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The Effect of Estradiol Valerate on Sex Reversal of Rainbow Trout, *Oncorhynchus mykiss*

¹Senol Güzel, ¹Kenan Güllü and ²Osman Özden

¹Department of Fisheries, Faculty of Agriculture, Yüzüncü Yil University, Van, Türkiye

²Department of Aquaculture, Faculty of Fisheries, Ege University, Izmir, Türkiye

Abstract: The effect of oral administration of 20 mg of estradiol valerate (E₂V) per kilogram of administered diet was studied on sex reversal in 35-day-old (average 0.32±0.02 g) rainbow trout, *Oncorhynchus mykiss*. The E₂V was treated for 56 days to fry. The research was carried out totally 355 days. At the end of this time, all fish were sampled, digested and analyzed for their sex determination and the Gonadosomatic Index (GSI) values of the E₂V and control groups were calculated. Compared to controls, there were changes in sex composition of E₂V treated fish. The sex composition of E₂V treated fish was found as 97% of female and 3% inter-sex. However it was found as 51% of female and 49% male in the control fish. Gonadosomatic indices of the E₂V and control groups were calculated as 0.12 and 4.21%, respectively. It was concluded that the determined application time, dose and period of E₂V in this study were effective in direct feminization of rainbow trout.

Key words: *Oncorhynchus mykiss*, estradiol valerate, sex reversal, gonadosomatic index

INTRODUCTION

In fish production, it is essential to get marketable fish size as soon as possible. In rainbow trout production, the early sex maturation in male fish is considered as a problem because this condition causes growth differences between female and male fish. It has been reported that mono-sex populations consisted of all females are preferred in rainbow trout because females grow faster have higher Food Conversion Rate (FCR), resist more to harsh living condition and have lower chance of being affected by disease than males. Male rainbow trout is not preferred in the commercial productions because males spend their energies for gonad development five to six months after egg hatching (Matty, 1985; Ingram, 1988). Therefore, rainbow trout producer in Turkey import female eggs in order to avoid the slow growth of males and to reach fast the marketable size with females (Özden and Güllü, 1996; Güner and Tekinay, 2000). The researches and efforts on the obtaining all female populations, therefore, are getting more important because of the mentioned reasons above. Estrogens (estradiol) are used directly in feminization, while androgens (methyl testosterone) are used indirectly in this process.

Some of the previous studies reported that the direct feminization by estrogens caused some increase in fish size (Schreck and Fowler, 1982; Degani, 1986). Some other researchers reported the opposite findings (Komen *et al.*, 1989; Parks and Parks, 1991). It has been reported by

several researchers that the effects of sex hormones on fish might vary based on the fish age (application time), dose of hormone and fish species (Matty, 1985; Degani, 1986; Yu *et al.*, 1979). Johnstone *et al.* (1978) reported that estradiol application with feed yielded 89% female, 9% male and 2% intersex in rainbow trout. Goetz *et al.* (1979) reported that the dietary administration of 17β-estradiol (10 mg kg⁻¹-food) yielded 54.2% female, 18.1% male and 27.7% inter-sex in Coho salmon, *Oncorhynchus kisutch*. Johnstone *et al.* (1979) reported 99% female and 1% inter-sex in *Salvelinus fontinalis*. Parks and Parks (1991) reported that the dietary administration of 17β-estradiol at rate of 40 mg kg⁻¹ for 77 days 87.5% female in *Salvelinus fontinalis*. Goryczko *et al.* (1991) reported that the dietary administration of 17β-estradiol (20 mg kg⁻¹ food, for 120 days) 95% female in rainbow trout.

In this study, the effect of estradiol valerate on the direct feminization in rainbow trout was investigated as different of previous studies. There are different results in effect on direct feminization of rainbow trout by estrogens treatment because of diversity of dose, treatment time and application period of the estrogens. Dose and treatment time (20 mg kg⁻¹ food and 56 days), generally used for estrogens, were chosen in this study too.

MATERIALS AND METHODS

This study was carried out in Yüzüncü Yil University Faculty of Agriculture Department of Fisheries Center for

Research and Practice and lasted 355 days. In the research 800 fry of rainbow trout (0.32 ± 0.02 g/fry, 35-days-old, post hatch) were used. Well water was used in the experiment and it was aerated with an aerator. In the research, fiberglass tanks with a volume of 1.4 m^3 and dimensions of $2.5 \times 0.8 \times 0.7$ m were used. Commercial granule feed (52% crude protein, $4000 \text{ kcal kg}^{-1}$ ME) was used for nourishing the fish.

Hormone treated diet was prepared according to rate of 20 mg kg^{-1} of diet by dissolving E_2 (estradiol-17-valerate, $C_{23}H_{32}O_3$, Schering, Germany) in 400 mL 95% of ethanol for each kg of diet. The hormone solution was added slowly into the diet while mixing continuously with an electric mixer. The control diet was prepared with the same method but without the hormone. Before cold storage at 4°C , the diets were air-dried overnight to evaporate the alcohol (Ingram, 1988; Gannam and Lovell, 1991).

In the experiment, Method of Random Coincidence Parcels was used (Yildiz and Bircan, 1991). Control and E_2V groups were formed in the experiment. As the study was planned with two repetitions, it was carried out in 4 groups in total. Each group included 200 fry.

The fish were fed 5-6 times per day in the fry period and 3 times a day, *ad libitum*, from fingerling period to market size. After feed with E_2V was given to the application group for 56 days, feed which was not processed was given to them for the remaining 299 days. The fish in the control group were given feed without E_2V and to which only Ethyl alcohol (95%) was sprayed and after this period normal feed was given until the end of the experiment (Ostrowski and Garling, 1988; Santandreu and Diaz, 1994).

In order to determine the sex reversal in rainbow trout, at the 105th day of the study, ten fish were randomly sampled twice and their histological gonad structures were investigated. Abdomens of fish were dissected carefully and their gonads were taken out for the histology study. Then, gonads were put into the Bouin fixation solution. After the 24 h fixation, tissues were checked, blocked with paraffin and cut into 6-7 μ sections. These samples were dyed with three dyes and were observed at microscopy and then were photographed (Kiermen, 1989). At the end of the study, all fish were sampled, digested and analyzed for their sex determination and the Gonadosomatic Index (GSI) values of the E_2V and control groups were calculated.

RESULTS AND DISCUSSION

The minimum, maximum and average water temperatures were 8.21 ± 0.05 , 17.12 ± 0.12 and $12.53 \pm 0.05^\circ\text{C}$,

respectively during the study. The soluble oxygen content and pH of the water were $6.2 \pm 0.11 \text{ mg L}^{-1}$ and 8.23 ± 0.01 , respectively. During the E_2V treatment period, the water temperature and the soluble oxygen amount of water were $10.32 \pm 0.30^\circ\text{C}$ and $6.70 \pm 0.01 \text{ mg L}^{-1}$, respectively.

At the end of the study, the E_2V treatment group had 97% of female and 3% inter-sex composition and the control group had 51% of female and 49% male composition (Table 1).

Table 1: The percentages of sex ratio and GSI of the experimental groups

Experimental groups	Sex ratio (%)			GSI (%)	
	Male	Female	Inter-sex	Male	Female
Control	49	51	-	8.41*	0.12
E_2V (20 mg kg^{-1} -diet)	-	97	3	-	0.12

* GSI values of mature male fish in the control groups

The similar findings in feminization rate were reported by some other researchers (Goryczko *et al.*, 1991; Johnstone *et al.*, 1978; Johnstone *et al.*, 1979; Garrett, 1989; Strüssmann *et al.*, 1996). Some other researchers had different feminization values: The feminization rate of Parks and Parks (1991), Lahav (1993) and Goetz *et al.* (1979) were 87.5, 82 and 54.2%, respectively. These differences might have been caused by the type, dose, application time and application period of the hormones used in the mentioned studies.

When we investigated the gonads of inter-sex fish (Fig. 1), a few of them had eggs, but most of them had sperms (a); some gonads had half eggs and sperms (b); some had almost completely eggs (c).

Johnstone *et al.* (1978) and Goetz *et al.* (1979) reported the inter-sex fish ratios in their studies as 2 and 27.7%, respectively.

The gonad maturation of fish were investigated and determined that approximately 50% of the male fish in the control group reached to sex maturation. Johnstone *et al.* (1979) reported the same trait as 20% in their study. The environmental differences, especially temperature variation might alter the maturation time and period. It was also determined that the female fish in both control and E_2V groups did not reach to the maturation and there was no difference between their GSI values. Johnstone *et al.* (1979) had similar findings. It is an expected result based on the general knowledge that there was no mature female fish at the end of the first year. At the end of the 355-day study, the GSI values of the male fish in the control group varied from 5.62 to 11.24% and its average did 8.41%. There was significant difference between the overall GSI values of the control (average 4.21%) and the E_2V group (0.12%) ($p < 0.05$).



Fig. 1: Gonad structures of inter-sex fish in the estradiol valerate group. a) Mostly sperms in gonads, b) Half sperms and half eggs in gonads, c) mostly eggs in gonads



Fig. 2: Gonad structure of feminized trout at the 105th-day of the study (200X) sp: spermatogonium, o: oocyte

It was determined by histology study that the oocyte formation in feminized fish by estradiol initiated at the 105th-day of the study (Fig. 2).

At the end of the present study, we concluded that the treatment of estradiol valerate, which is cheaper than other estrogens, applied with feed by the rate of 20 mg kg^{-1} for 56 days was effective in obtaining all female rainbow trout populations (97% female, 3% inter-sex). However, this finding was abstracted from a part of a thesis and all aspects of E_2V on sex reversal were not evaluated in the present study. The other results of E_2V

on the growth and survival rate, which were negative, were discussed in another study. Therefore, it is thought that E_2V should not be recommended in rainbow trout production due to its negative effects although it is highly effective obtaining all female populations.

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