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Effects of Oral Administration of Estradiol Valerate on Gonadal Sex Differentiation in the Rainbow Trout, *Oncorhynchus mykiss*

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Abstract: This study investigated the effects of administering Estradiol Valerate (E_2V) on gonadal sex differentiation in rainbow trout (*Oncorhynchus mykiss*, W., 1792). Rainbow trout fry of average weight 0.32±0.02 g, 65 days post fertilization (dpf), which had just started to feed were supplied with a feed containing E_2V at a rate of 20 mg kg^{-1} for 8 weeks (65-121 dpf). Fish samples were taken at random at 156 days (dpf,), 170, 191, 226 and 261 days and changes in fish gonads were analyzed histologically. At 156 dpf of the experiment, it was observed that oocyte formation had started in the fish gonads, suggesting feminization by the administration of the estradiol valerate. Intersex fish were observed at 170 dpf, while oocytes had gathered heads in gonads and sex transformation of some individuals had started at 191 dpf. Oocytes occupied the large areas of gonads and spermatogonia disappeared at 226 dpf as at 261 dpf. At the end of the experiment, sex compositions were 97% female, 3% intersex in the E_2V administration group and 51% female, 49% female in the control group. There were no apparent differences between control group fish and hormone-treated fish as regards oocyte size.

Key words: Estradiol valerate, rainbow trout, gonadal sex differentiation

INTRODUCTION

In commercial fish farming, it is essential to get fish of a marketable size as soon as possible. In rainbow trout production, early sexual maturation of male fish is considered a problem because males spend their energy on gonad development 5-6 months after hatching, with impaired growth as a result. It has been reported that mono-sex populations of females are preferred in rainbow trout farming because females grow faster, have a higher Food Conversion Rate (FCR), are more resistant to harsh living conditions and have a lower chance of being affected by disease than males (Matty, 1985; Ingram, 1988).

There are many benefits of sex control practices in fish breeding. Administering sex hormones in the first phase of fish life is one of the most effective methods of changing the relative distribution of the sexes within the population (Yamamoto, 1969). Much research into sex change has been reported for rainbow trout and salmon. For example, Johnstone *et al.* (1978) reported that estradiol administration with feed yielded 89% female, 9% male and 2% intersex fish in rainbow trout. Goetz *et al.* (1979) reported that the dietary administration of 17 β-estradiol (10 mg kg^{-1} food) yielded 54.2% female, 18.1% male and 27.7% intersex fish in coho salmon (*Oncorhynchus kisutch*). Johnstone *et al.* (1979) reported 99% female and 1% intersex fish in *Salvelinus fontinalis* after administration of 17 β-estradiol at a rate of 20 mg kg^{-1} food. Parks and Parks (1991) reported that dietary administration of 17 β-estradiol at a rate of 40 mg kg^{-1} for 77 days produced 87.5% females in *Salvelinus fontinalis*, while Goryczko *et al.* (1991) reported that the dietary administration of 17 β-estradiol (20 mg kg^{-1} food for 120 days) produced 95% females in rainbow trout. Blázquez *et al.* (1988) reported that the dietary administration of 17 α-ethynylestradiol (EE_2) at 10 mg kg^{-1} food from 60-260 days postfertilization (dpf) yielded 80% female and completely suppressed gonadal development (sterile fish) 20% in *Dicentrarchus labrax* Krisfalasu and Cloud (1999) reported that the immersion in 17 β-estradiol at 250 mg L^{-1} for 2 h periods during different stages of embryonic development beginning 30 dpf and continuing until 68 dpf yielded 44 and 51 dpf highest incidence of intersex (63%) in *Oncorhynchus mykiss*. Bjerregaard *et al.* (2008) reported that the

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administration of 17 β-estradiol (10-500 ng L^{-1}) and bisphenol A (50 μg L^{-1}) from 0-63 dpf founded in the interval of 60-40% and 40-60% females, males in S*almo trutta* and no significant difference in the Gonadosomatic Index between all groups.

The valerate derivative of estradiol is a natural estrogen that has a longer half-life and is less expensive than 17 β-estradiol. Although, there are studies of Estradiol Valerate (E_2V) use in pets, there are no reports of its effects in fish as valerate (Kalkan and Ocal, 1997).

The effect of estradiol valerate, on direct feminisation in rainbow trout has been investigated in previous studies but the results obtained have varied because of differences in dose, treatment time and application period of the estrogens. It has been reported by several researchers that the effects of sex hormones on fish might vary based on fish age (application time), dose of hormone and fish species (Yu *et al*., 1979; Degani, 1986).

The present study, aimed to determine sex differentiation in rainbow trout gonads as a result of estradiol valerate administration with feed in the ratio of 20 mg kg^{-1}.

MATERIALS AND METHODS

This study was carried out at the Department of Fisheries at Yuzuncu Yil University in Turkey and lasted 355 days. The experiment was carried out with 800 rainbow trout fry (0.32±0.02 g fry^{-1}, 65 dpf) reared in well-water aerated with an aerator. In the experiment, fiberglass tanks with a volume of 1.4 m^3 (dimensions 2.5×0.8×0.7 m) were used and the fish were fed on commercial granule feed (52% crude protein, 4000 kcal kg^{-1} Metabolic Energy).

Estradiol valerate, $C_{23}H_{32}O_3$, purchased from Schering Medicine and Medical Corporation, Germany (Istanbul, Turkey) was added to the diet of the experimental groups. 20 mg kg^{-1} feed of estradiol is a commonly used dose (Johnstone *et al*., 1978, 1979; Goryczko *et al*., 1991; Hunter and Donaldson, 1983). Therefore, it was used 20 mg kg^{-1} feed dose of E_2V in this experiment. The E_2V was dissolved in a few mL of butyl alcohol and then diluted in 400 mL of 95% ethanol for each kg of diet so that it could be sprayed onto the feed, while turning in a mixer. The feed used for the controls was sprayed with ethanol only. The feeding material was left to air-dry overnight to eliminate the alcohol and then stored at 4°C for use during the experiments (Ingram, 1988; Gannam and Lovell, 1991).

The method of Random Coincidence Parcels was used in the study (Yildiz and Bircan, 1991). A control group and an E_2V group were created and as each group was duplicated, there were 4 groups in total. Each group, composed of 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 marketable size. Feed with E_2V was given to the treatment groups for 56 days and then the normal feed was given for the remaining 299 days. The fish in the control group were given feed without E_2V but sprayed with 95% ethyl alcohol for 56 days 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 examine, the sex reversal in rainbow trout, 20 fish were randomly sampled twice at 156, 170, 191, 226 and 261 dpf of the study from both administration and control groups and their gonads were removed after careful dissection of the abdomen and gonad structure was investigated histologically. For this, the gonads were placed in Bouin's fixative solution for 24 h and then tissues were checked, blocked with paraffin and cut into 6 μm cross-sections. Six-micrometer-thick sections were stained with Mallory's triple stain (Kiermen, 1989). The preparations were evaluated by means of a bright-field microscope and photographed (Optiphot 2; Nikon, Tokyo, Japan). At the end of the study, all fish were sampled, dissected and analyzed and the sex ratios of the E_2V and control groups were determined.

RESULTS

At the end of the study, the fish in the E_2V group consisted of 97% females and 3% intersex, while those in the control group were 51% female and 49% male (Table 1).

Histological analysis of the gonads of control group fish at 261 dpf showed that there were no apparent differences in oocyte size in female fish in this group compared with the E_2V group and spermatogonium development was normal in the testis of male fish (Fig. 1a and b).

The results of histological observation of the gonads of the E_2V group at 156, 170, 191 and 226 dpf are shown in Fig. 2a-f. In the gonads of fish sampled at 156 dpf, there were some oocytes among spermatogonia in the fish gonads (Fig. 2a). In the gonads of fish sampled at 170 dpf (Fig. 2b), ovarian and testicular structures were apparent with the increased number of oocytes (Fig. 2c and d). The structure of gonad in the E_2V administration group at 191 and 226 dpf indicated that spermatogonia had

Table 1: Sex ratios of experimental groups (%)

	Sex ratio (%)		
Experimental group	Male	Female	Intersex
Control	49	51	-
E_2V (20 mg kg^{-1} feed)	-	97	3

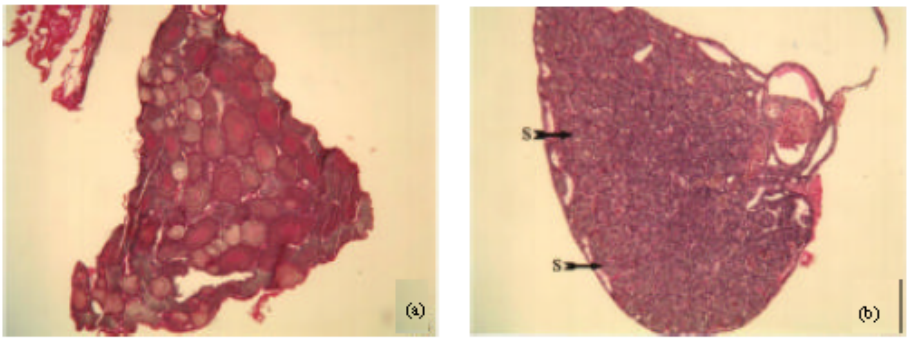

Fig. 1: Histological structure of control group fishes' gonads (a: ovary; b: testis, S: spermatogonium) at 261 dpf

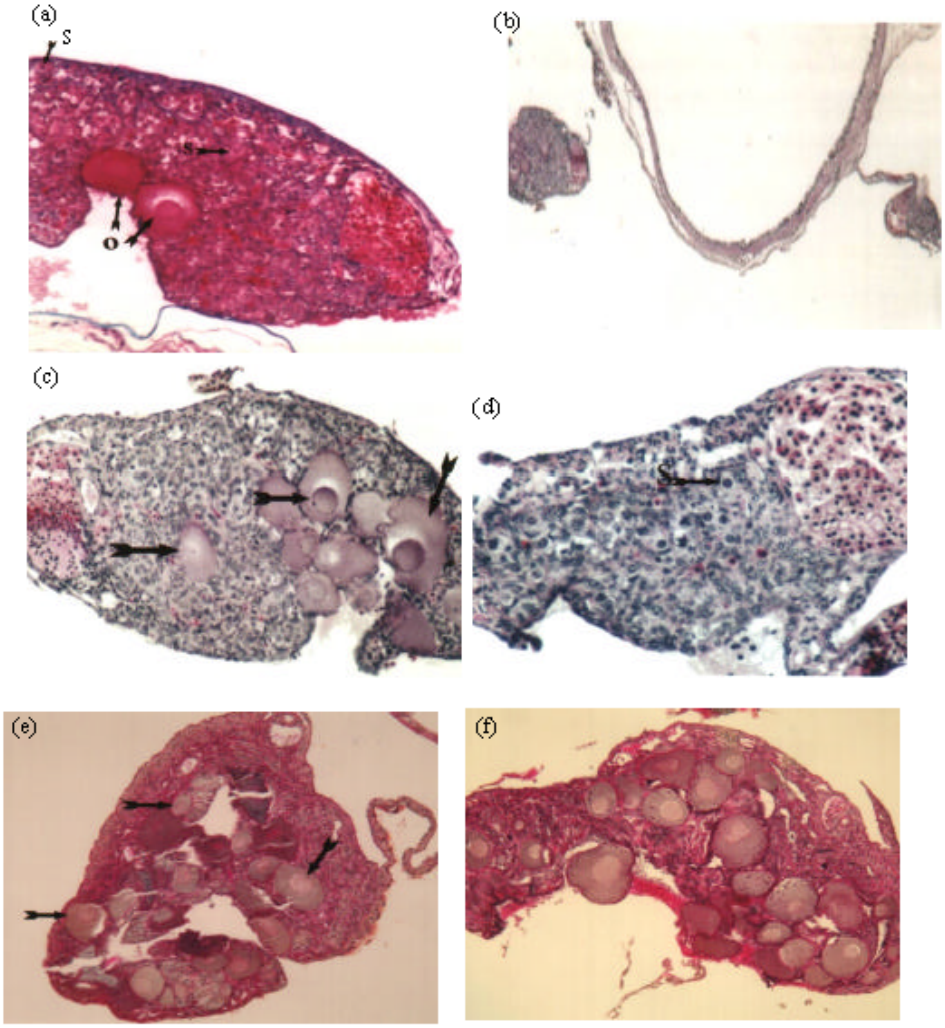

Fig. 2: Histological analysis that were made in the gonads of E_2V application group fishes. a: At 156 dpf, O: Oocytes and S: Spermatogonium. b: Intersex fish, at 170 dpf, c: Ovary; arrows: Oocytes. d: testis; S: Spermatogonium. e: Ovary; at 191 dpf; arrows: Oocytes. f: ovary; at 226 dpf

started to decrease, while oocytes developed the anterior portions of gonads at 191 dpf (Fig. 2e). Eventually, oocytes occupied the large areas of gonads and spermatogonia disappeared (Fig. 2f, 226 dpf) as 261 dpf.

DISCUSSION

The present study demonstrated that estradiol valerate administered at a rate of 20 mg kg^{-1} feed for 56 days was effective for feminizing rainbow trout (97% female and 3% intersex). Different studies on direct feminization of rainbow trout and salmon have found different sex ratios. Some of these reported results similar to the ratio of female (97%) found in the present study (Johnstone *et al*., 1978, 1979; Goryczko *et al*., 1991; Garret, 1989; Melard, 1995; Krisfalasu and Cloud, 1999). Melard (1995) reported that dietary administration of 17 α-ethynylestradiol at a rate of 100-150-200 mg kg^{-1} produced 94, 93 and 98% females in *Oreochromis aureus*. Krisfalasu and Cloud (1999) reported that dietary administration of 17 β-estradiol produced 90% females in *Oncorhynchus mykiss*.

Piferrer and Donaldson (1992) immersed newly hatched Chinook salmon (*Oncorhynchus tshawytscha*) fry in 400 μg L^{-1} E_2 solution for different periods and reported feminization rates of 72.2-100%. Nakamura (1984) immersed Masu salmon (*Oncorhynchus masou*) and chum salmon (*Oncorhynchus keta*) fry 5 days after hatching in E_2 solution for 18 continuous days and found that the groups exposed to E_2 were feminised in different ratios. Piferrer and Donaldson (1989) immersed Coho salmon (*Oncorhynchus kisutch*) eggs in 400 μg L^{-1} E_2 solution before and after hatching and discovered that the group immersed before hatching were feminised in the ratios of 82.5-84%, while the groups immersed after hatching were 46.3-73.7% feminised.

However, other studies found lower ratios for example Parks and Parks (1991) obtained 87.5% females, Lahav (1993) 82% and Goetz *et al*. (1979) obtained 54.2%. Different studies on direct and indirect feminisation of rainbow trout and salmon found different rate of intersex fishes. Johnstone *et al*. (1979) found a 1-12% rate of intersex fishes, Nakamura (1984) found 3% and Goetz *et al*. (1979) 27.7%. It is thought that those different sex ratios derived from dissimilarities of experimental conditions, type of hormone used, administration period, administrating timing and dose differences.

The present study, demonstrated that sex reversal had started in the gonads at 156 dpf of E_2V administration. Bjerregaard *et al*. (2008) found that determination of sex by histological evaluation of the gonads was possible from 155 dpf and onwards.

In this study, also found that oocyte formation had started in the fish gonads. In addition, it found that there were no differences in oocyte sizes between the gonads of control and hormone-treated fish. Furthermore, the study appeared that estradiol valerate can use to sex reversal of fish beside estradiol.

REFERENCES

Bjerregaard, L.B., C. Lindholst, B. Korsgaad and P. Bjerregaard, 2008. Sex hormone concentration and gonad histology in brown trout (salmo trutta) exposed to 17 β-estradiol and bisphenol A. Ecotoxicol., 17: 252-263. DOI: 10.1007/s10646-008-0192-2. http://www.springerlink.com/content/n5071 1816p 16 2188/.

Blázquez, S., M. Zanuy and F. Carrillo, 1988. Piferrer. Structural and functional effects of early exposure to estradiol-17 β and 17 α-ethynylestradiol on the gonads of the gonochoristic teleost Dicentrarchus labrax. Fish Physiol. Biochem., 18: 37-47. DOI: 10.1023/A:1007736110663. http://www.springerlink.com/Index/W08474212V5430J7.pdf.

Degani, G., 1986. Effect of combined dietary 17 β-estradiol and 17 α-methyltestosterone on growth and body composition of European eels (*Anguilla anguilla*). Aquacul., 59: 169-175. DOI: 10.1016/0044-8486(86) 90001-3. http://www.sciencedirect.com/science/journal/00448486.

Gannam, A.L. and R.T. Lovell, 1991. Growth and bone development in channel catfish fed 17 α-methyltestosterone in production ponds. J. World Aquacul. Soc. (WAS), 22: 95-100. DOI: 10.1111/j.1749-7345.1991.tb00721.x. http://www3.interscience.wiley.com/journal/119994287/abstract.

Garrett, G.P., 1989. Hormonal sex control of Largemouth Bass (*Micropterus salmoides*). The Progressive Fish-Culturist, 51: 146-148. DOI: 10.1577/1548-8640(1989) 051. http://afs.allenpress.com/perlserv/.

Goetz, F.W., E.M. Donaldson, G.A. Hunter and H.M. Dye, 1979. Effect of estradiol 17 β- and 17 α-methyltestosterone on gonadal differentiation in the coco salmon (*Oncorhynchus kisutch*). Aquacul., 17: 267-278. DOI: 10.1016/0044-8486(79) 90083-8. http:// www.sciencedirect.com/science/journal/00448486.

Goryczko, K., K. Bieniarz, S. Dobosz and J. Grudniewska, 1991. The effect of 17 β-estradiol on rainbow trout (*Oncorhynchus mykiss*, Walb.). Polskie Archiwum Hydrobiol., 38: 303-309. http://psjc.icm.edu.pl/psjc/cgi-bin/getpis.cgi?czas=Polskie+Archiwum+Hydrobiologii.

Hunter, G.A. and E.M. Donaldson, 1983. Hormonal Sex Control and its Application to Fish Culture. In: Hoar, W.S., D.J. Randall and E.M. Donaldson (Eds.). Fish Physiol., 9: 223-303. DOI: 10.1016/S1546-5098(08) 60305-2. http://www.sciencedirect.com/science.

Ingram, M., 1988. Farming Rainbow Trout in Fresh Water Tanks and Ponds. In: Lindsay Laird and Ted Needham (Eds.). Salmon and Trout Farming. 1st Edn. Ellis Horwood Limited. England, pp: 155-188. ISBN: 0-13-788266-1.

Johnstone, R., T.H. Simpson and A.F. Youngson, 1978. Sex reversal in salmonid culture. Aquacul., 13: 115-134. DOI: 10.1016/0044-8486(78)90106-0. http://www.sciencedirect.com/science/journal/ 00448486.

Johnstone, R., T.H. Simpson and A.F. Walker, 1979. Sex reversal in salmonid culture. Part III. The production and performance of all female populations of brook trout. Aquacul., 18: 241-252. DOI: 10.1016/0044-8486 (79)90015-2. http://www.sciencedirect.com/science/ journal/00448486.

Kalkan, C. and H. Ocal, 1997. Effect of External Estradiol Valerate or Diethylstilbestrol on Luteolysis During the Luteal Period in Cows. Turk. J. Vet. Anim. Sci., 21: 9-11. http://mistug.tubitak.gov.tr/bdyim/abs.php?dergi=vet&rak=96011.

Kiermen, J.A., 1989. Histological and Histochemical Methods. Theory and Practice 2nd Edn. Pergamen Press, Oxford, New York. ISBN: 0080362249. http://www.allbookstores.com/book/9780080362243.

Krisfalasu, M. and J.G. Cloud, 1999. Gonadal sex reversal in triploid Rainbow trout (*Oncorhynchus mykiss*). J. Exp. Zool., 284: 466-472. DOI:10.1002/(SICI)1097-010X(19990901)284:4<466:AID-JEZ13>3.0.CO;2-G. http://www3.interscience.wiley.com/journal/63002552.

Lahav, E., 1993. Use of sex reversed females to produce all-male tilapia (*Oreochromis aureus*) fry. The Israeli J. Aquacul. Bamidgeh (IJA), 45: 131-136. www.nature.com/hdy/journal/v92/n6/full /6800453a.html.

Matty, A.J., 1985. Fish Endocrinology. 1st Edn. Timber Press Ltd, Portland, pp: 220-241. ISBN: 0-88192-024-X.

Melard, C., 1995. Production of a high percentage of male offspring with 17 α-Ethynylestradiol sex-reversed Oreochromis aureus. I. Estrogen sex-reversal and production of F2 pseudofemales. Aquacul., 130: 25-34. DOI: 10.1016/0044-8486(94) 00313-D. http://www.sciencedirect.com/science/ journal/00448486.

Nakamura, M., 1984. Effects of estradiol-17 β on gonadal sex differentiation in two species of salmonids, the Masu salmon *Oncorhynchus masou* and the Chum salmon *Oncorhynchus keta*. Aquacul., 43: 83-90. DOI: 10.1016/0044-8486(84)90012-7. http://www.sciencedirect.com/science/journal/00448486.

Ostrowski, A.C. and D.L. Garling, 1988. Influences of anabolic hormone treatment and dietary protein: Energy ratio on condition and muscle deposition of rainbow trout. The Progressive Fish-Culturist 50: 136-140. DOI: 10.1577/1548-8640(1988)050<0136: IOAHTA>2.3.CO;2.http://afs.allenpress.com/ perlserv/.

Parks, L.M. and J.W. Parks, 1991. Sterilization and feminization of brook trout (*Salvelinus fontinalis*) by androgen and estrogen treatment. Bull. Aquacul. Assoc. Canada, 91: 34-35. http://www.aquaculture association.ca/bulletin/pub.html#bulletin.

Piferrer, F. and E.M. Donaldson, 1992. The comparative effect of the natural and a synthetic oestrogen for the direct feminization of Chinook salmon (*Oncorhynchus tshawytscha*). Aquacul., 106: 183-193. DOI: 10.1016/0044-8486(92)90202-V. http://www.sciencedirect.com/science/journal/ 00448486.

Piferrer, F. and E.M. Donaldson, 1989. Gonadal differentiation in Coho salmon, *Oncorhynchus kisutch,* after a single treatment with androgen or estrogen at different stages during ontogenesis. Aquacu., 77: 251-262. DOI:10.1016/0044-8486 (89) 90207-X. http://www.sciencedirect.com/science/ journal/00448486.

Santandreu, I.A. and N. Diaz, 1994. Effect of 17 α-methyltestosterone on growth and nitrogen excretion in Masu salmon Oncorhynchus masou Brevoort. Aquacul., 124: 321-333. DOI: 10.1016/0044-8486 (94) 90405-7. http://www.sciencedirect.com/science/ journal/00448486.

Yamamoto, T., 1969. Sex Differentiation. In: Hoar, W.S. and D.J. Randalls (Eds.). Fish Physiology, chap. 3. Academic Press. Inc. New York, Vol 3. ISBN: 0123504031. http://www.alibris.com/search/books/ isbn/0123504031.

Yildiz, N. and H. Bircan, 1991. Arastirma ve Deneme Metodlari. Ataturk Univ. Yay. No: 697, Zir. Fak. No: 305, Ders Kitaplari Serisi No: 57, Ataturk Univ. Zir. Fak. Ofset Tesisleri, Erzurum, pp: 1-277.

Yu, T.C., R.O. Sinnhuber and H.J. Dendricks, 1979. Effect of steroid hormones on the growth of juvenile Coho salmon (*Oncorhynchus kisutch)*. Aquacul., 16: 351-359. DOI: 10.1016/0044-8486(79)90074-7.http://www.sciencedirect.com/science/ journal/00448486.