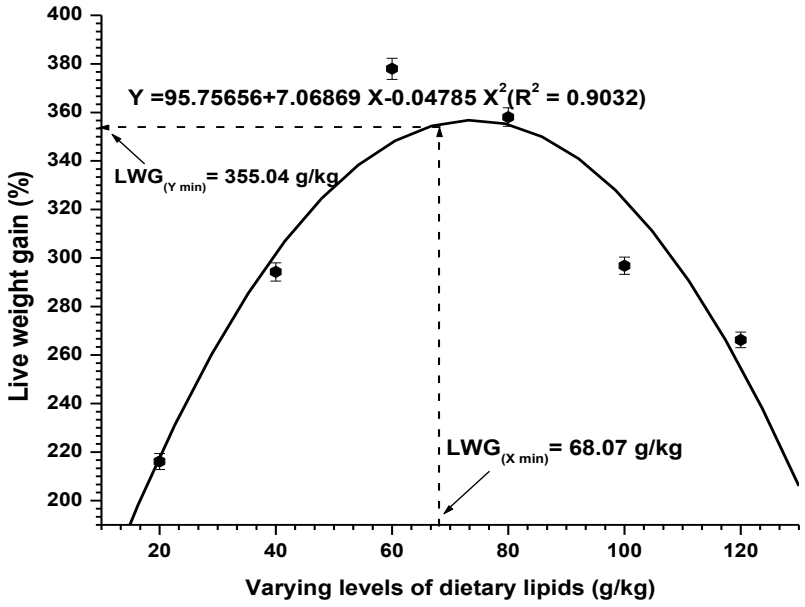

Fig. 3 Quadratic regression analysis of live weight gain (LWG%) against varying levels of dietary lipid levels in *C. carpio* var. *communis* fingerlings

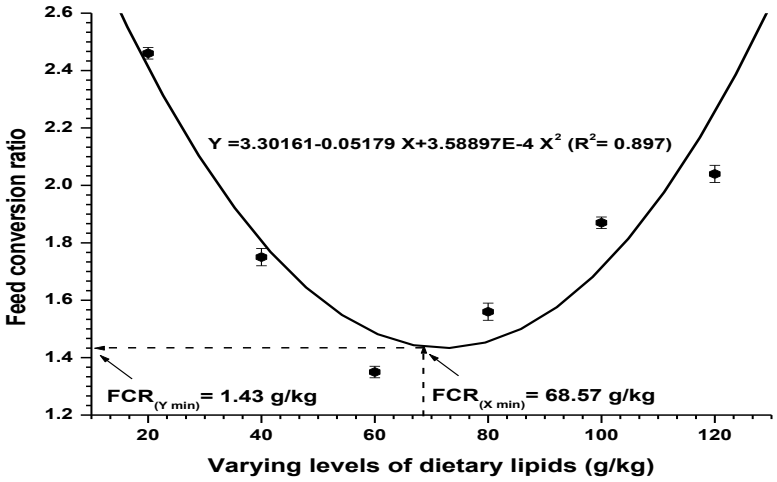

Fig. 4 Quadratic regression analysis of feed conversion ratio (FCR) against varying levels of dietary lipid levels in *C. carpio* var. *communis* fingerlings.

Evaluation of hematological parameters of fish is taken as the most effective approach in studying the physiological condition and health status of fish, irrespective of their presence in habitats (Kader et al., 2010; Ahmad et al., 2021). These metrics also provide important insights into fish physiology and act as markers of disease, stress, and environmental disruptions that arise in aquatic environments. In this study, significant ($P < 0.05$) variations in hematological parameters were observed in response to different levels of lipid inclusion in the diets. Hemoglobin (Hb), hematocrit (Hct%) content, and red blood cell (RBC) counts showed an increasing trend, peaking at 60.0 g/kg lipid inclusion level, thereafter a decreasing trend was observed. The higher Hb, Hct, and RBC values may be linked due to enhanced growth, which supports efficient oxygen transport in the blood. These findings align with earlier studies conducted on *Takifugu rubripes* (Kikuchi et al., 2009) and *Oreochromis niloticus* (Kasheif et al., 2011).

The concentrations of blood proteins, including globulin and albumin, offer valuable information about the overall health of fish (Varghese et al., 2020). Albumin levels in the serum significantly increased with higher dietary lipid content, peaking at the highest lipid level (120 g/kg). The fish groups fed with the D_6 and D_3 diets showed the highest albumin and lowest globulin levels, suggesting possible liver cell damage due to increased lipid intake. These results are consistent with previous studies on *Carassius carassius* (Wang et al., 2014; Paul et al., 2022). Serum protein profiles can indicate fish health, metabolism, and nutritional status (Maiti et al., 2023). The present study shows higher total serum protein levels in fish fed diets with increased lipid content, likely due to lipoproteins transporting the excess dietary lipids. Similar results were observed in *O. niloticus* (Lim et al., 2009). Triglycerides (TG) and total cholesterol (T-CHO) are key constituents of blood lipids and are primarily produced in the liver. Elevated blood lipid levels in the body can lead to various conditions, including hypertriglyceridemia (Tenenbaum et al., 2017). In the present study, it has been observed that serum TG and T-CHO levels significantly rise with fish fed at 120 g/kg of dietary lipid, suggesting high-lipid diets may elevate serum lipids and potentially cause various diseases. Similar findings have also been reported for *Acipenser baerii* (Ren et al., 2021) and *Acipenser baerii* × *Acipenser gueldenstaedtii* (Guo et al., 2011). Aspartate transaminase (AST) and alanine transaminase (ALT) are key enzymes involved in amino acid metabolism, primarily localized in liver cells but also present in the serum. Variations in their serum activity serve as crucial biomarkers for assessing liver function, with elevated levels typically signifying hepatocellular damage, degeneration, and necrosis (Yu et al., 2018). Fish fed D_1 and D_6 diets showed higher lipid levels compared to the D_3 diet, indicating that both insufficient and excessive lipid intake may impair liver function. These findings align with previous studies reported in *Brachymystax lenok* (Yu et al., 2018) and *Trachinotus ovatus* (Xun et al., 2021).

The overall body composition of fish serves as a key indicator of fish quality and nutritional value of feed, nutrient assimilation efficiency, flesh quality, and overall health status (Njinkoue et al., 2016). Fish fed a diet containing 60.0 g/kg (D3) lipid exhibited the highest protein content among all the groups. The highest values observed at this level may be attributed to improved efficiency in retaining protein content within the body. In contrast, body protein levels were low in the groups fed with both lower and higher lipid levels. A decrease in protein content with the inclusion of high dietary lipid levels has been observed in various fish species, *Scortum barchoo* (Song et al., 2009), *Solea senegalensis* (Borges et al., 2009), and *L. rohita* (Siddiqua and Khan, 2022). Existing literature indicates that elevated dietary lipid levels are also associated with increased lipid deposition throughout the fish's body while potentially reducing their moisture content (Shearer, 1994; Rasmussen et al., 2000; Xun et al., 2021). During the present

study, an increase in fat content and a reduction in moisture content were observed in scale carp fingerlings with respect to each incremental increase of lipid in the diet. This variation is likely due to the accumulation of fat within the fish's body. Similar observations regarding the lipid and moisture content relationship have been documented in other fish species, such as *Clarias batrachus* (Giri et al., 2000), *C. idella* (Du et al., 2005), and *Oreochromis niloticus* (Lim et al., 2009). The current study also shows that varying dietary lipid levels had no significant effect on the body ash content of scale carp fingerlings, and these results are in agreement with previous research findings on *Nibea cobor* (Huang et al., 2016) and *L. rohita* (Siddiqua and Khan, 2022).

Digestive enzyme activity and nutrient utilization are among the key factors involved in enhancing feeding and supporting fish growth. The enzyme profile and activity in a fish's digestive tract can be influenced by the type, source, and quantity of nutrients (Debnath et al., 2007; Mohantay et al., 2008). Besides, intestinal digestion and absorption play a crucial role in feed efficiency and fish growth. However, there is a direct relationship between fish growth and digestive enzyme activity (Hidalgo et al., 1999). Our findings indicate that protease and lipase activities significantly increased with dietary lipid levels up to 60 g/kg. However, higher lipid inclusion at 120 g/kg led to reduced growth and inefficient feed utilization in fish. Previous studies have shown that higher dietary lipid levels suppress the activity of protease and lipase enzymes in fish, including *Epinephelus coioides* (Li et al., 2016), *Brachymystax lenok* (Chang et al., 2018), and *Scophthalmus maximus* (Zhang et al., 2022). However, no significant differences in amylase activity were observed among fish groups fed diets with varying lipid levels. These findings are consistent with earlier research on *L. rohita* (Gangadhara et al., 1997), *P. gonionotus* (Mohanty et al., 2008), and *Acipenser baerii* (Ren et al., 2021).

Understanding the molecular processes and pathways involved in PUFA biosynthesis in economically significant freshwater carp species could facilitate enhanced consumption of plant-based lipid sources while maintaining optimal growth and PUFA accumulation in their fillets. LC-PUFAs are synthesized from shorter-chain PUFAs precursors through enzymatic processes facilitated by Fads and Elovl enzymes (Castro et al., 2016). In the current study an attempt has been made to evaluate the mRNA expression levels of essential genes involved in LC-PUFA biosynthesis, including FADS2 and ELOVL5 genes in scale carp fingerlings. The upregulation of all these genes was observed in fish that were fed diets supplemented with different levels of lipids, in comparison to the initial. The highest level of mRNA expression of FADS2 and ELOVL5 genes was observed in the fish group fed with 60.0 g/kg and 80.0 g/kg lipid diets. This could be attributed to the presence of LA and ALA acid in the experimental diets. Previous studies have indicated that higher levels of LA and ALA in the diet lead to the upregulation of desaturases and elongases (Turchini et al., 2006; Li et al., 2008). However, an excess of ALA in the diet may suppress the transcription of the FADS2 gene (Bell et al., 1993). The biosynthesis of LC-PUFAs was suppressed as dietary lipid levels increased beyond 80 g/kg, indicated by the reduced expression of desaturase and elongase genes. This down-regulation might result from elevated LC-PUFAs concentrations in groups fed with higher lipid levels. The present findings are in agreement with the findings of the other workers on freshwater fish species (Izquierdo et al., 2008; Ren et al., 2012; Xu et al., 2014; Nayak et al., 2018; Mir et al., 2020).

5. Conclusion

This study found that providing optimum lipid supplementation greatly increased growth performance in common carp, *C. carpio* var. *communis* fingerlings. Quadratic regression analysis revealed that the ideal amount of lipid for maximal growth was 68.0 g/kg of linseed oil in presence of 20.0 g/kg of cod liver oil, while both lower and excess lipid had a negative impact on growth. Lipid levels significantly altered hemato-biochemical parameters, serum metabolites, intestinal digestive enzyme activities, and expression of FADS2 and ELOVL5 genes. These findings will encourage the development of nutritionally balanced feed for intensive production of common carp, *C. carpio* var. *communis.*

Credit authorship contribution statement

Aamir Majeed: writing – review and editing, writing original draft, investigation, formal analysis. **Imtiaz Ahmad Khan:** writing – review and editing, Conceptualization, Methodology, Supervision, validation **Nazir A. Dar:** Visualization, writing – review and editing, Data curation, Conceptualization.

Declaration of Competing Interest

There are no conflicts of interest in this manuscript.

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Data Availability

The datasets used and/or analyzed during the current study will be available from the corresponding author upon reasonable request

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