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Impact of Varying Protein Levels on the Growth Rate of *Labeo rohita* Reared in Glass Aquaria

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ABSTRACT

Objectives. Aquaculture is an important technique used worldwide for the production of aquatic species. In this study, a monoculture experiment was performed to estimate the growth performance of *Labeo rohita* fingerlings under the influence of varying protein feeds used in various combinations in an intensive rearing system. It was found that different dietary protein levels significantly affected the growth rate of the above fish species, with an optimal protein level resulting in the highest growth performance. Previous studies showed that protein levels in the diet of *Labeo rohita* can influence growth, food conversion ratio, protein efficiency ratio, survival, and body composition. For instance, a diet containing 35% protein was found to significantly enhance growth, as compared to diets with lower or higher protein levels.

Methodology. Feeding trials were conducted to estimate the efficacy of the varying concentrations of dietary protein on the growth of *Labeo rohita* fingerlings in a glass aquaria for 75 days. The stocking density was 20 fish/aquarium. Diets containing 25%, 30%, and 35% protein were tested against a control containing 12% crude protein. The experiment was performed in the triplicate aquaria of glass. Fish were given feed at the rate of 4% of their body weight twice a day. Their growth performance was evaluated by measuring mean body weight gain (BWG), mean length gain (LG), feed conversion ratio (FCR), specific growth rate (SGR), and survival rate (SR) in different treatment groups. The proximate analysis of fish meat samples was conducted at the end of experiment.

Results. In the aquaria, fish fingerlings fed with 35% diet showed significantly greater weight gain (9.09 ± 0.09 g) as compared to those on other diets and control diet (6.8 ± 0.20). Simultaneously, a substantial increase in length gain was observed in fingerlings at 35% diet (8.2 ± 0.05), while the lowest weight gain (3.5±0.24g) was noted in those on control group diet. On the other hand, water quality variables, such as pH, temperature, dissolved oxygen, and electric conductivity remained constant.

Conclusion. It was concluded that a higher level of protein in the feed of *Labeo rohita* is directly related with the increased growth rate of fish.

Keywords: growth analysis, fish fingerlings, freshwater fish, *Labeo rohita,* length gain, proximate analysis, weight gain

1. INTRODUCTION

Aquaculture industry is a large source of income, food items, and also houses considerable global savings [1]. Aquatic harvesting is defined by Food and Agriculture Organization (FAO) as "the

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culture of aquatic animals such as shrimp, fishes, crustaceans and aquatic plants. People have come to recognize that their food choices may have a significant influence on their health, and as a result, there is a rising emphasis on eating a healthy diet across the world" [2, 3]. The success and sustainability of aquaculture depend significantly on proper nutrition and feeding practices [4, 5]. Fish are high in important fatty acids, especially omega-3 polyunsaturated fatty acids, which are required for optimal growth, development, and heart function during pregnancy and early childhood [6]. Due to their balanced amino acid profile and high protein content, fish products are good sources of protein [7].

The nutritional quality of fish, on the other hand, is not homogeneous and varies greatly between different fish species. It varies even within a single species, when produced in various settings and by using different culture techniques. *Labeo rohita* is one of the major carps that belong to the family Cyprinidae. It is also known as rohu or dumbra and found in South Asian and Southeast Asian rivers and freshwater lakes. It does not breed in captivity and is forced to breed under controlled settings [8]. Within the maximum documented age of 10 years, it can reach a maximum length of 200 cm and gain a maximum weight of 45 kg [9, 10]. Consumers choose it over other herbivorous fish species because of its distinct flavour and texture, while culturists prefer it because of its rapid growth, hardiness, and diverse feeding habits. This is why it is the most common fish in today's aquaculture operations [11].

The preparation of cost-effective fish feed from high-quality protein source is currently the most important challenge facing the aquaculture industry [12]. The rising need for fish meal in aquaculture, as well as other issues such as its higher prices and an unstable supply, necessitate the search for a fishmeal substitute [13]. Hence, researchers have concentrated on the use of plant-based protein to create viable fish diets. However, information regarding the formulation of artificial diets for productive and economically significant fish, such as large carps, can be challenging due to a variety of factors. Plant-based protein sources such as soybean, canola seeds, cottonseed meal, and sunflower meal have become increasingly popular as alternatives to fishmeal in aquaculture feeds [14, 15].

The kinds and amount of plant and animal protein sources used in diet formulation have a big influence on fish growth and feed conversion ratio (FCR). Animal-based protein sources can be replaced with plant-based protein sources with a protein concentration of 40% [16]. Based on the required growth rate and the harvested fish biomass, a diet consisting of rice bran, soybean meal, fish meal, vegetable oil, vitamin, and mineral combination influences the growth and survival of carp fingerlings [17].

This study involved the utilization of various meals of varying protein levels (25%, 30%, and 35%) as an energy source for rohu (*Labeo rohita*) fingerlings to monitor its possible effects on their growth. The purpose was to find out more about the efficacy of formulated feed which produced maximum growth in *Labeo rohita* at the lowest cost within the shortest possible duration.

2. MATERIALS AND METHODS

2.1 Experiment Layout

The project was performed in 40 liter glass aquaria in the chemistry laboratory of Fisheries Research and Training Institute,

Manawan-Lahore, Pakistan. Total 20 *Labeo rohita* (Rohu) fingerlings were placed in each aquarium filled with fresh water at random. Each treatment was tested in a triplicate. During the trial, fish fingerlings were given a 12-hour dark and light interval. They were categorized into four groups. Fish categorized in Group I were given a basal diet. While, fish categorized in Group II, Group III, and Group IV were fed on diets containing 25%, 30%, and 35% protein content, respectively. The experiment was performed in triplicate form. Fish were given feed twice daily at the rate of 4% of their body weight. Fish samples were taken on a periodical basis (3 fish/ group/ replicate) in order to adjust the size of ration. One quarter of aquaria water was changed every day to get rid of unused feed and waste products. The experiment was performed for 75 days.

The acceptance of the diet was monitored on a regular basis in each treatment group. Leftover feed was frequently cleaned from the aquaria bottom to meet the water quality requirements. The feed lost due to agitation and leaching of fish was not taken into account. FCR was calculated. On the water surface, there was no oily coating. When the feeding experiment ended, water level was reduced to the 3/4 of the total capacity. Then, all the fish fingerlings were taken and numbered from each tank to determine their survival rate. Moreover, their weight was calculated to determine the body weight gain.

2.2 Proximate Composition

The proximate analysis including moisture, fat, ash, and protein contents were recorded in carcass of fish [18].

2.3 Water Quality Parameters

Water quality parameters including Temperature, pH, and Dissolved Oxygen (DO) were recorded during this study; however, the average values were calculated on a monthly basis.

2.4. Data Analysis

One-way analysis of variance (ANOVA) was conducted to analyze the data in order to determine the effect of protein percentage on growth performance and nutritional digestibility of all treatments and test diets.

3. RESULTS

3.1 Growth Parameters

The current study was conducted to check the correlation between the articulated plant feed and the growth of fish fingerlings, as well as their feeding frequency and meat quality by using different treatment methods (T1-T3) differentiated with varying quantities of dietary proteins. The experiment aimed to assess the development reaction and body configuration of fish fingerlings, as well as to measure the nourishing influence of the formulated feed with varying protein levels of 25% (T1), 30%, (T2), and 35% (T3) on these fingerlings and also that of control diet (C), as given in Table 1. The maximum [$p \leq 0.05$] weight gain (WG) was attained with the diet (T3). The highest WG (9.09±0.09g) of *Labeo rohita* fingerlings was attained with 35% test diet (T3), as compared to other treatment diets.

The results indicate that the control group had the lowest weight gain, while the diet treatments with 30% and 25% showed higher weight gains. This suggests that dietary composition significantly influences growth performance.

3.2. Weight Gain Observations

3.2.1. Control Group (C). Weight Gain: (3.5 ± 0.24 g). Noted as the lowest

among all groups.

3.2.2. Diet Treatment 30% (T2). Weight Gain: (7.7 ± 0.10 g)**.** Indicates a substantial increase compared to the control group.

3.2.3. Diet Treatment 25% (T1). Weight Gain: (7.2 ± 0.30 g). Also shows a significant increase, though lower than T2.

3.2.4. Length Gain (LG) Analysis. Observed at (8.2 ± 0.05), indicating a positive response to dietary treatments.

3.3. Implications

The data suggests that higher dietary percentages lead to improved growth metrics, highlighting the importance of diet formulation in aquaculture practices.

By identifying the optimal protein level, fish farmers can improve the efficiency and productivity of *Labeo rohita* aquaculture, leading to better economic outcomes and sustainable fish farming practices.

Fish were fed daily 4% of their wet body weight twice a day in all treatments and control. In the control diet, the weight gain was significantly lower as compared to the other formulated diets, as shown in Table No. 1. This indicates that the control diet may lack essential nutrients or optimal formulations that promote growth, leading to subpar performance in terms of weight gain. The FCR (1.67) was considerably [$p \leq 0.05$] higher when the fingerlings were fed on 35% formulated diet (T3). It differed greatly [$p \leq 0.05$] from other diets including the non-treatment (control) diet. This revealed that the formulated feed efficiently transformed into fish muscle, indicating that these diets worked very well for fish growth. The poor value of FCR was noted for control diet (C), indicating that minimum diet was transformed into fish muscle. The maximum protein level of the fish fingerlings was observed in treatment (T3) diet. Soybean meal, fish meal, sunflower meal, corn gluten meal, wheat meal, canola meal, rice polish, mustard oil cake, vitamins, and many other ingredients gave positive results regarding fish growth.

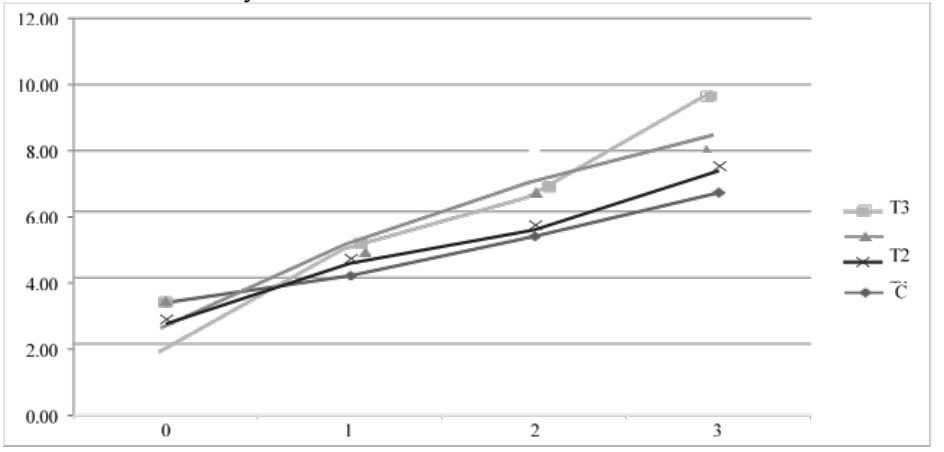

Figure 1. Growth of *Labeo rohita*
Note. Results are expressed as Mean ± SD values with error bars.
C= Control; T1= 25%; T2= 30%; T3= 35%

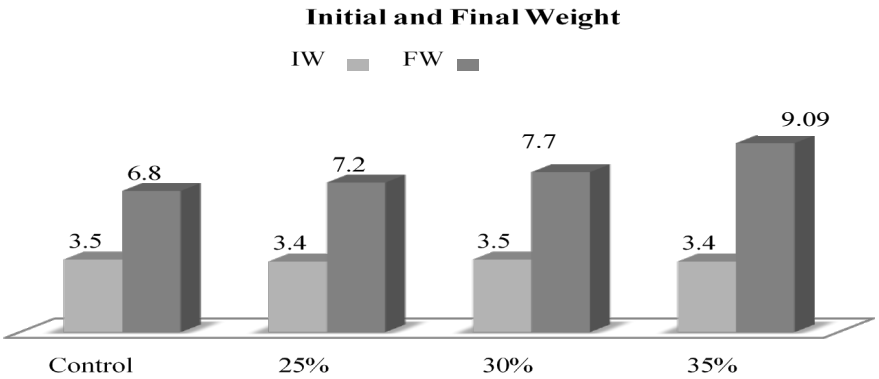

Figure 2. Length Parameters of *Labeo rohita*
Note. IL= Initial Length; FL= Final Length

Table 1. Comparison of Growth Parameters during Trial among Experimental Feed

Growth Parameters	Control Mean ± SD	T1 (25%) Mean ± SD	T2 (30%) Mean ± SD	T3 (35%) Mean ± SD
Initial Weight (g)	3.5 ± 0.00	3.4 ± 0.11	3.5 ± 0.10	3.4 ± 0.15
Final weight (g)	6.8 ± 0.20	7.2 ± 0.30	7.7 ± 0.10	9.09 ± 0.09
Average weight (g)	3.5 ± 0.24	3.6 ± 0.15	4.5 ± 0.12	5.4 ± 0.25
Daily Weight Gain (g)	0.04 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.08 ± 0.00
Specific growth rate SRG (g)	4.42 ± 0.01	5.06 ± 0.20	5.10 ± 0.16	7.58 ± 0.03
Initial Length (cm)	5.9 ± 0.14	6.0 ± 0.00	6.0 ± 0.07	6.0 ± 0.12
Final Length (cm)	7.3 ± 0.33	7.6 ± 0.19	7.9 ± 0.12	8.2 ± 0.05
Average gain length (cm)	0.09 ± 0.01	1.7 ± 0.01	2.0 ± 0.01	2.1 ± 0.00

Results are expressed as Mean ± SD values. Means with different superscripts within rows are significantly different at $p<0.05$.

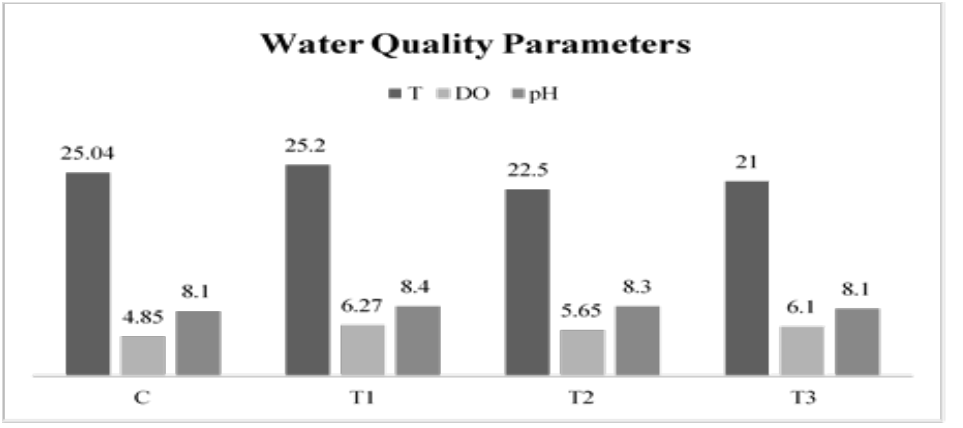

Figure 3. Water quality Parameters

3.4. Water Quality Parameters

Water quality parameters, such as temperature, pH, dissolved oxygen (DO), and electric conductivity (EC) remained constant throughout the study. In general, all water quality parameters were found to be congenial for the growth and survival of *Labeo rohita*.

Results are expressed as C= Control; T1= 25%; T2= 30%; T3= 35%; T= Temperature (°C); DO= Dissolved Oxygen (mgL^{-1}); pH= Concentration of hydrogen ions

3.5. Proximate Analysis

The maximum protein and fat in fish body were observed at 35% diet, that is, (74.05 ± 0.03) and (5.94 ± 0.00), respectively. The 2nd highest level of protein and fat in fish body was seen at 30% diet, that is, (73.34 ± 0.05) and (5.23 ± 0.04), respectively, in comparison to control diet. While, in fish fed on 25% diet, the values of different parameters showed the lowest amounts of protein and fat in carcass, that is, (73.05 ± 0.00) and (5.45 ± 0.04), respectively. According to the results of the current study, 35% diet has the ability to boost protein in comparison to control diet fish carcass protein level. When fingerlings were fed with 35% protein-based diet, ash contents were observed at the minimum level and the value of ash was higher than the fish fed on 25% protein-based diet. The results of total ash of Test diet T1, T2, and T3 were statistically diverse from each other. Also, the lowest moisture contents were recorded in fish fed on 35% diet, followed by 30% diet. Whereas, at 25% test diet the highest values of moisture contents were noted.

Table 2. Proximate Analysis *of Labeo rohita* Fingerlings under Different Treatments

Carcass Composition	Control Mean ± SD	25% Mean ± SD	30% Mean ± SD	35% Mean ± SD
Crude Protein	17.51±0.59	17.23±0.56	18.12±0.79	19.99±0.72
Crude Fat	4.45±0.00	5.45±0.04	5.23±0.04	5.94±0.00
Total Ash	2.24±0.01	2.12±0.01	2.05±0.04	1.87±0.12
Moisture	75.8±0.00	75.2±0.01	74.6±0.01	72.2±0.01

4. DISCUSSION

Labeo rohita fingerlings were found to have a substantially higher value [p<0.05] in terms of proximate composition of dry matter, ash, moisture, lipids, and proteins. The ideal amount of dietary protein for these fingerlings is 4% of their body weight. Diet C was nominated as control. Whereas, the rest of the diets (T1, T2, and T3) served as experimental/test diets, each formulated with 25% protein, 30% protein, and 35% protein, respectively. Treatment (T3) or 35% diet caused the maximum weight gain (9.09 ± 0.09). In this study, the influence of changing protein levels on several growth parameters, such as weight gain (WG), specific growth rate (SGR), and survival rate (SR) revealed that experimental feed had growth and production is considerably higher [p≤0.05] (25%, 30% and 35% protein). The results of fish growth showed that the fingerlings treated with T3 (35%) had significantly longer initial and final lengths than others. The current study also found that raising protein levels over the optimum level had a decreasing effect on fish growth, comparable to what was observed in eels. Previous studies regarding the influence of protein levels on fish development clearly demonstrated that when fed with diverse

protein-based diets, optimal growth occurred with 25% and 35% dietary protein levels. The current study also showed that *Labeo rohita* may achieve optimum development when fed on a 35% protein diet. This is in line with other researchers' findings for rohu and other fish [19, 20]. However, maximum SGR was observed for T3 (7.58 ± 0.03) [21]. The recorded highest growth in *Labeo rohita* fingerlings occurred when fed with 35% diet as compared to 25%, 30%, and control diet, though all these three diets were iso-nitrogenous.

The results revealed statistically significant differences ($p \leq 0.05$) in WG, LG, and SGR, when fish fingerlings were fed on 25%, 30%, and 35% diets, respectively. Significantly, a higher SGR was observed in T3 (23.33±0.37, 26.66±0.15) fed on 35% protein, as compared to all other treatments.[21]. *Labeo rohita* is a herbivore fish and prefers to feed on plant-origin feed ingredients which might be the reason for better growth as compared to control.

The results also showed that increased protein content has a significant [$p \leq 0.05$] effect on growth and can be safely used in this way. The results correlate with an experiment conducted previously, showing that a high level of crude protein content (up to 35-40%) has the potential to act as an alternative source of vegetable protein in the feed of livestock with good results.

4.1. Conclusion

This experiment aimed to determine the development response of the *Labeo rohita* fingerlings nourished with altered nourishing systems (25%, 30%, and 35% protein) to assess the efficacy of the nourishing rate on their development, existence, and body configuration over a three-month period of intensive rearing. After a three-month trial, all of the fish fingerlings were taken and the effects of the trial and control foods on their development were noted. Statistically significant [$p<0.05$] differences in length and weight gain were observed in the control group (wt. 6.8 ± 0.20 L, 7.3 ± 0.33), T1 (wt. 7.2 ± 0.30, L. 7.6 ± 0.19), T2 (wt. 7.7 ± 0.10, L 7.9 ± 0.12), and T3 (wt. 9.09 ± 0.09, L. 8.2 ± 0.05), respectively.

The lowest WG was noted in control group diet. Limnological studies showed that water quality variables, such as pH, temperature, dissolved oxygen, and electric conductivity remained constant. Hence, it was concluded that varying protein feeds enhanced growth rate, production, and meat quality in the aquarium.

CONFLICT OF INTEREST

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

Data will be provided by corresponding author upon reasonable request.

FUNDING DETAILS

No funding has been received for this research.

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