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Effect of Varying Levels of Lipids and Proteins on the Growth Indices and Fatty Acid Profile of *Labeo rohita* (Rohu)

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ABSTRACT

This project was a 2×2 factorial design executed for the evaluation of optimum lipid/protein level in pelleted diets for *Labeo rohita* (Initial weight; 2.87±0.01g). Four pelleted diets varying in their lipid/protein levels i.e. 7.5/25, 9.5/25, 7.5/30 and 9.5/30% were formulated and hand fed for 90 days to four groups of 10 fish each. Results of the present study showed that *L. rohita* attained significantly higher average wet weights, fork and total lengths of 4.79±0.04g, 66.63±0.04mm and 74.67±0.04mm, respectively due to 9.5/30% lipid/protein diet (D_4). None of the dietary lipid/protein levels in pelleted diet had a significant impact on either the condition or the survival of fish. Significantly higher ($p<0.05$) specific growth rate (SGR) and feed efficiency (FE) for *L. rohita* was also due to 9.5/30% lipid/protein diet (D_4). The pelleted diets upon fatty acid analysis showed almost complete absence of linoleic acid, α-linolenic acid, ecosapentanoic acid (EPA) and docosahexanoic acid (DHA). However, these fatty acids were found to be present in the flesh of *L. rohita* at the end of the experiment. The sum of saturated fatty acids (SFA) was higher as compared to the sum of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) in *L. rohita* flesh. Regardless of the feeding regimes there existed a higher proportion of n-3 fatty acids as compared to n-6 fatty acids in the flesh of *L. rohita*. Ratio of n-3/n-6 fatty acids in *L. rohita* flesh was significantly higher ($p<0.05$) due to 9.5/25% (D_2) lipid/protein level in pelleted diet. Moisture contents of *L. rohita* varied significantly among the treatments, showing an inverse relationship with body fats. The feeding regime D_4 (9.5/30% lipid/protein level) gave fish the highest flesh proteins i.e. 19.17±0.03%. The carcass lipid were generally higher in fish groups fed lower protein level whereas the fish groups fed higher protein level fetched comparatively lower body lipids. Body ash contents of *L. rohita* varied slightly among the treatments showing no obvious significant influence of varying lipid/protein levels.

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JZ designed the experiments and wrote the manuscript. ST and NS conducted the experiments. ZS and SA helped with the fatty acid profiling. YA and AZ dealt with data analysis.

Key words
Saturated fatty acids (SFA), Monounsaturated fatty acids (MUFA), Polyunsaturated fatty acids (PUFA), Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), *Labeo rohita*, Specific growth rate, GC analysis

INTRODUCTION

Table fish is a food commodity that provides a balanced profile of nutrients to humans worldwide. Fish meat serves as a valuable human resource; contributing towards a nation's economy and development (Teklu and Lema, 2015). Fish provides its consumers with an optimum mix up of essential fatty acids and all the crucial amino acids (Jabeen and Choudhary, 2011). Furthermore, is highly alimentative that provides energy required for daily human activities (Ljubojevic *et al*., 2013). Pakistan has been blessed with diverse fish fauna as well as fish habitats (Nazir *et al*., 2015). Unfortunately, least efforts have been exerted to channelize these abundant aquatic resources, towards commercial ventures.

Wild fishery is a natural resource that is renewable but at the same time it is highly vulnerable. Despite of their high rate of natural propagation these fisheries resources seems to be finite and limited (Sargent and Tacon, 1999). Fish catches in the natural environment are on the verge of decline. Only sustainable fish culture practices can bring this decline to compensation. Food production sectors such as poultry, livestock and fisheries need to be boosted through managerial practices for the provision of sustainable and secure animal proteins for human use (Nazir *et al*., 2015).

Labeo rohita is a commercial fish species, cultured throughout the sub-continent for its demand as food fish (Das *et al*., 2005). Flesh of *L. rohita* is also an important source of polyunsaturated fatty acids required for normal growth and development of humans (Memon *et al*., 2011). Taste acceptance, alimentative properties and commercial

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demand brings *L. rohita* substantial superiority over other locally reared fish species (Jabeen and Choudhary, 2011). Aquaculture is an expeditiously flourishing venture designed as a source of protein generation for human utilization (De Silva and Turchini, 2008). Intensive culture of fish causes a maximum yield per unit area at a minimum cost. In recent decades, intensive fish culture is preferred to be anticipated since it require fewer space and rapid fish tissue build up as compared to any other culture system. The main objective of this industrialized system is only to bring an increase in fish biomass within minimum possible time. Intensive culture of fish is a reliable means to promote food production in concomitant with human needs (Abid and Ahmed, 2009).

The ongoing aim in fish culture is to promote the growth of fish by supplying the fish with least cost artificial diets. Fish is a cold blooded animal that requires a higher level of nutrients in their diet that can be made available to the fish through artificial diets (Kadhar *et al*., 2012). However, care should be taken into account in order to develop a sustainable feeding regime that is economically beneficial as well as efficient growth promoter of fish (Srivastava *et al*., 2013). Besides proteins, dietary lipids are also of utmost importance for fish because they serve as an energy source and offers protection against environmental stresses (Hsieh *et al*., 2007). Fish meat is a known source of omega-3 fatty acids that are required by cardiovascular patients to lower their serum cholesterol (Kandemir and Polat, 2007; Hosseini *et al*., 2010). Apart from these aforementioned alimentative properties, fish oil has been found to be an enriched source of docosahexaenoic acid (DHA) (22:6; n-3) as well as eicosapentaenoic acid (EPA) (20:5; n-3) that are meant to counter metabolic and other related syndromes (Breslow, 2006). Linoleic acid and α-linolenic acid are the major essential fatty acids that are of special interest since the humans lack an enzyme for the synthesis of these essential fatty acids (Nelson and Cox, 2008). Linoleic acid and α-linolenic acid act as a precursor of docosahexaenoic acid (DHA) as well as eicosapentaenoic acid (EPA), both of which play important role as components of cell membranes, vital for the cell support, developmental purposes and strengthening of the immune system (Memon *et al*., 2011). (EPA) and (DHA) both are valuable because these fatty acids bring prevention from cardiovascular diseases and other health disorders (Rasoarahona *et al*., 2004).

This investigation was pursued for the evaluation of fatty acid profile of *L. rohita* flesh fed various lipid/protein graded diets. The purpose was also to test the ability of this fish species to synthesize the essential fatty acid viz. linoleic acid and α-linolenic acid and the resulting benefits these fatty acids confer upon human health and wellbeing.

MATERIALS AND METHODS

Fish species and feeding regimes

Labeo rohita (Rohu) fingerlings with an initial average body weight of 2.87±0.01g (purchased from Government Fish Seed Hatchery, Peer Mahal) were reared in the Aquaculture Research Laboratory, Government postgraduate college, Gojra from April to June 2016 under approximately 12/12 light dark period. Fish were acclimatized to laboratory conditions for 15 days and then randomly distributed into 12 homogenous groups of 10 fish each. Each fish group was reared in triplicate in 100L capacity glass aquaria supplied with electric aerators. Triplicate groups of fish were fed four isoenergetic pelleted diets, differing in their lipid and protein levels (Table I). The four pelleted diets fed to fish contained 7.5/25, 9.5/25, 7.5/30 and 9.5/30% lipid/protein level and were designated as D_1, D_2, D_3 and D_4, respectively. Fish were fed to visual satiation manually, twice daily for a period of 15 days. Amount of feed consumed and the growth exhibited by the fish were monitored fortnightly.

Table I. Formulation and proximate composition (%) of pelleted diets for *Labeo rohita*.

Ingredients (lipid/ protein %)	D_1 (7.5/25)	D_2 (9.5/25)	D_3 (7.5/30)	D_4 (9.5/30)
Wheat	15	17	15	13
Rice broken	15	8	1	1
Rice polish	8	12	8	8
Wheat bran	8	7	8	7
Canola meal	22	16	22	14
Sunflower meal	7	15	7	18
Corn glutton	5	5	5	5
Fish meal	18	18	32	32
Corn oil	1	1	1	1
Premix	1	1	1	1
Proximate composition (%)				
Moisture[a]	7.77	6.57	7.63	6.71
Crude protein[a]	25.09	25.34	30.20	30.33
Crude lipids[a]	7.65	9.45	7.59	9.43
Crude Ash[a]	4.57	5.5	5.94	6.70
Carbohydrates[b]	54.92	53.14	48.64	46.83
Energy (Kcal/kg)[c]	2534	2493	2520	2450

Note: Vitamin premix contains (gkg $^{-1}$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 performance

The following formulae were applied to calculate fish growth and feed efficiency.

$$\text{Condition factor (K)} = \frac{W \times 10^5}{L^3}$$

$$\text{Specific growth rate (SGR\%)} = \frac{\text{Ln (final fish weight)} - \text{Ln (initial fish weight)}}{\text{Days fish were fed}} \times 100$$

$$\text{Feed Efficiency (FE)} = \frac{\text{Weight gain (g)}}{\text{Feed Intake(g)}}$$

$$\text{Survival Rate} = \frac{\text{No. of stocked fish} \times 100}{\text{No. of recovered fish}}$$

Aftermath of the trial

For the determination of fish flesh proximate composition and fatty acid profile each of the four experimental groups of fish were starved for 24 h and then sampled randomly to begin the analysis procedure. Each group of fish were filleted, freeze dried, ground into fine powder and stored at -20ºC, packed in screw-top glass bottles until used for proximate composition or fatty acid profile.

Proximate composition of fish flesh and pelleted diets

Proximate composition of fish flesh and pelleted diets were analyzed through AOAC (2006). All the methods were run in triplicate and a very brief description of the methods is as follows. For the determination of moisture, the flesh and diet samples were oven dried at 102ºC for 24 h. Crude protein was determined by Kjeldhal method (Crude protein= Nitrogen×6.25). Crude lipids were estimated through extraction with chloroform: methanol (2:1); crude ash was determined in a muffle furnace at 550ºC for 6 h; carbohydrates were determined by subtracting the sum of moisture, crude protein, crude lipids and crude ash by 100.

Fatty acid methyl esters (FAME) preparation

Fatty acid methyl esters (FAME) synthesis method (Indrati *et al*., 2005) was employed for the fatty acid analysis of fish flesh and pelleted diets. 100mg powdered fish flesh and or diet in triplicate was taken into 10ml screw top glass bottles. 4ml mixture (1.7ml methanol: 0.3ml H_2SO_4: 2ml Chloroform) was added to it. Each bottle was vortexed for 20 seconds and tightly closed by a teflon cap to prevent any leakage. For the purpose of carrying out trans-esterification each bottle was placed in heating block at 90ºC for 90 minutes. After the completion of trans-esterification process each bottle was cooled to room temperature and leaking bottles were discarded. Now 1ml of distilled water was added to each bottle and allowed to stand for a minute. Two phases were developed the upper phase was discarded whereas the lower phase containing the FAME was dried with anhydrous Na_2SO_4. Each sample was transferred into polypropylene eppendorf tube that was tightly closed with a lid, labeled and refrigerated until GC (Gas Chromatography) analysis.

Gas chromatography (GC) analysis

Gas chromatography was performed at Central High-Tech Laboratory, University of Agriculture, Faisalabad, Pakistan. All tests were done on a gas chromatogram GC-17A (SHIMADZU) equipped with flame ionization detector and a fused silica capillary SGE column. Each sample was allowed to run in a gas chromatograph using nitrogen as a carrier gas on the mobile phase. The length of capillary column was 624 to 30 m and the diameter was 3.0µ to 0.32 id. The flow rate of the gas was 30cm per min. The peaks were identified by using external FAME standards (BP 1250mV, 10 samples, per second, peak width 0.020 min and the threshold level, 0.050 mV). The detection was done by FID at 250^{0}C and at a pressure of 5.57 psi. The temperature of column was set and held at 100ºC for 1 min. The temperature was then raised at 15ºC/minute up to 240ºC. The temperature of the injector port was set at 260ºC. Fatty acids were determined by comparing the retention times of FAME with a standard component FAME mixture. GC analyses were performed in triplicate and the results were expressed as mean value ± standard deviation.

Water quality attributes

For the purpose of maintaining a healthy environment for fish rearing the water quality attributes i.e. water temperature, pH and dissolved oxygen were measured through meters i.e. HANNA HI-8053, HI-8520 and HI-9146, respectively on daily basis.

Statistical analysis

The data were subjected to one factor and two factor analysis of variance (ANOVA) at a significance level of 0.05. Significance of means was further tested through Tukey's Honestly Significant Difference posthoc test. All the statistical analyses were performed through the statistical software, Statistix® (version 8.1; Analytical Statistix Software, Tallahassee, USA).

RESULTS

Fish growth and feed efficiency

Results pertaining to variations in fish average weight, fork length, total length, condition factor, specific growth rate, feed efficiency and fish survival are presented in Table II. The growth performance of the fish i.e. final average weight, fork and total length increased significantly

Table II. Growth and feed efficiency of *Labeo rohita* fed varying lipid/protein levels (%).

Parameters	D_1(7.5/25)	D_2(9.5/25)	D_3(7.5/30)	D_4(9.5/30)
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
Condition factor	1.10±0.00a	1.10±0.00a	1.13±0.00a	1.12±0.01a
Specific growth rate	0.80±0.05c	0.78±0.02c	0.97±0.06b	1.10±0.04a
Feed efficiency	0.14±0.01c	0.14±0.00c	0.17±0.01b	0.19±0.01a
Fish Survival	100±0.00a	100±0.00a	100±0.00a	100±0.00a

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

Table III. Fatty acid profile (g $100g^{-1}$) of pelleted diet offered to *Labeo rohita*.

Fatty acids	Formulae	D_1 (7.5/25)	D_2 (9.5/25)	D_3 (7.5/30)	D_4 (9.5/30)
Heptanoic acid	C 7:0	0.17	0.18	0.16	0.19
Decanoic acid	C 10:0	0.03	0.05	0.02	0.06
Dodecanoic acid	C 12:0	0.10	0.02	0.01	0.04
Myristic acid	C 14:0	0.43	0.52	0.44	0.59
Pentadecanoeic acid	C 15:0	0.07	0.06	0.09	0.10
Palmitic acid	C 16:0	8.40	7.33	7.45	8.56
Stearic acid	C 18:0	4.31	4.28	4.32	4.63
Arachidic acid	C 20:0	1.91	1.78	1.85	1.99
Docosanoic acid	C 22:0	2.00	1.97	2.12	2.35
Palmitoleic acid	C 16:1 n-7	0.04	0.01	0.05	0.06
Oleic acid	C 18:1 n-9	14.78	13.66	13.89	14.98
Erucic acid	C 22:1 n-9	28.45	27.97	27.58	28.65
Nervonic acid	C 22:1 n-9	2.30	2.00	2.36	2.45
Linoleic acid	C 18:2 n-6	0.01	0.01	0.01	0.01
α-linolenic acid	C 18:3 n-3	0.01	0.01	0.01	0.01
EPA	C 20:5 n-3	0.01	0.01	0.01	0.01
DHA	C 22:6 n-3	0.01	0.01	0.01	0.01
	Σ SFA*	17.42	16.01	16.46	18.51
	Σ MUFA¶	45.57	43.64	43.88	46.14
	Σ PUFA^{l}	0.04	0.04	0.04	0.04
	Σ n-3^{ǂ}	0.03	0.03	0.03	0.03
	Σ n-6^{l}	0.01	0.01	0.01	0.01
	n-3/n-6†	3.00	3.00	3.00	3.00

EPA, Ecosapentanoic acid; DHA, Docosahexanoic acid; *Sum of saturated fatty acids; ¶Sum of monounsaturated fatty acids; ^{l}Sum of polyunsaturated fatty acids; ^{l}Sum of omega 6 fatty acids; ^{ǂ}Sum of omega 3 fatty acids; †Ratio of omega 3/omega 6 fatty acids.

($p<0.05$) as the level of lipid/ protein ratio increased in the pelleted diets. None of the lipid/ protein level in the pelleted diet was able to cause any significant variation in the condition or survival of the fish. Both specific growth

rate and feed efficiency of the fish were significantly ($p<0.05$) higher as 1.10±0.04 and 0.19±0.01, respectively due to 9.5/30% lipid/protein level in pelleted diets.

Fatty acid profile of pelleted diets and fish flesh

The fatty acid profile of the pelleted diets is presented in Table III. The pelleted diets upon fatty acid analysis showed almost complete absence of linoleic acid, α-linolenic acid, EPA and DHA. Fatty acid profile of *Labeo rohita* flesh is presented in Table IV. The sum of saturated fatty acids (SFA), monosaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in the flesh of *L. rohita* plateaued at the highest lipid/protein level i.e. 9.5/30% (D_4). However, a gradual decrease in lipid/protein level of the pelleted diets caused a general decline in SFA, MUFA and PUFA of fish flesh. The principal omega-6 fatty acid recorded in the fish flesh was linoleic acid whereas principal omega-3 fatty acids recorded were α-linolenic acid, EPA and DHA. The concentrations of linoleic acid, α-linolenic acid, EPA and DHA in fish flesh increased significantly ($p<0.05$) with an increase in lipid/protein level of the pelleted diet. Ratio of n-3/n-6 fatty acids in *L. rohita* flesh was significantly higher ($p<0.05$) due to 9.5/25% (D_2) lipid/protein level in pelleted diet.

Proximate composition of pelleted diets and fish flesh

The formulation and proximate composition of the pelleted diets were in conformation to the each other with respect to lipid/protein levels (Table I). The proximate composition (moisture, crude protein, crude lipids, crude ash and carbohydrates) of *L. rohita* flesh are summarized in Table V. The moisture contents of *L. rohita* flesh plateaued when fed 7.5/30% lipid/protein diet (D_3). Crude protein content value of *L. rohita* flesh were highest as 19.17±0.03% as a result of feeding the fish 9.5/30% lipid/protein diet (D_4), followed by D_2, D_1 and D_3. On the other hand, lipid contents of fish flesh were generally higher in fish groups fed lower protein level i.e. D_1 and D_2 as compared to fish groups fed higher protein level i.e. D_3 and D_4. Ash contents varied slightly among the treatments showing no significant influence of varying lipid/protein levels in pelleted diets. Fish group fed lowest lipid/protein level i.e. 7.5/25% (D_1) in pelleted diet fetched significantly higher ($p<0.05$) carbohydrates in their flesh.

Water quality management

The temperature, pH and dissolved oxygen of the aquarium water did not varied significantly ($p>0.05$) across the treatments (Table VI).

Table IV. Fatty acid profile (g 100g^{-1}) of *Labeo rohita* flesh at final harvest.

Fatty acids	Formulae	D_1 (7.5/25)	D_2 (9.5/25)	D_3 (7.5/30)	D_4 (9.5/30)	p value
Dodecanoic acid	C 12:0	0.39±0.07c	0.42±0.07c	0.56±0.09b	0.91±0.05a	p<0.01
Myristic acid	C 14:0	7.14±0.04d	8.11±0.07c	8.62±0.06b	8.83±0.04a	p<0.01
Pentadecanoeic acid	C 15:0	1.51±0.13b	1.41±0.15b	2.00±3.60a	2.45±0.07b	p<0.01
Palmitic acid	C 16:0	29.68±0.10b	27.54±0.06d	28.16±0.09c	30.14±0.06a	p<0.01
Stearic acid	C 18:0	11.45±0.11d	12.11±0.03c	13.07±0.06b	13.79±0.10a	p<0.01
Palmitoleic acid	C 16:1 n-7	0.55±0.13b	0.63±0.05b	0.77±0.15b	1.10±0.04a	p<0.01
Oleic acid	C 18:1 n-9	10.42±0.11d	11.31±0.09b	10.57±0.11c	12.63±0.10a	p<0.01
Erucic acid	C 22:1 n-9	0.41±0.05c	0.52±0.12b	0.61±0.04b	1.16±0.07a	p<0.01
Linoleic acid	C 18:2 n-6	6.43±0.13c	5.31±0.04d	6.59±0.07b	7.48±0.09a	p<0.01
α-linolenic acid	C 18:3 n-3	2.15±0.06d	3.10±0.04c	3.42±0.13b	4.12±0.06a	p<0.01
EPA	C 20:5 n-3	3.11±0.03c	3.09±0.04c	3.77±0.09b	4.32±0.07a	p<0.01
DHA	C 22:6 n-3	4.99±0.01d	5.11±0.04c	5.81±0.08b	6.18±0.06a	p<0.01
	Σ SFA*	50.17c	49.59c	52.41b	56.12a	
	Σ MUFA¶	11.38c	12.46b	11.95c	14.89a	
	Σ PUFA‖	28.06d	29.07c	31.54b	36.99a	
	Σ n-3‡	10.25d	11.30c	13.00b	14.62a	
	Σ n-6‖	6.43b	5.31c	6.59b	7.48a	
	n-3/n-6†	1.59c	2.13a	1.97b	1.95b	

Similar alphabets in the same row are not significantly different ($p>0.05$). for abbreviations and other statistical details, see Table II.

Table V. Proximate composition of *Labeo rohita* flesh fed varying lipid/protein levels (%).

Flesh parameters	D_1(7.5/25)	D_2(9.5/25)	D_3(7.5/30)	D_4(9.5/30)
Moisture (%)	76.00±2.00c	78.00±2.00b	82.00±2.64a	78.00±2.64b
Crude Protein (%)	18.37±0.02ab	19.00±1.73a	15.24±0.02b	19.17±0.03a
Crude Lipids (%)	1.35±0.01a	1.32±0.03a	0.75±0.02c	1.02±0.01b
Crude Ash (%)	1.01±0.04b	1.03±0.04ab	1.05±0.03a	1.04±0.04ab
Carbohydrates (%)	3.27±0.01a	0.65±0.02d	0.99±0.01b	0.77±0.03c

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

Table VI. Water quality attributes during 90 days of the experiment.

Quality parameters	D_1(7.5/25)	D_2(9.5/25)	D_3(7.5/30)	D_4(9.5/30)
Water Temperature (°C)	29.01±0.30a	29.02±0.43a	29.16±0.09a	28.91±0.06a
DO (mgL^{-1})	6.170±0.02a	6.170±0.03a	6.162±0.03a	6.177±0.03a
pH	8.025±0.03a	8.005±0.03a	8.025±0.04a	8.072±0.04a

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

DISCUSSION

Fish growth and feed efficiency

In this investigation the growth performance i.e. final average weight, fork and total length of *L. rohita* treated with 9.5/30% lipid/protein diet (D_4) were significantly ($p<0.05$) higher than the fish fed with any other lipid/protein level. Satpathy *et al*. (2003) also obtained optimal growth of *L. rohita* when fed diet at 30% protein and 10% lipids therefore, this 9.5/30% level in pelleted diets may be deemed optimal for maximum yield of *L. rohita* under intensive culture condition. Condition factor is the growth parameter that represents the degree of well-being of cultured fish species, with respect to their wet body weight and total length (Javed, 2015). None of the dietary lipid/protein levels in pelleted diet had any significant impact on either the condition or the survival of fish in the present study, indicating the potential of all these lipid/protein levels to keep fish supple under each treatment. Kim *et al*. (2012) also demonstrated a lack of impact of varying lipid levels in olive flounder diet on its condition. Zeb (2016) also recorded 100% fish survival when pond raised cyprinids were fed pelleted diets varying in their protein contents. Specific growth rate of fish is the estimation of fish growth under specific period of time whereas feed efficiency is a growth parameter that explains relationship of feed with the growth of fish. Specific growth rate and feed efficiency of *L. rohita* were significantly ($p<0.05$) effected due to varying lipid/protein levels. Increase in dietary protein level along with an increase in dietary lipids caused a significant ($p<0.05$) increase in *L. rohita* specific growth rate and feed efficiency. Aminikhoei *et al*. (2015) also observed a significant improvement in the specific growth rate and feed efficiency of *Cyprinus carpio* with a unit increase in the lipid/protein level in pelleted diets.

Fatty acid profile of pelleted diets and fish flesh

The pelleted diets upon fatty acid analysis showed almost complete absence of linoleic acid, α-linolenic acid, EPA and DHA. However, these fatty acids were found to be present in the flesh of *L. rohita* at the end of the experiment. Paiko *et al*. (2010) also observed the occurrence of DHA in the flesh of *Channa striatus* when fed pelleted diets either deficient or contained very low levels of DHA. The occurrence of these fatty acids in finally harvested *L. rohita* flesh demonstrated the ability of this fish species to synthesize these fatty acids even when not provided in their diet. This might have occurred due to the possession of certain enzymes called desaturases and elongases by the fish that caused the desaturation and ultimate synthesis of these fatty acids (Zheng *et al*., 2009). Dietary lipid source is one of the major factors that influenced the fatty acids profile of fish flesh (Choi and Lee, 2015). In this investigation corn oil was used as a lipid source in the pelleted fish diets. The sum of SFA, MUFA and PUFA in the flesh of *L. rohita* plateaued at the highest lipid/protein level i.e. 9.5/30% (D_4). Sharma *et al*. (2010) also observed similar higher values of the sum of SFA, MUFA and PUFA in *L. rohita* fed with pelleted diet against wild *L. rohita*. The sum of SFA was higher as compared to the sum of MUFA and PUFA in *L. rohita* flesh in the present study. Similar findings were also reported by Ozparlak (2013) where he also observed a higher percentage of SFA in *Cyprinus carpio* flesh against the percentage of PUFA.

These findings suggest that these carp species possibly contain a higher SFA percentage in their flesh against MUFA or PUFA percentage. The n-3/n-6 fatty acids balance is one of the most important attribute of edible human food (Ghomi *et al*., 2012). Human di*et a*l over the world is susceptible to n-3/n-6 fatty acid imbalance; through containing a higher proportion of n-6 PUFA as compared to n-3 PUFA (Strobel *et al*., 2012). Any diet containing a higher proportion of n-3 PUFA and a lower proportion of n-6 PUFA are considered suitable for human health (Nelson and Cox, 2008). The results of the present study showed the occurrence of a higher proportion of n-3 fatty acids as compared to n-6 fatty acids in *L. rohita* flesh regardless of the treatments. This shows the significance of *L. rohita* flesh as an edible product containing most favorable fatty acid profile.

Proximate composition of pelleted diets and fish flesh

Proximate analysis of the pelleted diets generally conformed to the formulated lipid/protein levels. Moisture contents of *L. rohita* varied significantly among the treatments, showing an inverse relationship with body fats. Increase in moisture causes decrease in fat contents and vice versa. Similar inverse moisture/fat relation has also been reported by Ashraf *et al*. (2011) but for other carp species. The feeding regime D_4 (9.5/30% lipid/protein level) gave fish the highest flesh proteins i.e. 19.17±0.03%. Significantly higher ($p<0.05$) average body weight, fork and total length of *L. rohita* at this lipid/protein level i.e. D4 may be a direct consequence of accumulated flesh proteins. Kim *et al*. (2016) also observed similar results while studying the growth performance of juvenile parrot fish, *Oplegnathus fasciatus* in relation to carcass proteins. The carcass lipid were generally higher in fish groups fed lower protein level whereas the fish groups fed higher protein level fetched comparatively lower body lipids. Fingerlings of *Channa straitus* also accumulated higher body lipids when fed lower dietary proteins and vice versa (Paiko *et al*., 2010). Body ash contents of *L. rohita* varied slightly among the treatments showing no obvious significant influence of varying lipid/protein levels. Ash contents also did not show significant differences due to dietary protein for juvenile parrot fish (Kim *et al*., 2016) or showed slight differences in response to dietary lipids for Juvenile black fin sea bream (Rahim *et al*., 2015). The water quality parameters were within the permissible range for healthy fish culture.

CONCLUSIONS

The lipid/protein level i.e. 9.5/30% in pelleted diets may be deemed optimal for maximum yield, specific growth rate, feed efficiency and survival of *Labeo rohita* under intensive culture condition. This study also demonstrated the potential existence of desaturases and elongases in *L. rohita*, as this fish was able to synthesize the essential fatty acids without being provided in diet. There existed the occurrence of a higher proportion of n-3 fatty acids as compared to n-6 fatty acids in *L. rohita* flesh, indicating the significance of *L. rohita* flesh as an edible product conferring health benefits to humans. However, further research need to be sought in order to study the mechanism by which these desaturases and elongases operate in the fish.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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