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Optimum protein requirements for the intensive culture of *Labeo rohita* (Hamilton) in glass aquaria

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Abstract: To study the optimum protein requirements for the intensive culture of *Labeo rohita* (Hamilton) reared in glass aquaria during April to July 2009, three iso-caloric pellet feeds were prepared from locally available feed stuffs (rice protein, rice bran and wheat bran) of different protein levels such as 35%, 38% and 40% (dietary protein levels) of 2 mm dia. These feed stuffs were tested for proximate (bio-chemical composition) analysis and the amount of protein was found to be 13%, 12% and 40% respectively. Each feed was supplied at a rate of 8% of the body weight of fish twice a day. The results of the various growth parameters like suitability of protein level requirement, specific growth rate, mean total weight gain, percentage weight gain, feed conversion ratio, survival rate and production showed significantly ($p<0.05$) highest growth and production in feed B (38% gross protein) followed by feed C (40% gross protein) while significantly ($p<0.05$) lowest growth and production was recorded for feed A (35% gross protein). The water quality parameters were recorded throughout the study period and were found within the suitable ranges of fish culture. It is therefore concluded that the pellet feed with 38% (gross protein) is the optimum protein level for the better growth and production of major carp, *Labeo rohita*.

Keywords: Growth and Survival, Suitability of Dietary Protein, *Labeo rohita*.

1. INTRODUCTION

The success of intensive fish culture depends on the formulation of a fish feed that contains an optimum level of protein and energy necessary for the growth of fish and is also cheap. It is obviously necessary to formulate and manufacture fish feeds from locally available feed ingredients. Major carps are the most extensively reared (cultured) in Pakistan. These constitute nearly 40% of the local freshwater species of fish (Doha, 1967). Among the various species of carps, *L. rohita*, *C. catla* and *C. mrigala* have got a very high demand for their palatability. In spite of this, very little efforts have been made to undertake intensive culture of these fish. Various workers have reported the effect of different protein level on different fish species from elsewhere such as Cowey *et al*., (1972) in marine flat fish; Nose and Arai (1972) in Eel, *Anguilla japonica*; Garling and Wilson (1976) in channel catfish *Ictalurus punctatus*, Dabrosky and Wojno (1977) in rainbow trout, *Salmo gairdneri*, Anderson *et al*., (1981) small mouth Bass, Jauncey (1982), Mazid *et al*. (1987) in *Labeo rohita*. Recently Ashraf *et al*., (2008) studied effect of different feed ingredients on growth and survival on *Cirrhinus mrigala*. Present study was an attempted to supply the information on optimum protein requirements for the rearing and commercial farming of *Labeo rohita* from Pakistan.

2. MATERIALS AND METHODS

The ingredients for the experimental pellet were purchased from local markets and from Habib Industries, Hub, Balochistan. These ingredients were brought to the laboratory department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro. All the collected dietary ingredients were ground thoroughly with the help of grinder and sieved to pass through 0.5 mm mesh. The ingredients were mixed according to the formulae given in **(Table 1)**. The well-mixed ingredients were then put into the manually operated pellet machine for the preparation of pellet feed of size 2 mm. The pellets were then allowed to dry in the sun light, packed in air tight bottles in order to protect from moisture and were kept in three plastic bottles marked as Feed A, B and C.

Table 1. Formulation of the experimental diet

Ingredients %	Feed A	Feed B	Feed C
Rice Protein	50	50	50
Rice bran	30	35	40
Rice Milling	15	10	5
Wheat Flour	4	4	4
Salt+Vitamin premix	1	1	1
Total	100	100	100

(a) Proximate Composition of experimental Feeds

The prepared feeds were subjected to proximate composition analysis, according to the methods given in AOAC (1980). The analysis was done in the laboratory Institute of Bio-chemistry, University of Sindh, Jamshoro and the results are shown in **(Table 2)**. The fish feed were made isocaloric and different protein levels of 35%, 38%

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and 40% by different combination of ingredients. Theoretically obtainable percentage of metabolizeable energy obtained from carbohydrate, lipid and protein were calculated at the rate of 4.0 kacl/g of carbohydrate, 9.0 kacl/g of lipid and 4.0 kacl/g of protein as suggested by (Pike and Brown, 1967). The essential amino acid spectrum was determined by descending paper chromatographic method given by Saperstein (1966) modified by Gheyasuddin and Mohafez (1975). Quality of fish feeds in respect of growth rate was analyzed statistically using Duncan's multiple range test.

Table 2. Proximate composition of the experimental feeds

eeds	Moisture %	Crude protein	Crude lipid	Ash %	Crude fiber %	NFE* %
	11.60	35.00	10.50	10.80	10.00	22.10
	10.00	38.05	10.10	10.0	10.60	21.25
	10.00	40.00	10.00	10.80	10.00	19.20

*Nitrogen Free Extract calculated as:
100-% (Moisture + Protein +Lipid +Ash + Crude Fiber)

(b) Collection and Stocking of Fingerlings

The experimental fingerlings of carp, *L. rohita*, were collected from Government Carp Fish Hatchery, Badin where fry were produced by induced breeding. For the feed trial experiment 10 glass aquaria (size 90×30cm) were selected for a period of six months starting from April to July 2009. Three feed regimes (treatments) i. e. Feed A with 35%, Feed B with 38% and Feed C 40% (gross protein) were replicated thrice and one control with no prepared feed. All the experimental fish belonged to the same age group having mean length and weight of 5.5±1.40 cm and 6.4±1.6 g respectively. The experimental fish were stocked at a density of 10 fish/aquaria.

(c) Feeding and Sampling

The experimental feeds were supplied twice daily morning at 9.00 AM and evening at 5.00 PM at a rate of 8% of the body weight. Sampling was done at an interval of one month to adjust the feeding rate, by measuring the weight of fish and to observe the health condition of fish. The length of experimental fish was measured to the nearest mm with an ordinary scale graduated with tenth of centimeters. Weight was measured to the nearest g by a portable electronic balance (Model AK-3000H AFD).

(d) Water Quality Parameters

The water quality parameters like temperature, dissolved oxygen, pH, alkalinity, ammonia and nitrite were recorded monthly throughout the study period with the help of digital portable water quality meter model JENCO 3010 (made in Taiwan).

(e) Statistical Analysis

One way analysis of variance (ANOVA) was used to determine the effects of feed on the growth of carps. This was followed by Duncan's New Multiple Range Test (DNMRT), (Duncan 1995) at 5% level of significance to observe any difference among treatment means.

3. RESULTS

(a) Energy Input

The total obtainable energy (kcal) in 100 g of feed at various protein levels is shown in Table 3. The total energy coming from each of the feeds was 320.0 kcal/100g. In all the feed the major source of energy was protein. The fish feed were made isocaloric and different protein levels of 35%, 38% and 40% by different combination of ingredients. Theoretically obtainable percentage of metabolizeable energy obtained from carbohydrate, lipid and protein were calculated at the rate of 4.0 kacl/g of carbohydrate, 9.0 kacl/g of lipid and 4.0 kacl/g of protein as suggested by (Pike and Brown, 1967). Thus, these diets contained a range of protein: energy (P/ E) ratios from 93.77 to 125.0 mg protein/ kacl. The essential amino acid spectrum was determined by descending paper chromatographic method given by Saperstein (1966) modified by Gheyasuddin and Mohafez (1975) is shown in **(Table 4)**. The highest growth rate of experimental fish was obtained with feed B containing 38% protein with a P/E ratio of 109.37 (Table 5). The rate of growth of all the experimental fish increased almost proportionally up to the protein level and then decreased slowly beyond this level **(Table 5)**. This diet also showed the lowest feed conversion indicating the most efficient utilization of feed at this protein level. This indicated that the fish couldn't utilize excess levels of protein in the diet above the optimum. According to these results, a level of 38% protein in the diet with a P/E ratio of 109.37 is considered optimum for the growth of *L. rohita*,

Table 3. Energy input (kacl) in 100 g of feed at various protein levels.

Name of energy source	Feed A 35% protein	Feed B 38% protein	Feed C 40% protein
Protein	120.00 kacl	140.00 kacl	160.00 kacl
Fat	99.80 kacl	92.30 kacl	84.80 kacl
Carbohydrate	100.20 kacl	87.70 kacl	75.20 kacl
Gross energy content	320.00 kacl	320.00 kacl	320.00 kacl
P/ E ratio	93.75	109.37	125.0

Table 4. The essential amino acid composition of the three feeds

Amino acid	**Gram amino acid per 100 g protein**			
	Feed A 30% protein	Feed B 35% protein	Feed C 40% protein	FAO Reference protein
Lysine	4.26	5.20	6.30	5.50
Leucine	6.35	6. 90	7.20	7.00
Isoleucine	4.11	3.99	3.69	4.00

Valine	4.11	5.10	5.20	5.00
Arginin	4.93	5.00	5.15	----
Phenylalamine	3.64	4.55	5.86	4.00
Methionine	4.32	5.25	5.60	3.50
Histidine	2.00	1.80	1.29	----
Threonine	4.24	4.30	4.35	4.00
Tryptophan	1.29	1.50	1.68	1.00
Total	39.25	43.59	46.32	34.0

(b) Growth Performance of the Fish

The growth responses of carp fed with three different protein levels diets (iso-caloric) in terms of initial and final mean weight gain, percentage weight gain, specific growth rate (SGR), Food Conversion Rate (FCR), survival rate and production of the experimental fishes are presented in the Table 5. The fish with an initial average weight of 6.4 ± 1.6 g reached to a final weight of 135.50 ± 2.66, 110. 30 ± 1.44 and 120.50 ± 2.11 g in feed B with 38% gross protein. Results of these parameters indicated that the feed B containing 38% gross protein shows significantly ($p<0.05$) highest growth in terms all parameters like weight gain, percentage weight gain, specific growth rate, food conversion and production followed by feed C while significantly ($p<0.05$) lowest growth and production was recorded in feed A Table 5. No mortality was recorded (100% survival rate) in the experimental fish throughout the study period.

Table 5. Effects of various feeds on growth parameters of *Labeo rohita*.

Parameters	Feed A (35%)	Feed B (38%)	Feed C (40%)	Control* With out feed
Rearing Period (days)	180	180	180	180
Mean Initial Weight (g)	$6.4^{a1} \pm 1.6$	$6.4^{a1} \pm 1.6$	$6.4^{a1} \pm 1.6$	$6.4^{a1} \pm 1.6$
Mean final weight (g)	$88.29^{a} \pm 2.3$	135.50 ±2.66	100.0 ± 1.2	33.55 ± 1.66
Weight gain (g)	81.89^{a}	129.10	93.60	27.15
(%)Weight gain	1379^{a}	2117	1562	524
SGR(%per day)	1.46^{a}	1.69	1.52	0.92
FCR	4.50^{a}	3.80	4.10	----
Survival (%)	100^{a}	100^{a}	100^{a}	100^{a}
Production Kg/m^2/180 days	5.886^{a}	9.033^{a}	6.666	2.236

*Feed D = Control without feed, only with natural food.

Fig.1. in the same row having same superscripts are not significantly different ($p>0.05$).
Standard deviation

(c) Water Quality Parameters

The water quality parameters and their monthly fluctuations recorded throughout the study period were found with in the suitable ranges for the fish culture **(Table 6)**.

Table 6. Month-wise variation in water quality parameters in glass aquaria throughout the study period

Months	Parameters					
	Temperature (ºC)	pH	D.O. mg/L	Alkalinity mg/L	Ammonia mg/L	Nitrite mg/L
April	27.5	7.30	4.8	152	0.54	0.170
May	28.4	7.33	4.8	180	0.48	0.171
June	29.1	7.30	4.7	160	0.38	0.169
July	30.6	7.45	4.0	170	0.44	0.172

4. DISCUSSIONS

The proximate composition analysis in the present study showed much variation in protein content (35%, 38% and 40% protein) and slight variation in other parameters. Similar observations have been reported by several authors like Rahman *et al*., (1982), Sanaullah *et al*., (1986), Rahman (1989) and Rashid *et al*., (1996). The effect of different protein on various growth parameters like mean weight gain, percentage weight gain, specific growth rate, survival rate and production in the present study showed significantly ($p<0.05$) highest growth and production in experimental fish fed with feed B (38% gross protein) followed by feed C (40% gross protein) while lowest was recorded in fish fed with feed A (35% gross protein). Mazid *et al*. (1987) studied nutritional requirements of *Labeo rohita* and commented that major carp fed with 38% gross protein showed better growth in terms of weight gain. The similar results were obtained in the present study for *L. rohita*. The decreasing trend in growth of *L. rohita*, with the increasing level of protein above the optimum in the present study is similar to those reported for eel (Nose and Arai, 1972), plaice (Cowey *et al*., 1972) and grass carp (Dabrowsky, 1977). Various workers like Sanaullah *et al*. (1986) reported better weight gain in catfish, *Clarias batrachus* fed with 40% gross protein. Rashid *et al*. (1996) published information on *Pangasius sutchi* fed with 35% protein for highest growth and production in cage culture. Yesmin and Mollah (1997) reared African catfish *Clarias gariepinus* with 35% gross protein in low-cost feed and observed good growth, survival and production. Hossain and Parween (1998) reported better growth net gain and production in *Heteropneustes fossilis* fed with 38% gross protein. Nahar *et al*., (2000) studied effect of different food items on growth, survival and production of African catfish fed with 33% gross protein. Narejo *et al*., (2002) worked on the growth performance of snake eel *Pisodonophis boro* and found that the fish fed with 35% protein yielded significantly highest growth as compared to 40% and 45%. Narejo *et al.*, (2003) reported 35% gross protein for the better growth, survival and production for the

rearing of *Mastacembelus armatus* in cemented cisterns. All these above observations support the present study findings. The water quality parameters were recorded throughout the study period and were found with in the suitable ranges as reported by previous authors Rahman (1992), Narejo *et al*., (2002) and Narejo *et al.*, (2003).

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