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Growth performance of genetically male tilapia derived from YY male, sex reversed male tilapia and mixed sex tilapia of *Oreochromis niloticus* in earthen pond aquaculture system in Bangladesh

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Abstract
A study was conducted to compare the growth, survival and production performance of Genetically Male Tilapia (GMT) derived from YY male, Sex Reversed Tilapia (SRT) produced through the masculinization of sexually undifferentiated fry with 17-α-methyltestosterone and Mixed Sex Tilapia (MST) in six earthen ponds with a stocking density of 100 fish/decimal. Fishes were fed with supplementary feed containing approximately 30% crude protein. The initial weight of GMT, SRT and MST was 5.45±0.35, 4.35±0.35 and 4.70±0.50 g, respectively. Over the culture period of 105 days, the weights of GMT, SRT and MST were found 193.21±3.07, 176.36±0.94 and 154.82±2.57 g respectively and the performances were significantly different ($P<0.05$) from each other. The survival rate of GMT, SRT and MST was 91.68±1.35%, 87.38±1.38% and 88.16±0.73% respectively and they were not significantly different ($P>0.05$). The net production of GMT (4252.77±124.06 kg/ha) was significantly ($P<0.05$) higher than that of SRT (3712.56±71.46 kg/ha) and MST (3269.40±72.01 kg/ha).

Keywords: Tilapia, Growth, YY male, 17-α-methyltestosterone.

1. Introduction

The Nile tilapia, *Oreochromis niloticus* (Linnaeus) has been considered as one of the most important species of fish in tropical and sub-tropical aquaculture [13]. It is currently ranked second only to carps in global production and is likely to be the most important cultured fish in the 21st century [33]. In Bangladesh, commercial farming of tilapia has been found to develop rapidly since the introduction of Genetically Improved Farmed Tilapia (GIFT) from the Philippines in 1994 [2]. The success of using the GIFT strain of tilapia for commercial farming is due to its ability to produce millions of monosex male fry in hatcheries through hormone treatment. This practice has been found to considerably eliminate the problems relating production of mixed sex tilapia (MST) fry, those have slow growth and low production in a given culture facility [22]. The culture of mixed-sex tilapia can always give rise to unwanted reproduction that results in production of a significant number of unmarketable juvenile fish [14, 20], and overcrowding and stunted growth [28]. Numerous solutions to this problem have been proposed, including manual sexing and separation of the sexes, culture in cages, use of predator species, production of monosex hybrids, direct hormonal sex reversal and YY technology [22, 23, 31]. Moreover, sex-specific differences in growth were significant in *O. niloticus* where males grow significantly faster, larger and more uniform in size than females [23, 34, 8, 25]. The desirability of monosex male populations of tilapia is well established for increased production potential and low management requirements [32, 5, 12].

O. niloticus has an XX/XY chromosome sex determination system [4], but the process of sex differentiation is labile rendering sex reversal possible in the species [11]. Oral administration of exogenous male sex steroid hormones before the differentiation of primal gonadal cells can cause reversal of phenotypic sex [38, 6]. The success in the production of 100% sex-reversed males using 17α-methyltestosterone is also dependent on the method and frequency of feeding of hormone to the sexually undifferentiated fry and controlling all the factors that can affect the sex reversal process.

There are some concerns on environmental and human health due to the consumption of hormone-treated tilapia [24] but no evidence for any human health hazard was found by consuming the treated fish [15]. However, the use of testosterone for sex-reversal has a strong negative effect on the immune system of fish [17]. Through the application of relatively simple genetic manipulations, researchers are producing all- or nearly all-male progeny in the Nile tilapia [23] which is known as the "YY male technology". These YY males are known as "supermales" and have the unique property of siring only genetically male progeny. The YY male technology provides a robust and reliable solution to the problems related to early sexual maturation, unwanted reproduction and over population [23, 41, 1, 25]. The YY males are able to produce 95~100% males upon crossing with XX females. These males are termed as "genetically male tilapia" (GMT) and are separated from sex reversed male tilapia (SRT) and normal males. The GMT are all- male and supposed to have higher growth, delayed maturity and higher production than other types of male fish. Thus the study was aimed to evaluate the culture performance of YY males derived genetically male tilapia (GMT), by comparing with that of hormonally sex reversed male tilapia (SRT) and normal mixed sex tilapia (MST) in the earthen pond aquaculture system.

2. Materials and methods

This study was conducted in the Department of Fisheries Biology and Genetics, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh and Abrar Agro Fisheries and Hatcheries, Mymensingh, Bangladesh.

2.1 Pond preparation

The experiment was conducted in the earthen ponds with an area of four decimal each located at the Abrar Agro Fisheries and Hatcheries, Mymensingh, Bangladesh for a period of 105 days. Three treatments were tested in six ponds and there were two replications for each. Before initiating the experiment all the ponds were prepared by draining out of water. To disinfect these ponds and to stabilize pH of water, liming with calcium oxide (CaO) was done at the rate of 1 kg/decimal. Three days after the application of lime, the ponds were filled with ground water up to the level of 4 feet. This water level was maintained throughout the experimental period. All the ponds were fertilized with cow dung at the rate of 4 kg/decimal. Five days later inorganic fertilizers, urea and triple super phosphate (TSP) were applied at the rate of 50 and 75 g/decimal respectively. After three days of fertilization, stocking was done with the fingerlings of Genetically Male Tilapia (GMT), Sex Reversed Male Tilapia (SRT) and Mixed Sexed Tilapia (MST) at the stocking density of 100 fish/ decimal.

2.2 Origin of experimental fish

A good number of YY male and ordinary mixed sex tilapia fry originated from Swansea strain were collected from Central Luzon State University of Philippines and reared in ponds in Abrar Agro Fisheries and Hatcheries, Mymensingh up to maturity. These fish were used as brood fish.

2.3 Monosex (GMT & SRT) and mixed sex tilapia (MST) fry production

The Genetically Male Tilapia (GMT) fry was produced by crossing identified YY super males with ordinary XX females. The ordinary tilapia fry were collected and divided into two equal groups, one group of fry was fed with 17α- methyl testosterone treated diet (60 mg kg^{-1} feed) first at the rate of 50% body weightand then gradually reduced to 30% body weight per day for 30 days to produce Sex-Reversed Male Tilapia (SRT). While the other group was given a hormone untreated control diet at the same feeding rate to produce Mixed Sexed Tilapia (SRT). Hormone treated diet was prepared by the alcohol evaporation technique [36]. Fry of two groups were reared separately in the hapas set in a pond until stocked them in the experimental ponds.

2.4 Grow-out performance analysis

GMT, SRT and MST fingerlings with similar body weight of 5.45±0.35 g, 4.35±0.35 g and 4.70±0.50 g respectively, were stocked into six ponds, having two replicates of each with same stocking densities of 100 fish/decimal. During the experimental period (105 days), the fish were fed with the commercially formulated extruded pellet (floating) feed containing 30% crude protein at a rate of 10% down to 4% live body weight daily. The proximate composition of commercial feed was analyzed following the procedure of AOAC [3] in the Nutrition Laboratory of Aquaculture Department, BAU, Mymensingh. Experimental diets were manually introduced three times a day at 8 am, 1 pm and 5 pm.

Average body weight of stocked fish was measured bi-weekly to adjust feed quantity and to observe health condition. Growth parameters such as average weight gain (AWG), daily weight gain (DWG), specific growth rate (SGR), food conversion ratio (FCR), survival rate were calculated as follows [7, 29].

AWG (g/fish) = average final weight (g) - average initial weight (g),

$$\text{DWG (g per day)} = \frac{\text{average final weight (g)} - \text{average initial weight (g)}}{\text{experimental period (d)}}$$

$$\text{SGR (\% per day)} = \frac{(\ln \text{final weight} - \ln \text{initial weight})}{\text{experimental period (d)}} \times 100$$

$$\text{FCR} = \frac{\text{feed intake (g)}}{\text{live weight gain (g)}}$$

$$\text{Survival rate (\%)} = \frac{\text{final number of fish}}{\text{initial number of fish}} \times 100$$

Water quality parameters were measured weekly including temperature using a thermometer, pH by a portable pH meter (Jenway Ltd., Model 350) and dissolved oxygen by a portable dissolved oxygen meter (Jenway Ltd., Model 970).

2.5 Economic Evaluation

The gross revenue, production cost and net profit were estimated by simple economic analysis. Facility costs were not included in the analysis. The estimation was based on local market retail prices (at the current time) of feed, fertilizer, fish etc. on BDT.

2.6 Statistical Analysis

The data are expressed in terms of mean ± standard error. All growth parameters were analyzed by one-way ANOVA. Means were statistically compared using a Duncan multiple range test at 5% significance level. The statistical analyses were performed using SPSS 16.0 version for Windows.

3. Results

During this study period some physico-chemical parameters of water, such as temperature, dissolved oxygen and pH were measured and found them within the suitable range for fish culture (Table 1).

Table 1: Physico-chemical parameters of pond water during the study period.

Parameters	June	July	August	September
Water Temperature (^{0}C)	32.9 ± 1.92	32.0 ± 0.73	31.1 ± 0.68	32.3±0.14
Dissolved Oxygen (mg/l)	4.81 ± 0.59	4.48 ± 0.30	4.22 ± 0.33	4.67 ± 0.37
pH	7.73 ± 0.06	7.67 ± 0.04	7.59 ± 0.04	7.57 ± 0.04

Note: All data are presented as Mean ± SE

During the 105 days experiment, it was found that the growth rate of GMT was faster than that of SRT and MST. In the early rearing stage the fingerlings of GMT, SRT and MST had a fast growth which gradually declined in all cases with the progress of the culture period. The specific growth rate of GMT, SRT and MST fish ranged from 0.84 to 8.71, 1.08 to 9.70 and 0.77 to 9.54 percent per day respectively. Growth of fish during the first 15 days after stocking was extremely high in GMT, SRT and MST and rates were being 8.71, 9.70 and 9.45 percent per day, respectively (Table 2). Later on, the daily growth at fortnight sampling found decreased with a range from 0.84 to 5.22 percent per day for GMT, 1.08 to 4.58 percent per day for SRT and 0.77 to 4.49 percent per day for MST. It was seen from Table 2, GMT grew at a faster rate as compared to SRT and MST from the first month of rearing and it maintained the same growth trend all along the experiment. At the end of the experiment the average weight of GMT, SRT and MST became 193.21±3.07 g, 176.33±0.93 g and 154.82±2.57 g respectively. The specific growth rate decreased with the increase of rearing period and this gradual reduction of SGR continued up to the end of the experiment.

Table 2: Growth performances of three different groups of tilapia in ponds.

Days	GMT		SRT		MST	
	Average weight (g)	SGR (% per day)	Average weight (g)	SGR (% per day)	Average weight (g)	SGR (% per day)
0	5.45 ± 0.35	-	4.35 ± 0.35	-	4.70 ±0.50	-
15	20.08±0.46	8.71±0.28	18.62±1.31	9.70±0.07	19.55±0.65	9.54±0.49
30	41.76±2.24	5.22±0.22	35.31±0.98	4.58±0.30	36.88±4.35	4.49±0.61
45	69.40±0.72	3.39±0.43	56.58±1.20	3.14±0.04	53.86±1.70	2.67±1.00
60	99.43±3.43	2.24±0.15	91.82±4.79	3.02±0.19	96.30±1.20	2.62±0.74
75	136.85±2.15	2.13±0.34	125.30±3.77	2.08±0.15	119.65±1.60	1.45±0.01
90	168.85±2.15	1.40±0.02	148.29±2.25	1.13±0.10	136.92±3.03	0.90±0.06
105	193.21±3.07	0.84±0.02	176.33±0.93	1.08±0.06	154.82±2.57	0.77±0.03

The initial stocking weight of fingerlings of GMT, SRT and MST was similar but after 105 days of rearing the average final weight of GMT became significantly ($P<0.05$) higher than that of SRT and MST. At the end of the experiment the net production of GMT became significantly higher (4252.77 kg/ha) than that of SRT (3712.56 kg/ha) and MST (3269.40 kg/ha) (Table 3). The survival rates of GMT, SRT and MST were 91.68%, 87.38% and 88.16% respectively and these were not significantly different ($P>0.05$) from each other (Table 3). The food conversion ratios (FCR) of GMT, SRT and MST fish were 1.64, 1.78 and 1.89 respectively and FCR of GMT fish was significantly lower than that of SRT and MST (Table 3).

Table 3: Growth parameters of Genetically Male Tilapia (GMT), Sex-Reversed Male Tilapia (SRT) and Mixed Sex Tilapia (MST) in pond culture system during the study period

Growth parameters	Treatments		
	(GMT)	(SRT)	(MST)
Initial weight (g)	$5.45±0.35^a$	$4.35±0.35^a$	$4.70±0.50^a$
Final weight (g)	$193.21±3.07^a$	$176.36±0.94^b$	$154.82±2.57^c$
Weight gain (g)	$187.76±2.72^a$	$172.01±0.60^b$	$150.12±2.07^c$
% Weight gain (g)	$3456.10±172.03^a$	$3978.80±306.45^b$	$3225.87±299.13^c$
DWG (g per day)	$1.79±0.03^a$	$1.64±0.01^b$	$1.43±0.02^c$
Survival rate (%)	$91.68±1.35^a$	$87.38±1.38^a$	$88.16±0.73^a$
SGR (% per day)	$3.40±0.05^a$	$3.53±0.07^a$	$3.33±0.09^a$
Production (Kg/ha)	$4252.77±124.06^a$	$3712.56±71.46^b$	$3269.40±72.01^c$
FCR	$1.64±0.01^a$	$1.78±0.02^b$	$1.89±0.01^c$

Values with different superscripts in the same row indicate significant differences ($P<0.05$)

The result of the economic analysis showed that the net profit of GMT, SRT andMST fish were BDT 0.119±0.007 million, BDT 0.066±0.003 million and BDT 0.029±0.003 million respectively. The net profit of GMT fish was significantly ($P<0.05$) higher than that of SRT and MST (Table 4).

Table 4: Cost-benefit analysis per hectare of three different groups of tilapia.

Parameters	Treatments		
	(GMT)	(SRT)	(MST)
Gross revenue*	0.510±0.02^{a}	0.439±0.01^{b}	0.376±0.01^{c}
Production cost*	0.390±0.01^{a}	0.370±0.01^{ab}	0.345±0.01^{b}
Net return*	0.119±0.01^{a}	0.066±0.00^{b}	0.029±0.00^{c}

*BDT, Million; Figures with different superscripts in the same row indicate significant differences (P<0.05)

4. Discussion

The principal objective of this study was to compare the growth performance of fry of three different origin i.e. genetically male tilapia (GMT) derived from YY male, sex reversed tilapia (SRT) and mixed sex tilapia (MST) and at the end of 105 days experiment the GMT showed the highest average weight (193.21±3.07) compared to that of SRT (176.33±0.93) and MST (154.82±2.57). Similar result, was obtained by [25] who observed that after four months of rearing of GMT and SRT the average final weight of GMT became significantly higher than that of SRT and the average weight of GMT and SRT reached to 245.57 and 218.33 g respectively. The evaluation of the growth performance of three groups of Nile tilapia (Male, Female and Mixed-sex) in fertilized pond for 320 days experimental period showed the highest weight gain in male group followed by female and mixed-sex tilapia groups, i.e. the average weights were of 396.1, 344.3 and 291.8g respectively [16].

Increase in individual growth of Nile tilapia during monosex culture was observed in different studies [23, 10, 21]. In the present study significantly highest (P<0.05) weight gain was observed in the GMT fish group followed by SRT (172.01±0.60) and MST (150.12±2.07). Faster growth of monosex tilapia has been related to the lack of energy expenditure in egg production and mouth brooding by females and lower energy expenditure on courtship by males [10, 40]. [43] reported that hormone treated monosex tilapia achieved greater mean individual weight and length than mixed-sex fish. [27] Observed the all-male groups of *O. niloticus* were on an average 14.5% larger when compared with the whole mixed-sex groups and had a weight comparable to the males within the mixed-sex groups (+2.0%). [14] concluded that all male tilapia population have greater growth potential because no energy was shunted towards reproduction and no competition with younger fish occurred. When females become sexually mature after 4-6 months, they devote more energy and resources into egg production than into growth. [30] Evaluated the growth performance of two strains of Nile tilapia (GMT and mixed-sex) in a greenhouse recirculating aquaculture system (RAS) with feeding a 32% protein-riched floating catfish pellet for 73 days and found that the average weight of GMT and mixed-sex culture reached to 172 and 156 g, respectively. [26] concluded that the higher growth claimed for genetically improved farmed tilapia (GIFT) and genetically male Nile tilapia (GMNT) as compared with conventional Nile tilapia (CNT) (*Oreochromis niloticus*) but there were no significant differences (P>0.05) in average growth (82.2±7.2, 87.3±7.7 and 74.7±4.1 respectively for GIFT, GMNT and CNT. In contrast, [19] who evaluated the growth performance of three experimental groups of tilapia: mixed sex fish (control), hormone-treated fish and progeny of YY male tilapia in cage culture did not find any significant difference (P>0.05) in body weight and length among the three groups.

The survival rate of GMT, SRT and MST in this study were 91.68%, 87.38% and 88.16% respectively and they were not significantly different. Similar results were reported by [9] where the survival rate was not significantly different among mixed-sex and monosex fish and between different culture systems. [42] Reported that 17 α-methyltestosterone had no negative effect on survival of hormone treated monosex male nile tilapia. The survival rate of GMT was quite high (97.33%) compared to SRT (90.33%) but they were not significantly different [25]. Shaha DC [35] reported the survival rate of GIFT strain (*O. niloticus*) varied between 73 and 78%, whereas [38] recorded 95.75% and 81.25% survival rate for GIFT and existing Nile tilapia species respectively.

The initial stocking weight of GMT, SRT and MST was similar but after 105 days of rearing GMT produced significantly higher yields than MST (+30.1%) and SRT (+14.6%) in this study. Both GMT and SRT were monosex male tilapia and expected to have much higher production than any mixed-sexed tilapia culture. And it happened when the production of GMT, SRT and MST were compared by [23] and they found that GMT produced significantly higher yields than MST (+58.8%) and SRT (+31.03%) in ponds, and GMT yields were11.9% higher than MST in rice-cum-fish culture and 33.6-47.0% in recirculation systems.

The net production of GMT (4252.77±124.06) was significantly higher than that of SRT (3712.56±71.46) and MST (3269.40±72.01) in this study. Similar result was reported by [25] where GMT demonstrated significantly higher production (4427.87 kg/ha) than that of SRT (3653.45 kg/ha). [16] observed that the net fish production was 1148.0, 994.0 and 842.1 kg/ha/year in Male, Female and Mixed sex tilapia, respectively. Male tilapia culture contributed a significant increase in fish production than that of female and mixed sex tilapia in ponds.

The FCR of GMT (1.64±0.01) was significantly better than that of SRT (1.78±0.02) and MST (1.78±0.02) in the present study. Similar result was reported by [39] who observed better FCR for male tilapia populations compared to mixed-sex populations.

The net profit of GMT fish was significantly (P<0.05) higher than that of SRT and MST but on the production cost of GMT did not significantly differ from that of SRT. Kaliba AR [18] had the similar opinion as the production costs were relatively similar to mixed tilapia culture and all-male tilapia culture, but the revenue was significantly higher (about twofold) for all-male tilapia culture.

5. Conclusion

Genetically male tilapia (GMT) derived from YY male have been growing faster compared to sex reversed tilapia (SRT) and mixed-sex tilapia (MST). This YY-male technology is a viable and sustainable technology and can consistently produce monosex male tilapia in the existing hatchery systems without any special facilities and at minimal cost. Thus YY supermale production is a great achievement for the tilapia industry as they will be used to produce all-male tilapia in environment friendly condition without any hormone induction.

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