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EFFECTS OF DIFFERENT PROTEIN LEVEL FEEDS ON THE FINGERLINGS PRODUCTION OF RIVERINE ENDANGERED *Notopterus chitala* (Hamilton, 1882) IN PONDS

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This study was aimed to evaluate the efficiency of three protein level feeds on the fingerlings production of riverine endangered *Notopterus chitala* (Hamilton, 1882) for a period of two months in ponds. Chital fry (Initial wt, 5.6±0.19 g) were randomly stocked at the rate of 7410 per hector. Feeding trials were conducted under 3 treatments (T_1: feed contains 35% protein, T_2: feed contains 30% protein, T_3: feed contains 25 % protein) each with 3 replications. No significant (P<0.05) difference was observed for all the water quality parameters among the treatments. A suitable range of water quality parameters (temperature, water transparency (cm)), dissolved oxygen (mg/l), pH, alkalinity (mg/l), ammonia) were recorded with the environment of experimental ponds. The treatment had significant effects on weight gain, ADG, SGR among treatments. Highest weight gain (19.31 ± 0.23 g) was observed in T_1, lowest (13.96 ± 0.15 g) in T_3. SGR (1.65±0.02) and ADG (0.18±0.005) were significantly (P<0.05) highest in T_1 followed by T_2 and T_3. The *N chitala* exhibited highest survival rate (96.66%) in T_1 than T_2 followed by T_3 (88.89 %). The best FCR value (3.02±0.09) was recorded in treatment T_1 with 35% protein contain feed. Relatively, highest benefits (138690±175.36 BDT/ha) were found from treatment T_1 compared to other treatments. Similarly, the cost benefit ratio was highest in T_1 (1:1.23) and lowest in T_3 (0.95). Findings indicated that treatment T_1 (35% protein containing feed) was found best in terms of growth, production and economics of *N. chital* for fingerling production in ponds.

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INTRODUCTION

Notopterus chitala is widely distributed in deep and clear waters in the rivers, beels, reservoirs, haors, baors and ponds in Bangladesh (Islam and Hossain, 1983, Hafizuddin 1985, Azadi *et al.*, 1994). It is rich in nutritive value (Jafri *et al.,* 1994) and high valued species in Bangladesh. In recent years, the catch of this species has been declining fast due to environmental degradation (Hossain *et al.*, 2006). However, over exploitation, pollution and related man-made intervention on their natural habitats, have considerably reduced the population of this species and listed as endangered (IUCN, 2015). *Chitala* is one of the most important species that has been prioritized as a new candidate species (Ayyappan *et al*., 2001) for aquaculture. Actually, the success of commercial aquaculture operations depends on the availability of suitable diets that are efficiently digested and provide the required nutrients for optimum growth (Mokolensang *et al*., 2003). It is important to develop suitable fry rearing system with optimum protein containing feed for domestication and conservation of this species. Successful aquaculture operation of these high value species mostly depends on the application of nutrient rich feed (Dey *et al*., 2008). Fish feed generally constitutes 60–70% of the operational cost in intensive and semi- intensive aquaculture system (Singh and Balange.2005). An important approach to reduce feed costs in commercial aquaculture is to develop proper feed management and husbandry strategies (Lovell *et al*., 1998). Protein is the most expensive nutrient in fish diet (Pillay, 1990). Increasing protein levels in feeds can lead to improved fish production, but excessive dietary protein is not economical for fish culture (Erondu *et al*., 2006; Sheunn *et al*., 2003). Though this species has been reported quite favorable in pond habitat, but their monoculture technique with different protein-based feed has not yet been developed. Therefore, the present study was designed to evaluate the production performance of *Notopterus chitala* in monoculture management using different protein-based feed in northern area of Bangladesh.

MATERIALS AND METHODS

Study area and experiment pond

The experiment was conducted in the nine experimental nursery ponds situated on the north side of the department of Fisheries, University of Rajshahi for a period of 60 days. The average area of the ponds was 0.8 decimal (0.0032 hector). The water depth was maintained around 1.0-1.25 m by using pump machine at regular intervals. Prior to the experiment, the ponds were dried, aquatic vegetation and all fishes and other predatory species were completely removed. Lime (Calcium carbonate) was applied at the rate of 247 kg/ha. Three different diets for chital fry were tested in this experiment. Each diet of chital fry was considered as treatment and replicated thrice. The treatment assignment was as T_1(35 % protein), T_2(30% protein), T_3 (25% protein). The fry stocking rate per hectare was 7410 (30/deci)

Stocking and feed management of fry

The fry of Chital (*Notopterous chitala)* were collected from Padma River. Fry were transported to the experimental site through plastic bag with proper aeration. The chital fry (wt.5.6±0.19 g) was randomly stocked at the rate of 7410 per hector for each treatment. The stocked fries were fed with pellet diet containing different level of protein for different treatments (For T_{1} -35%, T_{2}- 30%, and T_{3}- 25%). Six ingredients were used in the formulated feed at different percentages and the ingredients were calculated for their inclusion rate and the results are shown in table-1&2. The proximate composition of feed ingredients was analyzed according to the methods given in Association of Official Analytical Chemists (AOAC, 1990). Experimental diets were formulated according to the Pearson square method. Feed requirement were calculated and adjusted after sampling of fish once in a fortnight. The feeds were initially fed at the rate of 10% of body weight for thirty days and the rate was reduced to 8% gradually next 30 days.

Table 1. Proximate composition of different feed ingredients

Ingredients	Moisture (%)	Crude Protein (%)	Crude Lipid (%)	Fibre (%)	Ash (%)	NFE (%)
Fish meal	16.63	58.5	7.62	2.54	24.89	6.45
Soyabean meal	13.46	48.0	13.44	11.12	9.73	17.71
Mustard oil cake	10.67	32.5	5.43	9.71	5.93	46.43
Maize bran	11.67	12.5	9.45	21.85	15.40	40.8.
Wheat bran	9.24	13.5	8.7	17.5	12.5	47.8
Rice polish	9.32	13.0	7.4	16.6	13.4	49.4

*NFE calculated as= 100%- (moisture +crude protein +crude lipid + fibre +ash)

Table 2. Composition of feed ingredients used for different treatments in the experiment. (by Pearson square method)

Treatments Feed ingredients	Inclusion level (%) of ingredients T_1	Inclusion level (%) of ingredients T_2	Inclusion level (%) ingredients T_3
Fish meal	24.17	18.68	13.18
Rice bran	9.15	14.64	20.14
Wheat bran	9.15	14.64	20.14
Soyabean meal	24.17	18.68	13.18
Maize bran	9.15	14.64	20.14
Mustard oil cake	24.17	18.68	13.18
Vitamin-Mineral premix	1.00	1.00	1.00
Protein level	**35%**	**30%**	**25%**

Growth sampling of fish

Fish were sampled fortnightly using cast net to assess their growth and health condition. At least 10% fish from each pond was taken to make assessment of growth trends and to readjust feeding rate. Length and weight of sampled fish were measured using a measuring scale and digital electric balance (OHAUS, MODEL No. CT-1200-S). Fishes was handled carefully to avoid stress during sampling.

Water quality monitoring

The different water quality parameters such as temperature (ºC), transparency (cm), pH, dissolved oxygen (mg/l), alkalinity, ammonia-nitrogen (mg/l) of the ponds were monitored between 8:30-9:30 A.M. in each fortnight to record the physic-chemical condition of the pond. A centigrade thermometer within the range of 0°C to 120°C was used to record the water temperature. A secchi disk (20 cm diameter) was used for the measurement of water transparency. pH of pond water was measured by the help of a pH meter (Hanna, Italy) at the pond site. The dissolved oxygen, total alkalinity and ammonia-nitrogen concentration of water was determined by the Winkler's titration method (APHA, 1992) and expressed in milligram per liter (mg/1) of water.

Growth parameters

Growth and feed utilization parameters (Mean weight gain, Average daily gain, Specific growth rate, Survival rate, Food conversion ratio) were used to evaluate the growth performance of fishes under different treatments. Data on growth collected from different treatments during the trials were calculated and analyzed using standard methods. (Brown, 1957: Ricker,1975, De Silva, 1989, Castell and Tiews,1980).

Profit and statistical analysis

Data on both fixed and variable costs were recorded to determine the total cost (BDT/ha/60 days). Total return determined from the market price of fish was expressed as BDT/ha/60 days. For the analysis of collected data, one-way analysis of variance (ANOVA) was performed using the SPSS (Statistical Package for Social Science, evaluation version-16.0) program and significance was assigned at the 0.05% level and tested Duncan's New Multiple Range Test (DMRT) to identify significant differences among the mean values. (Zar, 1984).

RESULTS AND DISCUSSION

Water quality parameters

Mean value of water quality parameters over the 60 days rearing of *Notopterus chitala* are presented in table 3. Physico-chemical and biological environment of a water body are mostly influenced by water temperature which is the most important physical factors. In present study, temperature varied from 26.70 to 33.60°C with the means of 30.67 ± 0.57 ºC, 29.73± 0.63, 30.75 ± 0.60 °C in treatments-1, 2 and 3, respectively. Samad *et al.* (2005) found water temperature varied from 29.00°C to 30.6 °C in nursery pond that was almost similar to those obtained in the present study. Rahman *et al.* (1992) stated water temperature ranged were 25.5°C to 30.0°C, which was favorable for fish culture

Water transparency is a gross measure of pond productivity. It acts as an index of productivity of a water body. It is closely related to the phytoplankton abundance. Wahab *et al*. (1995) suggested that the transparency of productive water should be 40 cm or less. Comparatively higher mean value (36.56 ± 0.44cm) of water transparency was found with the treatment T_3 and lower mean value (33.72 ± 0.21cm) was found with the treatment T_1. This might be due to the frequent application of feed and seasonal variation in plankton concentration. This finding strongly agreed with Boyd (1998) who found transparency between 30-45cm as good for fish culture. Rahman *et. al.* (2017) also measured transparency varied from 32.58 to 36.42cm in feed-based carp polyculture ponds.

Table 3. The mean values of water quality parameters under different treatments during the study period (60 days)

Treatments / Parameters	T_1	T_2	T_3
Temperature (°C)	30.75 ± 0.60 ^{a}	29.73± 0.63 ^{a}	30.67 ± 0.57 ^{a}
Transparency (cm)	32.72 ± 0.21 ^{a}	36.17 ± 0.41 ^{a}	36.56 ± 0.44 ^{a}
DO (mg/l)	5.29 ± 0.04 ^{a}	5.44 ± 0.05 ^{b}	5.46 ± 0.03 ^{b}
PH	7.53 ± 0.02 ^{a}	7.59 ± 0.02 ^{a}	7.53 ± 0.02 ^{a}
Alkalinity (mg/l)	143.06 ± 2.02 ^{a}	141.57 ± 1.76 ^{a}	140.75 ± 1.65 ^{a}
NH_3-N (mg/l)	0.105 ± 0.005 ^{a}	0.113 ± 004 ^{a}	0.110 ± 0.004 ^{a}

Values in a row bearing common letter(s) do not differ significantly ($p<0.05$)

In the present study, dissolved oxygen varied from 5.29 ± 0.04mg/1to 5.46 ± 0.03 mg/1(Table-2). The highest value was found with treatment T_3 whereas the minimum value of dissolved oxygen was found with treatment T_1. Wahab *et al.* (1995) reported low dissolved oxygen content (2.0 to 7.2 mg/1) during their experiment in Mymensingh. Begum *et al.* (2009) also estimated dissolved oxygen content lower (3.39-5.5 mg/1) in feed and fertilizer-based ponds. Ridha (2011) reported that the suitable DO of water body for fish culture would be 5-8 mg/1. Samad *et al* (2017) also measured dissolved oxygen varied from 4.76±0.04 mg/l to 4.91±0.04 mg/1 in *Notopterus chitala* ponds in Rajshahi area. Fish did not show any sign of oxygen deficiency throughout the study period. The concentration of dissolved oxygen was optimum due to exchange of water in ponds.

pH is considered as an important factor in aquaculture and treated as the productivity index of a waterbody. The pH value of the experimental pond varied from 7.53 ± 0.02 to 7.59 ± 0.02 which were within more or less acceptable range of 6.5-9.0 (Boyd, 1990). During the experimental period pH of the experimental pond was slightly alkaline, which indicated a good pH condition for fish culture. Similar findings were found by Saha *et al.* (2003) and Samad *et al.* (2004).

In alkaline waters essential nutrients are found in higher quantities and this is the most important reason for higher biological productivity in alkaline waters than in acidic waters. But highly alkaline condition is not favorable for biological production (Rahman, 1992). Total alkalinity values obtained during the study period were found to vary from 140.75 ± 1.65 mg/1 to 143.06 ± 2.02mg/1. Total alkalinity in the present study agreed with the findings of Samad *et al.* (2017) who recorded the values ranges from alkalinity 127.04±1.4 mg/l to 131.46±0.92 mg/1. The variations in total alkalinity in all the treatments were found in productive range for aquaculture ponds (Boyd 1982, Chakraborty and Nur*,* 2012 and Kohinoor *et al.* 2017).

The presence of ammonia in pond is normal due to natural fish metabolism and microbiological decay of organic matter. Chan *et al* (2007) found that lower than 1mg/l of NH_3 gas content in pond was good for fish culture. The value of NH_3-N was found to be ranged from 0.105 ± 0.005 mg/1 to 0.113 ± 004 mg/1. Ali *et al.* (2004), and Asaduzzaman *et al*. (2006) recorded ammonia nitrogen value ranged from 0.37, 0.01 to 0.82, 0.203 to 0.569 mg l^{-1}, respectively. Mensah *et al*. (2014) found ammonia nitrogen from 0.02 to 0.05 mg/l in tilapia culture pond. New (1987) reported that excessive use of feed or fertilizer caused sediments in the pond bottom which may produce ammonia and other gases. However, ammonia-nitrogen level in the rearing ponds was not toxic to cultured fishes.

Growth performance of *Notopterus chitala*

Growth parameters such as final weight, gain in weight and SGR% showed (Table-4) statistically significant differences (P<0.05) among three treatment groups**.** The effect of feed on the production of fingerling was significantly different (Hossain *et al.*, 2007). Dietary protein is used by fish for growth, energy and body maintenance (Kaushik and Medale, 1994). *N. chitala* was found to be fast-growing and reached 2 kg in 243 days (Rahmatullah *et al*., 2009). The highest final weight (19.31 ± 0.23 g) was found with the treatment T_1 (about 35% proteins) and the lowest final weight (13.96±0.15 g) was examined with the treatment T_3 (about 25% proteins). The growth in length was found to be very high in comparison with weight. It might be due to laterally flattened body of *N. chitala* which gain less weight with increase of body length (Azadi *et*. *al.,* 1994). Degain *et al*. (1989) established direct relationship between growth rate and protein content of diet. In the present studies also significantly higher growth rate was recorded in high protein (35%) diets than the low protein (25%) diets. Renukaradya and Varghese (1986) also concluded that daily growth rate of *Catla catla* was higher with diets having 30 and 40% protein which is similar to the present study. Samad *et al* (2005) obtained similar trends of growth rate of *Clarias batrachus* fingerlings in earthen ponds of Bangladesh.

Specific growth rate and average daily gain

The Specific growth rate (SGR, % bwd^{-1}), 1.65±0.02, 1.43±0.01 and 1.22±0.01 were found in T_1, T_2 and T_3 respectively (table 3.). SGR progressively decreased with decreasing protein level in feed. This finding is little bit lower than the findings of Samad *et al*. (2016) in case of *N. chitala*.fry under different stocking densities. Sarkar *et al*. (2006) found SGR in *Chitala chitala* fishes varied from 0.63±0.5 (used boiled egg-yolk) to 2.40±0.72 (used live *Tubifex* warm). In another experiment, Samad *et al*. (2017) pointed out that the SGR (% bwd-1) 3.84±.02 to 4.54± .01 of endangered *Notopterus chitala* (Hamilton, 1822) with *Oreochromis niloticus* culture in pond habitat.

The average daily gain (ADG, %) was also found higher in the treatment T_1 (0.18±0.005) whereas, the lowest value was found in T_2 (0.14±0.005) and T_3 (0.11±0.005). All values were significantly (P<0.05) different among the treatments. Samad *et al.* (2005) found the best ADG, (0.138g) in the experiment of *C batrachus*. Sangrattanakhul (1989) found the ADG of *A. testudineus* fish ranging from 0.100 to 0.120 g and this finding was lower than the findings of present study due feed and variation of species.Panase and Mengumphan (2015) also found the same trend as like in the present experiment. The present study showed that significant reduction in ADG resulted for reducing protein level in feeds*.*

Table 4. Growth and production performance of *N. chitala* under different treatment during the study period

Treatments parameters	T_1	T_2	T_3
Mean initial weight (g)	5.6±0.19 [a]	5.6±0.19 [a]	5.6±0.19 [a]
Mean final weight (g)	19.31±0.23 [a]	16.39±0.17 [b]	13.96±0.15 [c]
Mean weight gain (g)	13.71±0.23 [a]	10.79±0.17 [b]	8.36±0.15 [c]
Mean initial length (cm)	3.3±0.10 [a]	3.3±0.0.10 [a]	3.3±0.10 [a]
Mean final length (cm)	11.8±0.17 [a]	9.97±0.18 [b]	8.43±0.15 [c]
Mean length gain (cm)	8.50±0.17 [a]	6.67±0.18 [b]	5.13±0.15 [c]
SGR (%,bwd-1)	1.65±0.02 [a]	1.43±0.01 [b]	1.22±0.01 [c]
ADG (g)	0.18±0.005 [a]	0.14±0.005 [b]	0.11±0.005 [c]
FCR	3.02±0.09 [c]	3.68±0.07 [b]	4.51±0.20 [a]
Survival rate (%)	96.66±0.96 [b]	93.33±0.96 [ab]	88.89±2.00 [a]
Production (kg/ha/60 days)	138.32±1638 [a]	113.38±1172 [b]	89.65±966 [c]

Values in a row bearing common letter(s) do not differ significantly (p<0.05)

Survival rate

The survival rate (%) during the study period were 96.66 ± 0.96 %, 93.33±0.96 and 88.89 ± 2.00 % in the T_1, T_2 and T_3 respectively, which were significant different (P>0.05) among the treatments (Table-3). This survival rate is agreed with the survival rate of 88.00 to 94.00 %, recorded by Sarkar *et al.* (2006) in intensive recirculatory tank culture system of *Chitala chitala* (Hamilton). Rahmatullah *et al*. (2009) found survival rate for chital (*N. chitala*) was 47% which is too much lower than the present study due to the longer culture period with tilapia as a cospecies. Hossain *et al*. (2006) reported that, the highest survival rates of *N. chitala* fry (98.50%) was observed when fish spawn was used as feed for *N. chitala*. Samad *e t al.* (2016) also obtained that the survival rate of *Notopterus chitala* fry was significantly varied from 82.33±1.20% to 94.50±1.50% which is similar trend to the survival rate with the present study. The survival rate achieved in the present experiments were indicating that the healthy and suitable ecological nature of the ponds.

Food conversion ratio (FCR)

The food conversion ratio (FCR) values were found lowest in T_1 (3.02±0.09) followed by T_2 (3.68±0.07) and T_3 (4.51±0.20), which were significantly different among the treatments. The experiment showed that T_1 gave excellent results where the fish's feeds on high protein (35%) containing feed. Samad *et al*. (2014) recorded comparatively lower FCR value by using 30% proteins containing feeds for *Clarias batrachus*. The FCR value in T_3 was much higher than the findings of Haque and Mazid (2005). The improved FCR has important cost saving implication. This finding was concurred with those of Siddiky *et al.* (2015) in which the FCR value ranged from 1.50 to 2.00 for *Mystus gulio* and Paul *et al.* (2012) for *Ompok pabda* the FCR was of 2.17 at 33% protein level. Samad *et al*. (2004) also obtained the best FCR value for fry rearing of *H fossilis* was 3.25. Finally, all the above citation approved about the feed efficiency and better feed utilization in the culture unit.

Production and cost-benefit analysis of *N. chitala*

The maximum fish production (138.32±1638 kg/ha) was obtained in T_1 under the 35% protein contains feed was used (Table-4). The lowest fish production (89.65±966 kg/ha) was observed in T_3 which might be due to the lower protein level (25% protein) feed used. The annual yields of chital (*N. chitala)* were 0.92 t ha-^{1} year^{-1} (Rahmatullah *et al*., 2009). The present result is lower might be due to 60 days culture period and use of different diet in fry rearing. In another study, Samad *et al* (2016) obtained the highest production (300.78±1.62 kg ha-^{1}) under different stocking density for *N. chitala* fry culture in earthen ponds for a period of 75 days. This was probably due to the effect of feed with different inclusion level and variation of the culture period.

Table 5. Input cost and profit from *N. chitala* culture for 60 days in ponds of three different treatments

Items / **Treatments**	**T_1**	**T_2**	**T_3**
Pond preparation (BDT/ha)	22971	22971	22971
Feed cost (BDT/ha)	22250±150 ^{a}	18158±154 ^{b}	15548±236 ^{c}
Cost of seed (BDT/ha	59280	59280	59280
Operating cost (BDT/ha)	7500	7500	7500
Total cost (BDT/ha)	112000±150.44 ^{a}	107910±154.74 ^{b}	105300±236.79 ^{c}
Total income (BDT/ha)	250690±324.81 ^{a}	221440±161.75 ^{b}	205730±523.26 ^{c}
Net profit (BDT/ha)	138690±175.36 ^{a}	113530±59.89 ^{b}	100430±298.75 ^{c}
CBR	1.23±0.0002 ^{a}	1.05±0.002 ^{b}	0.95±0.001 ^{c}

Values in a row bearing common letter(s) do not differ significantly (p<0.05)
Input prices and fish prices were calculated according to Rajshahi fish market (Purchase price 8 BDT/fry, selling price 30 BDT/fingerling, 250 BDT/labour etc.)

The cost of different inputs and economic return from the sale of fishes in are summarized in table 5. Among the treatments, the highest production and total income 250690±324.81 BDT/ha/60 days were found in T_1 and consequently provides the highest net profit 138690±175.36 BDT/kg/60 days in $T_{1,}$ where fishes were fed 35% protein containing feed. Similarly, the net profit in treatment T_2 (113530±59.89 BDT/ha/60 days) and lowest in T_3 (100430±298.75 BDT/ha/60 days) which is statistically significant difference (P>0.05) among the treatment. Samad *et.al*., (2014) analyzed the maximum net profit of walking catfish (*Clarias batrachus*) culture was 713542 BDT/ha/120 days which is more than the net profit of the present study because of longer culture period with different diets.

The Cost benefit ratio (CBR) of *N. chitala* in different treatments of the present study was fairly high ranging from 1:1.23 (T_1) to 1:0.95 (T_3) and statistically significant different among the treatments. Samad *et. al.*, (2014) recorded that the CBR of *Clarias batrachus* culture was higher (1:1.24) when 30% protein containing feed used. In recent study, Samad *et al.* (2016) found best C.B.R (1:1.80) in the experiment of nursery rearing of *N. Chitala* and this finding was higher to the present outcome. The cause might be due to feed management, variation of study period and stocking size of the species. Considering overall production and economic analysis of the present study the best production obtained in treatment T_1 with 35% protein containing feed. Therefore, in *N. Chitala* farming using indigenous high protein containing feeds is suggested for commercial farmer in Bangladesh.

CONCLUSION

In the present study, feed which contains 35% protein was found the best among the treatments. Thus, the study indicated that feed has direct effects on the growth and production of *N. chitala* fingerlings under pond condition. A feed that contains more protein is best suited that the farmer can maximize their benefit by culturing *N. chitala* providing high protein feeds instead of commonly used low protein feeds. Therefore, further studies can be carried out with increasing protein level in feed to see the effect of the fingerling production of *Notopterus chitala* for longer period of time to evolve a definite pond culture technology of *N. chitala* in Bangladesh.

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