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Effects of Different Levels of Estradiol-17β on Growth, Survival and Sex-ratio of African Catfish (*Clarias gariepinus*, Burchell)

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Abstract: Four different doses such as 50, 100, 200 and 400 mg estradiol-17β/kg feed were administered for 40 days on African catfish bearing an average length 5.1 mm and weight 4.7 mg. Growth, survival and sex-ratios were evaluated. The mean length of fry after hormone feeding phase were found to be more or less similar in all the treatments. The mean weight of fry of the group fed on diet containing 100 mg estradiol-17β/kg feed was found to be significantly higher than those receiving 50, 200, 400 mg estradiol-17β/kg feed and control. The groups of fry treated with 400 mg estradiol-17β/kg feed and control group showed lower survival compared to those with other doses of hormone. No negative effect on growth and survival of estradiol-17β was however, observed in this study. The doses of 100 mg and 50 mg estradiol-17β/kg feed resulted in 87.76% and 80.85% female fish respectively which were significantly ($p < 0.01$) higher than that of the expected frequency of female fish in a normal population. The dose of 400 mg estradiol-17β/kg feed resulted in relatively lower frequency of female fish.

Key words: Estradiel-17β, growth, survival, sex ratio, *C. geriepinus*

Introduction

African catfish, *Clarias gariepinus* has been proved to be a very well known and popular fish species for aquaculture in a number of African, Asian and European countries (Hecht, 1985; Areerat, 1987; Huisman and Richter, 1987). Aquaculturists select this species for culture because of higher growth rate, easy culture system, high nutritive value and ability to tolerate poor water quality conditions, adaptability to over crowding, high muscle content and low bone content, diseases resistance, high tolerance of a wide range of environmental parameters and extremely high production. Considering its aquaculture potentiality, the Government of Bangladesh imported the fingerling of African catfish (*Clarias gariepinus,* Burchell) officially from Thailand in 1989. This exotic catfish has created a great sensation due to its fast growth rate under local conditions. It has been observed that the fish may grow up to 200 g within 60 days while local air breathing catfish, *Clarias batrachus* takes one year to reach that weight (Mollah and Karim, 1990).

Fish production can be improved by adopting mono-sex culture technique either by involving male or female fish, depending on the superiority of growth performance. The adequate supply of fry of a single sex is, however, the main prerequisite of this practice. In the fry or fingerling stages, it is usually not possible to separate physically the fish according to their sex. So, it is presumed that the whole population of fry be produced as a single sex. Monosex population can be produced by direct hormone treatment, by manipulating chromosome (gynogenesis/androgenesis), by hybridization and by indirect use of hormones involving induced phenotypic sex-reversal followed by selective breeding/progeny test. In the latter cases, depending on the sex determining mechanism, neo-female (phenotypic female but genotypic male) or neo-male (phenotypic male but genotypic female) are produced (Scott *et al.,* 1989; Melard, 1995). In case of *Clarias gariepinus* it is needed to produce all female population because of faster growth rate compared with male and to produce higher amount of stockable sized fry for aquaculture.

In present study, the naturally occurring estrogen hormone, estradiol-17β, has been administered orally with feed at four different levels in order to optimize the dose of hormone inducing a maximum sex-inversion in African catfish. The effects of estrogens on growth of fish have so far been reported to be either lacking or negative with a few exceptions (Donaldson *et al.*, 1979). In this study, the effects of different levels of estradiol-17β on growth, survival and sex-ratio of *Clarias gariepinus* fry have been evaluated.

Materials and Methods

Sources of fish fry and experimental design: The experiment was conducted in the Wet Laboratory at the Department of Fisheries Biology and Genetics under the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. A total number of 250 African catfish (*Clarias gariepinus*) fry used in the present experiment were collected from the outdoor culture pond. The fry were produced through induced breeding using human chorionic gonadotropin (HCG) and carp pituitary gland extracts as inducing agents. Initial average length and weight of fry were 5.1 mm and 4.7 mg respectively. Fifteen fiberglass tanks of size 0.92 x 0.46 x 0.46m^3 were arranged into 3 rows, and each tank contains 200 liters water. Fifty fry were released in each aquarium. The hormone was administered orally to the fry of treatments 1, 2, 3 and 4 (T_1, T_2, T_3, T_4) at the doses of 50, 100, 200 and 400 mg/kg feed respectively and a control was maintained. The fry of the control group were fed with the same but hormone free diet.

Preparation of hormone-mixed diet: The natural estrogen, estradiol-17β (sigma) collected from local market was dissolved in 95% ethanol and a stock solution with a concentration of 1 mg hormone/ml ethanol was prepared. To formulate the diet for this experiment, required amount of stock solution was mixed with a nursery feed manufactured by Saudi Bangla Fish Feed Ltd., Bangladesh. Additional 95% ethanol was used to equalize the volume of ethanol for all the treatments. Equal volume of ethanol without hormone was mixed with control diet. The mixture of feed was then air-dried at room temperature (30°C) overnight to remove the ethanol by evaporation. During the hormone feeding phase, the prepared feeds were stored in a refrigerator at 4°C.

Feeding trial and rearing: The fry were fed with prepared diet twice daily at morning and evening. They were fed with hormone mixed feed for about continuous 40 days. The uneaten feed, debris and feces were siphoned once daily at morning. At an interval of 10 days, 10 fries from each treatment were randomly sampled for measuring the length (mm) and weight (mg). Dead fry were removed from the fiberglass tanks. After completion of the hormone treatment they were further reared for 2 months in net cages with hormone-free artificial diet containing 60% fishmeal, 20% rice bran, 10% wheat bran, 10% oil cake and 3% molasses when sexing of the fish could be done easily. The net cages were set in a pond.

Collection and analysis of data: At the end of the experiment, when the male and female fish were sexually mature enough to identify the sex-ratio, fishes from different treatments were sexed morphologically by the genital papillae. Elongated and pointed genital papilla was identified the male as well as short and rounded genital papilla was identified the female of *Clarias gariepenus*. The sex of the relatively smaller fish were confirmed by examining the gonadal condition following dissection.

The data on different mean length and weight of the fry were analyzed by Analysis of Variance (ANOVA) following completely randomized design and Duncan's New Multiple Range Test (DMRT). The analysis was done by using a computer Package, STATGRAPHICS's version-7. The frequency of female/male fish obtained in different, treatments were compared with that of the expected frequency of 50% in a normal population by λ^2 test.

Results

Growth and survival of fry: The mean weight of fry of T_1, T_2, T_3, T_4 and control, at the end of the 40-days feeding phase of hormone were 975.4, 1053.0, 957.5, 957.7 and 864.4 mg respectively (Table 1). The mean weight of fry of T_2 was found to be significantly ($p< 0.05$) higher than those of T_1, T_3, T_4 and control. The mean weight of fry of T_1 is significantly ($p < 0.05$) higher than those of T_3, T_4 and control. However, no significant differences in mean weights were observed between T_3 and T_4. The mean weight of fry of control is significantly ($p< 0.05$) lower than those of T_1, T_2, T_3 and T_4. The periodic growth in weight of fry under the five treatments has been shown in Fig. 1.

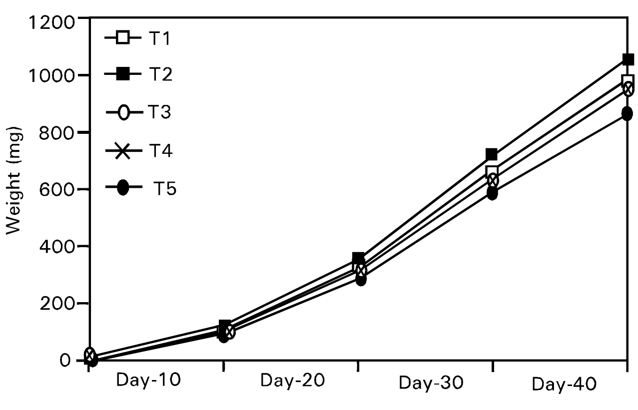

Fig. 1: Mean weight of *Clarias gariepinus* fry under different treatments during the 40 days hormone feeding phase. T_1, T_2, T_3, T_4 and control represents five different diets containing 50, 100, 200, 400 and 0 mg of estradiol-17β per kg feed respectively

The mean lengths of fry of T_1, T_2, T_3, T_4 and control, at the end of the hormone feeding phase were 52.52, 56.54, 51.22, 50.14 and 47.40 mm. No significant difference in mean lengths was observed among all the treatments.

Ten days after the hormone feeding trial, the mean weight of fry of T_2 was found to be significantly ($p< 0.05$) higher than those of T_1, T_3, T_4 and control. However, no significant differences in mean weight were observed between T_4 and control and among T_1, T_3 and T_4. The mean weight of fry of control was found to be the lowest. After 20 days, the mean weight of fry of T_2 was significantly ($p< 0.05$) higher than those of T_1, T_3, T_4 and control and no significant differences in mean weights were observed between T_3 and T_4. Thirty days after the hormone feeding trial, the mean weight of T_2 was found to be significantly ($p< 0.05$) higher than those of T_1, T_3, T_4 and control and no significant differences were observed between T_1 and T_3 and between T_3 and T_4.

In case of length, ten days after the hormone feeding phase, the mean length of fry of T_2 was found to be significantly ($p< 0.05$) higher than those of T_1, T_3, T_4 and control. No significant differences in mean lengths were observed among T_1, T_3, T_4 and control on that day. After 20 days the mean length of fry of T_2 was also to be significantly ($p< 0.05$) higher than those of T_1, T_3, T_4 and control and no significant differences were observed among T_1, T_3, T_4 and control.

The survival rate of fry of T_2 was the highest compared with T_4 and control, but nearer to T_1 and T_3. The survival rate of fry of T_1 and T_3 were almost similar. The lower survival rates were observed in T_4 and control and they were more or less similar but the control group observed lowest survival rate.

Sex-ratio: The data of sex-ratio of the fish of different treatment groups receiving different doses of estrogen are presented in Table 2. The frequencies of female fish obtained in the treatments T_1, T_2, T_3, and T_4 were found to be significantly ($p< 0.01$) higher than that of the expected frequency of female fish (50%) in a random normal population. The frequency of females in T_2 was found to be the highest (87.76%). On the other hand, the frequency of female fish in T_4 (400 mg/kg) was found to be the lowest and was not significantly different from that of the expected frequency of 50%. The frequency of female and male fish in the control group was also not significantly different from that of the expected ratio of male and female-1:1 in a normal population.

Discussion

Growth and survival: The data on growth of *Clarias gariepinus* fry fed on different levels of estradiol-17β in the present study revealed no clear relationship between the levels of hormone administered and the growth of fry. Anabolic steriods, both androgens and estrogens enhance the growth and feed conversion efficiency when administered at optimal level in fish (Matty 1985). The length of fry of T_2 (100 mg estradiol-17β/kg feed) was found to be higher than those feed on diets containing higher levels (200 and 400 mg/kg feed) of hormone and also the control. In case of weight gain, T_2 was significantly higher than those of T_1 T_3 T_4 and control. The mean weight of *Oreochromis niloticus* fry of the group fed on diet containing 100 mg estradiol-17β/kg feed was found to be slightly higher but not different from that fed on hormone-free diet (Alam *et al.*, 1998). The group of fry receiving the highest levels of estrogen (200 and 400 mg/kg feed) showed significantly lower growth than the relatively lower doses (50 and 100 mg/kg feed). These findings support that the higher doses of the hormone do not induce growth enhancement rather tend to be catabolic or exert deleterious effects which interfere with the normal life processes and related any gain in anabolic response expected by the increase in dose. The lower dose (50 mg/kg) has also no positive effect on growth and it is needed up to an optimal level. Though estrogens are frequently used as effective growth promoters in cattle, the growth promotive effects of estrogens in fish however, has been either lacking of negative with only few exceptions (Donaldson *et al.*, 1979; Hunter and Donaldson, 1983). Goetz *et al.* (1979) observed a dose-dependent reduction in weight of Coho salmon (*Oncorhynchus*

Table 1: Effects of different levels of estradiol-17β on growth and survival rates of *Clarias gariepinus* fry.

Parameters	Treatments				
	T_1 (50 mg/kg)	T_2 (100 mg/kg)	T_3 (200 mg/kg)	T_4 (400 mg/kg)	Control
Initial weight (mg± SD)	4.51 ± 0.94	4.51 ± 0.94	4.51 ± 0.94	4.51 ± 0.94	4.51± 0.94
Final weight (mg)	975.4^b	1053.0^a	957.5^c	957.7^c	864.4^d
Initial length (mm± SD)	5.12± 0.42	5.12± 0.42	5.12± 0.42	5.12± 0.42	5.12 ± 0.42
Final length (mm)	52.52	56.54	51.22	50.14	47.40
Survival (%)	52.67	53.33	52.67	47.67	45.33

Figures with different superscripts in a column differe significantly at P< 0.05

Table 2: Frequency of males and females in the group of fish grown from the fry fed on different levels of estradiol-17β.

Sex of fish	Numbers/frequency of fish in different treatments				
	T_1 (50 mg/kg)	T_2 (100 mg/kg)	T_3 (200 mg/kg)	T_4 (400 mg/kg)	Control
Male	38.00	43.00	32.00	28.00	18.00
Female	9.00	6.00	16.00	21.00	22.00
Total	47.00	49.00	48.00	49.00	40.00
Frequency of female (%)	80.85	87.76	66.67	57.14	45.00
Frequency of male (%)	19.15	12.24	33.33	42.86	55.00

kisutch) treated with estradiol-17β. Jensen and Shelton (1979) observed no change in growth of *Tilapia aurea* after treated with estradiol, estriol and estrone, three naturally occurring estrogens. Increased growth in exogenous estradiol-17β treated yellow perch (*Perca flavescens*) was reported by Malison *et al.* (1985). The periodic growth in length and weight were recorded with a view to observe the time when the difference (if any) started to be significant among the treatments. Both the mean weight and length of fry was significantly higher from the 10th day and it appeared significantly upto 20th day for mean length and upto the completion of the experiment for the mean weight.

It is however, important to examine whether the administered hormones have had negative effects on fry in terms of growth and survival. The groups of fry feeding on diet containing higher doses (200 and 400 mg/kg feed) and lower dose of hormone (50 mg/kg feed) feeds has negative effects (showed lower growth performance) than the treatments with moderate level of hormone (100 mg/kg feed). Since it has been reported that the higher doses of hormone could result in negative effects (Donaldson *et al.*, 1979; Hunder and Donaldson, 1983), it is needed to optimize the dose feeding in mind both the success of sex-reversal as well as the growth and survival of the treated fry. Around fifty percent survival in both the hormone feeding groups and control group indicate that the effects of estradiol-17β in the present study had no negative effect on survival of *Clarias gariepinus* fry. Alam *et al.* (1998) observed no negative effect of estradiol-17β on growth and survival of Nile tilapia (*Oreochromis niloticus* L.).

Sex-ratio: In the present study, the synthetic estradiol-17β was found to play a significant role in altering the sex of *Clarias gariepinus* into females. Among the four doses of hormone tested, the dose of 100 mg/kg feed was found to be most potent and resulted in 87.76% female fish compared to 45.00% of female fish in control group. None of the doses of estradiol-17β tested in the present study was found to be effective in producing all female populations of *Clarias gariepinus*. The physiological events that direct the onset of ovarian differentiation in salmonid involve the expression of the enzyme aromatase that produces estrogen including estradiol-17β. So, administration of aromatase inhibitor resulted in chromosomally female salmon to develop a normal functional male (Piferrer *et al.*, 1994). On the other hand, the use of antiestrogen resulted in conversion of female tilapia to male (Hines and Watts, 1995). Chang and Lin (1998) observed that treatment with exogenous estradiol-17β increased aromatage activity in gonads, increased estradiol level in the plasma and diminished levels of plasma 11 ketotestosterone which were associated with occurrences of sex-reversal in black porgy (*Acanthopagrus schlegeli*), a protandrous hermaphrodite. Therefore, the higher frequencies of female fish obtained in this study might have resulted from the effects of the exogenous estradiol-17β administered through diets.

It has been reported that the higher doses of hormone might not necessarily produced higher induction in sex-reversal (Guerrero 1975; Woiwode 1977; Ridha and Lone 1990). Alam *et al.*, (1998) observed in case of Nile tilapia that the doses of 200 mg and 150 mg estradiol-17β/kg feed resulted in 75.55% and 68.20% female fish respectively which were significantly (p< 0.01) higher than that of the expected frequency of female fish in a normal population. As the estrogenic hormone action is species specific, for maximum induction of sex-reversal the dose of a particular hormone to be optimised for each species individually. From the present study, it appears that the optimum dose of estradiol-17β in *Clarias gariepinus* lie with in the range of 50-100 mg/kg feed. But in consideration of both growth, survival and sex-ratio optimum level of dose i.e. 100 mg/kg feed in highly significant.

The objective of a sex-reversal experiment is to produce 100% mono population of a particular sex. The highest frequency of female fish obtained in the present study is 87.76% which indicates that it is possible to reverse sex significantly of dietary administration of estradiol-17β in *Clarias gariepinus*. Further research in this species is however, essential to produce 100% females.

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