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Effect of Estradiol Valerate Applied by Immersion and Oral Administration on Growth and Sex Reversal of Rainbow Trout, *Oncorhynchus mykiss*

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Abstract: This study aimed to investigate the effects of exogenous estradiol valerate (E_2V) applied by both immersion method and oral administration to produce monosex females on the growth of rainbow trout (*O. mykiss*). This study was conducted throughout 5 periods and 140 days. The rainbow trout fry (35 days old, post hatch) were immersed in a 400 μg L^{-1} of E_2V solution for 2 h, twice a week for 4 weeks. Then fry were fed with a diet containing 20 mg kg^{-1} of E_2V for 60 days. At the end of the study, E_2V treatments were found to be effective for altering sex ratios of rainbow trout. Female production rate was determined as 100% in the E_2V group. Results of the present study indicated that E_2V reduced the growth, the food conversion rate and the survival rate of rainbow trout fry. However, it was positively effective to produce all female population of the rainbow trout.

Key words: *Oncorhynchus mykiss*, estradiol valerate, hormonal sex reversal, growth

INTRODUCTION

One of the main objectives in fish production is to obtain products which include protein of high quality with minimum cost. This has been achieved through genetic selection, new feed formulation and new management techniques beforehand. However nowadays, the method of improving the effects of the endocrine system on sex reversal and growth through genetic manipulations has been applied since 1983. Agents which increase growth are widely used in researches with the aim of improving the performance of growth in pets[1]. This fact has encouraged the use of steroids (androgens and estrogens) and their synthetic analogues in fish culture[2,3]. Despite the fact that fish breeding sector has developed rapidly throughout the world in recent years, it has been dealing with the problems caused by traditional production methods. The fact that female rainbow trout make use of the feed given to them in a better way, which their feed conversion ratios are higher besides the fact that they grow up faster when compared to male rainbow trout has necessitated the formulation of monosex population by using steroids in the production of this type of fish[2,4].

Monosex population technology has been used in aquaculture to prevent unwanted reproduction and its consequences of overcrowding and stunting and/or increase yield by culturing the faster growing sex[5,6]. Among several monosex production techniques, hormonal sex reversal directly or indirectly, through breeding of sex-reversed fish, has commonly been used to produce monosex populations in aquaculture[7,8]. In male rainbow trout which are fed with estradiol, the formation of ovarium instead of testicle is obtained in gonads by providing a phenotypical change of sex. This means that male trout will use the energy, which they would spend for sexual maturity, for growth. Effects of sex hormones on fish vary based on the fish species and the time, the dose and the length of the application[2,9,10].

It is not known whether E_2V, like other estradiol derivatives, will have such an effect on growth and sex reversal of rainbow trout. This study is important in terms of illuminating this issue. Putting this forth will contribute to the production sector and to science. E_2V used in this study is a natural estrogen that has a longer halfing period and that is cheaper than 17 β-estradiol which is widely used. Although some studies exist with regard to the fact that it is used in pets, there is no research in which its effect on fish is examined. This study was conducted with an objective of investigating the effectiveness of different modes of E_2V administration on growth and producing monosex female populations of rainbow trout.

MATERIALS AND METHODS

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

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Research and Practice and lasted 140 days. In the research 800 fry of rainbow trout (0.32 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 x 0.8 x 0.7 m were used. Commercial granule feed (52% crude protein, 4000 kcal kg^{-1} ME) was used for nourishing the fish.

In the experiment, Method of Random Coincidence Parcels was used[11]. Two groups as control group and the E_2V group 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 values of water temperature and dissolved oxygen were recorded during the study. Water temperature varied from 9 to 17°C with an average of 13.8±0.09°C (except for immersion) and average dissolved oxygen level was about 6.2±0.1 mg L^{-1} during the experiment.

Hormone treated diet preparation: Hormone treated diet was prepared according to rate of 20 mg kg^{-1} of diet by dissolving E_2V (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[3,4].

Hormone immersion: Stock solution of E_2V was prepared according to rate of 400 μg L^{-1} by dissolving E_2V in 95% of ethanol. The alcohol concentration of the solution was arranged to 0.05 ml L^{-1}[12,13]. For the immersion treatment, fry were immersed in a 400 μg L^{-1} of E_2V solution for 2 h, twice a week for 4 weeks. The solution was aerated and kept at 10±1°C. Fresh and separate solutions were prepared for each treatment. Fish in control were immersed into the solution containing 0.05 ml L^{-1} of alcohol for the same length of time during the period of the immersion treatment.

For the feed treatment, fry were fed 20 mg E_2V kg^{-1} of diet ad-libitum for 60 days. In the control treatment, fry were fed only alcohol treated diet for 60 days. All groups were satisfactorily fed. Fish were weighed all together in the 30th and 60th days and their average weights were calculated. In the other measurements and weighing periods (the 90th, the 120th and the 140th days), fish samples (n=50) in each group were weighted in the 30-day periods[11,14]. Fish were anesthetized in the 10 mg L^{-1} of quinaldine solution before the measurements[15,16]. Weights are measured in grams and fork lengths were measured in centimeters. Growth parameters were calculated based on the common formulae[17].

Preserving and sexing fish: At the end of study, a total of 25 fish was sampled from each treatment replicate, anaesthetized by 2-phenoxy-ethanol (2-PE) and then killed. In the experimental and control group fish preserved in 10% buffered formalin or Bouin's for the histological sex identification. Gonads from these fish were paraffin embedded, sectioned (6-7 μm) and stained with Harris hematoxylin-eosin (HE) to study details of the gonad tissue. Then it was painted by ternary paints and investigated under a microscope[18]. Each fish sampled was categorized as male, female or intersex.

Data analysis: Data were analyzed using SAS Software. Multiple mean comparisons were made using Duncan test to estimate differences ($p<0.05$) among results obtained at trials. Sex ratios, mean percent survival and mean growth rates were analyzed using one-way ANOVA.

RESULTS AND DISCUSSION

The end of the 140-day long experiment, while the control fish reached to mean weight of 11.29±0.76 g, the fish in the treatment group (immersion and feeding with E_2V) reached to only 7.71±0.76 g of weights (Table 1). The condition factors of the control and the treatment group were calculated as 1.54±0.02 and 1.48±0.02, respectively. Differences among the values of lengths and weights were significant ($p<0.05$), but there was no significant difference among the values of the condition factors. Weight increases in the experimental groups as shown in Table 1.

At the end of the study, it was found that the combination of immersion and diet negatively affected the weight increase in rainbow trout. Some researchers also stated that the estradiol application retarded the growth of rainbow trout[7,19-21]. Some other researchers reported that the estradiol application had no effects on the weight increase in trout and salmons[19,22-24]. On the other hand, there are also some other researchers[9,21,25,26] reporting that more weight increases with the estradiol application. The condition factor was not affected and changed by the E_2V application in the present study. The values of the condition factors are similar with Piferrer and Donaldson[26].

Difference between of SGR% of the control and the treatment group was found insignificant (Table 1). The results of Johnstone[27] and Woo[28] are in agreement with the results of the present study. It was deduced that the application of E_2V negatively affected the feed conversion rate in rainbow trout (Table 1). Yu *et al.*[10] also reported that feed consumption was realized higher in the estradiol applied group. However, while Schreck and Fowler[25]

Table 1: Growth parameters of experimental groups; weight (W±SE), length (L±SE), condition factor (C±SE), specific growth rate (SGR%) and feed conversion rate (FCR), (n=50)

Periods (days)	Growth parameters	Experimental groups: Control	Experimental groups: E_2V (Immersion+diet)	F-test (p=0.05)
0		-	Immersion*	
30	W**	0.22	0.22	
60	W**	0.88	0.63	
	SGR (%)	5.01	3.49	
	FCR	1.07	1.75	
90	W±SE	2.82±0.16	2.17±0.16	0.004
	L±SE	5.85±0.12	5.22±0.12	0.001
	C±SE	1.37±0.03	1.50±0.04	0.007
	SGR (%)	3.47	4.14	
	FCR	1.08	1.19	
120	W±SE	6.46±0.25	4.72±0.25	0.001
	L±SE	7.38±0.09	6.36±0.23	0.001
	C±SE	1.59±0.02	1.60±0.03	0.834
	SGR (%)	2.47	3.59	
	FCR	0.67	0.81	
140	W±SE	11.29±0.76	7.71±0.76	0.001
	L±SE	8.90±0.22	7.94±0.19	0.003
	C±SE	1.54±0.02	1.48±0.02	0.118
	SGR (%)	3.22	2.45	
	FCR	1.13	1.73	
General	SGR (%)	3.54	3.42	0.851
	FCR	0.99	1.37	0.178

* Fry were immersed in 400 μg L^{-1} of E_2V solution for 2 h, twice a week for 4 weeks; ** Mean fish weights were calculated after weighting them together because of their small size

Table 2: Sex and survival rate of experimental groups

Experimental groups	Sex ratio (%): male	Sex ratio (%): female	Survival rate (%)
Control	55	45	90.5
E_2V (400 μg L^{-1}+20 mg kg^{-1}-diet)	-	100*	46.0*

* Significantly different from control ($p<0.05$)

reported a better feed conversion rate in the estradiol group, Woo *et al.*[28] stated that there was no difference in the feed conversion rates of the mentioned groups.

At the end of the study, it was detected that the fish applied in E_2V were all female, while proportions of female and males of the control group were 45 and 55%, respectively (Table 2). Some other researchers[19,22,24,26,29,30] reported similar results. However, there are some other researchers reporting the sex reversal rates in different proportions; Goryczko *et al.*[21] and Piferrer and Donaldson[31] reported the female ratios as 67 and 84%, respectively. It is thought that these different findings might have been resulted because of the variation in the dose and the length of application. None of the studies applying similar dose and application length as the present study reported inefficient of the application on the sex reversal, but there were differences in their sex reversal rates.

The combined application of E_2V negatively affected the survival rates in rainbow trout (Table 2). The other researchers working on this subject also reported that the estradiol application had negative effect on the survival rate[7,21,24,27]. However, some other researchers stated no effects of the estradiol application on the survival rate[25,30]. When we discuss the survival rate based on the application method, there was no difference in the survival rate between the control group and the group treated with immersion, while the treatment applied by feeding had more fish death compared to the control group. However, we have not reached into a clear conclusion for whether the increase in the death rates was caused by the feeding method or the continued effects of the immersion method.

Some observed differences between the present study and the other studies on the growth, the feed conversion rate and the survival rate could be explained with variation caused by the fish species and the time, the dose and the length of sex hormone applications[2,9,10].

At the end of the present study, it was concluded that rainbow trout which were first immersed in a 400 μg L^{-1} of E_2V solution for 2 h, twice a week for 4 weeks and then fed with 20 mg E_2V kg^{-1} of diet *ad libitum* for 60 days were negatively affected for their growth and feed conversion rate, but positively affected in their sex reversal for femaleness. Therefore, it was decided that the sex reversal by employing E_2V should not be advised due to its negative impacts on feed conversion and survival rates. However, the method of indirect sex reversal could be advised instead.

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