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EFFECTS OF FEEDING FREQUENCY ON GROWTH PERFORMANCES AND RNA : DNA RATIO OF THE GANGETIC MYSTUS (*MYSTUS CAVASIUS* HAMILTON, 1822) IN LABORATORY CONDITION

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Abstract

Experiments were conducted for a period of 90 days to estimate the effects of different feeding frequencies (one time per day: T1, two times per day: T2, three times per day:T3 and four times per day: T4) on growth performance of gulsha fish reared in laboratory facilities. Significantly higher condition factor was observed in T2 (1.61 ± 0.02) and lowest in T4 (1.40 ± 0.01). Significantly lowest SGR and ADG were recorded in T4 fish fed four times per day. No significant difference was obtained among different treatments for FCR and survival rate. The highest RNA : DNA ratio (0.93 ± 0.07) was observed in fish of T2 and the lowest ratio (0.57 ± 0.11) was observed in fish of T1. The results of the present study suggest that in laboratory rearing two meals per day could be supplied for better growth performances as per indication of condition factor and RNA:DNA ratio.

Introduction

The growth of fish at different stages of life mostly depends on the feed types, feeding regime, feed intake and its ability to digest and assimilation the nutrients. Role of feeding regime or frequency in commercial fish culture as well as early level survival and growth of fish has been reported as important factors to be considered [1-2]. Use of a judicious feeding frequency is also important to minimize water pollution and production cost of commercially important fish[3].

Fish feeding time and frequency have been studied by many authors to investigate feed intake and growth performance of *Labeo rohita*, *Heteropneustes fossilis*, *Oncorhyncus mykiss*, etc.[4-6]. Therefore, it is important to identify optimum frequency and feeding rate of economically important fish species in aquaculture for their better production. When a fish is fed according to their requirement, FCR and growth will be improved due to regulation of its food consumption in relation to energy requirement[7]. So, feed management in terms of standardization of feeding rate and frequency has become one

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of the vital areas of research in aquaculture. RNA:DNA ratio analysis is used to determine the growth condition of fish and it is based on the amount of RNA increase in the cell to systhesis protein where the amount of DNA is almost fixed in the cell. This approch has been successfully used in various aqatic organisms like fish, planktons and macroinvertebrates[8-9].

The Gangetic mystus (*Mystus cavasius*) is one of the most popular and economically important fish in inland aquaculture because of its high market demand and good taste. It is widely distributed in Bangladesh, Pakistan, Nepal, India, Sri Lanka and Myanmar[10]. Nowadays it is commercially cultured in fresh water ponds of Bangladesh. To improve the culture of *M. cavasius* there is a need for understanding the culture management method in the area of feeding and feeding frequency to produce fish within a short time and at minimum cost and quality. Many researchers studied the effects of feeding frequency on growth of some edible and aquarium fishes[4,11-13] but to the best of knowledge no information is available about the effect of feeding regime on growth and survival rate as well as RNA:DNA ratio regarding *M. cavasius*. So, the present study was undertaken to estimate the growth performances of *M. cavasius* under different feeding frequencies fed with micro-pelleted feed.

Materials and Methods

The Gangetic mystus (*Mystus cavasius*) was collected from the "Bhai Bhai Adarsho Matsho Hatchery" at Trishal, Mymensingh district. Fingerlings were transported with oxygenated polyethene bags and stocked in experimental tank at Aquatic Laboratory of Department of Fisheries, University of Dhaka.

The experiment was designed on four treatments (T1 : one meal/day, T2 : two meals/day, T3 : three meals/day and T4 : four meals/day) with three replications. Total 18 fish were randomly stocked in each tank with 30 liters water. The study was carried out for 90 days from August to October 2016. The fish were fed with commercial pellet diet named 'Optimum Micro Pellet' with 5% of their body weight. The ingredients of supplied feed were fish meal, wheat flour, yellow corn, shrimp meal, spirulina fish oil, vitamins and minerals. The supplied feed contains crude protein 32%, crude fat 4%, moisture 10% and crude fibre 4%.

Aeration was provided throughout the experiment. Water quality was monitored and the average values of dissolved oxygen, temperature, pH were found to be 7.7 ± 0.86 mg/l, 24.2 ± 2.45°C and 8.10 ± 0.89, respectively. After 90 days of rearing 30% fish were randomly collected by a fine mesh scoop net for growth measurement. During sampling, cold water was used for reducing the stress. After sampling, they were released carefully into the aquarium. To evaluate the growth of fishes and feed performances, average daily gain, specific growth rate, condition factor, feed conversion ratio and survival rate were assessed[14-15].

For the determination of RNA : DNA, nucleic acids were extracted using CTAB extraction method with slight modifications[16]. RNAse was not used in the procedure. First of all, an amount of fish tissue (0.48 g) was grinded to a fine paste with approximately 2000 µl of CTAB buffer. CTAB/fish extract mixture was transferred to a microcentrifuge tube. Proteinase K (100 µgm) was added and the sample was inverted to mix up. The mixture was then incubated in water bath for 2 hours at 55^{0}C. The sample was centrifuged for 5 minutes at 14000 rpm and the supernatant was transferred to another tube. An equal volume of 25 : 24 : 1 phenol : chloroform : isoamyl alcohol was added and mixed well with the sample. The mixture was centrifuged at 14000 rpm for 10 min. The supernatant was transferred to a clean microcentrifuge tube. To each tube, equal volume of isopropanol was added. The precipitate was isolated by spinning the tube at 14000 rpm for 5 min. The supernatant was removed and the DNA pellet was washed by adding two changes of ice cold 70% ethanol. All the supernatant was removed and DNA pellet was allowed to dry (approximately 15 min). The DNA was not allowed to over dry or it would be hard to re-dissolve. The DNA was then dissolved in 10 µl nuclease-free water and stored at 4^{0}C. Finally the quantity of DNA and RNA was measured by the Thermo Scientific Nanodrop 2000 Spectro-photometer with absorbance at 260/280 nm. After calibration, 1 µl of each sample was used for measurement of RNA and DNA. Average ratio of RNA and DNA was calculated for each frequency group and then compared.

All data were analyzed by using statistical package SPSS (Version 24; SPSS Inc., Chicago, IL, USA)with the level of significance at $p < 0.05$.

Results and Discussion

Highest average daily gain was obtained in T3 (0.30 ± 0.02 g/day) fish fed 3 meals per day and lowest was recorded in T4 (0.09 ± 0.001 g/day) fish fed 4 meals per day (Fig. 1). Significant difference ($p < 0.05$) in average daily gain was obtained among four treatments. The values of average daily gain of fish of T1 and T2 were (0.16 ± 0.014) and (0.27 ± 0.012), respectively.

Higher SGR was obtained in T1 (2.66 ± 0.11 %) and T2 (2.55 ± 0.24%) when compared with T3 (1.27 ± 0.02 %) and T4 (0.76 ± 0.02 %) (Fig. 2). Lowest SGR was measured in T4 fish feed four times per day. However, no significant difference ($p > 0.05$) was recorded between T1 (fish fed one meals per day) and T2 (fish fed two meals per day).

The fish of T2 was obtained highest condition factor (k) value (1.61 ± 0.02) and fish of T4 was obtained lowest k value (1.40 ± 0.01). The k values of T2 and T4 differ significantly. But there was no significant condition factor differences between T1 and T3 fish fed feed 1 meal per day and 4 meals per day (Fig. 3).

Lowest FCR was measured in T2 (1.78 ± 0.10) and highest was measured in T4 (2.69 ± 0.26). Highest survival rate was measured in T1 (92.20 ± 1.10 %) fish fed one meal per day

and lowest was recorded in T4 (87.75 ± 2.99 %) fish 4 meals per day. No significant difference in FCR and survival rate was measured in *Mystus cavasius* fish in four different treatments (Figs 4 and 5).

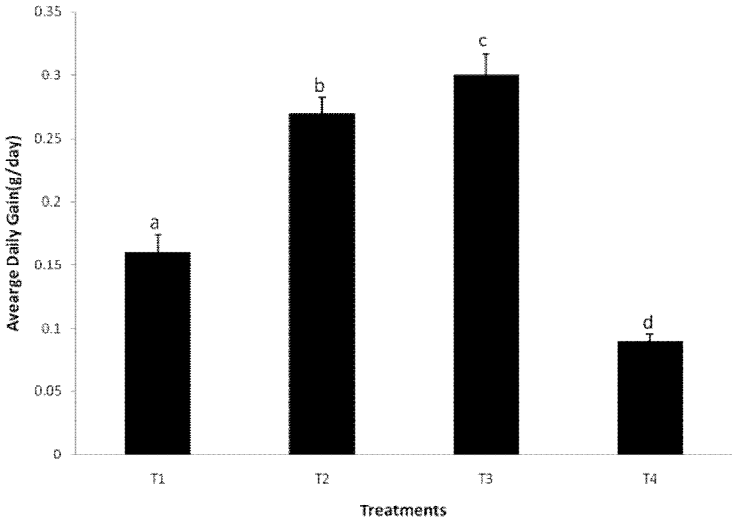

Fig. 1. Average daily gain (g/day) of *Mystus cavasius* in four different treatments cultured for 90 days. Bars with different letters are significantly different ($p < 0.05$).

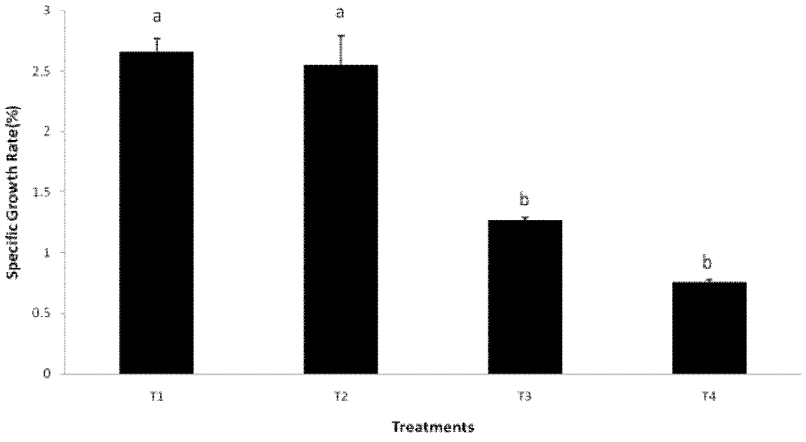

Fig. 2. Specific growth rate (SGR,%) (Mean ± SEM) of *Mystus cavasius* in four different treatments cultured for 90 days. Bars with different letters are significantly different ($p < 0.05$).

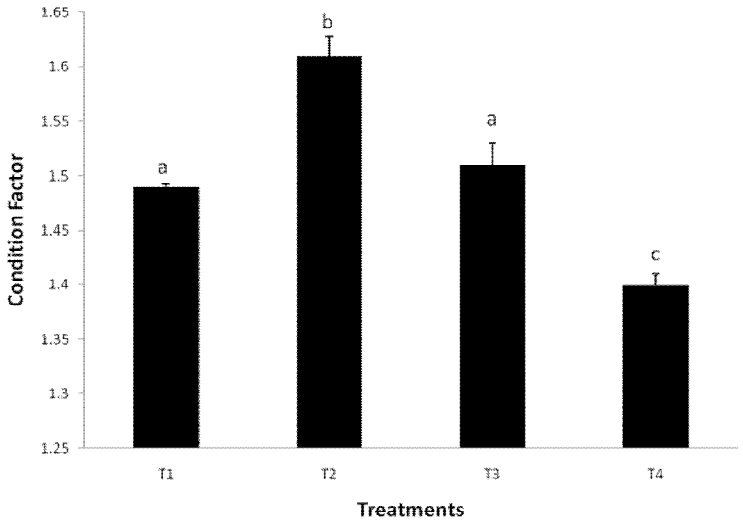

Fig. 3. Condition factor (Mean ± SEM) of *Mystus cavasius* in four different treatments cultured for 90 days. Bars with different letter are significantly different.

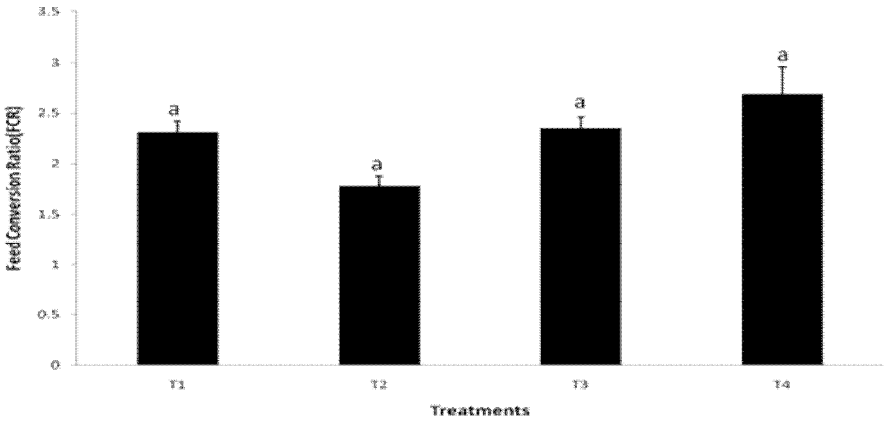

Fig. 4. FCR (Mean ± SEM) of *Mystus cavasius* cultured for 90 days with four different treatments. . Bars with same letter represent no significant difference ($p > 0.05$).

RNA and DNA levels in the tissue of *Mystus cavasius* in the different treatment groups are shown in Table 1. The highest RNA : DNA ratio (0.93 ± 0.07) was observed in T2 whereas the lowest (0.57 ± 0.11) RNA : DNA ratio was observed in T1 (Table 1). The values were significantly different.

This study investigates the growth performance and RNA/DNA ratio of Gangetic mystus fish with different feeding frequencies during a culture period of 90 days.

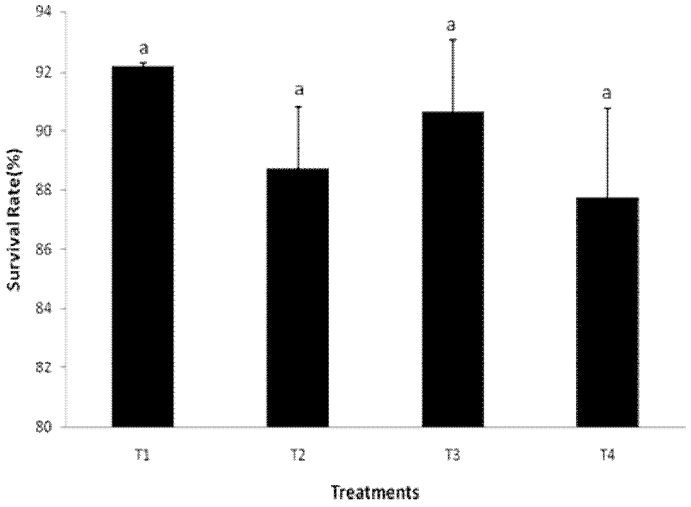

Fig. 5. Survival rate, % (Mean ± SEM) of *Mystus cavasius* cultured for 90 days with four different treatments. Bars with same letter represent no significant difference ($p > 0.05$).

Table 1. RNA, DNA content, and RNA : DNA in the muscle of *Mystus cavasius.*

Treatments	RNA (ng/µl)	DNA (ng/µl)	RNA : DNA
1	30.70 ± 2.67	55.96 ± 7.39	0.57 ± 0.11^{a}
2	19.50 ± 6.90	34.70 ± 14.20	0.93 ± 0.07^{b}
3	50.26 ± 13.35	62.33 ± 16.73	0.81 ± 0.004^{ab}
4	20.73 ± 3.38	25.43 ± 3.92	0.81 ± 0.008^{ab}

Values are mean ± SEM (n = 9). Means in the same column with different superscripts are significantly different at $p < 0.05$.

In average daily gain and specific growth rate, there are significant differences among different treatments. Lowest ADG and SGR are observed in T4 fish fed with 4 meals per day, whereas higher ADG and SGR are in T3 and T1, respectively. James and Sampath[18] observed highest growth and reproductive success in red swordtail (*Xiphophorus helleri*) that was fed twice a day in a cultured system, when compared with other feeding frequencies. But Guen-Up *et al*.[19] found that feeding to satiation once a day resulted in optimum growth of a commercial important black rockfish (*Sebastes schlegeli*).

Condition factor is one of the important growth parameters of this work and has significant difference among treatments. Highest condition factor value is observed in fishes fed with two meals per day and lowest with four meals per day. Thus, the results indicate that fish fed with two meals per day grows better than others. Actually, condition factor is an index to compare growth and fitness of fish based on the length and weight of fish. Rahman *et al.*[17] in a study on the survival and growth of catfish on

selected supplemental feeds reported the values of condition factor between 0.81 and 0.87. But Kasiri *et al.*[11] did not find any significant difference in condition factor among four different feeding frequencies of angel fish though k values were more than 1 in case of all treatments indicating fairly better condition of the fishes. Thus, condition factor is also used as an index to compare growth and well-being of fish based on the principle that heavier fish of a given length is in better condition.

In the present study, there is no significant difference in FCR value and survival rate among different treatments. Present observation of FCR is in agreement with previous reports on cage-reared channel catfish[20] and hybrid sunfish[21]. Kasiri *et al.*[11] also studied the effects of feeding frequency on growth performance and survival rate of angel fish *Pterophyllum scalare* and found no significant different of survival rate among four different treatments (four meals per day, two meals per day, one meal per day and every other day).

Quantitative nucleic acid analysis offers an important tool for the measurement of fish growth rate. The increased level of RNA in a tissue is expected to show increased growth of somatic tissues, whereas DNA, genetic carrier of information, remains constant in somatic tissues and principally acts as indictor of cell number or biomass[22]. The concentration of RNA has been proposed as an indicator of nutritional status and growth of fish[23]. Many authors, like Akhtar *et al*.[24] (*Labeo rohita*), Zehra and Khan[25] (*Catla catla*), Smith and Buckley[26] (*Gadus morhua*) etc. have suggested that RNA:DNA ratio is an index of growth of fish. In the present study, highest RNA : DNA is measured in T2 fish fed twice daily and lowest T1 fish fed once daily.

It is evident from this study that feeding frequency has significant effects on the growth performances and RNA/DNA ratio of Gangetic mystus fish and feeding frequency up to 3 meals per day could be used for better growth to this fish in culture condition. However, further investigation should be carried out to observe the effects of feeding regime on growth of Gangetic mystus (*Mystus cavasius*) fish in culture pond condition.

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