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Effect of 17 α-methyl testosterone on growth and sex-ratio of common carp, *Cyprinus carpio* var. *communis* (Linnaeus)

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Abstract

An experiment was conducted in laboratory and hapa, to observe the effect of 17 α-methyl testosterone on growth and sex-ratio of common carp, *Cyprinus carpio* var. *communis* (L.). Four treatments viz. T_1, T_2, T_3 and T_4 having 0, 150, 200 and 250 mg 17 α-methyl testosterone respectively in per kg nursery feed Starter-I (SABINCo) were used for a period of 35 days in laboratory system. Then the hormone treated fish were again reared in experimental hapa and fed nursery feed Starter-III (SABINCo) for another 90 days for the maturation of their gonad. After termination of the experiment the sex of all the fish were identified with gonad squashing method to identify male, female and sterile fishes. In terms of growth performances significantly higher (p<0.05) weight gain was found in T_3 but there was no significant variation in survival rate of *C. carpio* among the four treatments after 35 days of the experimentation. The highest percentage (74%) of male was observed in T_3 compared to other treatments after 90 days of the experimentation. The results indicated that the oral administration of 17 α-methyl testosterone at the dose of 200 mg per kg feed could be effective for the masculinization and sterilization of common carp with higher growth.

Keywords: 17 α-methyl testosterone, Growth, Sex-ratio, Common carp

Introduction

The common carp, *C. carpio* var. *communis* (L.) is widely cultured among cyprinid fishes because of its high tolerance to environmental fluctuation, high disease resistance and good taste. The common carp is believed to have originated in Central Eurasia (Balon, 1974) and then they are distributed over almost all the sub-tropical countries of the world (Jhingran *et al.,* 1985). Common carp, *C. carpio* var. *communis* (L.) were first introduced in Bangladesh by Department of Fisheries (DOF) in 1960 from Chaina and then second batch in 1995 from Vietnam (Rahman, 1985; Hussain, 1997). It is commonly cultured in combination with other carp species. The artificial propagation, growth and mortality of common carp have already been studied by several authors (Ahmed and Chowdhury, 1998; Hashem *et al.*, 1997). The desired production of this species is not achieved due to their early sexual maturation and spawning. As a results the occurrences of overcrowding is a common phenomenon in the framers culture pond which affects the growth and production. For this reason the aquaculture of common carp is still remain unsuitable in South East Asian countries including Bangladesh. Thus many scientists have conducted research work on common carp using methyl testosterone for yielding higher growth through sex inversion. The first success in inducing sex inversion in European strain of common carp, using methyl testosterone was achieved by Nagy *et al.* (1981). Asian strain of common carp was first subjected to sex manipulation in India in 1981 (Satyanarayan Rao, 1983), using methyl testosterone mixed diet. Sterilization and masculinization of common carp was achieved by Bharadwaj and Sharma, 2000 (74% sterile and 16% male fish) using dietary administration of methyl testosterone (Tablets) hormone @ 600 ppm. Thus this experiment was aimed to observe the effects of 17 α-methyl testosterone on growth and sex-ratio of common carp.

Materials and Methods

Brood fish collection

Mature males (10 pairs) and females (20 pairs) having average weight of 1.5 kg and 1.65 kg respectively were collected from Bangladesh Fisheries Research Institute, Mymensingh and reared in earthen pond. The brood fish were provided with a supplementary diet composed of fishmeal (20%), mustard oil cake (20%), sesame oil cake (12%), soybean oil cake (12%), wheat bran (15%), rice bran (15%), wheat flour (5%), and vitamin premix (1%) (Mollah, 2001). The supplemental feed was administered twice a day (at 9.00 and 16.00 h) at the rate of 3-5% of their body weight, which enhances the gonadal maturation in fishes.

Breeding and spawn collection

Five pairs of ready-to-spawn male and ten pairs of female common carp were collected from the stocking pond and kept in a hapa (152.4 x 106.7 x 121.9 cm^3) with water hyacinth for spawning. The fertilized eggs were collected from hapa and incubated in cistern having aeration facilities from porous plastic pipe for a period of 72 hours. Hatching was started after 58 hours of incubation and completed within 72 hours. After absorption of egg yolk the hatchlings were collected from cistern and transferred into the rectangular size tray (96.52 x 36.83 x 10.16 cm^3) and reared upto three days. Then the spawn were fed with boiled chicken egg yolk.

Experimental setup

The feeding experiment was divided into two phases; the first one was done in the Wet laboratory of Fisheries Faculty, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh for a period of 35 days. Three days old spawns of common carp were stocked in plastic bowl having water holding capacity of 10 liter and continuous flow of water from porous pipe and outlet facilities. The experiment was designed with 4 treatments (T_1, T_2, T_3 and T_4) and each treatment contained 3 replications. Each replicate bowl contained 200 larvae (average length 5.45±0.25 mm and weight 5.31±1.029 mg) at a stocking density of 20 larvae/L of water. The feed was formulated according to the different amount of 17 α-methyl testosterone hormone. At first defined amount of 17 α-methyl testosterone were dissolved in 250 ml ethanol and then were mixed with per kg nursery feed Starter-I (SABINCo).

The second trial was performed with the same larvae (35 days hormone treated common carp) reared for another 90 days in four experimental hapa (152.4 x 106.7 x 121.9 cm^3) that were placed in a pond of Field Complex, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh. During the rearing of post-hormone treated fry, they were fed a nursery feed (starter-III) at the rate of 8-12% of their biomass. The feed contained 92.21% dry matter, 23.39% protein, 11.49% lipid, 8.72% crude fibre, 29.98% NFE and 20.42% ash.

Rearing of larvae

The water of each bowl was exchanged by half of the volume with fresh deep tube-well water twice a day. The fecal output and waste of feed were removed from the bowl by siphoning twice a day. The feed was provided at the rate of approximately 8-12% body weight twice a day (at 9.00 and 16.00 h). The physico-chemical parameters such as dissolved oxygen (DO), free carbon-dioxide (CO_2), total alkalinity (carbonate and bi-carbonate), pH and temperature were estimated weekly in each bowl.

Sex identification

Sex of fish was determined by examining the gonads. The fish was dissected and gonad was stained with aceto-carmine solution and then squashed with gentle pressure on slide to observe their sex with a light microscope. Fish with recognizable ovarian and testicular portion were classified as female and male respectively. Fish with filiform (thread like) gonads were classified as sterile.

Analytical method

The physico-chemical parameters such as temperature, pH, dissolved oxygen (DO) and amoniacal nitrogen were estimated weekly in each aquarium with the help of Aqua mate water testing kit (Model WAQ-IA). A proximate composition of the feed ingredients was analyzed by standard methods (AOAC, 1980). The fish were sampled at weekly interval to determine the change in their growth (length and weight). Sampling was done in the early morning when the fish stomach was empty. The specific growth rate (SGR) was calculated as the percentage increase in body weight per day over given time interval by the following equation:

$$\text{Specific growth rate} = (\text{LnW2} - \text{LnW}_1) / (T_2 - T_1) \times 100$$

where, W_2 = final live body weight (g) at time T_2

W_1 = initial live body weight (g) at time T_1

Data analysis

The length gain (mm), weight gain (g), percent length gain, percent weight gain and specific growth rate and survival rate of larvae were tested using one way analysis of variance (ANOVA). Significant results ($P<0.05$) were further tested using Duncan's New Multiple Range Test (DMRT) to identify significant differences among means. This statistical analysis was performed with the aid of the computer software MSTAC program.

Result and Discussion

The initial length, final length, length gain, weight gain, specific growth rate (SGR) during the experimentation and survival rate of common carp with hormone treated feed are presented in Table 1. The highest length and weight gain (26.47±1.09 mm and 334.89±11.73 mg respectively) were observed in T_3 and the lowest length and weight gain (18.75±2.11 mm and 164.32±5.60 mg respectively) were observed in T_1. The highest SGR value (9.90±0.08) was also observed in T_3 and the lowest (8.24±0.08) was found in T_1. All the growth parameters of T_3 were significantly ($p<0.05$) different from other three treatments. The higher growth performances of common carp, *C. carpio* in hormone treated feed may be due to the entire reproductive energy was rechannelled for somatic growth, resulting in higher dressing weight (Das *et al.*, 1990). The lowest growth of common carp, *C. carpio* in control group (T_1) may be due to the early sexual maturation of the fish or decrease of genetic variation. The genetic control of sex differentiation was studied (doses were 200 ppm, 400 ppm and 600 ppm) by Bharadwaj and Sharma (2000). No significant differences were observed in survival rate for four different treatments.

Table 1. Growth and survival rate of common carp larvae after 35 days experiment

Treatment	Parameters							
	Initial wt. (mg) (M ± SD)	Final wt. (mg) (M ± SD)	Length gain (mm) (M ± SD)	Weight gain (mg) (M ± SD)	Length gain (%) (M ± SD)	Weight gain (%) (M ± SD)	Specific growth rate (SGR) (M ± SD)	Survival rate (%) (M ± SD)
T_1	5.31±1.029	169.6±6.80	18.75±2.11^a	164.32±5.60^a	344.73±38.67^a	3094.60±105.47^a	8.24±0.08^a	70.33±1.20^a
T_2	5.31±1.029	235.5±8.52	21.52±0.87^a	230.19±5.42^c	394.86±16.02^a	4335.02±102.15^c	9.02±0.5^c	72.00±3.00^a
T_3	5.31±1.029	340.2±15.12	26.47±1.09^b	334.89±11.73^d	485.74±20.05^b	6306.77±220.92^d	9.90±0.08^d	71.00±1.52^a
T_4	5.31±1.029	196.7±9.14	19.39±0.78^a	191.41±2.56^b	355.77±14.41^a	3604.77±48.39^b	8.60±0.03^b	68.66±2.03^a

Values of the parameter in each column with different superscripts (a, b, c & d) differs significantly (p<0.05)

The gonadal development observed in treatment T_1 (control) with maintaining normal sex ratio (male:female = 48.48:51.52) which are shown in Table 2. The gonadal development was varied from recognizable ovarian/testicular portion to completely suppressed (gonads were represented by thread like strands) i.e. sterile. The percentage of male was higher than females and sterile in all the hormone treated fishes. The highest percentage (74%) of male fishes were observed in the T_3 with 17 α-methyl testosterone at a concentration level of 200 mg/kg feed (Table 2). The highest percentage (36%) of sterile fish was observed in T_4 where inclusion of hormone at the dose of 250 mg/kg feed. Manzoor Ali (1985) recorded that 98% males and 2% sterile fish were found by dietary administration of methyl testosterone @ 400 ppm. The application of testosterone acetate @ 400 ppm resulted in 67% male and 33% female fish (Nagraja, 1986). Hackman (1974) also recorded that 70% male and 30% female fish were found by dietary administration of testosterone propionate @ 700 ppm. Bharadwaj and Sharma (2000) recorded that 16% male and 74% sterile fish were found by dietary administration of methyl testosterone (Tablets) hormone @ 600 ppm. In the present study, 74% male and 17% sterile common carps were found using 200 mg/ kg feed of 17 α-methyl testosterone. The differences of the results obtained may be the improper mixing of hormone with feed.

Table 2. Effects of dietary administration of 17 α-methyl testosterone on the sex ratio of common carp after 90 days of rearing

Treatments	No. of fish examined	No. of male (%)	No. of female (%)	No. of sterile fish (%)
T_1	33	16 (48.48)	17 (51.52)	-
T_2	32	21 (65.63)	4 (12.50)	7 (21.8)
T_3	32	26 (74.29)	3 (8.57)	6 (17.14)
T_4	28	16 (57.14)	2 (7.14)	10 (35.72)

The physico-chemical parameters during the experimental period in bowls are shown in Table 3. In case of physico-chemical parameters, no significant differences were observed during the experimental period in bowls. The temperature ranged from 27 to 30^{0}C, pH ranged from 7.4 to 7.8 and DO ranged from 5.6 to 6.1 ppm. The presence of ammoniacal nitrogen ranged from 0.29 to 0.35 ppm. Free carbondioxide were not measured in any bowl during the experimental period. The above mentioned physico-chemical parameters are suitable for larvae rearing of common carp. This indicated that all the experimental units had the same range of physico-chemical parameters, which also indicated the differences observed in growth and sex-ratio of common carp, *C. carpio* may be due to the effect of administration of 17 α-methyl testosterone. Thus inclusion of 17 α-methyl testosterone at the dose of 200 mg/kg feed may be effectiveness for higher sex-reversed ratio (maleness) in common carp.

Table 3. Physico-chemical parameters of water in the bowl during the experimental period

Parameter	Treatment			
	T_1	T_2	T_3	T_4
Temperature ^{0}C	27.3±2.56	27.50±2.1	28.10±1.85	28.3±2.45
pH	7.4±0.5	7.6±0.4	7.8±0.3	7.4±0.5
Dissolve oxygen (ppm)	5.6±0.5	6.1±0.6	6.1±0.6	5.7±0.5
Amoniacal nitrogen	0.35±0.02	0.33±0.05	0.29±0.05	0.33±0.05

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