Effect of Protein Diet on Growth Performance and Body Composition of *Labeo rohita* Reared in Glass Tank

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Authors' contributions

This work was carried out in collaboration among all authors. Author FA did acquisition of data, and analysis; author TN did acquisition of data and drafting the MS; authors SBH and MA did revision and proofreading; authors MMQ did interpretation of data; and author GS did conception and design of MS. All authors read and approved the final manuscript.

Original Research Article

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) and the influence of fish meal protein on growth of *Labeo rohita* 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

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control diet containing proteins, fats vitamins and fibres, T2 consisted of 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 60 days. 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.

1. INTRODUCTION

Aquaculture conditions are very important for fish production. Due to the increased fish 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 worldwide that 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 fish and 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 amount because fish 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 reported the effect of four different kinds of feed ingredient on growth of *Labeo rohita*. At the rate of 4% of body weight, fish fingerlings of *Labeo rohita* were provided with 30%, 35%, 40% and 45% of feed containing ground nut cake, azolla, rice ban and tapioca powder [5]. The growth and digestive enzyme activities of fingerlings of *Labeo rohita* can be controlled by different enzymes such as phytase, plant proteins and cellulose. For protein utilization, the phytase and cellulose are two most important enzymes while amylase, 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 being used for the survival, feed-conversion ratio, and growth of *Labeo rohita*. 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].

Another study reported that the consequence of plant protein, on fingerlings of *Labeo rohita*. In this study, asparagus, eichhornia, gliricidia, guar-gum binder, mineral-vitamin mixture, rice bran, groundnut-oilcake and fishmeal were used as plant protein mixture in 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

protein content 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 performed for sixty days to investigate different kinds of proteins on fingerlings of *Labeo rohita*. Ninety (90) fingerlings of *Labeo rohita* with different 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 Laboratory of Fisheries at Bahauddin Zakariya University (BZU), Multan, Pakistan in a plastic container.

2.2 Experimental Design

Three water tanks (T1, T2, and T3) containing thirty (30) specimen of *Labeo rohita* in each tank were used in this study. Before the start of experiment, the fingerlings were acclimatized by keeping them under controlled conditions for two weeks and 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 and the water flow was 300 ml /min in all aquariums. The individual weights of fingerlings (4.15 to 4.20 g) of *Labeo rohita* 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 conducted for sixty 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 diet 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 using Hanna-HI98193 Portable Metre.

2.3.2 Growth performance

Weight gain, % weight gain, length gain, feed-conversion ratio (FCR) and protein efficiency rate (PER) was recorded at the end of feeding trial using 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 Association of Official Analytical Chemists [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 powder of dry material was obtained in an electric temperature sensitive digital Moulinex (Ultrablend LM962B) blender at about 60 to 65°C. The residue substance in fish body 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 water was added. By kjeldahl method the amount of protein was determined. Both wet and

dry samples were processed for protein content analysis. To release nitrogen, meat was 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 was weighed.

2.4 Statistical Tool and Data Analysis

Data on specific growth rate, percentage survival and feed-conversion ratio of *Labeo rohita* 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 Intel based Statistical Package for the Social Science (IBMSPSS) Version 20 Programme was 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 increase in 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 (Fig. 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] ± 0.071	4.20[a] ± 0.056	4.15[a] ± 0.068
Final weight (g)	9.08[a] ± 0.069	9.16[a] ± 0.091	8.81[b] ± 0.14
Weight gain (g)	4.90[a] ± 0.094	4.96[a] ± 0.048	4.66[b] ± 0.039
% Weight gain	117.20[a]±7.06	117.90[a]±2.17	112.08[b]±2.69
Initial body length (inches)	5.28[ab] ± 0.94	4.97[b] ± 0.35	6.04[a] ± 0.28
Final body length (inches)	10.38[ab] ± 1.66	12.12[a] ± 1.78	9.26[b] ± 0.96
Length gain (inches)	5.10[b] ± 0.74	7.15[a] ± 1.36	3.22[c] ± 0.72
Specific growth rate	1.291[a] ± 0.160	1.296[a] ± 0.129	1.142[b] ± 0.069
Condition factor	0.577[a] ± 0.029	0.585[a] ± 0.008	0.545[b] ±0.011
PER	1.47[a] ± 0.034	1.54[a] ± 0.026	1.26[b] ±0.0181
FCR	1.63[b] ± 0.045	1.31[c] ± 0.053	2.06[a] ±0.039
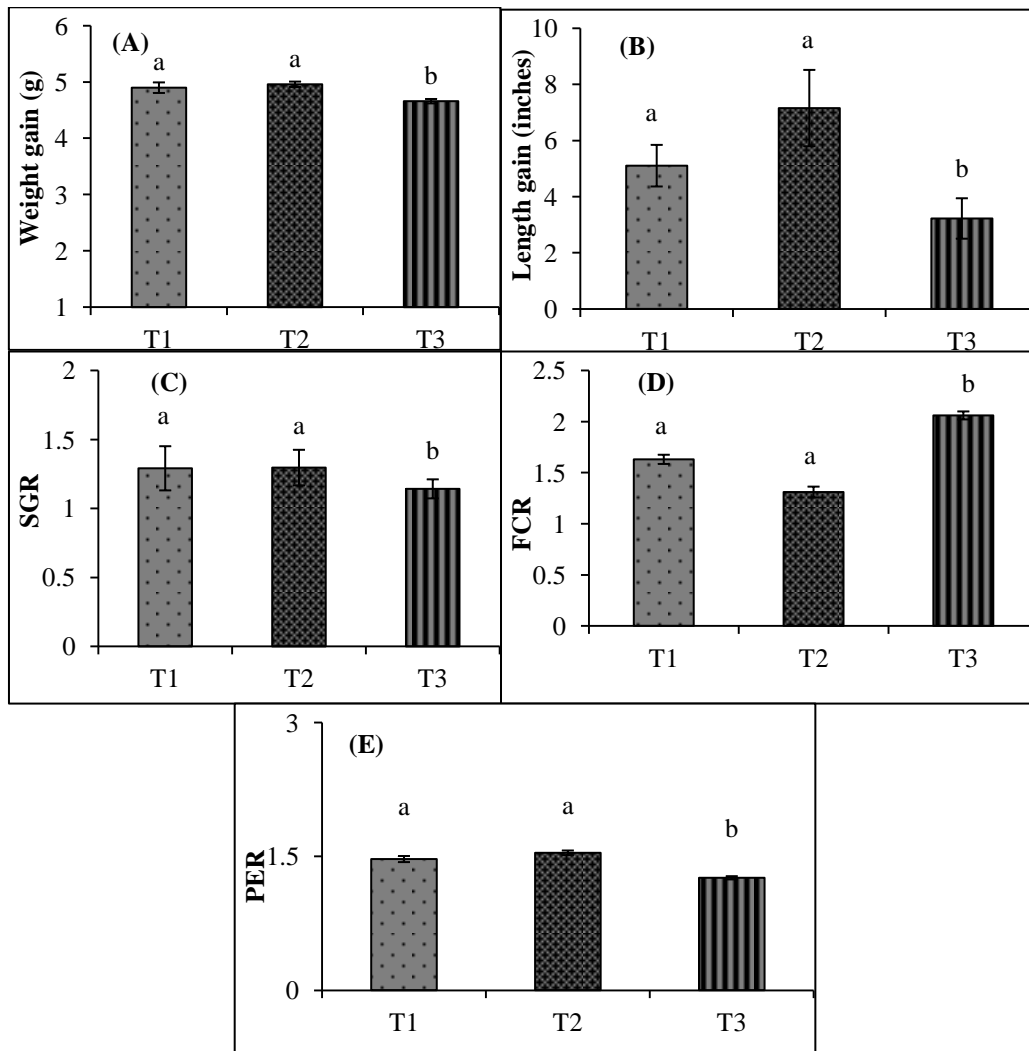
Fig. 1. Growth performance of *Labeo rohita* fish fingerlings fed with varying levels of proteins (A) Weight gain; (B) Length gain; (C) SGR; (D) FCR; and (E) PER. Values are mean ± Standard deviation

Table 2. Proximate body composition analysis 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]±0.705	78.380[c]±1.075	82.470[a]±0.521
2	Ash dry weight	14.630[b]±1.582	17.469[a]±1.779	11.481[c]±1.235
3	Ash wet weight	2.727[b]±0.372	3.119[a]±0.212	1.125[c]±0.787
4	Dry organic content	85.370[b]±1.582	82.530[c]±1.779	88.518[a]±1.235
5	Wet organic content	17.834[b]±0.712	14.570[c]±1.077	18.495[a]±0.525
6	Fat dry weight	45.061[b]±1.391	41.543[c]±1.285	55.061[a]±2.730
7	Fat wet weight	7.068[b]±0.870	5.770[c]±0.036	8.354[a]±0.661
8	Protein dry weight	54.569[b]±1.395	58.187[a]±1.296	50.750[c]±2.779
9	Protein wet weight	10.765[b]±0.817	12.799[a]±1.554	8.140[c]±0.462
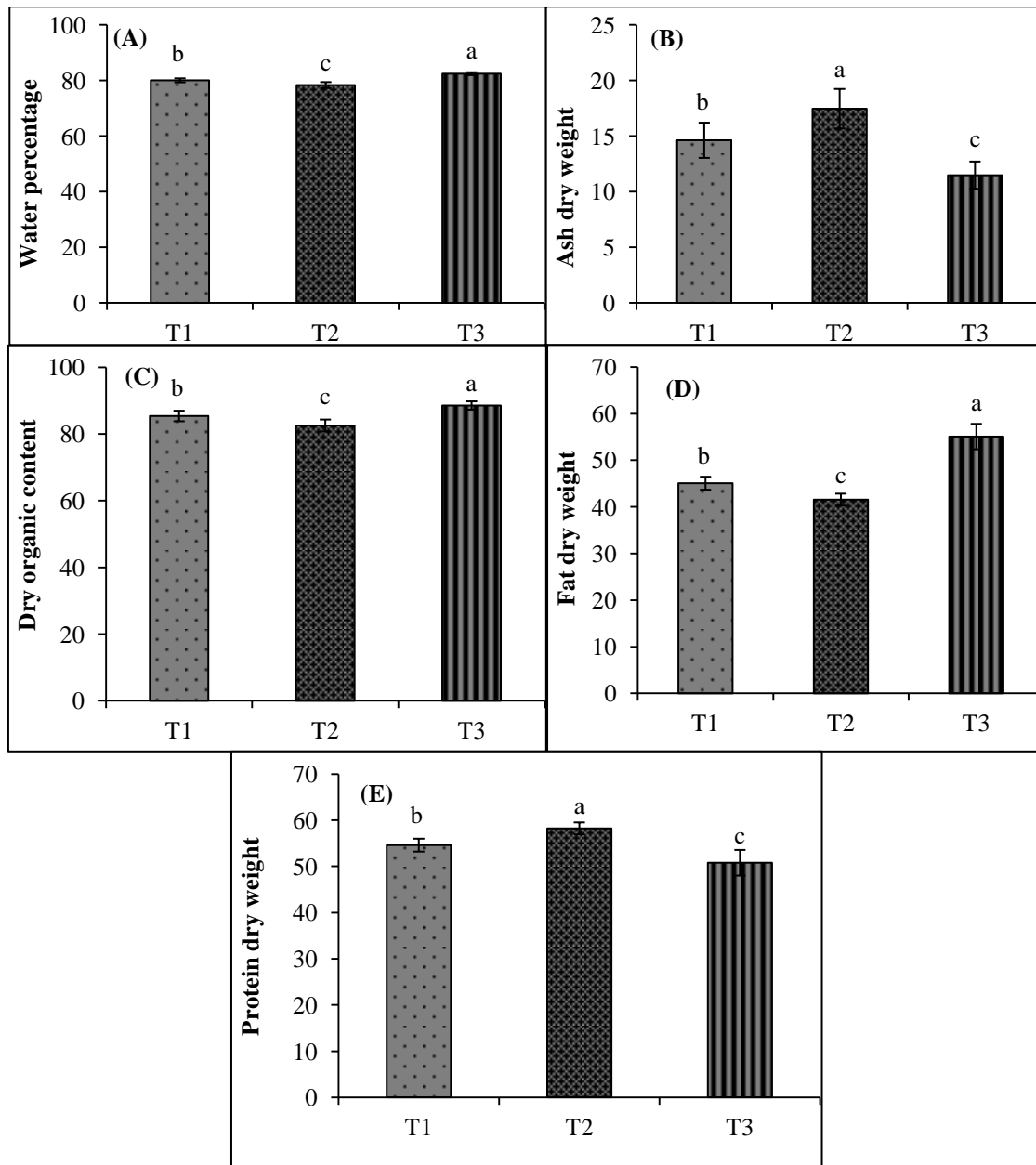
Fig. 2. Proximate body composition of *Labeo rohita* fish fingerlings fed with varying levels of proteins (A) Water percentage; (B) Ash dry weight; (C) Dry organic content; (D) Fat dry weight; and (E) Protein dry weight. Values are mean ± Standard deviation

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, Fig. 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 Fig. 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 T1 treatment was greater as compared to protein diet treatments (T1 and T2). However, the minimum water content 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 Figure 2B). 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 a 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.

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.

3. DISCUSSION

Fish is one of the important sources for increasing 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 of rapidly growing population [16].

Labeo rohita is one of the major carp found in Asian countries; therefore, efforts are being made to 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 as the increase in fish weight and fish length [18]. For this purpose, the physico-chemical parameters like temperature, dissolved oxygen, and pH of 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 protein in 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 this study showed 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 before its use for human consumption [28]. In this study, water percentage and fat content was higher in control group as compared to protein fed treatments while the protein and ash content was significantly higher in 35% crude protein diet as compared to other two treatments. The results 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 *Catla catla* (Ham.) and catfish which showed highest weight gain, significant growth ratio, feed conversion ratio, and protein efficiency ratio at highest protein diet [33, 34, 35]. Since the present research 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.

4. 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 appropriate for meeting the body requirement of fish. However, for determining the optimum level more investigations are needed.

DISCLAIMER

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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