

## Effect of ethylestrenol on growth and food utilization in *Channa striatus*

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**Abstract.** Juveniles of *Channa striatus* were fed twice a week with hormone supplemented diet at concentrations of 0, 1, 2.5, 5 or 10 mg/kg fish and the hormone effects on growth and food utilization were observed for a period of 75 days. Neither ethylestrenol nor the tested doses of the steroid appear to significantly change feeding rate. Administration of 5 mg of ethylestrenol/kg fish not only enhanced growth but also conversion efficiency.

**Keywords.** Steroid supplementation; ethylestrenol; appetite stimulant; anabolic steroid; conversion efficiency; *Channa striatus*.

### 1. Introduction

From a large number of recent publications on application of growth hormone in fish culture, it has been possible to identify 14 androgenic steroids, which were found to stimulate growth in fish (Donaldson *et al* 1979; Higgs *et al* 1982). However the response to each of these steroids is species specific; for instance,  $17\alpha$ -methyltestosterone promotes growth in *Oncorhynchus kisutch* (McBride and Fagerlund 1973, 1976; Fagerlund and McBride 1975, 1977, 1979). *Cyprinus carpio* (Lone and Matty 1980), *Tilapia aurea* and *Tilapia mossambica* (Guerrero 1975, 1976) but inhibited growth in *Salmo salar* (Simpson 1976) and *Salmo gairdneri* (Sower 1978). Conversely these two species positively respond to the treatment of ethylestrenol an anabolic androgenic steroid (Simpson 1976; Ince *et al* 1982; Lone *et al* 1982).

In India effects of growth hormones was tested mostly in air-breathing fishes (Nirmala and Pandian 1983; Sindhu and Pandian 1984). However, these authors have restricted the number and the duration of the experiment; further the hormone was injected on the first day of experiment. It is known that application of growth hormone through injection involves tedious labour in aquaculture practice. Secondly as the injected hormone gets rapidly metabolised and eliminated (Donaldson *et al* 1979), the desired positive response may not persist long. Hence long term studies on the effect of hormone, supplemented at lower doses through food are required. The present long term study was undertaken to ascertain the response of *Channa striatus* fed on supplemented diet with ethylestrenol.

### 2. Materials and methods

Juveniles of *C. striatus* ( $9.4 \pm 0.41$  g) were acclimated to laboratory conditions and divided into 5 groups. They were reared individually in cylindrical aquaria (14 L

capacity) for 75 days under natural L/D cycle (14/10 hr). Room temperature averaged  $28 \pm 2^\circ\text{C}$ . The aquarium water was changed every 3 days. At the start of the experiment and thereafter every 15 days, the test individuals were weighed to the nearest 1 mg in a 'Stanton' top pan balance.

The test animals were fed at 09:00 hr on weighed amounts of live *Lepidocephalichthys thermalis*, which were left in the aquaria for a maximum period of 2 hr, i.e. as it would happen in the natural habitat, the prey had to be actively predated by the fish. The uneaten prey remains were collected subsequently with a hand sieve and dried to weight constancy at  $90^\circ\text{C}$  for 6 hr. Faeces was collected by filtering the aquarium once in 3 days. The sacrifice method (Maynard and Loosli 1962) was followed to determine water and energy contents of the test individuals at the beginning of the experiment. Energy contents of food, faeces and test animals were estimated in a Parr 1411 Semi-micro bomb calorimeter.

The energetic equation in the present study is that of IBP formula (Petrusewicz and MacFadyen 1970) represented as:  $C = F + U + P + R$ ; where  $C$  is the food consumed,  $F$  the faeces,  $U$  the nitrogenous excretory waste,  $P$  the growth and  $R$  the energy released as heat due to metabolism. Rates of feeding, absorption and conversion were calculated using the following formulae:

$$\begin{aligned} \text{Feeding rate (Cr) (J/g/day)} &= \frac{\text{Average food consumed (J/day)}}{\text{Mid-body weight (g)}} \\ \text{Absorption rate (Ar) (J/g/day)} &= \frac{\text{Average food absorbed (J/day)}}{\text{Mid-body weight (g)}} \\ \text{Conversion rate (Pr) (J/g/day)} &= \frac{\text{Average food converted (J/day)}}{\text{Mid-body weight (g)}} \\ \text{Absorption efficiency (\%)} &= \frac{\text{Food absorbed (J)}}{\text{Food consumed (J)}} \times 100 \\ \text{Conversion efficiency (\%)} &= \frac{\text{Food converted (J)}}{\text{Food absorbed (J)}} \times 100 \end{aligned}$$

Mid-body weight of the test animal is the mid-point in the growth curve of the animal for the test period. It was calculated by the following formula:

$$\text{Mid-body weight} = \frac{\text{Initial live weight of the test fish (g)} + \text{Final live weight of the test fish (g)}}{2}$$

## 2.1 Hormone administration

The selected dosages of the hormone ethylestrenol (1, 2.5, 5 and 10 mg/kg fish) were dissolved in sesame oil (carrier solution). Hormone supplementation of diet was done by intramuscularly injecting the chosen dosage of the hormone into the prey organism *L. thermalis*, starved for 24 hr (to ensure the evacuation of gut), before being offered as food for the test individuals. As soon as the hormone treated *L. thermalis* was released into an aquarium, the fish consumed the entire prey, hence the hormone injected into the prey was received by the test individuals without any loss. After ensuring that *C. striatus* has consumed the entire prey supplemented with hormone, the remaining portion of the weighed *L. thermalis* was offered. The control fish was fed with *L.*

*thermalis* injected with sesame oil alone. Volume of the hormone solution injected was maintained constant at 25  $\mu$ l for all the tested dosages. For injection, a 25  $\mu$ l capacity sterilised tuberculin syringe (Top syringe, Bombay) was used.

The hormone supplemented diet was fed to the fish twice a week for a period of 75 days. Once in 15 days the quantity of the hormone injected into the prey organism was adjusted with new mean weight of the fish. All data were subjected to statistical analyses such as students test and single way analysis of variance.

### 3. Results

#### 3.1 Consumption

Feeding rate of individuals of *C. striatus* in the sham-treated group averaged to 228 Joules/g live fish/day. Neither ethylestrenol nor the tested doses of the steroid appear to significantly ( $P > 0.05$ , table 1) change the feeding rate, which averaged to 228 J/g/day in the 4 treated groups. Hence it may be said that ethylestrenol is not an appetite stimulant for *C. striatus*.

#### 3.2 Absorption

Trends obtained for the absorption rate of fish receiving different dosages of ethylestrenol followed that of feeding rate. Control group absorbed the food at a rate of 211 J/g/day. No difference was observed among the treated individuals, and also between control and treated ones. However the tested dosages of ethylestrenol (1, 2.5, 5 and 10 mg/kg fish) increases the absorption efficiency to 96 % from 92 % in the control. These were also in consistent with values obtained by the method of Pandian and Marian (1985). The minor differences among the hormone treated individuals were not statistically significant ( $P > 0.05$ ), but the difference between treated and sham groups was highly significant ( $P < 0.01$ ).

**Table 1.** Bioenergetics of *C. striatus* fed on diets supplemented with different dosages of the steroid hormone ethylestrenol. Each value ( $\bar{X} \pm SD$ ) represents the average performance of 3 individuals fed for a period of 75 days. Rates are expressed in J/g live fish/day and the efficiencies in percentage.

Parameters	Concentration of hormone (mg/kg fish)				
	0	1	2.5	5	10
Consumption rate	228 $\pm$ 17.0	236 $\pm$ 6.0	232 $\pm$ 14.0	224 $\pm$ 18.0	221 $\pm$ 14.0
Absorption rate	221 $\pm$ 16.0	225 $\pm$ 5.0	222 $\pm$ 10.0	214 $\pm$ 20.0	214 $\pm$ 14.0
Production rate	48 $\pm$ 5.0	79 $\pm$ 5.0*	78 $\pm$ 2.0*	83 $\pm$ 4.0*	69 $\pm$ 6.0*
Absorption efficiency	93 $\pm$ 1.0	96 $\pm$ 0.3*	95 $\pm$ 0.4*	96 $\pm$ 0.1*	96 $\pm$ 0.5*
Net conversion efficiency	23 $\pm$ 1.0	35 $\pm$ 2.0*	35 $\pm$ 1.8*	40 $\pm$ 0.7*	27 $\pm$ 2.0*

Values with asterisk are statistically different from the control (ANOVA) =  $P < 0.0005$ .

### 3.3 Growth

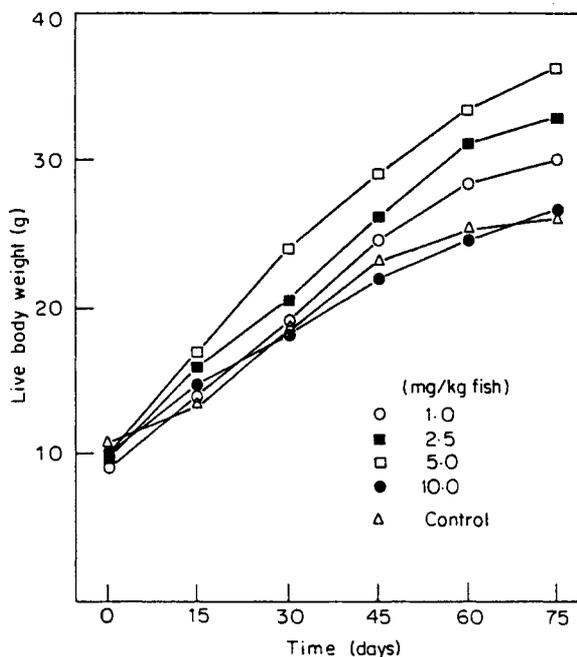
Trends obtained for growth of the tested 5 groups are shown in figure 1. During the 75 days, the fish grew from 10 g to 36, 33, 30, 27 and 26 g in the group receiving 5, 2.5, 1, 10 and 0 mg ethylestrenol/kg fish. All the experimental groups receiving low dosages of ethylestrenol (5, 2.5 and 1) were significantly ( $P < 0.001$ ) heavier than the control group (0 mg). The data clearly indicate that ethylestrenol is a growth promoter, when administered at about 5 mg/kg fish twice a week. Administration of 5 mg of ethylestrenol/kg fish is the optimal level in the fish *C. striatus*.

### 3.4 Conversion efficiency

Individuals in the control group converted the food at an average efficiency of 23%. Ethylestrenol supplementation with diet promoted the efficiency to 39% when supplemented at the optimum dose of 5 mg/kg fish. Conversion rate of the fish in the control group was around 48 J/g/day. Increasing the dosage 1, 2.5, 5 and 10 mg/kg enhanced the rate to 79, 78, 83 and 69 J/g/day.

## 4. Discussion

The present study has shown that ethylestrenol is not an appetite stimulant but an anabolic growth promoter of juvenile *C. striatus* and this observation confirms the



**Figure 1.** Effect of ethylestrenol supplemented with diet, on the growth of *C. striatus*. Each value represents the average performance of 3 individuals.

Table 2. Influence of steroid hormones on growth of commercially important fishes.

Species	Hormone	Optimum dose	Function of the hormone	Remarks	Reference
<i>Oncorhynchus kisutch</i>	17 $\alpha$ -methyltestosterone	10 mg/kg diet	Growth promoter	Cr not estimated	McBride and Fagerlund (1973)
<i>O. tshawytscha</i>	17 $\alpha$ -methyltestosterone	1 mg/kg diet	Growth promoter	Cr not estimated	McBride and Fagerlund (1973)
<i>O. kisutch</i>	17 $\alpha$ -methyltestosterone	2.5 mg/kg diet	Growth promoter	Cr not estimated	Yu <i>et al</i> (1979)
	Testosterone	2.5 mg/kg diet	Growth promoter	Cr not estimated	Yu <i>et al</i> (1979)
	Estradiol	2.5 mg/kg diet	Growth promoter	Cr not estimated	Yu <i>et al</i> (1979)
<i>O. kisutch</i>	17 $\alpha$ -methyltestosterone	1 mg/kg diet	Growth promoter	Cr not estimated	Fagerlund <i>et al</i> (1979)
<i>Cyprinus carpio</i>	17 $\alpha$ -methyltestosterone	2.5 mg/kg diet	Growth promoter	Cr not estimated	Lone and Matty (1980)
	Testosterone	10 mg/kg diet	Growth promoter	Cr not estimated	Matty and Lone (1979)
	Adrenosterone	1 mg/kg diet	Growth promoter	Cr not estimated	Matty and Lone (1979)
	Ethylestrenol	2.5 mg/kg diet	Growth promoter	Cr not estimated	Lone and Matty (1983)
<i>Channa striatus</i>	17 $\alpha$ -methyltestosterone	10 mg/kg fish	Growth promoter and appetite stimulant	Cr estimated	Nirmala and Pandian (1983)
	Testosterone	20 mg/kg fish	Growth promoter	Cr estimated	Nirmala and Pandian (1983)
	Testosterone propionate	10 mg/kg fish	Growth promoter	Cr estimated	Nirmala and Pandian (1983)
	Diethylstilbestrol	10 mg/kg fish	Growth promoter	Cr estimated	Nirmala and Pandian (1983)
	Docabolin	30 mg/kg fish	Growth promoter and appetite stimulant	Cr estimated	Nirmala and Pandian (1983)
	Diethylstilbestrol dipropionate	30 mg/kg fish	Growth promoter	Cr estimated	Nirmala and Pandian (1983)
	Estroid	30 mg/kg fish	Growth promoter	Cr estimated	Nirmala and Pandian (1983)
<i>Heteropneustes fossilis</i>	17 $\alpha$ -methyltestosterone	40 mg/kg fish	Growth promoter and appetite stimulant	Cr estimated	Sindhu and Pandian (1984)
<i>Channa striatus</i>	Ethylestrenol	33 mg/kg diet	Growth promoter	Cr estimated	Present study

Cr = Feeding rate

previous reports of Simpson (1976) and Lone and Matty (1983). Lone and Matty (1983) reported that a dose of 5 mg ethylestrenol/kg diet promotes maximum growth efficiency in juveniles of *Cyprinus carpio* without increasing feeding rate. Since Simpson (1976) and Lone and Matty (1983) fixed the dose of ethylestrenol with reference to unit weight of diet, there is a need to recalculate our data which are expressed in unit of dose per kilogram fish. Such calculation indicated that the optimum dose that maximises *C. striatus* growth is as high as 33 mg/kg diet. This high value may be compared with optimum doses reported for *C. carpio* (2.5 mg/kg diet: Lone and Matty 1983) and *Salmo gairdneri* (12.5 mg/kg diet: Simpson 1976). Clearly the anabolic property is realised in fishes, when ethylestrenol is supplemented but the optimum dose of ethylestrenol is a species specific character.

In *Salmo gairdneri* (Simpson 1976) and *C. carpio* (Lone and Matty 1983) the administration of optimum of ethylestrenol promoted the growth efficiency by about 17% higher than the control. An equal increase in efficiency was also observed in *C. striatus*. Irrespective of species specific variations in the efficiency, the optimum dose of ethylestrenol increased the growth efficiency by about 1.5 times over that of the control.

Available information on endocrine influence on somatic growth of commercially important fishes is presented in table 2. Pandian (1982) summarised the relevant information into 3 groups—(i) those which promote growth by acting as appetite stimulant (e.g. 17 $\alpha$ -methyltestosterone, docabolin and estroid), (ii) those which are anabolic growth promoter (e.g. ethylestrenol, diethylstilbesterol and diethylstilbesterol dipropionate) and (iii) those which act as anabolic growth promoter in one species but stimulate appetite in another species; testosterone acts as an appetite stimulant in *Oncorhynchus kisutch* (Yu *et al* 1979) but serves as an anabolic growth promotor in *C. striatus* (Nirmala and Pandian 1983). A large number of publications claim one or other steroid as anabolic; however these publications do not report feeding rates of the control and treated fishes. Hence it is difficult to know whether the same steroids indicated in table 2 acted as anabolic growth promoter or as an appetite stimulant. Based on the present observations, ethylestrenol served as an anabolic steroid in the young ones of *C. striatus*.

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