Original Research Article

Effect of protein diet on growth performance and body composition of *Labeorohita* reared inglass tank

Abstract

Fish is used as a great source of food and the quality of its meat can be indicated by fat and protein contents as well as low quantity of water in fish meat. Therefore, the present study was performed for improvement in weight, feed-conversion ratio, protein-efficiency ratio, and growth. The experiment was performed at commercial level in field (pond) andthe influence of fish meal protein on growth of *Labeorohita* was investigated in 60 days culture trials. 90 fingerlings(Size: 4.97 to 6.04; Weight: 4.15 to 4.20)were maintained in well aerated 280 L three glass tanks in triplicate. These glass tanks were named as T1, T2 and T3. T1 was consisted of 30% crude protein with control dietcontaining proteins, fats vitamins and fibres, T2 consistedof 35% protein with control diet,while T3 consisted of only control diet (rice polish). The fish were fed 4% percent of their body weight twice a day at twelve hours interval for 60days. There was a significant increase ($p<0.05$) in body weight, feed-conversion ratio, protein-efficiency ratio, and gross fish production of fish having 35% fish meal protein with control diet (T2) when compared with 30% fish meal protein with control diet T1 and control diet T3. These findings suggest that 35% fishmeal protein appear to be sufficient for obtaining optimum growth in fish.

Keywords: fish meal protein,fingerlings, protein-efficiency ratio, growth performance,

Labeo rohita

1. Introduction

Aquaculture conditions are very important for fish production.Due to the increasedfish demand, it is also cultured in farms or ponds. However, farmers face difficulty in producing large amount of fish because they lack information about the optimum physicochemical conditions of the pond. There are various kinds of fish worldwidethat are used for different purposes like sport fishing,ornamental purpose,*etc*. [1]. Fish is used as food worldwide because it contains large amount of protein while the amount of carbohydrates is very low.The quality of fish meat can be indicated by water proportion, fat and protein contents present in it. The quality of fish meat will be good if low quantity of water is present in fish meat. The amount of water present in fish body is 75 to 80 percent. Protein percentage in fish body is 25 to 30% and the lipid percentage is 2 to 12% [2].

Due to the presence of important nutrients; fish can fulfill the dietary requirements of human beings.Large amount of calcium is present in skeleton of fishand is very beneficial for human health. Smaller fish are the great source of calcium for human[3].Fish is also a rich source of vitamin A and D. The absence of vitamin D in old age people and children can be accomplished by using fish as a source of vitamin D.In diet of Japanese people, fish is present in great amountbecausefish meat prevents the deficiency of vitamin D[4].

In order to fulfill the increasing demand of fish, its production has to be increased. For this purpose, protein food is very important for maximum production of fish. One of the previous studies reportedthe effect of four different kinds of feed ingredient on growth of *Labeo rohita*. At the rate of 4% of body weight,fish fingerlingsof *Labeorohita*were provided with 30%, 35%, 40% and 45% of feed containingground nut cake, azolla, rice ban and tapioca powder[5]. The growth and digestive enzyme activities of fingerlings of *Labeorohita* can be controlled by different enzymes such asphytase, plant proteinsand cellulose.For protein

utilization, the phytase and cellulose are two most important enzymeswhileamylase, protease, and lipase are important for proper functioning of fish[6].

The acceptance for feed in fish increased due to plant protein mixture. Plant protein mixture is very important for fish health and growth[7].Nutritional composition, biological availability, energy content and digestibility of feedstuff ingredients are important criteria for inclusion of any plant or animal protein ingredient to formulate practical diets for fish[8].Different plants ingredients are beingused for the survival, feed-conversion ratio, and growthof *Labeorohita*.From plants, corn gluten meal, sunflower meal, canola meal, and rapeseed meal can be obtained to be used as fish feed. Corn is present in all plant foods. It is rich source of complex carbohydrate, which consists of simple sugars starch, and carbohydrates. Large amount of fats and carbohydrates decrease protein production. *Labeo rohita*has ability to use 43% carbohydrates in diets. This carbohydrate has no bad effects on fish health.They play important role in growth of fish and with these ingredients the length and weight of fish can also be increased[9]. These plant-based feed minimize the cost of fish and improve the growth and meat composition of fish. Feed represent 40 -50% of production costs so nutrition is very important in fish farming[10].

Anotherstudy reported thatthe consequence of plant protein, on fingerlings of *Labeorohita*.In this study, asparagus,eichhornia, gliricidia,guar-gum binder, mineral-vitamin mixture, rice bran, groundnut-oilcake and fishmealwereused as plant protein mixturein different concentrations (20, 30, 40, 50, 60 and 70 %)for 120 days. The highest growth performance was recorded in 50% diet as compared to all other diets. This experiment showed the importance of plant protein mixture on the growth and proteincontent of fish[11].Based on this, the present study is aimed to investigate the growth performance and body composition of *Labeo rohita* using various protein levels for economical production of fish.

2. Materials and Methods

2.1.Site Selection

This research was performedfor sixty daysto investigate different kinds of proteins on fingerlings of *Labeorohita*.Ninety(90) fingerlings of *Labeo rohita*withdifferent body size(4.15 to 4.20 g)were grown under extensive culture. These specimens were taken from Multan hatchery and were transported into the research Laboratory of Fisheries at Bahauddin Zakariya University (BZU), Multan, Pakistanin a plastic container.

2.2.Experimental Design

Three water tanks (T1, T2, and T3)containingthirty (30) specimen of *Labeo rohita*ineach tank were used in this study.Before the start of experiment, the fingerlings wereacclimatized by keeping them under controlled conditions for two weeksand were fed with control diet(rice polish) on erected iron stand fit glass aquaria (24 x 24 x 30 inches) which contain 280 liter water andthe water flow was 300 ml /min in all aquariums.The individual weights of fingerlings(4.15 to 4.20 g)of *Labeorohita* was determined initially and after two weeks, weight gain by fingerlings of *Labeo rohita*(4.15 to 4.20 g) was also determined to check that these fingerlings are acclimatizing the experimental conditions or not. After that experiment was conductedforsixty days (01-August to 30-September) in which variable ratio of proteins and control diet (3.7 Kcal/g of metabolizable energy, 3.4% fibre and 7.03% fat as well as vitamins and minerals in the form of dry pellets) was used. At the rate of 4% body weight, fingerlings were fed with three different levels(0, 30 and 35%)of crude protein (CP). The amounts of diets were adjusted according to individual weight of fingerlings of *Labeo rohita*. Feed sizes were 2nm because the mouth of fingerlings can swallow only the pellet of 2nm. The ratios of three different feeds were consisted of T1 (feed with CP 30% +

Control Diet), T2 (feed with CP 35%+ Control Diet) and T3 (feed with 100% control diet; rice polish). The weight of fish was determined with respect to different level of monthly interval.

2.3.Analysis

2.3.1 Water parameters

By using digital pH meter (Lutron PH-223 PEN), water pH was calculated and by means of a glass thermometer water temperature was recorded. Oxygen was continuously supplied in each glass aquaria and was measured usingHanna-HI98193 Portable Metre.

2.3.2Growth performance

Weight gain, % weight gain, length gain, feed-conversion ratio (FCR) and protein efficiency rate (PER)was recorded at the end of feeding trialusing the following formulas[12]. Survival rate and specific growth rate (SGR)of fingerlings was also determined at the end of feeding trial[13].

Weight gain = final weight –initial weight

Length gain = final length –initial length

FCR = Total dry feed fed /total wet weight gain

PER = weight gain / protein intake

2.3.3 Proximate composition of fish meat

The proximate composition of fish body was calculated using the methods described in Associationof OfficialAnalyticalChemists[14]. For water percentage, the initial weight for moisture substance determination was taken from fish body and then was placed in oven at about 105°C for 10 hours,until constant weight were reached. The sample was minced in grinder and moisture content was determined. For dry weight calculation, about 2g sample from fish body was taken. The sample was dried and placed in oven at 90 to 101 °C for 1

to 2 hours. The powderof dry material was obtained in an electric temperature sensitive digital Moulinex (Ultrablend LM962B) blender at about 60 to 65°C. The residue substance in fishbody was determined by taking 2g sample. In muffle furnace, the sample was heated at about 300 to 1100 °C,then for 6 hours cooling process was occurred at 5 °C. White substance or ash was obtained and through vacuum distillation organic substances from fish body were measured. Two gram sample from fish body was reacted with strong acid like HCl almost 2ml at high temperature 300 to 400 °C. Then this material was taken in flask at 40 °C and 50ml of distilled waterwas added. In Protein content analysis by kjeldahlmethod the amount of protein was determined. Both wet and dry samples were processed for protein content analysis. To release nitrogen, meatwas digested with strong acid which was determined by a titration technique. From the nitrogen concentration of the fish meat amount of protein was calculated. For conversion of nitrogen content to protein content a conversion factor of 6.25(equivalent to 0.16g nitrogen per gram of protein) was used. Fat was determined by using Soxhlet apparatus. For estimation of fat content, the dried sample left after moisture determinations were finely grinded and, the fat was extracted. After extraction, the solvent was evaporated and extracted material wasweighed.

2.4.Statistical Tool and Data Analysis

Data on specific growth rate, percentage survival and feed-conversion ratio of*Labeorohita*given with different diets were subjected to One Way Analysis of Variance (ANOVA) to determine the significant differences among the treatment means (5 % level of significance). The Intelbased Statistical Package for the Social Science (IBMSPSS) Version 20 Programmewas used in the statistical analysis. Correlation analysis was used to determine the relationship of temperature with the growth and survival of the experimental stocks.

3. Results

3.1 Water quality

During the experiment, water temperature varied from 20 to 25 °C, while water pH was 7.4 to 7.6. The concentration of oxygen was measured using portable oxygen detector and its concentration remained above 8.2 to 8.6 mg/l in each tank.

3.2 Survival

The survival of fingerlings varied among different treatments. 100% survival was observed in T2, 97% in T1 while T3 showed 90% survival rate (Table 1). The experimental fingerlings were highly adaptable to the aquaria they were placed in and the fingerlings remained active from the start to the end of the experiment.

3.3 Growth performance

The initial weight of fish fingerlings was 4.18g ± 0.056 (T1), 4.20g ± 0.071 (T2) and 4.15g ± 0.088 (T3). Despite of the little variation in initial weights among different treatments, the fish fingerlings fed with protein diet showed significant increase in final weight, weight gain and % weight gain. Maximum increase in weight (g) was observed in T2 treatment (9.16g), followed by T1 (9.08g) and T3 (8.81) treatments (Table 1). Similar trend was observed for increasein length. Initial length of fish fingerlings for all the three treatments were different from each other as shown in the Table 1. Maximum increase in length (inches) was observed in T2 treatment (12.12), followed by T1 (10.38) and T3 (9.26) treatments (Table 1).Thus, the maximum weight gain (4.96g), % weight gain (117.90%) and length gain (7.15inches) was shown by 35% protein diet treatment (Figure 1). The values of SGR were maximum in T1 (1.291) and T2 (1.296) treatments as compared to T1 (1.142) treatment. However, the value of condition factor was significantly increased in T2 (0.585) treatment as compared to T1 (0.577) and T3 (0.545) treatments (Table 1).

Table 1 Growth parameters of fish fingerlings fed with varying concentrations of protein diet

Parameters	Fish meal 30% (T1)	Fish meal 35% (T2)	Control group (T3)
Initial weight (g)	$4.18^{a} \pm 0.071$	$4.20^{a} \pm 0.056$	$4.15^{a} \pm 0.068$
Final weight (g)	$9.08^{a} \pm 0.069$	$9.16^{a} \pm 0.091$	$8.81^{b} \pm 0.14$
Weight gain (g)	$4.90^{a} \pm 0.094$	$4.96^{a} \pm 0.048$	$4.66^{b} \pm 0.039$
% Weight gain	$117.20^{a} \pm 7.06$	$117.90^{a} \pm 2.17$	$112.08^{b} \pm 2.69$
Initial body length (inches)	$5.28^{ab} \pm 0.94$	$4.97^{b} \pm 0.35$	$6.04^{a} \pm 0.28$
Final body length (inches)	$10.38^{ab} \pm 1.66$	$12.12^{a} \pm 1.78$	$9.26^{b} \pm 0.96$
Length gain (inches)	$5.10^{b} \pm 0.74$	$7.15^{a} \pm 1.36$	$3.22^{c} \pm 0.72$
Specific growth rate	$1.291^{a} \pm 0.160$	$1.296^{a} \pm 0.129$	$1.142^{b} \pm 0.069$
Condition factor	$0.577^{a} \pm 0.029$	$0.585^{a} \pm 0.008$	$0.545^{b} \pm 0.011$
PER	$1.47^{a} \pm 0.034$	$1.54^{a} \pm 0.026$	$1.26^{b} \pm 0.0181$
FCR	$1.63^{b} \pm 0.045$	$1.31^{c} \pm 0.053$	$2.06^{a} \pm 0.039$

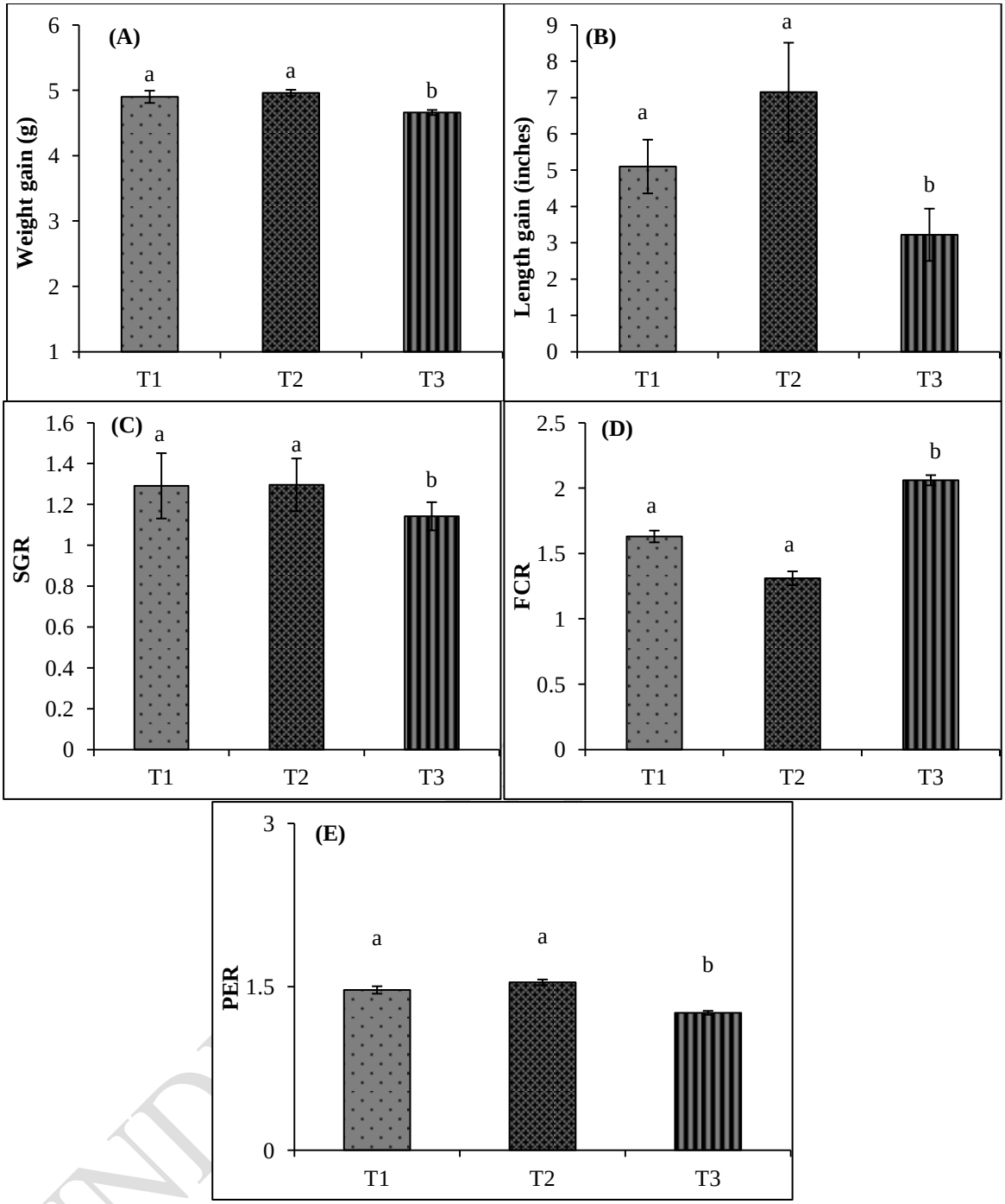

Figure 1 Growth performance of Labeo rohita fish fingerlings fed with varying levels of proteins (A) Weight gain; (B) Length gain; (C) SGR; (D) FCR; and (E) PER. Values are mean ± Standard deviation.

3.4 Feed utilization

The value of feed-conversion ratio was significantly different among different treatments of *Labeo rohita*(Table 1, Figure 2). The feed utilization significantly increased with increasing protein levels, suggesting the maximum FCR of 1.31 with 35% protein diet while the

minimum was observed with 2.06 for control diet treatment. However, the value of protein-efficiency ratio significantly increased as the level of protein increases. Maximum ratio was observed in T2(1.54) treatment while minimum was observed in T3 (1.26) treatment (Table 1, Figure 2).

3.5 Proximate body composition

Results of the various body composition parameters among the three treatments following ANOVA are shown in Table 2 and Figure 2A to 2E.The mean percentage of water content in T1,T2, and T3 were 80.099, 78.380, and 82.470, respectively. As shown in Figure 2A and Table 2, the percentage of water content in T1treatment was greater as compared to protein diet treatments (T1 and T2).However, the minimum watercontent was found in 35% fishmeal treatment (T2).There was a statistically noteworthy distinction $p<0.05$ of control diet (T3) when compared to T1 and T2.The percentage of ash dry weight in T1,T2 and T3 treatments were 14.630, 17.469, and 11.481, respectively. All these value were different from each other. So, there was a significant difference $p<0.05$ present among these entire three treatments (Table 1 and Figure2B).Mean values of dry organic content observed in T1, T2 and T3 treatments was 85.37, 82.530, and 88.518, respectively. All these values are different from each other. So therea significant difference $p < 0.05$ was present among all these three treatments (Table 1 and Figure 2C). The maximum organic content was found in control group (T1) while the minimum was found in T2 treatment.

Table 2 Proximate body compositionanalysis of fish fingerlings fed with varying concentrations of protein diet

Sr. No	Parameter	Fish meal 30% (T1)	Fish meal 35% (T2)	Control group (T3)
1	Water percentage	$80.099^{b}\pm0.705$	$78.380^{c}\pm1.075$	$82.470^{a}\pm0.521$

2	Ash dry weight	$14.630^{b}\pm1.582$	$17.469^{a}\pm1.779$	$11.481^{c}\pm1.235$
3	Ash wet weight	$2.727^{b}\pm0.372$	$3.119^{a}\pm0.212$	$1.125^{c}\pm0.787$
4	Dry organic content	$85.370^{b}\pm1.582$	$82.530^{c}\pm1.779$	$88.518^{a}\pm1.235$
5	Wet organic content	$17.834^{b}\pm0.712$	$14.570^{c}\pm1.077$	$18.495^{a}\pm0.525$
6	Fat dry weight	$45.061^{b}\pm1.391$	$41.543^{c}\pm1.285$	$55.061^{a}\pm2.730$
7	Fat wet weight	$7.068^{b}\pm0.870$	$5.770^{c}\pm0.036$	$8.354^{a}\pm0.661$
8	Protein dry weight	$54.569^{b}\pm1.395$	$58.187^{a}\pm1.296$	$50.750^{c}\pm2.779$
9	Protein wet weight	$10.765^{b}\pm0.817$	$12.799^{a}\pm1.554$	$8.140^{c}\pm0.462$

The percentage of fat dry weight content observed in T1, T2 and T3 were 45.061, 41.543 and 55.061, respectively. All the three treatments showed significant difference(Table 1 and Figure 2D). The highest protein diet showed minimum fat accumulation in the fish fingerlings. However, the mean percentage of protein content(dry weight)observed in T1, T2 and T3 was 54.569, 58.187 and 50.750, respectively. As represented in Figure 2E values of the T2 treatment (fed on fish meal 35%) were comparatively higher. Moreover, results of ANOVA showed significant difference ($p<0.05$) among the all the three treatments.

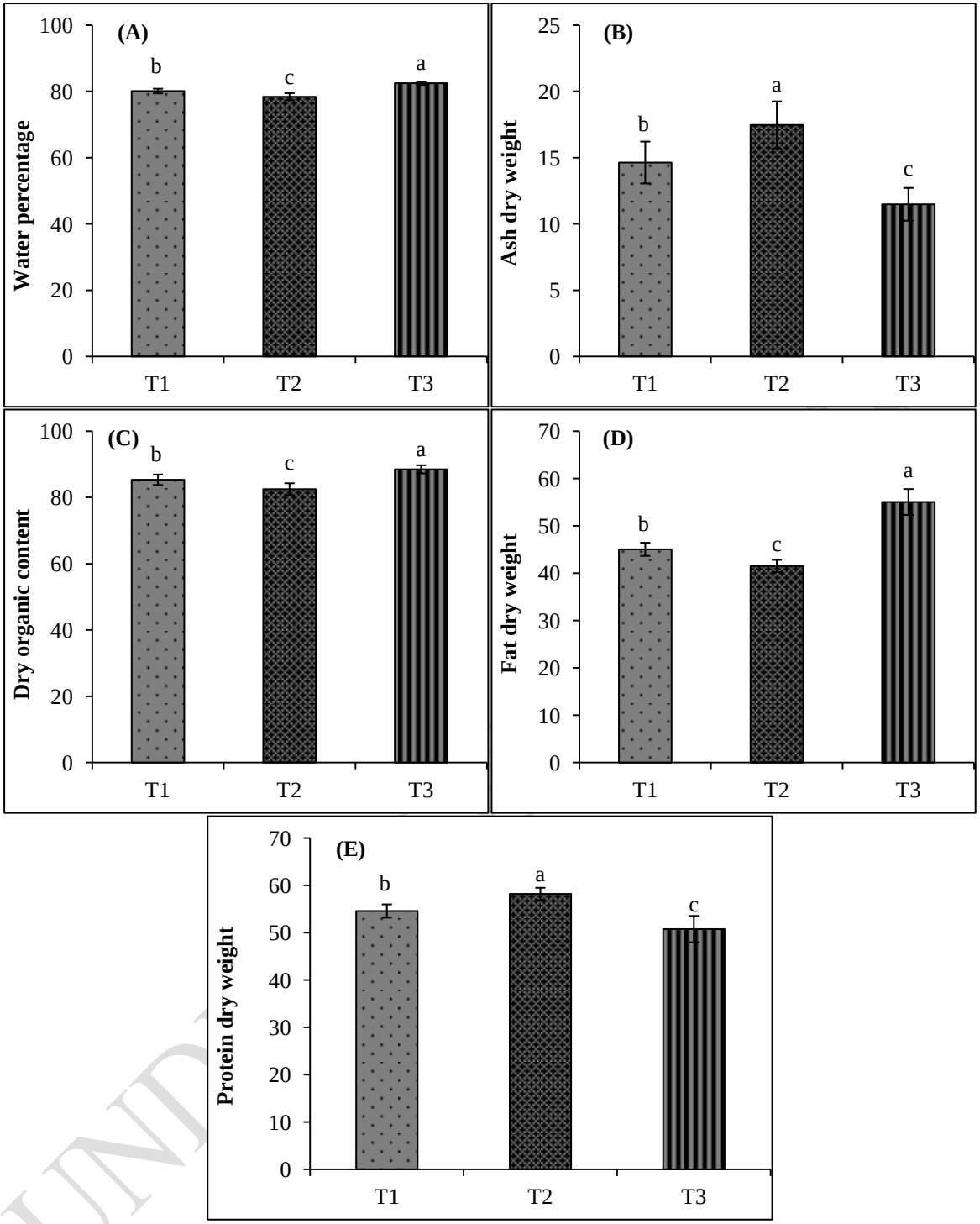

Figure 2 Proximate body composition of *Labeorohita* fish fingerlings fed with varying levels of proteins (A) Water percentage; (B) Ash dry weight; (C) Dry organic content; (D) Fat dry weight; and (E) Protein dry weight. Values are mean ± Standard deviation.

4. Discussion

Fishesare one of the important sources forincreasing the economy of Pakistan. Fisheries

department at various research and development organizations are actively contributing in developing high meat yielding varieties of fish. A large number of fish are exported from Pakistan to various continents of the world giving a huge boost to the GDP of Pakistan [15]. The main focus of scientists and researchers is to produce such varieties of fish that show maximum growth in limited time with cost-effective plant-based feed and can fulfill the food demands ofrapidly growing population[16].

Labeo rohita is one of the major carp found in Asian countries,therefore efforts are being madeto increase its quality through good nourishment and proper feed. With optimum environment and protein diet higher yield of fish meat in term of growth can be obtained[17]. Fish growth can be measured asthe increase in fish weight and fish length[18]. For this purpose, the physico-chemical parameters liketemperature, dissolved oxygen, and pHof water in tanks must be optimum for proper growth of fish fingerlings [19]. In the present study,water quality parameters were also measured and maintained to assure proper conditions for the growth of *Labeo rohita* fingerlings as suggested in the previous studies [20].

Moreover, studies also suggested that a significant aspect in production of aquafeed is the replacement of animal-based protein with cheap and readily available plant proteinin the diet of fish. Plant-based protein with an appropriate basic amino acid profile could be included for proper fish nutrition[21]. Therefore, the present study was conducted to investigate the growth performance and body composition of *Labeo rohita* fish fingerlings using varying levels of protein diet. The results of the present study showed that fish fingerlings fed with 35% crude protein showed significant increase in their weight, size, and specific growth rate. Previous studies also showed that the growth characteristics of fingerlings of *Labeo rohita* fed with various levels of crude protein were found to be increased significantly as the dietary protein levels were increased [22, 23].Moreover, the FCR and PER were increased as the

protein level increased which is similar to previous studies conducted on various fish including *L. rohita*[24].

Studies suggest that the proximate body composition of fish contributes in the evaluation of the feeding and physiological conditions of *L. rohita* fingerlings[25]. The results of proximate body composition obtained on water percentage, ash, organic, fat, and protein content, in thisstudyshowed approximately similar results as reported by different studies for *L. rohita*as well as for other species of fish [26, 27].

The information about these parameters should be considered beforeits use for human consumption[28].In this study, water percentage and fat content was higher in control group as compared to protein fed treatmentswhile the protein and ash content was significantly higher in 35% crude protein diet as compared to other two treatments.Theresults obtained are similar with the result of previous reports[29, 30].Furthermore, the dry organic content was higher in control group as compared to protein fed diets which is similar to previous studies[31, 32].

Similar explanations are reported in different studies using various species of fish such as *Catlacatla* (Ham.) and catfish which showed highest weight gain, significant growth ratio, feed conversion ratio, and protein efficiency ratioat highest protein diet[33, 34, 35].Since the presentresearch was a controlled study with fixed time duration so we need to interpret our results in a larger context and see if a more prolonged study would have shown different results.

Conclusion

The results of present study showed that plant protein an also be a possible replacement in diets of various species of fish. The highest protein concentration (35%) showed maximum growth performance and proximate body composition without any side effects.This showed that 35% dietary protein level is suitable and appropraite for meeting the body requirement of

fish. However, for determining the optimum level more investigations are needed.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

1. Shah SZH, Afzal M, Akmal A, Fatima M, Hussain SM. Effect of citric acid and phytase on growth performance and mineralization of *Labeo rohita* juveniles fed soybean meal based diet. Int J Agric Biol. 2016;18:111-6.

2. Fatma Abidi S, Khan MA. Dietary tryptophan requirement of fingerling rohu, *Labeo rohita* (Hamilton), based on growth and body composition. J World Aquac Soc. 2010;41(5):700-9.

3. Malik A, Kalhoro H, Shah SA, Kalhoro I. The effect of different stocking densities on growth, production and survival rate of pangas (*Pangasius hypophthalmus*) fish in cemented tanks at fish hatchery Chilya Thatta, Sindh-Pakistan. Int J Interdiscip Multidiscip Stud. 2014;1(10):129-36.

4. Lupatsch I, Kissil GW, Sklan D, Pfeffer E. Effects of varying dietary protein and energy supply on growth, body composition and protein utilization in gilthead seabream (*Sparus aurata* L.). Aquac Nutr. 2001;7(2):71-80.

5. Khan MA, Abidi S. Total aromatic amino acid requirement of Indian major carp *Labeo rohita* (Hamilton) fry. Aquac. 2007;267(1-4):111-8.

6. Stoner A. Effects of environmental variables on fish feeding ecology: implications for

the performance of baited fishing gear and stock assessment. J Fish Biol. 2004;65(6):1445-71.

7. Musharraf M, Khan MA. Dietary magnesium requirement for fingerlings of Rohu (*Labeo rohita*). Aquac. 2018;496:96-104.

8. Giri SS, Sen SS, Chi C, Kim HJ, Yun S, Park SC, et al. Effect of dietary leucine on the growth parameters and expression of antioxidant, immune, and inflammatory genes in the head kidney of *Labeorohita* fingerlings. Vet Immunol Immunopatho. 2015;167(1-2):36-43.

9. Zhao J, Liu Y, Jiang J, Wu P, Chen G, Jiang W, et al. Effects of dietary isoleucine on growth, the digestion and absorption capacity and gene expression in hepatopancreas and intestine of juvenile Jian carp (*Cyprinus carpio* var. *Jian*). Aquac. 2012;368:117-28.

10. Hardy RW. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquac Res. 2010;41(5):770-6.

11. Manjare S. Effect of plant protein mixture as fish meal on the growth performance of *Labeo rohita*. IJARIIE. 2016;1726:557-60.

12. Le Cren ED. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Percafluviatilis*). J Ani Ecol. 1951:201-19.

13. Parvez M, Sarker M, Azad M, Salequzzaman M. Effect on water quality, pond productivity and growth of carps in polyculture system by using homestead organic wastage as pond manure. Int J Sustain Agric Res. 2006;2(2):45-9.

14. Cunniff P. Association of Official Analytical Chemists. 1995.

15. Iqbal KJ, Ashraf M, Qureshi NA, Javid A, Abbas F, Hafeez-ur-Rehman M, et al. Optimizing growth potential of *Labeo rohita* fingerlings fed on different plant origin feeds. Pak J Zool. 2015;47(1).

16. Khati A, Danish M, Mehta K. Estimation of growth parameters in fingerlings of *Labeo rohita* (Hamilton, 1822) fed with exogenous nutrizyme in Tarai region of Uttarakhand,

India. Afr J Agric Res. 2015;10(30):3000-7.

17. Umer K, Ali M. Replacement of fishmeal with blend of canola meal and corn gluten meal, and an attempt to find alternate source of milk fat for rohu (*Labeorohita*). Pak J Zool. 2009;41(6).

18. Mishra K, Patra S, Samantaray K. Effect of algae based diets on growth performance, body composition and fatty acid profile of indian major carp, Rohu (*Labeo Rohita*, Hamilton). J Fish Sci. 2017;11(3):5.

19. Vani T, Saharan N, Roy S, Ranjan R, Pal A, Siddaiah G, et al. Alteration in haematological and biochemical parameters of *Catla catla* exposed to sub-lethal concentration of cypermethrin. Fish Physiol Biochem. 2012;38(6):1577-84.

20. Giri SS, Jun JW, Sukumaran V, Park SC. Evaluation of dietary *Hybanthusenneaspermus* (Linn F. Muell.) as a growth and haemato-immunological modulator in *Labeorohita*. Fish Shellfish Immunol. 2017;68:310-7.

21. Cho C, Bureau D. A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. Aquac Res. 2001;32:349-60.

22. Debnath D, Pal A, Sahu N, Yengkokpam S, Baruah K, Choudhury D, et al. Digestive enzymes and metabolic profile of *Labeorohita* fingerlings fed diets with different crude protein levels. Comp Biochem Physiol B: Biochem Mol Biol. 2007;146(1):107-14.

23. Abid M, Ahmed M. Growth response of *Labeorohita* fingerlings fed with different feeding regimes under intensive rearing. J Anim Pl Sci. 2009;19:45-9.

24. Lee SM, Kim KD. Effects of dietary protein and energy levels on the growth, protein utilization and body composition of juvenile masu salmon (*Oncorhynchusmasou* Brevoort). Aquac Res. 2001;32:39-45.

25. Shekhar C. Changes in muscle biochemical composition of *Labeorohita* (Ham.) in relation to season. Ind J Fish. 2004;51(3):319-23.

26. Jim F, Garamumhango P, Musara C. Comparative analysis of nutritional value of *Oreochromisniloticus* (Linnaeus), Nile tilapia, meat from three different ecosystems. J Food Qual. 2017;2017.

27. Ishtiaq A, Naeem M. Effect of dietary protein levels on body composition of *Catlacatla* from Pakistan. Sindh Univ Res J. 2019;51(2):309-18.

28. Rahman MM, Hossain MY, Jo Q, Kim S-K, Ohtomi J, Meyer C. Ontogenetic shift in dietary preference and low dietary overlap in rohu (*Labeorohita*) and common carp (*Cyprinuscarpio*) in semi-intensive polyculture ponds. Ichthyol Res. 2009;56(1):28-36.

29. Satpathy B, Ray A. Effect of dietary protein and carbohydrate levels on growth, nutrient utilization and body composition in fingerling rohu, *Labeorohita* (Hamilton). J Appl Ichthyol. 2009;25(6):728-33.

30. Banerjee S, Mitra T, Purohit GK, Mohanty S, Mohanty BP. Immunomodulatory effect of arsenic on cytokine and HSP gene expression in *Labeorohita* fingerlings. Fish shellfish Immunol. 2015;44(1):43-9.

31. Misra C, Das B, Mukherjee S, Pradhan J. Effects of dietary vitamin C on immunity, growth and survival of Indian major carp *Labeorohita*, fingerlings. Aquac Nutr. 2007;13(1):35-44.

32. Jorgensen C, Enberg K, Dunlop ES, Arlinghaus R, Boukal DS, Brander K, et al. Ecology-Managing evolving fish stocks. Sci. 2007;318(5854):1247-8.

33. Rahman M, Verdegem M, Nagelkerke L, Wahab M, Milstein A, Verreth J. Growth, production and food preference of rohu *Labeorohita* (H.) in monoculture and in polyculture with common carp *Cyprinuscarpio* (L.) under fed and non-fed ponds. Aquac. 2006;257(1-4):359-72.

34. Kumar N, Jadhao S, Chandan N, Kumar K, Jha A, Bhushan S, et al. Dietary choline, betaine and lecithin mitigates endosulfan-induced stress in *Labeorohita* fingerlings. Fish

Physiol Biochem. 2012;38(4):989-1000.

35. Hussain S, Afzal M, Salim M, Javid A, Khichi T, Hussain M, et al. Apparent digestibility of fish meal, blood meal and meat meal for *Labeorohita* fingerlings. JAni Plant Sci. 2011;21(2):807-11.