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Effect of steroid injection on food utilization in *Channa striatus*

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Abstract. Synthetic anabolic sex steroid hormones such as methyltestosterone (MT), testosterone (T), testosterone propionate (TP), estroid (ED), diethylstilbesterol (DES), diethylstilbesterol dipropionate (DES dipro) and docabolin (DN) were tested separately for their effect on food utilization in *Channa striatus*. Injection of low dosages of DES, DES dipro and TP acts as appetite depressors and Dn , MT, Ed and T as stimulators. MT, Ed and Dn induced not only increased feeding but also increased conversion. T promoted growth along with food consumption. Though DES and DES dipro suppressed feeding; individuals receiving these hormones showed increased growth with increasing dosage.

Keywords. Sex hormones; steroid injections, *Channa striatus*.

1. Introduction

The application of steroid sex hormones to enhance the rate and efficiency of food intake in animal husbandry resulted in substantial savings in production cost in United States (Monfort 1974). This finding has attracted the attention of aquaculturists, especially those producing highly esteemed but carnivorous fish. Unfortunately, the efficacy of several steroids on their positive growth effort is a matter of dispute, the experimental evidences being contradictory. For instance, DES, a steroid known for its high growth promoting ability in animal husbandry, is known to accelerate the growth rate and food conversion efficiency in the plaice *Pleuronectes platessa* (Cowey *et al* 1973). On the other hand, a number of workers have obtained contradictory results, indicating the growth depressing effect of DES (Bulkley 1972 : *Ictalurus punctatus*; Matty and Cheema 1978 : *Salmo gairdneri*).

A critical survey of literature reveals that to realize the growth-promoting ability, a suitable hormone and an appropriate dosage must be identified for the chosen species of fish; secondly, most of the previous workers have not attempted to experiment with an array of steroid hormones and a range of dosages to identify a suitable steroid; thirdly, previous studies have not identified whether the accelerated growth is due to enhanced appetite or improved food conversion efficiency or due to both. This paper reports the effect of a number of steroids on food consumption and conversion in the economically important air-breathing fish *Channa striatus* treated with different dosages of the selected hormones.

2. Material and methods

Juveniles of *C. striatus* (10 ± 2 g) were acclimated to laboratory conditions and feeding. For testing each hormone, five groups, each comprising a minimum of three individuals were used. The experiments were performed in round plastic

troughs (40 cm diameter, 14 l capacity) for 21 days. Room temperature averaged 28 ± 2°C. The aquarium water was changed every 3 days. The dissolved oxygen content of the aquarium water was estimated following the unmodified Winkler's method (Welch 1948).

The fish were fed everyday at 0900 hrs on weighed live *Lepidocephalicthys thermalis* which was left in the test aquaria for a maximum period of 2 hr and the residual prey-remains collected subsequently with a hand sieve and dried to weight constancy at 80°C.

Faeces was collected and dried every three days by filtering the entire aquarium. The sacrifice method (Maynard and Loosli 1962) was followed to determine water and energy contents of the test individuals before the experiment. All the experimental individuals were starved for 24 hr to ensure complete evacuation of gut, and then weighed to an accuracy of 0.1 mg. Caloric contents of fish, food and faeces were determined using a 1412 semi-micro bomb calorimeter (Parr Instrument Co., Moline, USA).

The bioenergetics components were determined using the following formulae :

$$\text{Feeding rate (Cr)} = \frac{\text{Food consumed (cal/day)}}{\text{Initial live weight of fish (g)}}$$

$$\text{Food absorbed (cal/day)} = \text{Food consumed (cal/day)} - \text{Faeces egested (cal/day)}$$

$$\text{Absorption rate (Ar)} = \frac{\text{Food absorbed (cal/day)}}{\text{Initial live weight of fish (g)}}$$

$$\text{Absorption efficiency (\%)} = \frac{\text{Food absorbed (cal/day)}}{\text{Food consumed (cal/day)}} \times 100$$

$$\text{Conversion rate (Pr)} = \frac{\text{Growth (cal/day)}}{\text{Initial live weight of fish (g)}}$$

$$\text{Conversion efficiency (\%)} = \frac{\text{Growth (cal/day)}}{\text{Food consumed (cal/day)}} \times 100$$

$$\text{Metabolic rate (Mr)} = \text{Absorption rate} - \text{Conversion rate}$$

Details of the chosen hormones are given below:

Common name	Systematic nomenclature	Manufacturers
Androgenic		
Testosterone (T)	17 β – Hydroxy-andrast-4 -ene-3	German Remedies, India
Testosterone propionate (TP)	17 β – Acetoxy-androse-4-ene-3 one	German Remedies, India
17 α-Methyl-testosterone (MT)	17 β-Hydroxy-17α-Methy-landrost-4-one-3-one	Sigma: St. Louis, USA
Estrogenic		
Diethylstilbesterol (DES)	4,4' (1,2-Diethyl-1, 2-ephenediyl bisphenol)	Sigma, St. Louis, USA

Diethylstilbesterol dipropionate (DES dipro)	Dipropionyl ester of x-x'-diethyl 4,4-stilbesterodiol	German Remedies, India
Docabolin (Dn)	17 β-(3-Phenylpropionyloxy oester-4 ene-3 one)	Organen (India Limited)
Estroid (Ed)	Dienesterol + Thyroid	Unichem Laboratories Limited

2.1 *Hormone administration*

The dosages of the chosen hormones administered were : 0,5,10,20 and 30 mg/kg fish. The hormones were dissolved and diluted with seasame oil (carrier solution) . Volume of the hormone solution injected into the fish was maintained constant at 60 µl for all the tested dosages. DES was administered intramuscular- ly, intra= peritoneally and orally through food. Nirmala (1981) found that intra- muscular administration of the hormone enhanced the conversion efficiency of *C. striatus* to the highest level of 47% compared with 28 and 31% when administered intra-peritoneally and through diet. Therefore, the chosen hormones were injected intramuscularly at the caudal region midway between the lateral line and ventral fin. Injections were given within a handling duration of 2 min without much disturbance to the individuals. Fish in the control group received sham injection of seasame oil alone. For injection, a 100 µl capacity sterilised tubercu- lin syringe (Scientific Glass Engineering, Australia) was used.

The results obtained were subjected to statistical analysis such as student's *t* test and two-way analysis of variance.

3. Results

3.1 *Feeding rate*

The data presented in table 1 reveals that *C.striatus* receiving MT, Dn and T injections consumed more food than those in the respective control groups. Individuals receiving Ed (except those in the 10 mg / kg dosage group) also displayed a higher feeding rate than those in the control group. Whereas the feeding rate of the individuals receiving Dn progressivley increased with dosage, the trend was not consistent in those treated with MT, T and Ed. In the T series, the lowest dosage of 5 mg/ kg evoked the highest feeding rate of 96 cal / g / day; at higher dosages of 10, 20 or 30 mg/ kg, the rate decreased to 82 and 77 cal/ g / day. DES and DES dipro especially in lower dosages depressed food consumption. Considering

Table 1. Feeding rate (cal / g / day) of *Channa striatus* as a function of dosage of the tested hormones.

Dosage (mg / kg fish)	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	72±2.9	72±2.4	91±2.1	78±2.0	75±2.2	71±2.7	77±2.7
5	80±2.1	83±2.2	110±2.6	73±2.4	65±2.3	96±2.4	67±2.8
10	110±1.6	69±1.2	112±2.3	66±2.4	65±2.3	82±2.1	89±2.1
20	92±1.7	77±2.5	118±2.5	80±2.8	64±2.0	77±2.0	56±2.4
30	113±1.4	82±1.9	129±2.8	77±2.2	80±2.1	77±2.7	71±0.2

Each value represents the mean (±SD) performance of 3-6 individuals.

Table 2. Absorption rate (cal/g/day) of *Channa striatus* as a function of different dosages of the tested hormones.

Dosage (mg/kg fish)	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	65±1.2	66±1.2	84±1.9	72±2.3	69±2.3	65±1.7	73±2.6
5	73±2.3	76±2.9	100±2.7	67±2.0	59±1.3	87±2.3	62±2.0
10	99±1.5	62±1.0	103±2.5	58±2.9	59±2.6	75±1.9	82±2.8
20	83±2.8	71±2.6	103±1.8	73±1.9	58±1.6	69±1.8	51±1.6
30	102±2.3	74±2.5	110±2.3	69±2.5	73±1.8	71±2.3	66±1.9

Each value represents the mean(± SD) performance of 3—6 individuals.

Table 3. Absorption efficiency(%) of *Channa striatus* as a function of hormones and dosages (mg/kg live fish).

Dosage	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	90	91	92	92	92	92	92
5	92	92	91	92	91	91	92
10	90	90	92	91	91	92	89
20	89	92	92	91	91	92	89
30	91	90	90	90	91	92	90

Each value represents the mean (±SD) performance of 3-6 individuals.

Hormones: $F(1)$ 6,24=1.08; $P>0.25$.Doses: $F(1)$ 4,24=1.43; $P>0.25$

$P > 0.05$ is not statistically significant.

all the dosages administered, 20 mg TP or DES dipro and 10 mg DES or DES dipro significantly decreased the feeding rate compared to those receiving sham injection in the respective groups (table 1).

3.2 *Absorption*

Absorption rate of the individuals receiving different dosages of the tested hormones was found to be dependant on feeding rate. Individuals displaying high feeding rate absorbed the food at a faster rate (table 2). For instance, absorption rate of the individuals receiving Dn was comparatively higher than those receiving other hormones and ranged between 100 and 110 cal/g/day for the different dosages. Corrspondingly, individuals whose feeding rate was depressed by DES (5 and 10 mg/kg) and DES dipro (5, 10 and 20 mg/kg) displayed low absorption rate ranging from 58 to 67 cal/g/day. Analysis of the data on absorption efficiency from (table 3) revealed that neither the tested hormones nor the different dosages have any significant influence on absorption efficiency, which ranged from 89 to 92%, and the difference was not statistically significant. This indicates that the steroids do not interfere with the mechanism of food absorption. Understandably, absorption efficiency of the individuals receiving different

Table 4. Weight gain (g) by *Channa striatus* as a function of the different dosages of the tested hormones.

Dosage (mg/kg fish)	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	3.0±0.2	2.8±0.1	4.4±0.4	2.9±0.2	2.6±0.2	2.7±0.3	2.4±0.3
5	4.3±0.3	3.6±0.3	4.6±0.4	4.3±0.3	2.4±0.2	5.2±0.4	4.3±0.3
10	8.1±0.9	5.2±0.4	5.9±0.6	5.9±0.5	2.8±0.3	4.5±0.5	6.5±0.5
20	3.5±0.2	6.5±0.5	7.9±0.5	5.8±0.5	4.4±0.4	6.0±0.5	2.8±0.2
30	5.3±0.2	5.8±0.3	9.3±0.8	4.3±0.3	6.4±0.5	1.3±0.2	2.6±0.3

Each value represents the mean (±SD) performance of 3–6 individuals.

Table 5. Conversion rate (cal/g/day) of *Channa striatus* as a function of different dosages of the tested hormones.

Dosage (mg/kg fish)	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	16±1.4	16±1.4	22±0.3	15±0.7	14±0.5	16±1.4	14±1.6
5	24±1.4	19±1.7	27±0.9	24±1.3	13±0.4	29±1.3	22±1.9
10	42±1.5	27±1.1	31±0.6	31±2.0	15±0.9	24±0.4	35±1.3
20	35±1.5	34±1.4	52±0.3	32±1.1	23±0.8	30±1.7	15±0.8
30	28±1.4	37±1.4	61±1.0	23±1.2	35±0.4	7±1.0	15±1.8

Each value represents the mean (±SD) performance of 3–6 individuals.

dosages of the tested hormones does not vary significantly from each other. The data revealed that neither the tested hormones nor dosages influence absorption efficiency (table 3).

3.3 *Weight-gain and conversion*

The initial live weight of the fish used in the feeding experiments in the different hormone series ranged from 9.2 to 10.6 g and the final weight from 11.7 to 19.2 g. Only in the Dn series, weight gain by the fish increased with increasing dosage and of all the tested dosages and hormones 30 mg Dn/kg induced the maximum weight gain of 9.3 g (table 4). In the DES or Ed series, weight gain by the fish increased with dosage up to 10 or 20 mg/kg and declined beyond this level, whereas DES dipro induced maximum weight gain at higher dosages, TP did so only at lower dosages, the lowest weight gain of 1.3 g was recorded for the fish receiving 30 mg T/kg.

Of the tested hormones MT, Ed and Dn induced increased feeding, increased conversion. Individuals receiving 30 mg/kg dosage of MT, Ed or Dn converted the food at the rate of 28, 37 or 61 cal/g/day compared to 16, 16 or 22 cal/g/day in the respective control groups. Individuals in the 20 mg dosage group of T displayed a two-fold increase in the conversion rate over that in the control group (table 5). Although lower dosages of DES and DES dipro suppressed feeding, individuals that received these hormones clearly indicated a tendency to increase

Table 6. Conversion efficiency (%) of *Channa striatus* as a function of different dosages of the tested hormones.

Dosage (mg/kg fish)	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	23±2.0	23±2.0	24±2.3	19±2.0	19±2.0	22±0.6	18±0.8
5	30±2.0	23±1.4	24±0.7	33±2.7	20±0.5	30±2.1	33±0.5
10	38±2.0	40±2.5	28±1.7	47±0.6	22±0.5	30±2.2	39±2.2
20	39±2.9	45±1.3	44±2.5	38±2.4	35±1.4	39±1.0	29±2.7
30	25±1.6	46±2.8	49±2.7	30±1.8	43±1.1	8±1.3	21±1.9

Each value represents the mean (±SD) performance of 3–6 individuals.

Table 7. Metabolic units (cal/g/day) of *Channa striatus* as a function of different dosages of the tested hormones.

Dosage (mg/kg fish)	Hormones						
	MT	Ed	Dn	DES	DES dipro	T	TP
0	49±2.4	50±2.4	62±1.9	57±1.6	55±2.9	49±1.8	59±2.1
5	49±2.2	57±2.3	73±2.8	43±2.8	46±0.9	58±2.4	40±2.1
10	57±2.6	35±2.9	72±2.1	27±2.8	44±2.7	51±2.2	47±2.9
20	48±2.0	37±1.5	56±2.9	41±1.3	35±2.7	39±2.8	36±2.6
30	74±2.9	37±2.5	49±2.3	46±2.3	38±1.4	64±2.0	51±2.4

Each value represents the mean (±SD) performance of 3–6 individuals.

conversion rate with increasing dosage. The conversion rate of 23 or 35 cal/g/day observed in the 30 mg dosage group of DES or DES dipro was significantly higher than 15 and 14 cal/g/day observed in the respective control groups. Briefly, while a few steroids enhance growth by increasing food consumption, a few other (DES or DES dipro) do so without increasing consumption.

3.4 *Conversion efficiency*

Data on conversion efficiency of *C. striatus* as functions of the tested hormones and dosages presented in table 6 reveal the following: (a) Administration of 10 or 20 mg dosage of MT, 20 or 30 mg dosage of ED, 30 mg dosage of Dn or DES dipro, 10 mg dosage of DES or TP and 20 mg dosage of T enhances the efficiency to a high level over their respective control groups. (b) Injection of 30 mg/kg of Ed or Dn enhanced the conversion efficiency to 46 or 49%; under similar conditions even 10 mg/kg of DES equally evoked conversion efficiency (47%). (c) The lowest efficiency of 8% is displayed by the individuals receiving 30 mg T dosage showing that at above optimal dosage (>20 mg), T functions as anti-anabolic agent. (d) Estrogens induce better conversion efficiency than androgens; the highest mean efficiency (47%), evoked by estrogens (Ed, Dn and DES) was significantly higher than that (39%) evoked by androgens (MT, T and TP). The lowest efficiency value of 8% observed in the T group was significantly lower than the lowest efficiency (20%) displayed by DES group.

3.5 *Metabolic rate*

As stated earlier certain dosages of estrogens (30 mg/kg Ed or Dn and 10 mg DES/kg) improved the growth efficiency of *C. striatus*. They achieve this by restricting the energy loss on metabolism and diverting more amount of absorbed food energy for growth compared to those receiving similar dosages of androgens. For instance, *C. striatus* receiving 30 mg/kg dosage of Ed or Dn lost 50 or 45% of the absorbed food energy on metabolism, as against 90 or 77% by those receiving 30 mg/kg dosage of T or TP. At the highest dosage (30 mg/kg), individuals receiving estrogens (ED, Dn, DES and DES dipro) displayed lower metabolic rates (37, 49, 46 and 38 cal/g/day, respectively; table 7) than those receiving androgens (MT, T and TP; 74, 64 and 57 cal/g/day, respectively). A dosage of 5 or 10 mg Dn (estrogen) per kg fish also significantly increased the metabolic rate from 62 cal/g/day in the control individuals to around 72 cal/g/day.

4. Discussion

A scanning of literature on the effect of hormones in fish revealed that earlier studies confined to growth increment (Pickford 1954a, b; Higgs *et al* 1975, 1976, 1977; Bulkley and Swihart 1973); some have also noted the impact of hormones on food consumption (Cowey *et al* 1973; Yamazaki 1976; Markert *et al* 1977; Fagerlund *et al* 1978, 1979; Yu *et al* 1979). The present study provides an integrated approach to explain growth response of fish to hormones. Steroid hormones have been proved to significantly influence feeding in fish. For instance, MT induces appetite and enhances food consumption in *Carassius auratus* (Yamazaki 1976), and *Oncorhynchus kisutch* (Fagerlund *et al* 1978, 1979) while DES depresses appetite and feeding rate in *Salmo gairdneri* (Ghittino 1970; Bulkley 1972; Fagerlund and McBride 1975 a, b). Growth can be increased either by enhancing feeding and/or restricting energy expenditure on metabolism (*e.g.* 20 mg dosage of Ed; tables 1, 4 and 7). Androgens like MT and T improve growth to a level less than that effected by estrogens through increased feeding. It is obvious that low dosages of estrogens especially DES and DES dipro (5 and 10 mg/kg) are capable of decreasing feeding and increasing growth. Observations of Fagerlund *et al* (1978) on *O. kisutch* reveal that MT administered individuals achieve maximum growth by mobilizing more fat and sparing sacrifice of proteins for energy production. Combined with this, increased feeding by these individuals was also responsible for the significant increase in growth. Hence, it is likely that DES and DES dipro administered *C. striatus* in the present study maximize growth by reserving more protein without consuming significantly more quantity of food. The significantly low rate of metabolism in these individuals supports the above conclusion.

The other estrogens ED and Dn cannot be neglected under the pretext that they induce maximum growth only by increasing the feeding and hence the enhanced cost. Individuals receiving optimal dosage (30 mg/kg fish) of Ed and Dn grew to larger size (15.6 ± 2.4g and 19.2 ± 2.3g) than those receiving other estrogens at even time. In aquaculture practices, producing fish of marketable size is of utmost importance (Bulkley 1972; Donaldson *et al* 1979). Therefore, in view of final body weight and marketable size of the individuals Ed and Dn are also useful as growth promotors, despite the high feed cost due to the administration of these hormones. Although the androgens MT and T increase feeding as well as growth of *C. striatus*, growth rate and final body weight (13.5 ± 2.0 and 11.7 ± 1.8g) of the individuals treated with these hormones are comparatively less than those

receiving Dn or Ed. Hence, based on conversion efficiency and marketable final body size of the test individuals; effectiveness of the tested hormones can be ranked in the following order Dn > Ed > DES > DES dipro > MT > T > TP.

Administration of combination of growth-promoting hormones with contrasting influence on feeding rate may yield surprising results. Combination of growth-promoting appetite stimulators such as Dn or Ed with appetite depressing growth promotors like DES or DES dipro may certainly decrease the feed cost considerably without sacrificing growth. In fact Higgs *et al* (1977) found that a combination of bGH and MT proved to be more effective in promoting growth than either bGH or MT independently. Similar studies involving combination of steroids are required.

Considering the impact of the tested dosages of each hormone on feeding and conversion rates, the hormones can be grouped under 3 different categories. (a) hormones which are effective at high dosages (*e.g.* Dn Ed : 30 mg dosage) (b) those which are effective at low dosages (*e.g.* DES, MT, TP : 10 mg dosage) and (c) those which are effective at a low dosage and unfavourable at higher dosage (*e.g.* T; 5 or 10 mg dosage favourable : 30 mg dosage unfavourable; see tables 1 and 5). Working on *Carassius auratus* Yamazaki (1976) found that 1 ppm dosage of MT was optimal for maximum growth; at 10 ppm dosage the growth rate was significantly less and at 30 ppm dosage the test individuals started losing weight. In the present study, 20 mg / kg dosage of T evoked maximum growth efficiency (39%) while at 30 mg / kg dosage, the efficiency was remarkably reduced to 8%. Reviewing the available information on hormonal enhancement of growth in fish, Donaldson *et al* (1979) proposed that at higher dosages a few steroids exert deleterious effects on various organs and cumulatively cancel the growth-promoting effect. Therefore, administration of arbitrary dosages of hormones may not lead to the realization of the property of the hormone. Optimal dosage for hormones should be fixed separately for each species treating different individuals with a wide range of dosages. For the order of preference of the tested hormones reported above the following are the optimal dosages : Dn : 30 mg; Ed: 30mg; DES: 10mg; DES dipro: 30mg; MT: 10mg pr 20mg; T: 20mg; TP: 10mg. These preferable dosages have been fixed considering the conversion efficiency of the test individuals in the respective groups (table 7).

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