J. Glob. Innov. Agric. Soc. Sci., 2016,4(2): 88-94.
ISSN (Online): 2311-3839; ISSN (Print): 2312-5225
DOI: ***10.22194/JGIASS/4.2.737***
http://www.jgiass.com

EVALUATION OF THE EFFECT OF DIETARY PROTEIN LEVELS ON GROWTH OF JUVENILE *Labeo rohita* UNDER INTENSIVE CULTURE SYSTEM

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Growth performance of *Labeo rohita* fingerlings was determined by using three iso-caloric diets with different protein levels against a control diet. Trial lasted for eight weeks. Fingerlings reared in triplicates under treatments viz. T1, T2, T3 and T4 (control) fed diets with 25%, 30%, 35% and 18% crude protein levels, respectively. Fingerlings under T2 showed significantly ($p<0.05$) higher weight gain (15.05g), length gain (40.42mm), condition factor (1.49±0.02), % specific growth rate (%SGR 2.62±1.65) followed by T3, T1 and T4, respectively. Mathematical computation of length weight relationship showed isometric growth pattern with maximum value of regression slope "b" for T2 (3.11) followed by T3, T1 and T4. Significantly ($p<0.05$) higher protein efficiency ratio (PER 1.93±1.11) and significantly ($p<0.05$) lower feed conversion ratio (FCR 2.48±1.59) was calculated for T2. Proximate analysis of carcass showed significantly ($p<0.05$) higher protein content for T2 (16.91±0.56). Physico-chemical parameters of water of aquaria were monitored weekly throughout trial period and were found to be within recommended ranges. It was concluded that *Labeo rohita* fingerlings fed diet having 30% crude protein exhibited better growth performance. Further increase in dietary protein level in T3 resulted in significantly ($p<0.05$) lowered values of growth indices.
Keywords: *Labeo rohita*, growth performance, dietary protein, condition factor.

INTRODUCTION

Fish farming is important and the most common form of aquaculture. Culturing species in water bodies has become a developing industry and requires much improvements, technically developed strategies and innovations. Many million people are engaged in this industry and a majority of them is especially involved in small scale projects of fish culture (Subasinghe *et al.*, 2012). Optimizing the feeding frequency is crucial for proper management of semi-intensive and intensive fish cultures (Dominic and Adetola, 2015).

The major carps i.e. *Labeo rohita*, *Catla catla*, *Cirrhina mrigala* and Chinese carps i.e. *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* are among the most important food fish in Pakistan. Developing nations, like Pakistan, are facing malnutrition especially of animal proteins as the per capita availability of protein is below the minimum average recommended daily requirements ((Rohankar *et al.*, 2012). Thus, aquaculture is a possible hope that can cater the emerging protein demands in the country (Haider *et al.*, 2015). Rohu (*Labeo rohita*) is a commercially important, herbivorous fish that has good growth potential and is widely consumed in Pakistan (Jabeen *et al.*, 2015).

It is necessary to provide fish with complete protein rich diet for healthy growth. Formulated feed could be supplemental or complete. Complete diet contains all necessary amino acids, vitamins, fats, carbohydrates, minerals. Locally procurable ingredients should be preferred for their use to formulate fish feeds (Rehman *et al.*, 2013). One of the major problems encountered by the fish breeders is the high cost of formulated fish diets (Sadiq *et al.*, 2015). In fish culture endeavors cost of the fish diets is 60% of total cost due to higher required protein levels in diets necessary for stabilizing fish health and growth rate (Zehra *et al.*, 2012).

Semi intensive and intensive culture practices depend on many factors like feeding regimes for achieving higher biomass production rate (Abid and Ahmed, 2009). In intensive culture, fish are fed with balanced diet (Ayyappan and Ali, 2007). Fish oil as a source of dietary lipids and fish meal as a source of dietary proteins are used two to five folds in quantities in many intensive aquaculture practices (Naylor *et al.*, 2000).

Artificial feeds are of great importance to sustain the aquaculture industry (Khan *et al.*, 2012). Optimal protein level in the artificial diets is a foremost preference in modern intensive fish rearing. Optimal level of protein in fish diets is the one that is just required to get maximum fish yield (Abidi and Mukhtar, 2008). The most important ingredient of fish feed is protein. Fish have high requirement for dietary protein level which usually ranges from 35% to 56% (Gandotra *et al.*, 2015). Present endeavor was therefore planned to optimize dietary protein level for *Labeo rohita* juveniles under intensive culture conditions.

MATERIALS AND METHODS

Three iso-caloric diets viz. Diet-1, Diet-2, Diet-3 having 25% crude protein (CP), 30%CP and 35%CP levels were formulated by using locally available ingredients. Mixture of rice polish and fish meal was used as control diet (Diet-4). Dry ingredients of all diets were blended with some quantity of water to make dough and finally prepared diets were obtained in the form of 2mm pellets. Table 1 presents the relative quantities of used ingredients and proximate composition of all formulated diets.

Labeo rohita fingerlings with an average weight 4.48±0.01g and average total length 67.92±0.10mm were procured from Fish Hatchery, Satiana Road, Faisalabad, Pakistan. All 120 fingerlings were brought live to the Research Laboratory at Govt. Postgraduate College, Gojra, Punjab, Pakistan. Fingerlings were distributed in triplicates in 12 glass aquaria of 100L capacity each, under four treatments viz. T1, T2, T3 and T4 (control). Fingerlings were acclimatized by feeding with control diet for two weeks. Water of glass aquaria was daily changed to maintain the cleanliness and general health of fingerlings. Moreover, aerators were used to maintain required dissolved oxygen level.

Table 1: Ingredients and composition of formulated diets (%).

Ingredients	**Diet – 1 (T1) 25% CP**	**Diet – 2 (T2) 30% CP**	**Diet – 3 (T3) 35% CP**	**Diet – 4 (T4) Control**
Wheat	26	15	3	0
Rice polish	25	25	25	70
Corn glutton 60%	7	18	30	0
Soya bean meal	10	10	10	0
Fish meal	25	25	25	25
Soya Oil	2	2	2	0
Vitamin mineral mixture	5	5	5	5
Proximate analysis of diets				
Moisture (%)	8.63	8.49	8.35	8.22
Crude protein (CP %)	25.37	30.10	35.26	18.95
Crude fat (CF %)	6.91	6.74	6.55	9.06
Ash (%)	6.61	6.55	6.48	9.89
Carbohydrates (%)	52.48	48.12	43.36	53.88
G.E. (kcal/kg)	3125.26	3132.45	3141.32	3101.15

CP = Crude Protein   CF = Crude Fat   NFE = Nitrogen Free Extract

Trial was conducted for eight weeks from 14-09-2015 to 08-11-2015. Fingerlings under different treatments were daily fed with experimental diets and control diet @4% of their average body mass in two portions. Fingerlings in glass aquaria under treatments viz. T1, T2, T3 and T4 received Diet-1, Diet-2, Diet-3 and Diet-4, respectively. Average fish weight and total lengths were recorded on weekly basis. The growth data thus obtained was used to calculate following growth indices for *Labeo rohita*.

Average Weight Gain (g) = Average final weight – Average initial weight

Average Length Gain (mm) = Average final length – Average initial length

Fulton's Condition Factor

$$K = W \times 100 / L^3$$

Specific Growth Rate (% day)

$$SGR(\%) = \left[(lnW_f - lnW_i) / T\right] \times 100$$

Feed Conversion Ratio

FCR = total feed intake (g)/ total wet weight gain (g)

Protein Efficiency Ratio

PER = wet weight gain (g)/ total protein intake

Survival Rate (%)= Final no. of surviving fish/ No. of stocked fish × 100

Gross Fish Production = No. of harvested fish × Average final weight of fish

Length-weight Relationship

The length-weight relationships of log transformed data were calculated by linear line regression analysis as per cube law (Le Cren, 1951).

$$W = aL^b$$

Where, W is Weight of fingerlings (g), L is total length (mm), "a" is the regression intercept and "b" is the regression slope. Logarithmically transformed formula derived from above equation is:

$$\log W = \log a + b \log L$$

***Proximate Analysis of Feed and Carcass*:** Artificial feeds (prior to trial) and carcass of *Labeo rohita* at final harvest were analyzed for their proximate composition (% moisture, % crude protein, % crude fat, % ash and % carbohydrates) by following standard methods of Association of Official Analytical Chemists (AOAC, 1995).

Water Quality Parameters: Temperature (°C), dissolved oxygen (mgL^{-1}), pH, electrical conductivity ($mScm^{-1}$) and ammonia (mgL^{-1}) of aquarium water affect the physiology and health of the fish. Temperature (°C), dissolved oxygen (mgL^{-1}), pH, electrical conductivity ($mScm^{-1}$) of aquarium water was measured by using digital meters viz. HANNA HI-8053, HI-8520, HI-9146 and HI-8733, respectively. Ammonia content of water was determined by following the method of A.P.H.A. (2005). These physico-chemical parameters were recorded on weekly basis.

Data on fish growth and proximate composition as well as physico-chemical parameters of aquarium water were subjected to statistical analysis using STATISTIX and MINITAB packages by following Steel *et al.* (1997). Analysis of variance (ANOVA) was performed and Tukey's Newman-Keul's test was used to find the statistical differences among mean values.

RESULTS

Among all the three graded protein levels and control diet the growth of *Labeo rohita* was significantly ($p<0.05$) higher due to T2 (30% CP). Regarding overall performance, the final average weight of 7.83±1.77g, achieved in control treatments was significantly lower than experimental diets. *Labeo rohita* attained a maximum average total length of 96.26±11.15mm due to T2 (30% CP). Significantly higher final average value of condition factor (K 1.49) was observed for T2 (30% CP) and T3 (35% CP), followed by fish reared under T1. Significantly higher (2.62±1.55%) value of specific growth rate (SGR) was found for *Labeo rohita* fingerlings fed 30% CP diet.

Significantly lower SGR of fish was observed in T4 (control) and was recorded as 1.54±0.65. Maximum value of FCR was recorded to be 3.09 in T3 and T4 (control) which showed non-significant difference between these two treatments for FCR value. The lowest FCR value was recorded to be 2.48±1.59 for T2 followed by 2.83±1.40 for T1. Non-significant differences existed between T1 and T4 for PER values. Maximum PER value was recorded for T2 to be 1.93±1.11. Maximum gross fish production at final harvest was calculated to be 585.9±0.09g followed by 490.2±0.11g, 407.4±0.03g and 322.5±0.22g under T2, T3, T1 and T4 (control), respectively. Survival rate for all treatment for whole experimental period sustained to be 100% (Table 2).

Labeo rohita fingerlings reared under T4 (control) depicted negative allometric growth while all other three experimental feeding groups showed positive allometric growth with maximum value of "b" under T2 i.e. 3.11 followed by T3 (3.08) and T1 (3.05) as shown in Table 3. Table 4 exhibits the proximate composition of fish carcass at final harvest. Fish reared under T2 deposited maximum crude protein and lipids. Water quality parameters during feeding trial remained within tolerable limits as shown in Table 5.

Table 2: Growth indices of *Labeo rohita* reared under treatments (T1-T4) receiving different dietary protein levels.

Parameters	T1 25% CP	T2 30% CP	T3 35% CP	T4 Control
Average initial weight (g)	4.47±0.05a	4.48±0.01a	4.48±0.03a	4.51±0.03a
Average final weight (g)	13.58±0.03c	19.53±0.08a	16.34±0.10b	10.75±0.25d
Average weight gain (g)	9.01	15.05	11.86	6.24
Average initial total length (mm)	67.99±0.11	67.79±0.48	67.9±0.12	68.01±0.11
Average final total length (mm)	96.81±0.07c	108.21±0.05a	103.13±0.09b	90.42±0.67d
Average Length gain (mm)	28.81±0.15	40.42±0.42	35.23±0.12	22.41±0.57
Condition factor (K)	1.46±0.01b	1.49±0.02a	1.49±0.01a	1.41±0.03c
SGR (% day)	1.96±1.30c	2.62±1.65a	2.30±1.55b	1.54±0.65d
FCR	2.83±1.40ab	2.48±1.59b	3.09±2.03a	3.09±0.77a
PER	1.79±1.07a	1.93±1.11a	1.46±0.93b	1.90±0.73a
Survival rate	100	100	100	100
Gross fish production (g)	407.4±0.03c	585.9±0.09a	490.2±0.11b	322.5±0.22d

Table 3: Final length-weight relationships of *Labeo rohita* reared under treatments (T1-T4).

Treatments	Regression Equation log W = log a + b log L (log 10 transformed data)	Correlation coefficient "r"	Coefficient of determination "r^2"	Regression slope 'b'	Probability
T1	W = -4.94+ 3.05L SE = 0.039	0.9993	0.9988	3.05	$p<0.01$
T2	W = -5.05 + 3.11L SE = 0.034	0.9995	0.9992	3.11	$p<0.01$
T3	W = -4.98 + 3.08L SE = 0.025	0.9998	0.9997	3.08	$p<0.01$
T4 (Control)	W = -4.725 + 2.93L SE = 0.101	0.9958	0.9918	2.93	$p<0.01$

Table 4: Proximate composition of meat of *Labeo rohita* reared under treatment (T1-T4).

Parameter	T1 25% CP	T2 30% CP	T3 35% CP	T4 (Control)
Moisture (%)	75.98±0.88bc	75.41±0.67bc	76.43±0.03ab	77.23±0.77a
Crude Protein (%)	16.41±0.66a	16.91±0.56a	16.01±0.72a	15.73±0.53a
Crude Fat (%)	4.73±0.53ab	4.81±0.50a	4.12±0.55ab	3.92±0.56b
Ash (%)	1.36±0.52a	1.39±0.54a	1.35±0.64a	1.33±0.65a
Carbohydrates (%)	1.52±0.68a	1.48±0.68a	2.09±0.59a	1.79±0.57a

P<0.05, Means labeled with different alphabets are significantly different. SD = Standard Deviation.

Table 5: Water quality parameters recorded for different treatments.

Parameter	T1 25% CP	T2 30% CP	T3 35% CP	T4 (Control)
Temperature (°C)	27.22±0.55d	28.78±0.51c	30.52±0.59b	31.17±0.65a
DO (mgL^{-1})	8.17±0.10a	7.80±0.29b	7.29±0.16c	7.04±0.07d
pH	7.62±0.37d	7.97±0.28b	7.86±0.36c	8.13±0.17a
EC (mScm^{-1})	2.59±0.48a	2.66±0.49a	2.68±0.54a	2.69±0.52a
Ammonia (mgL^{-1})	0.51±0.15c	0.86±0.34b	1.17±0.39a	0.39±0.21d

DISCUSSION

Results of present study revealed that *Labeo rohita* fingerlings fed on 30% CP level attained significantly higher average weight and total lengths as 13.93±4.65g and 96.26±11.15mm, respectively. However, at 35% CP level a decrease in average final weight and total length was noted and the recorded values were 11.90±3.90g and 91.55±10.64mm, respectively. *Labeo rohita* fingerlings showed maximum weight gain at 30% crude protein level when raised under laboratory conditions using slaughter house wastes as protein source (Singh *et al.*, 2005). *Labeo rohita* fingerlings experienced an increase in average body weight with an increase in crude protein level in the diets, up till 30% CP. However, a reduction in fish growth performance was noticed at any level of crude protein beyond 30% CP. Trend found in present results i.e. excessive proteins in feeds leads to decreased growth performance; may be attributed to deceased energy level available for fish growth since greater amount of energy is used in deamination of excess proteins in the diets (Kim *et al*., 2002).

Fish body tissues and organs have protein as a major component. When excessive protein is supplied in fish diet, only a fraction of it is converted into body flesh while other proteins are used to produce energy during metabolism. Significantly lower growth of control fish (7.83±1.77g) may be due to the fact that control diet had lower protein contents that alone did not fulfill the protein requirement of *Labeo rohita. Parachanna obscura* fingerlings also experienced reduced growth rate when fed diets with lower protein contents (Kpogue *et al.,* 2013). Environmental factors, climatic conditions and foraging differences lead to fluctuations in values of condition factor (K) within fish of same species or between fish of different species (Lizama *et al*., 2002). Condition Factor (K) is a growth parameter that represents the degree of fish wellbeing under a particular set of fish rearing condition (Javed, 2015). Level of crude protein in artificial diets significantly influenced the condition factor (K) of fish. Significantly higher values of condition factor (K) were recorded for 30% CP and 35% CP in artificial diets. Since *Labeo rohita* fingerlings fetched significantly higher weight gain at 30% CP level, so this dietary protein level may be regarded as ideal for the growth of fish under intensive culture conditions. Okon *et al.* (2014) also reported that fingerlings of *Clarias gariepinus* earned higher condition factor values and final average weight due to optimal level of protein in artificial diets i.e. 50.75% CP level. Specific Growth Rate (SGR) of *Labeo rohita* fingerlings was significantly higher due to 30% CP level in artificial diets. However, significantly lower SGR was recorded for fish fed diet with 35% CP level. Over all lower specific growth rate of fish fed lower protein diets was also reported by Chatta *et al.*

(2015). Gandotra *et al.* (2015) postulated that decrease in specific growth rate at protein level above the optimal may be due to reduction in the dietary energy for growth to deaminate and excrete excess absorbed amino acid.

Feed conversion ratio (FCR) by *Labeo rohita* fingerlings was significantly higher due to 30% CP level in artificial diets that caused the fish to secure significantly higher final average weight due to this feeding regime. *Parachanna obscura* fingerlings also attained high final average weight due to artificial diets that enabled the fish to earn significantly higher FCR (Kpogue *et al.,* 2013). Protein efficiency ratio also showed the same trend like Feeding efficiency. Highest value of protein efficiency ratio was recorded at 30% CP but it decreased with further increasing the crude protein level in artificial feed. Ahmad *et al.* (2012) described that PER is inversely effected by protein level.

Length weight relationship depicts isometric, positive allometric and negative allometric growth pattern. Isometric growth refers to no change in fish body shape during growth period. When fish attains length and its body becomes gradually deeper, this growth is called positive allometric. If fish body becomes slenderer and greater in weight during its growth, this is represented as negative allometric growth. All these growth patterns are represented by value of "n" in regression equation. Values of n=3, n<3 and n>3 depict isometric, positive allometric and negative allometric growth, respectively (Riedel *et al*., 2007). Results in present study show positive allometric growth pattern in T2, isometric growth in T1 and T3, while negative allometric growth in T4 (control). Value of "n" for fish reared under T2 (3.11) explains better productivity and weight gain due to Diet-2. Proximate composition of fish meat was significantly affected due to varying protein levels. Control *Labeo rohita* fetched highest moisture contents in its flesh. However, as compared to treated fish, protein contents of control fish and fish under T1 were the lowest and were non-significantly different. These results showed an inverse relationship between body moisture and protein accumulation. Parveen *et al.* (2012) also reported an inverse relationship between moisture and protein accumulation in the flesh of *Labeo rohita*. Around 30% CP level in artificial diets gave higher lipid contents to the fish. However, control fish accumulated lowest lipid contents in its flesh. This shows the incompetency of control diet to meet fish requirements. Fish fed control diet might have starved and started utilizing its own body lipids. Satpathy *et al.* (2003) also demonstrated the same results in his experiment while studying the impacts of varying dietary protein and lipid levels on the growth performance of *Labeo rohita*.

Ash contents were found to be non-significantly higher in T2 due to 30% CP. Ash contents in fish flesh serves as indicator for minerals availability to the consumer. In present study as protein contents are increasing ash contents are decreasing. Similar trend is explained by (Pham *et al.,* 2008) that as protein contents increases ash content decreases. In general, the metabolic processes indicate a flow of lipids into carbohydrates (gluconeogenesis) and vice versa. Thus, the two energy-producing nutrients remain in a reciprocal amount. Increasing the amount of protein in artificial feeds caused a decrease in the carbohydrate accumulated by the fish. This is the reason for the presence of highest amount of carbohydrate in fish reared under T3 (35%CP) as 2.096±0.30. Shah *et al.* (2016) reported that fingerlings of lemon fin barb show similar trend in carbohydrate with increasing level of carbohydrate beyond optimal level.

The water quality parameters observed during trial were found within the optimum range for the rearing of *Labeo rohita* fingerlings. Tolerable values of water quality parameters justify 100% survival rate of fingerlings under all treatments.

Dissolved oxygen concentrations of the aquarium water under all the treatments remained within range of 7.04 – 8.17 mg/L[1] (a limit considered safe for fresh water fish) as reported by Javed (1988). Ali *et al.* (2000) indicated that the suitable dissolve oxygen for fish growth was above 5ppm. The overall pH value under all treatments remained within range of 7.62-8.13 and differences among treatments are non-significant. Mahboob and Sheri (2002) while investigating the influence of artificial feeds on the performance of carps also reported non-significant pH values among the treatments. Electrical conductivity is the measure of total dissolved salts present in pond's water (Prajapati, 2014). Values of electrical conductivity for all treatments remained within 2.59-2.69 while that of ammonia content remained within 0.39-1.17.

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