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Effects of feeding rate on growth and biochemical parameters of Indian Major carp fingerlings, *Labeo rohita*

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Abstract
A 120 days experiment was conducted to determine the effects of feeding rate on growth, feed utilization and biochemical parameters of the *Labeo rohita*, fingerlings with an initial average weight of 58 ± 0.4 gm. The rohu fingerlings were randomly distributed into three treatment groups designated as, T-1, T-2, T-3 with one control (T-0) following completely randomized design (CRD). The fish were fed with iso-nitrogenous (25%) diet at the rate of 3% (T-0), 1% (T-1), 6% (T-2) and 8% (T-3) of their body weight (BW) day^{-1}. Weight gain (%) increased almost linearly with increasing feeding rates up to 6% BW day^{-1} beyond which no significant ($P > 0.05$) improvement in weight gain was observed. The specific growth rate (SGR) of rohu fed with 1% BW day^{-1} recorded 0.97% day^{-1} and increased significantly upto an average of 1.50% day^{-1} fed with 6% BW day^{-1}. Feed utilization did not differ significantly ($P > 0.05$) between fish fed at 3% and 6% BW day^{-1}; however, decreased when the rate were increased upto 8.0% BW day^{-1}. Protein efficiency ratio (PER) was o.73 for rohu fed at 1.0% BW day^{-1} compared to a rate of 3.04 for fish fed at 6% BW day^{-1}. Based on the weight gain (%), specific growth rate (SGR), feed conversion efficiency (FCR) and protein efficiency ratio (PER) the feeding rate of 6% BW day^{-1} may be recommended for *L. rohita* fingerlings. These findings indicated that rohu fingerling fed with 6% BW day^{-1} appears to be sufficient for obtaining optimum growth in Indian major carp fingerling, *Labeo rohita*.

Keywords: Growth parameters, feeding rate, IMC, *Labeo rohita*

Introduction
In the last few decades, aquaculture has become one of the fastest developing and promising sector among all the agricultural and allied sectors which provide a source of food, income and livelihood. Aquaculture production is continuing its pace to meet the global aquaculture demand of 119 MMT by 2050 [1] that can contribute to food security and sufficient nutrition for the growing population. However, feed-based culture system is the outmost important requirement to increase the fish production that can provide all the basic nutrients required for growth. Since feed costs are the largest operating expense in aquaculture enterprises [2, 3], optimizing feeding strategy is critical in order to optimize growth, feed utilization, minimize size heterogeneity, limit waste and cut costs [4, 5, 6, 7]. The substantial saving in farm operating cost could be reduced by adopting or optimizing the feeding strategy as feed requirement varies with size of the fish. Because of the high inclusion rate of many nutrients, commercial fish feeds are fairly costly to meet their growth requirements. Overfeeding results in deterioration of water quality, disease proliferation, fish mortality, reduction of feeding and production capacity [8, 6, 9], while underfeeding can lead to mortalities, diseases or decrease of fish growth [10, 11]. On the other hand, feeding less than the amount to achieve optimal growth of fish is also undesirable. Rearing condition, feeding rate, water quality and fish size are the most important factors affecting the growth of fish [12, 13, 14]. Therefore, determining the optimal feeding rate of feeding is important to increase the economic output from the culture activity [15]. Besides, the economic viability of feed and the establishment of feed requirements are crucial to increase production and operational benefits [16, 17, 18].
Among the carps *Labeo rohita* (rohu) is one of the suitable species for aquaculture because of its fast growth, tolerance to variable rearing conditions and fetching good market price. Consumer preference is also very high for this species and found to be tastier among the other Indian carps.

Two factors which determine the economic viability of aquaculture are the growth and feed utilization efficiency of the cultured species, and both are influenced by feeding rate [12, 19, 20]. The information about optimum feeding rates is important not only for promoting good growth and feed efficiency, but also for preventing water quality deterioration as the result of excess feeding. There is currently little information available on the effect of feeding rate on *Labeo rohita* fingerlings cultured under controlled conditions. Therefore, the present study was conducted to elucidate and optimize the feeding rate of *Labeo rohita* based on the growth, feed efficiency and body chemical composition.

Materials and Methods

Experimental design and fish diet

This study was performed for a period of 120 days in a rectangular cement concrete tank (5.5m x 4m x 1m) located at the college of Fisheries, AAU, Raha, Nagaon district of Assam. The tanks were provided with six inch of soil bed. Fingerlings of *Labeo rohita* were procured from the fish farm of the college of Fisheries, AAU, Raha. The experimental fish was acclimatized for a week in the experimental tank and fishes were fed with basal diet @ 5% of their body weight. After acclimatization a sample of 10 nos of fishes were randomly withdrawn from the stock, length and weight measured and recorded for baseline data. There were three treatments and one control designated as T-0 (control), T-1 (1% of BW day^{-1}), T-2 (6% of BW day^{-1}) and T-3 (8% of BW day^{-1}) with triplicate replication following completely randomized design (CRD). All the tanks were randomly stocked with fingerlings of rohu (*Labeo rohita*) at the rate of 8000 fingerlings/ha with average initial weight T-0 (58.0 ± 0.05 gm), T-1 (57.9 ± 0.92 gm), T-2(58.9 ± 0.40 gm) and T-3 (58.9 ± 0.56 gm) respectively. The treatment T-0 was considered as control with fish fed with 3% of their body weight daily. The supplementary feed was prepared containing 25% protein with conventional feed ingredients namely; rice bran, mustard oil cake, wheat flour and fish meal. The feed was fortified with a vitamin and mineral mixture (trade name, Agrimin forte) at 1% of total feed. Feed was given in the corner of the tank in the morning hour and evening hour through tray feeding method. Amount of feed to be given was adjusted at 15 days interval, based on sample weight taken on the basis of at least 50% of the fish stock.

Water quality monitoring

For analysis of different water quality parameters samples were collected from all tanks in the morning 6:00 - 09:00 hours at 15 days interval and analyzed by following the standard methods of APHA [21].

Estimation of proximate composition

The proximate composition of the experimental diets and fish was determined using the standard methods of AOAC [22] for moisture, crude proteins (CP), ether extract (EE), crude fiber (CF) and ash. Moisture was determined by drying samples in an oven at 102°C till to get a constant weight. Crude protein content was determined using an automated Kjeldahl (Kelplus, PELICAN). Crude lipid was determined by the ether extraction method using the Soxhlet apparatus (Model SD2, 1045, PELICAN). Ash content was determined by burning the samples in a muffle furnace (WIT; C and L Tetlow) at 550°C for 2 hr. The crude fiber was determined by Fibre tech (Tulin Equipment).

Calculations

Fish from each treatment groups were weighed and measured individually at the beginning and at end of the experiment. The following variables were calculated:

Weight gain (%)

Weight gain (%) = (Final weight of fish – Initial weight of fish/Initial weight of fish) x 100

Specific Growth Rate (SGR)

$$\text{Specific growth rate (SGR)} = \frac{\ln(W2) - \ln(W1)}{T} \times 100$$

Where, ln = Natural log
W1 = Initial weight of fish
W2 = Final weight of fish
T = Duration of feeding (days)

Feed Conversion Ratio (FCR)

$$FCR = \frac{\text{Mass of food consumed (dry)}}{\text{Increase in mass of animal produced (wet)}} \times 100$$

Protein efficiency ratio (PER) = Wet weight of fish (gm)/protein intake (gm).

Statistical analysis

One-way ANOVA was used to compare the effects of feeding rate on the growth performance and feed utilization. When a significant effect was found, a Duncan's test for multiple comparisons of means was performed. All statistical analyses were conducted using SPSS version 21 and assessed at a significance level of $p < 0.05$.

Results

Water quality

In this experiment the water temperature recorded a minimum of 23.26°C and a maximum of 34.33°C. Dissolved oxygen recorded a minimum value of 4.71 mg L^{-1} and a maximum of 7.25 mg L^{-1} and pH a minimum of 6.83 pH units and a maximum of 8.65 pH units. Regarding the dynamics of water nitrogen compounds, the minimum and maximum concentrations of nitrite and ammonium were as followed: 0.3 and 2.0 mg L^{-1}, 0.1 and 0.4 mg L^{-1} respectively. Hardness recorded a minimum value of 112.64 mg L^{-1} and a maximum of 141.65 mg L^{-1} and Alkalinity a minimum value of 111.07 mg L^{-1} and a maximum of 184.78 mg L^{-1}.

Weight Gain, Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER)

The initial mean weight of the *Labeo rohita* fingerling was not significantly different ($p > 0.05$) for each group. After 120 days of experiment, fish body weight from the treatment T-3 (165 ± 0.71 gm) and T-1 (186.3 ± 0.27 gm) showed no significant differences ($p > 0.05$), while the body weight of the fish from the treatment T-2 (339.03 ± 3.47 gm) is significantly ($p < 0.05$) higher than those from treatment T-0 (238.9 ± 0.75 gm). At the end of the trial, Duncan test divided the four experimental variants into three distinct groups: the body weight of fish from treatment T-3 (165 ± 0.71 gm) being significantly lower than those from T-1 (186.3 ± 0.27 gm), T-0 (238.9 ± 0.75 gm) and T-2 (339.03± 3.47 gm) (Table 1).

Table 1: Growth parameters of *Labeo rohita* fed with various feeding rate.

Growth Parameters	Experimental variants			
	T-0 (Control)	T-1 (1%)	T-2 (6%)	T-3 (8%)
Initial weight (g/fish)	58.9 ± 0.51	57.9 ± 0.92	58.9 ± 0.40	58.9 ± 0.56
Final weight (g/fish)	238.9 ± 0.75	186.3 ± 0.27	339.03 ± 3.47	165 ± 0.71
Weight gain%	311.89 ± 1.30^{c}	222.12 ± 0.45^{b}	508.72 ± 5.98^{a}	184.48 ± 1.22^{d}
SGR	1.17 ± 0.03^{c}	0.97 ± 0.01^{b}	1.50 ± 0.08^{a}	0.87 ± 0.03^{d}
FCR	1.64 ± 0.02^{b}	1.79 ± 0.03^{c}	1.422 ± 0.06^{a}	1.79 ± 0.06^{c}
PER	1.17 ± 0.001^{b}	0.73 ± 0.002^{c}	3.04 ± 0.008^{a}	0.33 ± 0.002^{d}

Data expressed as mean ± SE, n = 3; mean values in each row with different superscripts differ significantly ($p < 0.05$).

The influence of feeding rate on weight gain (WG%), specific growth rate (SGR), feed conversion efficiency (FCR), protein efficiency ratio (PER), are tabled in Table 1. All these indexes were affected significantly ($p < 0.05$) by feeding rate. Both WG and SGR increased significantly ($p < 0.05$) with the increasing feeding rate from 1 to 6%, and then decreased as the feeding rate increased from 6% to 8%. No significant difference was found on growth rate of fish fed 1% and 8% BW day^{-1}). Feed conversion ratio decreased significantly ($p < 0.05$) as the feeding rate increased. Protein efficiency ratio was increases as the feeding rate increased at an optimum level *i.e.*, fish fed with 6% BW day^{-1} and beyond in decreasing rate. Similarly, lower PER values were found in fish fed up to 1% BW day^{-1}.

Table 2: Proximate compositions of feed ingredients on dry matter basis.

Ingredients	Dry Matter (%)	Protein (%)	Lipid (%)	Fiber (%)	Moisture (%)	Ash (%)	NEF (%)
MOC	88.76 ± 0.01	31.50 ± 0.11	8.07 ± 0.05	12.77 ± 0.11	11.24 ± 0.02	6.78 ± 0.05	29.64 ± 0.10
Rice polish	89.85 ± 0.05	12.75 ± 0.02	8.22 ± 0.10	23.16 ± 0.01	10.15 ± 0.05	16.84 ± 0.03	28.88 ± 0.10
Wheat flour	89.84 ± 0.02	12.80 ± 0.01	1.78 ± 0.12	1.36 ± 0.02	10.16 ± 0.02	1.11 ± 0.10	72.79 ± 0.02
Fish Meal	91.45 ± 0.06	51.00 ± 0.05	5.58 ± 0.01	2.21 ± 0.15	8.55 ± 0.06	15.97 ± 0.01	16.69 ± 0.03

*Values of each parameter are mean ±SE of triplicate determinations

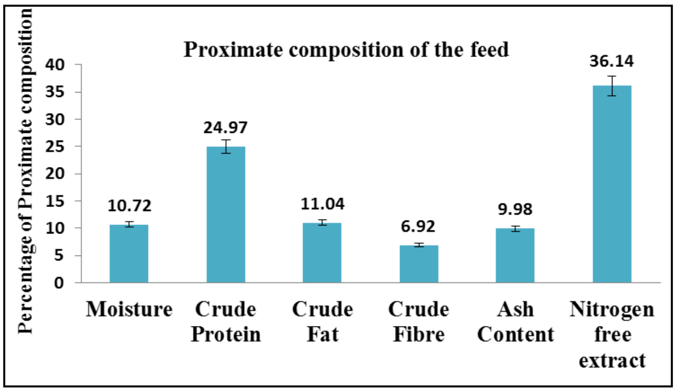

Fig 1: Graphical representation of Proximate Composition of feed.

Table 3: Proximate composition (g/kg wet weight basis) of Whole body, muscle and liver of *Labeo rohita* fed at different rates for 120 days

Parameters	Feeding level (% BW d^{-1})			
	T-0 (Control)	T-1 (1%)	T-2 (6%)	T-3 (8%)
Whole body				
Moisture	73.27 ± 3.6	73.31 ± 4.2	73.24 ± 5.3	73.17 ± 5.5
Protein	15.45 ± 2.4^{b}	16.13 ± 2.3^{a}	16.05 ± 1.7^{a}	14.98 ± 2.2^{b}
Lipid	5.36 ± 1.1^{a}	4.13 ± 1.4^{a}	5.27 ± 1.3^{a}	5.55 ± 1.7^{b}
Ash	5.10 ± 1.5	4.94 ± 1.3	5.16 ± 1.4	5.18 ± 1.4
Muscle				
Moisture	71.9 ± 0.40^{a}	71.7 ± 0.06^{a}	72.6 ± 0.24^{a}	71.5 ± 0.11^{a}
Protein	13.26 ± 0.33^{a}	12.9 ± 0.17^{a}	13.33 ± 0.19^{a}	13.43 ± 0.03^{a}
Lipid	1.5 ± 0.09^{b}	1.3 ± 0.12^{a}	1.5 ± 0.17^{b}	1.6 ± 0.12^{b}
Ash	0.7 ± 0.06^{a}	0.8 ± 0.09^{a}	1.3 ± 0.12^{a}	0.8 ± 0.04^{a}
Liver				
Moisture	63.5 ± 0.12^{a}	62.43 ± 0.03^{a}	62.8 ± 0.09^{a}	64.5 ± 1.6^{a}
Protein	10.67 ± 0.14^{a}	10.47 ± 0.09^{a}	10.83 ± 0.03^{a}	11.43 ± 0.12^{a}
Lipid	15.67 ± 0.15^{b}	9.6 ± 0.20^{a}	25.27 ± 0.23^{c}	28.57 ± 0.18^{d}
Ash	0.77 ± 0.9^{a}	1.3 ± 0.12^{a}	0.7 ± 0.06^{a}	1.3 ± 0.09^{a}

Data expressed as mean ± SE, n = 3; mean values in each row with different superscripts differ significantly ($p < 0.05$).

The proximate compositions of different feed ingredients are tabled in Table 2. Proximate composition of the feed found as 10.72 ± 0.09% moisture, 9.98 ± 0.01% ash, 24.97 ± 0.01% crude protein, 11.04 ± 0.02% crude fat, 6.92 ± 0.02% fiber and 36.14 ± 0.03 nitrogen-free-extract. Graphical representation of proximate composition of the feed is shown in Figure 1.

The proximate compositions of whole body, muscle and liver are shown in Table 3. Protein and ash contents of the whole body were not affected by feeding rate (p>0.05). Moisture in whole body were not significantly ($p < 0.05$) affected with the increasing feeding rate, and conversely, lipid increased significantly ($p < 0.05$) with the increasing feeding rate. Moisture content of muscle from fish fed 6% feeding rate was highest in all treatments and there had no significant differences among the other three treatments. Protein content of fish fed 1% feeding rate was lowest in fish muscle and liver and there was no significant differences among the other treatments. The lipid content of muscle was lowest in 1% and highest in 8% feeding rate treatment, and no significant differences were found among 3%, 6% and 8% feeding rate treatments. Liver lipid content increased with the increase of feeding rates, and especially when feeding rate exceeded 1%, liver lipid increased sharply and was even about three times more than lower feeding rate (1% BW day-^{1}).

Discussion

Feeding fish at required ration level promotes growth which is both economical and sustainable. Both underfeeding and overfeeding have serious economic concerns in aquaculture. In this study, lower feeding rates (1% BW day^{-1}) failed to support the growth performance than that achieved by the groups fed at 3% and 6% BW day^{-1}. The better growth rate accompanied with inferior protein gain is the indicator of overfeeding where feed wastage may cause water quality problems beside costs. It is well known that at higher feeding ratio fish can easily increase the chance to access food and use the provided energy for growth [23], while in lower feeding rate fish do not have enough energy to sustain their normal growth or the dietary nutrients are used for maintenance [24]. On the other side, according to Brett [12] and Cacho *et. al.*, [25] fish maximum growth occurs at the limit of voluntary food intake (satiation), while maximum feed efficiency occurs at some level below satiation. Our results are supported by the statements of these authors, being observed that in the treatment T-2, where fish were fed with 6% BW day^{-1}, the obtained FCR values and weight gain (%) were better than those obtained in other treatments. As in practice it is not possible to formulate diet that give 100% response, we have taken a percentage as 95% of the maximal quadratic plateau [26] to determine the optimum feeding rate for fingerling *L. rohita*. Based on the one way ANOVA optimum feeding rate of fingerling Rohu was found to be 6% BW day^{-1} which is higher than that reported for grass carp, *Ctenopharyngodon idella* [27], Nile tilapia, *O. niloticus* [28], tropical bagriid catfish, *M. nemurus* [29], walking catfish, *C. batrachus* [30], Clarid catfish hybrid, *Clarias gariepinus*, *Hetrobranchus bidorsalis* [31], yellow flounder, *L. ferruginea* [32] and lower than that reported for mrigal, *C. mrigala* [33], Cuneate drum, *N. miichthioides* [34], African catfish, *C. gariepinus* [35], Singhi, *Heteropneustes fossilis* [36]. The wide variations observed in the feeding rates of the fish species may be because of the methodological bias imposed by experimental design and conditions such as the model used to estimate requirements [37, 38], size/age differences of fish and experimental conditions such as stocking rate, feeding regimes or water quality [12, 39, 40]. The nature of the protein sources of test diets, the reference protein whose amino acid pattern is being emulated [41], fish size, culture protocols and different overall levels of performance by the fish [10] have direct influence on feeding rate of the fish. As has been reported that feeding fish ration greater than optimum level would increase the feed wastage, feed conversion ratio and deteriorate water quality [42, 43].

In this study, reduced growth at lower feeding rates (1% BW day^{-1}) suggested that the nutrients in such diets were just enough to support maintenance requirement only. This is in accordance to the findings of various other workers [8, 33, 36]. However, significant increase in growth of fish fed at 3% and 6% BW day^{-1} indicates that large portion of nutrients were utilized for growth and tissue building besides that utilized for maintenance. Growth retardation in fingerling rohu fed at 8% BW day^{-1} was due to over burden of dietary nitrogen in the body requiring its elimination which is done at the cost of deposited nutrients which is confirmed by the decline in protein at these feeding rates. The relationships between ingested nitrogen and excreted total ammonia nitrogen are well documented in teleost fish [44, 45, 46, 47] and the fate of the excess protein from the diet is its catabolism to produce ammonia.

The body moisture, ash and lipids were significantly affected by the feeding rate. Meat moisture contents were not affected by feeding rates whereas body lipid and ash contents had an increase with increase feeding rates. According to Love [48] the decrease of body moisture and increase of body lipid contents with increasing feeding rates to the optimum it is a well-established inverse relationship for many species of fish. Some authors showed that a lower body lipid content in fish meat is the result of choosing a lower feeding rate than optimum rate, while others suggests that a higher feeding rate than needed for maintenance requirement, lead to storage of excessive energy accumulates in the muscle and liver, mainly in the form of lipid [49, 50, 6]. In this study, body protein content wasn't affected by the chosen feeding rates. Although, the protein content in muscle tissue was lower in feeding rate of 1% BW day-^{1}, the supply of dietary protein was enough to maintain the body protein level as long as the fish from this experimental variant recorded an increase of body weight.

Conclusions

Considering the economic importance of cost in terms of feeding in culture system, an understanding of effective assimilation of feeding is very important. In conclusion, findings of the study suggests that the feeding rate of 6% BW day^{-1} is more efficient feeding strategy for the growth of *L. rohita*, since it provides better growth and production at low cost feeding.

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