INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
11–349/AWB/2011/13–6–971–975
http://www.fspublishers.org

Full Length Article

17α-Methyltestosterone Induced Masculinization and its Effect on Growth and Meat Quality of *Cyprinus carpio*

MUHAMMAD SAMEE MUBARIK[1], IFTIKHAR AHMED, ABDUL MATEEN AND TAHIRA IQBAL†
Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan
†Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan
[1]Corresponding author's e-mail: sameech2002@yahoo.com

ABSTRACT

The project was designed with the goals to determine the optimal dose rate of oral administration of the androgen 17α-Methyltestosterone (MT) for sex reversal fish seed of *Cyprinus carpio* as well as the meat quality (proximate composition). Sex reversal was completed in ten glass aquaria (70 L capacity each), where fish fed (30% crude protein) with 25, 50, 75 and 100 mg MT.kg^{-1} along with control (without MT hormone diet). In the glass aquaria feed was given @ 2% of wet body weight of fish twice daily for thirty days. After the treatment of MT, the fingerlings were shifted to earthen ponds for 281 days and feeding was done with 30% CP without hormone diet @ 2% body weight daily. All MT treated on maturation showed sex reversal in *C. carpio*, with a significantly higher male proportion than female as compared to control treatment. Dose rate of 100 mg MT.kg^{-1} of feed oral administration for 30 days resulted in maximum sex reversal i.e., 100% male population. Fish production was 1.42 times higher in 100 100 mg MT.kg^{-1} (2398.83 kg.ha^{-1}.yr^{-1}) as compare to control treatment (1693.58 kg.ha^{-1}.yr^{-1}) was estimated. Proximate body composition of control and treated fish showed a highly significant difference with respect to moisture, crude protein, fat, ash and gross energy, while it remain non-significant with respect to carbohydrates and nitrogen free extract. In treatment T_{25} the protein was minimum as 15.58%, while the maximum protein was found in that treatment in which MT was given @ 100 mg.kg^{-1} of diet. Sex reversal showed an increasing trend of crude protein and fat deposition as hormone (MT) concentration increased.

Key Words: *Cyprinus carpio*; 17α-methyltestosterone; Masculinization; Growth; Meat quality

INTRODUCTION

Fish plays an important role in the human diet, constituting the main and often irreplaceable source of animal based food protein constituents all over the world especially in Asia. The region has experienced the largest increase in human population and also has large reserves of water, which could be developed for aquaculture (Nanna *et al.*, 2003).

Carp culture practices ranges from intensive to extensive systems as ability to convert organic, domestic and agricultural wastes into high quality protein efficiently and at high growth rates. Culturing techniques are simple and require a low input from farmers, who can benefit easily from its culture. It is a good source of animal protein (Jian, 2002). Despite having many good characteristics, carp has a major constraint i.e., tendency to overpopulate a pond due to early maturation (±6 months old) at 400-500 g. It results in successive spawning during the growing season and hence in unwanted reproduction that usually leads to crowded condition in the ponds and consequently reduces growth in *Cyprinus capio* (Shelton, 1995). If fish do not spawn and produce fry, the gonadosomatic index (GSI) can exceed 20% of the harvested weight of an individual fish (Basavaraju *et al.*, 2002). The first method developed was to simply cull through a population, discard the females and keep the males. This system is obviously wasteful and inefficient (Hafeez-ur-Rehman *et al.*, 2008). The use of hybrid crosses was one of the primary methods of producing mostly male populations. The drawback to this method is that two separate pure brood lines must be maintained. The crossing must be done very carefully and meticulous records should be kept to ensure that the parent species are kept pure. Also, usually only one sex from each species is used for any particular cross, because the reciprocal cross (using the other sex from each species) is not as successful. Therefore, maintaining a commercial scale hatchery requires significant resources and staff (Fitzsimmons, 2008).

Production of mono-sex populations in several fish species require sex reversal, which is induced by steroid hormones (Shelton *et al.*, 1995). Of these androgens and oestrogens are used as masculinizing and feminizing agents, respectively. Steroid hormones are commonly applied by oral or immersion treatments. Oral administration of the synthetic androgen 17α-methyltestosterone (MT) has been effective in producing all male populations in carps. All

To cite this paper: Mubarik, M.S., I. Ahmed, A. Mateen and T. Iqbal, 2011. 17α-Methyltestosterone induced masculinization and its effect on growth and meat quality of *Cyprinus carpio*. *Int. J. Agric. Biol.,* 13: 971–975

male population increases the efficiency and feasibility of carp aquaculture. There have been numerous published attempts to optimize the methods of sex reversal by varying parameters such as hormone dose, treatment start time, duration of treatment and stocking density.

Androgens are commonly applied for hormonal stimulation of growth or sex reversal in fish (Colborn *et al.*, 1993; Damstra *et al.*, 2002). Among the androgens, MT is most commonly used. It is easily absorbed, does not accumulate in fish body and is readily excreted (Sumpter, 2005). Keeping in view all these problems, the present project was planned to determine optimal dose for oral administration of MT for sex reversal and the effect of androgen on growth and meat quality of *C. carpio*.

MATERIALS AND METHODS

Thirty five days old fingerlings collected from the earthen ponds were shifted to 10 aquaria (70 L capacity each) for oral administration of MT. In each aquarium 50 fingerlings were stocked and fed with 4 mm pellet size commercially formulated 30% crude protein aquaculture diet. Hormone MT was administrated at four different rates viz. 25, 50, 75 and 100 $mg.kg^{-1}$, while control was feed without hormone diet for 30 days @ 2% wet body weight twice a time daily. The other treatments were mixed with the hormone dissolved in absolute ethanol (1 mg 17-α MT/mL ethanol). From the standard, the treatment was diluted upto 75 mL and sprayed on all over the feed equally to homogenate. Then the hormone treated feed left over open at room temperate for 24 h, ethanol to evaporate leaving behind hormone. After 30 days fingerlings were then shifted in 5 earthen ponds having dimensions 25×8×1.5 m located at Fisheries Research Farms, University of Agriculture, Faisalabad, Pakistan. The supplementary feed (30% CP) was applied @ 2% of body weight daily while organic manure (poultry dropping) was applied @ 0.1 g N/100 g of body weight daily and was added on fortnightly basis. The growth parameters of fish viz. fork length, total length and wet body weight were measured and recorded to calculate specific growth rate (SGR), food conversion ratio (FCR), nitrogen incorporation efficiency (NIE), nitrogen conversion efficiency (NCE).

For limnological study, water samples were collected from all five ponds on fortnightly basis. The water temperature, light penetration, pH, dissolved oxygen, total alkalinity, carbonates, bicarbonates, total hardness, calcium, magnesium, total solids, total dissolved solids and planktonic biomass were determined for every treatment on fortnightly basis following APHA (1995). At the end of the experiment, all fishes from each treatment were dissected and their gonads on filter paper were examined under compound microscope for verification of their sex i.e., testis, ovary or inter-sex were noted. A representative sample of fish was homogenized using grinder following AOAC (1995) for determination of dry matter (DM), crude protein (CP), crude fat, ash contents and gross-energy. Finally data was subjected to analysis of variance (Steel *et al.*, 1997) and differences between means ($P<0.05$) were evaluated by Tukey's Honest Significance Test (HSD).

RESULTS AND DISCUSSION

In the present experiment, the control fish showed a normal sex ratio (male & female 1:1 ratio), while all other treatments receiving MT showed a significantly higher male proportion as compared to the control (Table I). The 100 mg MT kg^{-1} showed highest percentage of male 100 %, followed by 75, 50 and 25 $mg.kg^{-1}$ MT treatments with a male ration of 90, 76 and 60%, respectively. The female proportion also showed significant differences among control and groups that received MT treatments. The inter-sex population was absent in controls and 100 mg MT kg^{-1} treatment, while it was recorded as 10, 10 and 6% in 25, 50 and 75 $mg.kg^{-1}$ MT treatments, respectively. Statistical analysis of the sex reversal showed a highly significant difference ($P<0.01$) among all the treatments in respect of male, female and intersex ratio. Several studies of sex reversal have shown different results from various hormones given to different fish species. For instance, Lindsay *et al.* (2000) and Shalaby *et al.* (2006) observed that nile tilapia at dose of 60 $mg.kg^{-1}$ MT feed with 40% CP produced 100% males. Likewise, Arslan and Phelps (2003) reported that black crappie fry produced 96% male populations with oral administration of MT at dose rate of 60 $mg.kg^{-1}$. Iwamatsu *et al.* (2006) also successfully induced sex reversal in medaka (*Oryzias latipes*) by immersion in 30 $ng.L^{-1}$ MT for 3 weeks. Hybrid tilapia fry fed 100 $mg.kg^{-1}$ of fluoxymesterone yielded 96.1% sex reserved male population but higher than that dose rate of fluoxymeterone produced higher intersex and sterile fish (Manosori, 2004). In the present studies, the water quality parameters viz. water temperature, dissolved oxygen, pH and total dissolved solids under optimal limits and seemed to have no effect on the sex reversal, which is contrary to that reported by Beardmore *et al.* (2001) and Kang *et al.* (2008).

In the present experiment, survival of *C. carpio* was recorded as 100% during the growth phase, which clearly showed no significant relationship between survival and MT treatments (Table I). Likewise, Demska-Zakes (1997) reported no significant differences in pikeperch between the MT treated and control fish. Unlikely, survival progressively decrease up-to 76% with increase in MT immersion at a dose rate of 900 $\mu g.L^{-1}$ and 71% at the termination of experiment for fighting fish, *B. splendens* (Kirankumar & Pandian, 2002).

The final average total length showed marked differences among different treatments and fortnights. Application of MT at 100 $mg.kg^{-1}$ showed the highest gain in total length (28.4 cm) as compare to all other treatments. In this study, all MT treatments also showed higher average body weight of *C. carpio* than the control.

Table I: Sex percentage and different growth parameters of *Cyprinus carpio* under control and sex reversal treatments

Treatments	T_0	T_{25}	T_{50}	T_{75}	T_{100}
Male percentage	50 e	60 d	76 c	90 b	100 a
Female percentage	50 a	30 b	14 c	4 d	0
Intersex percentage	0	10 b	10 b	6 a	0
Initial weight (g)	16.2	16.4	16.2	13.5	16.6
Survival percentage	100	100	100	100	100
Final weight (g) for 281 days	521.1	573.2	653.9	622.2	738.1
Gross production ($kg.ha^{-1}.yr^{-1}$)	1693.58	1862.90	2125.18	2022.15	2398.83
Net production ($kg.ha^{-1}.yr^{-1}$)	1640.93	1809.60	2072.53	1978.28	2344.88
Food conversion ratio (FCR)	0.013	0.011	0.020	0.011	0.009
Specific growth rate (SGR)	0.536	0.549	0.572	0.561	0.586
Gonado somatic index (GSI)	17.46 a	9.07 b	7.71 c	7.46 c	2.47 d
Nitrogen incorporation efficiency (NIE)	1.66±0.56	3.92±1.15	4.32±1.21	4.23±1.27	5.22±1.63
Nitrogen conversion efficiency (NCE)	0.06±0.02	0.13±0.04	0.14±0.04	0.15±0.04	0.18±0.05

Values sharing similar letter in a column are statistically non-significant P>0.05

Table II: Analysis of variance on various parameters of growth of *Cyprinus carpio* control and treated

SOV	Fork Length		Total Length		Weight		Gain in Weight	
	MS	F. Value	MS	F. Value	MS	F. Value	MS	F. Value
Treatments	4.2290	60.05**	4.394	48.80**	28989	44.83**	337.310	40.29**
Fortnights	87.1385	1237.30**	100.399	1115.09**	209427	323.88**	345.106	41.22**

** = Highly significant (P < 0.01), * = Significant (P < 0.05)

Table III: Comparison of means proximate body composition of *Cyprinus carpio* under control and sex reversal treatments

Treatments	Moisture %	DM	Crude Protein %	Crude Fat %	Ash %	Carbohydrates	NFE	Gross Energy
MS F Value	**30.17 22.86****	**30.05 22.84****	**20.36 48.86****	**1.22 16.28****	**0.97 6.99****	**0.48 2.08^{NS}**	**0.15 0.37^{NS}**	**0.32 5.87 ****
SE	0.27	0.27	0.20	0.06	0.06	0.07	0.09	0.04
Control	80.43 a	19.58 d	15.58 d	3.11 b	0.10 c	0.78	0.88	1.95 b
25 mg/kg MT	79.29 b	20.71 c	16.32 c	3.17 b	0.89 a	0.31	1.20	1.93 b
50 mg/kg MT	78.02 c	21.98 b	17.79 b	3.16 b	0.60 ab	0.43	1.03	1.99 b
75 mg/kg MT	77.12 c	22.88 b	18.29 b	3.73 a	0.75 a	0.37	1.12	2.12 b
100 mg/kg MT	76.03 d	23.97 a	19.05 a	3.83 a	0.37 bc	0.76	1.13	2.36 a

** = Highly significant (P < 0.01), ^{NS} = Non-significant (P > 0.05), (Means sharing the similar letters in a column are statistically non-significant P > 0.05)

Table IV: Fortnightly observation means and analysis of variance of physcio-chmeical parameters

Variable	Mean ± SD					Treatments		Fortnights	
	Control	25 mg/kg MT	50 mg/kg MT	75 mg/kg MT	100 mg/kg MT	MS	F Value	MS	F Value
W Temp (ºC)	25.4±2.29	25.2±2.29	25.0±2.27	25.0±2.29	25.3±2.22	0.67	3.74*	257.28	1492.54**
LP (cm)	11.9±0.66	13.7±1.36	13.9±1.67	13.6±1.47	13.8±1.90	14.11	2.64*	87.27	16.30**
DO ($mg.L^{-1}$)	6.33±0.60	6.20±0.69	5.97±0.68	5.96±0.71	6.46±0.66	1.02	2.06^{NS}	20.30	41.10**
pH	8.71±0.04	8.61±0.05	8.66±0.05	8.63±0.06	8.68±0.04	0.03	2.58*	0.07	6.29**
TA ($mg.L^{-1}$)	552.5±20.95	544.2±18.94	532.6±18.45	524.2±16.59	504.1±14.41	7381.25	13.43**	14011.40	25.50**
Carb ($mg.L^{-1}$)	37.0±1.99	36.6±2.31	35.0±2.36	34.2±2.16	32.0±2.22	83.94	7.22**	197.66	17.01**
Bicarb ($mg.L^{-1}$)	515.5±20.38	507.6±18.40	502.3±18.88	490.0±16.46	471.6±14.19	6172.51	10.70**	13518.14	23.43**
TH ($mg.L^{-1}$)	357.9±19.51	361.6±19.51	331.0±15.65	326.6±14.85	333.3±21.24	5617.01	23.51**	15823.17	66.23**
Ca ($mg.L^{-1}$)	36.9±2.69	33.8±4.05	32.7±2.70	29.9±3.08	30.8±3.76	157.264	10.65**	486.91	32.98**
Mg ($mg.L^{-1}$)	80.3±4.65	82.1±4.46	74.6±3.63	74.3±3.38	75.6±4.60	269.26	20.50**	819.44	62.38**
TS ($mg.L^{-1}$)	1935.5±85.85	1857.0±74.89	1798.7±63.43	1837.5±84.49	1938.4±110.03	79915.99	6.53**	313543.20	25.64**
TDS ($mg.L^{-1}$)	1555.0±66.12	1579.4±63.29	1554.8±72.07	1564.0±70.96	1573.6±72.45	2555.87	0.48^{NS}	217340.08	40.99**
PB ($mg.L^{-1}$)	380.5±30.79	277.5±28.50	243.9±36.55	273.5±26.96	364.8±58.77	77222.27	5.71**	24982.81	1.85*

** = Highly significant (P < 0.01), * = Significant (P < 0.05), ^{NS} = Non-significant (P > 0.05)

MT applied at 100 $mg.kg^{-1}$ MT yielded the highest final gain in body weight (738.1 g) as compared to the control (521.1 g). Statistical analysis of the fork length, total length and body weight data showed a highly significant difference (P<0.01) among different treatments and fortnights (Table II), which is consistent with the findings of Manosori *et al.* (2004) for hybrid tilapia, while contrary to Demska-Zakes (1997) for *Stizostedion lucioperca*.

In earthen ponds, the final gross and net fish production of *C. carpio* showed a marked increase in all the MT treatments as compare to control, clearly indicating that MT enhanced the growth rate and biomass of *C. carpio* efficiently. The 100 mg MT kg^{-1} yielded the highest gross fish production of 2398.83 $kg.ha^{-1}.yr^{-1}$ (Table I) and it was

1.42 times greater than control fish (1693.58 kg.ha^{-1}.yr^{-1}). The inverse results of weight gain by fish fed with control diet (0 MT) were higher than that of fish fed treated diets (Davis & Ludwig, 2004).

In this experiment, Low food conversion ratio (FCR) and higher specific growth rates of *C. carpio* were observed with increase in MT (Table I). Earlier studies show that androgen-induced growth improvements were due to increased food conversion efficiency in several salmonids (Davis & Ludwig, 2004). FCR and protein efficiency ratio of tilapia fed 2 mg MT.kg^{-1} diet were significantly greater than control fish (Abdelghany, 1996).

In the present study, MT effect on meat quality was also studied. The moisture content of *C. carpio* meat showed a little difference among treatments. The control group showed the highest moisture contents (80.43%), while the treatment 100 mg MT kg^{-1} showed the lowest moisture contents (76.03%), while other three treatments showed intermediate values (Table III). The dry mass of *C. carpio* was the highest in 100 mg.kg^{-1} MT treatment and the lowest in control. The moisture content was inversely related to crude fat and ash content of meat of *C. carpio* in all treatments. Moisture contents were affected by MT levels but the increase in muscle protein of tilapia was increased with the increase in MT (Shalaby *et al.*, 2006). The protein contents of *C. carpio* were the highest in 100 mg.kg^{-1} MT treatment followed by 25, 50 and 75 mg.kg^{-1} MT and the lowest value was recorded in control (Table III). Ether extract of *C. carpio* meat was in the following decreasing order MT 100>75>50>25 mg MT kg^{-1} (Table III). In another other report, *C. carpio* moisture content increased, ash content decreased, while no effect on protein and fat content after treating with androgen (Lone & Matty, 1981). Abdelghany (1996) observed a greater retention of moisture, protein, fat and ash content of tilapia fed with 2 mg MT.kg^{-1} diet than the fish fed with control diet. Ash and carbohydrates contents of *C. carpio* meat varied widely under the different treatments ranging between 0.10-0.89% and 0.31-0.76%, respectively. The highest gross energy value of *C. carpio* meat was observed in 100 mg.kg^{-1} MT, which was in descending order in rest of the MT treatments. Nitrogen free extract carbohydrate from meat of *C. carpio* meat was lowest in control fish, which differed significant among various MT treatments (Table III). A non-significant effect on carbohydrates and NFE was reported with a rise in androgen in tilapia (Asad *et al.*, 2010).

In the earthen ponds, the limnological parameters were studied for their effect on fish growth. The overall the water temperature ranged between 13 to 35ºC and light penetration between 8 to 36 cm in all treatments. pH ranged between 8.43 to 8.93, 8.24 to 8.85, 8.42 to 9.07, 8.33 to 9.10 and 8.32 to 8.86 in control, 25, 50, 75 and 100 mg.kg^{-1} MT treatments, respectively. Average dissolved oxygen (DO) remained above 5 mg.L^{-1} (a limit considered safe for fresh water fisheries) for all the treatments. Low concentrations of dissolved oxygen (3.87 mg.L^{-1}) were recorded at higher water temperature (34.7°C), while high values (8.68 mg.L^{-1}) were recorded at lower water temperatures (15.4°C). Dissolved oxygen has an inverse relationship with water temperature of fish pond (Mateen & Ahmed, 2007). Analysis of variance for water temperature, LP, DO and pH of water showed a highly significant difference with respect to time and fortnights and a significant difference among treatments except DO (Table IV). The value of total alkalinity, carbonate and bicarbonate alkalinity ranged between 376 to 688, 20 to 53 and 322 to 652 mg.L^{-1}, respectively in the increased order of MT application (Table IV). The results of present investigation demonstrated that alkalinity showed no clear seasonal trends throughout study period. Total alkalinity, carbonate and bicarbonate concentrations were non-significantly positively correlated with planktonic biomass productivity under all the treatments (Abbas *et al.*, 2010).

Overall means of total hardness throughout the experiment are presented in Table IV. The seasonal fluctuations in calcium contents of pond water under different treatments ranged between 4.0 to 48.7 mg.L^{-1} and magnesium between 52.8 to 106.5 mg.L^{-1}. The patterns of seasonal variations in total alkalinity corresponded with the concentrations of total hardness (Asad *et al.*, 2010). The values of total solids, total dissolved solids and planktnonic biomass ranged between 891 to 2545 mg.L^{-1}, 721 to 1862 and 80 to 823 mg.L^{-1} in control and all treatments, respectively. The analysis of variance on total alkalinity, carbonates, bicarbonates, total hardness, calcium and magnesium, total solids, total dissolved solids and planktonic biomass showed a highly significantly difference among different treatments and fortnights except total dissolved solids varied non-significantly among different treatments (Table IV). Even though alteration in physical environment was observed, the growth, survival, body weight and protein contents significant increment suggested that the pond water has favored fish growth (Mateen & Ahmed, 2007).

CONCLUSION

There was an increasing trend of maleness with an increase in dietary MT dose. Use of such MT doses levels would help to meet the goal of controlling *C. carpio* reproduction with better quality and maximum survival.

Acknowledgement: Special thanks to Higher Education Commission (HEC), who provided me opportunity by funding for this study under Project No. 20-811/R&D/07/388.

REFERENCES

Abbas, S., I. Ahmed, M. Salim and K. Rehman, 2010. Comparative effects of fertilization and supplementary feed on growth performance of three fish species. *Int. J. Agric. Biol.,* 12: 276–280

Abdelghany, A.E., 1996. Effect of feeding17α-Methyltestosterone and withdrawal of feed utilization and growth of Nile Tilapia, *Oreochromis niloticus* L., fingerlings. *J. Appl. Aqua.,* 5: 67–76

AOAC, 1995. *Official Method of Analysis*, 16th edition. Association of Official Analytical Chemists, Arlington, Virginia

APHA (American Public Health Association), American Water Works Association and Water Environment Federation, 1995. *Standard Methods for the Examination of Water Wastewater*, 19th edition. APHA, American Water Works Association and Water Environment Federation, Washington, DC

Arslan, T. and R.P. Phelps, 2003. Production of monosex male black crappie, *Pomoxis nigromaculatus*, populations by multiple androgen immersion. *Aquaculture,* 234: 561–573

Asad, F., I. Ahmed, M. Saleem and T. Iqbal, 2010. Hormonal masculinization and growth performance in Nile tilapia (*Oreochromis niloticus*) by androgen administration at different dietary protein levels. *Int. J. Agric. Biol.,* 12: 939–943

Basavaraju, Y.G., C. Mair, H.M.M. Kumar, S.P. Kumar, G.Y. Keshavappa and D.J. Penman, 2002. An evaluation of triploidy as a potential solution to the problem of precocious sexual maturation in common carp, *Cyprinus carpio*, in Karnataka, India. *Aquaculture,* 204: 407–418

Beardmore, J.A., G.C. Mair and R.I. Lewis, 2001. Monosex male production in finfish as exemplified by tilapia: applications, problems, and prospects. *Aquaculture,* 197: 283–301

Colborn, T., F.S.V. Saal and A.M. Soto, 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ. Health Persp.,* 101: 378–384

Damstra, T., S. Barlow, A. Bergman, R. Kavlock and G.V.D. Kraak, 2002. *WHO Report on Global Assessment of the State-of-the-Science of Endocrine Disruptors*. http://www.who.int/ipcs/publications/en/frontcover.pdf

Davis, K.B. and G.M. Ludwig, 2004. Hormonal effects on sex differentiation and growth in sunshine bass *Morone chrysops* x *Morone saxatilis*. *Aquaculture,* 231: 587–596

Demska-Zakes, K., 1997. Effect of 17 alpha-methyltestosterone on gonadal differentiation in pikeperch, *Stizostedion lucioperca* L. *Aquacul. Res.,* 28: 59–63

Fitzsimmons, K., 2008. *Introduction to Tilapia Sex-Determination and Sex-Reversal (Report)*. University of Arizona. ag.arizona.edu/azaqua/ista/reports/sexreverse.doc

Hafeez-ur-Rehman, M., I. Ahmed, M. Ashraf, N. Khan and F. Rasool, 2008. The culture performance of mono-sex and mixed- sex tilapia in fertilized ponds. *Int. J. Agric. Biol.,* 10: 352–354

Iwamatsu, T., H. Kobayashi, R. Sagegami and T. Shuo, 2006. Testosterone content of developing eggs and sex reversal in the medaka (*Oryzias latipes*). *Gen. Comp. Endocrinol.,* 145: 67–74

Jian, Z., 2002. Status of Common carp verities under culture in China. *Aquacult. Asia,* 3: 27–28

Kang, I.J., H. Yokota, Y. Oshima, Y. Tsuruda, Y. Shimasaki and T. Honjo, 2008. The effects of methyltestosterone on sexual development and production of adult medaka (*Oryzias latipes*). *Aquat. Toxicol.,* 87: 37–46

Kirankumar, S. and T.J. Pandian, 2002. Effect on growth and reproduction of hormone immersed and masculinized fighting fish *Betta splendens*. *J. Exp. Zool.,* 293: 606–616

Lindsay, R.B., K.R. Kenaston and R.K. Schroeder, 2000. Low adult return of juvenile steelhead treated with 17α-Methyltestosterone to produce sterility. *North American J. Fish. Manage.,* 20: 575–583

Lone, K.P. and A.J. Matty, 1981. The effect of feeding androgenic hormones on the proteolytic activity of the alimentary canal of carp (*Cyprinus carpio*). *J. Fish Biol.,* 18: 353–358

Manosori, J., K. Petchjul and A. Manosroi, 2004. Effect of fluoxymesterone fish feed granule on sex reversal of the hybrid, Thai red tilapia (*Oreochromis niloticus* Linn.× *Oreochromis mossambicus* Linn.). *Asian Fish. Sci.,* 17: 323–331

Mateen, A. and I. Ahmed, 2007. Effect of androgen on sex reversal and growth of nile tilapia (*Oreochromis niloticus*). *Pakistan J. Agric. Sci.,* 44: 272–276

Nanna, R., M.M. Islam and S.H. Thilsted, 2003. Small indigenous fish species in Bangladesh: contribution to vitamin A, calcium and iron intakes. *J. Nutr.,* 133: 4021–4026

Shalaby, A.M.E., A.R. Ashraf and A.K.E. Yasir, 2006. *Sex Reversal of Nile Tilapia Fry using Different doses of 17α-Methyltestosterone at Different Dietary Protein Levels*, p: 13. http://ag.arizona.edu/azaqua/ista/ISTA7/Papers/Adel_et_al_sexreverse.doc

Shelton, W.L., V. Wanniasingham and A.E. Hiott, 1995. Ovarian differentiation in common carp (*Cyprinus carpio*) in relation to growth rate. *Aquaculture,* 137: 203–211

Steel, R.G.D., J.H. Torrie and D.A. Dinkkey, 1997. *Principles and Procedures of Statistics: A Biometrical Approach,* 3rd edition, p. 666. WCB/McGraw-Hill, New York

Sumpter, J.P., 2005. Endocrine disrupters in the aquatic environment: an overview. *Acta Hydrochem. Hydrobiol.,* 33: 9–16

(Received 01 June 2011; Accepted 09 July 2011)