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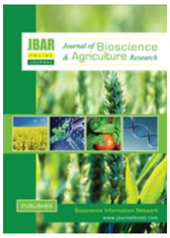

Effects of protein levels on growth of Monosex Tilapia (*Oreochromis niloticus* Linnaeus, 1758) fry in nursery ponds of Bangladesh

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ABSTRACT

An experiment was conducted for a period of 60 days to evaluate the effect of protein levels on growth performance, survivability and water quality parameters of monosex tilapia (Oreochromis niloticus) fry. The experiment was conducted under three treatments of dietary protein levels viz. T_1: 22% protein level; T_2: 24% protein level and T_3; 26% protein level with three replication each. Stocking density (249964 fish fry/ha) and initial weight 0.13±00g of fish was same in all treatments. Water quality parameter such as water temperature, dissolved oxygen, transparency, pH, alkalinity measured fortnightly. Mean value of water quality parameters showed no significant differences (P>0.05) among the treatments. Result showed that fry growth was significantly affected by protein level. The highest mean final weight gain was 26.79±1024g in T_3 and lowest mean weight gain was 16.23±0.21g in T_1. SGR (%, bwd^{-1})and FCR values showed significantly (P<0.05) different among the treatments. Feed with 30% protein level resulted in the highest production (P<0.05) in the T_3 treatments.

Key Words: *Oreochromis niloticus, Survivability, Monosex and Water quality*

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I. Introduction

Mozambique tilapia, *Oreochromis mossambicus* was introduced to Bangladesh from Thailand in 1954 (Rahman, 2005). But due to prolific breeding nature and vegetative feeding behaviour, the distribution and culture of this fish did not get that much popularity among the farmers as expected. Later on, Bangladesh Fisheries Research Institute (BFRI) imported Nile tilapia, *O. niloticus* from Thailand in 1986 (Gupta *et al.*, 1992) and developed culture technology of this fish (Hussain, 1989). Development of Genetically Improved Farmed Tilapia (GIFT) by ICLARM (Eknath *et al.*, 1993), and its introduction to Bangladesh in 1994 and further development of the strain by BFRI (Hussain and Kohinoor, 2003) paved the way of getting much popularity of tilapia throughout the country. GIFT *(O. niloticus*) is basically plankton feeder but gut analysis shows that the fish is omnivorous. GIFT is the most widely

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farmed variety and performs 60% better survival rate then the commercially available strain of tilapia (Sultana *et. al*. 1997). To get high production, fry of GIFT are converted to monosex tilapia (all male) by sex reversal method. Monosexing has been the key that has facilitated the development of tilapia culture in the global food fish area (Shelton, 2002). The presence of female tilapia leads to uncontrolled reproduction, excessive recruitment of fingerlings, competition for food, and stunting of the original stock, which may not reach marketable size. Culture of monosex tilapia is postulated to solve this problem (Guerrero, 1982). Therefore, the male monosex population of tilapia is being used for commercial culture. For fish supplementary diet, the continuous dependence on traditional feed ingredients like rice bran, oil cakes and soybean meal has led to an increase in the process of these components, which in turn influence profitability of aquaculture enterprises (Kumar, 2000). Aquaculture is a feed based industry with over 60% of the operational cost coming from feed sources alone (Pandian *et al.* 2001). The cost of feed is largely influenced by the level and sources of protein which is the most expensive component of a fish diet. The ultimate aim of artificial feedings in aquaculture is to achieve maximum protein deposition and growth within minimum inputs of feed at a minimum cost (Steven and Helfrich, 2002). In Bangladesh, a wide variety of agro based feed-stuffs, rich in protein, carbohydrate and energy are available and these ingredients can be better used for the formulation of cost effective fish feed. In this context, the present study was carried out to evaluate a suitable feed supplement rate of the production performances of male *O. niloticus* at high density in the ponds of northwestern part of Bangladesh.

II. Materials and Methods

Study location and pond facilities: The experiment was performed in nine earthen ponds of 0.004 ha in the hatchery complex, Department of Fisheries, University of Rajshahi for a period of 14th June to 13th August, 2013. The ponds were similar in shape, depth, basin configuration including water supply facilities. The water depth was maintained around 1.0 m using machine at regular interval.

Experimental design: Experiment was conducted under three treatments namely T_1, T_2, and T_3 each with three replications. Stocking density of *Oreochromis niloticus* the pond under each treatment was 249964 individual/ha (initial weight 0.13±00g). The differences among treatments were in feed and fertilizer level as shown in Table 01.

Table 01. Layout of the experiment

Treatments	Protein levels	Stocking density (individual/ha)
T_1	22 % protein	249964
T_2	24 % protein	249964
T_3	26 % protein	249964

Pond preparation: At first the bottom and sides of the selected ponds were repaired and all the aquatic weeds were removed manually by hand picking, uprooting and cutting from the nursery pond. All ponds were treated with lime at the rate of 247 kg/ha to disinfect the water. Then the experimental pond-1, pond-2 and pond-3 were fertilized by using cow dung-988 kg/ha, urea-24.7 kg/ha, TSP-12.35 kg/ha after 7 days of liming (DOF, 2002). The source of water of experimental ponds was rainfall and deep tube-well. During the introduction of water in each experimental pond, fine mesh (2 mm) nylon net hapa was used in the mouth of the pumped water to prevent predatory fish egg, spawns, fry and adult or larvae of aquatic harmful insects to inhabit their entrance. Then natural food production was tested and the water toxicity of the experimental ponds was checked. Netting was done to remove small frog and water bug from the experimental pond before 3 days of fry stocking.

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Collection and stocking: Larvae of Monosex tilapia were collected from Natore Govt. Hatchery, Natore. Larvae were kept inside the polythene bag with proper oxygen and the mouth of the polythene was bound tightly by rope. Then the larvae were brought and were transferred to the experimental pond and were acclimatized for about half an hour. Before releasing the larvae to the experimental pond the initial length and weight of 10 larvae were recorded with a sensitive portable electric balance (KD300kc: 0.01g-300g). Initial weight of larvae was 0.13±00g respectively. Fry were acclimatized with experimental pond water in plastic bag and released in each experimental pond at 8:00 am at the rate of 1012 individual/dec. in Treatment $T_{1,}$ 1012 individual/dec in Treatment T_2 and 1012 individual/dec in Treatment T_3.

Feed preparation and feeding: The required quantities of all ingredients mixed with hand (prepared feed) and spread it to the experimental pond surface. The supplemental feed was given to fry at the rate of 10%, 8% in 1st, 2nd Month respectively. Quantities of feed were adjusted every seven 15 days interval on the basis of increase in the average body weight of the stocked biomass. Half of the ration was supplied at 9.00 am and remaining half was supplied at 4.00 pm. Proximate composition of feed has been presented in the Table 02.

Table 02. Composition of feed ingredients used for different treatments in the experiment

Treatments / Feed ingredients	Amount (%) for T_1	Amount (%) for T_2	Amount (%) for T_3
Fish meal (58%)	14.06	17.18	20.31
Rice bran (12%)	23.95	21.87	19.79
Wheat bran (13%)	23.95	21.87	19.79
Maize bran (14%)	23.95	21.87	19.79
Mustard oil cake (32%)	14.06	17.18	20.31
Vitamin-Mineral premix	2	2	2
Protein level (%)	22	24	26

Sampling: Sampling was done on every fortnight interval in the morning (09:00 am to 10:00 am). Length and weight were recorded by random sampling of 10 fry from each experimental pond by using a small net. Weight was taken with an electric balance and length was recorded with measuring board. All the collected data were recorded in a note book and finally calculated the average length and weight of fry according to treatment on each sampling day.

Water quality monitoring: Physico-chemical parameters like water temperature (^{0}C), transparency (cm), Dissolved oxygen (mg/l), NH_3-N (mg/l), pH, Alkalinity (mg/l) of each experimental pond were measured at 15 days interval. Temperature (^{0}C), Transparency (cm), Dissolved oxygen (mg/l), NH_3-N (mg/l), pH, Alkalinity (mg/l) of water of each experimental pond under each treatment was recorded on sampling dates. Temperature was recorded by using a Celsius thermometer, transparency was recorded by secchi disc and other chemical parameters were recorded by using Hack kit box (DR/2010 model, HACH, Loveland, CO, USA, a direct reading spectrophotometer) at the pond site. Recording of water quality data were done between 09:00 am and 10:00 am.

Growth parameters: Growth, length in (cm) and weight in (g) was measured in every 15 days interval. To evaluate the fish growth the following parameters were measured: weight gain (g), length gain (cm), percent weight gain, percent length gain, SGR (%), survival rate, yield/kg/ha/2 months.

Following parameters were used to evaluate the growth:

i. Weight gain (g)=Average final weight — Average initial weight

ii. Length gain (cm)=Average final length — Average initial length

iii. Percent weight gain $= \frac{\text{Average final weight} - \text{Average initial weight}}{\text{Average initial weight}} \times 100$

iv. Percent length gain $= \frac{\text{Average final length} - \text{Average initial length}}{\text{Average initial length}} \times 100$

Specific growth rate (SGR) is the instantaneous change in weight of fish calculated as the % increase in body weight per day over a given time interval.

v. Specific growth rate (SGR) $= \frac{LnW_2 - LnW_1}{T_2 - T_1} \times 100$

Where, W_2 = Final live body weight (mg) at time T_2

W_1 = Initial live body weight (mg) at time T_1

vi. The survival rate was calculated by the following formula =

$$\frac{Initial\ number of\ larvae - Final\ number of\ larvae}{Initial\ number of\ larvae} \times 100$$

Production: At the end of the experiment most of the fishes were caught by net and the rest by drying the ponds. It was calculated as:

Production = No of fish harvest × Final weight of fish.

Statistical analysis: Weight gain (g), length gain (cm), percent length gain (cm), percent weight gain (g), final weight gain (g), final length gain (cm), and survival rate and production of fry during experimental period with same feeding & fertilization in different treatments were all tested using one way analysis of variance (ANOVA). Significant results ($P<0.05$) were further tested using Duncan's New Multiple Range Test (DMRT) to identify significant differences among means. This statistical analysis was performed with the support of the computer software SPSS (Statistical package for social sciences) program.

III. Results and Discussion

Water quality monitoring

Mean values of different physico-chemical parameters under different treatments by the total of all fortnights are presented in Table 3. No significant differences were observed for all the water quality parameters among the treatments.

Growth and production performances of *Oreochromis niloticus*

Variation in the mean values of different growth parameters under different treatments during study period are presented in Table 04. No significant ($P>0.05$) variation was recorded in initial weight of fishes among the treatments. The highest final weight was observed in T_3 (26.92±1.24g) and lowest in T_1 (16.36±0.21g). Final weight was statistically significant among the treatments.

Specific growth rate SGR (%, bwd^{-1}) 8.12±.02, 8.22±0.01 and 8.95±0.08 was found in T_1, T_2 and T_3 respectively. Experiment showed that treatment T_3 is the best result where the fish feed on mustard oil cake (15%), rice polish (25%), maize bran (20%), wheat flour (25%) fish meal (15%), cowdung, urea and TSP. Recorded specific growth rate of treatments T_1, T_2, and T_3 were 8.12±.02, 8.22±0.01 and 8.95±0.08 respectively, which were significantly (P<0.05) different among the treatments. Survival rates during the experiment period were 74.00±0.58, 83.33±0.88 and 88.33±0.88 in the T_1, T_2, andT$_3$respectively, which were not significantly different among the treatments. The mean net production was found as 3026.23±23.61, 3603.63±38.14 and 5944.03±59.34 kg/ha in T_1, T_2, and T_3 respectively, with the highest net production was observed in T_3 and lowest in T_1. The net productions are significantly (P<0.05) different among the treatments.

Table 03. Variation in mean values of physico-chemical characteristics under different treatments

Parameters	Treatments		
	T_1	T_2	T_3
Water temperature (°C)	31.97±0.09^a	32.10±0.12^a	31.94±0.09^a
Transparency (cm)	28.27±0.89^c	31.74±0.07^b	34.76±0.29^a
DO (mg/l)	6.10±0.11 ^{a}	5.94±0.08^b	5.67±0.98^b
pH	7.80±0.09^a	7.76±0.04^a	7.55±0.07^a
Alkalinity (mg/l)	112.20±0.53^a	98.36±0.45^b	93.47±0.84^c
NH_3-N (mg/l)	0.11±0.01^b	0.11±0.02^b	0.15±0.03^a

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

Table 04. Growth, survival and production performance of *Oreochromis niloticus*

Parameters	Treatments		
	T_1	T_2	T_3
Mean initial weight (g)	0.13±00^a	0.13±00^a	0.13±00^a
Final weight (g)	16.36±0.21^b	17.30±0.20^b	26.92±1.24^a
Weight gain (g)	16.23±0.21^b	17.18±0.20^b	26.79±1.24^a
SGR (%, bwd^{-1})	8.12±.02^b	8.22±0.01^b	8.95±0.08^a
Survival rate (%)	74.00±0.58^c	83.33±0.88^b	88.33±0.88^a
Yield (kg/ha/2 months)	3026.23±23.61^c	3603.63±38.14^b	5944.03±59.34^a

Figures in a same row having same superscript have no significant different (P<0.05).

Economic analysis

The mean value of CBR was found to be ranged from 1:1.55 to 1:2. 07. The minimum value of yield was recorded with treatment T_1 whereas the maximum value was recorded with treatment T_3. Total cost was found 10250 in (T_1), 12720 in (T_2) and 12970 in (T_3). Net benefit was found 15950 in (T_1), 21080 in (T_2) and 26930 BDTin (T_3) (Table 05).

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Table 05. Comparative economic analysis of monosex tilapia fry during 60 days in rearing system of three different treatments

Cost (Tk/ha)	T_1	T_2	T_3
Liming and Fertilizer cost	250	250	250
Feed cost	2345	2520	2820
Fry cost	8000	8000	8000
Pond operational cost	2000	2000	2000
Total cost	10250±245	12720±312	12970±213
Fry sale	26200±342 [a]	33800±234 [a]	39900±234 [a]
Net benefit	15950±123 [a]	21080±67 [a]	26930±76 [a]
Cost benefit ratio	1:1.55 [a]	1:1.65 [a]	1:2.07 [a]

Water quality parameters

Mean values of physico-chemical parameters are presented in Table 03. Parameters which were monitored are in suitable range for fish culture as reported by Boyd (1982) except water temperature which was slightly higher from the suitable ranges (25-30º). Rahman (1999) stated that water temperature ranged 25.5 C to 30.0 C, which is suitable for fish culture. In this study the temperature was found 31.97 ºC to 32.10 ºC which was similar to the findings of Kohinoor (2000) who suggested that the temperature ranged 18.5 ºC to 32.9 ºC is the best for culture. Alam *et al.* (2011) reported that temperature, dissolve oxygen and pH range of the water between 29.9 ºC to 32.5 ºC; 5.50mg/l to 6.60mg/l and 7.72 to 8.30 found the effect of stocking density on the growth and survival of monosex male tilapia . Chakraborty and Banerjee (2010) reported that the temperature, dissolve oxygen and pH of the water in monosex tilapia culture are 31 to 32.7 ºC; 7.1 to 7.4and 7.5 to 8mg/l. Findings are more similar with the present findings. Hussain (2004) stated that nile tilapia can easily grow from 2.0 to 8.0 mg/l dissolved oxygen range. Britz & Hechet (1987) stated that higher growth rates were obtained between 25 to 33ºC and the best was at 30 ºC. Values of dissolved oxygen obtained of the present study are coincide with the findings of Haque and Mazid (2005) who reported the dissolved oxygen ranged from 2.15- 6.74 mg/l. Boyd (1998) suggested that transparency between 30 to 45 cm as suitable for fish culture. Findings showed that transparency of the culture pond was 28.27-34.76 cm which was similar to the findings of Chakraborty and Banergee (2010). Wahab *et al*. (1995) suggested that the transparency of productive water should be 40 cm or less. Findings also agreed with Sarker *et al.* (2000) who reported that the water transparency was 27-35 cm which is less similar to the present study. Total alkalinity value recorded during the study period was found to vary from 93.47 ± 0.84 to 112.20 ± 0.33 mg/l. This finding agreed with Rahman (1999) who recorded total alkalinity of pond water from 71 to 175mg/1. This finding also agreed with Hossain *et al*. (2007) who reported the alkalinity of pond water as 81.25 to 145.5 mg/1. According to Boyd (1982) total alkalinity should be more than 20mg/l in natural fertilized pond. Ammonia-nitrogen value ranged from 0.11±0.01 to 0.15±0.03mg/1. These findings are suitable for fish culture which are supported by Boyd (1998) suggesting to keep the ammonia-nitrogen value in fish pond as less than 0.1mg/l.

Growth and production performances of *Oreochromis niloticus*

Growth performances (final weight, weight gain, specific growth rate, survival rate) of monosex tilapia (*Oreochromis niloticus)* revealed that T_3 was significantly higher (P<0.05) where the 26% protein level diets are maintained. Dietary protein is always considered to be of primary importance in fish feeding (Jauncey and Ross, 1982).Thus sufficient supply of dietary protein is needed for rapid growth (Lovell, 1989). Growth of *Oreochromis niolticus* obtained from the experiment indicated that the growth rate varied with different feed containing different protein levels. Average final weights were 16.36±0.21g; 17.3±0.2g and 26.92±1.24g in T_1, T_2, and T_3 respectively. The net weight gain of individual fish in T_3 was higher (26.79g) than those of treatments T_2 (17.30g) and T_1 (16.23g). Weight increments were statistically significant (P<0.05) among the treatments. The best results were obtained from where the fishes were supplied 30% protein supplement diet and followed by 25% and 22% protein diets, these findings are strongly support the Chuapoehuk (1987) who carried out experiment with 30%, 35% and

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40% protein diets and observed that the diet containing 30% protein produced optimum growth. Best growth of monosex fry tilapia occurred when 30% crude proteins were used. Chakraborty and Banerjee (2010) also supported these experimental findings. In cage culture system, in Kaptai lake Moniruzzaman *et. al.* (2015) reported that best outcomes of production of monosex tilapia were come in where 28.76% protein is used as feed which is similar with these findings.

Specific growth rate (SGR, % bw/day) as, 8.12±.02, 8.22±0.01 and 8.95±0.08 were found in, T_1, T_2, andT$_3$respectively. Findings strongly support the record of Zannatul *et al.* (2014), who recorded SGR (% bw/day) in 8.84 to 11.38 of fry of *Oreochromis niolticus*. Significantly highest specific growth rate (SGR) in T_3 might be due to the fact that the fish have utilized effectively the supplied feed enriched with 26% protein.

Survival rate (%) of *Oreochromis niloticus* in different treatments was fairly ranging from 74.00±0.58 to 88.33±0.88 which was similar to the range (70.62% to 93.45%) recorded by Alam *et al.* (2011). This findings also similar to the record of (Zannatul *et al.,* 2014) who recorded the survival rate of monosex fry tilapia in hapa are ranging from 79% to 92%.The highest survival rate is found with treatment at $T_3$where the fish is fed with 30% protein containing feed.

Economics

Mean value of CBR of tilapia farming with different treatment was 1:1.55 in (T_1), 1:1.65 in (T_2), 1:2.07 in (T_3). Significant differences were found under different treatments for the mean values of CBR. The highest CBR was found with T_3 whereas the lowest CBR was found with T_1. CBR varies with the variation in production cost and total return associated with market price for harvested fish. The finding is more or less similar to the finding with Bob-Manuel and Erondu (2010) who found CBR of nile tilapia *O. niloticus*as 1.60-2.03 and Ali *et al.* (2011) found CBR as 2.60 Tk. Data on economics indicated that the treatment T_3 was more profitable than that of treatment T_2 and T_1.

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