Research Article

Restricted feeding strategy in *Labeo rohita* fingerlings: Effects on growth, feed utilization and body composition

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ABSTRACT

A 90-day feeding trial was conducted to study the effect of qualitative and quantitative restricted feeding strategies on nutrient utilization and growth performance of rohu, *Labeo rohita*. Two experimental iso-caloric (355.82 - 357.58 kcal/100g) diets with two crude protein levels of 30% and 25% designated as diet A and B, respectively were prepared. One hundred and eighty fingerlings (3.95±0.06g)were distributed into five treatments in triplicates. The experimental design consists 1. Continuous feeding of diet A (T30, C); 2. Continuous feeding of diet B (T25); 3. Alternate feeding of diet A and diet B (T30/25); 4. Alternate day satiation feeding and starvation of Diet A (T30-1);and 5. Alternate day satiation feeding and starvation of Diet B (T25-1). Feeding was done twice daily throughout the experimental period. Results revealed that the dietary feeding regimes significantly affected growth parameters (P<0.05). However, growth performance in terms of specific growth rate (% SGR) and weight gain percent were similar among T30 (C), T30/25 andT30-1 groups. The FCR and PER of fish in dietary regimes T30-1 and T25-1 were better than feeding regime group of T30/25. Overall results of present study indicated that continuous feeding of 30% crude protein exhibited similar growth with that of 30% crude protein feeding followed by starvation(T30-1). From the economic point of view, feeding 30% of protein followed by one day starvation can be an alternative feeding strategy for grow-out culture of rohu.

Keywords: Alternate Feeding, Feed Management, *Labeo rohita*, Satiation Feeding, Starvation

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INTRODUCTION

Aquaculture remains a growing, vibrant and important production sector for high protein food. One of the major problem in rapidly growing aquaculture is the availability of fish feed, which accounts for more than 50% of the total production cost in intensified culture systems (Sehagal and Toor, 1991; De Silva, 1992). Diet modification approach to reduce cost of feed by replacing animal protein sources such as fish meal by plant protein sources have not resulted in comparable growth rates (Keembiyhetty and De Silva, 1993). Hence, feeding management strategies for improving the nutrient utilization, especially protein, can reduce feed cost per unit fish production (De Silva, 1985, 1989). Quantity and quality of feed consumed have a prominent effect on the growth rate, efficiency of feed utilization, and chemical composition (Lovell, 1989; Pickering, 1993). Considerable research effort has been made to determine the quantity and quality of dietary protein necessary to achieve optimum performance of fish. The significance of qualitative and quantitative feeds is well recognized (Luo et al., 2004; Tibbetts et al., 2005). Increase in dietary protein and feed ration has often been associated with higher growth rate in many species. However, there is a certain level beyond which further growth is not supported, and may even decrease (Gunasekera et al., 2000; Yang et al., 2002; Abbas et al., 2005; Debnath et al., 2007; Kvale et al., 2007) growth rate.

De Silva (1985) postulated that an improvement in nutrient utilization can be obtained by the application of a mixed protein schedule where a high-protein diet is alternated with a low-protein diet. This is because of protein digestibility varies on a day-to-day basis in a certain rhythmic manner in Nile tilapia (*Orechromius niloticus*) (De Silva and Perera, 1984). To reduce the feed cost and improve nutrient utilization, mixed protein schedules have been demonstrated for Indian carp (*Labeo rohita*) (Nandeesha et al., 1993, 1994). Thus, research in aquaculture nutrition is being directed towards the improvement of feeding practices and schedules (Eroldogan et al., 2004; Gomez-Requeni et al., 2004; Eroldogan et al., 2006a, 2006b). Fish farmers used to practice satiation feeding to get maximum production. However, stocking density, temperature and culture system affects the feeding behaviour of fish. Theoretically, the maximum feed utilization of fish occurs at a feeding rate above the maintenance feeding level but below the maximum or satiation feeding level (Eroldogan et al., 2004; Rowland et al., 2005). Under restricted feeding conditions, growth performance may change. Usually the best feed conversion efficiencies have been reported below satiation level (Van Ham et al., 2003; Eroldogan et al., 2004). For commercial fish farming, restricted feeding without growth suppression is preferable for economic and environmental reason, as well as for better final product quality. Furthermore, such feeding schedule could improve management of personnel, time and water quality, with reducing feed and labour costs (Gaylord and Gatlin, 2001; Eroldogan et al., 2006a).

Indian major carps (IMCs) and Exotic carps are considered to be the major aquaculture species in tropical countries, contributing to more than 97% of the total freshwater aquaculture production. Among IMCs, *Labeo rohita* has high demand and preferable fish in domestic market. Therefore,the present study aimed to evaluate the effect of qualitative and quantitative restricted feeding regimeson the growth performance and body composition of *Labeo rohita* fingerlings. Most of the work on fish nutrition has been in the area of mixed feeding schedules achieved by either alternate high and low protein diet or alternate high and low ration (Nandeesha et al., 1993, 1994, Toket al., 2017 and Kumar et al., 2012). However, minimal work has been done on combined alternate protein diet and ration. In view of this, the present study was conducted to address, whether adoption of qualitative and quantitative restricted feeding regimes can improve the nutrient utilization and feeding cost against a constant high protein daily feeding.

MATERIAL AND METHODS

Experimental animal

Labeo rohita fingerlings were procured from Hans Aquaculture Fish Farm, Raigad district, Maharashtra, India. The fish were transported in a circular container (500 L) with sufficient aeration to the wet laboratory of Central Institute of Fisheries Education, Mumbai. They were carefully transferred to another circular tank (1000 L capacity) and left undisturbed for overnight. In order to ameliorate the handling stress, the fish were given a mild salt treatment (3% NaCl) the next day. The fish stock was acclimatized under aerated conditions for a period of 15 days and fed with a practical diet containing 30% crude protein.

Experimental diet

Two experimental isocaloric (355.82 - 357.58 kcal/100g) diets with crude protein levels of 30% and 25% designated as diet A and diet B, respectively were prepared. Ingredients were finely ground and mixed thoroughly with water to make dough, and steam cooked in pressure cooker at 15 psi for 15 min. The vitamin mineral mixture was mixed after cooling dough. Finally, dough was pressed through a pelletizer having 2 mm diameter size. After air drying, the pellets were kept in a hot air oven at 400C till complete drying (final moisture level <12%). After drying the pellets were packed in polythene bags and were labeled according to the treatment codes. Formulation and proximate composition of the experimental diets is shown in Table 1.

Table 1: Formulation and proximate composition of the experimental diets (% DM basis)

Ingredient	Diet A‡	Diet B§
Fish Meal	20	15
Defatted Soya	14	11
GNOC	12.5	10
Rice Flour	13	18
Corn Flour	18	22
Wheat Flour	12	13.5
Oil mix	6	6
Premix*	2	2
CMC†	1.5	1.5
Betaine chloride†	0.9	0.9
BHT†	0.1	0.1
Proximate composition		
Moisture	9.40±0.10	10.35±0.05
Dry matter	90.59±0.10	89.65±0.05
Crude protein	30.05±0.06	25.04±0.08
Ether extract	6.01±0.05	6.03±0.02
Ash	9.14±0.01	7.73±0.01
Nitrogen free extract	40.56±0.43	45.63±0.18
Crude fibre	4.82±0.06	5.21±0.09
Digestible Energy**	355.82±0.19	357.58±0.35

* Composition of vitamin mineral mix (PREMIX PLUS) (quantity/2.5kg) Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6 mcg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 L- lysine, 10 g; DL- Methionine, 10 g; Selenium, 50 ppm; † Sd Fine Chemicals, Mumbai, India.‡ Diet - A (30% CP); § Diet - B (25% CP); ** Calculated Digestible Energy (K cal/100g) = (% CP x4) + (% EE x 9) + (% TC x 4) (Halver, 1976).

Experimental design

Experiment was conducted in 15 plastic rectangular tubs (65 X 48 X 45 cm, 140L capacity) covered with perforated lids. One hundred and eighty (180) fingerlings (3.95±0.06g)of *L. rohita* were randomly distributed into fiveexperimental groups in triplicates, following a completely randomized design (CRD). Twelve fishes with total weight ranging from 45.10 g to 46.90 g were stocked in each plastic tub with 100 L chlorine free bore well water. Aeration was provided for 24 h in all the experimental tubs. The total volume of the water in each tub was maintained at 100L throughout the experimental period. Feed was given to satiation twice in day at 8:00 and 18:00 hours. Feeding strategies for different treatment groups is shown in Table 2.

Water quality parameters

The water temperatures of all the experimental tubs were recorded using a temperature probe (MERCK, Germany). The pH was measured by a digital pH meter (LABINDIA) in all the experimental tubs. The dissolved oxygen was measured by membrane electrode method using dissolved oxygen meter (MERCK, Germany) for all the experimental tubs. The dissolved free carbon dioxide was measured by titrimetric method (APHA, 1998) and calculated using the following formula. Carbonate hardness was estimated by carbonate hardness test kit (Carbonate hardness test, MERCK, Germany) and expressed as mg L^{-1}.Un-ionized ammonia concentration was estimated spectrophotometrically at 635 nm wavelength as APHA (1998) and compared with standard graph. The concentration was expressed as mg L^{-1}. Nitrite concentration was estimated spectrophotometrically at wavelength of 543 nm as APHA (1998) and compared with standard graph. The concentration was expressed as mg L^{-1}. Nitrate concentration was estimated spectrophotometrically at wavelength of 543 nm as APHA (1998) and compared with standard graph. The concentration is expressed as mg L^{-1}. The physiochemical water parameters like temperature (22.5-28.3^{0}C), pH (7.5-8.4), dissolved oxygen (6.5-7.8mgL^{-1}), free carbon dioxide (not detectable), carbonate hardness (233-241mgL^{-1}), ammonia-N (0.07-0.10 mgL^{-1}), nitrite-N (0.001-0.005 mgL^{-1}), nitrate-N (0.02-0.05mgL^{-1}) were maintained within the optimum range as required for the *L. rohita* fingerlings. Growth was monitored at every 15-day interval by collectively weighing each group of fish. Fishes were starved overnight before taking the weight. The weight was taken in an electronic balance. Weight gain (%), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were determined for each group using the standard formula:

Weight gain (%) = [final weight- initial weight/initial weight]×100
SGR (%) = [ln final weight- ln initial weight/number of days]×100
FCR = feed given (dry weight)/body weight gain (wet weight)
FCE = net weight gain (wet weight)/feed given (dry weight)
PER = net weight gain (wet weight)/protein fed
Hepatosomatic index = [liver weight/weight of fish]×100
Gastrosomatic index = [gastrointestinal tract weight/weight of fish] ×100

Table 2: Feeding strategies during the experimental period

Treatment	Condition
T30 (C)	Diet A (30%) throughout the experiment
T25	Diet B (25%) throughout the experiment
T30/25	Diet A (30%) followed by Diet B (25%) in alternate days
T30-1	Diet A (30%) satiation throughout the experiment with alternate days starvation
T25-1	Diet B (25%) satiation throughout the experiment with alternate days starvation

Sampling and chemical analyses

At the beginning of the trial, a pooled sample of 10 fishes were collected to analyze initial carcass composition. At the end of 90 days, four fish, were randomly sampled from each tank were anaesthetized with CIFEcalm 0.03ml L^{-1} herbal anesthetic (developed at Central Institute of Fisheries Education, Mumbai) and then killed. Diet, ingredients and carcass samples were analyzed (Table 1 and Table 5) for dry matter (DM), ash, crude protein (CP, %N x 6.25) by the Kjeltec semi-automatic system (2200 Kjeltec Auto Distllation, Foss Tector and Sweden) and total lipids by Soxlet apparatus (AOAC, 1995) and Digestible energy content of samples was measured.

Statistical analysis

The data were statistically analyzed bystatistical package SPSS version 16. Comparison between two treatments was made using Duncan's Multiple Range Test (DMRT). Comparison among all the treatment was done by one way ANOVA. Comparisons were made at the 5% probability levels. All the observed responses were expressed as mean±SE.

RESULTS

The survival was not affected irrespective of feeding regime (P>0.05). A 100% survival was observed throughout the experimental period.The dietary feeding regimes significantly influenced growth parameters (P<0.05; Table 3). However, there was no significant difference in weight gain percentage and SGR among T30 (C),T30/25 andT30-1 groups. (Fig. 1).

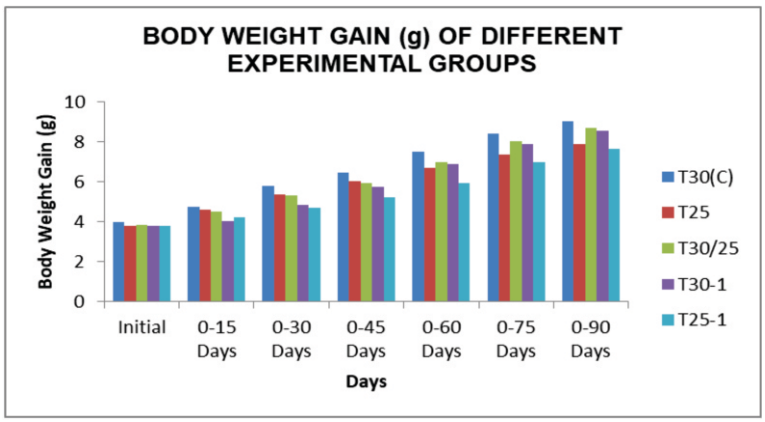

Fig 1: Body weight gain of different experiment group during 90 days of experimental period

The lowest FCR (2.55±0.02) was recorded in T30-1 feeding regime which was significantly different from all other treatment groups. Whereas no significant difference between T30 (C) and T30/25 groups. The highest FCR (3.35d±0.07) was found in T25 group which was significantly different from other groups.The higher PER values were recorded in T25-1 (1.44±0.01) and T30-1 (1.30±0.01) groups which were significantly differ from other groups ($P<0.05$). There was no significant difference between PER values of T30/25 (1.22±0.03) and T25(1.19±0.02). The lowest value was found in T30 (C) (1.10±0.01) group.The HSI and GSI of different experimental groups varied significantly ($P<0.05$) (Table 4). Higher values of HSI and GSI were observed inT30 (C), T25 andT30/25 which were significantly ($P<0.05$) different from T30-1 and T25-1 groups. Whole body moisture, crude protein, lipid and carbohydrate content were significantly different among all treatments except ash content (Table 5). The groups fed (T25 and T25-1) dietary regime showed lesser whole body crude protein than the groups fed (T30 (C), T30/25 and T30-1) dietary feeding strategy. Moisture content of restricted groups (T25-1 and T30-1) were higher than continuous feeding groups (T30 (C), T30/25 andT25) which is inversely related to whole body lipid content of fish.

Table 3: Growth parameters (% wt. gain, SGR, FCR, FCE, PER) of *Labeo rohita* fingerlings fed with different experimental diets

Treatments	% Wt. Gain	SGR*	FCR†	FCE‡	PER§
T30 (C)	128.36^{b}±2.84	0.91^{b}±0.01	3.00^{c}±0.04	0.33^{b}±0.00	1.10^{a}±0.01
T25	108.89^{a}±3.02	0.81^{a}±0.01	3.35^{d}±0.07	0.30^{a}±0.00	1.19^{b}±0.02
T30/25	125.99^{b}±3.71	0.90^{b}±0.02	2.98^{c}±0.08	0.33^{b}±0.00	1.22^{b}±0.03
T30-1	126.53^{b}±1.56	0.90^{b}±0.00	2.55^{a}±0.02	0.39^{d}±0.00	1.30^{c}±0.01
T25-1	102.30^{a}±3.92	0.78^{a}±0.01	2.77^{b}±0.03	0.36^{c}±0.00	1.44^{d}±0.01

Table 4: HSI and GSI of *Labeo rohita* fingerlings fed with different experiment diet

Treatments	HSI*	GSI†
T30 (C)	0.87^{ab}±0.03	1.68^{b}±0.07
T25	0.95^{ab}±0.03	1.78^{b}±0.07
T30/25	0.90^{b}±0.01	1.55^{ab}±0.02
T30-1	0.78^{a}±0.02	1.37^{a}±0.01
T25-1	0.76^{a}±0.02	1.34^{a}±0.05

Different superscripts in the same column signify statistical differences (P<0.05) (mean ± S.E.) (n = 6). * Hepato-somatic index = (weight of liver/weight of whole fish) x 100; † Gastro-somatic index = (weight of intestinal tract/weight of whole fish) x 100.

Table 5: Proximate composition of the whole body of *Labeo rohita* fingerlings fed with different experimental diets (% WM basis)

Treatments	Moisture	DM*	CP†	Lipid	Ash	TC‡	Digestible energy§
Initial	75.19±0.09	24.80±0.09	17.02±0.08	3.92±0.08	2.75±0.02	1.11±0.05	107.81±0.67
T_{30} (C)	71.17^{a}±0.06	28.82^{b}±0.06	18.22^{b}±0.14	6.56^{d}±0.06	2.48±0.05	1.55^{a}±0.10	138.16^{c}±0.40
T_{25}	71.19^{a}±0.00	28.80^{b}±0.00	17.12^{a}±0.29	6.13^{c}±0.14	2.36±0.07	3.18^{c}±0.20	136.44^{bc}±0.82
$T_{30/25}$	71.33^{a}±0.17	28.66^{b}±0.17	18.65^{b}±0.18	5.74^{b}±0.06	2.42±0.06	1.84^{ab}±0.21	133.65^{b}±1.07
T_{30-1}	71.92^{ab}±0.50	28.07^{ab}±0.50	18.42^{b}±0.23	5.11^{a}±0.07	2.29±0.06	2.25^{b}±0.31	128.68^{a}±2.34
T_{25-1}	72.25^{b}±0.07	27.75^{a}±0.07	16.57^{a}±0.16	5.00^{a}±0.06	2.46±0.01	3.71^{c}±0.06	126.16^{a}±0.33

Mean values in the same column with different superscript differ significantly (P<0.05).* DM- Dry Matter; † CP-Crude Protein; ‡ TC-Total Carbohydrate; § Calculated Digestible Energy (K cal/100g) = (% CP x4) + (% EE x9) + (% TC x4) (Halver, 1976).

DISCUSSION

Daily feeding with a constant rate of feed or protein is the most widespread practice in fish farming. However, (Crampton, 1991) argued that it may not be necessary to feed daily in order to obtain maximum growth rates. Protein level and feed ration are the most important factors that influence the growth, survival, and yield of fish as well as the economics of a farming industry, by influencing the feed cost, which is typically the largest operational cost. The result of the current study demonstrated that there was no significant difference in weight gain% among fish fed the diet containing 30% crude protein (T 30-C) or fish fed 30% and 25% crude protein (T 30/25) feed alternatively and 30% protein feeding followed by starvation for alternate days (T 30-1), as similar to the findings of (Amin *et al*., 2012) in sutchi catfish, this may be due to the increase in feed utilization during starvation episodes, a hyperphagic response during the re-feeding in (T 30-1) treatment and variation in digestibility of feed varies from day to day, following an apparent cyclic pattern (De Silva, 1985) in T 30-25 groups.

The results were similar to the findings of (Koppe *et al*., 1993) who reported that the feed conversion efficiency and protein and energy utilisation of alimented fish were superior to controls fed at satiation levels in *Piractus brachypomus*. Boujard *et al*., 2000 reported that compensatory growth caused by an increase in growth efficiency with no hyperphagic response in rainbow trout. Increased feed utilization can be evidenced from the values of FCR and PER. It is seen that lower feed conversion ratio is reported at feeding levels below the maximum or satiation (Eroldogan *et al*., 2004; Rowland *et al*., 2005). Under restricted feeding conditions, growth performance may change. Usually the best feed conversion efficiencies are obtained below satiation (Van Ham *et al*., 2003; Eroldogan *et al*., 2004). In the present study lower FCR was found in fish fed with 30% protein followed by fasting for alternate days (T 30-1) and 25% protein feeding followed by starvation for alternate days (T 25-1). This may be due to quantitative feed restriction in both treatments and higher digestive enzymatic activities which is likely to occur in restricted feeding regimes (Jobling, 1994; Eroldogan *et al*., 2008).

In the present study, the PER is not similar to the growth trend; whereas it varied inversely with amount of crude protein in the diet. Higher PER recorded in restricted feeding groups (T 30-1 and T 25-1) suggests that lower level of dietary proteins, efficiently utilized for growth rather energy production. Similar trends were also noticed in common carp (Nandeesha *et al*. 1995, 2002); sutchi catfish and silver carp (Ali *et al*., 2005). Higher PER in T 30-1 and T 25-1 treatments may be due to decreased protein input (Jauncey, 1982) or increase in protease activity. Several finding showed that restriction of food may lead to an increase in enzyme activity of different sections of intestine (Harpaz *et al*., 2005; Krogdhal *et al*., 2005). Metabolic demand for amino acids would be elevated due to higher rates of protein accretion during starvation. Similarly, a 3 days starvation and then 11 days re-feeding with apparent satiation increased rates of protein accretion in channel cat fish, *Ictalurus punctatus (*Gaylord and Gatlin, 2001*).*

Feeding regimes followed in the present study, results in a complex series of morphological and biochemical changes in body compositions of fish. The carcass protein level in different treatments correlates to the weight gain percentage and body lipid content of restricted feeding groups that are lower than the continuous feeding groups. This may be due to protein sparing effect by lipids during starvation. Accordingly, Weatherley and Gill, (1987) , suggested that the fish during starvation satisfy the energy demand by utilizing body lipid stores, results in increase in protein accretion. The present feeding regimes results in reduction of the percentage lipid content and an increase in the tissue water during starvation, indicating the inverse fat –moisture relationship (Xie *et al*., 2001; Xiao *et al*., 2012). because an increase in tissue hydration plays a role in the limitation of the loss or even the maintenance of wet body mass during starvation (Ali *et al*., 2003).

Lower HSI and GSI in restricted feeding groups (T 30-1 and T 25-1), in the current study, indicates that the maximum utilization of stored energy reserves (lipids or glycogen) during starvation to compensate energy metabolism. Our observation is in accordance with observations from several researchers with different species, suggests importance of the liver during short periods of starvation feeding regimes (Jobling, 1980; Ali and Jauncey, 2004; Eroldgan *et al*., 2008). Other possible reason for the values of HSI and GSI in different treatments may be related to dietary protein and carbohydrate levels. HIS and GSI values are believed to be inversely related to the dietary protein levels and positively related to the dietary carbohydrate levels (Brown *et al.*, 1992; Yang *et al.*, 2002; Chao *et al*., 2020) due to the deposition of glycogen in the liver.

CONCLUSION

The present study observed that all the fish subjected to qualitative and quantitative restricted feeding schedules were unable to maintain the same growth parameters as control fish. Taking the observed weight gain%, SGR, FCR, PER all feeding regimes in account, 30% crude protein feeding followed by starvation for one day may be an economical strategy for fish production in grow out ponds.

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