

Production of Female-Free Common Carp, *Cyprinus carpio* var. *communis* (L.) through Dietary Administration of the Androgen Mibolerone

S.K. DAS*, H.P.C. SHETTY and M.C. NANDEESHA

*University of Agricultural Sciences
College of Fisheries
Mangalore 575 002, India*

Abstract

An experiment carried out to produce all-male common carp, *Cyprinus carpio* var. *communis*, through dietary administration of mibolerone, a new androgen, indicated its high effectiveness in sex reversal at a very low dosage. Three-day old larvae treated with the androgen for 30 days at 5 and 10 mg·kg⁻¹ diet produced 95 and 99% sterile carp and 5 and 1% males, respectively, while 15 mg·kg⁻¹ diet resulted in 100% sterile fish. The sex ratio of control fish was almost 1:1. The gonadosomatic and viscerosomatic indices were significantly lower in treated fish than in controls.

Introduction

The exotic common carp, *Cyprinus carpio* var. *communis* is one of the most widely cultured species in India. This fish is either cultured alone or with other carps. One of the main constraints is its prolific breeding in Indian tropical conditions. The fish attains maturity within about six months and breeds naturally in the pond (Jhingran 1982). This phenomenon retards growth through competition for food and space. Further, in common carp, the gonads form 20-30% of body weight (Rao and Rao 1983).

To overcome these problems, there have been attempts in recent years to produce all-male or sterile carp populations. Considerable success has been achieved in producing male or sterile fish through the use of 17 α -methyltestosterone. However, the dosage required is

*Present address: Assam Agricultural University, College of Fisheries, Raha 782103 Assam, India.

above 200 mg·kg⁻¹ diet (Rao and Rao 1983; Basavaraja and Rao 1988). Recent investigations showed the possibility of producing a male-dominated population of *Oreochromis aureus* (Meriwether and Torrans 1986) and all-male populations of *O. mossambicus* (M.C. Nandeesh, unpubl. data) with mibolerone, a new androgen, at a dose less than 1 mg·kg⁻¹ diet. Preliminary studies conducted at this institute with common carp using this androgen indicated the possibility of producing sterile populations at less than 75 mg·kg⁻¹ diet (M.C. Nandeesh, unpubl. data). The present investigation was undertaken to study the minimum level of the hormone required to produce an all-male population of common carp.

Materials and Methods

Three-day old common carp larvae were used for hormone treatment. For each treatment, 450 larvae were stocked in four plastic pools of 1.8 m diameter and 0.9 m height. A minimum water depth of 40 cm was maintained. Three dosages of hormone were tried - 5, 10 and 15 mg·kg⁻¹ diet - while the hormone-free diet served as the control. The hormone was incorporated into the finely ground fish meal-based diet developed by Varghese et al. (1976) by spraying the hormone dissolved in 95% ethanol (Rao and Rao 1983), while for the control diet hormone-free ethanol was sprayed. The larvae were fed *ad libitum* with the hormone-incorporated diet for 30 days. At the end of 30 days, 100 fry from each treatment were taken and stocked in 50 m² ponds (10 x 5 x 1 m) and grown for 164 days. During this growout period, the ponds were fertilized with poultry manure (10 kg/pond/month) and the fish were fed daily at 5% body weight with a mixture of rice bran and groundnut oil cake at 1:1 ratio by weight. The fish were sampled once a month to assess their growth in length and weight. After every sampling, a part of the pond water was replenished with freshwater. The water quality with respect to temperature, pH, dissolved oxygen and dissolved carbon dioxide were measured at monthly intervals following APHA (1985) procedures.

On termination of the experiment, all the surviving fish were measured individually and sexed. The gonads and viscera were removed and weighed to calculate the gonadosomatic and viscerosomatic indices, respectively. Fish with fully developed ovaries or testes were classified as female or male, respectively, while those with filiform (thread-like) gonads were classified as sterile forms.

Samples of all gonads were processed for histological confirmation of sex following the procedure described by Gray (1964) employing Harris' hematoxylin and eosin stains.

Results and Discussion

The survival of fry treated with mibolerone for 30 days was found to be good in all the groups excepting the 10 mg·kg⁻¹ diet treatment, while the control group alone registered poor survival after the 164-day growout period (Table 1). The lower survival of the

Table 1. Effect of dietary administration of mibolerone on sex of common carp.

Treatment	Control	5 mg·kg ⁻¹	10 mg·kg ⁻¹	15 mg·kg ⁻¹
Number of larvae stocked	450	450	450	450
Number of fry recovered after treatment for 30 days	480	380	298	366
Survival (%)	95.5	84.4	66.2	81.3
Average weight (g)	0.14	0.28	0.36	0.29
Survival (%)*	56	91	86	97
Average weight (g) (Mean ± S.E.)	88.48 ± 4.28	39.36 ± 1.30	44.44 ± 2.10	37.58 ± 2.12
Sex composition (%)				
Male	30 (53.6)	5 (5.5)	1 (1.2)	-
Female	26 (48.4)	-	-	-
Sterile	-	86 (94.5)	86 (98.8)	97 (100)

Figures in parenthesis indicate percentage.

*Initial stocking for post-treatment grow-out period was 100 fry per treatment.

fish in the 10 mg·kg⁻¹ diet treatment and the control, 86% and 56%, respectively, is difficult to explain, since the various water parameters measured *viz.*, temperature (30.0-34.5°C), pH (7.4-7.8), dissolved oxygen (3.68-10.5 ppm) and carbon dioxide (0.5-2 ppm), were within the favorable limits for fish growth. Evidently because of poor survival in the control at the end of the growout period, the survivors in this treatment seem to have outgrown the survivors in other treatments to a significant degree, attaining about double the weight of the hormone-treated fish. Meriwether and Torrains (1986) recorded significantly higher growth in untreated (control) tilapia, *Oreochromis aureus*, than in those treated with mibolerone solution

during the treatment period. They also reported that with increasing concentration of mibolerone there was a corresponding negative effect on growth. The weights attained by the hormone-treated groups in the present study during the post-treatment period were almost equal, varying from 37.58 to 44.44 g, and the final body weights under the various treatments were negatively correlated with the survival rate.

Common carp larvae with high doses of 17 α -methyltestosterone over a period of time for sex reversal were reported to grow significantly faster than the untreated (control) larvae during the subsequent growout period, on account of the withdrawal effect of the hormone on growth promotion (Basavaraja and Rao 1988; Ali and Rao 1989).

The sex ratio of the fish in different treatments and control revealed interesting results (Table 1). No females could be seen in any of the hormone-treated groups, while males and females were present in the control at nearly 1:1 ratio.

Most of the fish in the hormone-treated groups were sterile, with their percentage increasing with increasing dosage of the hormone. At 15 mg·kg⁻¹ diet treatment, all the fish were sterile. The gonads of sterile fish were very much different in structure from those of males and females in the control, being only a thread-like structure consisting of connective tissue, devoid of any germ cells (Fig. 1). The few males encountered at the lower two dosages had normal testes.

Meriwether and Torrans (1986) reported that immersion of *O. aureus* fry in 10 ppm mibolerone solution resulted in total mortality within 24 hours, while immersion in 1.0 ppm mibolerone solution over four and six weeks resulted in 63.3% and 61.3% males, respectively, with the remainder having ovotesticular gonads. No individual with ovotesticular gonads (intersex) was observed in the present study.

Sterile common carp with filiform gonads as observed in the present study had earlier been obtained with 17 α -methyltestosterone treatment by Rao and Rao (1983), Basavaraja and Rao (1988) and Ali and Rao (1989). However, the dose required for the production of a female-free population of common carp with that hormone was above 200 ppm (Basavaraja and Rao 1988). This clearly indicates the higher potency of mibolerone for sex reversal of common carp. Lyster and Duncan (1963) have reported that mibolerone is 16 times more potent than methyltestosterone as an androgen in rats.

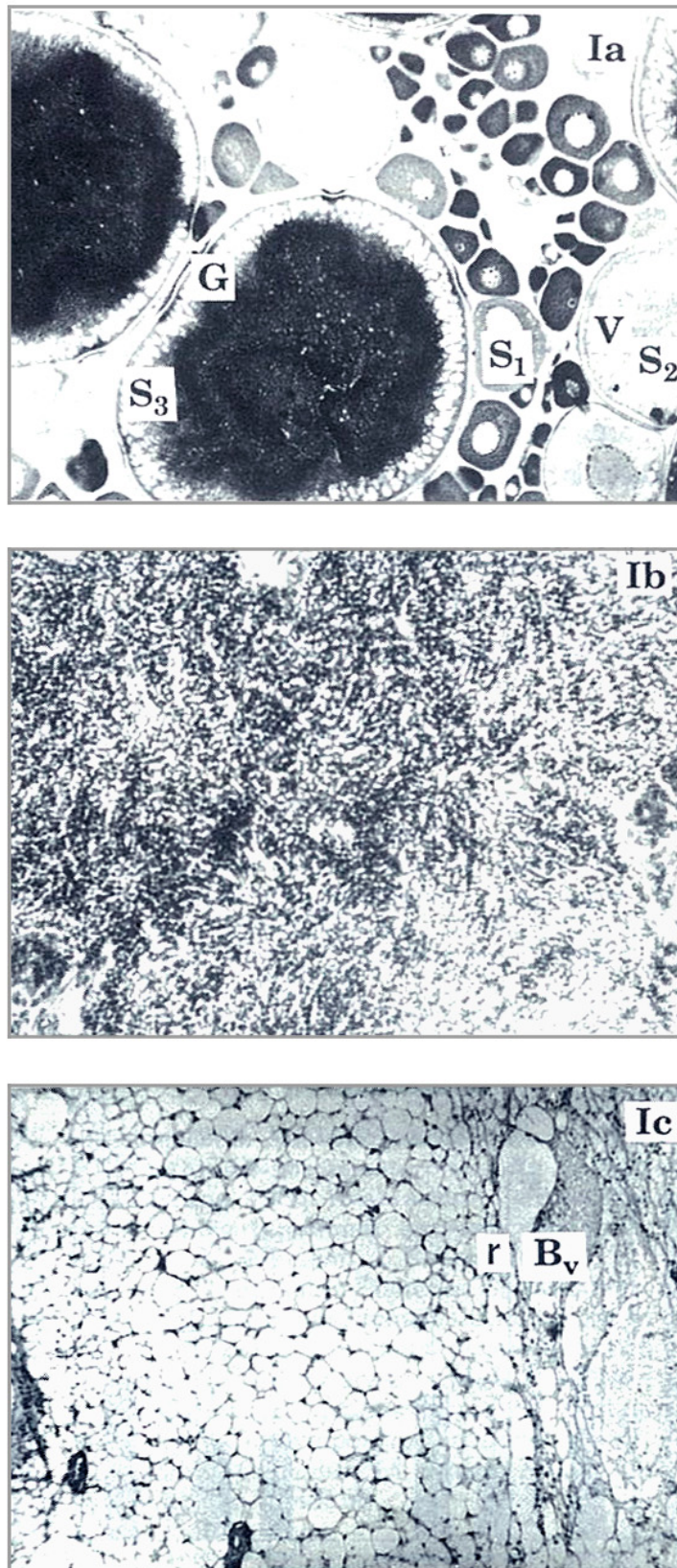


Fig. 1. Photomicrographs of transverse sections of gonads of the common carp, *Cyprinus carpio*. (a) Ovary of normal female showing oogenesis stages, S₁-S₃ with yolk vesicle (V) and yolk globule (G) (x10). (b) Testis of normal male showing different stages of spermatogenesis (x40). (c) Gonad of hormone-induced sterile fish showing only blood vessels (B_v) and connective tissue without any germ cells (x10).

It is well known that in sterile fishes, the entire reproductive energy is rechannelled for somatic growth, resulting in higher dressing weight. The gonadosomatic (GSI) and viscerosomatic (VSI) indices of sterile groups were significantly lower ($P < 0.05$) than those of the controls (Fig. 2). However, the difference in viscerosomatic indices of males in the control group and $5 \text{ mg}\cdot\text{kg}^{-1}$ diet treatment was not significant ($P < 0.01$). Observations of lower GSI and VSI values for hormone-treated fishes have been reported earlier (Rao and Rao 1983; Basavaraja and Rao 1988; Ali and Rao 1989).

The results of the present study demonstrate the effectiveness of mibolerone in producing a sterile dominated female-free population of common carp even at dosages as low as $5 \text{ mg}\cdot\text{kg}^{-1}$. Thus, this new androgen could be profitably employed for production of sterile common carp, if it is found to have no adverse residual effects.

At the present time, the only product containing mibolerone is "cheque drops" which is manufactured and sold by Upjohn Company,

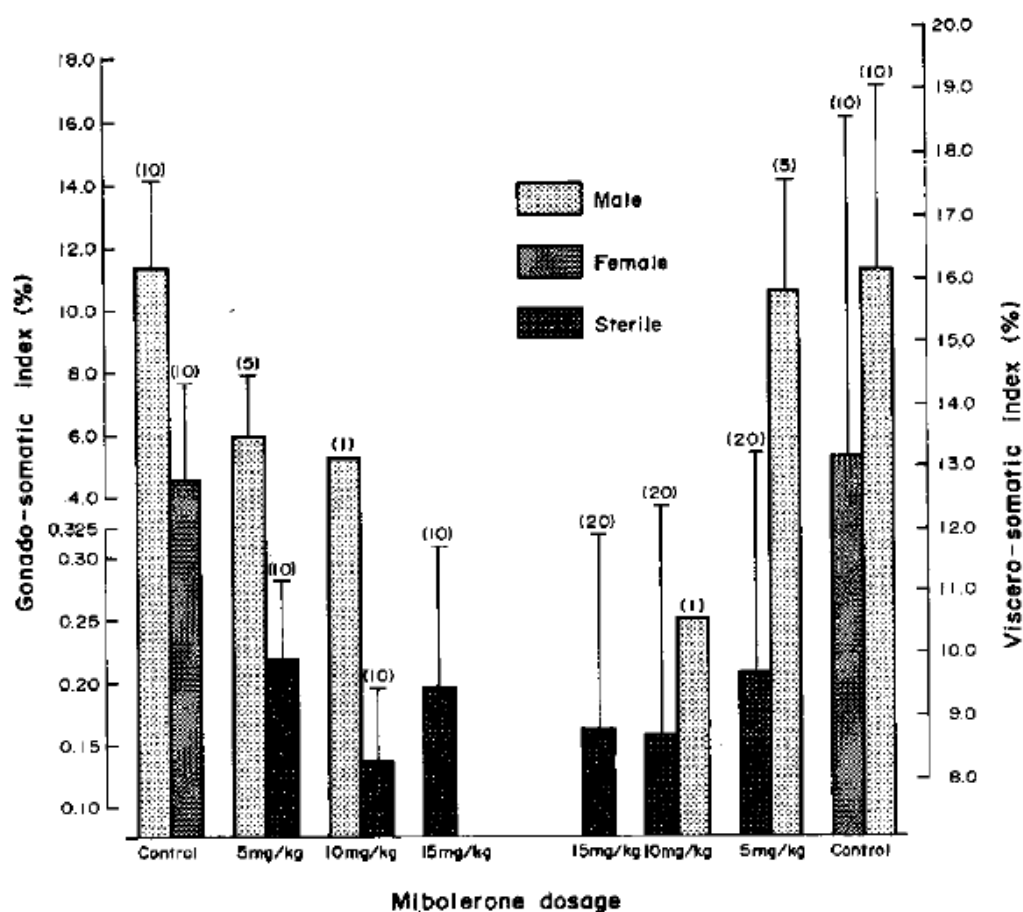


Fig. 2. Effect of dietary administration of mibolerone on the gonadosomatic and viscerosomatic indices of common carp. Vertical lines indicate standard deviation, and numbers in parenthesis represent sample sizes.

USA. It is reported that this veterinary product is administered orally on a daily basis to female dogs to delay estrus (E.F. Nicks, Upjohn Company, pers. comm.).

Acknowledgements

The award of a Junior Fellowship to the first author during the tenure of this study by the Assam Agricultural University is gratefully acknowledged. We are thankful to Prof. (Dr.) T.J. Varghese, Department of Aquaculture, for providing facilities and critical evaluation of the manuscript and to Mr. N. Basavaraja of the Department of Aquaculture for technical help. Grateful thanks are also due to Dr. F.H. Meriwether of the USA for kindly providing the hormones.

References

- Ali, P.K.M.M. and G.P.S. Rao. 1989. Growth improvement in carp, *Cyprinus carpio* (Linn.) sterilized with 17 α -methyltestosterone. *Aquaculture* 76: 157-167.
- APHA. 1985. Standard methods for examination of water and waste water. 16th Edition. American Public Health Association. Washington, DC.
- Basavaraja, N. and G.P.S. Rao. 1988. Induction of sterility in the common carp, *Cyprinus carpio* (Linn.) using 17 α -methyltestosterone. *Indian J. Anim. Sci.* 58(10): 1,248-1,251.
- Gray, P. 1964. Hand book of basic microtechnique. Third Edition. McGraw-Hill, New York.
- Jhingran, V.G. 1982. Fish and fisheries of India. Hindustan Publishing Corporation, Delhi.
- Lyster, S.C. and G.W. Duncan, 1963. Anabolic, androgenic and myotropic activities of derivatives of 17 α -methyl-19-nortestosterone. *Acta Endocrinol.* 43: 399-411.
- Meriwether, F.H. and E.L. Torrans. 1986. Evaluation of a new androgen (mibolerone) and procedure to induce functional sex reversal in tilapia *Oreochromis aureus*, p. 675-678. In J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum, Asian Fisheries Society, Manila, Philippines.
- Rao, H.N.S. and G.P.S. Rao. 1983. Hormonal manipulation of sex in the common carp, *Cyprinus carpio* var. *communis* (Linnaeus). *Aquaculture* 35: 83-88.
- Varghese, T.J., K.V. Devaraj, B. Shantharam and H.P.C. Shetty. 1976. Growth response of the common carp, *Cyprinus carpio* var. *communis* to protein-rich pelleted feed, p. 408-416. In Symposium on Development and Utilization of Inland Fishery Resources, Colombo (Sri Lanka), FAO Regional Office for Asia and the Far East, Bangkok, Thailand.