Investigation of sexual maturity and change in gonadosomatic index of sex reversed rainbow trout (*Oncorhynchus mykiss*) treated by oral 17 alpha methyl testosterone

Rastiannasab A.1*; Kazemi E.1

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Abstract

The study was carried out in the genetics and breeding research center of Yasouj. All eye-stage eggs were used for the experiment using vertical hatching units to complete hatching. A standard commercial granule feed was used in all experimental groups. The experiment consisted of control treatment and one dose of hormone 17α-Methyltestosterone added to the diet at the rate of 3 mg per kg food by dissolving in 200 ml of 95% ethanol per kg feed. The use of oral administration of 17α-methyltestosterone (17MT) at 3 mg 17MT/kg of feed for 70 days following the onset of feeding to sex reverse rainbow trout (*Oncorhynchus mykiss*) to produce XX males is well established. Examination of the results showed that 17α-methyltestosterone was effective in sex reversed rainbow trout. The results has shown in nearly 100% of the treated animals being males. The GSI index in female and male fish of control group were 0.04±0.1 and 0.07±0.01, respectively (Fig.3) and significantly different (*p*≤5%). But GSI index of 100% sex reversed male fish of MT treated was 0.06±0.01 and hadn't significantly different (*p*≥5%) with treated group (genetically male and female).

Keywords: Onchorynchus mykiss, 17α-Methyltestosteron, Sex reversal, GSI index, XX male

1-Shahid Motahary Cold-water Fishes Genetic and Breeding Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization, Yasouj, Iran

*Corresponding author's Email: a.rastian@areeo.ac.ir
Introduction
The sex reversal technique in fish is widespread in many countries. The development of these techniques is desirable because rainbow trout males reach their gonad maturity earlier compared to the females. The eggs were fertilized with the sperms taken from sex reversed males for producing all-female populations. Seasonal changes in carcass composition and differences in the size of farmed fish that occur with the onset of puberty make it difficult for breeders to achieve the desired quality and characteristics of the product in retail markets. Since these problems in rainbow trout are often related to males. Due to inconvenience caused by direct hormone usage related with human consumption, the most common method for sex reversal in rainbow trout is indirect method to obtain sex reversed brood stocks (Matty, 1985). All male trout stocks produced by this method have advantage in rainbow trout culture since the fish is used to generate brood stock. the eggs were fertilized with the sperms taken from sex reversed males for producing all-female populations.

The technique should increase efficiency of hatchery operations by allowing greater flexibility inherent in functional males, reduce the accidental harvesting of immature males, However, changes in the morphology of fish gonads after administration of sex steroids and after puberty have not received much attention. The aim of the present study was to establish masculinized brood stocks for producing all female populations in commercial rainbow trout farms.

Materials and methods
The study was carried out in the genetics and breeding research center of yasouj. All eye-stage eggs were used for the experiment using vertical hatching units to complete hatching. A standard commercial granule feed was used in all experimental groups. The experiment consisted of control treatment and one dose of hormone 17α-Methyltestosterone added to the diet at the rate of 3 mg per kg food by dissolving in 200 ml of 95% ethanol per kg feed. The use of oral administration of 17α-methyltestosterone (17MT) at 3 mg 17MT/kg of feed for 70 days following the onset of feeding to sex reverse rainbow trout (Oncorhynchus mykiss) to produce XX males is well established. A primary drawback to this protocol is that a high proportion of males are non-functional and milt is not released in them when pressure is applied to the abdomen, and therefore the males must be euthanized. Sex was determined at 420 dpf by sampling fish (N=28) from each group and examining gross gonadal morphology. Also sex reversal ratio and gonadosomatic index (GSI) were determined in hormone-treated and control (no hormone treated) groups. At the end of experiment, percent of sex reversed and neomale fish evaluated in comparison with sex ratio in normal rainbow trout population and required time from day of use hormone to achieving the results.
of production of sex reversed population was distinguished.

**Results**

The control group had natural testis and ovaries (Fig. 1). Examination of the results showed that 17α-methyltestosterone was effective in sex reversed of rainbow trout.

![Figure 1: Natural testis and ovaries of fish in control group.](image1)

The sex ratio in control group was 42% female and 58% male. The results have shown in nearly 100% of the treated animals being males (Fig. 2). The highest sex reversal ratio was observed in group treated with 3 mg/kg 17α-methyl testosterone (Fig. 2).

![Figure 2: All male gonads (testicles) from sex reversed male by oral administration of MT hormone.](image2)

The GSI index in female and male fish of control group were 0.04±0.1 and 0.07±0.01, respectively (Fig. 3) and significantly different (p<5%). But GSI index of 100% sex reversed male fish of MT treated was 0.06±0.01 and had not significantly differene (p≥5%) with treated group (genetically male and female).
**Discussion and conclusion**

Many studies have emphasized the positive effects of sex steroids on sex reversal in fish. Bye and Lincoln in 1986, used the hormone 17-alpha-methyltestosterone at a rate of 3 mg / kg of food for 60 days, they achieved 100% sex reversed of normal salmon larve and used xx male sperm with surgery. Oral administration of the hormone 17-alpha-methyltestosterone conducted at a dose of 3 mg / kg of food for 60 days to rainbow trout hatched from all female eggs. After puberty, 87% of the fish became sex reversed males and 13% of fish was maintained female and no intermediate sex was observed and feeding the fish at the same dose for 90 days, resulted in the production of 80% sex reversed male and 20% intermediate sex fish. The lowest effect of male hormone was observed in treat with 17 alpha-methyl testosterone at 3 mg / kg food for 30 days and only about 33% of fish were female due to reduced duration of hormone feeding (Atar et al., 2009). As results of reviewed. Oral administration of the hormone 17-alpha-methyltestosterone at a dose of 3 mg / kg for 70 days in this research resulted 100% sex reversed male from normal rainbow trout larve, whereas the sex ratio in control group was 42% female and 58% male. While sexual maturation occurs after about 24 months in females, it occurs at age of 10-18 months in males (Pickering, 1992). GSI ratio in female non MT treated fish was the lowest in comparison to MT treated fish group which is in agreement with Johnstone et al. (1978) and Bye et al. (1986) who report that male rainbow trout and Atlantic salmon reach maturity earlier than the females. Because of early maturation, sex reversed individuals could have higher GSI ratio against the control groups as it is reported by Baker et al. (1988),
Nakamura (1994), Pandian and Varadaraj (1987).

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