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Masculinization of African Catfish (*Clarias gariepinus*) Treated with Gokshura (*Tribulus terrestris*)

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Abstract

The present paper describes the effects of *Tribulus terrestris*, a masculinization agent, on the sex reversal of African catfish, *Clarias gariepinus*. One-day-old hatchlings were immersed in water containing an extract of *T. terrestris* at a rate of 0, 3, 6, or 9 g/30 l water for 30 days. The 9 g treatment resulted in 80.42% male populations. Morphological and histological examinations of the gonads in all groups revealed no intersex fish. Histological examination of fish treated with *T. terrestris* revealed no damage to the testes or ovaries. Despite differences in sex ratio, growth rate, ovaries, and testes, fish treated with *T. terrestris* were histologically similar to fish from the control groups. Survival ranged from 72% in the 3 g group to 80% in the 9 g during treatment and did not significantly differ from the control (p>0.05). The best growth was obtained in the 9 g treatment.

Introduction

Tribulus terrestris L. (Zygophyllaceae), also called gokshura, is a medicinal herb used in Bulgaria, eastern Europe, the Far East, China, and India to treat sexual deficiencies (Bucci, 2000). *Tribulus terrestris* has been reported to enhance libido sexualis, increase testosterone and LH levels, improve athletic performance, and increase the number and motility of spermatozoa (Adimolja, 2000; Bucci, 2000; Gauthaman et al., 2002). *Tribulus terrestris* contains a number of substances known as steroidal saponins. The saponin in *T. terrestris* thought to be responsible for its effect on testosterone levels is known as protodioscin (Adimolja and Adaikan, 1997; Ganzera et al., 2001). It is believed that *T. terrestris* affects androgen metabolism, significantly increasing testosterone or testosterone precursor levels (Neychev and Mitev, 2005).

Oral administration of *T. terrestris* increased sexual behavior in male rats (Gauthaman et al., 2003) and had a proerectile effect on rabbits (Adaikan et al., 2000).

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New-born guppies (*Poecilia reticulata*) treated with *T. terrestris* were sex reversed, underwent spermatogenesis, and had a better growth rate than untreated progeny (Cek et al., 2007a). Administration of *T. terrestris* extract to the zebra cichlid (*Cichlosoma nigrofasciatum*) also produced successful sex reversal (Cek et al., 2007b).

The sex of fish can be significant in aquaculture because of differences between males and females in growth rate, size, behavior patterns, and breeding time. In African catfish, *Clarias gariepinus*, males grow faster and reach a larger final size than females (De Kimpe and Micha, 1974). Therefore, it would be commercially advantageous to produce all-male catfish populations.

Administration of exogenous steroids can be effective in controlling sexual development (Al-ablani and Phelps, 2002). The synthetic steroid 17a-methyltestosterone is a male-specific hormone commonly used to induce sex reversal in teleost fish. However, steroid treatments have the disadvantages of being costly and suppressing growth in teleosts (Hunter and Donaldson, 1983), or producing sterile populations and occasional paradoxical feminization with prolonged duration at early stages of gonadal development in African catfish (Van Den Hurk et al., 1989; Turan 2005), channel catfish (*Ictalurus punctatus*; Goudie et al., 1983), rainbow trout (*Salmo gairdneri*; Solar et al., 1984), and cichlid (*Oreochromis mossambicus*; Varadaraj et al., 1994).

The aim of the current study was to investigate the effects of *T. terrestris* as an alternative masculinization agent in the African catfish.

Material and Methods

Experimental fish. The fish used in this study were one-day-old hatchlings of African catfish (mean wt 0.0028±0.0003 g), produced in the Mustafa Kemal University Fisheries Research Unit from gametes collected from sexually mature individuals. The hatchlings were randomly divided into twelve groups and stocked at a density of 100 fish per 30-l aquarium. The experiment was conducted in a controlled temperature of 25±1°C and photoperiod of 12-h light:12-h dark. After absorption of the yolk sac, catfish larvae were fed *Tubifex tubifex* (Muller) provided *ad libitum*. At ten days, the larvae were fed a trout diet (Aquamaks, Turkey: 48% protein, 18% lipid) three times a day *ad libitum*.

Tribulus terrestris treatment. From the age of one day, the hatchlings were subjected to sex-reversal treatment by immersion in water containing a *T. terrestris* commercial extract (supplied by Dietharmonie Medicinal Plants, France) for 30 days (Van Den Hurk et al., 1989) at levels of 0 (control), 3, 6, or 9 g extract per 30 l water. The extract was poured in the aquaria and the aquaria were aerated strongly to spread the solution throughout the water. The extract treatment was repeated three times weekly for 30 days. Before each treatment, the water in the aquaria was entirely replaced. After the four-week treatment period, the fish were kept in 100-l aquaria for further growth.

Sampling and histological procedures. Growth and survival were monitored throughout the experiment. Fish were individually anesthetized with 300 mg/l lidocaine-HCL\1000 mg/ $NaHCO_3$ (Park et al., 1988), weighed to the nearest 0.0001 g, and measured (total length) to the nearest 0.01 cm.

When the fish reached four months (mean wt 9.44±2.10 g), the age at which gonads in male and female catfish can clearly be distinguished, 50 randomly sampled fish from each group were sacrificed. The animals were anesthetized with 300 mg/l lidocaine-HCL\1000 mg/l $NaHCO_3$ (Park et al., 1988) and decapitated. The gonads were removed and examined to determine the sex ratio. For histological examination, the gonads were fixed in 10% neutral formalin and processed by routine dehydration and paraffin embedding procedures. Cross-sections (4-6 µ thick) were stained with Mayer's hematoxylin and eosin phloxine B solution, examined, and microphotographed. Testes were classified by developmental stage based on histological criteria adapted from Grier (1981).

*Statistical analysis***.** Chi-square (χ^2) test was used to determine whether the observed sex ratios differed from an expected 1:1.

Differences in growth were assessed by one-way ANOVA. The Duncan test was used to analyze which dosage caused the differences (Norusis, 1993).

Results

An approximately equal number of males and females was observed in the control (Table 1). However, the number of males was significantly higher than the number of females in all extract-treated groups with the best sex reversal in the 9 g/30 l treatment, indicating that masculinization is a dose-dependent process.

Morphological and histological examination of the gonads revealed no intersex fish and histological examination of the extract-treated testes and ovaries revealed no damage to the organ structure. Male gonads contained round premeiotic germ cells (primary spermatogonia) with round nucleii including several nucleoli (Fig. 1). There were many Leydig cells in the interstitium between the germ cells. Some of the germ cells of extract-treated fish lined up in small seminiferous tubules. Lobules containing numerous early stage spermatocytes (spermatogonia) were observed. Despite differences in sex ratio, growth rate, ovaries, and testes, fish treated with the extract were histologically similar to fish from the control groups.

At the end of four months, survival and total length in the extract-treated groups did not differ from the control (Table 2). The largest gain in body weight was observed in the 9 g/30 l group, indicating that *T. terrestris* has no negative effect on survival rate and does not seem to inhibit growth in African catfish.

Discussion

The present study revealed that an extract of *T. terrestris* was effective in increasing the proportion of males and improving growth performance in African catfish. Thus, it seems that *T. terrestris* could successfully be used as a natural alternative to synthetic hormones for sex reversal in fish culture. Prior to this study, our group obtained similar results using *T. terrestris* extracts to produce a male population in *P. reticulata* and *C. nigrofasciatum* (Cek et al. 2007ab).

Although the present research provides evidence that *T. terrestris* resulted in a high rate of masculinization, we cannot conclude that this potency was caused by an increase of testosterone since we did not measure plasma testosterone levels. The *T. terrestris* extract did not produce 100% males; a higher dose may have generated better results. Most literature on *T. terrestris* treatments in humans (Adimolja, 2000; Bucci, 2000) and rats (Gauthaman et al., 2002, 2003) demonstrate that it strongly affects androgen metabolism, significantly increasing testosterone or testosterone precursor levels. To our knowledge, this is the first report regarding the potential of *T. terrestris* as a masculinization agent in cultured food fish.

Survival rates were not influenced by the extract. Similarly, survival was high in studies

Table 1. Effects of *Tribulus terrestris* treatment on the sex ratio of African catfish (*Clarias gariepinus*).

Dose (g/30 l)	*Sex distribution (male:female)*	*Sex ratio (% male:% female)*	χ^2 *(compared to control)*
0	125:100	55.56:44.44	-
3	161:55	74.54:25.46	52.02*
6	183:48	79.22:20.78	78.90*
9	193:47	80.42:19.58	88.82*

* $p<0.001$

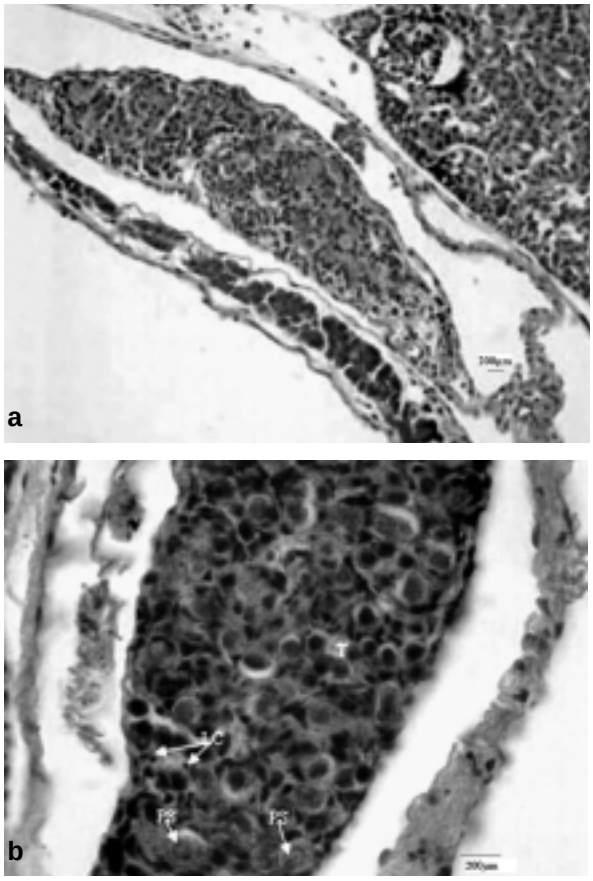

Fig. 1. Transversal sections of a testis from a 120-day-old African catfish treated with *Tribulus terrestris*, a masculinization agent: (a) general view of the bilobed testis, removed from the posterior region of the body cavity, (b) close-up of a testis containing primary spermatogonia (PS), many Leydig cells (LC), and germ cells lined up in small seminiferous tubules (T). Stained by hematoxylin and eosin.

Table 2. Effects of *Tribulus terrestris* treatment on survival and growth of African catfish (*Clarias gariepinus*) at four months.

Dose (g/30 l)	*Survival (%)*	*Total length (cm)*	*Body wt (g)*
0	75±4.58[a]	10.92±0.52[a]	8.60±1.31[a]
3	72±3.21[a]	11.57±0.58[a]	9.19±1.29[a]
6	77±3.22[a]	10.81±0.67[a]	8.96±0.76[a]
9	80±2.89[a]	11.46±0.82[a]	13.21±0.90[b]

* Values in a column with different superscripts are significantly different at $p<0.05$.

on *P. reticulata* and *C. nigrofasciatum* (Cek et al., 2007ab). The growth rate of extract-treated fish was faster than that of the control, similar to results obtained by Georgiev et al. (1988) in immature sheep, Gauthaman et al. (2002) in rats, and our group in *P. reticulata* and *C. nigrofasciatum* (Cek et al., 2007ab)

In summary, *T. terrestris* significantly enhanced sex reversal and growth in African catfish. Future studies should measure testosterone levels after *T. terrestris* treatment to provide more conclusive evidence of the effects of the herb on the sex ratio and whether it can successfully be used as a masculinization agent in fish culture.

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References

Adaikan P.G., Gauthaman K., Prasad R.N.V. and S.C. Ng, 2000. Proerectile pharmacological effects of *Tribulus terrestris* on the rabbit corpus cavernosum. *Ann. Acad. Med. Singapore*, 29(1):22-26.

Adimolja A., 2000. Photochemical and the breakthrough of traditional herbs in the management of sexual dysfunctions. *Int. J. Androl.*, 23(2):82-84.

Adimolja A. and P.G. Adaikan, 1997. Protodioscin from herbal plant *Tribulus terrestris* L. improves male sexual functions possibly via DHEA. *Int. J. Impotence Res.*, 9:64.

Al-ablani S.A. and R.P. Phelps, 2002. Paradoxes in exogenous androgen treatments of bluegill. *J. Appl. Ichthyol.*, 18:61-64.

Bucci L.R., 2000. Selected herbals and human exercise performance. *Am. J. Clin. Nutr.*, 72:624-636.

Cek S., Turan F. and E. Atik, 2007a. The effects of gokshura, *Tribulus terrestris*, on sex reversal of guppy, *Poecilia reticulata. Pakistan J. Biol. Sci.*, 10(5):718-725.

Cek S., Turan F. and E. Atik, 2007b. Masculinization of convict cichlid (*Cichlosoma nigrofasciatum*) by immersion in *Tribulus terrestris* extract. *Aquac. Int.*, 15:109-119.

De Kimpe P. and J.C. Micha, 1974. First guidelines for the culture of *Clarias lazera* in central Africa. *Aquaculture*, 4:227-248.

Ganzera M., Bedir E. and I.A. Khan, 2001. Determination of steroidal saponins in *Tribulus terrestris* by reversed-phase high-performance liquid chromatography and evaporative light scattering detection. *J. Pharm. Sci.*, 90:752-1758.

Gauthaman K., Adaikan P.G. and R.N. Prasad, 2002. Aphrodisiac properties of *Tribulus terrestris extract* (protodioscin) in normal and castrated rats. *Life Sci.*, 71(12):1385-1396.

Gauthaman K., Ganesan A.P. and R.N.V. Prasad, 2003. Sexual effect of puncturevine (*Tribulus terrestris*) extract (protodioscin): an evaluation using a rat model. *J. Altern. Complement. Med.*, 9:257-265.

Georgiev P., Dimitrov M. and S. Vitanov, 1988. The effect of the preparation Tribestan on the plasma concentration of testosterone and spermatogenesis of lambs and rams. *Veterinarna Sbirka*, 3:20-22.

Goudie C.A., Redner B.D., Simco B.A. and K.B. Davis, 1983. Feminization of channel catfish by oral administration of steroid sex hormones. *Trans. Am. Fish. Soc.*, 112:670-672.

Grier H.J., 1981. Cellular organization of the testis and spermatogenesis in fishes. *Am. Zool.*, 21:345-357.

Hunter G.A. and E.M. Donaldson, 1983. Hormonal sex control and its application to fish culture. pp. 223-303. In: W.S. Hoar, D.J. Randall, E.M. Donaldson (eds.). *Fish Physiology, Vol. IX, Part B: Behavior and Fertility Control.* Academic Press, New York.

Neychev V.K. and V.I. Mitev, 2005. The aphrodisiac herb *Tribulus terrestris* does not influence the androgen production in young men. *J. Ethnopharmacol.*, 101:319-323.

Norusis M.J., 1993. *SPSS for Windows Advanced Statistics*, release 6.0. SPSS Inc., USA. 578 pp.

Park I.S., Kim J.M., Kim Y.M. and D.S. Kim, 1988. Influence of lidocaine as an anesthetic for marine fishes. *J. Fish Pathol.*, 1:123-130.

Solar I.I., Donaldson E.M. and G.A. Hunter, 1984. Optimization of treatment regimes for controlled sex differentiation and sterilization

in wild rainbow trout (*Salmo gairdneri* Richardson) by oral administration of 17α-methyltestosterone. *Aquaculture*, 42:129-139.
Turan F., 2005. *Effects of Natural (Androstenedione and Red Clover) and Synthetic (17α-Methyltestosterone and β-Estradiol) Hormones on Sex Reversal, Gonadal Development and Growth Performance in the African Catfish Clarias gariepinus (Burchell, 1822).* Ph.D. Thesis, Institute of Natural Sciences, Mustafa Kemal Univ., Hatay.

Van Den Hurk R., Richter C.J.J. and J. Janssen, 1989. Effects of 17 alpha-methyltestosterone and 11 beta-hydroxyandrostenedione on gonad differentiation in the African catfish. *Aquaculture*, 83:179-191.
Varadaraj K., Kumari S.S. and T.J. Pandian, 1994. Comparison of conditions for hormonal sex reversal of Mozambique tilapias. *Prog. Fish-Cult.*, 56:81-90.