Effect of Feeding Frequency on Growth and Nutrient Retention in Cirrhinus Mrigala (Daud) Fingerlings under Laboratory Conditions-Effect on Nitrogen Retention and Excretion of Metabolites

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Abstract:

Studies were conducted to determine the effects of feeding frequency on growth performance and some bioenergetic parameters on fingerlings of Indian major carps. First experiment was conducted on the fingerlings of Cirrhinus mrigala (Mean body weight 4.30±0.050g) under laboratory conditions. Four feeding frequency groups were set up: feeding once (T1), twice (T2), thrice (T3) and four (T4) times d-1. At the end of 75 days, higher mean body weight (4.4±0.22g), specific growth rate (0.95 ± 0.0g) and nutrient retention (GPR=30.85 ± 1.77 and GER=26.06 ± 1.17) were observed in treatment T4 which were significantly (P0.05) affected. Water quality, nutrients and productivity status of ponds revealed favourable levels and appear to have been affected by feeding frequency. Based on these observations, it appears that, feeding three times a may be accepted as sufficient for growing Indian major carp species.

Keywords:

Mrigal, Feeding Frequency, Growth performance, Feed Conversion Ratio. Carcass Composition, Excretion of Metabolites, Water Quality, Pond Productivity

1. Introduction

If aquaculture is to continue to grow, it is imperative that an eco-friendly and economically viable system has to be developed. For developing such a system, delivery of the inputs in the form of feeds and fertilizers have to be regulated. In fish farming, feeding presents the largest part of expenses in intensive and semi-intensive aquaculture as feed represents more than 40-70% of the total operational expenditure of the operating cost of an aquaculture enter-prise. Fish farmers are striving hard to minimise feed losses to improve farm profitability and minimize adverse effects on water quality and fish health. Even though many models are available for calculating daily feed ration [1] but the farmers are still facing the problem of how and when to deliver the ration so that the fish are able to utilise it in an optimal way.

The amount of the daily feed intake, frequency and timing of the delivery of ration are the key factors of feed management strategies, influencing growth and feed conversion efficiency [2, 3]**.** Further, optimal feeding frequency may vary depending on species, age, size, environmental factors, husbandry and feed quality [2]**.** Several studies have been conducted to evaluate the effects of feeding frequency on growth, survival, feed utilization and body composition in many edible and non-edible fish species [4-10].

Feeding and ingesting are the final result of a number of interacting factors between the fish (senses and hormonal systems) and its environment (stock density, size range or variability, season, temperature, day length and time of the day). Times of feeding and feeding frequency have been reported to affect feed intake and growth performance in a number of fish species [11-13]. Feed management in terms of optimization of feeding rate and frequency has emerged as one of the crucial areas of research in the field of aquaculture. Overfeeding and leftover food disrupts the water quality [14], while inadequate food supply has direct impact on production cost. Studies have revealed that increasing feeding frequency has been shown to improve growth, however, when feeding rate is high or intervals are shorter than the time required for regaining appetite is insufficient, it may lead to gastric overload and the food passes through the digestive tract more quickly, without effective digestion and absorption[6,8,15].

In practice, when fish are fed frequently manually or with automated feeders, it is difficult to ensure that the delivery of ration will allow each individual to feed to satiation during each feeding. During lowfrequency feeding a portion of the feed is not consumed by the fish as some food is likely to fall or sink to the bottom. This is especially true with fish that feed in the water column. . In this case, feeding frequencyis increased and the feed is delivered in a greater number of rations throughout the daily cycle.These studies indicate that feeding frequency is an important factor required to fix an appropriate feeding management program. And for proper aquaculture management, the feeding time and amount have to be set appropriately. Therefore, more attention is required to be given to the optimum feeding management in order to reduce overfeeding, feed wastage, maintenance of water quality, ensure better FCR (feed conversion ratio), optimal weight gain, survival and fillet composition of cultured organism.

Despite the great potential of Indian major carps, information regarding the effects of feeding strategies and management practices on Indian major carp species are limited[4,16,17]. Therefore, experiments were conducted to investigate and identify the optimum number of feeds d^{-1} which could be given so as to maximize growth performance without deteriorating the culture environment.

The aim of the present studies was to determine the effect of feeding frequency (manual delivery of the ration) on growth performance and some bioenergetics parameters in Indian major carps. Two experiments were conducted. First experiment was conducted under laboratory conditions and C *mrigala* fingerlings were fed at four frequencies: one, two, three and four times a day. Growth parameters, feed conversion efficiency and effect on excretion of metabolites were studied. The second experiment was conducted under field conditions and the fingerlings of all the three Indian major carp species were grown in polyculture and were fed at five frequencies: one, two, three four and six times a day. The objectives of this experiment were to examine the impact of feeding frequencies on growth performance of Indian major carp fingerlings (i) Physico-chemical characteristics of pond water, their nutrient status and (iii) impact on pond productivity.

2. Materials and Methods

Experiment I: Effect of four different feeding frequencies (one, two, three and four feedings d^{-1}) on growth performance, and bioenergetic parameters in the fingerlings of *Cirrhinus mrigala* under laboratory conditions-75 day treatment.

2.1. Experimental Design and Diet Preparation

Ingredient contents and proximate composition of the diet is given in Table 1.

Diet preparation: Before grinding, ingredients were dried in an oven at 60^{o}C. Dough of the flour, using distilled water was made and prepared in pelleted form using a mechanical palletizer (0.5mm). Pellets were oven dried (60^o–62^{o}C) and stored for later use. Diet was fortified using a mineral and amino acid (methionine + lysine) premix. To eliminate or inactivate anti nutritional factors(ANFs), soybean seeds were hdrothermicallytreated for 15 min (at 15 lbs) at 121^{o}C ; 1% chromic oxide (Cr_2O_3) was added as an external indigestible marker for digestibility estimations**.**

Table 1. *Ingredient content (%) and proximate composition (% dry weight basis)of experimental diet.*

Ingredients	**% inclusion**
Groundnut oilcake	60
Rice bran	23
Processed soybean[a]	10
Carboxymethyl cellulose	5
Mineral premix and amino acids[b] (MPA)	1
Chromic oxide	1
Proximate composition (%)	**Mean ± S.E. of mean**
Dry matter	92.4 ±0.03
Crude protein	35.0 ±0.53
Crude fat	8.0 ±0.055
Ash	6.2 ±0.015
Crude fibre	9.8 ±0.026
Gross energy kJg^{-1}	17.3 ±0.03

All values are mean ± S.E. of mean.

a Hydrothermically processed in an autoclave at 121^{o}C (15 Lb) for 15 minutes. to remove anti nutrient factors (ANF)

b Each kg contained: Copper 312 mg, Cobalt 45 mg, Magnesium 2.114g, Iron 979 mg, Zink 2.130 g, Iodine 156 mg, DL-Methionine 1.920 g, L-Lysine Mono Hydrochloride 4.4 g, Calcium 30%, Phosphorus 8.25%

2.2. Collection and Care of Animals

Fingerlings of *C. mrigala* were obtained from the local suppliers and maintained in transparent glass aquaria (60×30×30 cm), stacked in an air-conditioned wet laboratory facility, aquahouse where the temperature was maintained at $25^{o}\pm1^{o}$C and the lighting schedule of 12 h of light (08^{oo}-20^{oo} h) alternating with 12 h of darkness (20^{oo}-08^{oo} h) controlled by an electric timer. Fingerlings were acclimated to the laboratory conditions for a period of 15 days prior to the initiation of the experimental treatments and fed *ad libitum* between 08^{oo}-09^{oo} h on the experimental diet containing 35 per cent protein (Table 1 for ingredient contents and proximate composition).

2.3. Experimental Fish and Feeding Trials

At the start of the experiment 120 fingerlings of *C mrigala* (mean body weight 4.30±0.050g) were randomly distributed to 4 treatment groups of 30 fish each. Each treatment was replicated three times with 10 fish peraquarium. The experiment was conducted in transparent glass aquaria with a capacity of 60 L containing 30 L chlorine free fresh water. Before the initiation of feeding schedules, fingerlings were individually weighed using a digital balance and randomly distributed. The fish were fed at following four different frequencies (one, two, three and four meals/d) and were fed at 3% BW d^{-1}.

T 1: Fed once a day at 08^{oo}h at 5% BW

T 2: Fed twice a day at 08^{oo}h and 12^{oo}h at 2.5% BW per feeding

T 3: Fed three times a day at 08^{oo}h, 10^{oo}h and 12^{oo}h at 1.6 % BW per feeding

T 4: Fed four times a day (08^{oo}, 10^{oo}, 12^{oo} and 14^{oo}h) at 1.25% BW per feeding

Meals timings were equally spaced throughout the day from 08^{00} to 20^{00} h and the fish were fed as per the feeding schedule. The feeding rate was same in all treatments but frequency was different. The fish were hand fed at the fixed time interval on a supplementary diet containing 35% protein (Table 1) for the whole duration of 75 days.

Fish were bulk weighed every 15th day and amount of feed to be given was adjusted accordingly. Each group of fish was exposed to the diet for 3 hours; thereafter, uneaten feed was siphoned out, stored and dried separately for calculating feed consumption per day. Faecal matter voided by the fish was collected and oven dried (at 60 ℃) for subsequent analysis for digestibility estimations [18]**.** The water in the aquaria was renewed daily with water adjusted to the laboratory temperature (25^{o}C). Water parameters such pH and dissolved oxygen of the aquaria water were measured daily. pH of the aquarium water fluctuated between 7.25–7.55, and dissolved oxygen content ranged between 5.0–7.0 mg L^{-1}. At the termination of experiment fish from all the treatments was weighed individually and processed for subsequent analyses. At the start of the experiment 10 fish from the stock were randomly taken for analysis of proximate composition and considered as initial carcass composition. At the end of the experiment, nine fish from each treatment were taken and sacrificed for final carcass analysis.

2.4. Analytical Techniques

At the end of the feeding trial of 75 days, water samples (i.e. on 76^{th} day) from each aquarium were collected at two-hourly interval over a period of 24 h for the estimation of excretory levels of total ammonia (N-NH_4) and reactive orthophosphate (o-PO_4) production following APHA [19] and calculated following[20]**.** The quantity of nitrogen and phosphate excreted by the fish in aquaria water was calculated by summing the values obtained at two-hour intervals over a period of 24 h. as follows:

$$\text{Total N-NH+4/o-PO4- excretion (mg kg-1 BW day-1)} = \frac{\text{N-NH+4/o-PO-4 (mg L-1) in water}}{\text{Fish biomass (kg) per L of water}} \quad (1)$$

The feed ingredients, experimental diets, faecal samples, fish carcass (initial and final) were analysed following [21]. Dry matter (after desiccation in an oven at 105°C for 24 h), ash (incineration at 550°C for four hours in a muffle furnace), and nitrogen were determined using the micro-kjeldahl method; the crude protein content was estimated by multiplying nitrogen by a factor of 6.25. Crude fat contents were determined by petroleum ether extraction (Soxhlet's apparatus) and crude fibre by treating moisture and fat free sample successively with dilute acid and alkali. Phosphorus in feed and carcass was determined spectrophotometrically after acid digestion (nitric acid: perchloric acid 10:1). Nitrogen-free extract (NFE%) was calculated by subtracting the total percentage of crude protein, crude fat, ash, moisture, and crude fibre from 100. Chromic oxide levels in the diets as well as in the faecal samples were estimated by nitric acid-perchloric acid digestion method using UV visible spectrophotometer measuring optical density at 350 nm [22]. Growth and nutrient retention parameters were calculated following [23]. Apparent protein digestibility (APD) of the diets was calculated according to the methods of [24). Live weight gain (g), percent weight gain, specific growth rate, feed consumption per day in percentage of body weight, feed conversion ratio (FCR), gross protein retention (GPR), and gross energy retention (GER) were calculated using standard method [23]. Gross energy content of the diet and fish carcasses was calculated using the average caloric conversion factor of 0.3954, 0.1715, and 0.2364 Kj g^{-1} for lipid, carbohydrate, and protein, respectively [25]**,** whereas metabolizable energy in diets and feeds was calculated using caloric conversion factors: 0.335, 0.138, and 0.188 KJ g^{-1} for lipid, carbohydrate, and protein, respectively[26].

2.5. Growth Performance and Bioenergetic Parameters

Weight gain (g), growth % gain in body weight, specific growth rate (SGR), feed conversion ratio (FCR), gross protein retention (GPR), gross energy retention (GER), apparent protein digestibility (APD) were calculated as follows:

(1) Weight Gain (BWg) = final fish weight (g) – initial fish weight (g)

(2) Growth per cent gain in body weight was measured by the following formula:

$$\text{Growth \% gain in body weight} = \frac{W2-W1}{W1} \times 100$$

Where,

W1= Initial weight

W2= Final weight

(3) Specific growth rate (SGR) = $(\text{Ln } W_t - \text{Ln } W_0) \times 100\ t^{-1}$

(4) Feed conversion ratio (FCR) = $P/(W_t - W_0)$

(5) Gross protein retention (GPR)
Gross protein retention was calculated by using following formula:

$$\text{GPR (\%)} = \frac{\text{(Protein gain, g)}}{\text{(Protein intake, g)}} \times 100$$

(6) Gross energy retention (GER) was calculated by using the following formula:

$$\text{GER (\%)} = \frac{\text{(Fish energy gain, kJ)}}{\text{(Energy intake, kJ)}} \times 100$$

$$\text{viii) APD} = 100\text{-}100 \times \left(\frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\% \text{ protein in faeces}}{\% \text{ protein in diet}}\right)$$

2.6. Statistical Analysis

In order to determine significant differences among different treatments, results were analyzed by one-way Analysis of variance (ANOVA). Duncan's multiple range tests and Student's t-test [27] using SPSS (version 11.5 for windows) was applied to assess the significance of the differences among treatments. The data are presented as mean ± standard error (SE) of the mean of the replicate groups. Treatment effects were considered significant at the $P < 0.05$ level.

Experiment II: Effect of five feeding frequencies on growth performance of Indian major carp fingerlings in polyculture-90 days

Studies were conducted at the fish pond facility of the Department of Zoology and Aquaculture, CCS Haryana Agricultural University, Hisar (Lat. 29^o, 10' N, Long. 75^{o}46' E), India. The experiment was conducted in 10 earthen ponds (each 0.0001 ha) from September to November (90 days). Prior to the commencement of treatments, ponds were cleaned, lime was applied @ 200 kg ha^{-1} and filled with fresh water (EC 509-622 μS cm^{-1}) and allowed to stabilize for about two weeks. Semi dry cow-dung @ 10000 kg ha^{-1} y^{-1} was applied to fertilize the ponds at biweekly intervals. Manure was diluted in pond water (in the ratio 1:3 w/v) before application. To maintain the desired depth of water in the ponds (90 cm), water was replenished as often as required.

2.7. Stocking and Feeding

Two weeks after the application of organic fertilizer, six week old farm reared Indian major carp (*Catla catla*, *Cirrhinus mrigala* and *Labeo rohita)* fingerlings at 50 fingerlings per pond were stocked. During the acclimation period of two weeks, Fishes were fed only once daily between 08^{00}-09^{00}h at 5 percent of body weight d^{-1} on the same diet used in experiment 1 (Table 1) containing about 35 per cent crude protein. At the expiry of acclimation period, experimental feedings were initiated**.** The fingerlings were fed at five different frequencies (one, two, three, four and six meals d^{-1}) with the same total meal intakes (5% body mass) and the fish were fed as per the feeding schedule given in Table 2. The feed was broadcasted over the water surface at a predetermined site of the pond as per schedule. About 20% of the total fish were sampled fortnightly by a seine net to monitor the growth and the amount of feed to be given was adjusted accordingly.

Table 2. *Feeding frequency under field conditions.*

Expt.2:Feeding frequency of three species of Indian major carp fingerlings under -polyculture - 90 days treatment			
S.No	Feeding frequency	Time of feeding	Rate per feeding (%)
T1	Once a day	08^{oo}h	At 5% BW
T2	Two times a day	08^{oo}h and 10^{oo}h	At 2.5% BW per feeding
T3	Three times a day	08^{oo}h, 10^{oo}h and 12^{00}	At 1.7% BW per feeding
T4	Four times a day	08^{oo}h, 10^{00}h , $12^{00}$and 14^{oo}	At 1.25% BW per feeding
T5	Six times a day	08^{oo}, 10^{00},12^{00}, 14^{00}, $16^{00}$and 18^{00}h	At 0.83% BW per feeding

2.8. Water Quality Monitoring

Water samples were obtained in replicate of four from each pond (i.e. 8 samples from each treatment) before sunrise and analysed following APHA [19]**.** Water temperature (^{o}C) and pH were recorded daily. During the study period a total of two samplings (at the end 45 and 90 days) were done. Planktons (phytoplankton and zooplankton) were analysed quantitatively according to the standard methods [28]. For estimating the total assimilation rate by the producer organisms in ponds, net primary productivity (NPP) was determined following the light and dark bottle method. For qualitative and quantitative estimations of phyto- and zooplankton, water samples were also collected in replicates of four from each pond (i.e. 8 samples per treatment) at the end of 45 and 90 days interval. Plankton samples were obtained by passing 20 L of water taken from five different locations through plankton net (mesh size 125 µm). Plankton densities were estimated using a Sedgwick-Rafter cell [28] under a binocular microscope. Plankton species diversity ($\bar{d}$) was determined using Shanon and Weaver's diversity index formula [29]**.** For the determination of chlorophyll ^{a} concentrations, a known amount (10L from five different locations from each treatment) of water was filtered through Whatmanfilter paper (No. 40) and was determined following Boyd [30]. Ninety days post-stocking ponds were completely drained and the fish were harvested, counted and individually weighed to the nearest gram. At the start of the experiment 15 fish from the stock were randomly taken for analysis of proximate composition and considered as initial carcass composition. At the end of the experiment, 15 fish (five of each species) from each replicate treatment were taken and sacrificed for final carcass analysis.

2.9. Analytical Techniques

Proximate analyses of feed ingredients, experimental diets, and fish carcass (initial and final) was done following the standard methods of AOAC [21].

2.10. Sediment Analysis

At the end of the experiment, sediment samples from the bottom of each pond were collected using a cone sampler (Area 858.3Sq cm) and analysed. For the study of macro zoo benthos, the mud samples from each pond were separated using 0.5 mm mesh size sieve (Hovgaard, 1973). Organisms were sorted out manually using a pair of forceps for qualitative and Weaver's diversity index [29]**.**

2.11. Statistical Analysis

Data were subjected to multivariate analysis following Prein, *et al*, [31]. Coefficient of correlation between different parameters and multiple regression between independent (hydrochemical parameters) and dependent variables (biological/productivity indicating parameters) was determined. In order to determine significant differences, results were analyzed by one-way Analysis of variance (ANOVA) followed by Duncan's multiplerange tests and Student's t-test [27] using SPSS (version 11.5 for windows) was applied to assess the significance of the differences among treatments. The data are presented as mean ± standard error of the mean (SE) of the replicate groups. Treatment effects were considered significant at the $P < 0.05$ level.

3. Results and Discussion

Experiment I: Effect of four different feeding frequencies (one, two, three and four feedings d^{-1}) on growth performance, and bioenergetic parameters in the fingerlings of *Cirrhinus mrigala* under laboratory conditions-75 day treatment

3.1. Fish Growth (Figure 1)

Percentage survival was found to be independent of the treatment effects. ANOVA showed that mean fish weight and growth rates (growth per day and SGR) were significantly (P<0.05) affected by the feeding frequency.

At the end of 75 days, a higher mean body weight (4.4±0.22g) and specific growth rate (0.95 ± 0.0g) were observed in treatment T4 which were significantly (P<0.05) higher than T1 but were not significantly (P<0.05) different when compared with T2 and T3 (Table 3).

Table 3. *Effect of four different feeding frequencies on growth, performance, digestibility, nutrient retention and carcass composition (% wet weight) in C. mrigala fingerlings under laboratory conditions (75 days).*

Parameters		T_1	T_2	T_3	T_4
Live weight gain (g)		3.43^{b} ± 0.09	3.76^{ab} ± 0.17	3.83^{ab} ± 0.44	4.44^{a} ± 0.22
Growth (per cent gain in body weight		78.47^{b} ± 1.79	86.57^{ab} ± 4.70	90.75^{ab} ± 11.47	105.00^{a} ± 4.55
Specific growth rate (SGR%d^{-1})		0.77^{b} ± 0.013	0.83^{ab} ± 0.03	0.86^{ab} ± 0.08	0.95^{a} ± 0.03
Food conversion ratio (FCR)		2.06^{a} ± 0.030	1.73^{ab} ± 0.10	1.78^{ab} ± 0.22	1.51^{b} ± 0.08
Gross protein retention (GPR)		21.64^{b} ± 0.63	26.55^{ab} ± 1.46	27.15^{ab} ± 3.08	30.85^{a} ± 1.77
Gross energy retention (GER)		18.53^{a} ± 0.41	22.60^{ab} ± 1.08	22.94^{ab} ± 2.44	26.06^{a} ± 1.17
Apparent protein digestibility (%)		78.78^{a} ± 0.62	80.48^{a} ± 0.34	81.23^{a} ± 0.71	81.68^{a} ± 0.27
Total ammonia (mg kg^{-1} BW d^{-1})		503.09^{a} ± 13.07	337.42^{b} ± 3.84	287.17^{c} ± 3.78	245.35^{a} ± 9.13
Reactive phosphate (mg kg^{-1} BW d^{-1})		393.36^{a} ± 11.02	308.36^{b} ± 10.93	302.31^{b} ± 8.76	265.93^{c} ± 2.19
Carcass composition (%)	Initial value				
Moisture	77.5 ± 0.08	76.3^{a} ± 0.14	76.0^{b} ± 0.01	75.7^{bc} ± 0.07	76.0^{c} ± 0.02
Crude protein	12.1 ±	14.0^{c} ± 0.12	14.2^{b} ± 0.03	14.5^{a} ± 0.05	14.5^{a} ± 0.03

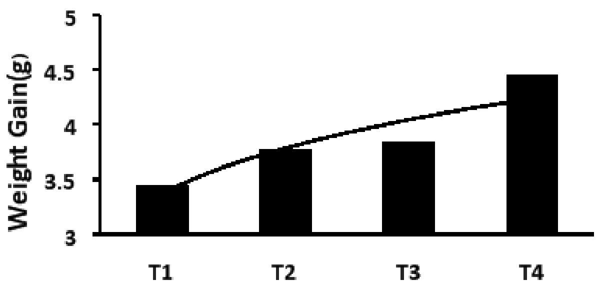
	0.03				
Crude fat	3.7 ± 0.02	5.3^{c} ± 0.04	5.4^{b} ± 0.06	5.5^{a} ± 0.02	5.5^{a} ± 0.01
Ash	3.5 ± 0.03	3.07^{a} ± 0.02	3.0^{b} ± 0.01	3.0^{b} ± 0.01	3.0^{b} ± 0.01
Phosphorus	0.5 ± 0.01	0.5^{a} ± 0.01	0.5^{a} ± 0.01	0.5^{a} ± 0.01	0.5^{a} ± 0.01

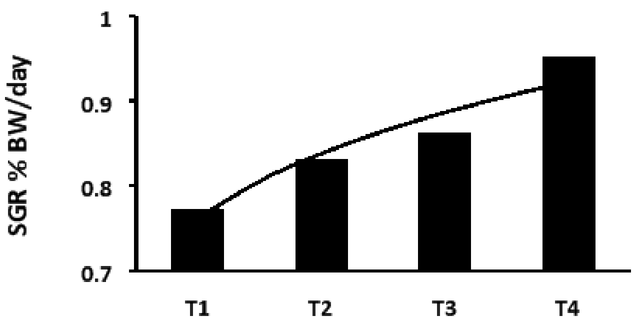
All values are mean ± S.E. of mean, Means with the same letter/s in the same row are not significantly (P 0.05) different.

T_1 = Feeding once a day (08^{oo} h), T_2 = Feeding twice a day (08^{oo} and 12^{oo} h), T_3 = Feeding three times a day (08^{oo}, 12^{oo} and 16^{oo} h), T_4 = Feeding four times a day(08^{oo}, 12^{oo}, 16^{oo} and 20^{oo} h).

Like weight gain parameters, digestibility and nutrient retention also gradually increased with increase in number of meals d^{-1} (Tables 4). *C mrigala* fed four meals d^{-1} had higher values of GPR and GER though differences were not statistically significant (P<0.05) from the groups given two or three meals d^{-1}, though significantly (P<0.05) higher than the fish fed only once a day. Not many variations in apparent protein digestibility (%) were observed among the four treatment groups. Food conversion ratio (FCR) decreased with each increase in the frequency of feeding and significantly (P<0.05) lower values were observed in fingerlings fed four times a day, however, these values were not significantly (P<0.05) different from the fish fed 2 or 3 times daily, though were significantly lower (P<0.05) than those fish fed once a day only (Fig 1).

Nitrogen retention as indicated by GPR values increased, while excretion of metabolites (N-NH_4 and o-PO_4) decreased with each increase in the frequency of feeding. Lower values in nitrogen retention (GPR) and higher values in excretion of metabolites were observed in the group fed only once a day (Table 3). A review of the data indicated that the excretory levels of metabolites and nitrogen retention appeared to be affected by the frequency of feedings.





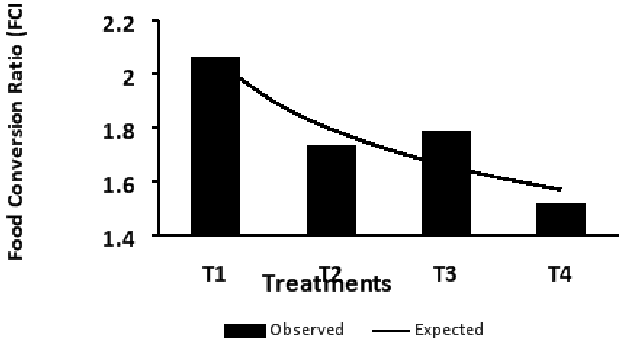


Figure 1. *Weight gain (g), Specific Growth Rate (SGR) and Feed conversion ratio of Cirrhinus mrigala and related orthogonal polynomial fit curve at four different feeding frequencies.T1 = Feeding once a day (08oo h), T2 = Feeding twice a day (08oo and 12oo h), T3 = Feeding three a day (08oo, 12oo and 16oo h)and T4 = Feeding four times a day (08oo, 12oo, 16oo and 20oo h) under laboratory conditions.*

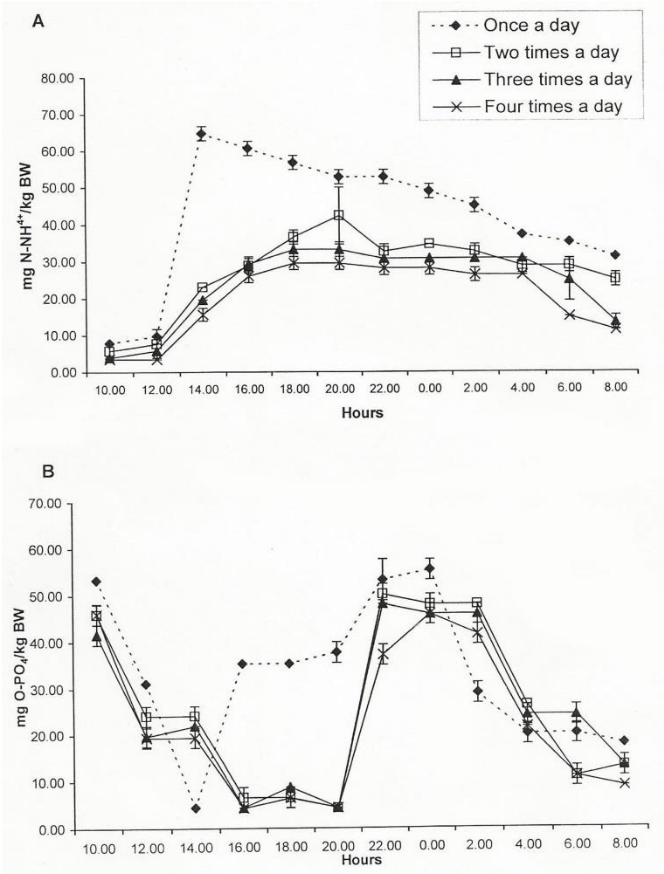


Figure 2. *Effect of four different feeding frequencies on postprandial patterns of total ammonia excretion (A) and orthophosphate (B) production (mg kg1 BW) by Cirrhinus mrigala fingerlings in holding water. All values are mean ±SEM of six observations.*

Postprandial excretory levels of ammonia and reactive phosphate decreased with each increase in frequency of feeding. Significantly ($P<0.05$) lower concentrations of metabolites were observed in the holding water where the fish were fed four times a day, while significantly ($P<0.05$) higher values were observed where the fish were fed once or twice a day (Table 3). Peak values in $N\text{-}NH_4^+$ excretion were observed 6h post-feeding in the group fed once a day, while not much variations in excretory patterns of $N\text{-}NH_4^+$ were observed in treatments fed twice, thrice or four times a day.

After attaining maximum levels, a plateau persisted for 10-20 h post-feeding and thereafter, only a slight decrease in N-NH_4^+ levels was observed. Concentration of soluble P **(**o-PO_4**)** in the aquaria water was high 2h post-feeding, returning to basal level. Again an increase after 14h post-feeding was observed at all the feeding frequencies (Fig 2).

3.2. Fish Carcass Composition

Significantly ($P<0.05$) higher accumulation of protein and fat and lower ash contents were observed in the groups fed three and four times a day in comparison with the groups fed once and twice a day. No variations inmoisture and phosphorous contents were observed among the different treatment groups (Table 3).

Experiment II: Effect of five feeding frequencies on growth performance of Indian major carp fingerlings in polyculture-90 days.

3.3. Growth Performance (Figure 3)

No fish disease was encountered during the experimental period and the survival varied from 87.50 to 96.08%. ANOVA revealed that irrespective of the species stocked a significant ($P<0.05$) increase in final biomass, mean fish weight gain, growth per cent gain in body weight and specific growth rate was observed with each increase in the frequency of feeding and highest values were observed in fish fed six times a day, though these values were not significantly different than the groups fed three or four meals per day. Low growth performance was observed in the groups fed once or twice a day (Table 4).

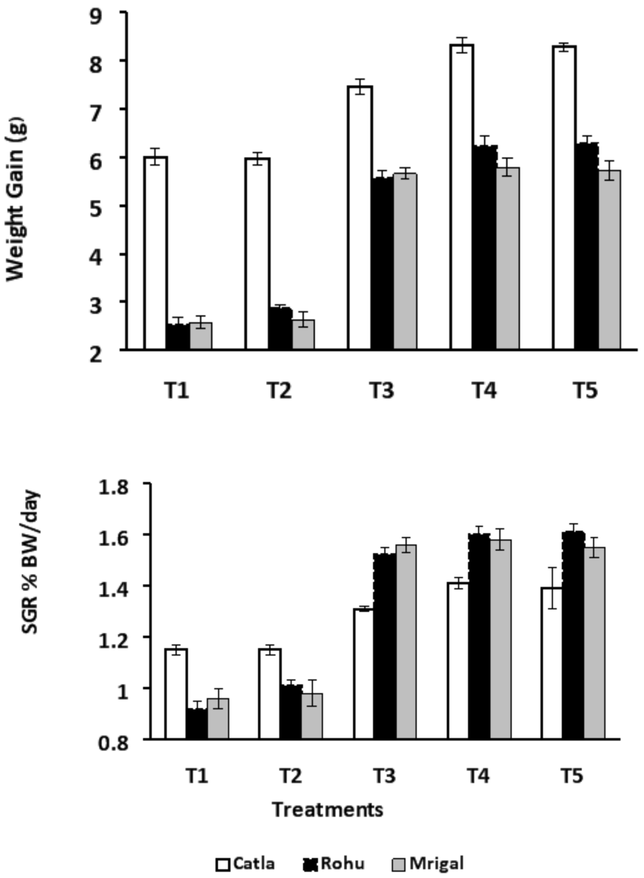


Figure 3. *Weight gain (g) and specific growth rate (SGR) of catla, rohu and mrigal at five different feeding frequencies when cultured under polyculture under field conditions .T1 = Feeding once a day (08oo h), T2 = Feeding twice a day (08oo and 12oo h), T3 = Feeding three a day (08oo, 12oo and 16oo h), T4 = Feeding four times a day (08oo, 12oo, 16oo and 20oo h)and T5=Feeding six times a day (08oo, 12oo ,16oo h and 20oo h).*

Table 4. *Effect of five different feeding frequencies (1,2,3,4 and 6 feedings d-1) on growth performance of Indian major carp fingerlings under field conditions (composite fish culture) -90 days treatment.*

Treatment	Fish Species	Initial mean fish wt. at stocking 0f each species (g)	Survival rate (%)	Final biomass (g)	Final mean fish wt. (g)	Mean fish wt. (g) gain	Growth per cent gain in BW	SGR% gd^{-1}
T1	Catla	3.29 ±0.01	96.08	151.84 ± 4.12	9.30^c ± 0.17	6.01^c ±0.18	182.89^c ±6.02	1.15^c ± 0.02
	Rohu	1.96 ±0.01	96.08	73.28 ± 2.88	4.49^c ± 0.16	2.53^c ±0.15	129.24^c ±6.96	0.92^c ± 0.03
	Mrigal	1.88 ±0.02	89.58	63.93 ± 2.08	4.46^b ± 0.10	2.58^b ±0.12	137.38^b ±7.72	0.96^b ± 0.04
T2	Catla	3.27 ±0.02	94.11	147.77 ± 4.72	9.24^c ± 0.12	5.97^c ±0.14	182.62c ±5.20	1.15^c ± 0.020
	Rohu	1.91 ±0.02	90.20	77.14 ± 3.08	4.82^c ± 0.04	2.88^c ±0.06	148.53c ±4.00	1.01^b ± 0.020
	Mrigal	1.86 ±0.03	87.50	62.91 ± 1.94	4.49^b ± 0.14	2.63^b ±0.16	141.45^b ±10.20	0.98^b ± 0.050
T3	Catla	3.31 ±0.01	96.07	175.97 ± 4.42	10.77^b ±0.16	7.46^b ±0.15	225.13^b ±3.86	1.31^b ± 0.010
	Rohu	1.91 ±0.02	96.08	122.21 ± 3.85	7.48^b ± 0.13	5.57^b ±0.15	291.80^b ±10.20	1.52^a ± 0.030
	Mrigal	1.85 ±0.02	91.67	110.25 ± 3.84	7.51^a ± 0.10	5.66^a ±0.12	280.80^a ±3.44	1.56^a ± 0.030
T4	Catla	3.25 ±0.01	94.11	185.08 ± 5.69	11.57^a ±0.16	8.32^a ±0.16	255.77^a ±5.48	1.41^a ± 0.020
	Rohu	1.91 ±0.02	92.16	127.74 ± 6.19	8.15^a ±0.19	6.23^a ±0.20	323.25^a ±11.17	1.60^a ± 0.30
	Mrigal	1.85 ±0.02	95.83	116.95 ± 3.21	7.64^a ± 0.17	5.79^a ±0.19	313.22^a ±13.23	1.58^a ± 0.040
T5	Catla	3.32 ±0.01	94.12	185.53 ± 7.47	11.59^a ±0.11	8.28^a ±0.10	249.54^a ±2.45	1.39^a ± 0.080
	Rohu	1.94 ±0.02	90.20	126.34 ± 6.29	8.24^a ± 0.13	6.29^a ±0.14	324.75^a 10.20	1.61^a ± 0.030
	Mrigal	1.88 ±0.02	95.83	116.58 ± 1.43	7.61^a ± 0.19	5.73^a ±0.21	305.04^a ±13.86	1.55^a ± 0.040

All value are mean ± S.E. of mean. Means with the same letter/s in the same row are not significantly ($P<0.05$) different. Feeding frequency: T1= Once a day. T2 = Twice a day, T3 = Three times a day, T4 = Four times a day, T5 = Six times a day.

3.4. Fish Carcass Composition

In general, body composition showed a decrease in moisture and ash contents and an increase in protein and fat accumulation with each increase in the frequency of feeding. A review of the data had revealed that though highest levels of protein, fat and energy were observed in the group given six feeds per day, however, these values were not significantly ($P<0.05$) different than the groups given three or four feedings per day. Significantly ($P<0.05$) lower levels of protein, fat and energy contents were observed in the group fed only once or twice a day (Data not shown).

3.5. Physico-chemical Characteristics of Pond Water (Table 5)

Feeding frequency has also affected the physico-chemical characteristics of pond water in all the treatments. Dissolved oxygen (DO) concentration remained at optimal levels (5.92-7.55 mg l^{-1}) and free CO_2 was absent in all the treatments. pH remained alkaline and varied from 8.84 to 9.19. BOD_5 and NH_4-N decreased with increase in the frequency of feeding and thus significantly ($P<0.05$) lower values were observed in the ponds where the fish were fed four or six times a day. Total hardness and turbidity decreased with increase in the frequency of feeding, however, significantly ($P<0.05$) higher values were observed in ponds where the fish were fed only once a day.

In general the concentration of nutrients (o-PO_4, total-P, and NO_2-N) were significantly ($P<0.05$) higher in ponds where the fish were fed only once a day except NO_3-N which increased with increase in the frequency of feeding (Table 6).Net primary productivity (NPP) and plankton (both phytoplankton and zoo plankton) population (No l^{-1}) were higher in ponds where the fish were fed four or six times a day which coincided with higher growth. A simple correlation revealed that interrelationship among the independent variables were often highly significant ($P<0.05$), e.g. alkalinity was significantly correlated with turbidity and nutrients (Total Kjeldahl nitrogen, orthophosphate and NO_2-N). Available nitrogen (NO_3-N) was significantly and positively correlated with plankton population and NPP. Studies have revealed a negative correlation of turbidity, alkalinity, nutrients, NH_4-N and BOD_5 with weight gain, clearly indicating that feeding regimes had also affected the physico-chemical characteristics of pond water and fish growth, which appears to depend on the availability of food in treatments where the fish were fed at multi time intervals, than feeding only once a day for the satiation and growth of fishes.

Table 5. *Effect of five different feeding frequencies (1,2,3,4 and 6 feedings d-1) on physio-chemical and biological characteristics of pond water.*

Parameters	**45days**					**90 days**				
	T1	**T2**	**T3**	**T4**	**T5**	**T1**	**T2**	**T3**	**T4**	**T5**
Dissolved oxygen mg l^{-1}	5.93^{a} ± 0.07	6.28^{a} ± 0.05	7.28^{b} ± 0.06	7.07^{b} ± 0.03	7.55^{b} ± 0.03	5.98^{a} ± 0.05	5.92^{a} ± 0.01	6.08^{a} ± 0.05	6.19^{a} ± 0.05	6.73^{b} ± 0.03
pH	9.17^{a} ± 0.06	9.13^{a} ± 0.06	$9.0\ 8^{a}$ ± 0.05	9.17^{a} ± 0.03	9.19^{a} ± 0.02	8.84^{a} ± 0.02	8.86^{a} ± 0.04	8.93^{a} ± 0.02	8.87^{a} ± 0.03	9.00^{a} ± 0.01
Total alkalinity mg l^{-1}	80.67^{a} ± 0.70	78.67^{a} ± 1.33	76.00^{a} ± 1.15	72.67^{b} ± 0.66	72.00^{b} ± 1.15	102.00^{c} ± 1.15	100.0^{c} ± 01.15	100.67^{c} ± 1.76	96.00^{c} ± 1.15	94.67^{c} ± 0.66
Total hardness mg l^{-1}	95.33^{a} ± 0.66	94.00^{a} ± 1.15	96.00^{a} ± 1.15	96.00^{a} ± 1.15	93.67^{a} ± 0.33	118.67^{b} ± 0.61	111.33^{b} ± 0.67	110.67^{b} ± 0.67	109.33^{b} ± 0.67	108.67^{b} ± 0.67
BOD mg l^{-1}	3.93^{a} ± 0.97	2.60^{b} ± 0.36	2.31^{b} ± 0.21	2.21^{b} ± 0.02	2.18^{b} ± 0.02	2.34^{b} ± 0.03	1.40^{c} ± 0.44	1.03^{c} ± 0.01	1.11^{c} ± 0.07	1.02^{c} ± 0.22
Turbidity (NTU)	1.57^{a} ± 0.03	1.63^{a} ± 0.03	1.53^{a} ± 0.03	1.50^{a} ± 0.06	1.47^{a} ± 0.03	2.57^{b} ± 0.03	2.17^{b} ± 0.03	2.03^{b} ± 0.03	1.97^{b} ± 0.03	1.93^{b} ± 0.03
Chlorides mg	11.67	12.99	12.66	12.32	12.32	12.32	10.67	10.32	10.32	10.00

l^{-1}	^{a} ± 0.53	^{a} ± 0.58	^{a} ± 0.33	^{a} ± 0.33	^{a} ± 0.33	^{a} ± 0.33	^{a} ± 0.33	^{a} ± 0.33	^{a} ± 0.33	^{a} ± 0.02
Total phosphate mg l^{-1}	0.20^{a} ± 0.01	0.16^{a} ± 0.02	0.13^{a} ± 0.01	0.16^{a} ± 0.01	0.15^{a} ± 0.00	0.35^{b} ± 0.01	0.30^{b} ± 0.010	0.17^{a} ± 0.01	0.16^{a} ± 0.01	0.15^{a} ± 0.01
Orthophosphate mg l^{-1}	0.10^{a} ± 0.01	0.07^{a} ± 0.02	0.04^{a} ± 0.07	0.04^{a} ± 0.01	0.03^{a} ± 0.01	0.11 ± 0.00	0.08^{a} ± 0.00	0.06^{a} ± 0.01	0.05^{a} ± 0.00	0.04^{a} ± 0.01
Total K nitrogen	3.49^{a} ± 0.01	3.51^{a} ± 0.05	3.44^{a} ± 0.06	3.38^{a} ± 0.06	3.34^{a} ± 0.09	3.96^{b} ± 0.04	3.81^{b} ± 0.07	3.63^{b} ± 0.16	3.51^{a} ± 0.18	3.27^{c} ± 0.06
Ammonia mg l^{-1}	0.10^{a} ± 0.01	0.05^{a} ± 0.01	0.05^{a} ± 0.01	0.04^{a} ± 0.00	0.04^{a} ± 0.00	0.14 ± 0.00	0.08^{a} ± 0.01	0.08^{a} ± 0.01	0.06^{a} ± 0.01	0.06^{a} ± 0.01
Nitrate mg l^{-1}	1.39^{a} ± 0.10	1.58^{b} ± 0.14	1.43^{a} ± 0.14	1.36^{a} ± 0.07	1.33^{a} ± 0.04	2.22^{c} ± 0.07	2.44^{c} ± 0.14	2.51^{c} ± 0.07	2.58^{d} ± 0.00	2.61^{d} ± 0.03
Nitrite mg l^{-1}	0.01^{a} ± 0.00	0.01^{a} ± 0.00	0.01^{a} ± 0.00	0.01^{a} ± 0.00	0.01^{a} ± 0.00	0.01^{a} ± 0.00	0.01^{a} ± 0.00	0.01^{a} ± 0.00	0.01^{a} ± 0.00	0.01^{a} ± 0.00
Phytoplankton Nos L^{-1}	333.00^{a} ± 8.00	364.00^{b} ± 4.00	372.00^{b} ± 4.00	392.00^{c} ± 7.00	413.00^{c} ± 4.00	313.30^{a} ± 7.00	495.67^{d} ± 4.00	504.00^{d} ± 4.00	516.00^{d} ± 7.00	541.00^{e} ± 5.00
Zooplankton Nos L^{-1}	84.00^{a} ± 4.00	196.00^{b} ± 4.00	217.00^{b} ± 5.00	237.00^{c} ± 0.00	266.00^{c} ± 5.00	192.66^{b} ± 7.33	392.00^{c} ± 4.00	416.33^{c} ± 7.00	441.33^{d} ± 4.33	466.66^{d} ± 9.00
NPP mg C L^{-1}d^{-1}	0.73^{a} ± 0.04	0.73^{a} ± 0.02	0.74^{a} ± 0.19	1.15^{b} ± 0.10	1.25^{b} ± 0.10	1.43^{b} ± 0.08	1.65^{c} ± 0.15	1.70^{c} ± 0.07	1.88^{d} ± 0.03	1.92^{e} ± 0.03

All values are mean ± SE of mean, Temperature during the experimental period varied from 13.60 to 31.60 ^{o}C.Means with the same letter/s in the same row are not significantly (P 0.05) different. Feeding frequency: T1= Once a day. T2 = Twice a day, T3 = Three times a day, T4 = Four times a day, T 5 = Six times a day.

3.6. Sediment (Table 6)

No variations in moisture contents, organic carbon and hardness were observed among the different treatments. pH of the sediment remained alkaline and fluctuated between 7.47±0.09 to 7.58±0.18. A slight increase in electrical conductivity (EC) and a decrease in chlorides and o- PO_4 levels were observed with increase in the frequency of feeding. Nitrates (NO_3-N) were totally absent.

Table 6. *Effect of five different feeding frequencies (T1,T2,T3,T4 and T5 feedings d-1) on physico-chemical characteristics of sediment.*

Parameters	**Treatments No.**				
	T1	**T2**	**T3**	**T4**	**T5**
Moisture (%)	70.21 ±0.02	68.12 ±0.120	68.16 ±0.16	68.00 ±0.50	67.69 ±0.09
pH	7.47 ±0.09	7.51 ±0.110	7.57 ±0.12	7.56 ±0.16	7.58 ±0.18
EC µs cm^{-1}	211.50 ±0.96	212.0 ±2.005	214.0 ±4.01	217.5 ±2.50	217.5 ±2.50
Hardness mg g^{-1}	1.16 ±0.02	1.14 ±0.005	1.13 ±0.01	1.08 ±0.01	1.14 ±0.03
Chloride mg g^{-1}	0.41 ±0.05	0.49 ±0.00	0.41 ±0.08	0.33 ±0.00	0.25 ±0.08

o- PO4 mg g^{-1}	0.10 ±0.02	0.10 ±0.025	0.13 ±0.00	0.08 ±0.00	0.07 ±0.01
NO_3 - N mg g^{-1}	±0.00	±0.000	±0.00	±0.00	±0.00
Organic carbon %	0.27 ±0.01	0.28 ±0.00	0.28 ±0.00	0.27 ±0.00	0.28 ±0.01

All values are mean ±S.E. of mean.

4. Discussion

4.1. Effect of Feeding Frequency on Growth Performance and Bioenergetic Parameters

Feeding frequency had a significant effect on growth performance in C mrigala which increased with increase in feeding frequency. At the end of 75 days, weight gain parameters in *C mrigala* fed four meals d^{-1} though were higher, however, their values were not significantly (P<0.05) different from the groups given two or three feedings d^{-1}, but were significantly higher than the fish fed only once a day. The orthogonal polynomial fit curve, fitting to the data of weight gain and SGR also indicated a clear frequency-dependent trend line curve being higher at multi feedings d^{-1}.The R^2 values of regression were also higher depicting a significant frequency-dependent response. Under field conditions also all the three Indian major carps species responded very well to the frequency of feeding. At the end of 90 days, a significant (P<0.05) increase in final biomass,weight gain and bioenergetic parameters took place with each increase in the frequency of feeding and highest values were observed in fish fed six times a day, however, these values were not significantly different than the groups fed three or four meals per day. Low growth performance was observed in the groups fed once or twice a day. These results are similar to those reported by [32] Grayton and Beamish (1977), who had fed rainbow trout fry and observed that three meals a day were better than one and six meals per day.[33] Kasiri et al. (2011) reported that feeding angelfish juvenile four meals a day was better than two or one meal perday. [34]Asuwaju *et al.* (2014) reported that feeding *Clarias gariepinus* one time daily did not improve the growth; however, five times daily reduced mortality and improved feed conversion raito. According to Zakaria*et al.*[9](2016) maximum growth in an endangered temoleh, *Probarbus jullieni* was observed when fed three times a day which was better than fish fed two or one meal per day. According to Biswas *et al.*[17] (2006) feeding the Indian major carps (catla, rohu and mrigal) three times daily led to higher growth than those fed twice or once a day, however, differences were not statistically significant. Choudhury *et al.*, (2002)[16] and Abid and Ahmed (2009)[4] have also suggested that feed utilization capacity of *Labeo. rohita* fingerlings at three times feeding frequency was higher under intensive rearing than one or two times feeding indicating a direct relationship between the feeding frequency and growth performance. These results indicate that multiple feeding regimes appear to be more effective in efficient utilization of feed than a single feeding.

Laboratory studies on *C mrigala* and field investigations on Indian major carps had also suggested that when feeding frequency is high (3,4 or 6 d^{-1}) and the intervals between meals are short, no differences or insignificant differences were observed in growth and also in bioenergetic parameters. This amply supports the findings that food passes through the digestive tract more quickly without effective digestion. According to De Silva and Anderson (1995)[35] that beyond a certain level, excessive feeding has no influence on growth rather resulted in poor growth performance. According to He *et al*., (2015)[36], frequent feedings resulted in a prolonged digestion process and reduced the net energy gain and dietary protein efficiency. In other words, the net energy gain of juvenile snakehead, *Channa argus* fed four meals daily was lower than the fish fed at other frequencies and thus frequent feedings with smaller meals proved to be less beneficial for food energy utilization. Consistent with this hypotheses, results suggest that frequent feeding the fishes (>2) may be less beneficial for food energy utilization. A study conducted by Alanärä et al.[1](1992) had also confirmed that frequent feeding with an automatic feeder had a negative effect on growth performance of rainbow trout. This species exhibits a rapid increase in activity during feeding; this may suggest that frequent feeding is a stress factor that elicits greater expenditure of energy leading to reduction in fish growth. Even though no research has been conducted on the activity pattern during feeding in *C mrigala* or other Indian major carp species, it is presumed that low growth or insignificant differences during multiple feedings or at higher feeding frequency may be attributed to higher activity and thus expanding more energy.

Like weight gain and SGR, digestibility and nutrient retention (GPR and GER) also gradually increased with increase in number of meals d^{-1} (Tables 3). *C mrigala* fed four meals d^{-1} though had higher GPR and GER, however, their values were not significantly ($P<0.05$) different from the groups given two or three feedings d^{-1}, but were significantly higher than the fish fed only once a day. In general, the food conversion rate (FCR) decreased with growth. *C mrigala* fingerlings fed four times a day had the least FCR (1.51 ± 0.08), which was not significantly ($P<0.05$) different from the groups fed two or three times a day, though significantly ($P<0.05$) lower than the group given only one meal a day. The orthogonal polynomial fit curve, fitting to the food conversion ratio (FCR) also showed a frequency-dependent trend line curve being lower at higher feeding frequencies. R^2 values of regression were also high depicting a significant frequency-dependent response. These results are in agreement with the findings of Wu *et al*. (2015), who have also reported that in rare minnow, FCR decreased with growth for most of the groups. According to Nekoubin and Sudagar (2012)[37] the best feed conversion ratio (FCR) in Grass Carp (*Ctenopharyngodon idella)* was obtained in the group given four meals d^{-1}, however these values were not significantly different from the groups given two or three ($P>0.05$) feeding d^{-1}. These studies indicate that excessive feed intake above what the fish really needs results in high FCR.

4.2. Fish Carcass Composition

Feeding frequency affects feed utilisation and hence influences fish carcass composition [38, 11, 8, 39]. Significantly ($P<0.05$) higher values of protein and fat and lower values of ash contents were observed in the groups fed four and three times a day in comparison with the groups fed once or twice a day. It shows that the experimental fish converted and utilized the protein from the feed into their body protein. These results coincidewell with higher growth observed in *C mrigala* and also with the other three Indian major carp species grown under field conditions. Present results are similar with some previous findings such as; whole-body crude protein and crude lipid content increased with increase in feeding frequency in juvenile red spotted grouper [40[(Kayano et al.,1993), juvenile Korean rockfish[45] (Lee, *et al.*, 2000)and *Heterobranchus bidorsalis* X *Clarias gariepinus* [42] (Obe and Omodara, 2014).

Even though, the same amount of feed was given in all the treatments, higher growth performance and higher values in most of the bioenergetic parameters were observed at higher/multi feeding frequency treatments rather than when given a single or two feedings per day.This may be attributed to the fact Indian major carp require the availability of feed at definite time intervals or at a specific time of the day, rather than delivering the ration in one instalment. Studies on scheduled meal timings conducted on *C mrigala* had revealed that higher growth parameters, low FCR and low excretion of metabolites (N-NH_4 and o-PO_4) were observed when feed was delivered between 12^{00} h and 16^{00} h. in comparison to the fish fed at other times of the day[13] (Garg and Kalla 2017). In the present studies low growth was observed in the groups fed a single meal (fed at 08^{00}h) or two meals (fed at 08^{00} h and 12^{00}h d^{-1} respectively) delivered in the morning hours. This may be attributed to low utilization of feed perhaps of the timings of delivery of ration. Present studies have thus shown that *C mrigala* like many other fish species utilize the feed more efficiently when delivered at a specific time of the day. This agrees well with some earlier findings reported by many workers [6,12,13 [43,44,45-47] (see: Biswas *et al*., 2010; Caldini *et al*. 2013; Marinho *et al*., 2014; Mizanur and Bai 2014, Garg and Kalla, 2017; Kitagawa *et al*.,2015; Boerrigter *et a*l.,2016; Mattos *et al*., 2016). These investigations have shown that along with the feeding frequency, the time of delivery of ration might also be a significant factor in obtaining production.

4.3. Nitrogen Retention and Postprandial Excretory Levels of Total Ammonia (N-NH4+) and Reactive Phosphate (o-PO4) (Figure 2)

A review of the data indicated that the excretion of metabolites appeared to have been affected by the frequency of feedings and showed a negative correlation with growth and digestibility parameters and also with nitrogen retention. These studies have revealed that higher growth in *C mrigala* shows a negative correlation with post prandial excretion of ammonia (N-NH_4) and phosphate production (o-PO_4). These results are similar to those of Marinho *et al* (2014) who have also observed that higher growth and higher nitrogen retention in Senegalese sole (*Solea senegalensis*) showed a negative correlation with total ammonia excretion. According to He *et al* [36], ammonia excretion significantly increased with an increase in feeding frequency; however, no significant differences were observed because of larger variation for each group. Higher gross protein retention (GPR) further indicate that Indian major carp fingerlings appear to use the supplementary feeds more efficiently for somatic growth when fed at least three times d^{-1}. This may suggest that a feeding regime which extends from 12^{oo}h to 16^{oo} h favours higher growth and thus appears to be more effective in higher production.

According to Marinho *et al* (2014)[45] time of delivery of ration and its size play a determinant role in regulating feed intake, improved growth and waste outputs. According to Phillips *et al*., (1998)[48], feeding frequency may influence fish growth both directly and indirectly as it can affect water quality through oxygen and ammonia concentrations.

4.4. Effect of Feeding Frequency on Hydrobiological Characteristics of Pond Waters

The recorded physico-chemical parameters like temperature, salinity, conductivity, dissolved oxygen (DO), and pH are considered to be important and critical water quality parameters in aquaculture[30,44,49,50].aterquality factor interacts with and influences other parameters, sometimes in complex ways. Higher DO level is needed to support an increase in metabolic rates and other physiological parameters. In the present studies, Dissolved oxygen (DO) concentration remained significantly ($P<.05$) higher in ponds, where the fish were fed four or six times d^{-1} in comparison to the treatment where the feed was given once a day only. Statistically, DO showed a significant positive correlation with NPP ($r=0.56$) and fish weight gain ($r=0.73$, 0.69, 0.63) respectively for catla, rohu and mrigal clearly indicating that high DO favours high productivity of fish ponds. These observations are similar to those of Randolph and Clemens [51]. High BOD, nitrogen, phosphate and low NO_3-N levels in ponds where the fish were fed once a day indicated an accumulation of biodegradable material due to the accumulation of unconsumed feed.

Statistically nitrate or available nitrogen (NO_3-N) showed a significant and positive correlation with zooplankton ($r=0.74$), phytoplankton ($r=0.76$), NPP ($r=0.46$), whereas, significant and negative correlation with BOD_5($r=-0.55$) indicate a higher trophic status of ponds, where the fish were fed six times a day. Further, fish weight gain also showed a significant positive correlation with phtoplankton ($r=0.67$, 0.74, 0.69), zooplankton ($r=0.73$, 0.74, 0.75), NPP ($r=0.77$, 0.79, 0.76) for catla, rohu and mrigal respectively. These results clearly indicate that fish growth is also significantly and positively correlated with the exogenous environmental factors such as trophic status of the ponds, temperature and oxygen. Similar results have also been reported by many other workers [42,49-50-52-53].

Application of multiple regression models [31] indicate that amongst all the independent variables, fish growth appears to depend more on total phosphate ($r2=0.80$, 0.92, 0.88). Statistically N-NH_4^+ ($r=-0.71$, -0.79, -0.72) and o-PO_4 ($r=-0.84$, -0.90, -0.86) showed a significant and negative correlation with fish weight gain, clearly explaining that low growth in ponds where the fish were given a single meal a day be attributed to low DO and high N-NH_4^+ and o-PO_4. Cuenco *et al*. [54] in a study of factors affecting fish population and growth showed that besides available food and DO, ammonia in water is another important factor which affects fish growth. Low values of total phosphate and NO_3-N in sediment also coincided with maximum utilization of nutrients and high fish growth in ponds where the fish were fed four or six time's d^{-1}.

According to Giberson and Litvak [55] single ration feeding produces a substantial, short-term decrease in water quality that can be a significant stress factor. According to Phillips *et al*. [48], feeding frequency may influence fish growth both directly and indirectly as it can affect water quality through oxygen and ammonia concentrations. The maximum ammonia excretion and oxygen consumption and fluctuations in these parameters in the daily feeding cycle when one ration is delivered can be significantly higher than when feed is delivered more frequently or in multiple frequencies. This was confirmed in European perch, *Percafluviatilis*, ([56] and in pikeperch [57]. Therefore, to obtain higher and rapid growth and also high feed efficiency in aquaculture, it is necessary to set feeding ration and frequency with respect to the capacity of digestion of the fish, which should coincide with the “preferred feeding frequency of the target species. These results indicate that feeding frequency plays an important role in food utilization and growth performance in Indian major carp species as it not only affects fish growth but also alters water quality and nutrient status of ponds. Therefore, it is imperative that the feed may not be given in one instalment; rather it should be fairly distributed during the day time for the diurnal fish species like Indian major carps. These findings have practical significance in maximizing growth and survival of fingerlings for feed managers during rearing. Feeding the fingerlings thrice a day should be adopted for optimum growth, survival and efficient nutrient utilization.

5. Conclusions

Assuming that the cost of labour is not usually a limiting factor in most of the developing countries including India where mostly semi intensive fish farming is practiced. From these studies it can be concluded that feeding the Indian major carp fingerlings thrice a day should be adopted for optimum growth, survival and efficient nutrient utilization. If possible, delivery of ration should coincide with the feeding rhythm of the fish when the fish are able to use the feed efficiently [13, 44]. By controlling the optimum feeding frequency, farmers can successfully reduce the feed cost, maximize growth, increase profits and also would be able to manage water quality which is important in rearing fish in culture conditions.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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