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Effect of Diethylstilbestrol and Norethindrone on Sex Ratio and Body Indices of Common Carp, *Cyprinus carpio* (Linn.)

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Abstract

A synthetic estrogen diethylstilbestrol (DES) and a synthetic androgen, norethindrone (NE) were evaluated for their efficacy on sex ratio and body indices of common carp, *Cyprinus carpio* (Cyprinidae). Five-day-old fry of common carp were fed diets incorporated with DES at 400, 500 and 600mg kg^{-1} diet for 30 days in circular fiberglass tanks; this was followed by 120 days rearing on hormone free-diet in fertilized concrete tanks. All the treatments significantly altered the sex ratio; DES at 400mg kg^{-1} was found to be the best as it produced the lowest percentage of males (7.7). At 400 and 500mg kg^{-1} the production of females was significantly higher. Interestingly, the percentage of intersex fish increased as the dosage of DES increased. The control group had 60.6% males and 39.4% females. In another experiment, NE fed at 50 mg kg^{-1} diet to different age groups (10-, 20- and 30-d-old) of common carp for 50 days, produced 100% male population in all the groups. In control, 52-55.55% males and 44.5-48% females were observed. This is the first report on the complete masculinization of common carp with NE. In both the experiments, the average final weights of common carp varied slightly depending on the survival, which was generally good.

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In general, DES and NE treatments resulted in a slight variation in the gonadosomatic index and hepatosomatic index of common carp. Hormone treatment resulted in lower viscerosomatic index in all the groups excepting 20- and 30-d-old fry. The condition factor of females was higher in all the hormone treated fish, while the values were lower in DES-treated males than that of control. The histological examination of gonads indicated the presence of males, females, sterile and intersex fish in all the DES treated fish, whereas it revealed the presence of only males in all the NE-treated common carp. The oral administration of the gonadal hormones did not significantly affect the proximate composition of the muscle of common carp. The benefits of stocking grow out ponds with monosex or sterile carp fry are also discussed.

Introduction

The common carp, *Cyprinus carpio* (Linn.) is one of the most important freshwater species cultured in the world and is ranked 3rd among the cultured fish species (Crespi 2004). It is widely used in Indian carp polyculture. It grows faster than rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) (Bhimachar and Tripathi 1968). *C. carpio* has advantages over other carp species for aquaculture since it can breed throughout the year and has a shorter generation time. However, early maturation and prolific breeding is a major problem in culture ponds in tropics as this carp attains maturity within 5-6 months (Jhingran 1982) and thus upsets stocking density due to autostocking, which adversely affects final production. Hence, there is a need to produce monosex population which, when stocked in culture ponds, would help overcome undesirable spawning. The gonadal hormones (androgens and estrogens) are widely used for the manipulation of sex in fish. The steroid hormones that are capable of inducing sterility or sex reversal in common carp are 17 α-methyltestosterone (17 α-MT) (Nagy et al. 1981; Sathyanarayana Rao and Satyanarayana Rao 1983; Basavaraja and Satyanarayana Rao 1988; Manzoor Ali and Satyanarayana Rao 1989; Komen et al. 1989, 1993), 17 β-estradiol (17βE_2) (Sathyanarayana Rao and Satyanarayana Rao 1983; Komen et al. 1993), testosterone acetate (Nagaraj and Rao 1988), mibolerone (Das et al. 1990; Sobhana and Nandeesha 1994), norethindrone (Basavaraja et al. 1997a; Manjappa 2004), methyldihydrotestosterone (MDHT) (Basavaraju et al. 2001) and diethylstilbestrol (Basavaraja et al. 1997b).

Nagy et al. (1981) were the first to successfully induce sex reversal in common carp (European strain) using 17α-MT. Later, sex reversal in the same strain was induced using 17α-MT and 17βE_2 (Komen et al. 1989). In India, Sathyanarayana Rao and Satyanarayana Rao (1983) were the first to produce a female free population of common carp (Asian strain) through the dietary application of 17 α-MT. A progeny consisting of 100% sterile common carp was produced by oral administration of NE at 50mg kg^{-1} diet for 50 days starting from first feeding and the hormone was found to be 8-10 times more potent than 17 α-MT (Basavaraja et al. 1997a). On the other hand, DES did not significantly alter the sex ratio at 100mg kg^{-1} diet, but partially induced sterilization at 300mg kg^{-1} for 30 days administered from 5 days post-hatching (Basavaraja et al. 1997b). Production of all-female common carp offspring is preferred since females grow slightly faster than males. It has so far not been possible to produce a 100% male population of the Asian strain of common carp through administration of androgens, barring one

study by Basavaraju et al. (2001) who claims to have achieved 100% masculinization using MDHT. Hence, this study was aimed at achieving complete masculinization and feminization of common carp with NE and DES respectively, as sex reversed males (genetic females, XX) produce an all-female progeny when crossed with normal females.

Materials and Methods

The present study was conducted at the College of Fisheries, Mangalore (India) to evaluate the effect of two steroid hormones on gonadal development and body composition of common carp, *Cyprinus carpio*. The two steroid hormones used in the study, i.e. norethindrone (19- norethisterone; 19-nor-17-alpha-ethynyl-4-androstene-17 beta-ol-one) and diethylstilbestrol (4, 4'-(1-2 diethyl-1, 2-ethenediyl) bisphenol) were obtained from Sigma, USA.

Production of common carp seeds

The common carp fry were produced following the method described by Jhingran and Pullin (1985). Mature male and female common carp were selected based on their external body characters and kept for breeding in a nylon hapa (2x1x1m) fixed in a concrete tank (5x5x1m) at a sex ratio of 1:2 (female:male). Hydrilla, a submerged aquatic weed, was used as substratum for egg collection. The eggs were hatched in nylon hapas (2x1x1m).

Preparation of basal diet and hormone incorporation

The basal diet used for incorporating the hormones contained the following ingredients:rice bran(40%), fish meal(25%), groundnut oil cake(24%), tapioca flour(24%), and mineral and vitamin mix (1%) (with 26% protein). The required quantity of dried pellet was powdered and the powder was sieved to obtain a particular size of 500μm. The required quantity of hormone was dissolved in absolute alcohol and the hormone solution was sprayed on powdered diet with chromatogram column sprayer and mixed so as to achieve uniform distribution of hormone. It was later dried at ambient temperature to obtain dry feed particles. The control diet was prepared in the same manner using only the solvent (ethanol). After drying, the diets were packed in air tight bottles and stored in a dry place.

Administration of hormone

Experiment 1

Five-day-old common carp fry (weight 2.6mg, length 6-7mm) were divided into four groups of 150 each and stocked in circular FRP tanks (mouth diameter 53cm and height 45 cm). The first three groups were fed with diets containing DES at 400, 500 and 600mg kg^{-1} feed, respectively. The fourth group, which received the hormone-free diet, served as control. During the hormone treatment period, the respective groups of fry were fed at 200% of their body weight in split doses (morning and evening) twice daily for the first fifteen days and were given diet at

100% of their body weight for the next fifteen days. The total duration of hormone treatment was 30 days.

Fry/ fingerling rearing

For post-treatment rearing, the fry from respective groups were transferred to prepared concrete tanks (10x5x1m). The tanks (with soil base) were first drained, dried and limed (400kg/ha) and then filled with fresh water up to a depth of 0.8m. The tanks were fertilized with poultry manure at 2000 kg/ha (10 kg/tank). During the grow-out period, the fish were fed on a fish-meal based pelleted diet (26.23% protein) at 5% of their body weight during the first two months, after which they were fed at 2.5% of their body weight, twice daily, morning and evening. The duration of post-treatment period was 120 days.

Experiment 2

Ten-, twenty- and thirty-day-old fry/fingerlings of common carp were used for oral administration of NE. Ten-day-old fry (100 numbers) were stocked in FRP tanks (mouth diameter 53 cm and height 45 cm) and fed on NE at 50mg kg^{-1} diet for 50 days at a feeding rate of 10% of their body weight twice daily, morning and evening. Similarly, twenty and thirty-day-old fry (100 numbers each) were fed with diets containing NE at 50ppm for 50 days. The fourth group (10-day-old fry), which received a hormone-free diet, served as control.

Fry/fingerling rearing

After the specified periods of hormone administration, the surviving fry/fingerlings from respective groups were transferred to nylon hapa (1.5x1x1m) fixed in prepared concrete tanks (5x5x1m) for grow-out purpose. During grow-out period, the fish were fed on a fish meal based pelleted diet (26.23% protein) at 5% of their body weight during the first two months and at 2.5% of their body weight during the rest of rearing period. The duration of post-treatment period was 120-150 days.

Fish were sampled once a month to assess the growth and to readjust the quantity of feed to be given. A minimum of 25% of the total population was sampled each time to record increment in length and weight.

Water quality

During hormone treatment the tank water was changed once every three days to prevent accumulation of metabolites of the steroids used. Fecal matter and left over feed were removed by siphoning to maintain the required water quality. Since *Aeromonas* infection was observed in the experimental tanks, formalin treatment (bath) at 10ppm was given to prevent mortality of fish. The tanks were aerated using a vortex blower.

Evaluation of hormone treatment

Upon termination of the experiment all the surviving fish in the tanks and hapas were harvested, counted and measured individually for length and weight. They were anaesthetized with quinaldine at 10 ppm and dissected out for gonadal examination. Fish with recognizable ovarian and testicular portions were classified as female and male, respectively. Fish with filiform (thread like) gonads were classified as sterile and gonads possessing both ovarian and testicular portions were grouped as intersex fish. Body indices such as gonadosomatic index (GSI), hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor (K) were determined using standard methods. On termination of the experiments, fish meat was collected from all the treatments as well as control and analyzed for proximate composition (moisture, crude protein, crude fat, ash and nitrogen free extract (NFE)) following AOAC (1975) methods.

Histological examination

When the sex of fish could not be determined by macroscopic observation of gonads, the same was confirmed by histological examination wherein the samples of gonads (immature, intersex and filiform gonads) were collected and fixed in buffered formalin for 24 hrs, dehydrated using alcohol, required gonad portions were taken and embedded in paraffin wax. Sections of 5 µ were cut using a microtome and fixed on slides. The sections were stained with Ehrlich haematoxylin-eosin following the procedure described by Gray (1964). The gonadal sections were then examined under a research microscope (Olympus).

Statistical analysis

To test the equality of sex ratio in different groups, Chi-square test was employed. The significant difference between mean values of various parameters of hormone treated and control fish was tested using a computerized statistical package MINITAB.

Results

Experiment 1

Survival and growth

Data on hormone treatment, survival, mean size and sex ratio of common carp fed on DES are presented in table 1. Survival varied both during hormone treatment and post treatment periods. When compared to control, DES-treated groups recorded lower survival (survival decreased with increasing dosage) during hormone treatment and post-treatment, barring 400 ppm. The survival was higher during grow-out. At harvest, the hormone treated groups showed significantly higher growth (Fig. 1) than control.

Sex ratio and gonads

The control group showed a sex ratio of 1:0.65 (M: F). All the treatments resulted in the production of male, female, sterile and inter-sex fish (Table 1). The percentage of males significantly decreased in DES treated group leading to the sex ratio being different from the expected 1:1. While the proportion of sterile fish did not differ much between treatments, the percentage of intersex fish increased as the dosage of DES increased. The males and females of the control group showed normal gonadal development, whereas the DES administered groups showed abnormal testis, thread-like (filiform) and inter-sex gonad (Figs. 2, 3, 4 and 5).

Experiment 2

Survival and growth

Table 2 shows data on the initial number of fry, survival, mean size and sex composition of different group of common carp treated with NE. At the end of NE treatment period, the average survival of fry ranged between 14% (10-d-old) and 86.5% (30-d-old). During post-treatment rearing period, the average survival of fish was higher and it varied between 68 and 100%. At the end of the grow-out period in hapas, the length and weight of fish were found to be low in control and 10-d-old groups, where as they were better (due to extended rearing period) in the 20-d-old and 30-d-old groups (Table 2).

Sex ratio and gonads

The ratio between male and female was normal, i.e. 1:0.86 in control, while only males were encountered in all the three groups treated with NE, indicating 100% masculinization (Table 2). No females were observed in any of the hormone treated groups.

Body indices

The ovarian development was suppressed significantly in all the DES treated groups (indicated by lower GSI values) compared to control (Table 3). The testicular development was stimulated in 500 and 600 mg kg^{-1} groups, while it was inhibited in the 400 mg kg^{-1} group, compared to control. The HSI of both male and female of control and treated groups were comparable. The VSI values of all the treatments were lower than that of control in both males and females, with the 600 mg kg^{-1} group showing the least VSI. The condition factor of female was higher in all the treatments, whereas it was lower in males than that of control (Table 3).

The GSI of males of the NE treated and control was comparable (values are similar) (Table 3). Females were absent in the treated groups. While the HSI and VSI of males of all the groups did not differ significantly, the condition factor was found to be higher in the NE treated groups than the control (Table 3).

Table 1. Initial number stocked, survival, mean size and sex composition of common carp treated with DES

Treatment	No. of fry stocked at the start of the experiment	End of hormone treatment		Post-treatment rearing in tanks					Sex composition					Chi Square test
		No. of fish obtained	Survival (%)	No. of fish stocked	No. of fish recorded	Survival (%)	Average weight (g) Mean ± SE	Average length (cm) Mean ± SE	Male %	Female %S	Sterile	Inter sex	Sex ratio M:F	
Control	150	85	56.7	60	36	60	88.9^{a} ± 3.2 (33)	16.8^{a} ± 0.3 (33)	60.6 (20)	39.4 (13)	0	0	1:0.7	1.1
400 mg.kg^{-1}	150	75	50	60	39	65	80.3^{a} ± 2.6 (39)	16.4^{a} ± 0.2 (39)	7.7 (3)	69.2 (27)	15.4 (6)	7.7 (3)	1:9	19.2*
500 mg.kg^{-1}	150	64	42.7	60	29	48.3	95.8^{a} ± 3.5 (29)	17.5^{a} ± 0.2 (29)	13.8 (4)	55.2 (16)	13.8 (4)	17.2 (5)	1:4	7.2*
600 mg.kg^{-1}	150	30	20	30	15	50	194.3^{b} ± 7.6 (15)	21.8^{b} ± 5.4 (15)	13.3 (2)	40 (6)	20 (3)	26.7 (4)	1:3	2

Numbers in parenthesis under fish weight and length and sex composition denote sample size. * Significant (P<0.05).
Values with different superscripts in same column differ significantly (P<0.05).

Table 2. Initial number stocked, survival, mean size and sex composition of common carp treated with NE

Treatment	No. of fry stocked at the start of the experiment	End of hormone treatment		Post-treatment rearing in tanks					Sex composition					Chi Square test
		No. of fish obtained	Survival (%)	No. of fish stocked	No. of fish recorded	Survival (%)	Average weight (g) Mean ± SE	Average length (cm) Mean ± SE	Male %	Female %	Sterile	Inter sex	Sex ratio M:F	
Control	200	50	25	50	39	68	8.7 ± 2.94 (39)	8.2 ± 0.8 (39)	53.6 (21)	46.3 (18)	0	0	1:0.9	0.23
10-d-old	200	28	14	28	24	85.7	9.4 ± 0.7 (24)	8.35 ± 0.2 (24)	100 (24)	0	0	0	1:0	24*
20-d-old	200	143	72.5	60	60	100	35.7 ± 1.7 (53)	11.9 ± 0.2 (53)	100 (53)	0	0	0	1:0	53*
30-d-old	200	173	86.5	60	60	100	40.0 ± 3.1 (53)	12.3 ± 0.2 (53)	100 (53)	0	0	0	1:0	53*

Numbers in parenthesis under fish weight and length and sex composition denote sample size. * Significant (P<0.05).

Table 3. Influence of DES and NE on body indices of common carp (mean ± SE)

Body indices		DES				Age of fry			
		Control	400 mg.kg^{-1}	500 mg.kg^{-1}	600 mg.kg^{-1}	Control	10-d-old (50ppm NE)	20-d-old (50ppm NE)	30-d-old (50ppm NE)
GSI (%)	Male	3.4^{b} ± 0.13 (10)	2.3^{a} ± 0.32 (3)	3.7^{b} ± 0.88 (4)	5.59^{c} ± 1.30 (2)	0.6 ± 0.16 (20)	0.48 ± 0.18 (10)	0.41 ± 0.07 (20)	0.65 ± 0.09 (20)
	Female	3.4^{b} ± 0.08 (10)	1.2^{a} ± 0.32 (10)	0.8^{a} ± 0.15 (10)	1.45^{a}± 0.29 (6)	0.4 ± 0.12 (20)	0	0	0
HSI (%)	Male	1.0^{a} ± 0.03 (10)	0.9^{a} ± 0.14 (3)	1.0^{a} ± 0.07 (4)	0.6^{a} ± 0.23 (2)	1.4 ± 0.12 (20)	1.56 ± 0.09 (10)	1.58 ± 0.07 (20)	1.52 ± 0.30 (20)
	Female	1.0^{a} ± 0.07 (10)	1.2^{a} ± 0.10 (10)	1.1^{a}± 0.08 (10)	0.9^{a} ± 0.11 (6)	1.4 ± 0.11 (20)	0	0	0
VSI (%)	Male	15.5^{c} ± 0.74 (10)	11.4^{a} ± 0.71 (3)	12.7^{b} ± 0.74 (4)	11.8^{ab} ± 1.56 (2)	15.0 ± 1.16 (20)	13.5 ± 1.29 (10)	15.2 ± 0.95 (20)	15.8 ± 0.72 (20)
	Female	11.8^{b} ±0.72 (10)	11.5^{b} ± 0.82 (10)	11.3^{b} ± 1.19 (10)	6.5^{a} ± 0.98 (6)	13.7 ± 0.95 (20)	0	0	0
K (%)	Male	5.6^{b} ± 0.75 (10)	3.3^{a} ± 0.13 (3)	4.0^{a} ± 0.22 (4)	3.2^{a} ± 0.0 (2)	2.6 ± 0.12 (20)	3.1 ± 0.19 (10)	4.0 ± 0.17 (20)	3.7 ± 1.50 (20)
	Female	1.6^{a} ±0.21 (10)	3.3^{b} ± 0.12 (10)	3.3^{b} ± 0.13 (10)	2.6^{b} ± 0.18 (6)	2.5 ± 0.14 (20)	0	0	0

Numbers in parenthesis indicate sample size. Values with different superscripts in the same row differ significantly ($P<0.05$).

Histology

The histological examination of gonads from control fish revealed the presence of testis and ovary in different stages of spermatogenesis and oogenesis (Fig. 6 and Fig. 7). The intersex gonads showed both the ovarian and testicular portions (Fig. 8). The sterile gonads (filiform) revealed the absence of any germ cells, but constitute of mostly connective tissue (Fig. 9).

Proximate composition

Data on the proximate composition of whole fish muscle of carp fed on DES diets is presented in table 4. The result did not reveal any definite trend in the proximate composition of common carp, excepting NFE content which showed an increase with an increase in the dosage of DES.

Data on the proximate composition of muscles of different age groups of common carp fed on NE is depicted in table 4. There was a slight variation in moisture, protein, and NFE contents of hormone treated fish compared to control. Fat and ash contents showed a slight increase in the groups that were fed on NE.

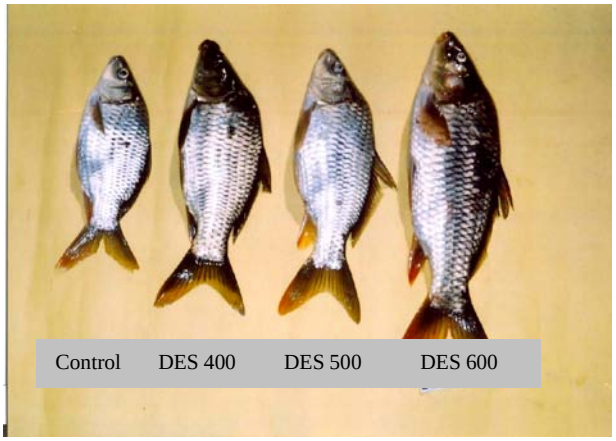

Fig. 1. Photograph showing control and DES treated (400, 500 and 600 mg kg^{-1}) fish at the end of 150 days post-treatment rearing. Note the larger size of steroid treated fish

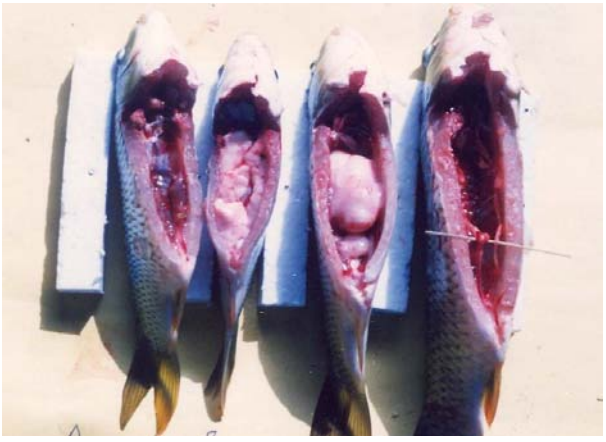

Fig. 2. Photograph showing normal ovary, normal testis, abnormal testis and filiform gonad, respectively, of DES treated fish. Note the thicker musculature of sterile fish.

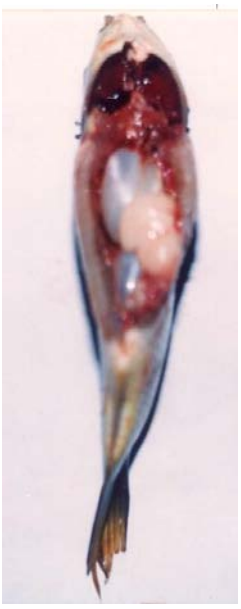

Fig. 3. Photograph showing two lumps of abnormal testis (whitish)

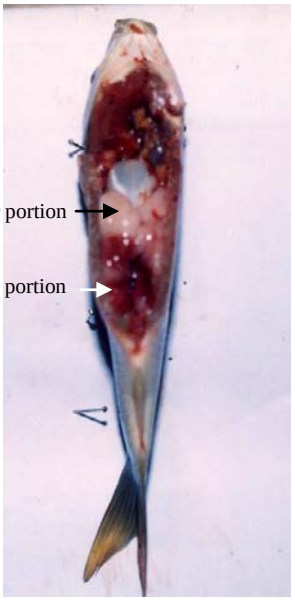

Fig. 4. Photograph showing intersex carp (DES 600). Note the testicular (whitish) portion interspersed amidst ovarian portion (reddish)

Table 4: Effect of DES and NE on proximate composition of whole fish (carcass)[1]

Proximate composition	DES				Age of fry			
	Control	400 mg.kg^{-1}	500 mg.kg^{-1}	600 mg.kg^{-1}	Control	10-d-old (50ppm NE)	20-d-old (50ppm NE)	30-d-old (50ppm NE)
Moisture (%)	80.1 ± 0.1	79.5 ± 0.1	80.0 ± 0.4	78.4 ± 0.4	79.1 ± 0.6	79.7 ± 0.3	78.5 ± 0.4	78.5 ± 0.2
Protein (%)	13.9 ± 0.7	14.3 ± 0.5	13.0 ± 0.7	14.4 ± 0.7	14.3 ± 0.6	13.7 ± 0.6	13.8 ± 0.6	14.3 ± 0.6
Fat (%)	2.0 ± 0.10	2.0 ± 0.11	2.1 ± 0.06	2.1 ± 0.1	2.3 ± 0.3	2.5 ± 0.2	2.7 ± 1.8	2.5 ± 0.2
Ash (%)	3.4 ± 0.6	3.4 ± 0.3	3.6 ± 0.2	3.5 ± 0.5	2.6 ± 0.5	2.7 ± 0.3	2.9 ± 0.4	3.2 ± 0.2
NFE (%)	0.6 ± 0.2	0.8 ± 0.3	1.3 ± 0.2	1.6 ± 0.3	1.7 ± 0.2	1.4 ± 0.2	2.1 ± 5.3	1.5 ± 0.4

[1]Means of three estimations ± SE
There was no significant (P<0.05) difference between the mean values of different treatments.

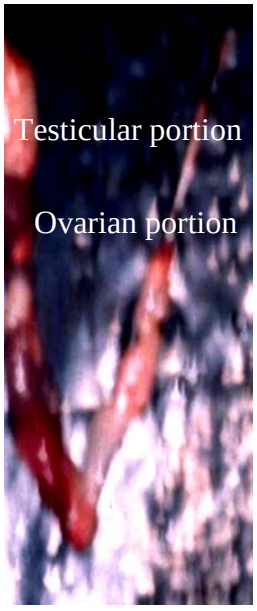

Fig. 5. Photograph showing the mounted intersex gonad of DES treated carp. Note the abnormal lobes containing testicular (whitish) and ovarian (reddish) portions

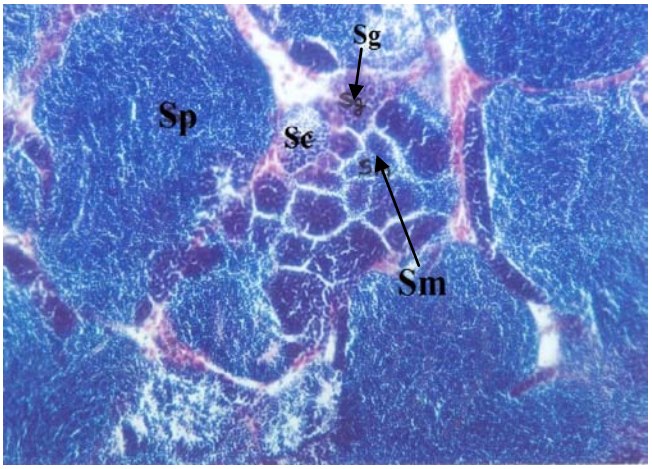

Fig. 6. Photomicrograph of testis of control fish showing different stages of spermatogenesis (x 200): spermatogonium (Sg), spermatocyte (Sc), spermatids (Sm) and spermatozoa (Sp)

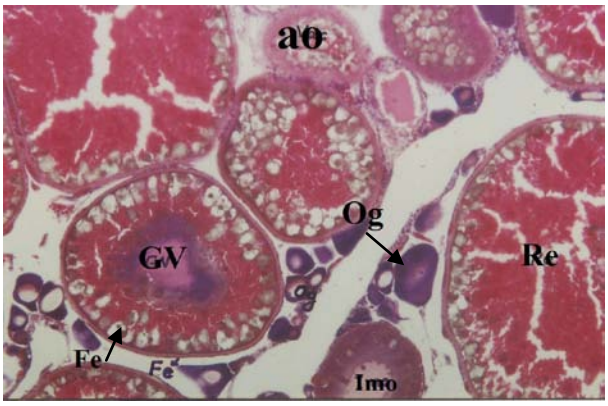

Fig. 7. Photomicrograph of ovary of control fish showing different stages of oogenesis (x 200): germinal vescicle (GV), immature oocyte (Imo), follicular epithelium (Fe), ripe egg ready to ovulate (Re), atretic oocyte (ao) and oogonia (Og)

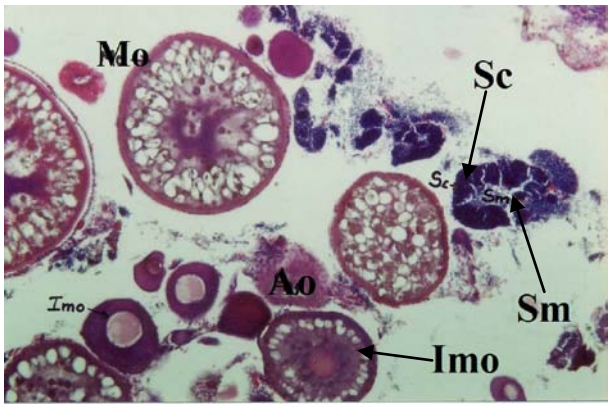

Fig. 8. Photomicrograph of DES treated inter-sex gonad showing testicular and ovarian portions (x200): spermatocyte (Sc), spermatids (Sm), immature oocyte (Imo), maturing oocytes (Mo) and atretic oocyte (Ao)

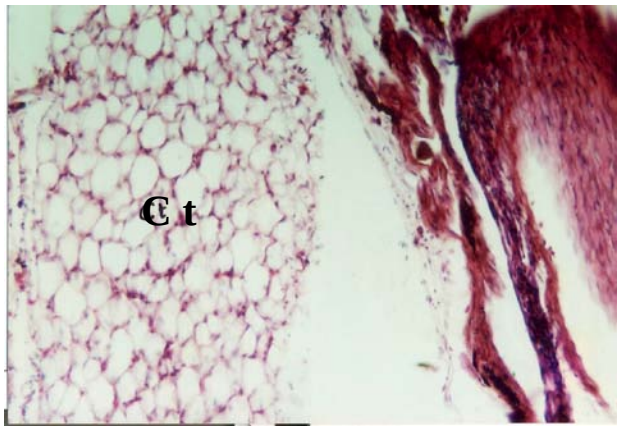

Fig. 9. Photomicrograph showing sterile gonad of DES treated carp. Note connective tissue (Ct) without any germ cells (x 400)

Discussion

The results demonstrate that it is possible to alter the sex ratio of common carp, *Cyprinus carpio* by oral administration of synthetic gonadal hormones, diethylstilbestrol and norethindrone. It is evident that the percentage of males in all the DES treated groups has been reduced significantly leading to the production of higher percentage of females, barring 600 mg kg^{-1}, and sterile and intersex fish. Earlier studies conducted in this laboratory and elsewhere revealed that different estrogens $17\beta E_2$, $17\beta E_2$ benzoate and DES failed to induce complete sex reversal/sterilization, but induced partial sterilization/sex reversal in the Asian strain of common

carp (Sathyanarayana Rao and Satyanarayana Rao 1983; Nagaraj and Rao 1988; Komen et al. 1989; Basavaraja et al. 1997b) albeit high doses (as high as 800mg kg^{-1}) were employed. Sathyanarayana Rao and Satyanarayana Rao (1983) obtained 15.20% male, 41.80% female and 43% sterile fish at 200mg kg^{-1} 17βE_2 fed for 131 days, while Nagaraj and Rao (1988) reported the occurrence of 64% females and 36% males when 800mg kg^{-1} 17βE_2 benzoate treated diet was given for 30 days. This indicates that common carp is more resistant to oral administration of estrogens than androgens. Sehgal and Saxena (1997) obtained up to 82.5% female common carp after the dietary administration of estrone at 200mg kg^{-1} for 60 days. Varadaraj and Pandian (1989) reported that feeding of 9-d-old fry *adlibitum* with DES supplemented diet at 100mg kg^{-1} for 11 days, resulted in an all-female population of tilapia (*Oreochromis mossambicus*). Similarly, Basavaraja et al. (1990) reported 100% sex reversal in *O. mossambicus* through dietary administration of DES at 50 and 100 ppm for 30 d to the yolk sac absorbed fry. On the other hand, a population consisting of a 16.67% sterile fish, 60.00% males and 23.3% female common carp was obtained by the oral administration of DES at 300mg kg^{-1} diet for 30 days from first feeding (Basavaraja et al. 1997b).

In the present investigation, NE administered at 50 $mg.kg^{-1}$ diet for 50 days to 10-, 20- and 30-day-old fry produced all-male population of common carp, indicating 100% masculinization. This is the first report on the successful sex reversal of the common carp using norethindrone. Manjappa (2004), however, observed different proportion of males, females and sterile fish when 40-, 50-, 60- and 70-d-old common carp were fed with NE at 50 mg kg^{-1} for 50 days. The differential sexual types obtained in the two studies may be attributed to the timing of NE treatment, wherein younger and older fry were used in the present and earlier studies, respectively. The all male progeny observed in the present study is probably the result of the timing of treatment (10 to 30-d-old fry and above) compared to all the earlier studies, which reported 100% sterile fish or sterile dominated population, wherein the yolk-sac absorbed fry was used for steroid treatment. Basavaraja et al. (1997a) successfully produced 100% sterile or sterile dominated population of common carp using 6-day-old fry, treated with NE at 5, 25 and 50mg kg^{-1} diet for a period of 50 days. In *O. mossambicus*, Varadaraj (1990) observed 100% masculinization employing norethindrone acetate at a minimum dose administered 10-20 d following 5-d post-hatching. Kumaraswamy (2002) produced a predominantly male dominated population of red tilapia by oral administration of NE at 30 and 40mg kg^{-1}. Our findings are useful to Indian carp freshwater aquaculture since stocking pond with monosex male common carp fry would help overcome undesirable spawning.

Our study also indicates that the oral administration of DES resulted in the production of females, males, sterile and intersex fish. This observation suggests that the sex ratio of common carp was altered not only by sex reversal from male to female (higher percentage of female is indicative of this), but also by the suppression of testicular development leading to sterilization and inter-sexuality; intersex fish proportion increased as the dosage of DES increased.

Inter-sexuality in the European strain of common carp was observed when 17α-MT and 17βE_2 were administered at different concentrations (Nagy et al. 1981; Komen et al.1989, 1993). In the present investigation, the DES treatment at various dosages produced different proportions

of intersex common carp, showing an increasing proportion of intersex fish with an increase in the dosage of DES, which probably indicates the sub-optimum dosage level of DES treatment for complete feminization. The NE treatment did not yield any intersex fish in any of the treatment groups.

The administration of gonadal hormones has been reported to cause sterility by the suppression of gonadal development in tilapia (Basavaraja et al. 1991), common carp (Sathyanarayana Rao and Satyanarayana Rao 1983; Das et al. 1990; Basavaraja et al. 1997a and Bharadwaj and Sharma 2000; Manjappa 2004), salmon (Hunter et al. 1982; Donaldson and Hunter 1982) and red tilapia (Raghavendra 2002; Kumaraswamy 2002). In the present investigation dietary administration of DES at 400, 500 and 600 mg.kg^{-1} diet induced 15.4, 13.8 and 20% sterility, respectively, while NE at tested doses did not produce any sterile fish. This may be attributed to the sub-optimum dose of the former hormone. The differential responses of the two hormones can be substantiated by the fact that androgens are generally more potent than estrogens in sex manipulation in fish.

Abnormal gonads were observed in large-mouth bass treated with DES (Salam et al. 2001). The effect was most profound when DES was fed at 400mg kg^{-1} (ovarian deformity of 7-11%). In the present study, DES at 600mg kg^{-1} produced 20% abnormal testis/ovaries, whereas at 400 and 500mg kg^{-1} the abnormality was 13.7 and 7.7%, respectively. Our results also reveal that NE treatment did not result in any apparent gonadal deformity.

In the common carp, the gonads account for nearly 26-30% of body weight (Jhingran 1982) and lead to the major loss of weight due to evisceration. Manzoor Ali and Satyanarayana Rao (1989) reported that loss of weight due to evisceration was only 5.6-7.5% in the 17α-MT treated fish, compared to 14.95% in control. Basavaraja et al. (1997a) recorded higher dressing weight in hormone (NE) treated fish than the control. In the present study, the GSI of DES treated fish showed a slight variation. In the NE treated groups, the testicular development was slightly suppressed, while the ovarian development was completely inhibited. Administration of NE at 5mg kg^{-1} diet slightly suppressed testis and significantly suppressed ovaries in common carp (Basavaraja et al. 1997a). Bharadwaj and Sharma (2000) observed completely suppressed gonads in common carp fed with 17α-MT at 600mg kg^{-1}. On the contrary, Manjappa (2004) reported that the ovarian development was stimulated when NE was fed at 50mg kg^{-1} for 50 days to 40-60 days old common carp.

The results of the present study also reveal no definite trend in the hepatosomatic index of DES treated groups. Manjappa (2004) found no significant difference in the HSI of male and female common carp treated with NE. However, the viscerosomatic index (VSI) of males and females of treated groups was lower than that of control, owing to gonadal suppression. The higher condition factor of females of DES treated groups could be attributed to slower rate of increase in length in relation to fish weight gain. Similar results were reported in rainbow trout and diploid and triploid male tilapia (Lincoln and Scott 1984; Hussain et al. 1995).

Administration of synthetic hormones has been reported to affect the biochemical composition of test animals. The results of the present study did not indicate any definite trend with regard to moisture, protein, fat, ash and nitrogen free extract content between treated and untreated fish.

The present investigation reveals the possibility of manipulating the sex ratio in common carp by oral administration of DES and NE. However, further research needs to be conducted to produce a male-free population of *Cyprinus carpio* using DES. Oral administration of NE at 50mg kg^{-1} for 50 days to 10-, 20-, and 30-d-old fry results in 100% sex reversal in common carp. Further research on progeny testing of the sex reversed fish and production of an all-male population through selective breeding is required.

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References

AOAC. 1975. Official methods of analysis of the Association of Analyitical Chemists. 12th edition, Washington, DC. Pp. 1094.

Basavaraja, N. and G. P. Satyanarayana Rao. 1988**.** Introduction of sterility in the common carp *Cyprinus carpio* (Linn) using 17α -methyltestosterone. Indian Journal of Animal Sciences 58(10): 1248-1251.

Basavaraja, N., B. Gangadhar and K. S. Udupa. 1997a. success full induction of sterilization and growth improvement in common carp, *Cyprinus carpio* through dietary administration of the androgen Norethindrone. Asian Fisheries Science 10: 29-40.

Basavaraja, N., B. Gangadhar and K. S. Udupa. 1997b. Effect of diethylstilbestrol-incorporated diet on sex ratio and body composition of common carp, *Cyprinus carpio*. Journal of Aquaculture in the Tropics 12: 209– 218.

Basavaraja, N., M. C. Nandeeesha, T. J. Varghese and P. Keshavanath. 1991. effect of feeding high-levels of 17α-MT on the sex ratio and growth of two sizes of *Oreochromis mossambicus* (Peters). Indian Journal of Animal Sciences 61(7): 776-779.

Basavaraja, N., M. C. Nandeeesha, T. J. Varghese, P. Keshavanath and G. K. Srikanth. 1990. Induction of sex reversal in *Oreochromis mossambicus* by diethylstilbestrol. Journal of Applied Ichthyology 6: 46–50.

Basavaraju, Y., G. C. Mair, D. J. Penman, S. A. S. Ahemad, H. M. Mohankumar and S. Pradeep kumar. 2001. Masculinization in sex reversal in common carp (*Cyprinus carpio*). An approach for the indirect production of monosex female carp as a possible solution to control unwanted reproduction. 6th Asian Fisheries Forum, Kooshiung (Taiwan), 25-30 No 2001, pp. 23**.** (Abstract).

Bharadwaj, R. and L. L. Sharma. 2000. Effect of methyltestosterone (tablets) in sterilization and masculization of common carp, *Cyprinus carpio communist (L*). Indian Journal of Fisheries 47(4): 377-381.

Bhimachar, J. E. and S. D. Tripathi. 1968. A review of culture fisheries activities in India, FAO Fisheries Report 44(2): 14-17.

Crespi, V. 2004. FAO Fact sheets on Aquaculture, FAO Aquaculture News Letter, 32: 9-11.

Das, S. K., H. P. C. Shetty and M. C. Nandeesha. 1990. Production of female-free common carp, *Cyprinus carpio var. communis (L.)* through dietary administration of the androgen mibolerone. Asian Fisheries Science 3: 197–203.

Donaldson, E. and G. Hunter. 1982. Sex control in fish with particular reference to salmonids. Canadian Journal of Fisheries and Aquatic Sciences 39: 99– 110.

Gray, P. 1964. Handbook of Basic Microtechnique, ed. 3, McGraw Hill Book Company, New York. 302p.

Hunter, G. A., E. M. Donaldson, F. W. Goetz and P. R. Edgell. 1982. Production of all-female and sterile coho salmon, and experimental evidence for male heterogamety. Transactions of the American Fisheries Society 111: 367–372.

Hussain, M. G., G. P. S. Rao, N. M. Humayun, C. F. Randall, D. J. Penman, D. Kime, N. R. Bromage, J. M. Myers and B. J. McAndrew. 1995. Comparative performance of growth, Biochemical composition and endocrine profiles in diploid tilapia *Oreochromis niloticus*. Aquaculture (138): 87-97.

Jhingran, V. G. 1982. Fish and Fisheries of India. Hindustan Publishing Co., Delhi, India, 666 pp.

Jhingran, V. G. and R. S. Pullin. 1985. A hatchery manual for common Chinese and Indian major carps. Asian Development Bank and ICLARM, Manilla, Phillipines, 199p.

Komen, J., E. H. Eding, H. B. J. Bonger and C. J. J. Richfer. 1993. Gynogenesis in common carp *Cyprinus carpio* IV. Growth, phenotypic variation and gonadal differentiation in normal and MT-treated homozygous clones and F1 hybrids. Aquaculture, 111: 271-280.

Komen, J., P. A. J. Lodder, F. Huskins, C. J. J. Richter and E. A. Huisman. 1989. Effects of oral administration of 17α-methyltestosterone and 17β-estradiol on gonadal development in common carp, *Cyprinus carpio* L. Aquaculture, 78: 349-363.

Kumaraswamy, A. R. 2002. Effect of an androgen norethindrone, on sex reversal in red tilapia. M.F.Sc., Thesis submitted to University of Agricultural Sciences, Bangalore pp. 1-57.

Lincoln, R. F. and A. P. Scott. 1984. Sexual maturation in triploid rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Biology 25: 385-392.

Manjappa, N. 2004. Manipulation of sex in the common carp, *Cyprinus carpio* (LINN) using norethindrone, M.F.Sc Thesis, submitted to University of Agricultural Sciences, Bangalore pp. 1-67.

Manzoor Ali, P. K. M. and G. P. Satyanarayana Rao. 1989. Growth improvement in carp, *Cyprinus carpio* (Linnaeus), sterilized with 17alpha-methyltestosterone. Aquaculture 76: 1– 2.

Nagaraj, C. G. and G. P. S. Rao. 1988. Effect of testosterone acetate and estradiol benzoate on sexuality and growth of *Cyprinus carpio* (Linn). In: M. Mohan Joseph (ed). *The First Indian Fisheries Forum, Proceedings.* Asian Fisheries Society Indian Branch, Mangalore. pp.115-117.

Nagy, A., M. Bercsenyi and V. Csanyi. 1981. Sex reversal in carp (*Cyprinus carpio*) by oral administration of methyltestosterone. Canadian Journal of Fisheries and Aquatic Science 38: *725– 728.*

Raghavendra, C. H. 2002**.** Induction of masculinization in red tilapia by endocrine sex reversal. M.F.Sc thesis, submitted to University of Agricultural Sciences, Bangalore pp.1-53.

Salam, A. Ai-Ablani, P. Ronald and Phelps. 2001. Induction of feminization in large mouth bass (*Micropterus Salmoides*) by oral administration of Estradiol-17β or Diethylstilbestrol and associated Pathological changes. Journal of Aquaculture in the Tropics 16 (2): 185-195.

Sathyanarayana Rao, H. N. and G. P. Satyanarayana Rao. 1983. Harmonal manipulation of sex in the common carp. *Cyprinus carpio var. Communis* (Linnaeus). Aquaculture 35: 83-88.

Sehgal G. K. and D. K. Saxena. 1997. Effect of estrone on Sex composition growth and flesh composition in common carp, cyminus carpio communis (Lim). Journal of Aquaculture in the Tropics 289-295.

Sobhana, K. S. and M. C. Nandeesha. 1994. Standardization of mibolerone dosage for production of female-free common carp (*Cyprinus carpio var. communis* L.) and the impact of the hormone of growth and flesh composition. Journal of Aquaculture in the Tropics 9: 133–139.

Varadaraj, K. and T. J. Pandian. 1989. First report on production of supermale tilapia by integrating endocrine sex reversal with gynogenetic technique. Current Science (Bangalore) 58: 434–441.

Varadaraj, K. 1990. Production of monosex male Oreochromis mossambicus (peters) by administering 19-norethisterone acetate. Aquaculture and Fisheries Management 21 (1): 133-135.

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