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TRIAL OF SEX REVERSE PERCENTAGE ON FEMALE SHING *(Heteropneustes fossilis)* BY USING 17β-ESTRADIOL SEX HORMONE

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ABSTRACT

A study was conducted to evaluate the production of monosex (female) shing (*Heteropneustes fossilis*) by using a sex hormone (17β-Estradiol). The experiment was composed of three treatments T_1 (70 mg 17β-Estradiol /kg feed), T_2 (80 mg 17β Estradiol /kg feed) and T_3 (90mg 17β- Estradiol /kg feed) in duplicate were fed shing fishes in cemented cistern. The hormone was administered orally at variable doses with Tiger brand nursery fish feed (Eon Group) for 30 days. The fry were fed with the hormone mixed feed five times in a day. Every seven days intervals 50% water of the tanks were changed. A high mortality rate (47.6% to 67.4%) was observed in different treatments. The occurrence of female fish at the end of the experiment in treatment T_I, T_2 and T_3 were 75%, 86%, and 96% female respectively. T_3 (90 mg 17β-Estradiol /kg, feed) showed the best performance with 96% sex reversal to female progeny.

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INTRODUCTION

Hormonal sex reversal is a technique of changing the sex either from male to female or from female to male in fish by administering synthetic steroid hormones before and/or during the period of organogenesis. In this technique, the first feeding fry are treated with female hormones or Estrogens (i.e.17β Estradiol) which develops ovary and female sexual characteristics in fish (Hussain, 2004) and on the other hand, treatment with male hormones androgens (i.e. 17α methyl testosterone), which develops testes and male sexual characteristics at maturity. The sex reversal technique is very simple, economic, low cost involving and ensures high- production and high net profit which can be done by a technician without sophisticated laboratory and equipments. Stinging catfish is native to Bangladesh, Pakistan, and India including the Andaman Islands, Nepal, Srilanka, Burma, Thailand, Indus Basin and Laos (Talwar and Jphijioraii, 1992*). Heteropneustes fossilis is* one of the cultivable species in Southeast Asia. Due to its high price aqua culturist are enthusiastic to culture it. The muscle of this fish is very easily digestible and people of the region take it as a replenished diet for the malnourished or sick people. Fish consumption rate is gradually decreased due to human interference and environmental degradation. Therefore, we are searching to adopt some aqua cultural technique to increase production. The choice of conversion of sexes (either all males or all females) is one of the important tools for the increase of the production depends on growth characteristics of individual sexes of fish species. For *Heteropneustes fossilis* a rationale is that the females of this species are considerably larger in size than the males of the same age (Bhatt, 1968). It has been found that administering estrogen hormones for some period in life time can reverse the entire or at least the majority of the fry population into effective female in salmonids and cyprinids, Same technique might be used for the production of all female of *Heteropneustes fissilis.*

The production of sex reversed female fry population by feeding the fishes with steroid hormone treated diet. Donaldson and Hunter (1982) reported that hormone treatment ensured maximization of growth by diverting nutrients and ensured better meat quality. The hormone treated fish yield higher standing crops than the controls at if corresponding stocking rate (FAO, 1976). The objective of this research is to determine the optimal dose of estrogen hormone (17β-Estradiol) for the production of mono-sex female *Heteropneustes fossilis.*

MATERIALS AND METHODS

Study area

The experiment was conducted in six 4m^2 rectangular cemented cisterns. The experiment was carried out in “Noha Aqua Farm and Hatchery” at Muktaghasa region of Mymensingh district.

Preparation of cisterns

Cisterns were cleaned with Active blue (anti-fungal) and potassium permanganate solution thoroughly and two times washes the cistern. Then the cisterns were filled with water. All the cisterns were maintained with a water level of 40 cm depth throughout the period of 33 days. Then the water level was maintained 75 cm depth. Each cistern was provided with pieces of PVC pipes (4cm diameter) as shelter for fish.

Spawning of *Heteropneustes fossilis*

Spawning of *Heteropneustes fossilis* was performed in the Noha Aqua Farm and Hatchery at Muktaghasa region of Mymensingh district. Brood fishes were collected from outside of this hatchery. Only good looking, conspicuous, healthy and uninjured fishes were selected for induced breeding. Sexes were determined by careful examination. Handling of fish was done very carefully to avoid injury and secondary infection. Human Chorionic Gonadotropin (HCG) was used to induce breeding and injected at 1000 IU/kg body weight of both male and female fishes. For all the treatments, the hormone was administered by intra-muscular injection on muscles beneath the dorsal fin slightly above the lateral line. After injection, the brooders were kept in separate breeding tanks for each treatment.

Experimental designs

After hatching 500 spawns were kept in tray (30 cm x 50 cm x 12 cm; 12.6 liter capacity) for 3 days. The experiment was designed with three treatments (T_1, T_2 and T_3) in two replication. Each replicate contained 500 fry. T_1, T_2 and T_3 were steroid treatment orally. Steroid (17β - Estradiol) treatment consisted of three doses (70 mg 17β -Estradiol /kg feed, 80 mg 17β - Estradiol /kg feed, 90 mg 17β- Estradiol /kg feed in duplicate were used.

Table 1. Dose of hormone used in different treatments

Treatment	Replication	17β - Estradiol hormone (mg) /kg feed
T1	R_I	70
	R_2	
T2	R_1	80
	R_2	
T3	R_I	90
	R_2	

Diet formulations

The experimental diet with different doses of 17β - Estradiol (i.e. 70 mg/kg feed, 80 mg/kg feed, 90 mg/kg feed) were prepared through ethanol evaporation method (Nair and Santiago, 1994). The experimental diet was used Tiger brand nursery fish feed (Eon Group) 55% protein for the sex reversal purpose.

Process of diet formulation and preservation

The required amount of hormones and feed were measured by electrical balance and ethanol was measured by measuring cylinder. At first 500 mg of 17β - Estradiol was diluted with 200 ml ethanol to prepare stock solution. After that, for 70 mg estradiol /kg feed, 14 ml stock solution was taken into measuring cylinder and ethanol was added to prepare 100 ml solution and mixed with 1 kg feed. Accordingly for 80 mg estradiol /kg fired, 16 nil stock solution was taken into measuring cylinder and ethanol was added to prepare 100 ml solution and mixed with 1 kg feed. In case of 90 mg estradiol/ kg feed, 18 ml stock solution was taken into measuring cylinder and ethanol was added to prepare 100 ml solution and mixed with 1 kg feed. Then the feed was stirred vigorously for homogenous mixing of hormone. After completion of the diet preparation these were air dried by fanning and spread out on the floor become fully dry. The hormone treated diet was then kept in a dry and cold place up to 7 days at 10^0 C. Diets were prepared at every 7 days.

Starting of the experiment

After hatching the spawns were kept in 6 trays (30 cm x 50 cm x 12 cm) for 2 days. Then spawns were transfer from Noha Aqua Farm and Hatchery to the cisterns. The first feeding was starting at 48 hours later of hatching. The first feeding was hormone (17β -Estradiol) mixed Tiger brand nursery fish feed. The experiment was comprised of three treatments viz. T_1 70 mg/kg feed, T_2 80 mg/kg feed and T3 90 mg/kg feed doses of 17β –Estradiol.

Rearing of fry

The spawns were fed with the hormone mixed feed five times with three hour interval in a day. 50% of the water was changed every seven days interval. Faecal matter and unused feed was siphoned out every day just after feeding and replaced water was added. The hormone treatment was conducted for 30 days. During hormone treatment period, first 15 days they fed with 200% of their body weight and rest 15 days 100%.

Sex identification

The fishes were sexed by gonad squashing and aceto-carmine staining method (Guerreo and Shelton, 1974). The fish was killed and the viscera were removed. The gonads were lying along the surface of the body cavity on either side of the fish just above the kidney. The gonads were removed and placed on a clean glass slide A few drop of aceto-carmine stain were added and the gonads squashed with a cover slip. The slides were examining under a microscope (Novex Holland, K-RANGE; WF10 X, and SPL 10/0.25). Ovarian tissue was identified by the presence of scattered oocytes and the testicular tissue was identified by the presence of spermatocytes with a uniform background and the intermingling of spermatogonia and oocytes in the same gonad.

RESULTS AND DISCUSSION

In the present experiment, the initial weights of 500 fry were taken. The average initial weight was 0.0010 g. The result of mortality in different treatment is given in Table 2. A high rate of cumulative mortality was observed in all treatments range from 47.6 to 67.4% and comparatively more mortality was observed in first seven days. T_3 showed the highest mortality rate (67.4%). However the average mortality rate of T_1 (70mg of 17β -Estradiol / kg feed) demonstrated the lowest mortality (47.6%) which was significantly different from other treatments. Fish from three treatments fed with l7β -Estradiol hormone treated feed and sexed at the end of the experiment. The results of sex ratio in different treatment are given in Table-2. T_1 (70 mg/kg feed), T_2 (80 mg/kg feed) and T_3 (90 mg/kg feed) showed 75%, 86% and 96% female sex respectively. Some intersex individuals were found in the T_1, T_2 and T_3 showed 3%, 2% and 2% intersex individuals respectively.

In the present study, the oral administration of hormone (17β -Estradiol) treated feed can significantly alter the sex ratio towards female in *Heteropneustes fiossilis* has been expected. The variations in the percentage of female as obtained from different hormone treated groups were found to different doses, viz. T_1, (70 mg 17 β -Estradiol /kg feed), T_2 (80mg 17β -Estradiol /kg feed), T_3 (90mg 17β -Estradiol /kg feed) showed femininity 75%, 86%, and 96% of the total respectively. Intersex individuals were characterized by the intermingling of spermatogonia and oocyte in the same gonad. Intersex individual was T2, T3 and T4 showed intersex individual 3%, 2% and 2% respectively. The presence of intersex individuals in hormone induced sex reversal was not surprising, since it has been reported in previous studies (Piferrer *et al.,* 1994) on different fish species. Carrilon *et al*. (1993) studied sex reversal of Nile Titapia *(Oreochromis niloticus)* by administering diethyl stilbestrol and ethynyl estradiol and noted that ethanyl estradiol treatment resulted in 57.59 to 65% female; 32 to 35.1% male and 3.0 to 9.4% ovotestes, hearing fish while diethyl stilbesterol treatment resulted in 60.3 to 80% females, 12.7 to 37% males and 0.7 to 7.3% ovotestes bearing fish. Duration of hormone treatment might be taken as an important consideration for induction of sex-reversal in fish. Various durations of hormone treatment were mentioned by many authors. George and Pandian (1995) administered β -estradiol or diethyl stilbestrol to Poecilia sphenops fry for 30 days. Rosenstein and Hulata (1992) administered hormone for a period of 7 to 30 days for feminizing *Oreochromis* fry. The present duration of hormone treatment for 30 days was within the range reported by the above authors. A high rate of cumulative mortality was observed in all treatments range from 47.6 to 67.4% and comparatively more mortality was observed in first seven day. High mortality was also reported by Rahman and Sarder (2002) in both hormone and non-hormone (control) treatments in case of *Oreochromis nilolicus*. Average high mortality found in the treatment, T3 which was 77.6%. Lower mortality rate was found in T_l, However the mortality rate significantly different from treatment T_2 and T_3.

The present study indicates that there is a positive relationship between the (loses or estrogen and the sex reversal to female progeny. Increasing frequency of female and decreasing number of males and ovotestcs bearing fish indicates that 100% female fish can be obtained by optimizing the hormone dose and the duration of the hormone treatment. For the standardization of dose and treatment duration further experiments are to be conducted in different field situation by different persons to achieve precise reproducibility of the experiment.

CONCLUSION

The experiment was carried out in six rectangular cemented cisterns (4m2 each) located in the Noha Aqua Farm and Hatchery at Muktaghasa region of Mymensingh. Estrogen hormone (17β -Estradiol) was administered orally at 3 different doses viz.70, 80 and 90 mg per kg with Tiger brand nursery fish feed were used for a period of 30 days (15th March to 13th April, 2015). Feeding was done 5 times with three hours interval in a day. The experiment was designed with three treatments viz. T_1 (70 mg/kg Iced), T_2 (80 mg/kg feed) and T_3 (90 mg/kg feed) with two replications. For each treatment 500 fry were used. The experimental diet with different doses 17β -Estradiol (i.e. 70 mg, 80 mg and 90 mg / kg feed) was prepared through ethanol evaporation method (Mair and Santiago, 1994). The diets were preserved in dry and cold place 7 days and temperature maintain at 10^0 C. 50% of the water in the cisterns was changed every seven days interval prior to feeding, to remove fecal matter and unfed materials. After completion of the rearing, sex identification was done by dissecting the fish. A high rate of mortality was observed in all treatments. Average mortality rate ranged from 47.6% to 67.4%. Highest mortality was observed during the first 7 days. In case of feminization the average percentage of female in different treatments, T_I, T_2 and T_3 were 75%, 86% and 96% respectively. T_3 showed the best performance with 96% sex reversal to female progeny, which was significantly different from other treatment. Increasing frequency of female and decreasing number of males and ovotestes bearing Fish indicates that 100% female fish can be obtained by optimizing the hormone dose and the duration of the hormone treatment.

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