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Effect of Protein Levels on Growth and Digestibility in the Indian Major Carp, *Labeo Rohita* (Hamilton) Using Slaughter House Waste as the Protein Source

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ABSTRACT

In order to determine protein requirements for optimum growth, four diets with varying protein levels (25 to 40%) using slaughter house waste as the major protein source were formulated and hand fed to the fingerlings of *L. rohita* maintained under laboratory condition. Studies have revealed an increase in live weight gain (g), growth percent in body weight, specific growth rate, apparent protein digestibility, protein efficiency ratio and survival in the fingerlings fed on a diet containing 30% Crude protein in comparison with the fish fed on other diets containing low or high protein levels. Carcass composition also revealed high accumulation of protein and fat in fish fed on a diet containing 30% protein, while those of moisture content remained low. These findings suggest that about 30% Crude protein with 367 kcal/g energy content in diet appears to be sufficient for obtaining optimum growth in *L. rohita* when slaughter house waste (@ 243g/kg of diet) is used as the major source in practical diet.

Key Words: Protein level; Digestibility; Growth; *Labeo rohita* fingerlings

INTRODUCTION

The optimization of fish production requires research into feeding techniques, which promote growth and at the same time reduce the quantity of waste products released in the water. Dietary protein is one of the major determinates of fish growth. Protein constitutes 45-75% of tissue dry matter. The capacity of the fish to synthesis protein *de nova* from carbon skeleton is limited and thus it has to be supplied in diet. Protein levels in successful feeds range from 20-60% to accommodate for differences in the physiological needs of different fish species (Hepher, 1988; Wilson 1989). Further, optimum protein requirements vary with the protein source. Slaughter house waste contains fairly high protein levels. Therefore, in the present study diets were formulated by incorporating slaughter house waste for obtaining different protein levels and attempt was also made to study the effect of feeds on growth and digestibility in the carp, *Labeo rohita***.**

MATERIALS AND METHODS

Experimental fish and acclimation. Fingerlings of *Labeo rohita*, procured from Fish Farm, Dept. of Fisheries, I.G.A.U. were given a prophylactic dip dilute $KMnO_4$ solution before stocking in glass aquaria. After 15 days acclimation to the formulated diet consisting of rice bran and mustard oil cake, the fingerlings were sorted out to almost identical size (Ave. wt. 1.83± 0.02 g) group.

Preparation of experimental diets. Four diets (D_1-D_4) with varying protein levels were prepared by finely powdered, mustard oil cake, rice bran, slaughter house waste, vitamin-mineral mixture and soybean oil. Slaughter house waste in the diets was incorporated at levels of 14.62, 24.32, 34.04 and 43.75% to replace equal proportion of the other ingredients. Physiological fuel values of 3.5, 4.5 and 8.5 kcal/g for carbohydrate, protein and lipid respectively were used for dietary energy calculations (Jauncey, 1982). The proximate composition of the ingredients used in formulated of the diets is given in Table I. The formulation and proximate composition of the test diets are displayed in Table II. Dietary ingredients were cleaned, milled and mixed in definite proportions. Therefore, thick dough was made using lukewarm water. Carboxymethyl cellulose was added as binder, while 1% Cr_2O_3 was added as an indigestible external marker for digestibility estimations. Using a hand pelletizer, 0.5 mm. thick pellets were obtained, while using in feeding experiments.

Experimental design. At the beginning of the feeding trial, the fingerlings of *Labeo rohita*, (Ave. wt. 1.83±0.02 g) were stocked at a density of 10 in each of the specially designed

Table I. Proximate composition of feed ingredients used in formulation of different diets (g/ 100g, as fed basis)

Ingredients	Moisture	Crude protein	Crude fat	Crude fibre	Total ash	NFE
Rice bran	9.0± 0.16	13.56± 0.36	10.22± 0.11	19.33± 0.19	10.53± 0.07	37..36
Mustard oil cake	8.5± 0.10	38.00± 0.50	8.44± 0.12	7.5± 0.10	14.06± 0.04	23..50
Slaughter house waste	7.8± 0.20	65.00± 1.88	6.11± 0.25	2.33± 0.17	7.93±0.02	14. 58

± SEM

glass aquaria (60X30X30 cm.) containing 60 L of dechlorinated bore water. Each dietary treatment consisted of four replications and the experiment continued to 49 days. The initial weight of the fish and proximate composition of the muscle were determined prior to commencement of the experiment. Each group of fish was fed with the respective diet @ 4% of the total body weight twice daily at 8.00-8.30 h. and 16.00-16.30 h. The water from each aquarium was changed daily and replenished with fresh water. The fish were weighed on every 7th day and the amount of ration adjusted accordingly. Any left over food was collected after 3 h of feeding fish, separately from each aquarium and weighed after drying in an oven to determine the feed consumption. The faecal matters voided by the fish were also collected by siphoning method separately from each aquarium. The pooled faecal samples were dried in hot air oven at 60^{0}C and were subsequently analysed for digestibility estimations. At the termination of the experiment, the fish from all the treatments were weighed individually and processed for subsequent analysis. At the end of the experiment, 5 to 7 fish from each treatment were sacrificed and analyzed for proximate composition of whole skeletal muscle.

Analytical technique. Feeding ingredients, experimental diets, faecal matter sample and the fish muscle were analysed for proximate composition following AOAC (1989) procedures. Chromic oxide in the diet and faecal samples were determined spectrophotometrically following Bolin *et al.* (1952).

Water Quality. Water quality was monitored at weekly interval for temperature, pH, dissolved oxygen, free carbon di- oxide and total alkalinity (APHA, 1990)

Data collection.

Percent live weight gain = 100× (BW_f –BWi) / BWi

Specific growth rate (SGR) (% day^{-1}) = [(In BW_f – InBWi) / day on trial] ×100

Where, BWi and BWf were initial and final body weights of the fish, respectively

Average daily gain (ADG) =Duration of experiment (days) / Net weight gain (mg)

Protein efficiency ratio (PER) = Wet weight gain of fish (g) / protein consumed (g)

$$\text{Apparent protein digestibility (APD) (\%)} = 100 - 100 \frac{(\%\ Cr_2O_3 \text{ in diet} \times \%\text{ protein in faeces})}{(\%\ Cr_2O_3 \text{ in faeces} \times \%\text{ protein in diet})}$$

Statistical analysis. Data were tested for significance employing one-way analysis of variance (Snedecor & Cochran, 1968) and Duncans (1955) multiple range test.

RESULTS

Growth. The growth response of rohu fed on diets containing different protein levels (1-4) are shown in Table III. Growth in terms of live weight gain (g), percent gain in body weight and specific growth rate (SGR) were significantly ($p<0.01$) enhanced in fish fed on D_2 containing 30% crude protein. With further increase in dietary protein levels a decrease in live weight gain was observed.

Table II. Ingredients (%) and proximate composition of the experimental diets (% on dry matter basis)

Ingredients (g 100 g^{-1})	Diets			
	D_1 (25%)	D_2 (30%)	D_3 (35%)	D_4 (40%)
Rice bran	58.38	48.68	38.96	29.25
Mustard oil cake	20.00	20.00	20.00	20.00
Slaughter house waste	14.62	24.32	34.04	43.75
Soybean oil	2.0	2.0	2.0	2.0
Vitamin-Mineral mixture*	2.0	2.0	2.0	2.0
Carboxy methyl cellulose	3.0	3.0	3.0	3.0
Proximate composition**				
Moisture	8.30	8.20	8.15	8.35
Crude protein	25.00	30.00	35.00	40.00
Crude fat	10.55	10.15	9.74	9.16
Crude fibre	13.12	11.47	9.82	8.17
Total ash	10.10	9.95	9.61	9.36
Nitrogen free extract	32.93	30.23	27.68	24.96
Metabolizable Energy (k cal 100g^{-1})	363.34	367.22	369.13	369.93

* Vitaminate forte, Roche India Ltd.; ** On dry matter basis

Table III. Growth, Feed utilization efficiency, Digestibility and Muscle composition of *Labeo rohita* fingerlings fed the control and test diets for 49 days

Parameters	D_1	D_2	D_3	D_4
Initial individual wet weight of fish (g)	1.82±0.01	1.83±0.02	1.81±0.01	1.81±0.01
Final individual wet weight of fish (g)	3.79±0.01	4.63±0.02	4.51±0.01	4.27±0.02
Individual net weight gain (g)	1.96±0.01^{d}	2.80±0.01^{a}	2.69±0.01^{b}	2.44±0.02^{c}
Percentage live weight gain	107.39^{d}	153.27^{a}	148.52^{b}	133.52^{c}
SGR (% day^{-1})	1.73±0.01^{d}	2.48±0.01^{a}	2.38±0.02^{b}	2.16±0.07^{c}
FCR	2.50±0.02^{a}	1.89±0.01^{d}	1.93±0.01^{b}	2.07±0.01^{c}
PER	1.61±0.03^{b}	1.75±0.07^{a}	1.48±0.06^{c}	1.19±0.11^{d}
Survival (%)	97.25±2.5^{a}	100±0.0^{a}	100±0.0^{a}	95.0±2.88^{a}
APD	79.76±0.03^{c}	85.03±0.07^{a}	84.52±0.29^{b}	79.01±0.02^{d}
Muscle composition				
Moisture (Initial value: 83.42)	78.64±0.01^{c}	78.24±0.68^{d}	78.75±0.56^{b}	78.94±0.62^{a}
Protein (Initial value: 55.71)	58.34±0.1^{b}	59.21±0.2^{a}	58.34±0.1^{b}	57.77±0.2^{c}
Lipid (Initial value: 10.90)	13.53±0.08^{a}	13.21±0.05^{b}	12.99±0.12^{c}	12.52±0.17^{d}

± SEM; Values in the same rows with different superscripts differ significantly ($P < 0.01$)

Water parameters. Temperature, pH, dissolved oxygen, free CO_2 and alkalinity of the water in the experimental aquaria ranged from 18.7-26, 7.45-7.82, 6.65–7.20 mg L^{-1}, 0.0-2.32 mg L^{-1} and 80-100 mg L^{-1}, respectively.

Nutrient utilization. Feed conversion ratio (FCR) remained significantly low (1.89) in fish fed on D_2 containing 30% crude protein in comparison with the fish fed on low (25%) or high protein (35 and 40%) diet on the other hand, protein efficiency ratio (PER) was significantly ($p<0.01$) high in fish fed on D_2.

Carcass moisture was high in fish fed on D_4, while highest values of proteins were observed in fish fed on D_2, which decreased on further increase in the dietary protein content.

Digestibility. These result have also revealed that digestibility of protein, significantly ($p<0.01$) higher in D_2

and D_3 containing 30 and 35% crude protein, however, with further increase or decrease in protein level a significant decrease ($p<0.01$) in apparent protein digestibility was observed.

DISCUSSION

Present studies on Indian Major Carp *Labeo rohita* has revealed high growth, high apparent protein digestibility and high accumulation of carcass protein in fish fed on a diet containing 30% protein. Significantly ($p<0.01$) low live weight gain, decrease in APD, increase in FCR values was observed in fish fed on diet containing 25% crude protein.

Results on optimum protein levels for *Labeo rohita* are broad agreement with those of Gangadhara *et al*. (1997), Chakraborty *et al.* (1999), Rangacharyulu *et al.* (2000) and Paul and Mohanty (2002) on *L. rohita*. These authors obtained high growth and feed efficiency in fish when fed on a diet containing 30-35% crude protein. Further, growth depressing effect of high protein levels has been reported for rohu (Khan & Jafri, 1992; Chakraborthy *et al*., 1999; Saha & Ray, 1998; Nandeesha *et al*., 2002).

Protein levels above the optimum requirements may result in decreased growth rates because of a reduction in dietary energy available for growth due to the energy required to determinate and excrete the excess of absorbed amino acid (Jauncy, 1981). Present studies, therefore, suggest that optimum protein levels not only reduce the waste input in the holding water but also results in higher growth and better feed efficiency. The results are in agreement to those of Deepak and Garg (1999) on catfish and Kalla (2002) on *C. mrigala*.

In conclusion, the results of this study clearly demonstrate that slaughter house waste supplemented with vitamin-mineral mixture can be recommended as a protein rich supplementary diet for the rohu up to inclusion level of 340 g /kg without sacrificing growth and feed efficiency of the fish.

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