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Optimization of hormone treated diet for masculinization of red tilapia (*O. niloticus*)

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Abstract
In the present study, optimum feeding rate of hormone (17α-MT) treated diet was evaluated in relation to higher male production and growth of fish. The present study was conducted for a total period of 6 months 2016. Initially for a period of one month the yolk sac observed fry was fed on hormone treated (@50 mg/kg) diet in glass aquaria and later for 5 months in cemented cisterns (3X3X1 m) for observing growth and sex ratio. For this purpose, four feeding rates, such as 10% (T1), 15% (T2), 20% (T3) and 25% (T4) were tested in triplicate. A control group without hormone treatment and 10% feeding rate was also used as control. The male population was significantly higher in treatments than control. As such the highest (100%) male population was noticed in T2, T3 and T4. In control it was only 60%. However, the weight gain was higher (1.86 g) in higher feeding rate of 15% body weight was found suitable.

Keywords: *Oreochromis niloticus*, Hormone, Sex ratio, feeding rate, Growth

Introduction
The decline in the catch of tilapia in freshwater ecosystems and its early sexual maturity in culture systems are well-recognized problems in tilapia farming [1]. The Nile tilapia (*Oreochromis niloticus*), one of the most popular aquaculture species, despite having many good characteristics, one of the main impediments in tilapia production at commercial scale is its precocious reproduction. It attains sexual maturity at an early age and reproduces after every 4-6 weeks in the pond. The mono-sex culture technique can be used to control this unwanted reproduction of tilapia by culturing all male tilapia in pond. Tilapia has sexual growth dimorphism in which males grow faster and have more standard size than females [2].
The world-wide harvest of farmed tilapia has now surpassed 1 MMT, and tilapias are second only to carps as the most widely farmed freshwater fish in the world. The Nile tilapia (*Oreochromis niloticus*) is one of the first fish species cultured. Illustrations from Egyptian tombs suggest that Nile tilapia were cultured more than 3,000 years ago. Tilapia has been called Òsaint PeterÕs fishÓ in reference to Biblical passages. The Nile tilapia is still the most widely cultured species of tilapia in Africa. Positive aquacultural characteristics of tilapia are their tolerance to poor water quality and the fact that they eat a wide range of natural food organisms. Biological constraints to the development of commercial tilapia farming are their inability to withstand sustained water temperatures below 50 to 52º F and early sexual maturity that results in spawning before fish reach marketable size. Following is a discussion of the characteristics and culture of non hybrid tilapia.

- Tilapias (*Oreochromis* spp) have become increasingly important as cultured fish species worldwide.
- However, the problem of overpopulation in ponds caused by prolific breeding is still a major constraint in the development of tilapia aquaculture.
- The current strategy is to emphasize production of males only groups because they have faster growth than females.
- Production of monosex tilapia has been achieved through the use of androgens, hybridization, chromosomal manipulation and heat treatment of embryos and larvae.

Tilapia is the generic name of a group of cichlids endemic to Africa. The group consists of three aquaculturally important genera - *Oreochromis, Sarotherodon* and *Tilapia*. Several characteristics distinguish these three genera, but possibly the most critical relates to reproductive behavior.

All *tilapia* species are nest builders; fertilized eggs are guarded in the nest by a brood parent. Species of both *Sarotherodon* and *Oreochromis* are mouth brooders; eggs are fertilized in the nest but parents immediately pick up the eggs in their mouths and hold them through incubation and for several days after hatching. In *Oreochromis* species only females practice mouth brooding, while in *Sarotherodon* species either the male or both male and female are mouth brooders. Considering the aspect of tilapia, the present was conducted to work out the optimum feeding rate of hormone (17α-MT) diet for higher male production.

Materials and methods

For conducting this study, the fish "Red Tilapia" was choosen as an experiment fish. The fish belongs to family Cichlidae, which is basically originated from Africa. The original red tilapia is genetic mutants. The first red tilapia was produced in Taiwan in the late 1960s, by a cross between a mutant reddish-orange female Mozambique tilapia and a normal male Nile tilapia [3]. The seed (just yolk sac absorbed fry) of this fish was procured from the fish farm of MPUAT Udaipur. In this study 50 post-hatch larvae were stocked in each aquarium. A total of four treatments (T_1, T_2, T_3 and T_4) and one control.

To standardize the feeding rate of hormone treated diet for the production of higher percentage of male population, 17α-MT 50 mg MT/kg diet was mixed in basal diet. This dose was selected through a preliminary study. The hormone treated diet was fed to post-hatching (just after the yolk sac absorption) fry @ 10 (T_1), 15 (T_2), 20 (T_3) and 25% (T_4) body weight and 10% body weight in control without hormone for 30 days. The mean length, weight and survival of the fish in each treatment were recorded. After 30 days of growth parameters were calculated and using following formula;

Net weight gain (g) = Final weight (g) – Initial weight (g)

Per cent weight gain (%) = [{Final weight – Initial weight}/ Initial weight] X 100

SGR (%) = [(log final weight – log initial weight) / time (days)] X 100

Water quality parameter *viz*., temperature, pH, dissolved oxygen, electrical conductivity, total dissolved solids and nitrate-nitrogen in experimental systems were analyzed following standard methods [4].

Further, on the completation of hormone treatment of 30 days. All the fishes were transferred to cemented cisterns (3x3x1m) where they were reared for 5 month. During this period fry were fed on commercial feed (Growel pelleted feed) @10% of body weight. At the end of grow out phase 30% fishes were used for sexing.

Results

The data pertaining to sex of experimental fish are depicted in Table 1. The control group had 60% male and 40% female population, while all other treatments received methyl testosterone showed a greater and significant higher male proportion as compared to the control. The female proportion also showed significant differences among control and treatments. In treatments, the lowest (80%) male population was in T_1 (10% feed) while T2 (15% feed), T3 (20% feed) and T4 (25% feed) have 100 per cent male population.

The statistical analysis of the results (ANOVA) for sex reversal data showed a highly significant ($p<0.01$) difference as compared to control. The mean values of male and female populations were also significantly different between control and treatments. However, the differences within treatments were non significant. Thus, the results indicated that the feeding rate of 15% is sufficient for producing 100% male population in red tilapia.

The feeding rates significantly affected the growth of red tilapia. All the treatments received MT showed more average body weight (Table 2) and gain in body weight than the control. Treatment T_4 (25% feed) showed the highest gain in weight (1.86 g) followed by T_3 (1.48g), T_2 (1.14g), T_1 (1.14g) and control (0.79g). Treatment T_4 showed the highest per cent gain in weight (618.78%) followed by T_3 (493.0%), T_2 (380.11%), T_1 (2.62.33%) and control (154.33%). The maximum SGR of 7.04 per cent was observed in T_4 followed by T_3, T_2 and T_1 with respective values of 6.36, 5.60 and 4.60%. The minimum of 4.60 per cent was observed in control. In general, the growth significantly increased with increasing feeding rates. The results of statistical analysis (ANOVA) on net weight gain in different treatments was highly significant at 5% level of probability ($p<0.05$). The MT treated diets fed fish showed higher gain in total length than control. As such the highest gain in length (1.54 cm) was noticed in T_4 followed by treatment T_3 (1.52 cm), T_2 (1.50 cm) and T_1 (1.48 cm). The lowest (1.45 cm) length gain was recorded in control. The length gain in different treatments was significantly different as compared to control. However, it was non significantly different among treatments ($p<0.05$).

Survival: The survival of fish among treatments was more or less same (Table 2). Treatment T_4 and T_3 showed the maximum survival of 86.67 %, while control showed the minimum survival of 73.33 %. In treatment T_2 and T_1 the survival was 83.33 and 80.00% respectively. Statistically, the survival was not significant ($p>0.05$) between treatments and control.

Physico-chemical Parameters of Water

The water temperature ranged between a minimum of 19.32ºC and a maximum of 28.86 ºC in T_1. The average temperature was lowest (22.91ºC) in T_3 and highest (23.31ºC) in control (Table 3). pH fluctuated between 7.18 to 8.21 with minimum in T_1 and maximum in control. Further, the minimum (7.56) and maximum (7.70) mean values of pH was noticed in T_2 and T_1 respectively. The highest (2.35 mMoh) and lowest (1.68 mMoh) values of EC were in T_2 and T_1 respectively. The highest (7.82 mg/l) values of dissolved oxygen was observed in T_2, while the minimum (5.41 mg/l) was noticed in T_3. The average dissolved oxygen concentration ranged between 6.52 and 6.99 mg/l in T_2 and T_3 respectively.

TDS ranged between a minimum of 967.0 mg/l in T_2 and a maximum of 1060.5 mg/l in T_3. The highest (1026.16 mg/l) and lowest (992 mg/l) mean values of TDS were noticed in T_4 and T_1 respectively. The TDS values were quite high in all the treatments because the water source (bore well) used had a high TDS value. The range and average values nitrate-nitrogen have been presented in Table 3. The highest (0.031 mg/l) was seen in T_3 and lowest (0.016 mg/l) in T_3 and control. Further, the mean values of nitrate-nitrogen were highest (0.021 mg/l) in T_1 and lowest (0.020 mg/l) in T_2, T_3, T_4 and control.

Discussion

The present study was aimed to find out the optimum feeding rate of 17α-methyl testosterone treated diets to obtain all male population of red tilapia (*Oreochromis niloticus*). Results of

this study showed that 50 mg MT.kg^{-1} hormone treated diet with different feeding rates (i.e. 10, 15, 20 and 25%) gave a significantly higher male/female ratio as compared to control. The 100 per cent male population was recorded in all treatments (T_2, T_3 and T_4) except T_1 (80%). Obtained 100% male *O. niloticus* at a daily dose rate of 4, 5, and 6% of body weight with 60 mg/kg of 17α-methyl testosterone (MT) for 14 days [5]. Fed hormone mixed feed at 15 % of body weight daily to obtain 99.3% males for 30 mg MT/kg of feed and 97 % male for 60 mg MT/kg of feed. The oral administration of 17α-MT at 40-60 mg.kg^{-1} feed from first feeding for a duration of 3-4 week produced about 98-100% male tilapia [6]. The studied on two strains, grey tilapia (TG) and red tilapia (TR) of *O. niloticus*, fed with the androgen, 17α-methyl testosterone at 60 mg/kg feed. Hormone treatment resulted in the production of 97% males in the TG strain and 96% males in the TR strain. There was no significant difference in percentage sex direction in the two strains [7].

In the present study, different feeding rates with single dose of MT significantly influenced the growth of red tilapia. All the treatments (T_1, T_2, T_3 and T_4) registered higher average body weight and gain in body weight of red tilapia than the control. The mean weight of 2.16 g to 0.76 g and mean length of 1.14 cm to 0.05 cm were observed in T_4 (50 MTmg/kg 25% feed) and control respectively. The channel catfish (*Ictalurus punctatus*) were fed diets containing MT at concentrations of 0, 2.5 and 10 mg.kg^{-1} for 123 days and found that weight gain in fish fed on control diet (0 MT) was higher ($p<0.05$) than that fed the MT treated diets [8]. The studied the growth and survival of two strains of *O. niloticus* (Strain A = Grey tilapia and Strain B = Red tilapia) fed on diets treated with the androgen 17α-methyl testosterone at 60 mg/kg feed for 8 weeks. The mean weight of strain A at the end of the experiment was 1.82 g and 0.96 g for treated and untreated fish respectively; corresponding results for strain B were 2.72 g and 1.12 g respectively. Mean length of strain A was 3.39 cm for treated and 2.87 cm for untreated fish, while strain B recorded mean length of 5.29 cm for treated and 2.67 cm for untreated fish. Mean percentage survival for strain B was 92% and 97% for treated and untreated groups and, 90% and 89.33% respectively for strain A. Survival did not differ between treated and control fish [9]. In the present study, the specific growth rate of red tilapia ranged between 3.33 % (control) to 7.04 % (T_4). However, a slightly higher SGR of 6.31 % (control) and 9.51% (70 mg MT/kg) were reported for Nile tilapia [10]. The results of this study on sex and growth had shown the positive impact of hormone and feeding rate. A feeding rate of 15% body weight was found more favorable for all male production. Hence, a feeding rate of 15% is recommended for commercial production of all male population of red tilapia.

Water Parameters: The water quality status monitored throughout the experimental period is summarized in Table 3. The parameters such as pH, dissolved oxygen (mg/l), total dissolved solids (mg/l), nitrate-nitrogen (mg/l) and electrical conductivity (mMoh) were measured and found congenial for the growth red tilapia. DO concentration should remain above 4 mg l^{-1} and the optimum temperature between 26- 28^{o}C for ideal fish culture. The values of DO and temperature remained well within this limit which could be considered good for tilapia growth [11].

Table 1: Per cent populations of male and female in different treatments.

Treatments	Sample size (Nos)	Male		Female		Sex ratio M:F
		Nos	%	Nos	%	
Control	30	18	60.0^{c}	12	40.0^{a}	0.6:0.4
T_1	30	24	80.0^{b}	6	20.0^{b}	0.8:0.2
T_2	30	30	100.0^{a}	0	0.0^{c}	1:0
T_3	30	30	100.0^{a}	0	0.0^{c}	1:0
T_4	30	30	100.0^{a}	0	0.0^{c}	1:0

Mean in the same column with same superscripts are not significantly different

Table 2: Growth performance of red tilapia fed with experimental diet (After 30 days).

Treatments	Control	Treatment T_1	Treatment T_2	Treatment T_3	Treatment T_4
Net weight gain	0.46±0.009^{e}	0.79±0.027^{d}	1.14±0.033^{c}	1.48±0.038^{b}	1.86±0.043^{a}
Per cent weight gain	154.44±2.940^{e}	262.33±9.045^{d}	380.11±11.163^{c}	493.00±12.702^{b}	618.78±14.338^{a}
SGR	3.33±0.041^{e}	4.60±0.089^{d}	5.60±0.083^{c}	6.36±0.077^{b}	7.04±0.071^{a}
Length gain	1.45±0.0 18^{e}	1.48±0.015^{d}	1.50±0.012^{c}	1.52±0.012^{b}	1.54±0.018^{a}
Survival	73.33±1.667^{b}	80.00±2.887^{d}	83.33±1.667^{c}	86.67±1.667^{a}	86.67±1.667^{a}

Table 3: Range and mean values (± Standard error) of selected water quality parameters during the experimental period.

Parameter	Treatments				
	Control	Treatment T_1	Treatment T_2	Treatment T_3	Treatment T_4
Temperature (◦C)	19.5-25.5 (23.31±0.77)	19.32-28.86 (23.12±0.85)	19.55-25.45 (23.19±0.79)	19.75-24.85 (22.91±0.67)	19.5-25.65 (23.18±0.80)
DO (mg/l)	6.03-7.26 (6.84±0.16)	5.79-7.38 (6.88±0.20)	6.06-7.82 (6.52±0.23)	5.41-7.54 (6.99±0.15)	5.78-7.48 (6.76±0.20)
TDS (mg/l)	983-1011 (999.9±3.69)	979.5-1005.5 (992±3.43)	967.0-1046.5 (1006.4±9.67)	993.05-1060.5 (1021.81±8.66)	994.3-1038.5 (1026.16±5.76)
EC (mMoh)	2.00-2.12 (2.04±0.02)	1.68-2.00 (1.89±0.04)	1.94-2.35 (2.10±0.05)	1.95-2.25 (2.06±0.04)	2.07-2.16 (2.11±0.01)
pH	7.25-8.21 (7.69±0.14)	7.18-8.16 (7.60±0.12)	7.19-8.08 (7.56±0.12)	7.21-8.15 (7.63±0.13)	7.23-8.19 (7.70±0.15)
Nitrate-nitrogen (mg/l)	0.016-0.025 (0.020±0.001)	0.018-0.029 (0.021±0.001)	0.017-0.030 (0.020±0.002)	0.016-0.031 (0.020±0.002)	0.017-0.027 (0.020±0.001)

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