

# Successful Induction of Sterilization and Growth Improvement in Common Carp *Cyprinus carpio* Through Dietary Administration of the Androgen Norethindrone

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## Abstract

Six-d-old fry of the carp, *Cyprinus carpio* (Linn.) were fed diets incorporated with 5, 25 and 50 mg·kg<sup>-1</sup> diet of norethindrone (NE) for 50 d in fiberglass tanks; this was followed by 180 d rearing in fertilized cement cisterns where hormone-free diet was given.

NE at 5 mg·kg<sup>-1</sup> diet produced 40.00% male, 31.40% female and 28.60% sterile fish. While 25 mg·kg<sup>-1</sup> NE induced 96.30% sterile carp and 3.70% males, 50 mg·kg<sup>-1</sup> produced 100% sterile carp. The sex ratio of the untreated control group was almost 1:1.

Administration of NE at 5 mg·kg<sup>-1</sup> slightly suppressed the gonads (both ovaries and testes). At higher doses, the steroid produced sterile carp which lacked any visible ovarian/testicular portions. After 180 d post-treatment grow-out, the increase in mean final weights of hormone-treated fish was 19.80, 11.30 and 216.32%, respectively, when compared with the control. Loss of weight due to evisceration was 16.40-17.50% in normal fish, and only 8.4-8.90% in steroid-treated groups, suggesting the availability of 8% more edible meat per unit weight from sterile fish compared to normal fish. Results of biochemical analysis revealed that the hormone treatment had a positive effect on fat deposition, and significantly increased RNA/DNA ratio.

The results suggest that it is possible to produce a 100% sterile progeny of common carp, and to improve growth and dressing weight of NE-treated fish. This is the first report on the successful induction of sterilization with NE treatment in this carp. The benefits of stocking grow-out ponds with sterile carp fry are also discussed.

## Introduction

The common carp (*Cyprinus carpio*, Cyprinidae) is one of the most important freshwater fishes used in aquaculture. It occupies third place in aquaculture production after silver carp and grass carp. Its total yield in 1992 was over 1,200,435 metric tons (FAO 1992). The common carp is distributed throughout tropical, sub-tropical and temperate regions. The variety found in India is of Asian origin and is widely used in polyculture/monoculture of carps all over India and other Asian countries. It grows fast and accounts for nearly 46% of the total yield in polyculture (Anon. 1977).

In India, this carp attains maturity within 5-6 months and breeds naturally in the pond (Jhingran 1982). Its early maturation and prolific breeding habit retard growth through competition for food and space. A considerable quantity of metabolizable energy is thus wasted which adversely affects production. In fully mature carp, the gonads comprise 26-30% of body weight (Jhingran 1982). In India, the gonads are normally removed and discarded.

Stocking grow-out ponds with monosex or sterile carp fry would help overcome this problem; research in this area has received considerable attention (Nagy et al. 1981; Basavaraja and Satyanarayana Rao 1988; Komen et al. 1989; Manzoor Ali and Satyanarayana Rao 1989; Das et al. 1990; Sobhana and Nandeeshha 1994). Nagy et al. (1981) were the first to successfully induce sex reversal in common carp (European strain) using  $17\alpha$ -methyltestosterone ( $17\alpha$ -MT). Similarly, sex reversal in the same strain was induced by Komen et al. (1989), and in the Israeli common carp by Gomelsky et al. (1994). These studies reported the production of male-dominated progenies of common carp by oral administration of  $17\alpha$ -MT. Other workers have produced progeny consisting of predominantly sterile fish in the Asian strain of common carp by feeding  $17\alpha$ -MT-incorporated diets (Sathyanarayana Rao and Satyanarayana Rao 1983; Basavaraja and Satyanarayana Rao 1988; Manzoor Ali and Satyanarayana Rao 1989) and by mibolerone treatment (Das et al. 1990; Sobhana and Nandeeshha 1994). The minimum effective dose of  $17\alpha$ -MT to produce all-male or sterile fish varies with strain. Nagy et al. (1981) and Komen et al. (1989) produced a male-dominated population using  $17\alpha$ -MT at 50-100 mg·kg<sup>-1</sup> diet. The minimum effective dose of  $17\alpha$ -MT required to produce a female-free population (Asian strain) is over 200 mg·kg<sup>-1</sup> (Sathyanarayana Rao and Satyanarayana Rao 1983; Basavaraja and Satyanarayana Rao 1988), while that of mibolerone is low, i.e., 2 mg·kg<sup>-1</sup> diet (Sobhana and Nandeeshha 1994). Nevertheless, mibolerone is currently available for non-research applications by veterinary prescription only, and relative cost comparisons are not available (Meriwether and Torrains 1986). In general, cyprinids are highly resistant to hormonal sex manipulation.

Information on the use of 19-norethynyltestosterone (NE) for sex reversal in fish is scanty. The sex manipulation potential of this synthetic androgen was investigated in *Oryzias latipes* by Kawamoto (1969) who found this hormone to be 110 and 15 times more potent than 11-ketotestosterone and  $17\alpha$ -MT, respectively (see Yamazaki 1983). Varadaraj (1990) obtained monosex male *Oreochromis mossambicus* using 19-norethisterone acetate (NE acetate). The present investigation was conducted to evaluate the sex manipulation potential of NE, and its impact on meat yield and body composition in the Asian strain of common carp.

## Materials and Methods

Norethindrone (19-norethisterone; 19-nor-17-alpha-ethynyl-4-androstene-17 beta-ol-one; Sigma) was administered at three levels, 5, 25 and 50 mg·kg<sup>-1</sup> diet. Six-d-old common carp fry, obtained by induced

breeding (*Hydrilla* was used as spawning inducer), were divided into four groups of 100 fry each (average weight 4 mg; average length 7 mm), stocked and reared in circular fiberglass tanks (1.0 m in diameter x 0.60 m height) with a water volume of about 400 l, placed outdoors. Three groups were fed daily on diets containing 5, 25 and 50 mg·kg<sup>-1</sup> NE; the fourth group, which received a hormone-free diet, formed the control. The fry were fed twice daily, morning and evening. Feed consumption was estimated at 200% bw·d<sup>-1</sup> for the first 15 d, 100% bw·d<sup>-1</sup> for the second 15 d and 20% bw·d<sup>-1</sup> for the next 20 d, a total duration of 50 d hormone administration. The tanks were cleaned and water replenished once every 5 d to avoid plankton growth. Hormone incorporation into the diet was accomplished by dissolving 5, 25 or 50 mg NE in 50 ml of 95% ethanol. The stock diet used in the study contained about 30% protein (composition: 40% rice bran, 24% fish meal, 25% groundnut oil cake, 10% tapioca flour and 1% mineral and vitamin mix). The required quantity of powdered stock diet was placed in a plastic basin and sprayed with the hormone solution by means of a chromatogram sprayer to ensure uniform distribution of the hormone. The solvent was allowed to evaporate at room temperature. The control diet was prepared in the same manner using the solvent (ethanol) only.

After 50 d treatment, surviving fish from each group were weighed and transferred to fertilized (10 kg poultry manure·25 m<sup>-2</sup>) outdoor cement cisterns (5 x 5 x 1 m; 0.8-1.0 m water depth) for rearing on a hormone-free diet. During the post-treatment rearing in cisterns, all the groups received a mixture of groundnut oil cake and rice bran (1:1 ratio) containing about 25% protein at a reduced feeding rate of 2-3%. Once every 21 d, the fish were sampled to assess their growth and to readjust the feed quantity. Water in the cisterns was partially replenished once a month to maintain water quality. All the cisterns were re-manured with poultry manure at 5 kg per cistern at monthly intervals.

After 180 d rearing in cisterns, surviving fish were harvested and individual weights recorded. They were sexed by gonadal examination. Fish showing recognizable ovarian and testicular portions were classified as females and males, respectively. Fish with filiform (thread-like) gonads were classified as sterile. The gonadosomatic index (GSI), expressed as gonad weight·total fish weight<sup>-1</sup> x 100, for each treatment was determined and the maturity stage of gonads was assessed visually. Weight loss due to evisceration was determined. The proximate composition of fish muscle, and muscle RNA and DNA contents were determined by the methods of Ceriotti (1955) and Giles and Myers (1965), respectively.

The chi-square test was used to test the equality of the number of males and females in each group, excluding sterile fish. The mean values of different parameters of treated and control groups were subjected to the student's 't'-test. The significant difference between different pairs was tested using ANOVA (Snedecor and Cochran 1968) and the multiple range test (Duncan 1955).

The study was carried out between November and May when air and water temperatures in the tanks and cisterns ranged between 26 and 32°C.

## Results

### *Survival and Growth*

Data on hormone treatment, survival, mean size and sex ratio are presented in Table 1. Survival varied both during hormone treatment and post-treatment periods. Compared to the control, NE-treated groups recorded lower survival (survival decreased with increasing dosage) during hormone treatment, and higher survival during grow-out. At harvest, all hormone-treated groups attained significantly higher growth than control fish ( $P < 0.05$ ), even though the former recorded much higher survival (Table 1 and Fig. 1).

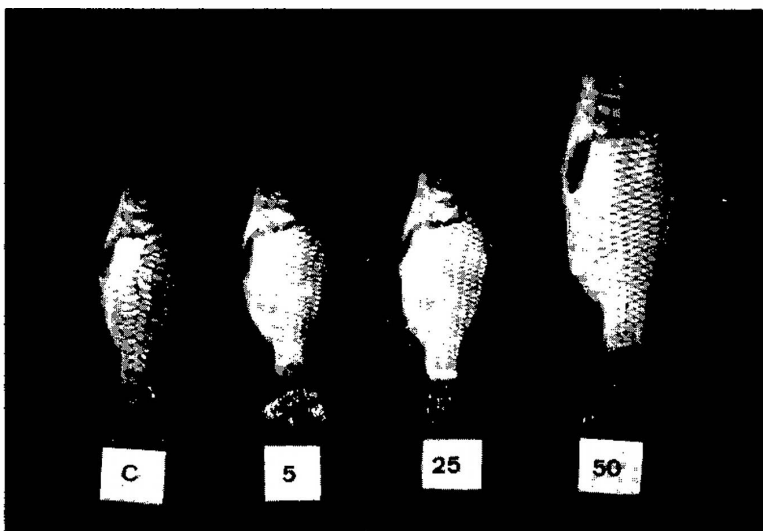


Fig. 1. Photograph showing NE-treated (5, 25 and 50 mg·kg<sup>-1</sup> diet) and control (extreme left) fish at the end of 180-d post-treatment rearing. Note the larger size of the hormone-treated carp.

### *Sex Ratio and Gonads*

The control group showed a sex ratio of 1:1.3 (M:F). The lowest dose tried (5 mg) resulted in the production of male, female and sterile fish (Table 1). At this dose, males to a lesser extent and females to a greater extent appear to have been converted into sterile carp due to the suppression of gonadal development. The next higher dose (25 mg) yielded 3.70% male and 96.30% sterile fish, whereas the highest dose (50 mg) produced only sterile carp, devoid of ovary or testis. The significant dose-dependent increase in the percentage of sterile fish in NE-treated groups was a result of the suppression of gonadal development in both sexes.

The males and females of the control group showed normal gonad development and were found to be either maturing (10%) or mature (90%). Among the males and females in the 5-mg group, about 15% were maturing

Table 1. Initial number stocked, survival, mean size and sex ratio of common carp recorded during the experiment.

No. of fry stocked at the start of experiment	End of hormone treatment		Post-treatment rearing in cisterns				Sex composition			
	Average weight of fry (g)	Survival (%)	No. of fry obtained and stocked	No. of fish re-corded	Survival (%)	Average weight of fish (g) Mean $\pm$ SD	Male	Female	Sterile	Sex ratio
Control	0.21	63	63	23	37	67.40 <sup>a</sup> $\pm$ 22.20 (23)	10 (43.50)	13 (56.50)	0	1:1.30
5 mg·kg <sup>-1</sup>	0.28	40	40	35	88	84.00 <sup>c</sup> $\pm$ 27.32 (35)	14 (40.00)	11 (31.40)	10 (28.60)	1:0.80
25 mg·kg <sup>-1</sup>	0.28	33	33	27	82	75.00 <sup>b</sup> $\pm$ 26.61 (27)	1 (3.70)	0	26 (96.30)	1:0
50 mg·kg <sup>-1</sup>	0.29	20	20	13	65	213.20 <sup>d</sup> $\pm$ 55.78 (13)	0	0	13 (100.00)	0

Numbers in parentheses under fish weight and sex composition denote sample size and percentage of fish, respectively. Values with different superscripts differ significantly ( $P < 0.05$ ).

and 85% were mature. In sterile fish, the development of gonads was almost completely suppressed and they were represented by thread-like (filiform) strands (Figs. 2 and 3). Administration of NE at  $5 \text{ mg}\cdot\text{kg}^{-1}$  slightly suppressed the testes and significantly suppressed ovaries, as indicated by the GSI values (Table 2).



Fig. 2. Photograph showing ovary (left) and testis (center) in control fish, and the filiform gonad (right) in NE-treated sterile fish. Note the thin, thread-like gonad lifted with a spatula in NE-treated carp. Note also the thick musculature in the abdomen in sterile fish, the testis and ovary occupying the entire abdominal cavity, and the thin, sagging musculature clearly discernible in normal fish.



Fig. 3. Photograph showing the exposed gonads. Note the small, thin, filiform sterile gonad of NE-treated fish (center) and the large well-developed ovary (left) and testis (right) of control fish.

Table 2. Influence of NE on the gonadosomatic index (%) of common carp (mean  $\pm$  SD).

Treatment	Male	Female
Control	10.40 $\pm$ 1.58 (8)	10.19 $\pm$ 4.36 (12)
5 mg·kg <sup>-1</sup>	9.55 $\pm$ 2.95 (10)	5.35 <sup>1</sup> $\pm$ 3.34 (8)
25 mg·kg <sup>-1</sup>	14.00 (1)	0
50 mg·kg <sup>-1</sup>	0	0

<sup>1</sup>P<0.05

Numbers in parentheses indicate sample size.

### Evisceration of Fish

Data on total fish production and percentage weight loss due to evisceration are given in Table 3. Total fish production in all NE-treated groups was higher than in the control at harvest. A significant dose-dependent decrease in percentage weight loss due to evisceration was observed in all the sterile carp, the lowest being recorded in the 50-mg group. Even the 'normal fish' of the group treated with 5 mg NE recorded lower weight loss compared to the control.

Table 3. Effect of NE on total fish production and percentage weight loss due to evisceration.

Treatment	Total body weight (g)	Mean body weight (g)	Increment in mean body weight (%) over control	Percentage weight loss due to evisceration (mean $\pm$ SD)	
				Normal fish	Sterile fish
Control	1,550.00	67.40 <sup>a</sup> $\pm$ 22.20 (23)	-	17.50 <sup>a</sup> $\pm$ 4.20 (20)	0
5 mg·kg <sup>-1</sup>	2,940.00	84.00 <sup>b</sup> $\pm$ 27.32 (35)	$\pm$ 19.80	16.40 $\pm$ 4.02 (21)	8.90 <sup>b</sup> $\pm$ 2.65 (9)
25 mg·kg <sup>-1</sup>	2,025.00	75.00 <sup>c</sup> $\pm$ 26.61 (27)	$\pm$ 11.30	0	8.60 <sup>b</sup> $\pm$ 2.55 (19)
50 mg·kg <sup>-1</sup>	2,771.60	213.20 <sup>d</sup> $\pm$ 55.78 (13)	$\pm$ 216.32	0	8.40 <sup>b</sup> $\pm$ 2.03 (9)

Numbers in parentheses denote sample size.

Values with different superscripts differ significantly (P&lt;0.05).

### Biochemical Analysis

Data on the proximate composition of whole fish and muscle nucleic acid levels is presented in Table 4. The results did not reveal any definite trend in the proximate composition of whole fish, barring fat content which showed a progressive increase with increase in the dose of NE (P<0.05). Muscle RNA/DNA ratio was higher in all hormone-treated groups.



Table 4. Effect of NE on the proximate composition of whole fish (carcass)<sup>1</sup> and muscle nucleic acid levels<sup>2</sup>.

Treatment	Proximate composition (%)				Nucleic acids (mg·g <sup>-1</sup> muscle)			
	Moisture	Protein	Fat	Ash	RNA	DNA	RNA/DNA	
Control	75.0 ± 1.17	15.46 <sup>b</sup> ± 0.21	3.79 <sup>a</sup> ± 0.08	3.20 <sup>a</sup> ± 0.25	3.45 <sup>a</sup> ± 0.21	0.72 <sup>c</sup> ± 0.0	4.77 <sup>a</sup> ± 0.30	
5 mg·kg <sup>-1</sup>	72.00 ± 0.49	17.29 <sup>c</sup> ± 0.19	4.49 <sup>b</sup> ± 0.16	4.21 <sup>b</sup> ± 0.11	3.83 <sup>b</sup> ± 0.32	0.43 <sup>a</sup> ± 0.0	8.81 <sup>c</sup> ± 0.73	
25 mg·kg <sup>-1</sup>	72.67 ± 1.23	15.57 <sup>b</sup> ± 0.25	6.28 <sup>c</sup> ± 0.21	3.35 <sup>a</sup> ± 0.12	4.88 <sup>d</sup> ± 0.32	0.58 <sup>b</sup> ± 0.0	8.43 <sup>c</sup> ± 0.55	
50 mg·kg <sup>-1</sup>	72.28 ± 1.02	14.90 <sup>a</sup> ± 0.20	6.81 <sup>d</sup> ± 0.19	3.24 <sup>a</sup> ± 0.09	4.43 <sup>c</sup> ± 0.32	0.72 <sup>c</sup> ± 0.0	6.12 <sup>b</sup> ± 0.44	

<sup>1</sup> Means of three estimations ± SD<sup>2</sup> Means of two determinations ± SD

Values with different superscripts in the same column differ significantly (P&lt;0.05).

## Discussion

The results indicate that 100% sterile common carp can be produced by dietary administration of NE. Under the given experimental conditions, this was successfully achieved at a dietary concentration of  $50 \text{ mg}\cdot\text{kg}^{-1}$  for 50 d, starting 6 d post-hatching. This is the first report on successful inducement of sterility in this species employing NE. Oral administration of other synthetic androgens has earlier been found to induce sterilization and yield progeny consisting of only sterile or female-free populations in the Asian strain (Sathyanarayana Rao and Satyanarayana Rao 1983; Basavaraja and Satyanarayana Rao 1988; Manzoor Ali and Satyanarayana Rao 1989; Das et al. 1990; Sobhana and Nandeesh 1994). In *Oreochromis mossambicus*, Varadaraj (1990) observed 100% masculinization employing NE acetate at a minimum dose of  $3 \text{ mg}\cdot\text{kg}^{-1}$ , administered 10-20 d following 5-d post-hatching.

Predominantly sterile fish of the European strain have also been obtained with  $50\text{-}100 \text{ mg}\cdot\text{kg}^{-1}$   $17\alpha\text{-MT}$  treatment (Komen et al. 1989). With the exception of the study of Sathyanarayana Rao and Satyanarayana Rao (1983), who obtained female-free (having only male and sterile fish) *C. carpio* by treating 1-d-old hatchlings with  $17\alpha\text{-MT}$  over a longer period of 131 d, other workers successfully produced sterile fish (Asian strain) by restricting the hormone treatment period to only 30 or 50 d, starting from 1-6 d post-hatching. Similar observations have also been made in rainbow trout (Jalabert et al. 1975), the European strain of common carp (Komen et al. 1989), coho salmon (Donaldson and Hunter 1982; Shelbourn et al. 1992; Piferrer et al. 1994) and tilapia (Basavaraja et al. 1991).

Results of this study indicate that dietary NE treatment does not induce intersexuality (ovo-testicular condition) in common carp. Earlier studies conducted in this laboratory on hormonal sex control in this strain did not report any intersex carp after  $17\alpha\text{-MT}$  or mibolerone treatment. On the other hand, Nagy et al. (1981), Komen et al. (1989) and Komen et al. (1993) found 38.50, 5.20 and 97% intersex fish (European strain), respectively, with  $17\alpha\text{-MT}$  treatment. However, Gomelsky et al. (1994) did not find any intersex fish (Israeli common carp) although they observed up to 21% sterile fish after treating 40-d-old fry with  $100 \text{ mg}\cdot\text{kg}^{-1}$   $17\alpha\text{-MT}$  for 40 d.

The sterility induced by NE was stable 6 months of post-treatment rearing, and all the sterile fish showed filiform gonads. Our earlier work with  $17\alpha\text{-MT}$  and mibolerone-treated fish reared for 9-12 and 5-7 months, respectively, also revealed that the sterile condition is stable.

Certain anabolic androgens enhance growth at low doses (1-5 ppm) in fishes such as rainbow trout (Yamazaki 1976), common carp (Lone and Matty 1980; Basavaraja et al. 1989), coho salmon (Fagerlund et al. 1983) and Indian major carps (Deb and Varghese 1988). However, information on the growth-promoting effect of NE is lacking. A significant increase in growth of *C. carpio* was observed by Lone and Matty (1980) with various androgens at doses of 1-5 ppm. They also found a retardation of growth with  $17\alpha\text{-MT}$  when the dosage was increased to 10 ppm. Similar effects of  $17\alpha\text{-MT}$  at higher doses have been reported by Clemens et al. (1966),

Yamazaki (1976) and Jensen et al. (1983). In the present study, high doses of androgen resulted in much faster growth (11-20% at comparable stocking densities with the exception of 50 mg NE where highest average growth was obtained due to low survival as a result of *Microcystis* blooms) than in control fish. This may be attributed to the fact that the retarding effect of high doses of androgen ceases after the withdrawal of hormone treatment as reported by Lone and Matty (1980), Basavaraja and Satyanarayana Rao (1988) and Manzoor Ali and Satyanarayana Rao (1989). The faster growth rate of sterile fish in this study may be due to the direction of metabolizable energy (15-20%) from gonadal development to somatic growth. Manzoor Ali and Satyanarayana Rao (1989) recorded 40-47% more growth in  $17\alpha$ -MT induced sterile carp. However, Das et al. (1990) and Sobhana and Nandeeshha (1994) found no increment in the growth of mibolerone-induced sterile carp.

In this study, NE-treated carp not only grew faster, but also showed significantly lower weight loss from evisceration than control fish. The sterile fish, devoid of gonads, yielded about 8% more dressing weight compared to control fish. Similarly, Manzoor Ali and Satyanarayana Rao (1989) obtained 7-8% more meat yield per unit weight upon evisceration in sterile carp in comparison with control fish.

Only the fat content increased in NE-treated groups. While Sobhana and Nandeeshha (1994) found higher protein and fat values in mibolerone-treated common carp, Basavaraja et al. (1988) did not observe any correlation between  $17\alpha$ -MT feeding and proximate composition. In this study, NE feeding also enhanced RNA/DNA ratio. Since growth is accomplished primarily by protein synthesis, the RNA/DNA is considered an effective index for monitoring growth in fish (Love 1980; Khan and Jafri 1991; Mathers et al. 1993). Similar findings of hormonal enhancement of RNA and RNA/DNA has been reported by Lone and Matty (1983) in ethylestrenol-fed common carp. The present observation of comparatively higher RNA and lower DNA contents (per gram of muscle) of NE-fed fish suggests that growth caused by NE is mainly by hypertrophy. In carps, it is reported that hypertrophy claims a major share in growth (Matty and Lone 1985).

Based on hormone dosage, it can be stated that oral administration of NE to common carp fry is 8-10 times more potent than feeding  $17\alpha$ -MT for inducing complete sterility (Basavaraja and Satyanarayana Rao 1988; Manzoor Ali and Satyanarayana Rao 1989). Both hormones were tested under almost identical conditions. In conclusion, our study reveals that the minimum dose of NE required to produce a predominantly sterile (female-free) and 100% sterile progeny of common carp is 25 and 50  $\text{mg}\cdot\text{kg}^{-1}$  diet, respectively, to be fed over 50 d, 6 d post-hatching. Stocking grow-out ponds with only sterile fish would control unwanted reproduction in culture ponds. Higher growth and meat yield of sterile carp obtained with NE may improve economic efficiency.

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