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Reproductive characteristics of adult channel catfish treated with trenbolone acetate during the phenocritical period of sex differentiation

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Abstract

Channel catfish fry fed for 60 days with 0, 50 or 100 mg/kg trenbolone acetate (TBA) and judged by dissection of fingerlings to be males were grown to sexual maturity in ponds. Body weight and gonadal development were compared when the fish were 18 months old. Trenbolone-treated fish were significantly lighter, shorter and the gonads less developed than control males. Three-year-old fish were visually examined for external sex characteristics, and sampled for gonadal development and plasma hormone concentrations. Gonad weight, GSI and plasma testosterone were significantly higher in control fish than in either of the trenbolone-treated groups. Twenty fish from each treatment group were placed in spawning cages with normal female fish. Five spawns were obtained from each of the treatment groups; however, all 10 spawns were composed of infertile eggs. TBA interferes with normal gonadal development of both the testis and ovary but does not functionally masculinize channel catfish. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Channel catfish; Sex differentiation; Trenbolone acetate; Reproduction

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1. Introduction

Channel catfish have a homogametic female sex determination system (Davis et al., 1990). Gonadal phenotype can be manipulated with external hormones during the phenocritical period of gonadal commitment (Davis et al., 1992). However, this species, unlike most other fish (Hunter and Donaldson, 1983), is functionally feminized by treatment with an extensive number of sex hormones, including estrogens, aromatizable and nonaromatizable androgens and aromatase inhibitors (Goudie et al., 1983; Davis et al., 1990). Fish feminized by exogenous hormones are viable, fertile, spawn and have characteristics similar to normal (XX) females, except that the hormonally sex-reversed females (XY) produce spawns with a 3:1 (male: female) sex ratio (Davis et al., 1991). One third of the phenotypic males has a YY sex genotype (Davis et al., 1991) and produces only male offspring. Production of sexually competent phenotypic males with the XX genotype has not been demonstrated. Trenbolone acetate (TBA) is a synthetic anabolic androgen which increases weight gain in beef cattle (Roche and Quirke, 1986), and has been reported to masculinize channel catfish (Galvez et al., 1995) and tilapia (Galvez et al., 1996). If the apparent masculinization results in fertile male fish, one half of the fish should be XX males. This is important because unique channel catfish sex genotype/phenotype combinations of XY and YY females and YY males have been produced. All of these unique phenotype/genotype combinations have been demonstrated to be viable and fertile; however, no success in producing reproductively competent XX males has been demonstrated (Davis et al., 1995). The only method of determining the sex genotype of catfish is progeny testing by mating the hormone-treated fish with normal fish of known genotypes. Molecular identification of the sex genotype would be a very valuable tool, but has not been accomplished for catfish. This paper reports reproductive characteristics and spawning success in mature channel catfish which were fed TBA during the phenocritical period of sex determination.

2. Materials and methods

Channel catfish fry were fed in May and June of 1994 with either 0, 50 or 100 mg TBA (Hoechst-Roussel Agri-Vet, Somerville, NJ, USA). Diets were prepared as previously reported (Galvez et al., 1995) and feeding continued for 60 days beginning at first feeding. Untreated fish had an equal number of males and females. Both groups of TBA-treated fish were originally judged to be all males by dissection at 120 days of age (average weight = 27.7 g). Details of the experimental conditions during hormonal treatment can be found in Galvez et al. (1995). Remaining fish were placed in 0.05 ha ponds at 100 fish/pond and fed to satiation daily from April through October until the third year. Each treatment was placed in a separate pond and a similar number of males sexed by visual examination of the external genitalia from the control group were placed in a third pond. During October of 1995, when the fish were about 18 months old, 45 fish from each of the three groups were sampled for weight, standard length and gonad weight. On May 20, 1997, 3-year-old fish from each group were sampled. Ten control

fish and 14 fish from each TBA-treated group were sampled. Fish were visually examined for male external sex characteristics such as shape and muscularity of the head and development of the genital papilla. Fish were anesthetized with MS222, the body and gonad weights were taken, and a blood sample collected in heparinized syringes. Plasma was separated and stored frozen at -80°C . Gonadal weight was expressed as gonadal percent of the body weight (GSI). Plasma testosterone and estrogen concentrations were determined by RIA (Testosterone MAIA and Estradiol MAIA, Polymedco, Cortland Manor, NY). A plasma sample was also taken from untreated females of the same age and strain for comparison of estrogen concentrations. Twenty fish from each TBA treatment group were placed in spawning cages with mature, normal females and spawning cans. Spawning cans were checked three times a week and any spawn

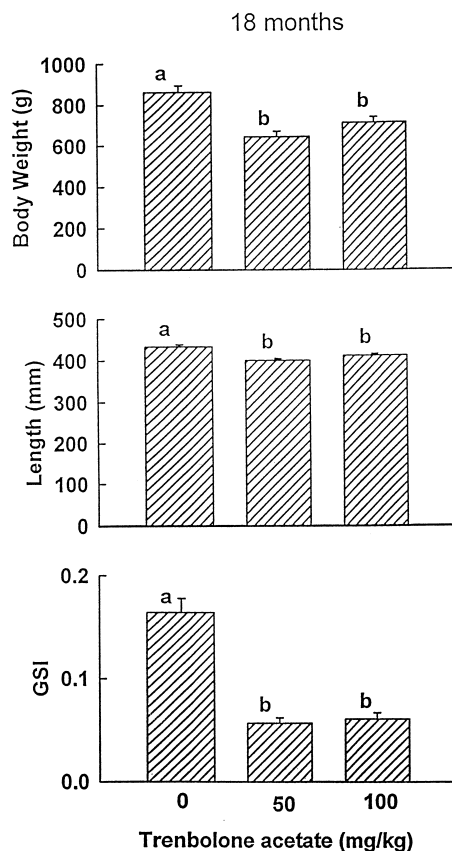


Fig. 1. Body weight, standard length and GSI of 18-month-old channel catfish. Treatment groups were fed 50 or 100 mg of TBA per kilogram of food for the first 60 days of feeding and fed normal food thereafter. Controls were visually selected males from the control fed fish. Groups were raised in separate 0.05 ha ponds at 100 fish/pond. Values are means \pm S.E. of 45 fish in each group. Different letters are significantly different subsets ($P < 0.05$) by Tukey's multiple range test.

removed to a hatching system. When all fish were removed from the spawning cages, a second group of at least 13 fish per treatment was sampled on June 23. Most spawnings have been completed by this time in channel catfish.

Statistical analyses were done by analysis of variance followed by Tukey's multiple range test when significance ($P < 0.05$) among group variation was indicated (Statistix for Windows, 1996).

3. Results

Body weight, standard length and GSI of the control fish sampled in 1995 when the fish were about 18 months old were significantly ($P > 0.05$) greater than both TBA

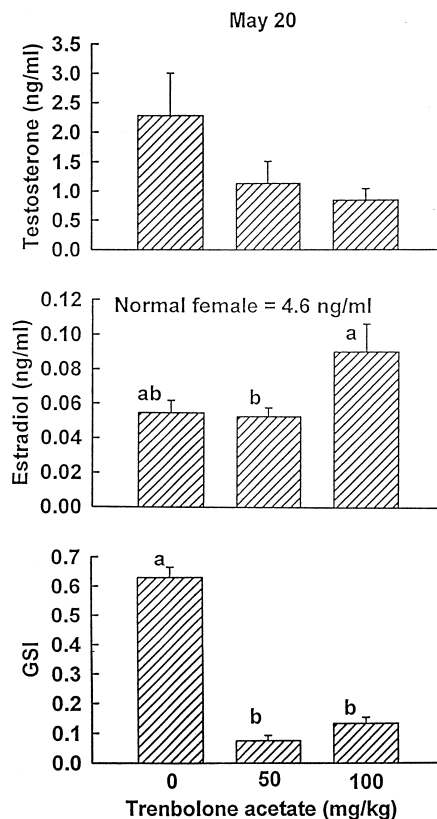


Fig. 2. GSI, plasma testosterone and estrogen concentrations of 3-year-old fish sampled on May 20. Treatment groups were fed 50 or 100 mg of TBA per kilogram of food for the first 60 days of feeding and fed normal food thereafter. Values are means \pm S.E. Different letters are significantly different subsets ($P < 0.05$) by Tukey's multiple range test.

treatment groups which were statistically similar to each other. The most dramatic difference was in GSI where the values for TBA-treated fish were about half that of the controls (Fig. 1). These fish were not expected to be sexually mature at this age and the measurements were done outside of the reproductive season.

Fish sampled in 1997 were 3 years old and were expected to be sexually mature and the samples were taken during the peak spawning period. External morphology of all three groups was similar. Distinct muscular pads were present on the head and a well-developed male genital papilla was present in all fish examined.

The GSIs of both treatment groups from May 20 were significantly smaller than controls. Control GSI was over 3.5 times larger than the GSI of 18-month-old fish while the TBA-treated fish had a GSI barely twice that of 18-month-old fish. Histological

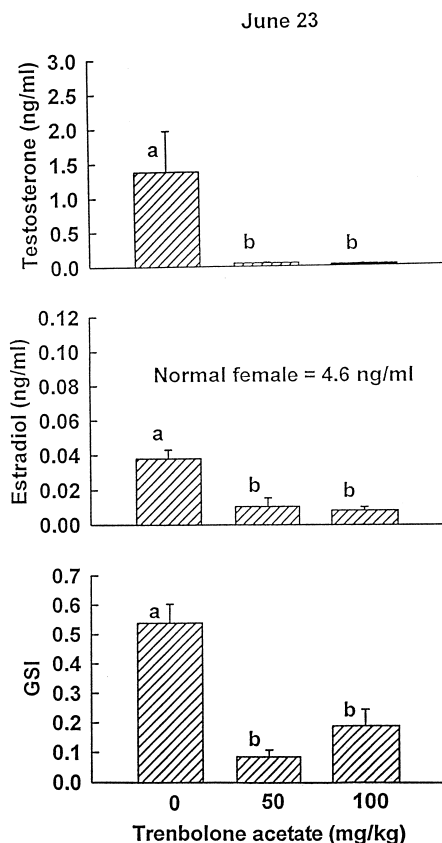


Fig. 3. GSI, plasma testosterone and estrogen concentrations of 3-year-old fish sampled on June 23. Treatment groups were fed 50 or 100 mg of TBA per kilogram of food for the first 60 days of feeding and fed normal food thereafter. Values are means \pm S.E. Different letters are significantly different subsets ($P < 0.05$) by Tukey's multiple range test.

examination of the gonads was not done; however, the gross appearance of TBA-treated fish was less discrete than fish which had not completed maturation or that of immature fish. Testosterone concentrations from both treatment groups were about one half that of controls but were not found to be significantly different at the 0.05 level of probability. Testosterone concentrations of normal spawning female fish are about 19 ng/ml compared to concentrations in normal spawning male fish of between 1 and 2 ng/ml found here and reported elsewhere (Davis et al., 1995). Estrogen concentrations were less than 0.10 ng/ml in all groups compared with 4.6 ng/ml in normal spawning female catfish (Fig. 2). Direct examination of the hormonal data shows no suggestion of a bimodal distribution of individual values which resulted in quite small standard errors. Gross gonadal appearance of the control group was normal for mature testes. Gonads in the TBA-treated groups did not show signs of fingerlike testicular fimbriations typical of mature testes. The gonads of two fish from the 50 mg/kg group and four from the 100 mg/kg group appeared to have some ovarian characteristics. One of the four fish in the 100 mg/kg group had very large ovaries with a GSI of 9.41% and a plasma testosterone concentration of 10.68 ng/ml. These ovaries had lobules or segments down the length unlike normal ovaries which are cylindrical structures with no segmentation. This fish was excluded from the data in Fig. 2.

TBA-treated fish occupied and cleaned the cans in preparation for spawning similar to normal male fish. After 1 month in the spawning cages, five spawns from the 50 mg group and five from the 100 mg group were found. All of the spawns appeared normal when removed from the cans; however, none of the eggs developed. The eggs were inspected microscopically each day and no signs of fertilization or development were evident.

Control fish sampled on June 23 (Fig. 3) had significantly higher GSI, testosterone and estradiol concentrations than either of the TBA treatment groups which were similar to each other. Gonadal weight and GSI for the control group were similar to the control group sampled in mid-May. None of the 50 mg/kg TBA group was judged to have ovotestis; however, gonads from 10 of the 15 fish from the highest TBA group had at least some follicle-like components and were judged to be ovotestis. The degree of follicle-like masses ranged from gonads with only a few masses with a GSI of 0.037% to larger ovarian-like masses with a GSI of 0.716%. None of the gonads from either treated group was typical of testes or ovaries.

4. Discussion

Treatment with TBA had no beneficial effect on the growth of channel catfish as 4-month-old fingerlings (Galvez et al., 1995) or as 18-month-old market-size fish as reported here. In fact, market-size (over 500 g) TBA-treated fish were significantly smaller than male controls after 18 months. No comparisons were made with normal females or with mixed sex groups. Channel catfish have not been shown to respond to other androgens in an anabolic fashion. Growth was decreased in channel catfish fed methyl testosterone, 11-ketotestosterone, estradiol or triiodothyronine in the diet (Gan-

nam and Lovell, 1991). A number of anabolic steroids, growth hormone and thyroxine have been shown to be anabolic in many species, especially salmonids (Donaldson et al., 1979; Higgs et al., 1982; Schulte et al., 1989) and carp (Lone and Matty, 1980, 1982). In yellow perch, females are larger than males and estrogen has been shown to promote growth in that species (Malison et al., 1985).

Commitment of the presumptive gonadal tissue in channel catfish occurs during the first 21 days of feeding (Goudie et al., 1983; Davis et al., 1992). In addition, hormonal treatment with estradiol, aromatizable and nonaromatizable androgens and aromatase inhibitors during that period has been found to feminize the gonad or to have no effect on the developing gonad or sex ratio. Fish feminized with hormones have been shown to be viable and fertile. Adult fish feminized with hormones, or produced by back crosses of such fish, have been shown to have a variety of unique sex genotype/phenotype combinations. The only unique sex phenotype not described is XX masculinization (Davis et al., 1995).

TBA-treated channel catfish were originally reported (Galvez et al., 1995) to be all-male populations by visual examination of very young fish (about 4 months of age). At this age, the testes are barely visible by gross examination while the ovaries of such fish are distinct and clearly recognizable. Ovaries can also be rendered opaque with acetic acid and are easy to detect. This procedure assumes that if no ovaries are present, the fish are males. Ovaries differentiate long before testes develop (Patino et al., 1996) and this technique has been useful and extensively verified in experiments which have induced feminization by hormones and the production of all-male populations by crossing YY males with XX females (Davis et al., 1995). Data in the present paper suggest that the TBA-treated channel catfish reported by Galvez et al. (1995) as males were sterile fish with no (or little) gonadal development. Other treatment protocols with TBA have recently been reported to increase the number of males in Nile tilapia following a single 3-h immersion during days 12–15 post-fertilization (Contreras-Sanchez et al., 2000), and feeding TBA in the diet to 9-mm average-length blue tilapia for 28 days has also been reported to masculinize the fish. However, most of these experiments did not report complete population masculinization, and did not report spawning of the TBA-treated progeny. Treatment with TBA in our experiments continued for 60 days, which is well beyond the established phenocritical period for hormonal feminization in channel catfish (Davis et al., 1992). Therefore, it is possible that a reduced exposure in concentration or treatment period may be effective in functionally masculinizing channel catfish.

TBA has both androgenic and antiestrogenic activity in mammals and has been reported to be 10–50 times more metabolically active than testosterone (Neumann, 1976). Treatment with TBA in boars and bulls results in reduced plasma testosterone concentrations (Silcox et al., 1986; Ventanas et al., 1991).

In our experiments, treatment of channel catfish with TBA during the phenocritical period of gonadal commitment apparently interfered with the normal development of both testes and ovaries regardless of the sex genotype. The normal sex ratio of channel catfish spawns is an equal number of males and females. Since the sex determination model is homogametic for females (Davis et al., 1990), one half of the fish should be XX and the other half XY regardless of the sex phenotype. There was no apparent

differential response to TBA by different sex genotypes since the responses were similar in most individuals and none of the data indicated a bimodal distribution. Since half of the fish were expected to be genetic females, it is not possible to determine that any ovary-like appearance in the gonad was due to genetic-directed gonadal development or a possible paradoxical feminization as previously described for many compounds in channel catfish (Davis et al., 1990). Similarly, the sex genotype of the single fish with very large ovarian-like gonads could not be determined since the sex genotype can only be deduced by the sex ratio of the progeny. Treated groups had gonads which are best described as undifferentiated with occasional development of ovarian tissue. The use of TBA in the culture of channel catfish might be of some use where sterile fish are needed or such fish might have a higher dressout percentage due to suppressed gonadal development. Interference of normal maturation of the reproductive tract in mammals treated with TBA has also been reported. Treatment of beef heifers with TBA delayed puberty and retarded reproductive tract development (Moran et al., 1990).

The mechanism of TBA interference with gonadal commitment is not clear. It is generally thought that hormonal feminization during development in catfish results from the active steroids interacting with estrogen receptors. TBA may interfere with the natural sex-determining mechanism by binding, but not activating, a receptor (steroid or otherwise) which results in an inhibition of the normal sex-determining pathway(s). However, the external morphology of adult fish suggests that either TBA treatment or adequate endogenous testosterone synthesis partially masculinizes these fish. This possibility is further supported by spawning can cleaning behavior and induction of spawns from some females. Female fish do not spawn when held in aquaria alone, with other females, or with males of closely related species such as blue catfish (Goudie et al., 1995).

Verification of functional masculinity and successful hormonal sex reversal should be verified by examination of the gonadal function of adult animals and by spawning success. The appearance of juvenile gonads may not accurately reflect a complete or functional aspect of the presumed sex-reversing effect of a treatment. This is particularly true of fish like channel catfish where visual appearance of the testes occurs much later than that of the ovaries.

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