Role of Dietary Endrogen (17 Alpha methyltestosterone) on growth rate, feed conversion efficiency and body composition in *C. punctatus*

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Abstract— Fish were fed diet containing 17 Alpha methyltestosterone (MT) at doses 0 (control), 0.5, 1.0 and 2.0 mg/kg of diet for 80 days. Fish administered with 17 Alpha methyltestosterone (MT) in feed exhibited increased growth (in terms of mean increase in length, live weight gain, growth percent gain in body of weight and specific growth rate) with increase in the dose of MT from 0.5 to 1.0 mg/kg body weight and thereafter further increase in the dose of MT no improvement in growth was observed indicating 1.0 mg of MT/kg of diet appear to be optimum for growth performance in C. punctatus. Significantly (P<0.05) highest percent gain in body weight was observed in fish fed on diet containing 0.1 mg of MT (108.64%), which was only marginally low (103.66%) in the fish fed on 2.0 mg of MT. FCR and food consumption improved with increase in the MT levels in the diet. PER, GPR, GER and APD values also increased with increase in the doses of steroid upto the highest dose. Fish fed with MT at the rate 1.0 or 2.0 excreted significantly (P<0.05) low amounts of Ammonia and Phosphate in the holding water in comparison to the fish fed on low dose of MT (0.5 mg) or control. Significantly highest VSI values were observed in the diet containing MT at the rate 1.0 mg/kg and HSI value decreased with each increase in steroid level in diet. MT treated fish had high carcass protein, fat, phosphorus and energy as compared to the control. Moisture and ash content of carcass in steroid treated fish also remained low in comparison to control. Economy in this connection can be achieved through the use of anabolic androgenic steroids as food additives for increased growth rate and food conversion efficiencies in aqua-culturally important slow growing fish species.

I. INTRODUCTION

Administration of hormones to a wide variety of fish generally results in a dose-dependent increase in growth reviewed by Donaldson et al., 1979; McLean and Ž Donaldson, 1993. Recently, in aquaculture industry, there has been considerable interest in oral administration of hormones. Many types of hormones can be administered by this route. Three types of hormones have been shown to increase growth rate in fish both alone and also in combination. These are (i) pituitary growth hormones, (ii) the anabolic steroid hormones, (iii) and the thyroid hormones. In addition to these, the insulin is a fourth group of hormones which play a significant metabolic role and may be capable of growth promotion alone or in combination with other hormones (See Higgs et al., 1982; Hunter and Donaldson, 1983). Androgens are probably actual anabolic hormones as they are known to affect the muscle cells directly (Towers and Florini 1975). Hormone acts as an appetite stmulant in fishes has been demonstrated in Carassius auratus (Yamazaki 1976); in Oncorhynchus kisutch (Fagerlund and McBride 1975; McBride and Fagerlund 1976); in Chanria striatus (Nirmala and Pandian 1983) and in Heteropneustes fossilis (Sindhu and Pandian 1984).. The effects of growth hormone on the fish growth have been studied worldwide by various workers and this is the most recent field including the use of genetic manipulated growth hormone.

Markert et al. (1977) proposed that the exogenous growth hormone may stimulate appetite in salmon by a direct action on centers in the hypothalamus that influence feed intake or by inducing a number of metabolic changes that feedback on the hypothalamic centers to affect appetite. These workers also suggested that exogenous growth hormone may improve feed and/or protein conversion in salmon by one or more of the following possible mechanisms: (1) stimulation of lipid mobilization and oxidation; (2) an action on the rate of protein synthesis and/or breakdown; and (3) stimulation of insulin synthesis and release.

Since feed accounts more than 50 percent of the operational costs of commercial production of carnivorous fish species like murrels, any improvement of growth rate and / or feed conversion efficiency could potentially decrease the costs of fish production. Therefore, the current study was undertaken to determine the effect of oral administration of 17 α methyltestosterone (MT) on growth rate, feed efficiency and body composition in the fingerlings of C. punctatus. Effect of MT was also examined on VSI ,HSI, liver glycogen and proteolytic enzyme activity.

II. MATERIAL AND METHODS

Specimens of C. punctatus (BW 4.80-4.86) were obtained from fish dealers of Hissar. Fish were placed in transparent glass aquaria (60x30x30 cm) kept in laboratory where the temperature was maintained at 250C±1('C and the lighting schedule at 12h of light (08°°-20°°h) alternating with 12h of darkness (20°°- 08°°). The average intensity of light inside the laboratory was approximately 1000 lux. Fish were acclimated in the laboratory for a minimum of seven days prior to the initiation of experimental treatments and were fed ad libitum on a feed containing fishmeal as the protein source between 16°° and 19°°h. The water in the aquaria was renewed daily with water which had been previously equilibrated to the desired temperature (25°C).Experiments were conducted in an air-conditioned laboratory under a light regimen of LD 12:12 at 25°C.

Preparation of diets: Ingredients were ground to pass through 0.5 mm sieve prior to the addition of hormones and analysis for proximate composition. Soybean seeds were cleaned , autoclaved for 15 minutes at 121°C (15 Ibs) to remove ANFs and used as the protein source without adversely affecting the growth (Garg et al., 2002), dried in an oven maintained at 60°C. Other source of diet were mixed in certain ratios (Groundnut oilcake 375.5),(Processed soybean 112.5),(Rice bran 25.0),(Wheat flour, 25.0). Simultaneously proximate analysis was also conducted for moisture, crude protein, crude fat, crude fiber, ashNFE and phosphorus. Each kg contains: Copper 312 mg. Cobalt 45 mg, Magnesium, 2.114 g, Iron 979 mg, Zinc 2.130 g, Iodine 156 mg, DL-Methionine 1.920g, L-Lysine Mono Hydrochloride 4.4 g. Calcium 30%, Phosphorus 8.25%. The pelleted diet supplemented with MT hormone at doses of 0.5, 1.0 and 2.0 mg/kg of diet and fish were fed at the rate of 3% per day body weight for 80 days. Control group was also fed with diet without hormone for 80 days. The ration size was adjusted at every 15 days intervals after bulk weighing of the fish. Individual weight and length of fish was recorded at the beginning and at the end of experiment for the determination of condition factor. Liver and viscera were extripated for the calculation of hepatosomatic index (HSI) and viscerosomatic index (VSI). Liver was also processed for the estimation of glycogen (Dubois et al., 1956). Intenstine was dissected out while keeping the fish at 4°C for the determination of proteolytic enzyme activity (Kunitz, 1947). Fish carcass was processed for proximate composition following AOAC (1995). Water samples were collected 6-8 hr post-feeding for the determination of Ammonia and Phosphate levels from the holding waters following APHA (1998). Duncan Multiple Range Test (Duncan, 1995) was used to evaluate the differences among treatment groups at the 0.05 level of significance. Group means were compared by Students 't' test (Snedecor and Cochran, 1982).

III. RESULT

Fish administered with 17 α methyltestosterone (MT) in feed exhibited increased growth (in terms of mean increase in length, live weight gain, growth percent gain in body weight and specific growth rate) with increase in the dose of MT from 0.5-1.0 mg per kg, and thereafter, with further increase in the dose of MT no improvement in growth performance was observed, indicating 1.0 mg of MT per kg of diet appear to be optimum for growth performance in *C. punctatus*. Significantly, ($P<0.05$) highest percent gain in body weight was observed in fish fed on a diet containing 1.0 mg of MT (108.64%), which was only marginally low (103.66%) in the fish fed on 2.0 mg of MT (Table 1).

FCR and food consumption improved with increase in the MT levels in the diet. PER, GPR, GER and APD values also increased with increase in the doses of steroid up to the highest dose used in the studies. However, no significant ($P>0.05$) differences were observed among higher doses of MT used in the experiment (1.0 and 2.0 mg per kg diet) (Table 1).

Per cent increase in length increased with each increase in hormonal level in diet and thus, mean length SGR values were significantly ($P<0.05$) high in MT fed fish as compared to the control. Condition factor (k) remained significantly ($P<0.05$) low in hormonal treated groups as compared to the controls (Table 2).

Effect on $N\text{-}NH^{+}_{4}$ excretion and $O\text{-}PO^{-}_{4}$ production: fish fed on MT @ 1.0 or 2.0 mg per kg of diet, excreted significantly ($P<0.05$) low amounts of $N\text{-}NH^{+}_{4}$ and $O\text{-}PO^{-}_{4}$ in the holding water in comparison to the fish fed on low dose of MT (0.5 mg) or controls fed on hormone free diet (Table 3).

Effect on VSI and HSI: Significantly ($P<0.05$) highest VSI values were observed in the group fed on diet containing MT @ 1.0 mg per kg. No significant differences between the control and the lowest (0.5 mg per kg) dose of MT used in the studies were observed. On the other hand, HIS values significantly ($P<0.05$) decreased with each increase in the steroid level in the diet (Table 4).

Proximate carcass composition: MT treated fish had high carcass protein, fat, phosphorous and energy as compared to the control. Significantly ($P<0.05$) high values however, were observed in groups treated with MT at 1.0 – 2.0 mg. Moisture and ash contents of carcass in steroid treated fish also remained low in comparison with the controls (Table 4).

Effect on liver glycogen and intestinal proteolytic enzyme activity: Depletion in liver glycogen was observed in groups fed on diet containing high concentration of MT (1.0-2.0 mg per kg), while glycogen synthesis was enhanced in group fed on low (0.5 mg per kg) concentration of MT (Table 5).

IV. DISCUSSION

17 α methyltestosterone (MT): Effect on growth: The results of the present study clearly show that MT when given per os, significantly increased the growth in *C. punctatus*. The growth was more pronounced with lower doses of steroid than with the higher dose. On the basis of percentage increase in weight gain over the controls, MT at 1.0-2.0 mg per kg of diet appears to be an optimum dose for growth in these species. The fish receiving the optimum dose 1.0-2.0 mg were heavier but there is no significant difference in weight gain in fish receiving MT @ 1.0 and 2.0 mg per kg of body weight. These results substantiate the earlier findings that MT although increase growth at lower doses, became deleterious and detrimental to growth at higher doses (Yamazaki, 1976; Lone and Matty, 1980; Degani, 1985; Lone, 1989; gannam and Lovell, 1991; Santandreu and Diaz, 1994; Abdelghany, 1995). A decrease in growth rate at higher doses is probably due to a catabolic action and decrease in appetite of the organism, an effect seen also in mammals (Kochakia, 1976).

Physiology : Condition factor (k) Viscero-somatic index (VSI), Heapato-somatic Index (HSI), liver glycogen and proteolytic enzyme activity: A decrease in condition factor (k) on treating *C. punctatus* with steroid is similar to those reported on salmonids (McBride and Fagerlund, 1973, 1976; Fagerlund and McBride, 1975, 1977; Higgs et al., 1977). According to Killian and Kohler (1991), the condition factor in red tilapia was depressed for the steroid fed groups. Carp exhibited no change in condition factor when treated with MT for 90 days, but after the cessation of treatment the experimental groups had significantly higher condition factors than control (Lone and Matty, 1980).

Effect on tissue (VSI and HSI) : In the resent studies the hepatosomatic index (HSI) decreased in all the treated groups. A decrease in HSI was also noted when carp were fed with methyltestosterone (Lone and Matty, 1980). A probable reason for decrease in HSI was the mobilization of fat from liver to the muscle, as muscle lipids were increased after treatment. Simpson (1976) observed that livers of his experimental trout were without any fatty infiltration compared with controls which had abundant fat stores in their livers. A significant increase in viscerosomatic index (VSI) was noted in all the groups after 80 days. These observations are similar to those of Lone and Matty (1980) in carp and Killian and Kohler (1991) in red tilapia, Increase in VSI may be attributed to the proliferation of internal viscera as a result of steroid treatment. Therefore, a small percentage of the growth increase may be attributed to an increase in weight of viscera in *C. punctatus*.

After 80 days of feeding on MT containing diet, the amounts of muscle protein were higher than the controls. Considerable information is available regarding the effect of growth on protein content of the whole fish and / or different body organs (Love, 1970) and it has been stated that the protein level of muscle increase with growth (Shulman, 1976) in the prematurity stage, as during spawning much energy is channeled towards the gonads for sexual products. Since fish growth is generally due to enhanced protein synthesis, a possible change in muscle gross composition and protein content would seem likely and this factor becomes all the more important when growth under the influence of an anabolic agent is considered. It has been argued (Love, 1970) that muscle lipids increase during the growth of fish, and the cholesterol content of the muscle is independent of total lipid content of the fish (Wurtziger and Hensel, 1976). In the present study total lipids increased in all experimental groups after 80 days of steroid feeding. Deposition of fat muscle in response of steroid feeding has been reported many a times in salmonids (See Higg et al., 1977) and in nile tipaia (Abdelghany, 1995).

The moisture content of the muscle is said to have an inverse relationship with lipids and proteins (Love, 1970). The changes in moisture content in *C. punctatus*, in the present study support earlier observations. Low moisture content as a result of MT treatment in *C. Punctatus* is similar to those of Ostrowski and Garling (1986), they observed that MT treatment significantly decreased moisture content in whole body and empty carcass samples of rainbow trout. Methyltestosterone induces growth by acting probably in three different ways that is increase food conversion, activation or secretion of other endogenous anabolic hormones. It is also possible that steroid hormones activate the secretion, and consequently act in an additive fashion with other anabolic hormones. For instance, MT activates the thyroid and pancreas in fish (McBride and von Oberbeeke, 1971; Higgs et al., 1977; Hunt and Eales, 1979; Milne and Leatherland, 1980). Improvement in the food conversion efficiency has been reported in rainbow trout and coho salmon (Simpson, 1976; Matty and Cheema, 1978). In the present study also food conversion values for experimental groups were higher than the controls. Increased food conversion is probably the product of increased digestion and assimilation of food. MT treatment stimulates hepatic glycogen depletion through the reduction in liver glycogen synthease activity (Leung et al., 1991). The decrease in glycogen synthesis from glucose or hepatic storage may result in increased plasma glucose available to spare the dietary protein normally metabolized and used as a source of energy resulting in high accumulation of protein in muscle. Depletion of hepatic glycogen at higher doses of steroids was also observed in *Salmo gairdneri* (Hirose and Hibiya, 1968).

Effect on nutrient retention: MT treatment generally enhances nutrient utilization which is reflected by improvement in FCR and PER. Different authors have found similar responses (Simpson, 1976; Matty and Cheema, 1978; Fagerlund et al., 1979 and 1980; Schreck and Fowler, 1982), while less marked responses have been observed by Yu et al. (1979) and Ince et al. (1982). The values noted for FCR and PER during this trial were progressively changed proportionally to the weight gains obtained for each hormonal dosage except at the highest

dosage of steroid (6.0 mg kg^{-1}). These observations suggest that there could be an other mechanism different from nutrient assimilation interacting to increase the observed growth. The most probable is a general increase in food intake because of less food wastage. During the present study, fish administered the lower MT doses had elevated appetite relative to that of the control group. The administration of more than 1.0 mg/kg of MT was observed to decrease the excretion of ammonia and increased nitrogen retention in *Juvenile O. masou* (Santandreu and Diaz, 1994). Present results in *C. punctatus* has also revealed a decrease in the excretion of ammonia and $O\text{-}PO^{-}_{4}$ production as result of MT administration (0.5-1.0 mg kg^{-1} of diet).

The results of the present study also show that administration of MT significantly reduced ammonia excretion and increased nitrogen retention and hence suggest a reduction in the degradation of body proteins. In mammals also similar responses have been observed in relation to the decrease in the excretion of nitrogenous compounds (Kochakian, 1935; 1950a.n; Lewis et al., 1981; Michelsen et al., 1982; Harrison et al., 1989).

In conclusion, the use of 17 α-methyltestosterone incorporated into the diet of *C. punctatus* appears promising from the standpoint of improving growth and feed conversion under optimal and advantageous for fish culture. An increase in VSI in treated fish could result in lower dressout weights. Nevertheless, the fact that the steroid is rapidly excreted by fish and is essentially removed after one month (Goudie et al., 1986) makes its use for food-fish production feasible. The shorter growing season in temperate zones could be partially ameliorated by use of 17 α-methyltestosterone on early life stages and continued as a feed additive to stimulate growth.

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Table1: Effect of feeding 17 α methyltestosterone (MT) on growth and feed efficiency in C. punctatus -80 day treatment (LD 12:12 at 25°C)

Parameters	Dietary level (mg per kg)			
	Control	0.5	1.0	2.0
Initial live weight (g)	9.11	9.08	9.11	9.08
Final live weight (g)	15.05	15.56	19.02	18.50
Live weight gain (g)	5.94^{D}±0.05	6.47^{C}±0.03	9.90^{A}±0.00	9.42^{B}±0.07
Growth percent gain in body weight	65.16^{D}±0.82	71.29^{C}±0.55	108.64^{A}±0.31	103.66^{B}±1.20
Specific growth rate (SGR % per day)	0.63^{D}±0.01	0.67^{C}±0.004	0.91^{A}±0.0	0.89^{B}±0.01
Food consumption per day in % body weight	1.56^{B}±0.02	1.52^{B}±0.02	1.82^{A}±0.01	1.79^{A}±0.01
Feed conversion ratio (FCR)	2.55^{A}±0.002	2.32^{B}±0.02	2.06^{C}±0.01	2.10^{C}±0.02
Protein efficiency ratio (PER)	0.90^{C}±0.01	1.08^{B}±0.02	1.24^{A}±0.01	1.22^{A}±0.01

Values are mean ±SE of mean of three observations. Means with the same letter/s in the same row are not significantly (P<0.05) different. Data were analysed by Duncan's multiple range test.

Table2: Effect of feeding 17 α methyltestosterone (MT) on final mean length, percent increase in length, mean length specific growth rate and condition factor (k) in C. punctatus -80 day treatment (LD 12:12 at 25°C)

Parameters	Dietary level (mg per kg)			
	Control	0.5	1.0	2.0
Initial mean length (cm)	9.93	9.80	9.90	9.90
Final mean length (cm)	11.03	11.83	12.96	12.94
Percent gain in length	11.21^{D}±0.67	21.23^{C}±0.72	30.63^{B}±0.33	33.90^{A}±0.82
Mean length specific growth rate	0.133^{C}±0.00	0.24^{B}±0.01	0.33^{B}±0.00	0.37^{A}±0.01
Condition factor (k)	1.12^{A}±0.01	0.92^{B}±0.01	0.86^{C}±0.57	0.85^{C}±0.01

Values are mean ±SE of mean of three observations. Means with the same letter/s in the same row are not significantly (P<0.05) different. Data were analysed by Duncan's multiple range test.

Table3: Effect of feeding 17 α methyltestosterone (MT) on nutrient retention and postparandial excretory levels of total ammonia (N-NH+4) and reactive phosphate production (O-PO-4) in C. punctatus -80 day treatment (LD 12:12 at 25°C)

Parameters	Dietary level (mg per kg)			
	Control	0.5	1.0	2.0
Gross protein retention (GPR)	17.80^{C}±0.36	22.25^{B}±0.45	28.94^{A}±0.20	28.34^{A}±0.34
Gross energy retention (GER)	12.37^{C}±0.06	16.17^{B}±0.19	22.37^{A}±0.17	22.13^{A}±0.20
Apparent protein digestibility (APD%)	80.92^{D}±0.00	81.87^{C}±0.00	88.71^{A}±0.003	88.42^{B}±0.003
$N\text{-}NH^{+}_{4}$ (mg KG^{-1} BW d^{-1})	56.18^{A}±0.951	50.47^{B}±0.47	15.44^{C}±0.33	15.40^{C}±0.20
$O\text{-}PO^{-}_{4}$ (mg KG^{-1} BW d^{-1})	42.78^{A}±0.06	39.82^{B}±0.87	22.45^{C}±0.70	23.18^{C}±0.72

Values are mean ±SE of mean of three observations. Means with the same letter/s in the same row are not significantly ($P<0.05$) different. Data were analysed by Duncan's multiple range test.

Table4: Effect of feeding 17 α methyltestosterone (MT) on viscera-somatic index (VSI), hepato-somatic index (HSI) and carcass composition (% wet weight basis) in C. punctatus -80 day treatment (LD 12:12 at 25°C)

Parameters	Initial value	Dietary level (mg per kg)			
		Control	0.5	1.0	2.0
Visero-somatic index (VSI%)	3.21±0.01	3.48^{C}±0.03	3.52^{C}±0.01	4.24^{A}±0.02	4.15^{B}±0.03
Hepato-somatic index (HSI%)	0.82±0.01	1.09^{A}±0.01	0.94^{B}±0.01	0.79^{C}±0.02	0.80^{C}±0.02
Carcass composition (%)					
Moisture	78.93±0.02	77.39^{A}±0.01	76.28^{B}±0.01	72.04^{D}±0.04	72.16^{C}±0.03
Crude protein	12.40±0.00	14.64^{C}±0.11	15.79^{B}±0.00	18.07^{A}±0.01	17.89^{B}±0.07
Crude fat	2.31±0.01	2.94^{C}±0.00	3.86^{B}±0.03	4.08^{A}±0.05	4.04^{A}±0.006
Ash	3.15±0.005	3.21^{A}±0.01	3.09^{B}±0.006	2.01^{D}±0.03	2.06^{C}±0.006
Phosphorous	0.48±0.003	0.56^{C}±0.001	0.65^{B}±0.00	0.76^{A}±0.01	0.74^{A}±0.02
Energy (kJ g^{-1})	4.39±0.001	4.93^{D}±0.01	5.41^{C}±0.004	6.53^{A}±0.01	6.48^{B}±0.006

Values are mean ±SE of mean of three observations. Means with the same letter/s in the same row are not significantly ($P<0.05$) different. Data were analysed by Duncan's multiple range test.

Table5: Effect of feeding 17 α methyltestosterone (MT) on liver glycogen and protelytic enzyme activity in C. punctatus -80 day treatment (LD 12:12 at 25°C)

Parameters	**Dietary level (mg per kg)**			
	Control	**0.5**	**1.0**	**2.0**
Liver glycogen (mg g^{-1})	3.34^{B}±0.18	4.16^{A}±0.13	2.13^{C}±0.04	2.24^{C}±0.07
Total enzyme activity (mg g^{-1} h^{-1})	1825.13^{C}±47.5	2340.74^{B}±59.2	3037.03^{1}±30.2	2962.96^{A}±0.37
Specific activity (mg of tyrosine mg^{-1} of protein h^{-1})	1.99^{B}±0.003	2.06^{B}±0.05	2.43^{B}±0.06	2.40^{A}±0.10
Values are mean ±SE of mean of three observations. Means with the same letter/s in the same row are not significantly ($P<0.05$) different. Data were analysed by Duncan's multiple range test.				