Research Article

Impact of graded dietary protein on growth parameters of hybrid (*Labeo rohita* ♀ and *Catla catla* ♂) from Southern Punjab, Pakistan

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Abstract | This study was conducted to analyze the impact of varying levels of plant based protein diets (15%, 20%, and 25% crude protein) prepared from cheaper plant proteins, to keep minimum use of fish meal, on growth performance, survival and production of hybrid fry (*Labeo rohita*♀ x *Catla catla* ♂). The hybrid fry of mean 1.05±0.08 g body weight and 4.36±0.40 cm mean length were acclimatized and transferred to 8 X 6 X 3 ft. hapas. Fry were fed with fish meal at the rate of 10 % of body weight during acclimatization period and experimental feed at the rate of 5 % of body weight up to the end of the 90 days experiment in duplicate at the fish farms facility. The growth performance of test fishes was observed at monthly interval and accordingly the fish feed was readjusted. During the study period, water quality parameters were maintained within safe limits for fish growth. Ten fish specimens from each treatment were randomly collected at the end of trial for growth parameters analysis. Different growth parameters, such as mean weight gain, mean length gain, specific growth rate, protein efficiency ratio, feed conversion ratio and production were determined. Results revealed maximum gains in T3 (25%) feed group as compared to T1 (15%) and T2 (20%) feed groups. The lowest food conversion ratio (FCR) was also noted in the T3 (25%) group showing best feed composition of T3 (25%) feed by the tested fish fry. ANOVA analysis showed highly significant (P<0.001) difference in the final weight, final length and production among three treatment groups (T1, T2, and T3), while no difference (P>0.05) was observed in SGR, FCR and PER values of the three treatment groups. Hence, a protein diet containing 25% dietary protein is not only cost-efficient, economical but also nutritional and wholesome for a fish's health condition.

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Introduction

For successful fish farming, proper feed formulation is required. Aquaculture business gains profits, when fish feed provide maximum growth. Growth is most determining factor for fish cultivation success (Muhammadar *et al*., 2021). Combating disease, enhancing growth rates, producing efficient feed and finally a product having nutritional and safety standards are predictive gears to determine Aquaculture success. Nonetheless, one of the big obstacles is the expense of fish feed ingredients, which is about 80% of the production budget in the flourishing fish industry (Cheikyula *et al*., 2020). Protein is considered most expensive ingredient in feed formulation. If proteins content increase in fish feed than the optimum required level, they can results in increased energy cost,also increased excretion of nitrogenous material and retarded growth (Monentcham *et al*., 2009; Abdel-Tawwab *et al*., 2010). Being an overpriced

ingredient, proteins must be exercised cautiously because it's uncontrolled use is wasteful and economical freight (Deng *et al*., 2011; Wang *et al*., 2017).

Protein has various roles and functions, including collagen, which is a fibrous connective tissue having importance to form fish muscles (Subandiyono and Hastuti, 2011). In determining growth process, protein is valuable content because most of the fish's body (45-75% dry weight) consists of protein (Iahtiaq and Naeem, 2019). Throughout the world, fish is used as a valuable source of protein (Ahmad *et al*., 2017).

Fish meal having growth potential, palatability and balanced amino acids composition, is a preferred ingredient in fish feed but the drawback of using fish meal is its limited supply and high cost, therefore, fish feed industry and growers need to search for alternate source (s) for fish feed (Kaushik and Troell, 2010; Radhakrishnan *et al*., 2016). In this context, the presence of balanced amino acids and low costs, plant protein sources are best alternative to fish meal. The plants-based sources of proteins can cope the need of the day because of increasing fish culture with increasing population (Mahboob, 2014; Daniel, 2018; Zettl *et al*., 2019). The best of hybrid characters: *Catla* and *Labeo*, with a small head, deep body, more flesh as weighed up with both of parents, hence, evidenced the best prospect of culturing than either of parents (Basavaraju *et al*., 1995).

The purpose of the study was to review three crude protein diets (15%, 20%, and 25% CP) and regionally convenient plant-sourced ingredients and evaluate their outcomes on growth of the hybrid (*L. rohita x C. catla*) fish.

Materials and Methods

The present study was conducted from June to August 2017 for 90 for 3 months at Tawakal Tilpia Fish Hatchery, Muzaffargarh, Punjab, Pakistan.

Preparation of feed

By using weighted quantities of different cheaper and easily available plant protein contents (e.g. sarson meal, sunflower meal, wheat bran, canola meal, rice polishing, corn glutton, vitamin premixes, fish meal in limited amount), three crude protein diets [TI (containg 15% crude proteins), T2 (contaiing 20% crude proteins), and T3 (containing 25% crude proteins)] were prepared at Institute of Pure and Applied Biology (IPAB), Bahauddin Zakariya University, Multan, Pakistan feed preparing feed lab. Thorough mixing of weighted quantities of all the feed components was done and finally ground to powder form for its easy ingestion. For proper storage of feed, polythene bags were used.

Feeding trial

The hybrid fry were selected for this experiment to observe effect of the three proteins diet on growth of the hybrid fry. Eighteen days old fry of hybrid were collected from Tawakkal Tilapia Fish Hatchery. The fry had mean weight of 1.05±0.08 g and mean length 4.36±0.40 cm and were kept in hapas (each *8x6x3 ft.)* in single stagnant earthen pond. Fish meal diet was given to fry @ 10% body weight during acclimatization of 2 weeks in nursery tanks. Experiment was performed in duplicate for each treatment. Feed was provided once daily between 8:00-9:00 am at the rate of 5% of fish body weight. At the end of feeding trial, 10 fish samples from each treatment were randomly selected for growth analysis. Various growth parameters were calculated by using standard formulae following; Sawhney and Gandotra (2010) and Ishtiaq and Naeem (2019), given below

Specific growth rate (SGR)

It was calculated by the following expression:

$$\%\ \text{specific growth rate} = \frac{100(\text{LnW2} - \text{LnW1})}{\text{no. of days}}$$

Feed conversion ratio (FCR)

It was calculated by the formula given below:

$$\text{FCR} = \frac{\text{feed intake (in grams)}}{\text{weight gain (in gram)}}$$

Protein efficiency ratio (PER)

The following formula was used for the measurement of PER:

$$\text{PER} = \frac{\text{gain in weight (in grams)}}{\text{intake of protein (in grams)}}$$

Survival rate % (SR)

Survival rate of the treated fish was calculated using the following formula:

$$\text{Survival rate (\%)} = \frac{\text{Nf X 100}}{\text{Ni}}$$

Table 1: *Different growth parameters of the hybrid (Labeo x Catla) fish with ANOVA and t-test comparison.*

Growth parameters	Treatment 1 Mean±S.D.	Treatment 2 Mean±S.D.	Treatment 3 Mean±S.D.	ANOVA P value
Experimental period (weeks)	90 days	90 days	90 days	
No. of hybrid fry	150	150	150	
Mean initial weight (g)	1.5±0.07^{ac}	1.10±0.01^{c}	1.05±0.08^{ac}	0.000***
Mean final weight (g)	10.60±2.01^{bc}	14.60±4.67^{bc}	3.80±2.25^{c}	0.000***
Live weight gain (g)	9.60±1.56^{bc}	13.5±1.99^{bc}	22.75±3.39^{c}	0.000***
Mean initial length (cm)	4.37±0.40^{a}	4.35±0.39^{a}	4.36±0.40^{a}	0.495^{ns}
Mean final length (cm)	7.37±0.81^{bc}	8.16±0.63^{bc}	9.44±0.69^{c}	0.000***
Length gain (cm)	3.00±1.36^{bc}	3.81±1.40^{bc}	5.08±1.50^{c}	0.000***
Survival (%)	100±0.00	100±0.00	100±0.00	
Mean Final Condition Factor	2.37±0.38^{ab}	2.40±0.27^{ab}	2.86±0.33^{b}	0.004**
Feed conversion ratio	2.15± 0.90^{a}	1.89±0.09^{a}	0.99±0.10^{a}	0.189^{ns}
Protein efficiency ratio	4.86±0.77^{a}	5.70±0.91^{a}	6.98±0.68^{a}	0.758^{ns}
Specific growth rate	0.97±0.07^{a}	1.33±0.32^{a}	1.65±0.01^{a}	0.274^{ns}
Production g/m2/90 days	3.90±0.88^{b}	6.28±1.12^{b}	16.68±1.92^{b}	0.001***

*S.D.: Standard Deviation Highly significant correlation =***P <0.001; Significant correlation =**P<0.01; Non-significant correlation P >0.05.*

Where;
Nf = final number of fishes and Ni = initial number of fishes

Production (P)
Productivity was calculated using the following formula:
Production = (weight gain in g / Area) /days

Fulton condition factor (K)
It was measured by the following formula:

$$\text{Fulton condition factor} = \frac{\text{fish weight (in grams)}}{\text{fish length (in cube centimeter)}} \text{X100}$$

Physico-chemical Analysis
On daily basis, different parameters were observed. Major were dissolved oxygen, temperature and pH level of water, while on fortnightly basis, total hardness and transparency were noted. Transparency was measured with the help of Sacchi disc. Dissolved oxygen was monitored using dissolved oxygen meter LT-Lutron DO-5510 Taiwan. Monitoring of pH and hardness was carried out using digital pH meter KL-009 (1) made in China and p24-565714 made in Germany respectively.

Data analysis
By using MS-Excel, ANOVA was performed. If a significant difference (ANOVA, $p< 0.05$) was observed, t-test was used to determine the differences between three treatment means. Multiple regression analysis was performed by using MINITAB for total length, wet weight and condition factor. F-statistics p values also calculated for total length, wet weight and condition factor.

Results and Discussion

No mortality of the experimental fish was observed during the entire study period. Results of average weight gain, average length gain, FCR, PER, SGR and several other growth parameters for the three protein diets are given in Table 1.

Weight gain (WG)
The hybrid fish showed highest weight gain (22.75±3.39 g) in T3 (25% crude proteins) feed group, followed by T2 (20% crude proteins) (13.05±1.99 g), and T1 (15% crude proteins) (9.60±1.56 g) (Table 1).

Length gain (LG)
Maximum mean length gain was observed in T3 (25%) feed group (5.08±1.50 cm), followed by T2 (20%) feed group (3.81±1.40 cm), and T1 (15%) feed group (3.00±1.36 cm) feed (Table 1).

Feed conversion ratio (FCR)
The FCR mean values revealed increasingly poor trend than T3 (25%) feed group (0.99±0.10), in T2

Table 2: *Multiple regression analysis among total length (TL), wet body weight (W) and condition factor (K) of the hybrid (Labeo x Catla) fish.*

Relationships	Treatment Groups	r	a	b1± S.E.	b2± S.E.	r^2	F-test P value
TL= a + b1 W+ b2 K	Treatment 1	0.995***	7.016	0.3023±0.0108	-1.0772±0.0719	0.992	0.000***
	Treatment 2	0.998***	8.070	0.22617±0.00587	-1.1894±0.0452	0.997	0.000***
	Treatment 3	0.999***	11.143	0.10435±0.00585	-1.4649±0.0398	0.999	0.000***
W = a + b1TL+ b2 K	Treatment 1	0.995***	-22.94	3.279±0.117	3.542±0.249	0.991	0.000***
	Treatment 2	0.997***	-35.43	4.401±0.114	5.224±0.270	0.995	0.000***
	Treatment 3	0.996***	-103.63	9.377±0.526	13.62±1.10	0.993	0.000***
K= a+ b1 TL+ b2 W	Treatment 1	0.984***	6.380	-0.9002±0.0601	0.2729±0.0192	0.970	0.000***
	Treatment 2	0.994***	6.745	-0.8323±0.0316	0.18789±0.00972	0.990	0.000***
	Treatment 3	0.998***	7.5971	-0.6791±0.0185	0.07024±0.00567	0.998	0.000***

*(Coefficient of correlation (r); Intercept (a); Regression coefficients (b1, b2); Standard error (SE); Highly significant correlation=***P<0.001; Significant correlation=**P<0.01; Non-significant correlation=>0.05)*

(20%) feed group (1.89±0.09) and in T1 (15%) feed group (2.15± 0.90) (Table 1).

Proteins efficiency ratio (PER)

Highest mean PER value was noted in T3 (25%) feed group (6.98±0.68), followed by T2 (20%) feed group (5.70±0.91), and T1 (15%) feed group (4.86±0.77) (Table 1).

Specific growth rate (SGR)

Highest mean SGR value was observed in T3 (25%) feed group (1.65±0.01), followed by T2 (20%) feed group (1.33±0.32), and lowest in T1 (15%) feed group (0.97±0.07).

Production

The production mean values analysed in the three feed groups confirmed the highest production (16.68±1.92) was gained in T3 (25%) feed group, followed by T2 (20%) feed group (6.28±1.12) and T1 (15%) feed group (3.90±0.88) (Table 1).

ANOVA and t-test Analysis

Mean Initial length, feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR) and % survival of hybrid showed no significant difference (p>0.05) between different treatments. Significant difference (p<0.01) among different treatments was found in mean initial weight, final weight, final length, weight gain, length gain, condition factor (K) and production of hybrid. Highly significant difference (p<0.001) existed in mean final weight, final length, weight gain and length gain of hybrid in T3 than T1 and T2. Values of initial weights were similar in T1 and T3 but significantly differ in T2.

Multiple regression analysis

Multiple regression analysis data between total length, body weight and condition factor are given in Table 2 revealed highly significant correlation (p<0.001) between these growth parameters and F-statistics p value also confirmed strong relationship among above mentioned growth parameters.

Water quality parameters

Different water quality parameters studied and maintained during the experiment are shown in Table 3.

Table 3: *Water quality parameters data.*

Water quality Parameters	Treatments groups	Mean ± S.D	Range
Water temperature(°C)	Treatment 1	27.78±1.00	27-30
	Treatment 2	27.11±0.67	27-30
	Treatment 3	27.44±0.77	27-30
pH	Treatment 1	8.11±0.31	7.6-8.4
	Treatment 2	8.28±0.31	7.6-8.3
	Treatment 3	8.14±0.26	7.6-8.1
Dissolved oxygen (mg/l)	Treatment 1	6.49±0.33	5.1-6.6
	Treatment 2	6.48±0.21	5.1-6.7
	Treatment 3	6.50±0.33	5.1-6.8
Total hardness(mg/l)	Treatment 1	155.16±5.82	147-163
	Treatment 2	155.19±6.19	147-164
	Treatment 3	156.91±4.00	148-164
Transparency(cm)	Treatment 1	24.01±1.90	20-27
	Treatment 2	23.34±1.19	20-27
	Treatment 3	24.07±1.87	20-27

S.D:Standard Deviation.

An essential step in the formulation and preparation of fish feeds is search for protein sources that are cheaper and can be obtained from plant proteins (Hussain *et al*., 2018). Also, it is necessary to gain

knowledge about proteins requirement of different fish species.

In the present study, the fish fed with T3 (25% crude proteins) feed indicated relatively more increase in length, weight, specific growth rate, protein efficiency ratio and feed conversion ratio as compared to T2 (20% crude proteins) and T1 (15% crude proteins) feeds. Normally, higher growth rate in fishes has been obtained with increasing dietary protein levels but after a certain limit growth is suppressed (Ghulam *et al.*, 2005; Kvale *et al.*, 2007). The present study showed significant increase (P<0.01) in weight and length of three feed groups of the hybrid, similar to the results reported by Ahmed and Maqbool (2017), Zeng *et al.* (2021) and Muhammadar *et al.* (2021) showing significant increase in body weight and body length with increasing dietary protein levels, but contrary to the findings of Hasan *et al.* (1997) who had concluded no difference in growth performance of *Cyprinus carpio* when fed upon different plant origin feeds and Khalid and Naeem (2018). In the present study, maximum growth was achieved with 25% crude proteins diet but results may change if further levels of proteins, such as 30%, 35%, etc. are used because many researchers (Bahnasawy, 2009; Giri *et al.*, 2011; Khan *et al.*, 2013; Opiyo, 2014) had concluded maximum growth in fish from 30 to 35% proteins level in feeds, whereas some researchers had also observed the highest growth even above 35% to 40% and uptil 45% protein level in some fish species (Baruah *et al.*, 2015).

The differences in protein requirement among the fish species may be due to difference in methodology of feed formulations, fish size, feeding level and frequency, stocking density, water quality and protein sources in the diet (Kim *et al.*, 2001; Tibbetts *et al.*, 2005). Variations in growth parameters at different protein levels in different stages of fish may be due to several factors, such as fish size, dietary protein quality, stocking density, feeding methodology and environmental impacts (Bahnasawy, 2009). An important fact regarding proteins utilization by fish body is that they cannot metabolize proteins if the level of proteins is above their body requirement (Jauncey, 1982); thus, extra proteins are wasteful for fish. The growth rate of fish becomes reduced because extra proteins metabolization requires higher energy than their deposition in body, so fish body weight decreases due to extra proteins level in feed due to unavailability of necessary non-proteins source which are essential for the deamination of high proteins diet (Kim *et al.*, 2002).

Results of the present study revealed an improving trend in FCR values with increasing dietary protein levels from 15% to 25% proteins diets and this increasing trend is similar to the findings of many researchers (Ahmed and Maqbool, 2017; Ishtiaq and Naeem, 2019; Ahmad and Ahmad, 2020). However, no statistically significant difference (P>0.05) was observed in FCR values of the three (T1, T2, T3) treatment groups and this observation is similar to the findings of Bharadwaj *et al.* (2002) and Khan *et al.* (2013). The FCR value in the present study was lowest for the T3 feed group (0.99±0.10) than T2 feed group (1.89±0.09) as well as T1 feed group (2.15±0.90) and also lower than the value of FCR obtained in the hybrid (*Labeo x Catla*) reported by Kalsoom *et al.* (2009), Najia (2003) in *L. rohita*. In the lowering of the FCR value, important factors suggested are fish health, feed quality, environmental impact and Aquaculture management, so that more flesh will be developed by proper feed contents utilization (Pirali *et al.*, 2014).

Protein efficiency ratio values in the present study revealed an increasing trend with increasing dietary proteins levels, similar to the findings of Daudpota *et al.* (2014), Ahmed and Maqbool (2017) and Ishtiaq and Naeem (2019) who had reported an increase in PER values with increasing dietary proteins level from 25% to 30%; however, this finding is contrary to the reports of many researchers (Wafa, 2002; De Silva *et al.*, 2016) showing a significant decrease in PER with increasing dietary protein levels. Generally, best FCR and highest PER values can be obtained at high protein level feeds (Ahmed and Maqbool, 2017). The values of PER in the present study increased with increasing dietary protein levels but this increase was statistically not different (P>0.05) between treatments; research results of Hasan *et al.* (1997) and Khan *et al.* (2013) had indicated similar trend. Dietary proteins intake impacts both FCR and PER and its conversion into fish weight (Koumi *et al.*, 2009).

An increasing trend in SGR with increasing dietary protein level was observed in the present study was in general agreement with the findings of many researchers (Wafa, 2002; Choudhary *et al.*, 2017). However, in the present study no statistically significant difference (P>0.05) among the three treatment groups was noted. In a previous study, Daudpota *et al.* (2014) had shown decrease in SGR value which is

contrary to present study findings. The present study revealed 100% survival of the hybrid fish. This finding has similarity to many researchers (Iqbal *et al*., 2015; Choudhary *et al*., 2017).

Multiple regression analysis data confirmed highly significant correlations between total length, wet weight and condition factor, similar to the findings of Iqbal and Naeem (2018).

Conclusions and Recommendations

The higher growth rate and lowest FCR in T3 (25%) feed group indicates that the hybrid fish had better growth at higher proteins level than the 20% and 15% proteins feed groups. The study confirms that plant-based protein sources are suitable in the formulation of fish feed. Further investigations using higher proteins levels feed are suggested to elucidate any further improvement in the hybrid weight and mass gain. Further studies should also be conducted in polyculture ponds rather in hapas to study the difference in growth pattern of hybrid.

Novelty Statement

This study will be helpful in the formulation of low cost feeds for fish industries to propagate the culture of hybrid fish (*Labeo rohita* ♀ and *Catla catla* ♂).

Author's Contribution

Rabia Iqbal: Performed experiments, did statistical analysis, and wrote the manuscript.
Muhammad Naeem: Provided guidelines to design the experiment, supervised the research work and helped in reviewing the manuscript.

Conflict of interest
The authors declare that they have no conflict of interest**.**

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