



17 α -Methyltestosterone can induce masculinisation and sterilisation in amur carp *Cyprinus rubrofasciatus* Lacepede 1803

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ABSTRACT

To find a suitable solution to the problem of early sexual maturation and unwanted reproduction during grow-out, the present study was aimed at production of sterile or monosex population of amur carp through endocrine manipulation. In this investigation, 24 day old amur carp fry were fed with diets containing 17 α -methyltestosterone (17 α -MT) at dosages of 200-1000 ppm for 30 d. While 17 α -MT incorporation at 400 ppm resulted in complete masculinisation, 600 ppm induced 100% sterilisation. 17 α -MT at 200 ppm induced the production of intersex and sterile fish. At 800 ppm, it produced 8.33% male, 8.33% female and 83.34% sterile fish, whereas 1000 ppm yielded 63.63% male, 9.10% female and 27.27% sterile fish. After 168 days post-treatment rearing, increase in fish weight was 16.01- 233.15%, over control. Loss of weight due to evisceration was 8.75-12.43% in control, while it was 7.31-9.78% in sterile fish, indicating the availability of more edible meat in the latter. MT-treated carp showed a slight variation in survival, gonadosomatic index (GSI), hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor. The histology of gonads revealed normal development of ovary and testis in control fish, but in 17 α -MT treated fish the gonadal development was either normal or abnormal in male and slightly suppressed in female. Intersex fish had both ovarian and testicular tissues, while sterile fish possessed mostly connective tissue.

Keywords : Amur carp, Masculinisation, 17 α -Methyltestosterone, Reproduction, Sterilisation

Introduction

In India, the exotic common carp (an Asian strain that was introduced into India in 1957) is an important aquaculture species, contributing significantly to enhanced fish production. In a tropical country like India, it attains early sexual maturation (<6 months at a weight of 50 to 100 g) and spawns in grow-out ponds before attaining marketable size of about 1000 g. This results in poor growth due to competition for food and space and the gonadosomatic index can exceed 20% of the harvested weight (Basavaraju *et al.*, 2008). Hence, there was a need to utilise a late maturing common carp breed like amur carp (*Cyprinus rubrofasciatus* Lacepede 1803) for small-scale farming in monoculture as well as in polyculture along with other carps.

The amur carp is an original wild form, which was originally found in the Western Asian and Eastern European rivers. Amur strain of common carp, introduced into India a decade ago, performed consistently superior to all other stocks, including local stocks, in all trials across culture systems and environments in India (Basavaraju, 2013). The increase in weight of amur strain

over the local stocks was 13.2 to 50.1%, with a mean increase of 27.3% and it matures at the end of first year in hinterland. However, even the amur carp was known to attain maturity (male in particular) at 6 months in culture ponds in coastal Karnataka (Basavaraju, pers. comm.). One possible solution to the problem of early maturation is masculinisation and/or sterilisation of the fry prior to stocking in culture pond. Application of monosex/sterile fish production technique will not only help reap higher benefits, but also prevent exotic fish like common carp from breeding in culture ponds and natural water bodies.

The first success on hormonal sex reversal in common carp (European strain) was achieved with 17 α -methyltestosterone (17 α -MT) (Nagy *et al.*, 1981). Sathyanarayana Rao and Satyanarayana Rao (1983) were the first to obtain a female-free population of the Asian strain of common carp using 17 α -MT. Subsequent studies on the hormonal manipulation of sex indicate that all-male or all-sterile progenies of the Asian strain were successfully produced (Basavaraju and Satyanarayana Rao, 1988; Manzoor Ali and Satyanarayana Rao, 1989; Das *et al.*, 1990; Komen *et al.*, 1993; Sobhana and

Nandeesh, 1994; Basavaraja *et al.*, 1997). The minimum effective dose of 17α -MT to produce an all-male population is 50-100 mg kg⁻¹ diet (Nagy *et al.*, 1981; Komen *et al.*, 1989) and to produce a female-free population (Asian strain) is 200-400 mg kg⁻¹ diet (Sathyanarayana Rao and Satyanarayana Rao, 1983; Basavaraja and Satyanarayana Rao, 1988; Manzoor Ali and Satyanarayana Rao, 1989). However, Bharadwaj and Sharma (2000) were not successful in producing a female - free progeny using 17α -MT. Basavaraju *et al.* (2008) were successful in inducing 100% sex reversal (masculinisation) in the Asian strain by the oral administration of methyl dihydrotestosterone (MDHT) to 50 day old fingerlings for 40-50 days. Vinod and Basavaraja (2010) were successful in producing 100% male population through oral administration of NE (Norethindrone) at 50 ppm for 50 days to 10, 20 and 30 day old fry. An all-male population was also produced by feeding 17α -MT (100 ppm) to 35 day old fry (Mubarik *et al.*, 2011). Singh (2013) reported production upto 82.5% male common carp following administration of tamoxifen (200 ppm) to 35 days post-hatch fry for 60 days. So far no attempt has been made to manipulate sex ratio of amur carp using hormonal sex-reversal or any such technique.

Inter-sexuality was also observed in the European as well as Asian strains of common carp following feeding with 17α -MT, 17β -estradiol ($17\beta E_2$) and diethylstilbestrol (DES) (Nagy *et al.*, 1981; Komen *et al.*, 1989, 1993; Vinod and Basavaraja, 2010). In the Asian strain, Basavaraju *et al.* (2008) obtained intersex (3.0-29.3%), with 17α -MT and MDHT. Vinod and Basavaraja (2010) observed an increasing proportion (7.70-26.7%) of intersex common carp with an increase in the dosage of DES, while Vasanthakumar *et al.* (2011) observed 2.63-4.40% intersex fish when NE was fed at 20-30 ppm for 50 days.

The present study was carried out with the objective to produce a female-free population of amur carp by oral administration of 17α -MT and to evaluate its effect on gonadal development as well as body indices.

Materials and methods

The current study was carried out at College of Fisheries, Mangaluru, wherein the amur carp was used as an experimental animal. The synthetic steroid hormone 17α -methyltestosterone used in the present work was obtained from Sigma-Aldrich (Merck, Germany).

Procurement of amur carp fry

A stock of 20 day-old amur carp fry maintained at Inland Fisheries Research and Information Centre (I), Hesaraghatta, Bengaluru (originally sourced from Hungary) was transported to the fish farm of College of Fisheries, Mangaluru and was stocked in circular

(2 m dia.) FRP tank for acclimatisation. After 4 days of acclimation to the laboratory conditions, the fry were employed for experimentation.

Incorporation of 17α -MT into diet

The required quantity of fish meal based floating pellets (30% protein and 5% fat) were powdered and the powder was sieved to obtain a particle size of 500 μ . Incorporation of hormone into the basal diet was accomplished by weighing appropriate quantity of 17α -MT and by dissolving in absolute alcohol. The powdered feed was flooded with alcohol containing hormone using a chromatography column sprayer to ensure adequate and uniform dispersal of the hormone. The solvent was then allowed to evaporate by drying the hormone-incorporated diet in the sun. The control diet was prepared in the same manner using only the solvent (alcohol).

Administration of 17α -MT to fry

Twenty four day-old amur carp fry was divided into six groups of 200 each and was stocked in concrete tanks (3×3×3 ft), where continuous aeration was maintained using a vortex blower (0.5 hp). The first five groups were fed with diets containing 17α -MT at 200, 400, 600, 800 and 1000 ppm, respectively. The sixth group which received a hormone-free diet, served as control.

During the hormone treatment period, the respective groups of fry were fed at 20% of their body weight twice daily (morning and evening) for the first 15 days and later they were fed at 10% of their body weight for the subsequent 15 days. After 30 days of 17α -MT treatment, the fish were fed on a hormone-free diet in the respective tanks (3×3×3 ft) for a month after which they were transferred to grow-out ponds.

For maintenance of water quality, faecal matter and left over feed particles were siphoned out daily from the bottom of each experimental tank and 50% of water volume was exchanged at weekly intervals.

Grow-out rearing of fingerlings

For post-treatment rearing, all the fish which survived in the respective groups were transferred to prepared outdoor concrete ponds (5×5×1 m), with 8-10" soil base. Prior to stocking, the ponds were drained, dried, limed and then filled with freshwater, followed by fertilising with poultry manure at 3 kg per pond. During grow-out period, the fish were fed with a fish-meal based pelleted diet (protein content 30%) once daily at 5% of their body weight and monthly sampling was carried out to record weight and length of fish.

Evaluation of hormone treatment

After 90 days of grow-out rearing in ponds, the fish were dissected out for gonadal examination. Fish with recognisable ovarian and testicular portions were classified as female and male, respectively. Fish with filiform (thread like) gonads were classified as sterile, while those with both ovarian and testicular tissues were categorised as intersex fish.

The sex of fish was also confirmed by histological examination of gonads. For this purpose, gonads were dissected out and fixed in 10% neutral buffered formalin, dehydrated using alcohol, required gonad samples were embedded using molten wax and sections (5 μ m thick) were taken following the standard histological steps, as described by Gray (1964). The stained sections were then examined under a research microscope (Olympus).

Body indices

Body indices such as gonado-somatic index (GSI), hepato-somatic index (HSI), viscero-somatic index (VSI) and condition factor (K) were estimated following standard methods.

Statistical analysis

Length, weight, survival and body indices were analysed statistically using Duncan's multiple range test to determine significant difference ($p < 0.05$) between means by using the Statistical Software SPSS for windows 20.0. Chi-square test was employed to test the equality of sex ratio in different groups.

Results

Effect of 17 α -MT on survival

Data on survival of amur carp during hormone treatment and post-treatment periods is presented in Table 1. During the 17 α -MT treatment period of 30 days and post-treatment rearing in cement tanks (3x3x3 ft) for

one month, the survival obtained in different treatments was low to moderate. Data on survival of fish recorded at the end of post-treatment rearing is also given in Table 1. The survival of fish was better during grow-out rearing than during treatment period. When compared to control, 17 α -MT treated groups recorded higher survival at the end of the grow-out period. At harvest, the hormone treated groups (200, 400, 600 and 800 ppm) showed 100% survival, barring 1000 ppm where it was 78.57%, with control registering 80% survival (Table 1).

Effect of 17 α -MT on growth

The results of the administration of 17 α -MT on weight and length of amur carp during treatment period are given in Table 2. Average weight increased from 0.27 to 0.44 g and length increased from 2.06 to 2.12 cm during the period, with the maximum weight and length recorded at 1000 ppm at the end of 30 days. At 30 days after stocking in grow-out ponds, the average weight ranged between 19.0 g (1000 ppm) and 104.5 g (400 ppm), whereas the average length varied between 10.87 cm (1000 ppm group) and 16.97 cm (600 ppm group). Similar trend in weight and length was maintained till the end of the 90 days rearing period; the weight ranged between 81.63 g (1000 ppm) and 323.83 g (400 ppm) and length varied between 18.10 and 26.27 cm (Table 3). The hormone treated groups (200, 400, 600 and 800 ppm) showed significantly higher growth than control except 1000 ppm groups.

Effect of 17 α -MT on sex composition and gonads

The results of the effect of feeding diets containing 17 α -MT on the sex composition of amur carp are presented in Table 4. In control, the gonadal development was normal (Figs. 1a, b) and the sex ratio was 1: 0.71 (M:F), which was not different from the expected 1:1 sex ratio (Table 4). The testicular development was either normal (Fig. 1c) or suppressed to various

Table 1. Survival of the fish fed on different doses of 17 α -MT

Treatment	Beginning of hormone treatment		End of hormone treatment		Post-treatment rearing		
	No. of fry stocked	No. of fry obtained	Survival (%)	No. of survived fish stocked in grow-out ponds	No. of fish obtained at the end of the experiment	Survival (%)	
Control	200	35	17.5	15	12	80	
200 ppm	200	34	17.0	8	8	100	
400 ppm	200	40	20.0	3	3	100	
600 ppm	200	54	27.0	4	4	100	
800 ppm	200	48	24.0	12	12	100	
1000 ppm	200	47	23.5	14	11	78.5	

Table 2. Mean body weight (g) and length (cm) attained by the fish during 17 α -MT treatment period (mean \pm SE; n = 10)

Days Treatment	0		10		20		30	
	Weight	Length	Weight	Length	Weight	Length	Weight	Length
Control	0.023 \pm 0.002	1.19 \pm 0.01	0.033 \pm 0.002	1.355 \pm 0.205	0.215 \pm 0.025	1.675 \pm 0.025	0.265 \pm 0.025	2.065 \pm 0.065
200 ppm	0.024 \pm 0.002	1.20 \pm 0.02	0.034 \pm 0	1.54 \pm 0.02	0.226 \pm 0.031	1.69 \pm 0.01	0.325 \pm 0.125	2.115 \pm 0.005
400 ppm	0.022 \pm 0.001	1.205 \pm 0.005	0.033 \pm 0.002	1.545 \pm 0.035	0.292 \pm 0.024	1.695 \pm 0.015	0.30 \pm 0.02	2.12 \pm 0.01
600 ppm	0.023 \pm 0.001	1.202 \pm 0.008	0.033 \pm 0	1.542 \pm 0.021	0.253 \pm 0.029	1.702 \pm 0.008	0.395 \pm 0.057	2.092 \pm 0.031
800 ppm	0.023 \pm 0	1.175 \pm 0.005	0.033 \pm 0	1.535 \pm 0.025	0.257 \pm 0.022	1.695 \pm 0.15	0.408 \pm 0.038	2.06 \pm 0.06
1000 ppm	0.023 \pm 0	1.21 \pm 0.01	0.033 \pm 0	1.525 \pm 0.015	0.264 \pm 0.027	1.705 \pm 0.005	0.44 \pm 0.035	2.12 \pm 0.01

Table 3. Mean body length and weight (g) attained by the fish during post-treatment rearing mean \pm SE

Days Treatment	30		60		90	
	Length	Weight	Length	Weight	Length	Weight
Control	11.52 \pm 0.56 (4)	22.50 \pm 3.40 (4)	15.62 \pm 0.90 (4)	58.50 \pm 11.95 (4)	19.22 \pm 0.31 (12)	97.20 \pm 4.30 (12)
200ppm	16.10 \pm 0.65 (4)	64.50 \pm 6.60 (4)	20.92 \pm 0.51 (4)	132.25 \pm 3.22 (4)	25.25 \pm 0.34 (8)	221.50 \pm 6.95 (8)
400ppm	15.13 \pm 4.83 (4)	104.00 \pm 50.84 (4)	23.16 \pm 1.61 (4)	213.33 \pm 56.96 (4)	27.53 \pm 2.27 (3)	323.83 \pm 73.76 (3)
600ppm	16.97 \pm 1.05 (4)	83.00 \pm 14.27 (4)	21.37 \pm 0.52 (4)	166.75 \pm 3.19 (4)	26.27 \pm 1.37 (4)	244.75 \pm 33.69 (4)
800ppm	13.67 \pm 0.35 (4)	45.00 \pm 3.69 (4)	17.52 \pm 0.47 (4)	48.50 \pm 6.13 (4)	22.71 \pm 0.58 (12)	164.81 \pm 12.65 (12)
1000ppm	10.87 \pm 0.41 (4)	19.00 \pm 3.31 (4)	13.82 \pm 0.56 (4)	93.00 \pm 3.10 (4)	18.10 \pm 0.51 (11)	81.63 \pm 6.84 (11)

Numbers in parentheses indicate sample size

degrees (Figs. 1d, e, f), with 400 ppm 17 α -MT inducing 100% masculinisation (Table 4). While males were absent at 600 ppm, their proportion was 8.33 and 63.63% at 800 and 1000 ppm. The females were completely absent at 200, 400 and 600 ppm, but were present in the groups fed with 17 α -MT at 800 (8.33%) and 1000 ppm (9.10%) (Fig. 1g; Table 4).

The steroid administration led to the production of varying proportions of sterile fish (27.27-100%), with 600 ppm inducing complete sterilisation (Table 4), resulting in the production of fish with filiform (thread-like) gonads (Fig. 1h). On the other hand, the lowest dose tested (200 ppm) produced one intersex fish (Table 4).

Body indices

The GSI of male of control fish (5.02) and that of 1000 ppm treatment (4.77) was not significantly different ($p < 0.05$), but it was significantly lower in 400 and 800 ppm fed groups (Fig. 2). However, the ovarian development was slightly inhibited (as indicated by lower GSI) in both the treatments wherever a female was encountered. Similarly, the GSI of sterile fish was negligible in all the

treatments, barring 400 ppm where no sterile fish was found (Fig. 2a). Fig. 2b did not indicate a significant variation in HSI of males, females or sterile fish. Data on the effect of 17 α -MT on VSI is presented in Fig. 2c. No significant variation in VSI was observed among different treatments. Data on the condition factor of the hormone treated and control fish is given in Fig. 2d. The condition factor of male, female and sterile fish did not show any significant difference between the control and treated groups.

Fish biomass and evisceration loss

Data on fish biomass and evisceration loss of the hormone treated and control fish is shown in Table 5. The maximum fish biomass (1318.5 g) was recorded in the group treated with 17 α -MT at 800 ppm, whereas the minimum biomass was observed in the group fed with 17 α -MT at 200 ppm. While the increment in body weight ranged between 69.6% (800 ppm) and 233.15% (400 ppm) over the control, the group fed on 17 α -MT at 1000 ppm recorded negative growth, *i.e.* -16%, compared to control. The weight loss due to evisceration in males was

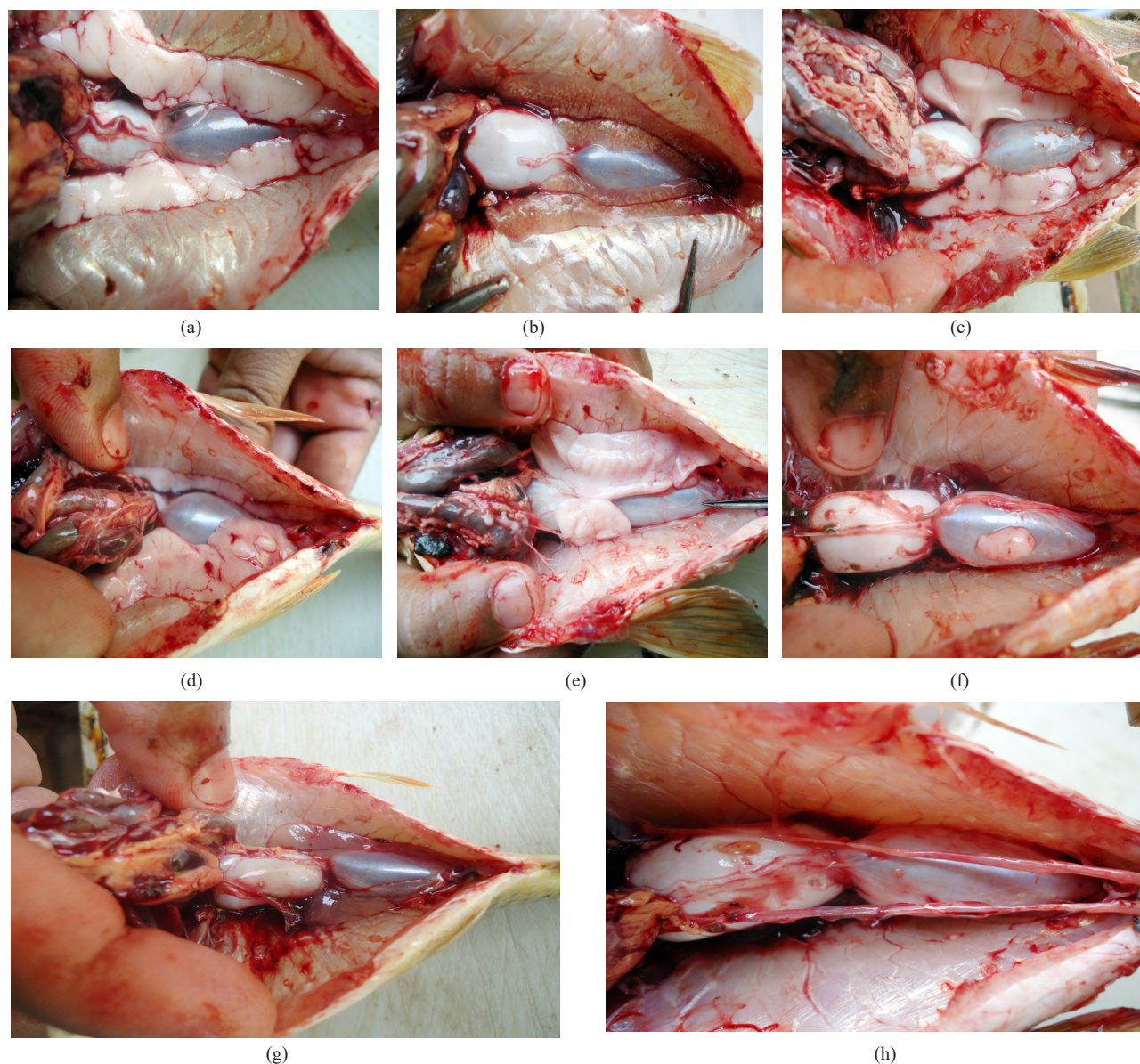


Fig. 1. (a) Untreated fish showing normal development of testis; (b) Advanced maturing ovary (Control) showing granular oocytes; (c) Fish treated with 17 α -MT (400 ppm) showing normal development of testis; (d) Fish treated with 17 α -MT (1000 ppm) showing a single well developed (right) and less developed testicular lobe (left); (e) Fish fed with 17 α -MT (400 ppm) showing unequal testicular lobes; (f) Fish treated with 17 α -MT (800 ppm) showing a small lump of testicular tissue (arrow) and filiform (thread-like) gonad; (g) Fish fed with 17 α -MT (1000 ppm) showing maturing ovary; (h) Fish orally administered with 17 α -MT (800 ppm) showing filiform gonad (both lobes).

significantly lower than that of control and 1000 ppm (Table 5). The loss due to evisceration is much less in sterile fish *vis-à-vis* males of control (Table 5). The sterile fish exhibited thicker musculature than untreated fish.

Histology

Histological examination of gonads revealed the presence of ovary and testis in different stages of development (Fig. 3a,b, c). The filiform gonad showed

the presence of only connective tissue, free from male and female germ cells (Fig. 3d). The intersex fish had both testicular and ovarian tissues (Fig. 3e). On the other hand, the histology of one thread-like gonad (sterile fish) indicated the presence of a cyst of immature oocytes as well as connective tissue (Fig. 3f). In contrast, the histology of testes showed no significant differences in the number of testicular cells between the control and hormone treated fish.

Table 4. Effect of oral administration of 17 α -MT on sex composition of amur carp

Treatment	No. of fish sexed	Sex Composition				Sex ratio (M:F)	Chi square value
		Male	Female	Sterile fish	Intersex		
Control	12	7 (58.33)	5 (41.67)	0	0	1:0.71	0.32
200 ppm	8	0	0	7 (87.5)	1(12.5)	0	-
400 ppm	3	3 (100)	0	0	0	1:0	-
600 ppm	4	0	0	4 (100)	0	0	-
800 ppm	12	1 (8.33)	1 (8.33)	10 (83.34)	(0)	1:1	-
1000 ppm	11	7 (63.63)	1(9.10)	3 (27.27)	(0)	1:0.14	4.94*

Numbers in parentheses indicate percentage; * Indicates deviation from the expected 1:1(M:F) sex ratio.

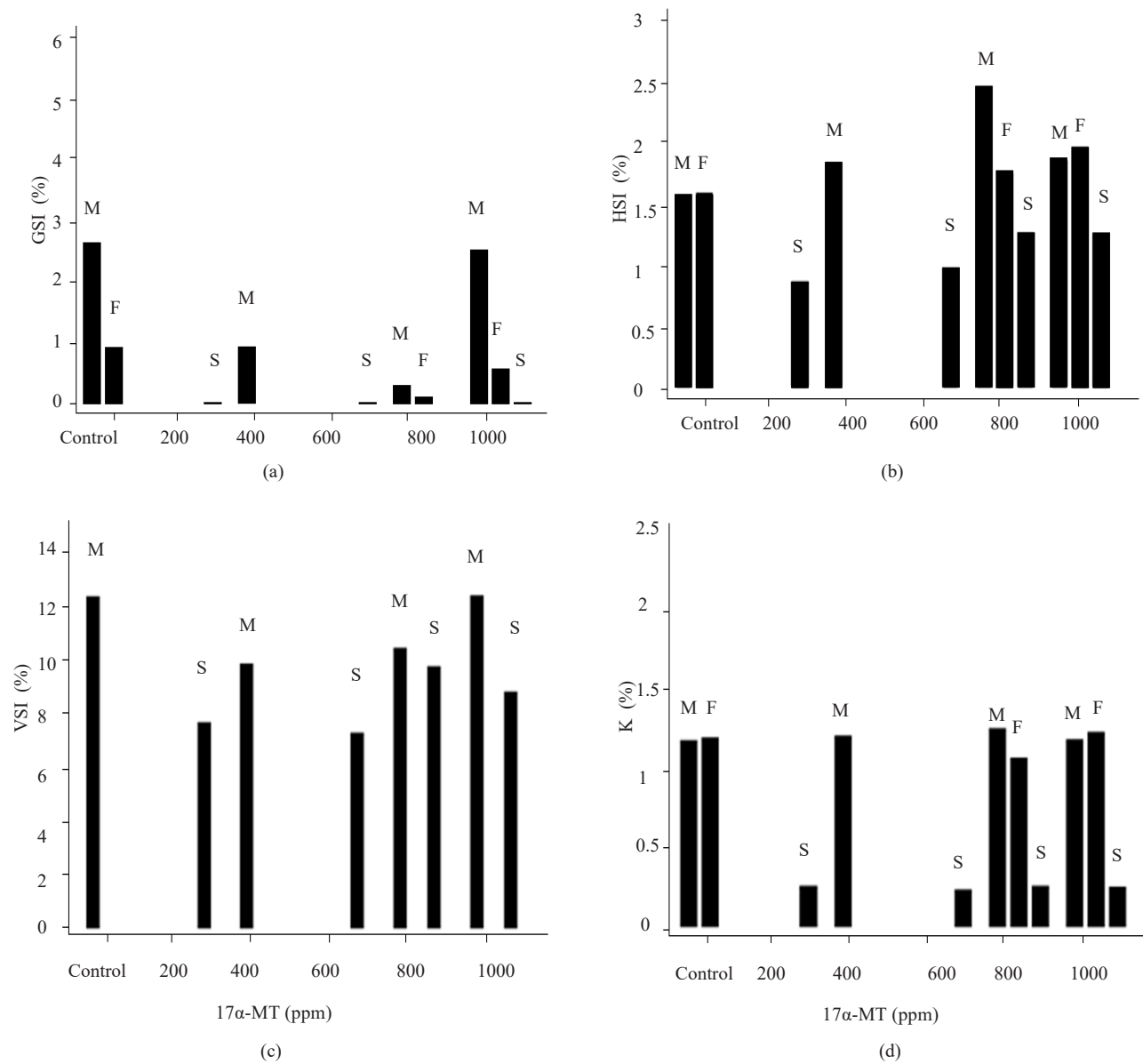


Fig. 2. Effect of 17 α -MT in amur carp. (a) GSI, (b) HSI, (c) VSI, (d) Condition factor. M - Male, F - Female, S - Sterile

Table 5. Effect of 17 α -MT on total fish biomass and weight loss due to evisceration (Mean \pm SE)

Treatment	Total biomass (g)	Mean body weight (g)	Increment in mean body weight over control (%)	Weight loss due to evisceration (%)			
				Male	Female	Sterile fish	Intersex fish
Control	1166.5	97.20 \pm 4.30 (12)	-	12.39 \pm 0.50 ^a (7)	9.30 \pm 0.77(5)	0	0
200 ppm	886.0	221.50 \pm 6.95 (8)	127.88	0	0	7.71 \pm 0.46 ^{bc} (3)	8.75 (1)
400 ppm	971.5	323.83 \pm 73.76 (3)	233.15	9.88 \pm 1.10 ^b (3)	0	0	0
600 ppm	979.0	244.75 \pm 33.69 (4)	151.80	0	0	7.31 \pm 0.51 ^c (4)	0
800 ppm	1318.5	164.81 \pm 12.65 (12)	69.55	11.46 (1)	10.022 (1)	9.78 \pm 1.23 ^a (10)	0
1000 ppm	898.0	81.63 \pm 6.84 (11)	-16.01	12.43 \pm 0.86 ^a (7)	9.92 (1)	8.83 \pm 0.51 ^{ab} (3)	0

Numbers in parentheses indicate sample size; * Means with only one observation was not considered to estimate significant difference

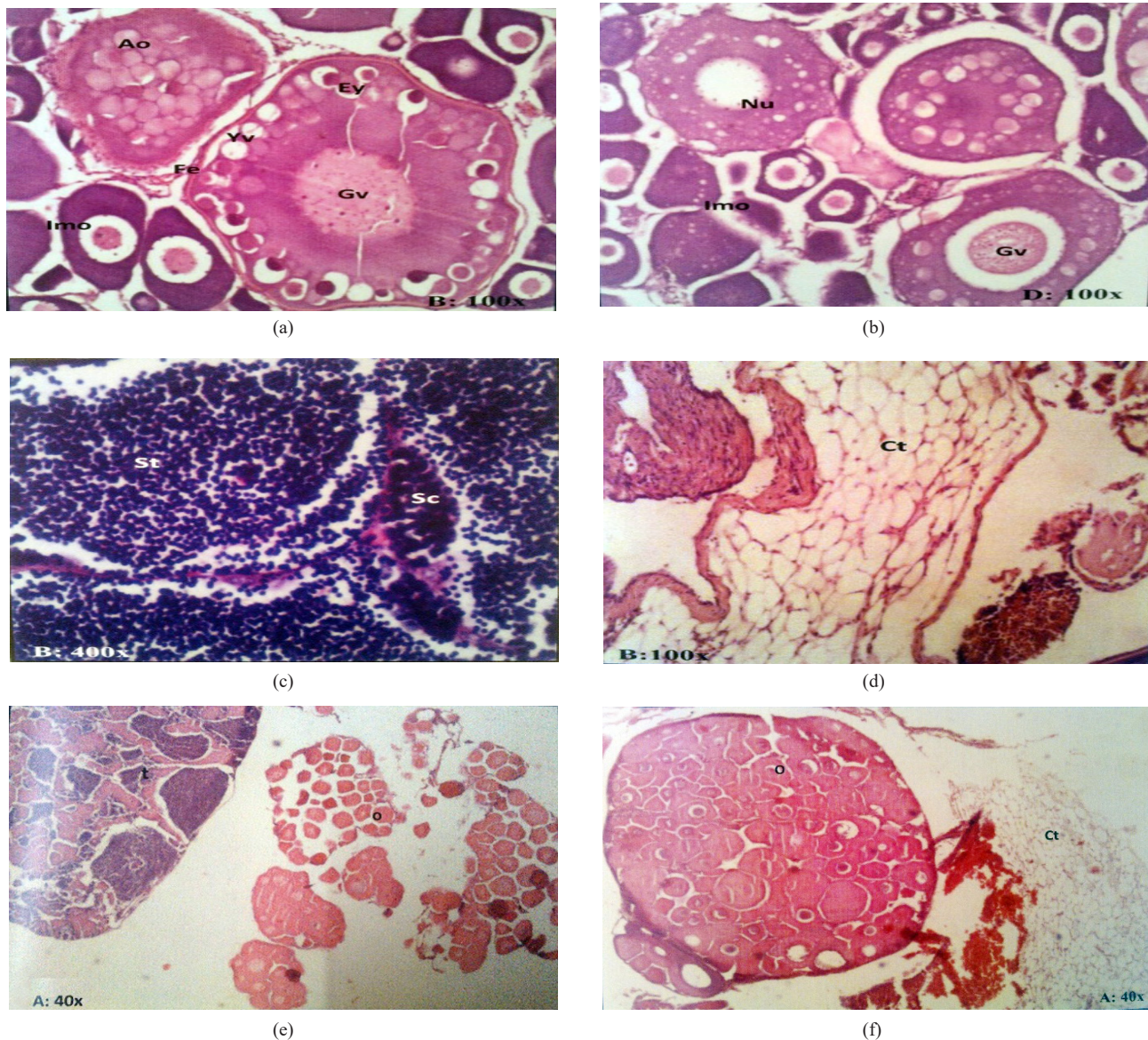


Fig. 3. Histological sections of gonads. (a) Ovary of control fish showing maturing stage: yolk vesicle (Yv), germinal vesicle (Gv), follicular epithelium (Fe), immature oocyte (Imo), early-yolk (Ey) and atretic oocyte (Ao), (b) Ovary of 17 α -MT treated fish showing maturing stage: nucleolus (Nu), Imo and Gv; (c) Testis of 17 α -MT treated fish showing spermatocytes (Sc) and spermatids (St); (d) Filiform gonad of 17 α -MT induced sterile fish showing connective tissue (Ct) without any germ cells; (e) Intersex gonad (200 ppm) showing testicular (t) and ovarian (o) tissues, showing testicular cells in different stages of development (left) and immature oocytes (right); (f) Sterile gonad of 17 α -MT treated fish showing the presence of a cyst of immature oocytes (o) and Ct.

Discussion

The results of the present investigation demonstrated that it is possible to induce complete masculinisation or sterilisation, resulting in the production of a female-free population of amur common carp by oral administration of 17α -MT. This is the first report on hormonal masculinisation or sterilisation of amur carp. These results conform with those findings reported earlier for the Asian strain of common carp. Androgen-induced masculinisation and/or sterility was observed in the Asian strain, *viz.*, 17α -MT (Nagy *et al.* 1981; Sathyanarayana Rao and Satyanarayana Rao, 1983; Basavaraja and Satyanarayana Rao, 1988; Manzoor Ali and Sathyanarayana Rao, 1989; Gomelsky *et al.*, 1995; Bharadwaj and Sharma, 2000), MDHT (Basavaraju *et al.*, 2008) and norethindrone (Basavaraja *et al.*, 1997; Manjappa *et al.*, 2006; Vinod and Basavaraja, 2010; Vasanthakumar *et al.*, 2011). For the first time, a population consisting of males and sterile fish (Asian strain) was produced by feeding 17α -MT (200 ppm) for 131 d, starting from first feeding (Sathyanarayana Rao and Satyanarayana Rao, 1983), while in the European strain, an all-male progeny was obtained by feeding 17α -MT (100 ppm) incorporated diet for 36 days, beginning at 8-80th day after hatching (Nagy *et al.*, 1981). In the latter strain, oral administration of 17α -MT at 100 ppm induced 61.5-94.5% masculinisation, while the discharge water of the MT-fed tanks resulted in higher masculinisation (81.2-100%) of gynogenetic offspring (Hulak *et al.*, 2008).

In the present study, administration of 17α -MT resulted in male dominated (400 and 1000 ppm) or sterile fish dominated (200, 600 and 800 ppm) population and this conforms with most of the earlier reports on Asian strain. Complete sex reversal of gynogenetic female common carp was achieved when MDHT was fed at 50 and 100 ppm for 50 days during 51-100 days after hatching (Basavaraju *et al.*, 2008). Similar complete masculinisation of carp was achieved by Vinod and Basavaraja (2010) by oral administration of NE at 50 ppm for 50 days to 10, 20 and 30 day-old fry. Vasanthakumar *et al.* (2011) obtained 100% males following oral administration of NE at 40 ppm to 20 day-old fry for 50 days. Based on our findings, it could be inferred that oral administration of 17α -MT at 400 ppm or 600 ppm for 30 days is sufficient to induce 100% masculinisation or sterility in amur carp. The differential efficacy of androgens to induce masculinisation or sterility may be attributed to the fact that 17α -MT is aromatisable, while MDHT is non-aromatisable (Devlin and Nagahama, 2002). In *C. carpio*, aromatase inhibitors, anastrazole (200 mg kg⁻¹) treated fish had 98.1% males and fadrozole treatment at the same dose resulted in 97.1% masculinisation (Singh and Ruchi Singh, 2013).

In the present investigation, dietary administration of 17α -MT at 600 ppm produced 100% sterile fish, but higher doses (800 and 1000 ppm) led to lower proportions of sterile fish, 87.50 and 27.27%, respectively. The lowest dose tested did not totally induce sterilisation under the given experimental conditions. Similar findings were reported earlier in our laboratory and elsewhere in India wherein administration of gonadal hormones caused sterility by the suppression of gonadal development in common carp (Basavaraja and Satyanarayana Rao, 1988; Manzoor Ali and Satyanarayana Rao, 1989; Das *et al.*, 1990; Sobhana and Nandeesh, 1994; Basavaraja *et al.*, 1997; Vinod and Basavaraja, 2010).

Sub-optimum dose of steroids may induce the production of intersex fish as has been observed in common carp (2.2-5.2%) following administration of 17α -MT (Komen *et al.*, 1989). On the other hand, Basavaraju *et al.* (2008) observed a slightly higher level of intersexuality (up to 29.3%) in common carp when fed with 17α -MT at 50-100 ppm. In this study, only one intersex fish was observed at the lowest dose (200 ppm), which may have been insufficient to reverse sex. Vinod and Basavaraja (2010) reported intersexuality (17.24-26.66%) after feeding common carp with DES, while Vasanthakumar *et al.* (2011) reported the presence of intersex fish (<5%) in NE-fed common carp. Nagy *et al.* (1981) attributed the occurrence of ovo-testis (intersex) in some of the MT treated fish to incomplete sex reversal.

17α -MT is an aromatisable synthetic androgen and as such can be metabolised to estrogens (Crim *et al.* 1981). In many species, administration of high dosages of MT for short durations has been observed to cause paradoxical feminisation (Pandian and Sheela, 1995). Our results show that lower doses of 17α -MT did not induce feminisation, only higher doses (800 and 1000 ppm) produced 8.33 and 9.10% females, which may be due to conversion of 17α -MT to estrogen by aromatase enzyme. Similar findings were reported by Piferrer *et al.* (1994) who found that higher doses of 17α -MT induced feminisation due to the aromatisation of MT.

In the present study, abnormalities in testicular development of 17α -MT-treated carp (more pronounced at 400 and 1000 ppm (Fig. 1) were observed and this could be attributed to partial inhibition of testicular growth by the hormone and /or short period of hormone administration. However, it did not result in any apparent morphological deformities in this species. No abnormal ovaries were found in any of the treatments where females were encountered. Similar observation was made by Vasanthakumar *et al.* (2011) in NE treated groups. Vinod and Basavaraja (2010) also reported upto 20% abnormal testis/ovaries when DES was fed to common carp. In this

investigation, comparison of growth rates of hormone fed amur common carp and untreated fish could not be made owing to non-uniform stocking densities.

In the present study, the steroid was found to retard gonadal development of amur carp, barring 1000 ppm where testicular growth was unaffected. It is in agreement with earlier studies of Satyanarayana Rao and Satyanarayana Rao (1983), Basavaraja and Satyanarayana Rao (1988), Manzoor Ali and Satyanarayana Rao (1989), Bharadwaj and Sharma (2000), Basavaraja *et al.* (1997), Manjappa *et al.* (2006), Vinod and Basavaraja (2010) and Vasanthakumar *et al.* (2011), who generally observed lower GSI values of male and female common carp, following administration of different steroid hormones. Our study indicates no significant variation in HSI, VSI and condition factor between control and 17 α -MT fed groups, conforming with the results of most of the previous studies carried out on common carp.

Gonads account for nearly 26-30% of body weight in mature common carp (Jhingran, 1982), which can lead to a major loss of weight due to evisceration. In the present study, the amur carp fed with 17 α -MT showed lower weight loss from evisceration than the control fish. The sterile fish devoid of gonads had a lower weight loss of 7.31-9.78%, compared to normal fish (9.30-12.39%). Similarly, Manzoor Ali and Satyanarayana Rao (1989) reported that the loss of weight due to evisceration was only 5.59-7.42% in the 17 α -MT treated fish, while it was 14.95% in control fish. Basavaraja *et al.* (1997) recorded higher dressing weight of 8% in NE treated fish than the control.

In the present investigation, the histology of gonads of control fish showed normal oogenesis and spermatogenesis. While some filiform gonads of 17 α -MT treated amur carp had only connective tissue, others possessed both degenerating ovary and connective tissue. Das *et al.* (1990) as well as Sobhana and Nandeesh (1994) reported that the ovary showed different stages of oogenesis with yolk vesicle and yolk globules, and the testis revealed different stages of spermatogenesis and the filiform gonads showed only the connective tissue without any germ cells, in common carp treated with the androgen, mibolerone. Vinod and Basavaraja (2010) reported the presence of only spermatogenic cells in common carp treated with NE and the presence of intersex fish, possessing both ovarian and testicular tissues when treated with DES. Intersex fish observed in our study also showed both ovarian and testicular tissues. Vinod and Basavaraja (2010) reported the presence of only connective tissue in filiform gonad.

Orally administered steroids like 17 α -MT (a synthetic derivative of natural androgen, testosterone) get rapidly metabolised and eliminated from the body

(Lone and Matty, 1981; Johnstone *et al.*, 1983; Goudie *et al.*, 1986; Satyanarayana Rao *et al.*, 1990). Macintosh (2014) reported that a great majority of tilapia traded internationally, is obtained from sex ratio manipulation using MT. Hence, consumption of such hormone-treated fish (after a withdrawal period of 3-4 weeks) would not cause any health hazard.

Oral administration of 17 α -MT successfully produced a population consisting of 100% males or 100% sterile fish or male, female and sterile fish. The production of monosex male or sterile fish could be used to prevent undesirable spawning during grow-out and is likely to produce more yield and increase dressing weight, particularly from sterile fish. However, further studies are needed to determine the critical minimum dose of 17 α -MT required to induce 100% masculinisation or sterilisation in amur carp and improve survival during hormone treatment period.

Acknowledgements

We thank the Dean, College of Fisheries, Mangaluru for providing facilities to carry out the research and Dr. Y. Basavaraju, FRIC (I), Bengaluru, for a gift of live amur carp fry. We thank the anonymous reviewers for their inputs which have greatly improved the quality of the manuscript.

References

- Basavaraja, N. and Satyanarayana Rao, G. P. 1988. Induction of sterility in the common carp *Cyprinus carpio* (Linn.) using 17 α -methyltestosterone. *Indian J. Anim. Sci.*, 58(10): 1248-1251.
- Basavaraja, N., Gangadhar, B. and Udupa, K. S. 1997. Successful induction of sterilization and growth improvement in common carp, *Cyprinus carpio* through dietary administration of the androgen, norethindrone. *Asian Fish. Sci.*, 10:29-40.
- Basavaraju, Y. 2013. Amur - a new breed of common carp. *Mysore J. Agri. Sci.*, 47 (1) : 119-123.
- Basavaraju, Y., Mohan Kumar, H. M., Pradeep Kumar, S., Umesha, D., Srivastava, P. P., Penman, D. J. and Mair, G. C. 2008. Production of genetically female common carp, *Cyprinus carpio*, through sex reversal and progeny testing. *Asian Fish. Sci.*, 21: 355-368
- Bharadwaj, R. and Sharma, L. L. 2000. Effect of methyltestosterone (tablets) in sterilization and masculinization of common carp, *Cyprinus carpio communis* (L). *Indian J. Fish.*, 47(4): 377-381.
- Crim, L. W., Peter, R. E. and Billard, R. 1981. Onset of gonadotropic hormone accumulation in immature trout pituitary gland in response to estrogen or aromatizable androgen steroid hormones. *Gen. Comp. Endocrinol.*, 44: 374-381. doi: 10.1016/0016-6480(81)90015-0.
- Das, S. K., Shetty, H. P. C. and Nandeesh, M. C. 1990. Production of female-free common carp, *Cyprinus carpio*

- var. *communis* (L.) through dietary administration of the androgen mibolerone. *Asian Fish. Sci.*, 3: 197-203.
- Devlin, R. H. and Nagahama, Y. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture*, 208:191-364. doi:10.1016/S0044-8486(02)00057-1.
- Gomelsky, B. I., Cherfas, N. B., Hulata, G., Peretz, Y. and Bendom, N. 1995. Hormonal sex inversion in the common carp, *Cyprinus carpio* L. *Aquaculture*, 129: 215-219. doi:10.1016/S0990-7440(03)00085-8.
- Goudie, C. A., Shelton, W. L. and Parker, N. C. 1986. Tissue distribution and elimination of radiolabelled methyl testosterone fed to sexually undifferentiated blue tilapia. *Aquaculture*, 58: 215-226. [https://doi.org/10.1016/0044-8486\(86\)90087-6](https://doi.org/10.1016/0044-8486(86)90087-6).
- Gray, P. 1964. *Handbook of basic microtechniques*, 3rd edn. McGraw-Hill Book Company. New York USA.
- Jhingran, V. G. 1982. *Fish and fisheries of India*. Hindustan Publishing Co., Delhi, India., 666 pp.
- Johnstone, R., Macintosh, D. J. and Wright, R. S. 1983. Elimination of orally administered 17 α -methyl testosterone by *Oreochromis mossambicus* (tilapia) and *Salmo gairdneri* (rainbow trout) juveniles. *Aquaculture*, 35: 249-257. [https://doi.org/10.1016/0044-8486\(83\)90095-9](https://doi.org/10.1016/0044-8486(83)90095-9).
- Komen, J., Eding, E. H., Bongers, H. B. J. and Richter, C. J. J. 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., Lodder, P. A. J., Huskens, F., Richter, C. J. J. and Huisman, E. H. 1989. Effects of oral administration of 17 α -methyltestosterone and 17 β -estradiol on gonadal development in common carp (*Cyprinus carpio* L.). *Aquaculture*, 78: 349-363. [https://doi.org/10.1016/0044-8486\(89\)90111-7](https://doi.org/10.1016/0044-8486(89)90111-7).
- Lone, K. P. and Matty, A. J. 1981. Uptake and disappearance of radio-activity in blood and tissues of carp (*Cyprinus carpio*) after feeding H-testosterone. *Aquaculture*, 24: 315-326. [https://doi.org/10.1016/0044-8486\(81\)90066-1](https://doi.org/10.1016/0044-8486(81)90066-1).
- Macintosh, D. J. 2014. *Risks associated with using methyl testosterone in tilapia farming* (a white paper), p.12.
- Manjappa, N., Basavaraja, N. and Vasanthakumar, A. 2006. Studies on sex ratio and body indices of different age groups of common carp fed on norethindrone. *J. Aquac. Trop.*, 21(3-4): 181-192.
- Manzoor Ali, P. K. and Satyanarayana Rao, G. P. 1989. Growth improvement in carp *Cyprinus carpio* (Linnaeus) sterilized with 17 α -methyltestosterone. *Aquaculture*, 76: 157-167.
- Mubarik, M. S., Ahmed, I., Manteen, A. and Iqbal, T. 2011. 17 α -methyltestosterone induced masculinisation and its effect on growth and meat quality of *Cyprinus carpio*. *Int. J. Agric. Biol.*, 13: 971-975.
- Nagy, A., Bercsenyi, M. and Csanyi, K. 1981. Sex reversal in carp (*Cyprinus carpio*) by oral administration of methyltestosterone. *Can. J. Fish. Aquat. Sci.*, 38: 725-728.
- Pandian, T. J. and Sheela, S. G. 1995. Hormonal induction of sex reversal in fish - A review. *Aquaculture*, 138: 1-22.
- Pifferer, F., Zanuy, S., Carrillo, M., Solar, I. I., Delvin, R. H. and Donaldson, E. M. 1994. Brief treatment with aromatase inhibitor during sex differentiation causes chromosomally female salmon to develop as normal, functional males. *J. Exp. Zool.*, 270: 255-262.
- Sathyanarayana Rao, H. N. and Satyanarayana Rao, G. P. 1983. Hormonal manipulation of sex in common carp, *Cyprinus carpio* var. *communis* (Linnaeus). *Aquaculture*, 35: 83-88. [https://doi.org/10.1016/0044-8486\(83\)90073-X](https://doi.org/10.1016/0044-8486(83)90073-X).
- Satyanarayana Rao, G. P., Sharma P. V. and Keshavappa, G. Y. 1990. Elimination of testosterone in the fry of common carp, *Cyprinus carpio* (Linn.). In: *Proceedings of the Second Indian Fisheries Forum*, 27-31 May 1990, Asian Fisheries Society, Indian Branch and College of Fisheries, Mangalore, India, p. 87-89. [https://doi.org/10.1016/0044-8486\(83\)90073-X](https://doi.org/10.1016/0044-8486(83)90073-X).
- Singh, A. K. 2013. Introduction of modern endocrine techniques for the production of monosex population of fishes. *Gen. Comp. Endocrinol.*, 181:146-155. DOI: 10.3923/jfas.2016.1.11.
- Singh, A. K. and Ruchi Singh, 2013. *In vivo* response of melatonin, gonadal activity and biochemical changes during CYP19 inhibited sex reversal in common carp *Cyprinus carpio* (L). *Anim. Repr. Sci.*, 136 (4) : 317-325. DOI: 10.1016/j.anireprosci.2012.11.004.
- Sobhana, K. S. and Nandeesh, M. C. 1994. Standardisation of mibolerone dosage for production of female free common carp (*Cyprinus carpio* var. *communis* L.) and the impact of hormone on growth and flesh composition. *J. Aquac. Trop.*, 9: 133-139.
- Vasanthakumar, A., Basavaraja, N., Manjappa, N. and Vinod, B. H. 2011. Manipulation of sex and progeny testing in common carp, *Cyprinus Carpio* (Linn.). *J. Aquac. Trop.*, 26(1-2): 63-81.
- Vinod, B. H. and Basavaraja, N. 2010. Effect of diethylstilbestrol and norethindrone on sex ratio and body indices of common carp, *Cyprinus carpio* (Linn.). *Asian Fish. Sci.*, 23: 100-115.

Date of Receipt : 11.04.2018
 Date of Acceptance : 18.06.2019