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HEPATIC ENZYME AND METABOLIC RESPONSES OF *LABEO ROHITA* (ROHU) TOWARDS VARYING DIETARY LIPID/PROTEIN RATIOS IN PELLETED DIETS

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ABSTRACT
A 75-day feeding trial was conducted in a 2×2 factorial design to evaluate the effects of varying dietary lipid/protein ratios on the growth performance, hepatic enzymes, and metabolic responses of *Labeo rohita* (initial weight: 2.87 ± 0.01 g). Four pelleted diets containing protein levels of 250 and 300 g kg^{-1} and lipid levels of 75 and 95 g kg^{-1} were formulated and fed to fish (n = 10) randomly distributed into four triplicate groups (T_1–T_4). Fish receiving the 95/300 g kg^{-1} lipid/protein diet (T_4) attained significantly higher ($p<0.05$) average wet weight, fork length, total length, nitrogen conversion ratio, and protein efficiency ratio. However, the same diet also induced significantly elevated ($p<0.05$) hepatic alkaline phosphatase, alanine transaminase, and aspartate transaminase activities, indicating enhanced metabolic load on the liver. Bilirubin concentrations remained consistently low across all treatments. In contrast, fish fed the 75/250 g kg^{-1} lipid/protein diet (T_1) showed significantly higher ($p<0.05$) serum globulin levels, suggesting an immunological benefit. Nitrogen incorporation efficiency, hepatic albumin, total protein, and gamma-glutamyl transferase did not vary significantly ($p>0.05$) among treatments. Overall, these findings suggest that a 95/300 g kg^{-1} lipid/protein diet improves growth performance of *Labeo rohita* but may impose hepatic stress, while lower lipid/protein levels support certain immune parameters.

INTRODUCTION

Fish farming in Pakistan is a means to provide valuable animal protein for human utilization (Iqbal *et al*., 2016). The significance of nutritional attributes of farmed fish has been proven to be more reliable than those of their wild counterparts (Mahboob *et al*., 2004). The fish farming industry exerts serious efforts to provide high-quality fish commodities for consumers (Karakatsouli, 2012). Fish meat has been proven to be alimentary for humans as it has high nutritional and biological value due to its desirable protein and lipid contents (Mahboob *et al*., 2015). Rearing fish has become an important protein manufacturing enterprise due to human dependence on it. *Labeo rohita* is amongst the most important freshwater omnivorous fish species, ranked high due to its low rearing cost and commercial importance as a food animal. It is cultured throughout the sub-continent (Kausar and Salim, 2006) and occupies a chief position amongst the three major carp species (Luhariya *et al*., 2014) due to its better flesh quality and consumer preference.

Aquaculture practices through fish rearing are directed towards the production of food fish for satisfying the nutritional demands of the world's growing population (Adabu *et al*., 2014). In recent years, a utilization shift of aquatic products from wild fishing to sustainable aquaculture development has been observed (Zhu *et al*., 2010). The demand for aquatic products is on the rise as a substitute for other animal protein sources. Such rising demands can only be satisfied through fish rearing under appropriate culture conditions. Employing the mechanism of intensive fish rearing has become inevitable due to the present-day higher animal protein demand (Abid and Ahmed, 2009). The intensive fish rearing method is expected to bring fish production to competent levels. However, intensive fish rearing is a feed-based venture that requires the development of processes curtailing feeding costs and improving the market demands of the reared fish. Feeding fish with appropriately formulated diets is the major requirement for their rearing under intensive culture conditions (Mahanand *et al*., 2013). Least-cost and nutritionally balanced feed stuff is required to attain the maximum fish yield. Offering the fish with pelleted diets helps convert leftover feed ingredients' protein to high-value fish somatic proteins required for human utilization (Zeb and Javed, 2016). Formulated fish diet consists of indispensable constituents like protein, carbohydrate, fat, minerals, and vitamins that keep fish targeted to put on body tissue and build up a healthy biomass (Craig and Helfrich, 2002).

Fish liver is the central metabolic station that performs multiple, critically vital activities, i.e., detoxification, storage, excretion, conversion, and synthesis of various tissue proteins. Liver enzymes, i.e., Alkaline phosphatase (ALP), Aspartate transaminase (AST), Alanine transaminase (ALT), and Gamma-glutamyl transferase (GGT), have antioxidant properties and help regulate the detoxification process, thus keeping fish physiological processes homeostatic towards any dietary intake (Kaur and Kaur, 2015). Liver metabolic responses of fish, such as bilirubin, globulin, albumin, total protein, and triglycerides, also provide reliable information on animal health and dietary intake.

In this investigation, variations in hepatic enzyme concentrations [i.e., Alkaline phosphatase (ALP), Aspartate transaminase (AST), Alanine transaminase (ALT), and Gamma-glutamyl transferase (GGT)] and liver metabolic responses of *Labeo rohita* (viz., Bilirubin, Globulin, Albumin, Total protein, and Triglycerides) have been exploited as tools to access the impact of varying lipid/protein ratios in pelleted diets on the growth indices of the *Labeo rohita*.

MATERIALS AND METHODS

Experimental Fish, Station, and Duration

This investigation was carried out from April 15, 2016, to July 01, 2016, at the Fish Biochemistry Laboratory, Department of Zoology, Government Postgraduate College, Gojra. Fingerlings of *Labeo rohita* were purchased from the Government Fish Seed Hatchery, Peer Mahal, District Toba Tek Singh, and brought to the Fish Biochemistry Laboratory, Department of Zoology, Government Postgraduate College, Gojra, where they were acclimatized for a period of 10 days in glass aquaria.

Experimental Conditions

Triplicate groups of fingerlings with an initial average wet weight of 2.87±0.01g were randomly distributed in glass aquaria (100-liter water capacity) supplied with electric aerators. Fish (n=10) were assigned to four triplicate groups of feeding regimes designed to contain a variable lipid to protein ratio, viz. 75/250, 95/250, 75/300, and 95/300 g kg^{-1}diet and designated as T_1, T_2, T_3, and T_4, respectively.

Pelleted Diets

Pelleted fish diets were prepared by mixing the appropriate amount of ingredients that were purchased from Pakistan Feeds Limited, Gala Mandi, Gojra. An adequate amount of water was added to make a dough that was pelletized with the help of a hand pelletizing machine. Pellets (3mm) were sun-dried and stored in (5kg capacity) airtight plastic jars until used. Each artificial diet was chemically analyzed in triplicate for its moisture, crude protein, crude fats, and total Ash, according to the methods outlined in A.O.A.C. (2006). The formulation and chemical composition of all four different fish diets are shown in Table I.

Fish Management and Feeding

Each glass aquarium was siphoned using a suction pump to remove fish feces and uneaten fish feed. Following siphoning, the water from each glass aquarium was completely drained out and replaced with fresh tap water. Each triplicate group of fish was fed with isocaloric diets (2500 Kcal Kg^{-1}) that differed in their protein and lipid ratios to complete visual satiation @ 5% of their wet body weight twice daily between 09:00 and 16:00 hours, respectively, whereas fish diet consumption and growth performance were evaluated on a fortnightly basis.

Table I: Formulation and proximate composition of pelleted diets (g kg^{-1}).

Lipid/protein ratio (g kg^{-1})	T1 (75/250)	T2 (95/250)	T3 (75/300)	T4 (95/300)
Wheat	150	170	150	130
Rice broken	150	80	10	10
Rice polish	80	120	80	80
Wheat bran	80	70	80	70
Canola meal	220	160	220	140
Sunflower meal	70	150	70	180

Lipid/protein ratio (g kg $^{-1}$)	**T1 (75/250)**	**T2 (95/250)**	**T3 (75/300)**	**T4 (95/300)**
Corn glutton	50	50	50	50
Fish meal	180	180	320	320
Corn oil	10	10	10	10
Premix	10	10	10	10
Proximate Composition (g kg $^{-1}$)				
Moisture[a]	78	66	77	67
Crude protein[a]	251	253	302	303
Crude fat[a]	76	95	76	94
Ash[a]	46	55	59	67
NFE[b]	549	531	486	469
Energy (Kcal/kg)	2534	2493	2520	2450

Note: Vitamin premix contains (g kg^{-1}dry weight): Vit A, 3600000IU: Vit D_3, 1200000IU: Vit E, 4200mg: Iron, 10000mg: β Carotene, 40mg: Zinc, 14000mg: Manganese, 16000mg: Casein, 1800mg: Cobalt, 160mg: Selenium, 60mg: Copper, 2400mg: Nicotinamide, 2400mg: Dicalcium phosphate, 80000mg.[a]Estimated in triplicate; [b]Estimated by difference (Moisture + Crude protein + Crude fat + Ash) – 100;[c]Estimated through Bomb Calorimeter (Parr Instrument Company Moline, USA)

Fish Growth Evaluation

The growth performance of *Labeo rohita* was evaluated through monitoring the following fish growth and feeding parameters, viz., nitrogen conversion ratio, nitrogen incorporation efficiency, protein efficiency ratio, and fish survival.

$$\text{Nitrogen conversion ratio} = \frac{\text{Increase in fish yield (g)}}{\textit{Nitrogenadded}} \times 100$$

$$\text{Nitrogen incorporation efficiency} = \frac{\text{Nitrogen intake}}{\text{Increase in body mass}} \times 100$$

$$\text{Protein Efficiency Ratio} = \frac{\text{Gain in body The gain (g)}}{\text{Protein intake(g)}}$$

$$\text{Survival rate (\%)} = \frac{\text{No. of stocked fish} \times 100}{\text{No. of recovered fish}}$$

Hepatic studies

Liver enzymes, i.e., Alkaline phosphatase (ALP), Aspartate transaminase (AST), Alanine transaminase (ALT), and Gamma-glutamyl transferase (GGT), and some liver metabolic parameters, i.e., bilirubin, globulin, albumin, total protein, and triglycerides of *Labeo rohita* were estimated at the end of this experimentation. For this purpose, three fish from each treatment were sacrificed, and their livers were excised, and equal weight (1g) aliquots were made. An electric tissue homogenizer (Polytron PT 1200 E Kinematica, Switzerland) was used to homogenize the fish liver tissues in a buffer, i.e., sodium dodecyl sulfate (SDS-0.7%) in order to obtain a homogenate. All the aforementioned biochemical parameters were determined through Kits from Randox Laboratories, Limited (UK), spectrophotometrically as per the manufacturer's instructions.

Data Analysis

The data obtained during this investigatory period were subjected to statistical analysis by one-way and two-way analysis of variance (ANOVA) after Sokal and Rohlf (1981). Differences among treatment means were determined by the Scheffe post hoc test at a significance level of $p<0.05$. Data analyses were performed using the statistical software Statistix® (version 8.1; Analytical Statistics Software, Tallahassee, USA).

RESULTS

Fish Growth Performance: The data regarding the growth performance of *Labeo rohita* towards varying dietary lipid/protein ratios in pelleted diets are presented in Table II. *Labeo rohita* attained significantly ($p<0.05$) higher average body weight (4.79±0.04g) due to T_4 (95/300 g kg^{-1} lipid/protein diet), followed by T_3, T_2, and T_1. However, non-significant differences existed between T_1 and T_2. Final average fork length of *Labeo rohita* was significantly ($p<0.05$) higher (66.63±0.04mm) due to T_4 (95/300 g kg^{-1} lipid/protein diet), followed by T_3, T_2, and T_1. *Labeo rohita* reared under T_4 (95/300 g kg^{-1} lipid/protein diet) also gained significantly ($p<0.05$) higher average total length of 76.67±0.04mm, followed by T_3, T_2, and T_1.

Nitrogen Conversion Indices: Nitrogen incorporation efficiency of the pelleted *Labeo rohita* diets showed non-significant differences among them (Table II). Among the treatments, T_4 (95/300 g kg^{-1}lipid/protein diet) gave the pelleted diets the highest nitrogen conversion ratio value of 4.18±0.15, which was non-significantly different from T_1 and T_2, whereas non-significant differences also existed among T_1, T_2, and T_3. Likewise, significantly ($p<0.05$) higher average value of protein efficiency ratio, i.e., 0.71±0.08, was achieved due to T_4 (95/300 g kg^{-1} lipid/protein diet). However, non-significant differences for the protein efficiency ratio of the pelleted diets existed among T_1, T_2, and T_3 (Table II).

Table II: Growth and nitrogen indices of *Labeo rohita* fed varying lipid/protein ratios in pelleted diets (g kg-1).

Parameters	T_1(75/250)	T_2(95/250)	T_3(75/300)	T_4(95/300)
Initial weights (g)	2.87±0.01	2.87±0.01	2.85±0.01	2.87±0.01
Final weights (g)	4.22±0.03c	4.23±0.03c	4.69±0.03b	4.79±0.04a
Initial fork lengths (mm)	56.3±0.03	58.4±0.03	56.3±0.03	57.90±0.04
Final fork lengths (mm)	64.22±0.03d	65.02±0.03c	65.37±0.04b	66.63±0.04a
Initial total lengths (mm)	65.1±0.07	63.8±0.02	62.2±0.03	63.6±0.04
Final total lengths (mm)	72.42±0.04d	72.50±0.02c	73.90±0.03b	74.67±0.04a
Nitrogen Conversion Ratio	3.69±0.21ab	3.56±0.08ab	3.26±0.22b	4.18±0.15a
Nitrogen Incorporation Efficiency	0.39±0.05a	0.72±0.12a	0.78±0.43a	0.27±0.01a

Protein Efficiency Ratio	0.59±0.04b	0.57±0.01b	0.60±0.06b	0.71±0.08a
Fish Survival	100±0.00a	100±0.00a	100±0.00a	100±0.00a

Similar alphabets in the same row are not significantly different ($p>0.05$).

Liver Enzyme Assay: Data regarding the mean values of liver enzymes, i.e., Alkaline phosphatase (ALP) (IU L^{-1}), Aspartate transaminase (AST) (IU L^{-1}), Alanine transaminase (ALT) (IU L^{-1}), and Gamma-glutamyl transferase (GGT) (IU L^{-1}) of *Labeo rohita* are presented in Table III. ALP levels in the liver of *Labeo rohita* were significantly ($p<0.05$) higher (78±0.32 IU L^{-1}) due to T_4 (95/300 g kg^{-1} lipid/protein diet), whereas non-significant differences existed among T_1, T_2, and T_3 (Table 3). The concentration of ALT in *Labeo rohita* liver was significantly ($p<0.05$) higher, i.e., 38±2.12 IU L^{-1}, due to T_4 (95/300 g kg^{-1} lipid/protein diet), followed by the T_1, T_3, and T_2 (Table III). Significantly ($p<0.05$) higher AST level of 9.00±0.21 IU L^{-1} was also caused by T_4 (95/300 g kg^{-1} lipid/protein diet), while non-significant differences also existed among T_1, T_2, and T_3 (Table III). GGT levels in *Labeo rohita* liver followed the order: $T_1=T_2>T_4>T_3$. GGT levels of *Labeo rohita* were significantly lower (0.60±0.26 IUL^{-1}) due to T_3 (75/300 g kg^{-1} lipid/protein diet).

Table III: Liver enzyme concentrations of *Labeo rohita* fed varying lipid/protein ratios in pelleted diets (g kg-1).

Liver enzymes (IUL^{-1})	T_1(75/250)	T_2(95/250)	T_3(75/300)	T_4(95/300)
Alkaline phosphatase (ALP)	54.00±02b	47.00±02b	51.00±0.26b	78.00±0.32a
Alanine transamianse(ALT)	24.00±03b	7.00±02d	17.00±03c	38.00±2.12a
Aspartate transamianse(AST)	4.00±02b	4.00±2.64b	4.00±03b	9.00±0.21a
Gamma-glutamyltransferase (GGT)	3.00±02a	3.00±02a	0.60±0.26b	2.00±0.32a

Similar letters in the same row are not significantly different ($p>0.05$).

Liver Metabolic Responses: Data regarding the mean values of liver metabolic parameters such as bilirubin (mg dl^{-1}), Globulin (g dl^{-1}), Albumin (g dl^{-1}), Total protein (g dl^{-1}), and Triglycerides (mg dl^{-1}) of *Labeo rohita* are presented in Table IV. The concentration of bilirubin was significantly lower in the liver of *Labeo rohita* reared under T_1 (75/250 g kg^{-1} lipid/protein diet) (Table IV). However, bilirubin concentrations in the liver of *Labeo rohita* did not vary significantly among T_2, T_3, and T_4. *Labeo rohita* fetched significantly ($p<0.05$) higher value of globulin, i.e., 2.00±0.36 g dl^{-1} due to T_1 (75/250 g kg^{-1} lipid/protein diet). However, non-significant differences in globulin concentrations in *Labeo rohita* liver existed among T_2, T_3, and T_4 (Table IV). Liver albumin concentrations of *Labeo rohita* did not vary significantly ($p>0.05$) among the treatments (Table IV). *Labeo rohita* liver accumulated significantly ($p<0.05$) higher triglyceride levels due to T_1 (75/250 g kg^{-1} lipid/protein diet) as 229±3.74, followed by T_4, T_3, and T_2. Liver total protein concentrations of *Labeo rohita* also did not vary significantly ($p>0.05$) among the treatments (Table IV).

Table IV: Metabolic responses of *Labeo rohita* fed varying lipid/protein ratios in pelleted diets (g kg-1).

Metabolic Profile	T_1(75/250)	T_2(95/250)	T_3(75/300)	T_4(95/300)
Bilirubin (mg dl^{-1})	0.20±0.1b	0.30±0.2a	0.30±0.26a	0.30±0.14a
Globulin (g dl^{-1})	2.00±0.36a	0.90±0.20b	1.10±0.36b	1.10±0.14b
Albumin (g dl^{-1})	2.10±0.26a	2.20±0.45a	1.80±0.53a	2.10±0.28a
Triglycerides (mg dl^{-1})	229.00±3.74a	85.00±2.45d	131.00±3.74c	152.00±2.45b
Total protein (g dl^{-1})	4.10±0.26a	3.10±0.30a	2.90±0.26a	3.20±0.32a

Similar letters in the same row are not significantly different (p>0.05).

Water Quality Attributes: Data regarding the water quality attributes, i.e., water temperature, dissolved oxygen, and pH, are presented in Table V. The temperature and dissolved oxygen levels of the water contained in the glass aquaria did not vary significantly ($p > 0.05$) among the treatments (Table V). Water pH value was considerably higher due to T_4 (95/300 g kg^{-1} lipid/protein diet). However, non-significant differences also existed among T_1, T_2, and T_3 for their pH values (Table V).

Table V: Water quality attributes during the experimental duration.

Quality Parameters	T_1(75/250)	T_2(95/250)	T_3(75/300)	T_4(95/300)
Water Temperature (°C)	29.01±0.30a	29.02±0.43a	29.16±0.09a	28.91±0.06a
Dissolved Oxygen (mgL^{-1})	6.170±0.02a	6.170±0.03a	6.162±0.03a	6.177±0.03a
pH	8.025±0.03b	8.005±0.03b	8.025±0.04b	8.072±0.04a

Similar letters in the same row are not significantly different (p>0.05).

DISCUSSION

The lipid/protein requirements of fish vary considerably with size, age, and species of fish under question, as well as with rearing system and nutrient quality of the pelleted diets. Adequate lipid/protein ratio in fish feed enhances fish growth, while feed containing inadequate or excessive lipid/protein ratio may result in fish growth reduction and poor feed utilization.

Fish Growth Performance: *Labeo rohita* showed a variable growth response towards varying lipid/protein ratios in pelleted diets. Results of the present study showed that *Labeo rohita* attained significantly higher average weights, fork and total lengths of 4.79±0.04g, 66.63±0.04mm, and 74.67±0.04mm, respectively, due to a 95/300 g kg-1 lipid/protein diet (T_4). Optimal lipid requirements for aquarium-reared *Labeo rohita* fed pelleted diets have also been reported to be approximately 100 g Kg^{-1} dietary lipid (Satpathy *et al*., 2003). Debnath *et al*. (2007), while evaluating the optimal crude protein level in pelleted diets, also recommended 300 g Kg^{-1} protein as a growth promoter for intensively reared *Labeo rohita.* Thus, a 95/300 g kg-1 lipid/protein pelleted diet may be deemed most suitable for rearing *Labeo rohita* under intensive culture conditions.

Nitrogen Conversion Indices: Nitrogen incorporation efficiency (NIE) of the pelleted diets for *Labeo rohita* was not affected by either any dietary lipids or protein level. Nitrogen conversion ratio (NCR) is a ratio between a gram of nitrogen supplied to the fish through diet and a gram of weight attained by the fish in a specific period of time. The results of the present study showed that the highest nitrogen conversion ratio i.e., 4.18±0.15, of the pelleted diet for *Labeo rohita* was also due to 95/300 g kg-1 lipid/protein diet (T_4). Parveen *et al*. (2012) also reported a similar trend of nitrogen conversion ratio for pond-reared *Labeo rohita* and *Cirrhinus mrigala* fingerlings fed a pelleted diet containing 300g kg $^{-1}$dietary protein. In terms of protein efficiency ratio, the results of this study also showed that the pelleted diet at 95/300 g kg^{-1} lipid/protein ratio gave significantly ($p<0.05$) higher average value of protein efficiency ratio i.e., 0.71±0.08; thus causing the fish to gain significantly higher body weight, fork, and total lengths at this lipid/protein combination. Singh *et al*. (2008), while studying the influence of temperature and protein level on the growth performance of *Cirrhinus mrigala* fry, demonstrated significantly ($p<0.05$) higher protein efficiency ratio due to a diet containing 280g Kg^{-1} protein level. This shows that this protein level in pelleted diets is more committed to giving higher fish yield for these cyprinid species.

Liver Enzyme Assay: Liver is a fish organ that can produce the highest concentration of the enzyme alkaline phosphatase (ALP) as compared to any other fish organ (Shahsavani *et al*., 2010). Alkaline phosphatases are located in the plasma membrane of most animal cells, meant to improve growth and cell differentiation as well as absorption and nutrient transportation (Mazorra *et al*., 2002). Results on hepatic enzymes of *Labeo rohita* revealed significantly ($p<0.05$) higher (78.00±0.32 IUL^{-1}) ALP level in the liver of the fish fed 95/300 g kg-1 lipid/protein diet (T_4). The occurrence of higher ALP levels in the liver may be attributed to the excessive metabolic loads imparted on the liver fed a 95/300 g kg^{-1} lipid/protein diet. *Labeo rohita* liver metabolic performance might have been stimulated abruptly and channelized towards the formation of nuclear proteins, phospholipids, and nucleic acids, thus mobilizing such higher lipid/protein levels of the pelleted diets (Sadiq, 2008), causing the fish liver to produce such higher ALP levels.

Aminotransferases i.e., Alanine transaminase (ALT) and Aspartate transaminase (AST) are the intracellular, non-plasma specific enzymes that are present in the liver, kidney, skeletal muscles, heart, and gills (Shahsavani *et al*., 2010). Fish are known for their preferential ability to utilize amino acids for gluconeogenesis. Higher concentrations of ALT and AST in liver tissue are the indicators of alanine and aspartate (amino acids) directed towards glucose production via gluconeogenesis (Tejpal *et al*., 2009). The results of the present study show that the concentration of ALT and AST in *Labeo rohita* liver was significantly ($p<0.05$) higher at 38.00±2.12 IU/L and 9.00±0.21IU/L, respectively, due to a 95/300 g kg^{-1} lipid/protein diet in T_4. This higher transaminase activity suggests the preference of *Labeo rohita* to utilize dietary protein as an energy source when fed with a higher protein diet; therefore in this particular instance, these enzymes helped the excess amino acid to be metabolized for energy purposes. Higher ALT and AST activities were also reported in *Labeo rohita* when fed diets containing 350gkg^{-1} protein diet (Kumar *et al*., 2009). Gamma-glutamyl transpeptidase (GGT) is an enzyme whose higher concentrations are meant to report any tissue damage activities in the fish organ (Nanda *et al*., 2010). In the present study *Labeo rohita* liver, GGT levels were generally lower and non-significantly different among all the executed treatments except for (T_3), suggesting elimination of any chance of liver tissue damage in the fish due to any feeding regime.

Liver Metabolic Responses: Bilirubins are pigmented compounds synthesized in the liver by the breakdown of hemoglobin, reporting liver impairments when present in higher concentrations (Nelson and Cox, 2000). Results of the present study showed very low concentrations of bilirubins in *Labeo rohita* liver across the treatments that were recorded as 0.20±0.10, 0.30±0.20, 0.30±0.26, and 0.30±0.14 mg dl^{-1} in T_1, T_2, T_3, and T_4, respectively. Such lower concentrations of bilirubins in *Labeo rohita* liver may be attributed to the liver fitness of fish fed any lipid/protein combinations.

Globulin and albumin are the proteins synthesized in the liver and are advantageously used by the animal in various immune responses (Alexander *et al*., 2010). The present study showed that *Labeo rohita* fetched significantly ($p<0.05$) higher value of globulin i.e., 2.00±0.36 g/dl, due to 75/250 g kg^{-1} lipid/protein diet (T_1). Such significantly higher production of globulin might have occurred due to feeding *Labeo rohita* a poor lipid/protein combination that caused the fish to suffer through stress and produce higher concentrations of globulin. Such increased concentration of globulin in fish liver fed lower protein concentration may be attributed to possible intestinal inflammatory response in fish (Lopez *et al*. 2015). Liver albumin concentrations did not vary significantly ($p>0.05$) among the treatments, suggesting varying lipid/protein combinations have no impact on liver albumin concentrations of *Labeo rohita*.

The present study also showed that *Labeo rohita* liver accumulated significantly ($p<0.05$) higher triglyceride, i.e., 229.00±3.74 mg $dl^{-1,}$ due to the 75/250 g kg^{-1} lipid/protein diet (T_1). Such higher levels of triglycerides might have been generated in the fish liver due to their mobilization for fulfilling the energy requirements of the fish (Das *et al*., 2009) fed lower lipid/protein ratios. A trend of decrease in total protein contents of the fish often signals the breakdown process that might have been imparted due to stress (Pakhira *et al*., 2015). Total protein concentration in *Labeo rohita* liver did not vary significantly among the treatments. Therefore, it might be concluded that the total protein contents were not likely to be subjected to any breakdown during this investigatory period. Water quality parameters, i.e., water temperature, dissolved oxygen, and pH, were within the permissible range for fish rearing (Dhawan and Kaur, 2002). In conclusion, this study has revealed that intensively reared *Labeo rohita* requires a lipid/protein combination of 95/300 g kg-1 in the pelleted diet. Such a higher lipid/protein requirement of *Labeo rohita* was due to the sole dependence of the fish on pelleted diets under intensive conditions. Liver enzyme and metabolic assay eliminated the possibility of stress imparted on fish due to any feeding regime.

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Author's contributions

Jhan Zeb contributed to designing the experiment and drafted this manuscript; Ahmar helped in the collection, handling of the data, and statistical analysis of the data. Aatar Yaqoob helped with fish liver analysis. All of the authors approved the final manuscript.

No conflict of interest

The authors declare no conflict of interest.

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