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Hormonal Masculinization and Growth Performance in Nile Tilapia (*Oreochromis niloticus*) by Androgen Administration at Different Dietary Protein Levels

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ABSTRACT

The study was planed to determine the effects of different doses of hormone 17α-methyltestosterone (MT) and different protein level of feeding on masculinization and growth performance in Nile tilapia (*Oreochromis niloticus*). The experiment was conducted in two phases. In phase-I, two experiments were performed in six glass aquaria with two replicates. In each aquarium, 50 fry of tilapia were stocked. In experiment A, three glass aquaria were used having the size of 3.0 × 1.5 × 1.0 ft. In this experiment, aquarium -1 was used as control (no hormone given) while in aquaria-2 and 3, the MT were given@ 60 and 70 mg MT/kg of feed containing 30% crude protein. In experiment B, aquarium - 4 was used as control (no hormone added) while in aquaria-5 and 6, the MT were given@ 60 and 70 mg MT/kg of feed containing 40% crude protein. Both the experiments were run parallel for 30 days. In phase-II, the fry were shifted to 6 earthen ponds in which feed was given without hormones on the same protein level as in phase-1, to monitor their growth performance for 183 days. At the final harvest frequency of males and females was determined by morphomatric characteristics and examination of gonads. Results showed that MT treated groups had significantly higher number of males than control and maximum fish growth was recorded in treatments fed with 40% CP level as compared to 30% CP level. The highest percentage of male (93-100%) was recorded at the hormone dose of 70 mg MT/kg of feed with 30 and 40% protein levels. Masculinized tilapia showed maximum growth performance (final body weight 162.4 g & weight gain 160.4 g) with maximum fish production i.e., 2000.7 kg/ha/year at 40% CP diet with 70 mg MT/kg of feed. Among all the sexes (male, female & inter sex), females showed the highest gonadosomatic index (GSI), which was lowest in males. In conclusion, higher hormonal dose and higher dietary protein level increased masculinization and growth in fish.

Key Words: Androgen; Dietary protein; Masculinization; Growth; Nile tilapia

INTRODUCTION

Fast growth rate at high density, palatability to artificial feed and controlled reproduction are some of the most important characteristics in a successful fish culture. Tilapia is likely to be the most important of all aquaculture fish in 21st century (Fitzsimmons, 2000). Tilapia has certain favourable characteristics, which make its cultivation, like most tolerant to adverse environmental conditions, can survive at low dissolve oxygen, euryhaline, relatively fast growth and efficient food conversion. All these characteristics make tilapias one of the best choices for farmers (Yi *et al.,* 1996; Penna-Mendoza, 2005).

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 early age and reproduces after every 4-6 weeks in the ponds. The monosex 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 (Mair & Little, 1991). There are four strategies for monosex male culture i.e., manual process by visual examination; hybridization; gene manipulation and masculinization via steroid hormone.

At the time of hatching tilapia fry are still sexually undeveloped. Hence, during the early period of gonad differentiation, changes in sex hormone level can affect the final sex independently of the genetic sex (Andersen *et al*., 2003). Oral administration of steroid hormone by incorporation in feed has a long history of use and is more successful for masculinization than other methods. In tilapia sex steroid hormones play a promising role in directing the gonad differentiation process (Piferrer, 2001). In tilapia culture, 17-α-methyltestosterone (MT) is the most commonly used synthetic androgen to alter the sex ratio of

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fish. The MT promotes both muscle growth and development of male sexual characters (Adel *et al*., 2006). However, it is important to identify the optimal level of MT, type as well as duration and timing of treatment for consistent, successful sex reversal (Dunhan, 1990). When tilapia fry were fed diets with 17-α-methyltestosterone all male populations were obtained (Green *et al*., 1997). Succession in this method at the rate of 100% in each application depends on efficiency of hormone, dosage, method of administration, time and duration of treatment and species (Altun *et al*., 2006). The dosage rate ranges from 10-70 mg hormone/kg of diet for Nile tilapia and duration of administrating MT for masculinization of Nile tilapia varies from 14 to 60 days (Abucay & Mair, 1997).

Growth in fish is affected not only by quality of dietary protein but also by protein levels in diet. Dietary protein requirements of several species of tilapia range between 20% and 56% (El-Sayed & Teshima, 1991). Fry growth improves with increasing protein levels. In aquaculture diets, protein is the most expensive and critical ingredient from the stand point of cost and growth response. In aquaculture a lot of work has been done on tilapia growth and its culture but little is known about the efficacy of androgen hormone and dietary protein on masculinization and growth. Keeping this in view, this study was undertaken to produce masculinized tilapia by oral administration of MT and to examine the effect of different dietary protein levels.

MATERIALS AND METHODS

For studying the masculinization and growth of Nile tilapia (*Oreochromis niloticus*) under different treatments the experiment was conducted in two phases at Fisheries Research Farms, University of Agriculture, Faisalabad, Pakistan. In phase-1, fertilized eggs were collected from the mouth of female tilapia and shifted to cemented circular tank, where they were kept till hatching. After fourth day of hatching, the fry were randomly distributed to six glass aquaria (two controls & four treatments) at the density of 50 fry per aquarium with two replicates of each treatment. Fry were orally administrated with different dose rates of 17-α-methyltestosterone (MT) and dietary protein levels in 6 treatments under two experiments. In experiment A, aquarium-1 was used as control in which no hormone was given while in aquaria 2 and 3, the MT were given @ 60 and 70 mg MT/kg of feed containing 30% crude protein. In experiment B, aquarium - 4 was used as control in which no hormone was given while in aquaria 5 and 6, the MT were given @ 60 and 70 mg MT/kg of feed containing 40% crude protein. Feeding was done at the rate of 10% of the total fish biomass for 30 days. During the experimental period water quality parameters were monitored regularly.

After 30 days, the fry were shifted to six earthen ponds (phase II) for a culture period of 183 days. In this phase, feed was given without hormones on the same protein level as in phase-1, to monitor their growth performance i.e., body weight on fortnightly basis. At the end of phase-II on final harvesting, all fish were taken by draining out the pond water to determine the survival rate and for morphological and histological examination of experimental fish. After measuring the processing traits each fish was dissected and the paired gonads were removed with the help of forceps, weighed and then placed on a microscope slide, stained with fast green, covered with another slide and squashed. The tissues observed under microscope were recorded as testes (male), ovaries (female) and ovo-testis (inter-sex). The sex of each fish and weight of gonad was recorded to determine the gonadosomatic index (GSI). The GSI was estimated with the help of formula given by Hopkins (1979).

Gonadosomatic index (GSI) = (Gonad weight) × 100/(body weight)

To find out the relationship between hormone and sex ratio (sex reversal) the 'Logistic Regression' was applied. To compare the growth parameters and means of different variables analysis of variance (ANOVA) and Duncan's Multiple Range (DMR) test (Duncan, 1995) was used. Regression and correlation among various variables were computed to find out particular trends and relationship by following Steel *et al*. (1997). M-Stat and SPSS (1999) packages of the computer were used for the statistical analysis of the data. All statistical computations were performed at the $P \leq 0.05$ probability level.

RESULTS AND DISCUSSION

The present project was planned to determine the dose of 17-α-methyltestosterone and optimum dietary protein level in order to obtain all masculinized Nile tilapia (*O. niloticus*) with higher growth potential. Sex ratio, gonadosomatic index (GSI) and survival rate of Nile tilapia are summarize in Table I. In all treatments survival rate of experimental fish remained 100%, which showed that 17-α-MT did not affect survival rate of fish, which may be due to rapid hormonal excretion in fish via faeces and gills (Cravedi *et al*., 1993). Among the hormone treated groups, the highest frequency of male tilapia at 100% was recorded in treatment T_6 in which fry was orally administrated with 70 mg MT/kg of feed at 40% CP. From the results it is very obvious that oral administration of 17-α-MT was effective in producing masculinized tilapia. On contrary, lowest ratio (68%) was recorded in treatment T_5 which were fed with diet containing 60 mg MT/kg of feed at 40% dietary protein level. In treatment T_3, 92% male population was obtained when fry was given diet with 70 mg MT/kg of feed at 30% CP. There was a direct relationship between masculinization and dose rate of hormone in all treatments. Response curve obtained during regression analysis showed probability of male proportion increased with increasing hormone dose rates (Fig. 1). It is clear from the results dietary protein levels have no influence on the sex reversing activity in Nile

Table I: Percentage and gonadosomatic index of male, female and inter sex *O. niloticus* under different treatments in both experiments

Protein levels	Treatments	No. of analyzed Fish	Survival rate (%)	Male			Female			Inter Sex		
				Number	%	GSI %	Number	%	GSI %	Number	%	GSI %
Exp. A 30% CP	0 mg MT/kg	50	100	20	40	0.37	30	60	1.57	0	0	0
	60 mg MT/kg	50	100	38	76	0.35	10	20	1.41	2	4	0.89
	70 mg MT/kg	50	100	46	92	0.34	4	8	1.38	0	0	0
Exp. B 40% CP	0 mg MT/kg	50	100	23	46	0.38	27	54	1.52	0	0	0
	60 mg MT/kg	50	100	34	68	0.32	15	30	1.39	1	2	0.99
	70 mg MT/kg	50	100	50	100	0.31	0	0	0	0	0	0

Table II: Fortnightly observations on average and increase in body weight of *O. niloticus* under different treatments in both experiments

Fort-nights	Experiment A 30 %CP						Experiment B 40 %CP					
	0 mg MT/kg		60 mg MT/kg		70 mg MT/kg		0 mg MT/kg		60 mg MT/kg		70 mg MT/kg	
	Av. body wt.	Inc. in Body wt.	Av. body Wt.	Inc. in Body Wt.	Av. body wt.	Inc. in body wt.	Av. body wt.	Inc. in body wt.	Av. body wt.	Inc. in body wt.	Av. body wt.	Inc. in body wt.
Stocking	2.0	-	1.8	-	1.8	-6.8	1.6	-	1.9	-	2.0	-
1	7.7	5.7	7.7	5.9	8.6	6.8	7.9	6.3	9.0	7.1	10.2	8.2
2	18.4	10.7	19.9	12.2	20.4	11.3	18.9	11.1	22.7	14.5	24.7	14.8
3	27.3	8.9	31.6	11.7	34.8	14.4	28.7	9.8	39.1	16.4	44.6	19.9
4	44.8	17.5	50	18.4	53.2	18.4	45.3	16.6	60.6	21.5	69.3	24.7
5	56.6	11.8	67.3	17.3	71.7	18.5	60.9	15.6	80.9	20.3	93.0	23.7
6	72.1	15.5	86.6	19.3	88.0	16.3	77.6	16.7	97.6	16.7	112.6	19.6
7	83.7	11.6	99.5	12.9	101.9	13.9	89.8	12.2	111.8	14.2	127.5	14.9
8	92.2	9.2	111.3	11.8	112.6	10.7	98.7	8.9	122.2	10.4	139.5	12.0
9	98.4	5.5	120	8.7	121.3	8.7	105.9	7.2	130.7	8.5	147.1	7.6
10	104.0	5.6	124.2	4.2	125.4	4.1	112.8	6.9	136.8	6.1	152.9	5.8
11	109.3	5.3	127.4	3.2	130.5	5.1	116.6	3.8	141.7	4.9	157.6	4.7
12	112.4	3.1	131.4	4.0	135.2	4.7	120.0	3.4	146.5	4.8	162.4	4.8

Table III: Different growth parameters of *O. niloticus* in treatments fed at different dietary protein and hormone levels

Growth characteristics	Experiment A 30 % CP			Experiment B 40 % CP		
	0 mg MT/kg	60 mg MT/kg	70 mg MT/kg	0 mg MT/kg	60 mg MT/kg	70 mg MT/kg
No. of stocked fish	50	50	50	50	50	50
Initial average body weight (g)	2.0	1.8	1.8	1.6	1.9	2.0
Final average body weight (g)	112.4	131.4	135.2	120.0	146.5	162.4
Increase in average body weight (g)	110.4	129.6	133.4	118.4	144.6	160.4
Specific growth rate % (SGR)	2.201	2.344	2.360	2.359	2.374	2.402
Fish production/ pond/183 days (kg)	5.62	6.57	6.76	6.00	7.32	8.12
Fish production/ pond/year (kg)	11.20	13.10	13.48	11.96	14.60	16.20
Fish production/ acre/year (kg)	562	655	674	598	730	810
Fish production/ ha/year (kg)	1388.4	1617.8	1664.7	1477.0	1803.1	2000.7

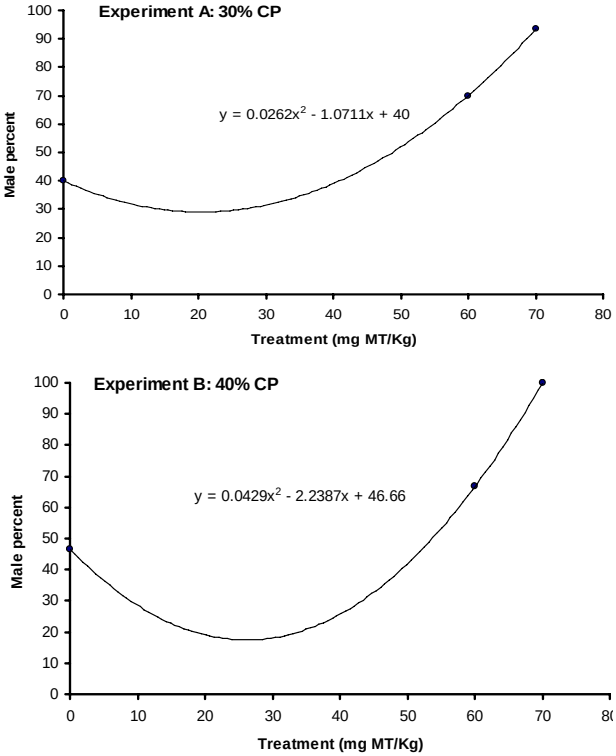
tilapia. However, it is very certain from the findings that masculinization in Nile tilapia increased with increasing the hormone doses in diets. Nevertheless, these results are in agreement with Ekwu and Sikoki (2001), who obtained 97% males in TG strain (Grey tilapia) and 96% males in TR strain (Red tilapia) when fry were fed with 17-α-MT at 60 mg/kg of feed. The highest proportion of males (90.3%) was obtained by Ridha and Lone (2008) when *O. spilurus* fry was orally administrated with 70 mg/kg of 17-α-MT. Female ratio of 20 and 30% was obtained in treatments fed with hormone dose of 60 mg MT/kg of feed at 30 and 40% dietary protein. Lower concentration increased the frequency of female, which may be due to rapid metabolism and excretion of hormone causing no anabolic effect. No inter-sex fish was observed in control and highly treated groups.

Hormone doses and sex showed a promising affect on gonadosomatic index (GSI) in tilapia. Females displayed significantly higher dressing out performance than males given by significantly lower value of GSI. In males lower GSI resulted from the large somatic mass of the animals. Akel and Moharram (2007) also found high GSI in female over male. As far as concern is about the effect of dietary proteins on GSI in *O. niloticus* there are reports of GSI not being affected by variations in dietary protein levels (Gunasekera & Lam, 1997).

The highest growth in terms of final weight (162.4 g) of Nile tilapia was obtained at 40% protein diet with 70 mg MT/kg of feed and lowest growth (112.4 g) was obtained with diet contain 30% CP without hormone. In terms of fish production, the results showed that different growth

Fig. 1: Response curves showing the probability of occurrence of males in an *O. niloticus* population for both experiments

Y = occurrence of male



parameters (final body weight, weight gain & SGR) of Nile tilapia were significantly affected by protein level and hormone doses incorporation in feed (Table III). Among the treated groups the treatment which fed with 60 mg MT/kg of feed at 30% CP showed the lower growth rate. Highest yield over the 183 day experimental period was 2000.7 kg/hectare/year of Nile tilapia in treatment T_6, and it was due to availability of higher protein diet (40%) at 70 mg MT/kg (Table III). Statistical analysis showed highly significant variations ($P<0.01$) in gain in body weight among all six treatments, similar variations were also recorded among fortnights through the experimental period. The increase in growth by hormone may be due to the fact that MT induced the feed digestion and absorption rate causing increase in body weight (Adel *et al*., 2006). Similarly Sparks *et al.* (2003) found that *O. mossambicus* fry fed with MT added to their feed grew significantly larger than their respective controls. Significant increase in growth in 17-α-MT treated group of tilapia was also recorded by Ridha and Lone (2008). In the present study the economic dietary protein level for tilapia growth was found to be higher (40%) than that of 30%, whereas specific growth ranged from 2.20 (control at 30% CP) to 2.40 (70 mg MT/kg feed at 40% CP) at 2% of body weight feeding level. According to Ogunji and Wirth (2000) SGR increased with higher dietary protein content which may be due to a reduction in dietary energy available for growth.

CONCLUSION

There was an increased trend towards the growth with an increase in dietary protein level and MT dose. Use of such MT doses and protein levels would help to meet the goal of controlling tilapia reproduction with fast growth.

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