A Feasibility Study on Sex Reversal of Nile Tilapia *(Oreochromis niloticus)* Fry using Different Doses of 17- Methyl Testosterone at Different Protein Levels

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Abstract

This research was undertaken to investigate the effect of two protein diets (30% and 40%) and two hormonal doses 40 and 60 mg 17- methyl testosterone (MT)/ 1 kg diet on the sex reversal of Nile tilapia, *Orechromis niloticus* using validated microscopic examination of the gonads and sex determination of the fish. Nile Tilapia fry (seven days post hatching) receiving following diets for 28 days. Diet A – containing 30% CP (crude protein) without hormones as control 1; Diet B –40 mg MT with 30% CP; Diet C-60 mg MT with 30% CP; Diet D – containing 40% CP without hormones as control 2; Diet E- 40 mg MT with 40% CP; Diet F – 60 mg MT with 40% CP. The frequency data of males and females after treatment and microscopic characteristic showed that the % of males obtained by B (82%) , C (94%), E (92%) and F (98%) treatments were higher than the control groups (A and D) , and a dose of 60 mg MT/ 1 kg of diet C and F were more efficient in sex reversal , resulting in 94 -98% males. Among diets, the treatment F (60 MT / kg and 40% CP) was better than the treatment C (60 mg MT with 30% CP). These results indicated that male sex reversal increase with increasing dose of MT and crude protein. The water quality parameters, recorded during the present study in rearing hapas such as Water Temperature (29– 35)°C,water pH(7.2– 8.5), Dissolved Oxygen content (3–.5.6)mg/L,Alkalinity (75-100) mg/L, Hardness (85-111) mg/L, and Ammonia(0.365-0.658) mg/L in the optimal water quality ranges for Nile tilapia. Results showed that fish growth was significantly affected by protein level and 17 methyl testosterone. The highest growth performance (final weight, and growth rate (GR) of fry were obtained with the 40% protein diet with 60 mg MT/kg.

Key words: 17- Methyl testosterone, Sex reversal, Nile tilapia, Growth, Protein

Introduction

The Nile Tilapia, *Orechromis niloticus* (L1758) is a widespread species used in tropical countries for aquaculture. In addition to high growth rate of Nile tilapia and the consumer performance, Nile tilapia is also resistant to considerable levels of adverse environmental and management conditions. Natural populations of these fish occur in Africa and these species *O. niloticus* has been introduced to almost every tropical country in the world for aquaculture. Tilapia culture has increased in fresh water since its introduction to Sri Lanka in 1950s (De silva 1997). One of the main problems of Nile tilapia culture is their early maturation (4-5 months old) as they maturate at 20-30g. This result, in successive spawning during the growing season and unwanted reproduction that usually leads to crowded condition in rearing ponds and consequently reduces growth (Varadukaj and pandium 1987).

Nile tilapia males grow bigger and faster than females. In order to avoid unwanted spawning in a production tank, all male population is preferred. Several methods are used to skew sex ratios and increase the percentage of males in a Nile tilapia population. The first method was culling through population, discarding the females and keeping the males. Common method of generating mostly male populations is through the use of steroid hormones fed to sexually undifferentiated fry (phelps *et al.* 1995). Exposing the fish to different forms of testosterone or estrogen may lead to sex reversal. Hormones are generally included in the diets for several weeks when the fish start eating, other hormones have been tested and sex reversal has also been achieved by immersion in a solution (Obi and Shelton 1983).

Control of reproduction in tilapia is possible through mono-sex culture, which may be achieved by various method including manual sexing, hybridization and hormonal sex reversal to produce all male tilapia population (Guerrero, 1982).

Table 1: Composition of the experimental diet containing 30 and 40% crude protein

Ingredients	30%	40%
Fish meal	17.9	25.6
Soybean meal	30	45
Wheat bran	5	5
Yellow corn	40.5	18.9
fish oil + corn oil(1:1)	2.0	2.0
Vitamin and mineral premix	1.5	1.5
Ascorbic acid	0.06	0.06
starch	1.97	0.87
Carboxy methyl cellulose	1.0	1.0
Total	100	100

Table 2: Frequency of *O.niloticus* males and females identified by validated microscopic examination of the gonads after 28 days of hormone treatments and different levels of protein.

Treatment	Number of analyzed fish from each treatment	Male %	Female %
A	100	60	40
B	100	86	18
C	100	94	6
D	100	62	38
E	100	92	8
F	100	98	2

Table 3: Average values of weight, growth rate, feed conversion ratio, and survival rate of *O.niloticus* fry after 28 days of hormone treatments and different levels of protein.

Treatment	Initial weight (g/fish)	Final weight (g/fish)	Growth rate(g/fish)	FCR	Survival rate (%)
A	0.015	0.94^{c}	0.033^{c}	1.44^{a}	60
B	0.016	1.13^{asc}	0.039^{ad}	1.31^{bc}	65
C	0.016	1.18^{b}	0.0415^{ab}	1.29^{c}	70
D	0.016	1.03^{a}	0.036^{ac}	1.36^{b}	65
E	0.016	1.27^{b}	0.044^{bc}	1.29^{c}	68
F	0.015	1.44^{c}	0.054^{b}	1.24^{c}	70

The same letter in the same row is not significantly different at P>0.05

Table 4: Water quality parameters in hapas during the study period

Parameter	Treatment					
	A	B	C	D	E	
Water Temp: (°C)	29 - 35	29 - 35	29 - 35	29 - 35	29 - 35	29 - 35
pH	7.5 -8.3	7.2 - 8.5	7.2 - 8.2	7.2 - 8.1	7.4 - 8.2	7.2 - 8.3
Disso: Oxygen (mg/L)	3.0 - 5.44	3.0 - 5.23	3.0 - 5.62	3.0 - 5.36	3.0 - 5.42	3.0 - 5.60
Alkalinity (mg/L)	75 -80	85 - 95	75 - 85	80 - 100	85 - 90	82 - 90
Hardness (mg/L)	90 - 111	85 - 96	90 - 98	92 - 103	96 - 104	93 - 102
Ammonia (mg/L)	0.365	0.501	0.410	0.358	0.652	0.658

Materials and Methods

Nile tilapia fry were stocked at the rate of 10000 individuals hapa (2x5x1.2m) in fresh water ponds. The Nile tilapia fry were 12 mm-15 mm in initial length and 0.015g -.0.016g in initial weight at the times of stocking. 17-Metheyl testosterone (MT) stock solution was made by dissolving 1g of hormone in 1L of 95% ethanol. Treatments were made by taking the accurate amount of the hormone from stock solution and brought up to 100ml by adding 95% ethanol. This solution was evenly sprayed over 1 kg of the diet and mixed. The mixture was mixed again and this was repeated to ensure an equal distribution of the MT throughout the feed. Treated diets were dried in room temperature at 25°C for 24 hours them kept in freezer till use. Eighteen haps were randomly allocated in to six treatments.

group A: Fry were fed with a diet containing 30% cp (as a control diet 1)

group B: Fry were fed with a diet containing 30% cp with 40mg MT/kg

group C: Fry were fed with a diet containing 30% cp with 60mg MT/kg

group D: Fry were fed with a diet containing 40% cp (as a control diet 2)

group E : Fry were fed with a diet containing 40% cp with 40 mg MT / Kg

Group F : Fry were fed with a diet containing 40% cp with 60 mg MT/kg

Nile Tilapia fry were fed the diets twice / day for 6 days a week, at a rate of 15% of the total fish biomass and left over removed by siphoning. The experiment lasted 28 days; in July month 2009. The water quality parameters were recorded each hapa every week. At the end of the experiment period, growth and survival parameters were calculated.

Growth performance was calculated as follows:

Growth rate(g/fish) = W2-W1/T

Where W1 and W2 are the initial and final fish weight, respectively and T is the number of days in the feeding period.

Results and Discussion

17 -methyl testosterone was effective in producing phenotypic male of tilapia. Doses of 60mg MT/kg feed with 40% cp/kg diet produced 98% male population. 92 % males was obtained in Nile Tilapia fry fed with 40% cp with 40mg MT/kg diet when compared with the control group (40% cp) without hormone (62%) males. The lowest ratio of male in hormone treated fry was 86% when fed with diet containing 40 mg MT with 30%cp/kg diet compared to 60% males when fed with diet containing 30 % cp without hormones (Table 2). These results are in agreement with Phelps and Cerezo (1992). Similarly, Book et el. (1992) showed that phenotype male increased with increasing of hormones in diets of Nile tilapia.

Average weight at end of 28 day period were larger (1.44g / fish) for fry group fed with high level of hormones (60 mg /kg diet)and 40 % crude protein. These results indicate that the inclusion of protein and hormones in fish diet is beneficial for the growth. The increase of fish growth may be because of that the androgenic steroids may promote release of growth hormone from the pituitary somatotrops fish (Higgs et al. 1976). However, sex reversed tilapia has been found to be faster than all male tilapia produced by manual sexing or hybridization (Hanson et al. 1983)

The data on survival rate of fry fed with different levels of hormone and protein for 28 days are shown in (Table 3). The different growth rate parameters (final body weight, growth rate (GR),and feed conversion ratio (FCR)) of O *niloticus*fed with 30 and 40% protein diets at low and high dose of 17 -methyltestosterone(40 and 60 mg/kg feed) are shown in table (3). These results indicate that the inclusion of protein and hormones in fish diet is beneficial for fish growth. The increase in fish growth may be because of that MT induce the feed digestion and absorption rate causing increase in body weight (Yamazaki, 1976), or may be MT administration increased the proteolytic activity of the gut as the case in mirror carp loading to increase the growth rate (Lone and Matty 1981). These results are in agreement with Khouraiba (1997) who found that the hormones significantly(P0.05) increased the final weight of fish Nile tilapia as compare to untreated fish. The interaction of both factors was affected on the growth parameters. The highest growth (final body weight, growth rate (GR), and feed conversion ratio (FCR)) of Nile tilapia was obtained with the 40% protein diet with 60 mg (MT)/kg feed and the poorest growth performance of Nile tilapia fry was obtained with diet contain 30% cp without hormones. In this study, there was significant increase in growth parameters (P0.05) with increasing of protein and hormonal levels. These are in agreement with Khattab et al. (2001) who showed that the final body weight, weight gain and specific growth rate (SGR) were positively enhanced by protein level. Also, Al-Hafedh (1999) found that the better growth rate of Nile tilapia was obtained at high dietary protein levels (40%- 45%) than at 25 -35% protein. Feed utilization of Nile tilapia was significantly affected by protein levels and hormones levels.

The water quality parameters, recorded during the present study in rearing such as water temperature (29- 35)°C, water pH (7.2- 8.5), dissolved oxygen content (3-.5.6)mg/L, alkalinity (75-100)mg/L, hardness (85-111)mg/L, and ammonia(0.365-0.658)mg/L in the optimal water quality ranges for Nile tilapia. Therefore, water quality parameters may not be the limiting factor for the growth of Nile tilapia.

Conclusion

In conclusion, these results indicated that sex reversal of Nile tilapia increase with increasing dose of MT and crude protein.

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