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EFFECT OF PROTEIN TO ENERGY (P/E) RATIOS ON GROWTH PERFORMANCE, BODY COMPOSITION AND DIGESTIVE ENZYME ACTIVITIES IN ROHU, *Labeo rohita* FINGERLINGS

Muhammad Javed Iqbal[1], Muhammad Afzal[1,*], Khalid Abbas[1] and Muhammad Shahid[2]

[1] Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad 38040 Pakistan;
[2] Department of Biochemistry, University of Agriculture, Faisalabad 38040 Pakistan
***Corresponding author's e-mail: afzal160@uaf.edu.com**

A 4 x 3 factorial experiment was performed to determine the effect of protein-to- energy (P/E) ratios for *Labeo. rohita*(rohu)fingerlings. Twelve experimental diets with four dietary protein (DP) levels (24, 26, 28, and 30 %) and three dietary energy (DE) levels (2400,2700, and 3000 kcal / kg) ranging from 80.00 mg / kcal to 125.00 mg /kcal P / E ratio were formulated. Each diet was randomly assigned to each triplicate group of seventeen fish averaging 6.5 ±0.6 g (mean ± SD) for 10 weeks. The results showed that growth performance was highest in terms of weight gain (WG), feed conversion ratio (FCR), feed intake (FI), protein efficiency ratio (PER), specific growth rate (SGR) and digestive enzyme activities of lipase (0.567±0.05), protease (1.16±0.02) and amylase (3.43±0.02) U/mg were observed in rohu, *L. rohita* fingerlings fed diet containing 26% protein and 3000 kcal/kg energy, with 86.67 mg /kcal P/ E ratio. The crude protein, lipid and ash contents of fish were significantly ($p > 0.05$) affected by changing dietary P / E ratios. However, dry matter (DM) and gross energy (GE) contents of the fish did not improve by changing the dietary P / E ratios. In conclusion, the best protein-to-energy ratio to culture *L. rohita* fingerlings is estimated in 86.67 mg/kcal.

Keywords: Growth, enzymatic activity, body composition, *Labeo rohita*.

INTRODUCTION

The development of an efficient aquaculture industry is primarily dependent on the availability of inexpensive feed ingredients in terms of quality and quantity. Fish meal is an excellent source of nutrients such as essential amino acids, indispensable fatty acids, vitamins, minerals (Zhou *et al.*, 2004), and growth factors. However, its limited supply, increasing demand and high cost has driven interest of fish farmer to use less expensive plant-based ingredients, such as canola meal, sunflower meal and soya-bean meal, in aquaculture feeds (Lim *et al*., 2011).

Dietary protein is utilized for the maintenance processes, reproduction and growth of fish (Hossain *et al.*, 2010). Optimal dietary protein levels, therefore, are important to maximize the fish growth. Similarly, adequate dietary energy is important for the maintenance of life processes. Moreover, lipids and carbohydrates are cheap sources of dietary energy and may reduce protein utilization as a dietary energy source (Mohseni *et al.*, 2013). On the other hand, inadequate levels of dietary energy can increase fish production cost (Okorie *et al.*, 2007). However, excessive dietary energy can reduce feed consumption, inhibit feed utilization, increase lipid deposition and reduce fish growth (NRC 2011). Therefore, the optimum ratio between protein and non-protein sources of energy in fish diets, is associated with higher nutrients retention (Ai *et al.*, 2004) and reduce organic pollutants in the culture environment. *Labeo rohita* commonly known as rohu, is an important fish because of higher growth rate, market values, quality meat and consumer preference and is being cultured in Asia especially in the Indo Pak subcontinent (Khan *et al.*, 2004) The estimated production of this fish species in subcontinent was 9, 45,233 metric tons (FAO, 2014).

The optimization of protein to energy (P/E) ratios may differ with fish species, age, protein sources, diet formulation, seasonal factors and experimental design (Teshima *et al.*, 2006; Okorie *et al.*, 2007). In some fish species, P/E ratios have been reported 98.3 mg/kal for Singhi, *Heteropneu stesfossilis* (Khan *et al.*, 2012); 92.25 mg/kcal for Persian sturgeon, *Acipenser persicus* (Mohseni *et al.*, 2013); 150.75 mg/kcal for parrot fish, *Oplegnathus fasciatus* (Kim *et al.*, 2016); 97.35 mg/kcal for lemon fin barb hybrid larvae, *Hypsibarbus wetmorei* × *Puntinus gonionotus* (Anizah *et al.*, 2017).

Because limited information is available on dietary P/E ratios for *L. rohita* fingerling, the current investigation was undertaken to study the influence of varying P/E ratios on growth parameters, digestive enzymes activities and body composition of *L. rohita* fingerlings. The ultimate objective of the study was to establish optimum dietary P/E ratios for commercial applications.

MATERIALS AND METHODS

***Experimental design and diet preparation*:** Twelve experimental diets with 24, 26, 28, and 30% dietary protein levels and 2400, 2700, and 3000 kcal/ kg dietary energy levels with P/ E ratio from 80.00, 86.7, 88.9, 93.3, 96.3, 100.0, 100.0, 103.7, 108.3, 111.1, 113.7 and 125.00 mg protein/kcal energy were formulated (Table 1).The feed ingredients were taken from animal feed market. The composition of feed ingredients was chemically analyzed by following AOAC (1995) before diet formulation. All feed ingredients were ground to a fine powder to the desired particle size (0.05 mm) using a grain grinder; (Jimo disk mill (model FFC-45, China). Fish meal, canola meal, soybean meal and sunflower meal were used in the diet as a protein source while soybean oil and fish liver oil were used as a lipid (energy) source. However, wheat flour was used as a source of carbohydrates to adjust the dietary energy content of the experimental diet. All the experimental diets were also fortified by adding 1% vitamins premix and 1% minerals premix. The ingredients (dried) were weighed using an electric balance and mixed well in an electric mixer for 10-12 minutes according to the composition ratios of each diet (Table 1). The mixture of fish liver oil and soybean oil was added gradually while mixing constantly. Suitable dough of each diet was prepared by slowly adding 15% water. The dough was pelleted using lab Extruder (Model SYSLG 30-IV) for making floating pellets and then dried in an open air at 22 °C for 24 h (Lovell, 1989).All diets were packed in plastic bags, sealed, and stored in the refrigerator at -20 ^{0}C until fed.

***Fish and Feeding Conditions*:** *Labeo rohita* with 7-8 cm size were purchased from a Public Sector Fish hatchery (Satyana Road, Faisalabad, Pakistan). The fish were maintained for 2 weeks in V-shaped steel tanks. The *L. rohita* fingerlings were fed on basal diet during acclimation period. 17 fish with an averaging 6.5 ± 0.06 g (mean ± SD); randomly distributed into 36 V-shape tanks of 70 L capacity. Before feeding trial, fish were bathed with 5 g/L sodium chloride to kill pathogenic

Table 1. Formulation and proximate analyses of the experimental diets (% dry matter basis).

	Protein level (%)											
	24			26			28			30		
	2400	2700	3000	2400	2700	3000	2400	2700	3000	2400	2700	3000
Ingredients (%)												
Fish meal*	20	20	20	24	24	24	28	28	28	32	32	32
Soya bean meal*	9	11	13	10	12	14	13	15	17	14	16	18
Canola meal*	3	3	3	3	3	3	3	3	3	3	3	3
Sunflower meal*	3	3	3	3	3	3	3	3	3	3	3	3
Corn gluten*	14	14	14	14	14	14	14	14	14	14	14	14
Wheat bran*	22	20	18	20	18	16	17	15	13	14	12	10
Rice polish*	10	8	6	9	7	5	6	4	2	5	3	1
Wheat flour*	9	7	5	7	5	3	6	4	2	5	3	1
Fish oil*	3	3	3	3	3	3	3	3	3	3	3	3
Soya oil*	1	5	9	1	5	9	1	5	9	1	5	9
Vitamins***	1	1	1	1	1	1	1	1	1	1	1	1
Minerals**	1	1	1	1	1	1	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1	1	1	1	1	1	1
Citric acid	3	3	3	3	3	3	3	3	3	3	3	3
Total	100	100	100	100	100	100	100	100	100	100	100	100
Proximate composition (% dry matter)												
Dry matter	91.0	91.4	91.7	91.0	91.3	91.3	90.9	91.3	91.6	90.9	91.3	91.6
Crude protein	24.3	24.4	24.6	25.8	26.0	26.2	28.0	28.2	28.3	29.6	29.8	29.9
Lipid	11.7	15.0	18.3	11.1	14.4	17.6	10.3	13.6	16.9	9.7	13.0	16.2
Ash	6.7	6.5	6.4	7.03	6.8	6.6	7.4	7.0	6.8	7.6	7.2	7.0
Gross energy	2.5	2.7	2.9	2.4	2.7	3.0	2.5	2.8	3.0	2.5	2.8	3.0
P/E ratio†	100.0	88.9	80.0	108.3	96.3	86.7	113.7	103.7	93.3	125.0	111.1	100.0

Note: *Fish meal, soybean meal, canola meal taken from Nishat poultry and fish feed mill (Faisalabad, Pakistan), sunflower meal, corn gluten meal, wheat bran, wheat flour, fish oil, soya oil purchased from Qaderi animal feed (Faisalabad, Pakistan). ** Minerals mixture (mg/kg diet) calcium (Ca)=155 gm, phosphorus (P) =135 gm, magnesium (Mg)=55 gm, sodium (Na) =45 gm, Zn(Zinc)= 3000 mg, manganese (Mn) =2000 mg, iron (Fe) =1000 mg, copper (Cu)= 600 mg, cobalt (Co)= 40 mg, iodine (I)=40 mg, potassium (K)=2000 mg. *** Vitaminspremix (mg / kg diet) Vit A=15 MIU; Vit B_1=15000 mg; Vit K =3 4000 mg; Vit B_{12} = 9000 mg; Vit D=33 M IU; VitE =6000 IU; Vit B_6= 4000 mg; Vit C= 15000 mg; Vit B_3=25000 mg; Vit B_2= 6000 mg;Vit B_9=750 mg;Vit B_5=10000 mg; † Protein to energy ratio in mg protein / kcal energy.

bacteria and fungi if any as described by Rowland and Ingram (1991). All tanks were equipped with an aeration system. The oxygen was supplied in the range of 5.6-7.2 mg/L through this system round the clock. Water temperature and pH were adjusted between 25.1-28.9 ^{0} C and 7.3-8.5, respectively. Each test diet was assigned to three replicate tanks. Fish were fed twice daily (0900 and 1700 hours) to apparent satiation (2% wet weight/ day) for 10 wk. After 2 hours of feeding uneaten diet was siphoned manually.

***Growth Study*:** All fingerlings of each treatment were weighed fortnight, during whole experimental period. Growth rate was measured according to the following formulae.

Weight gain (%)

$$= \frac{Final\ weight\ of\ Fish - Initial\ weight\ of\ Fish}{Initial\ weight\ of\ Fish} \times 100$$

$$SGR = \frac{(In\ final\ weight - In\ initial\ weight)}{Total\ Experimental\ days}$$

$$\text{Feed conversion ratio (FCR)} = \frac{Total\ feed\ intake\ (g)}{Fish\ weight\ gained\ (g)}$$

$$\text{Protein efficiency ratio (PER} = \frac{Wet\ weight\ gain\ (g)}{Protein\ intake\ (g)}$$

$$\text{Feed intake (\%)/day)} = \frac{Feed\ consumption\ (g)}{\left[\frac{Final\ weight + Initial\ weight}{2}\right] \times days} \times 100$$

***Chemical analysis of whole body*:** On termination of feeding trial, 5sample fish from each group were collected, starved for 24 hours and immersed in 3000 mg/ L clove oil for 40-60 seconds to anesthetize (Khajepour *et al.*, 2012). All collected fish were killed with a sharp blow on head. They were then frozen at -20 ^{0}C until proximate analyses. The fillets of fish were analyzed following AOAC (1995). Dry matter of fish fillets was analyzed by oven drying at 105 ^{0}C for 12-13 h, crude protein (N × 6.25) by Kjeldahl method, lipid by ether extraction procedure (Bligh and Dyer, 1959) of Soxtec HT_2 1045. The gross energy of fish was determined through Oxygen Bomb Calorimeter (Parr Instrument Company, street No. 211 Molin, united state America). Muffle furnace was used to determine ash.

***Digestive Enzyme Essay*:** At the termination of experiment, 7 fish were killed from each treatment and dissected within 2 hours of capture to collect visceral contents. The intestinal contents were frozen to -20 °C until chemical analysis of digestive enzyme activity. All frozen intestinal samples of *L. rohita* were homogenized separately. Then, a 5% solution of all replicates of 1 g homogenate in a cold 0.25 M sucrose solution in an electric homogenizer (HG-15D, DAIHAN scientific brand) was prepared. The homogenates were then centrifuged (Hettich D-78532, Germany) at 5000×g for 15 minutes at 5°C. The supernatants were collected in vials (100-200 µl) and stored in -20°C until used for spectrophotometric determination of three digestive enzyme activities.

***Protease activity*:** Protease activity was determined by following Kuntiz (1947). Enzyme activity is expressed as specific activity (U/ mg). One unit protease activity is expressed as µmol of *p*-nitrophenol released per minute.

***Amylase activity*:** The digestive activity of amylase was determined by Rick and Stegbauer (1974) using a 2 (w / v) ratio of the starch solution as a substrate. The maltose standard curve was drawn to calculate amylase activity that expressed as µmol maltose released from starch per min per mg protein at 37°C.

***Lipase activity*:** Lipase activity was measured using p-nitrophenyl palmitate as a substrate (Mahadik *et al*., 2002). One unit lipase is expressed as the enzyme that is required to catalyze substrate and release 1 µmol of fatty acids per min at 37°C.

***Statistical analysis*:** Finally, one-way and two way analysis of variance were performed on different types of data to test whether the differences between treatments were significant (Steel *et al.*,1996). When significant the differences were observed, Tukey's multi-range test at a $p < 0.05$ was used to compare differences between treatment values (Snedecor and Cochran, 1991). Data from all treatment groups were statistically analyzed using Minitab software, version 8.1.1 (College Park, PA).

RESULTS

***Growth performance*:** The gain in weight (%), FCR, daily feed intake, PER and SGR of the fingerlings fed diets with varying P/E ratios are given in Table 2. The dietary protein, energy levels and their ratios significantly affected the weight gain (%), FCR, feed intake (%/fish/d), and SGR of *L. rohita* fingerling while dietary energy levels non significantly affected the PER of *L. rohita* fingerlings. The higher values of WG, PER and SGR were observed in the group of fish fed D6 (26% protein and 3000 kcal/kg energy) with P / E ratio of 86.67 mg / kcal. The gain in weight and specific growth rate were gradually increased in fish fed D4, D5 and D6 at each energy level. FCR value was lower in fish fed D6. Nevertheless, WG and SGR for the fingerlings fed 24% protein and 2400 kcal/kg energy levels were less than those fed same level of energy at each DP level. However, the interactions of both DP and DE levels did not significantly ($p>0.05$) affect WG, FCR, FI, PER and SGR of *L. rohita* fingerlings. Factorial plots of interactions of WG (%), FCR, SGR, FI and PER with protein and energy levels are shown in Figure 1.

***Whole body composition*:** The significant ($p< 0.05$) differences in crude protein, lipid, and ash contents were observed between dietary treatments. However, no differences were recorded for whole body dry matter and gross energy contents in relation to DP levels (Table 3). Significant ($p< 0.05$) differences were recorded for lipid, and ash with dietary energy levels. Moreover, the interactions of protein and energy did not show any significant differences ($p>0.05$) in dry matter, crude protein, ash and gross energy

Table 2. Growth responses of *L. rohita* fingerlings fed different P/E ratio diets.

Diet (P/E) (mg/kcal)	Weight gain (%)	FCR[1]	FI[2](%/fish/d)	PER[3]	SGR[4]
D1 (24/2400)	230.13±1.72^{c}	2.41±0.19^{a}	4.74±0.06^{a}	1.73±0.27^{bc}	1.363±0.002^{c}
D2(24/2700)	240.71±1.72^{bc}	2.01±0.24^{abc}	4.02±0.49^{abc}	2.10±0.26^{abc}	1.376±0.001^{abc}
D3(24/3000)	240.43±4.72^{bc}	1.80±0.02^{bc}	3.60±0.04^{bc}	2.26±0.03^{ab}	1.379±0.007^{abc}
D4(26/2400)	251.10±2.61^{abc}	1.99±0.06^{abc}	3.92±0.14^{abc}	1.95±0.06^{abc}	1.366±0.003^{bc}
D5(26/2700)	261.80±14.1^{ab}	1.75±0.01^{bc}	3.60±0.08^{bc}	2.20±0.01^{ab}	1.419±0.016^{ab}
D6(26/3000)	273.23±3.53^{a}	1.59±0.05^{c}	3.33±0.11^{c}	2.41±0.07^{a}	1.425±0.006^{a}
D7(28/2400)	257.35±2.71^{ab}	1.87±0.07^{abc}	3.77±0.16^{abc}	1.91±0.07^{abc}	1.379±0.012^{abc}
D8(28/2700)	263.27±1.55^{ab}	1.90±0.06^{abc}	3.82±0.08^{abc}	1.87±0.06^{abc}	1.392±0.012^{abc}
D9(28/3000)	269.52±3.97^{a}	1.91±0.05^{abc}	3.83±0.13^{abc}	1.85±0.25^{bc}	1.374±0.023^{abc}
D10(30/2400)	242.80±5.06^{bc}	2.18±0.04^{ab}	4.37±0.09^{ab}	1.55±0.03^{c}	1.391±0.008^{abc}
D11(30/2700)	230.63±0.75^{c}	2.21±0.05^{ab}	4.39±0.14^{ab}	1.52±0.03^{c}	1.389±0.012^{abc}
D12(30/3000)	243.23±2.58^{bc}	2.26±0.18^{ab}	4.53±0.37^{ab}	1.50±0.11^{c}	1.387±0.003^{abc}
ANOVA (p- value)					
Protein	0.000***	0.000***	0.000***	0.000***	0.012*
Energy	0.014*	0.028*	0.045*	0.052^{NS}	0.032*
P×E[5]	0.216^{NS}	0.060^{NS}	0.068^{NS}	0.144^{NS}	0.065^{NS}
P/E[6]	0.000***	0.001**	0.001**	0.000***	0.000***

Note: Data are means of triplicate, (Mean±SE) Means sharing the same superscripts in column are non-significantly different by Tukey s test. * and NS indicates significant (p < 0.05) and non-significant (p> 0.05).1. Feed conversion ratio; 2. Feed intake; 3. Protein efficiency ratio; 4. Specific growth rate. 5. Interaction of protein and energy; 6. Protein and energy ratio.

Table 3. Body composition of *L. rohita* fed practical diets with various P/E ratios.

Diet (P/E) (mg/kcal)	Dry matter(%)	Crude protein(%)	Lipid(%)	Ash(%)	Energy(kcal/g)
D1 (24/2400)	24.41±1.29^{a}	15.19±0.05^{bc}	3.74±0.05^{cd}	2.61±0.09^{a-d}	3.57±0.06^{b}
D2(24/2700)	24.80±0.88^{a}	15.15±0.35^{bc}	3.94±0.04^{c}	2.82±0.04^{ab}	3.59±0.05^{b}
D3(24/3000)	25.10±0.30^{a}	15.10±0.02^{c}	4.64±0.04^{a}	2.85±0.21^{ab}	3.70±0.08^{ab}
D4(26/2400)	23.73±1.45^{a}	16.27±0.01^{abc}	3.46±0.03^{ef}	2.79±0.16^{abc}	3.66±0.08^{ab}
D5(26/2700)	24.28±0.25^{a}	15.78±0.04^{abc}	3.59±0.01^{de}	2.94±0.02^{ab}	3.71±0.04^{ab}
D6(26/3000)	25.37±0.08^{a}	15.70±0.33^{abc}	4.15±0.03^{b}	3.07±0.02^{a}	3.76±0.07^{ab}
D7(28/2400)	24.19±0.23^{a}	16.48±0.28^{a}	3.23±0.06^{gh}	2.77±0.08^{abc}	3.67±0.06^{ab}
D8(28/2700)	23.22±0.34^{a}	16.24±0.28^{abc}	3.40±0.03^{efg}	2.57±0.13^{bcd}	3.75±0.08^{ab}
D9(28/3000)	21.75±1.15^{a}	15.92±0.30^{abc}	3.92±0.03^{c}	2.57±0.03^{bcd}	3.80±0.07^{ab}
D10(30/2400)	24.73±1.84^{a}	16.88±0.38^{a}	3.12±0.04^{h}	2.33±0.02^{cd}	3.54±0.06^{b}
D11(30/2700)	22.46±0.79^{a}	16.38±0.07^{ab}	3.31±0.03^{fgh}	2.22±0.06^{d}	3.71±0.06^{ab}
D12(30/3000)	21.28±0.47^{a}	16.16±0.20^{abc}	3.67±0.05^{d}	2.15±0.04^{d}	3.98±0.07^{a}
Protein	0.037*	0.000***	0.000***	0.000***	0.117^{NS}
Energy	0.407^{NS}	0.033*	0.000***	0.879^{NS}	0.001**
P× E	0.151^{NS}	0.885^{NS}	0.002**	0.085^{NS}	0.185^{NS}
P/E	0.231^{NS}	0.003**	0.009**	0.000***	0.541^{NS}

Note: Data are means of triplicate, (Mean± SE)Means sharing the same superscripts in column are non-significantly different by Tukey s test.*and NS indicates significant (p< 0.05) and non-significant (p > 0.05).

contents of whole body except lipid. The P/E ratio significantly affected the crude protein, lipid and ash content of body but non-significantly affected the dry matter and gross energy contents of whole body of *L. rohita* fingerling. Factorial plots of interactions of DM, CP, lipid, ash (%) and gross energy (kcal/g) with protein and energy levels are shown in Figure 2.

Digestive enzyme activities: Amylase, lipase and protease activities of the *L. rohita* fingerlings fed diets of varying P/E ratios is shown in Table 4. Amylase, lipase and protease activities were significantly affected by DP level while DE level only affected protease and lipase activities. The interactions of protein and energy were significantly affected the amylase and protease activities. The higher values of proteases and lipase were recorded in *L. rohita* fed D6 diet. However, amylase activity was maximum in fish fed D7 diet. The protease and amylase activities were increased with increased DP level from 24% to 28% at each DE level. The lipase activity was decreased with increasing protein level from 24% to 28% at each DE level. Factorial plots of

Table 4. Digestive enzyme activities performed by *L. rohita* fingerlings fed practical diets with various P/E ratios

Diet (P/E)(mg/kcal)	Amylase(U/mg)	Proteases(U/mg)	Lipase(U/mg)
D1 (24/2400)	2.42 ± 0.20^{de}	0.78 ± 0.01^{d}	0.507 ± 0.012^{ab}
D2(24/2700)	2.68 ± 0.15^{cde}	0.83 ± 0.01^{cd}	0.557 ± 0.052^{a}
D3(24/3000)	2.78 ± 0.05^{bcde}	0.75 ± 0.01^{d}	0.510 ± 0.052^{ab}
D4(26/2400)	2.78 ± 0.11^{bcde}	0.88 ± 0.02^{cd}	0.497 ± 0.057^{ab}
D5(26/2700)	2.89 ± 0.07^{bcd}	0.95 ± 0.02^{bc}	0.557 ± 0.055^{a}
D6(26/3000)	3.43 ± 0.02^{a}	1.16 ± 0.02^{a}	0.567 ± 0.057^{a}
D7(28/2400)	3.51 ± 0.03^{a}	0.95 ± 0.02^{bc}	0.373 ± 0.014^{ab}
D8(28/2700)	3.25 ± 0.08^{ab}	1.05 ± 0.01^{ab}	0.423 ± 0.049^{ab}
D9(28/3000)	2.16 ± 0.08^{abc}	0.75 ± 0.02^{d}	0.373 ± 0.012^{ab}
D10(30/2400)	2.62 ± 0.16^{de}	0.82 ± 0.09^{cd}	0.470 ± 0.052^{ab}
D11(30/2700)	2.54 ± 0.06^{de}	0.83 ± 0.03^{cd}	0.557 ± 0.061^{a}
D12(30/3000)	2.29 ± 0.03^{e}	0.73 ± 0.03^{d}	0.306 ± 0.0467^{b}
ANOVA *(P value)*			
Protein	0.000^{***}	0.000^{***}	0.002^{**}
Energy	0.476^{NS}	0.018^{*}	0.049^{*}
P× E	0.000^{***}	0.000^{***}	0.155^{NS}
P/E	0.001^{**}	0.003^{**}	0.059^{NS}

Note: Data are means of triplicate, (Mean± SE). Means sharing the same superscripts in column are non-significantly different by Tukey's test. * and NS indicates significant ($p < 0.05$) and non-significant ($p > 0.05$).

interactions of amylase, lipase and protease (U/mg) with protein and energy levels are shown in Figure 3.

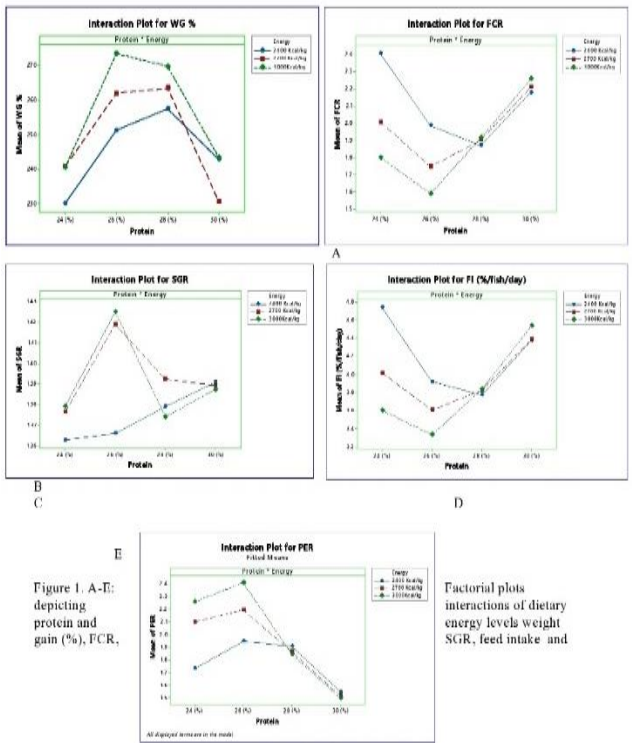

Figure 1. (A-E) Factorial plots depicting interactions of dietary protein and energy level weight gain (%), FCR, SGR, feed intake of *Labeo rohita*.

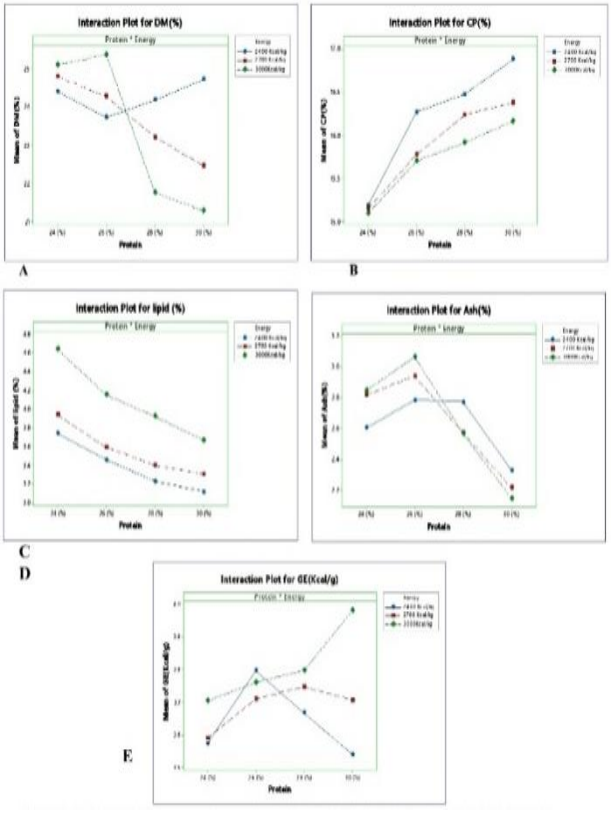

Figure 2. (A-E) Factorial plots depicting interactions of DP and DE levels on whole body dry matter (%), crude protein (%), lipid (%), ash (%) and gross energy (kcal/g) of *Labeo rohita*.

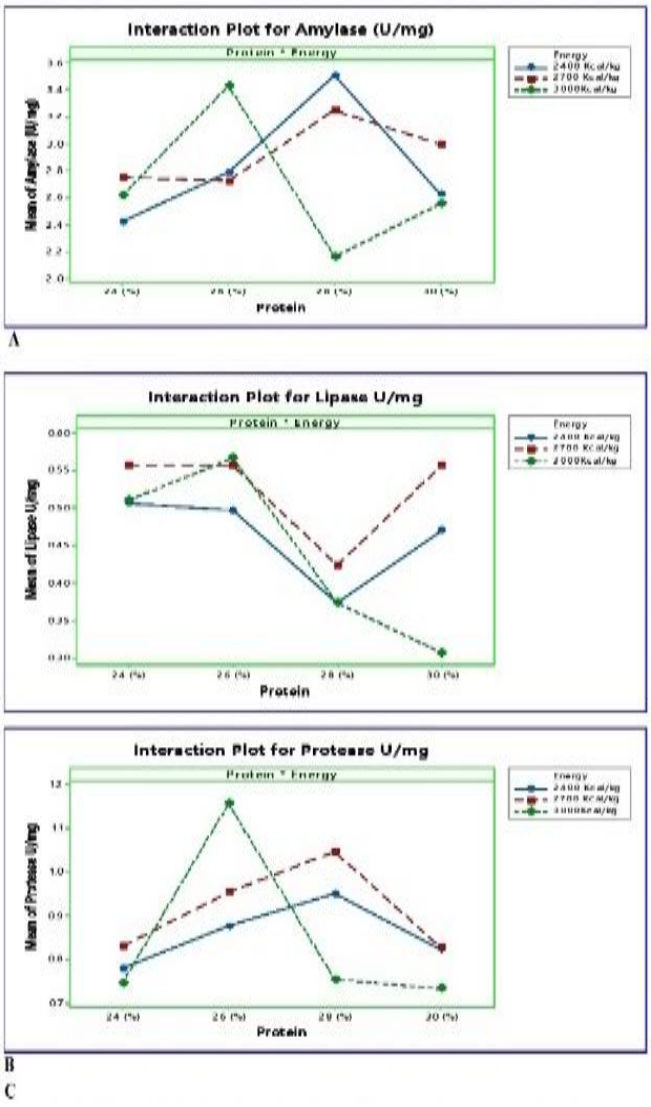

Figure 3. (A-C) Factorial plot depicting interactions of dietary protein and energy levels on amylase, lipase and protease activity (U/mg) of *Labeo rohita*.

DISCUSSION

Labeo rohita fish gained maximum weight when fed diets containing 26% protein and 3000 kcal/kg with P/E ratio 86.67 mg/kcal. The optimization in P/E ratio is generally considered as a means to spare protein thereby increasing growth at reduced cost (Khan *et al.,* 2012). In general, fish fed more dietary protein led to higher growth performance (NRC, 1993). However, the growth rate of fish remains constant or decreases with increasing dietary protein levels because excess protein is metabolized (Hossain *et al.,* 2010). The average gain in weight, feed conversion efficiency, protein efficiency and specific growth rate were increased by increasing protein levels in the diet up to 28%. However, all these growth parameters remained constant at 30% DP level with each DE level. Jauncey(1982) reported that the growth performance in some fishes were reduce when fed diet with excess protein levels. Moreover, higher WG (%) was observed in fish fed high lipid(energy) diets compared to the fish fed a low dietary energy. It seems that the low protein diets were efficiently utilized by *L. rohita* for protein synthesis, which led to high protein retention in the body. FCR in *L. rohita* was efficiently improved by increasing DP levels at each DE level. The feed intake (%/fish/d) ability of the fish was reduced by increasing DP levels at each DE level. The present study thereby agrees with the findings of Kim *et al*. (2016).

The higher values of dry matter and ash were observed in diet-6 (26% protein and 2700 kcal/kg) with 86.67 mg /kcal ratio, whereas the higher content of crude protein was observed in fish fed D10 (30% protein and 2400 kcal/kg) with 125 mg /kcal ratio. The fish fed D3 (24% protein and 3000 kcal/kg) with 80.00 mg / kcal ratio showed higher lipid content. The highest energy content was observed in fish fed on D12 (30% protein and 3000 kcal/kg) with 100.00 mg /kcal ratio.

Proximate analysis of whole body indicated that CP, lipid and ash were significantly improved (p<0.05) while dry matter and gross energy were non-significantly improved by changing P/E ratio. Based on this study, whole body dry matter of fish increased by increasing the DE levels at each DP level. The protein content of fish increased by increasing DP levels at each DE level. The results of this study are in line with the findings of Mohseni *et al.* (2013). In contrast, Schulz *et al.*(2007) observed non-significant effects on fish CP contents by increasing and decreasing protein levels in the diets. Therefore, these findings might be due to the differences in protein sources, dietary protein levels, laboratory animals or environmental situations used in various studies.

The contents of lipids in *L. rohita* fingerlings were increased with increasing DE levels at each DP level. The results of present study are similar to the findings of Li *et al.* (2010) but differ to the results reported for some other aquatic species where high lipid (energy) diets were used (Li *et al.,* 2013). The different observations might be due to the differences in aquatic species and DP and DE levels used in the diets. The fish body ash content was increased by increasing dietary protein levels from 24% to 26% at each DE level and decreased by increasing protein level from 28% to 30% at each dietary energy level. These findings confirm the results of others (Khan *et al.,* 2012; Mohseni *et al.,* 2013; Rivas-Vega *et al.,* 2013). However, body ash content was increased by increasing dietary protein and dietary energy levels (Li *et al.,* 2013; Kim *et al.,* 2016).

Whole body gross energy content was increased by increasing DP levels at each DE level. These findings are agreed with the results of Ali *et al.* (2008), although contradictory finding

have been described by Lemos *et al.* (2014), who observed that body gross energy content was decreased by increasing DP and DE levels. The increase in GE content of fish might be due to increasing level of DE content, with higher dietary lipid subsequently improving energy retention efficiency in fish.

Higher values of protease and lipase were observed in diet having 26% protein and 3000 kcal/kg energy with (P/E) ratio 86.67 mg/kcal except amylase, where the higher values were observed in the diet containing 28% protein and 2400 kcal/kg energy with (P/E) ratio 113.67 mg/kcal. The lower values of protease and lipase were observed in diets containing 30% protein and 3000 kcal/kg energy with (P/E) ratio 100.00 mg /kcal while the lower value of amylase was noted in the diet having 28% and 3000 kcal/kg with (P/E) ratio 93.33 mg / kcal). Thus, amylase (U/mg) and protease (U/mg) were significantly affected by P/E ratios in the diets while lipase activity remained unaffected. In the present study, amylase (U/mg) and protease (U/mg) activities of *L. rohita* were first increased by increasing DP levels from 24% to 28% at each DE level and then decreased in fish fed diet with 30% DP level at each DE level. The amylase (U/mg) and protease activities were increased by increasing DE level at each DP level. These observations are thus in line with the results of others (Khan *et al.*, 2012; Rivas-Vega *et al.*, 2013). The increasing activity of protease might be due to increasing DP levels used as a substrate for protease activity. Lipase activity was first decreased and then increased by increasing dietary protein levels at each dietary energy level. At each protein level, there was no clear pattern of lipase activity as the level of DE increased. This is similar to the observations of Dong *et al.,* (2007), but contrasts to the report of Mohanta *et al.,* (2007). This variability may be due to difference in the experimental species, dietary protein sources and rearing condition. The lipase activity of *L. rohita* species is significantly improved by dietary energy levels. When the energy of the diet increases, the digestive enzyme activity of the lipase increases first and then decreases. These results are agree with the findings of Xiang *et al.*(2008). Thus, overall, digestive enzyme activities in *L. rohita* fingerlings were significantly affected by increasing and decreasing levels of protein and energy / protein and energy ratios in experimental diets.

***Conclusion*:** We concluded that the growth performance of *Labeo rohita* fingerlings is significantly affected by changing protein and energy ratios in the diets. Digestive enzyme activities of *L. rohita* were significantly improved by increasing and decreasing protein energy levels and their ratios in experimental diets. Meanwhile, fish protein, lipid and ash contents were significantly affected whereas DM and GE contents of *L. rohita* were not affected by changing dietary P/E ratios. The best growth performance of *L. rohita* was observed when fed diet with 26% protein level and 3000 kcal/kg energy of P / E ratio 86.67 mg/kcal energy.

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