Journal of the Indian Fisheries Association
23,1993, 65-71

FIELD APPLICATION OF ANABOLIC STEROIDS IN CARP SEED PRODUCTION I : REARING OF FRY TO FINGERLING STAGE

K. VENKATESHVARAN*, G.VENUGOPAL, AND R.R.K. REDDY
Freshwater Fish Farm (CIFE),
Balabhadrapuram - 533 360, Andhra Pradesh

ABSTRACT

Ethylestrenol (17 β - Hydroxy-17 α -ethyl-estr-4-en-3-one) and Stanozolal (17β - Hydroxy 17 α -methy l-5 α -androstano - (3,2-C)- pyrazole), both synsthetic androgenic steroids, were fed via diet at 3 ppm to the fry of catla,rohu and silver carp which were reared upto fingerling stage over a period of 167-172 days in earthen ponds. Ethylestrenol enhanced growth in silver carp and rohu but retarded growth in catla. Stanozolal depressed growth in all the 3 species. Length-weight relationship for these fry had been worked out and the relative condition factor in all the cases was very close to or slightly above 1.0.

INTRODUCTION

Aquacultural scientists for a number of years were trying to improve the efficiency of food conversion. The use of anabolic promoting drugs in animal hasbandry to enhance growth rate and realise substantial savings in production costs promoted investigations into the use of sex hormones and their synthetic analogues in fish.

Hormonal and nonhormonal growth promoters have been used in the husbandry of birds and mammals for a long time, but their use in aquacultural activities is quite recent. According to Donaldson *et al.* (1979) three factors which enter into yield per unit cost are amenable to alteration by the use of hormonal growth promoters : i) the time to reach the appropriate size for release can be shortened; ii) the fish can be grown to a larger size prior to release at the normal time, thus increasing survival after release; and iii) the food conversion efficiency can be improved, an important factor considering the cost of feed.

Donaldson *et al.* (1979) and Higgs *et al.* (1982) have chronicled the advances in the use of steroid hormones and their derivatives in aquaculture. Matty (1985), summarising earlier works, suggested that the hormonal growth promoters to be used in fish farming must be incorporated into the feed.

Works on these lines from India have been carried out on mahseer (Shyama and Keshavanath, 1988), rohu (Nanjundappa and Varghese, 1988), fingerlings of catla, rohu, mrigal and common carp (Deb and Varghese, 1988) and the fry of mrigal and common carp (Omprakash, 1990). As most of these studies are *in vitro,* and as information on the anabolic effects of hormones on various life history stages of fishes is scanty, the present study was taken up to examine the anabolic activity of two synthetic steroids on the fry of catla, rohu and silver carp.

MATERIAL AND METHODS

Study area : The study was conducted at the Freshwater Fish Farm of CIFE at Balabhadrapuram, East Godavari District, Andhra Pradesh between October, 1990 and January, 1991.

Test hormones : Ethylestrenol (17 β-Hydroxy-17 α-ethyl-estr-4-en-3-one) marketed under the brand name Orabolin by M/s Infar (India) Limited, Calcutta and Stanozolal (17 β-

*Central Fisheries Extension Training Centre, Kakinada;
Present Address : Central Institute of Fisheries Education, Versova, Bombay - 400 061.

Hydroxy-17 α -methyl -5 α - androstano - (3,2-C) pyrazole) marketed under the brand name Menabol by M/s CFL Pharmaceuticals Limited, Bombay, were the two hormones used. These were selected due to their ready availability in the market in tablet form at inexpensive rates.

Test animals : The fry of catla *(Catla catla),* rohu *(Labeo rohita)* and silver carp *(Hypophthalmicthys molitrix)* were used. These fry were reared in the same nursery ponds where they were reared from spawn stage, due to certain functional constraints at the farm.

Experimental design : 15 earthen nursery ponds, each measuring 20 m x 10 m (0.02 ha) were used. Table 1 provides details of stocking and other relevant particulars. Pre- and post-stocking management practices adopted were those of CIFE (Venugopal and Venugopal, 1988). Artificial feeding was @ 10% of body weight during the first month which was then reduced to 5% of body weight. The hormonal tablets were finely powdered and premixed to the feed at 3 mg/kg of feed (@ 3 ppm),a dose arbitrarily arrived at based on Matty (1985).

Water quality : Analysis of water quality parameters, and of plankton population were carried out once in a fortnight following standard methods (APHA, 1975).

Growth Studies and Relative Condition Factor : Growth of the test animals was measured once a month. The fingerlings were harvested by repeated drag-netting with a cotton drag net.

Following Lagler (1956) the relative condition factor K_{TL} (K Total Length) was computed from the pooled-up length - weight data using the formula

$$K_{TL} = \frac{W}{a.Ln}$$

RESULTS

The range of variation observed in the various water quality parameters and plankton volumes are presented in Table 2. Qualitatively cyanophycean members, especially *Anacystis* dominated the phytoplankton population while rotifers such as *Keratella* and *Brachionus* dominated the zooplankton population.

Results of the growth studies are presented in Table 3 and those of relative condition factor in Table 4.

Table 1 : *Details of test animals stocked in the ponds and nature of hormone treatment given.*

Pond No.	Species	No. Stocked	Density (no/ha)	Av. size stocked (length (cm) weight (g))	Date of stocking	Hormone received	Date of harvest
KNP 48	Catla	80	4000	16.5 - 70.0	23.10.1990	Ethylestronol	12.4.1991
KNP 49	Catla	80	4000	16.5 - 70.0	23.10.1990	Ethylestrenol	12.4.1991
KNL 50	Catla	80	4000	16.5 - 70.0	23.10.1990	Stanozolal	12.4.1991
KNP 51	Catla	80	4000	16.5 - 70.0	23.10.1990	Stanozolal	12.4.1991
KNP 66	Catla	80	4000	16.5 - 70.0	23.10.1990	Nil (Control)	12.4.1991
KNP 54	Silver Carp	80	4000	16.5 - 70.0	23.10.1990	Ethylestrenol	12.4.1991
KNP 55	Silver Carp	80	4000	5.5 - 1.8	28.10.1990	Ethylestrenol	12.4.1991
KNP 56	Silver Carp	80	4000	5.5 - 1.8	28.10.1990	Stanozolal	12.4.1991
KNP 57	Silver Carp	80	4000	5.5 - 1.8	28.10.1990	Stanozolal	12.4.1991
KNP 67	Silver Carp	80	4000	5.5 - 1.8	27.10.1990	Nil (Control)	12.4.1991
KNP 60	Rohu	80	4000	9.5 - 9.5	28.10.1990	Ethylestrenol	12.4.1991
KNP 61	Rohu	80	4000	9.5 - 9.5	28.10.1990	Ethylestrenol	12.4.1991
KNP 62	Rohu	80	4000	9.5 - 9.5	28.10.1990	Stanozolal	12.4.1991
KNP 63	Rohu	80	4000	9.5 - 9.5	28.10.1990	Stanozolal	12.4.1991
KNP 68	Rohu	80	4000	9.5 - 9.5	28.10.1990	Nil (Control)	12.4.1991

Table 2 : *Showing Variations in physico-chemical parameters of water and plankton volume during the study period.*

Pond No.	Temp.(^{O}C)		DO (ppm)		Free CO_2(ppm)		pH		Tot.Alk CaCo3(ppm)		Plankton (cc/50 1)	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
KNP 48	27.0	29.0	6.0	12.0	0.0	16.0	8.2	8.6	160.0	180.0	0.1	0.7
KNP 49	27.0	28.0	8.0	10.0	2.0	6.0	8.0	8.3	170.0	180.0	0.2	0.9
KNP 50	26.5	30.0	6.4	10.0	2.0	18.0	8.0	8.3	160.0	180.0	0.1	0.9
KNP 51	26.5	30.0	8.4	12.0	2.0	18.0	8.0	8.3	170.0	200.0	0.1	1.3
KNP 66	27.0	29.5	8.0	16.4	0.0	16.0	8.0	8.4	170.0	180.0	0.2	1.1
KNP 54	27.0	29.5	8.4	10.8	2.0	16.0	8.0	8.3	164.0	180.0	0.3	1.0
KNP 55	26.5	28.5	6.4	8.0	2.0	18.0	8.2	8.3	172.0	190.0	0.2	1.2
KNP 56	26.5	29.5	7.2	10.4	2.0	20.0	8.2	8.3	156.0	194.0	0.2	1.2
KNP 57	26.0	29.0	4.0	9.6	0.0	4.0	8.2	8.6	162.0	200.0	0.2	1.1
KNP 67	26.0	29.5	4.0	8.8	0.0	16.0	8.0	8.4	178.0	194.0	0.2	1.2
KNP 60	26.0	29.5	5.2	8.0	0.0	18.0	8.0	8.6	162.0	190.0	0.2	1.3
KNP 61	26.0	29.0	4.0	8.0	2.0	16.0	8.0	8.3	152.0	188.0	0.1	1.1
KNP 62	26.0	29.0	4.8	8.0	2.0	14.0	7.9	8.3	164.0	212.0	0.2	1.4
KNP 63	27.0	29.0	6.0	8.0	0.0	18.0	8.2	8.6	180.0	210.0	0.1	1.1
KNP 68	26.0	29.0	4.8	8.0	0.0	2.0	8.2	8.6	168.0	190.0	0.1	1.2

Table 3 : *Survival and growth of test animals over the study period*

Pond No.	Species	Hormone	Duration in days	No. harvested	Survival (%)	Av. length (cm)	Av. daily increment (cm)	Av. weight (g)	Av. daily increment (g)
KNP 48	Catla	E	172	72	90.00	25.3	0.05	213.8	0.92
KNP 49	Catla	E	172	78	97.50	26.0	0.06	259.8	0.91
KNP 50	Catla	S	172	69	86.25	26.5	0.06	249.0	1.05
KNP 51	Catla	S	172	65	81.25	25.7	0.05	251.0	0.96
KNP 66	Catla	C	172	72	90.00	28.0	0.06	290.0	0.92
KNP 54	Silver carp	E	167	49	61.25	25.2	0.12	175.0	1.00
KNP 55	Silver carp	E	167	66	82.50	29.3	0.14	229.0	1.34
KNP 56	Silver carp	S	167	49	61.25	18.5	0.08	88.0	0.45
KNP 57	Silver carp	S	167	44	55.00	14.5	0.05	29.0	0.18
KNP 67	Silver carp	C	168	59	73.75	23.0	0.10	132.0	0.76
KNP 60	Rohu	E	167	75	93.75	29.0	0.12	238.0	1.36
KNP 61	Rohu	E	167	77	96.25	27.2	0.12	210.0	1.17
KNP 62	Rohu	S	167	76	95.00	24.3	0.09	186.0	0.90
KNP 63	Rohu	S	167	76	95.00	26.8	0.10	192.0	1.11
KNP 68	Rohu	C	167	78	97.50	26.7	0.09	205.0	0.97

(E = Ethylestrenol; S = Stanozolal; C = Control)

Table 4 : *Length-weight relationship and relative condition factor of test animals during study period*

Pond No.	Species	Hormone	Length-weight relationship	K_{TL}
KNP 48	Catla	E	$W = 0.0300\ L^{2.7517}$	1.01
KNP 49	Catla	E	$W = 0.0494\ L^{2.6057}$	0.97
KNP 50	Catla	S	$W = 0.0163\ L^{2.9398}$	1.02
KNP 51	Catla	S	$W = 0.0437\ L^{2.6141}$	1.03
KNP 66	Catla	C	$W = 0.0727\ L^{2.4800}$	1.01
KNP 54	Silver carp	E	$W = 0.0052\ L^{3.2405}$	1.04
KNP 55	Silver carp	E	$W = 0.0281\ L^{2.6906}$	1.03.
KNP 56	Silver carp	S	$W = 0.0075\ L^{3.1429}$	1.05
KNP 57	Silver carp	S	$W = 0.0201\ L^{2.6942}$	1.09
KNP 67	Silver carp	C	$W = 0.0167\ L^{2.8665}$	1.00
KNP 60	Rohu	E	$W = 0.0429\ L^{2.5754}$	1.00
KNP 61	Rohu	E	$W = 0.1009\ L^{2.2868}$	1.02
KNP 62	Rohu	S	$W = 0.0445\ L^{2.5683}$	1.01
KNP 63	Rohu	S	$W = 0.1000\ L^{2.3009}$	1.00
KNP 68	Rohu	C	$W = 0.0497\ L^{2.5433}$	1.00

E = Ethylestreno; S = Stanozolal; C= Control ; K_{TL} = K (Total Length)

DISCUSSION

Physico-chemical and biological parameters of water : The regime of physico-chemical parameters showed different degrees of fluctuations (Table 2); however, these had always been within the optimum levels (Alikunhi, 1957) and are characteristic of eutrophicated tropical ponds associated with aquacultural activities (Stickney, 1979). Similarly indicative of eutrophicated water bodies are the Cyanophyceae (Strom, 1924) and the Rotatoria (George *et al.* 1986).

Anabolic response of fish to hormones :

Ethylestrenol : Ethylestrenol had been fed via diet to *Tilapia melanopleura* (Hutchinson and Campbell, 1964) and to Atlantic salmon *Salmo gairdneri* at 2.5 ppm (Simpson, 1976 a). Matty (1985) also reported ethylestrenol to enhance the growth of carps and salmonids.

Table 3 indicates that ethylestrenol at 3 ppm during the present study had retarded growth in catla but promoted growth in the case of silver carp and rohu. The growth rate had also been depressed in the fry receiving the treated feed. Survival was as good as, or better than, that in the control pond except in KNP 54 (silver carp) where it was lesser. Matty (1985) stated that growth retardation takes place at elevated doses of hormones but in an overall way suggested that 3 ppm of most hormones would be an optimum dose for most fishes. Thus, it is possible that 3 ppm of ethylestrenol may be a high dose for catla fry. Moreover, Simpson (1976 b) had shown ethylestrenol treatment in Atlantic salmon to be most effective in promoting growth in the slower growing mode during the first year of growth and that the growth of the faster growing mode was depressed by hormone treatment when the weight exceeded 10 g.

Stanozolal : Matty (1985) reported that

Stanozolal improves weight gain in carp and salmonids, while Bulkley and Swihart (1973) reported enhanced growth of the goldfish, *Carassius auratus* when they were fed stanozolal via diet at doses ranging between 8.3 and 83.3 mg/kg feed. *In vitro* studies of Omprakash (1990) showed an anabolic responses in the fry of mrigal and common carp and lack of such a response in silver carp; an optimum dose of 0.9 ppm for mrigal fry and of 0.9 - 1.8 ppm for common carp fry was suggested.

The present data (Table 3) display growth retardation in all three species and a poorer survival when they were fed stanozolal via diet at 3 ppm. Growth retardation has been maximum in silver carp; Omprakash (1990) had shown a lack of anabolic response by the fry of this species to stanozolal.

Relative condition factor : Increase in weight (= soft tissue growth) does not correspond to increase in length (= bone growth) of hormone treated fish and hence the relative condition factor is decreased to levels less than 1.0 (Matty, 1985). No such decrease was observed in the present study in any experimental pond including controls (Table 4) irrespective of positive or negative response to the hormone by the fish.

In Coho salmon, feeding various hormones in doses of 0.2 to 1.0 ppm resulted in condition factors which were inversely proportional to the dose (Fagerlund and Mc Bride, 1975 & 1977; Mc Bride and Fagerlund, 1976). However, a 10 ppm dose of 17 α-methyltestosterone (Fagerlund and Mc Bride, 1975) reversed this relationship and brought about a condition factor substantially greater than those obtained with lower doses.

It is also possible that the present data on relative condition factor is an indirect proof that the hormone dosage was on the higher side.

Tissue residues of orally administered anabolic steroids : No efforts were made to study the tissue residues in the present case. Johnstone *et al.* (1983) and Matty (1985) have shown the rapid elimination of these compounds in carp and pacific salmon. On the basis of 11 - day concentrations and half-lives determined in plasma and tissue (Fagerlund and Mc Bride, 1978) one may anticipate that, when hormone treated juveniles are released to the ocean and later recaptured as adults or precociously matured males, residues will be below the levels which can be detected by any known methods (Donaldson *et al.* 1979). Applying the same logic, hormone treated spawn or fry when harvested as adults will be expected to have residues well below normal tissue levels or below detectable levels and are hence safe for human consumption.

If, on the other hand, the hormones are to be applied in the culture of impounded fish, which will be used for human consumption, then a hormone withdrawl period will have to be imposed at least when synthetic 17 α - methyltestosterone is used (Donaldson *et al.* 1979; Matty,1985).

ACKNOWLEDGEMENTS

The authors are thankful to Dr.G.Gopalkrishna, formerly Principal, Central Fisheries Extension Training Centre, Kakinada and presently Scientist-in-Charge, Freshwater Fish Farm, Balabhadrapuram for facilities provided; and to Prof.Y. Sreekrishna, Director, Central Institue of Fisheries Education, Bombay for permission to publish the paper.

REFERENCES

APHA, 1975. *Standard Methods for the Examination of Water and Wastewater.* 14th Ed. APHA, AWWA, WPCF, Washington D.C., U.S.A. : 1193 p

Alikunhi,K.H. 1957. Fish Culture in India. *Fm. Bull. Indian Coun. Agri. Res.,* **20** : 144 p

Bulkley, R.V., and Swihart G.L. 1973. Effects of the anabolic steroid stanozolal on growth of channel catfish, *Ictalurus punctatus* and goldfish *Carassius auratus. Trans. Am. Fish. Soc.,* **102** : 444 - 446.

Deb, D. & Varghese T.J. 1988. Effect of 17 α - methyltestosterone on the growth and food conversion of major carps. *Proc. First Indian Fish. Forum* : 69 - 71.

Donaldson, E.M., Fagerlund U.H.M., Higgs D.A., and Mc Bride J.R. 1979. Hormonal Enhancement of Growth p.255 - 597 In : Hoar, W.S., Randall, D.A. and Brett, J.R.(eds). *Fish Physiology* Vol. VIII : *Bioenergetics and Growth.* Academic Press, New York : 786 p

Fagerlund, U.H.M. and Mc Bride, J.R. 1975. Growth increments and some flesh and gonad characteristics of juvenile coho salmon receiving diets supplemented with 17 α - methyltestosterone. *J. Fish Biol.*, **7** : 305 - 314.

Fagerlund, U.H.M. and Mc Bride J.R. 1977. Effect of 17 α - methytestosterone on growth, gonad development, external features and proximate composition of muscle of steelhead trout, coho and pink salmon. *Fish. Man. Serv. Tech. Rep.* No.716

Fagerlund, U.H.M. and Mc Bride J.R. 1978. Distribution and disappearance of radioactivity in blood and tissues of Coho salmon *(Oncorhynchus kisutch)* after oral administration of 3H - testosterone. *J. Fish. Res. Board Can.*, **35** : 893-900.

George, J. P., Venugopal, G. and Venkateshvaran K. 1986. Anthropogenic eutrophication in a perennial tank : Effect on growth of *Cyprinus carpio communis. Indian J. Environ. Hith.*, **28** (4) : 303 - 313.

Higgs, D.A., Fagerlund U.H.M., Eales, J.G. and Mc Bride J.R. 1982 Application of thyroid and steroid hormones as anabolic agents in fish culture. *Comp. Biochem. Physiol.*, **73** (B) : 143- 176.

Hutchinson.R.E. and Campbell, G.D. 1964 An attempt to increase the weight of *Tilapia melanopleura,* a fish used in fish farming, with an anabolic steroid ethylestrenol.*S. Afr. Med. J.*, **38** : p640

Johnstone, R., Macintosh, D.J., and Wright R.S. 1983. Elimination of orally administered 17 α - methyltestosterone by *Oreochromis mossambicus* (tilapia) and *Salmo gairdneri* (rainbow trout) juveniles. *Aquaculture,* **35** : 249-257.

Lagler, K.F. 1956. The effect of ethylestrenol on the growth, food conversion and tissue chemistry of the carp, *Cyprinus carpio. Aquaculture,* **32** : 39-55.

Matty, A.J. 1985. *Fish Endocrinology.* Croom Helm, Timber Press, Portland, U.S.A. : 267 p

Mc Bride, J.R. and Fagerlund, U.H.M 1976. Sex steroids as growth promoters in the cultivation of juvenile coho salmon *(Oncorhynchus kisutch). Proc. World Maricult. Soc.*, **7** : 145 - 161.

Nanjundappa, T. and Varghese T.J., 1988. Effect of diethyl stilbestrol on growth and food conversion of *Labeo rohita* (Ham.) *Proc. Fist Indian Fish. Forum* : 79-80.

Omprakash. 1991. *A Study on Growth Amplification Activity of Synthetic Androgenic Steroid Hormone in Three Species of Carp Fry.* Unpubl. Dissertation CFETC (CIFE/ICAR) Kakinada : 45 pp.

Shyama, S. and Keshavanath, P. 1988. Growth responses of Mahseer *(Tor khudree)* and Silver carp *(Hypophthalmichthys molitrix)* to steroid incorporated diets. *Proc. First Indian Fish. Forum,* 73 -75.

Simpson, T.H. 1976 a. Cited in T. Loftas, Day of the Farmed Scotch salmon. *New Sci.*, **70** : 20 - 21.

Simpson, T.H. 1976 b. Endocrine aspects of

salmonid culture. *Proc. R. Soc. Edinburgh,* Sect. B. 75 : 241 - 252.

Stickney, R.R. 1979. *Principles of Warmwater Aquaculture.* John Wiley & Sons, New York : 375 pp.

Strom, K.M. 1924 Studies on the ecology and geographical distribution of freshwater algae and plankton. *Rev. Algalog. T.I.,* 2 : 127-155.

Venugopal, G. and Venugopal N. 1988. Nursery Pond Management practical guidelines. Pap 17. In : *Background Information for Short Term Training Programme on Management of Freshwater Fish Farms.* Fresh Water Fish Farm of CIFE, Balabhadrapuram, A.P.