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Effect of Feeding 19-Norethisterone on Growth and Body Composition of Rohu, *Labeo Rohita*

B. GANGADHAR, M.C. NANDEESHA, T.J. VARGHESE and P. KESHAVANATH

Department of Aquaculture
College of Fisheries
University of Agricultural Sciences
Mangalore 575 002, India

Abstract

A growth trial with fingerlings of rohu, (*Labeo rohita* Cyprinidae) fed on pelleted diets supplemented with 19-norethisterone at doses of 0, 0.25, 0.50, 0.75 and 1.0 ppm was conducted over a period of 120 d. The steroid significantly improved weight gain at 0.75 ppm level of inclusion. At this dosage, specific growth rate, food conversion ratio, protein efficiency ratio and net protein retention were also better. An increase in carcass protein and fat content and a decrease in moisture content were recorded in the steroid-fed fish. Protein digestibility of the diets also showed an improvement in steroid incorporation. The results indicate that 19-norethisterone can be used as a potent anabolic steroid to enhance growth in rohu at 0.75 ppm.

Introduction

The beneficial effects of anabolic steroids on growth and protein accretion in farm animals have prompted investigations on their possible benefits in fish culture. The more important steroids used for growth promotion in aquaculture include 17α-methyltestosterone (MT), 11 keto-testosterone and ethylestrenol. The growth promoting effect of MT has been investigated extensively in goldfish (Yamazaki 1976), common carp (Lone and Matty 1980 1983), coho salmon (Fagerlund *et al.* 1983), catfish (Sindhu and Pandian 1984; Mukhyopadhyaya *et al.* 1986), tilapia (Macinthosh *et al.* 1985; Pandian and Varadraj 1987; Basavaraja *et al.* 1991), murrel (Arul 1986), Indian major carps (Deb and Varghese 1988), grouper (Chua *et al.* 1989) and mahseer (Gogoi and Keshavanath 1990). In the present study, an attempt was made to evaluate the effect of the synthetic androgen, 19-norethisterone (Norethindrone; 17à-ethynyl-19-nortestosterone; 19-nor-17α-ethynyl-4-androsten-17α-ol-3-one), on growth and carcass proximate composition of the Indian major carp, rohu (*Labeo rohita*).

Materials and Methods

The standard fish meal-based pelleted feed developed at the College of Fisheries, University of Agricultural Sciences, Mangalore, India (Varghese *et al.* 1976), was used as the dietary medium for hormone administration

Table 1. Composition and proximate analysis of basal feed.

Ingredient	%	Proximate analysis	%
Fishmeal	26	Moisture	6.63 (0.10)
Groundnut oil-cake	25	Protein	30.40 (0.63)
Rice bran	38	Fat	3.50 (0.26)
Tapioca flour	10	Ash	19.38 (0.35)
Vitamin and mineral mix*	1	Fibre	13.03 (0.37)
		Nitrogen free extract	27.06
		Energy (kJ/g)	11.62

Note: Figures in parenthesis indicate standard deviation.
*Supplevite-M - Sarabhai Chemicals Ltd., India.

(Table 1). The hormone was incorporated at four levels (viz. 0.25, 0.50, 0.75 and 1.0 ppm, respectively) and the hormone-free diet served as the control. Fifty mg of the hormone was first dissolved in 100 ml of ethanol and the required volume of this solution was mixed with an excess (100 ml•kg^{-1} diet) of ethanol and added to cooked and cooled dough in order to get the desired concentrations. The dough was mixed thoroughly to ensure uniform dispersal of the hormone before pelletizing. The pellets were dried to less than 10% moisture and stored in dry polythene bags. The control diet was also prepared in the same manner, but without the addition of the hormone. The experiment was carried out using 15 cement tanks, with triplicate tanks for each treatment over a period of 120 d. Each of the 25 m^2 (5 x 5 x 1 m) tank was stocked with 25 rohu fingerlings of an average weight of 3.20 g. The fish were fed once daily in the morning at 5% body weight. The fish were sampled once every two weeks and the quantity of feed was adjusted based on the growth. Water quality parameters such as temperature, dissolved oxygen, pH and total alkalinity were measured every two weeks using APHA (1985) methods. Quantitative planktonic assay was carried out by filtering 100 l of water from each cistern using 60 μ bolting silk cloth. On termination of the experiment, individual weights of fish were recorded and samples from each treatment were taken for carcass proximate analysis (AOAC 1975). Specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and net protein retention (NPR) were calculated according to the following equations:

$$SGR = \frac{\text{Log}_e \text{ Final weight} - \text{Log}_e \text{ Initial weight X 100}}{\text{Experimental duration in days}}$$

$$FCR = \frac{\text{Dry weight of feed given (g)}}{\text{Wet weight gain (g)}}$$

$$PER = \frac{\text{Gain in wet weight of fish (g)}}{\text{Dry weight of protein fed}}$$

$$NPR = \frac{\text{Gain in carcass protein (g) X 100}}{\text{Dry weight of protein fed (g)}}$$

A short-term experiment was also conducted in 10 glass aquaria (77 x 38 x 38 cm) with static water system to study the digestibility of the diets. In each aquarium, five individuals (average weight = 20 g) were stocked and acclimatized to the control and test diets for a period of 10 d. Fish in two aquaria were fed with each of the diets at 5% body weight, once daily at 1000 hr. The left over feed was removed at 1600 hr. The fecal samples were collected through siphoning at 0900 h the following day. After collection of fecal matter, water in the tanks was completely changed. Fecal samples collected over a period of 20 d from each treatment were pooled together and protein digestibility was determined employing crude fiber as the marker using the following formula:

$$100 - 100 \text{ X } \frac{\text{\% marker in diet}}{\text{\% marker in feces}} \text{ X } \frac{\text{\% protein in feces}}{\text{\% protein in diet}}$$

Duncan's multiple range test (1955) was used to rank the treatment means tested for significance ($P<0.05$) employing analysis of variance (ANOVA) for the different parameters.

Results and Discussion

Water quality parameters, viz., temperature (26-29°C), dissolved oxygen (6.10-8.76 ppm), pH (7.20-8.90) and total alkalinity (33.46-66.25 ppm) were within the desirable limits for the growth of carps. Since the ponds were without soil base and not fertilized, dry weight of plankton obtained was negligible (0-12.5 $mg \cdot 100^{-1}$) in the different treatments.

The growth rates of rohu fed with the control and test diets are presented in Fig.1. Although there was an overlap in growth during the early part of the experiment, marked differences could be noticed between the different treatments after 45 d. The weight attained under 0.75 ppm treatment was consistently better than in other treatments almost throughout the study period and growth recorded in this treatment on termination was higher than in the other treatments. A decline in growth was recorded under 1.0 ppm steroid treatment (Table 2).

While there have been no published reports on the usage of 19-norethisterone in carps, excepting a recent study on common carp (Gangadhar *et al.* 1996), there are reports available on the usage of closely related androgen, methyltestosterone in carps and other species. Lone and Matty (1980) recorded the best growth of common carp when treated with MT at 2.5 - 5.0 ppm. However, Deb and Varghese (1988), in the common carp, catla and rohu, and Yamazaki (1976), in gold fish (*Carassius auratus*), respectively, obtained better growth at the 1 ppm level of dietary MT. Against these results, 19-norethisterone proved more potent than MT. Gangadhar *et al.* (1996) observed

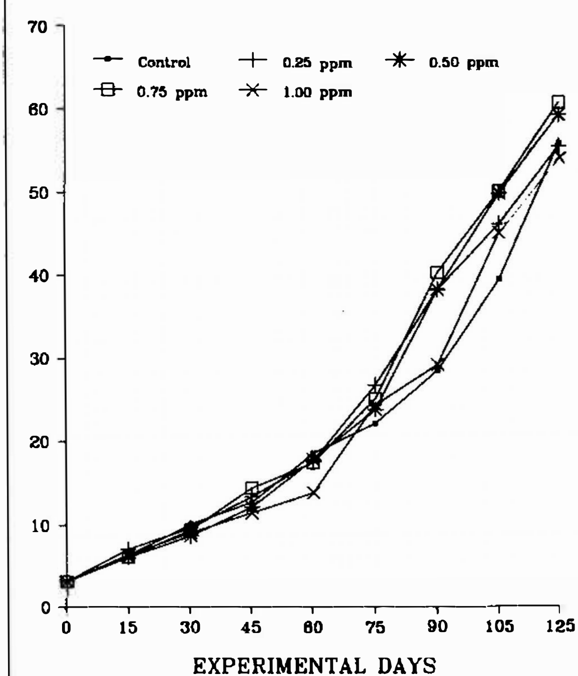

Fig. 1. Growth of rohu reared on experimental diets containing different doses of 19-norethisterone.

Steroids can activate the release of, or act additively with, the endogenous hormones to bring about growth promotion. Evidence suggests that MT activates the thyroid, interrenal and pancreas in fish (Higgs *et al.* 1977). Synergistic effect of MT with thyroid hormones has been reported by Higgs *et al.* (1977) and Fagerlund *et al.* (1980). Growth depression observed in the present study at 1.0 ppm of androgen supplementation is probably due to the catabolic action of the steroid at higher levels of supplementation (Lone and Matty 1980, 1983; Nirmala and Pandian 1983; Basavaraja *et al.* 1989; Satoh and Nimura 1991).

Although SGR, FCR and PER were better in treated fish up to 0.75 ppm level of steroid incorporation as compared to control, there was no significant difference between the two treatments statistically. Improvement in the food conversion efficiency with steroid feeding has been reported in coho salmon (Fagerlund *et al.* 1978), rainbow trout (Matty and Cheema 1978), common carp (Lone and Matty 1980), rohu (Deb and Varghese 1988), mahseer (Gogoi and Keshavanath 1988) and red sea bream (Woo *et al.* 1993) through better utilization of feed. Steroid-treated fishes showed better net protein retention in comparison with the control; this is reflected by higher protein accretion in the body. Anabolic steroids have been shown to facilitate in vivo incorporation of [C-14]-leucine into the muscle proteins of rainbow trout (Matty and Cheema 1978).

The digestibility study revealed that steroid incorporation improved dietary protein digestibility in all the treatments (Table 2). The highest protein digestibility coefficient was recorded with the diet containing 0.50 ppm norethisterone. Higher protein and fat digestibility has been observed in common carp fed 19-norethisterone (Gangadhar *et al.* 1996). Improved food assimilation and better digestibility of nutrients with MT feeding has been reported in common carp (Lone and Matty 1980), Indian major carps (Deb 1986) and mahseer (Gogoi and Keshavanath 1988). Ince *et al.* (1982) found that dietary ethylestrenol increased apparent digestibility and assimilation in rainbow trout. Hormones affect the central nervous system and therefore, feeding behavior

Table 2. Growth indices and protein digestibility in rohu fed hormone-supplemented diets. Initial weight 3.20 + 0.27 g; length 5.18 + 0.23 cm.

Parameter	Diet (ppm)				
	Control	0.25	0.50	0.75	1.00
Final weight (g)	55.39[ab] (1.42)	55.07[ab] (2.40)	58.85[bc] (2.37)	60.31[c] (1.85)	53.98[a] (0.63)
Final length (cm)	15.47 (0.96)	15.21 (0.98)	15.65 (0.98)	15.77 (1.12)	15.33 (1.19)
Specific growth rate (%)	2.37 (0.02)	2.37 (0.06)	2.43 (0.03)	2.44 (0.02)	2.35 (0.01)
Food conversion ratio	2.27 (0.06)	2.24 (0.17)	2.09 (0.08)	2.04 (0.06)	2.27 (0.02)
Protein efficiency ratio	1.17 (0.03)	1.15 (0.09)	1.24 (0.05)	1.27 (0.04)	1.14 (0.01)
Net protein retention (%)	8.23[a] (0.14)	10.80[c] (0.62)	9.24[ab] (0.66)	10.98[c] (0.26)	10.44[bc] (0.81)
Overall survival (%)	76.00	80.00	76.00	78.66	76.00
Apparent digestibility of protein (%)	73.03 (0.33)	79.52 (0.20)	82.14 (0.13)	76.90 (0.30)	79.76 (0.46)

Note: Figures in the same row having different superscript are significantly different ($P<0.05$).
Figures in parenthesis indicate standard deviation.

Table 3. Carcass proximate composition (%) of rohu fed experimental diets supplemented with 19-norethisterone.

	Diets (ppm)				
	Control	0.25	0.50	0.75	1.00
Moisture	79.00 (0.72)	75.38 (2.18)	77.39 (1.39)	76.67 (2.18)	75.80 (1.86)
Crude protein	13.92a (0.14)	14.96c (0.28)	14.26ab (0.29)	15.04c (0.12)	14.80bc (0.36)
Fat	2.44a (0.02)	5.06c (0.08)	3.55b (0.02)	3.32b (0.11)	5.36c (0.14)
Ash	2.53ab (0.01)	2.47a (0.06)	2.59b (0.06)	2.56b (0.01)	2.54b (0.05)

Note: Figures in the same row having different superscripts are significantly different ($P<0.05$).
Figures in parentheses indicate standard deviation.

(Matty and Lone 1985). Histological observations of gut of masu salmon treated with MT revealed hypertrophy and hyperplasia of acinus and granular cells, suggesting increased digestion and assimilation of feed (Yamazaki 1976). Hormone treatment affected the carcass proximate composition of rohu (Table 3). A reduction ($P>0.05$) in moisture and an increase in protein and fat content ($P<0.05$) of the treated fish were observed. In a similar study, Gangadhar *et al.* (1996) obtained no difference in moisture content of 19-norethisterone-fed common carp, although there was an increase in fat content. In coho salmon (Fagerlund *et al.* 1978), rohu (Deb 1986; Konda Reddy *et al.* 1987), catla (Deb 1986), common carp (Lone and Matty 1980) and mahseer (Gogoi and Keshavanath 1990), higher carcass protein content was obtained with MT treatment. However, Chua *et al.* (1989) found no effect of MT treatment on the tissue protein content of estuarine grouper. It has been argued that muscle lipids increase during the growth of fish (Love 1970). Deposition of fat with steroid treatment as observed in the present study has been reported earlier in salmonids (Fagerlund and McBride 1975; Higgs *et al.* 1977; Yu *et al.* 1979), estuarine grouper (Chua *et al.*1989), common carp (Lone and Matty 1980; Shobhana and Nandeesha 1994), catla and rohu (Deb 1986). Fishes treated with 19-norethisterone showed no difference in ash content compared with that of the control. Feeding juvenile coho salmon with three steroids, MT, testosterone and estradiol did not significantly affect the body ash content (Yu *et al.* 1979). A decrease in moisture and ash content with steroid feeding has been observed by Lone and Matty (1983), Deb (1986), Gogoi and Keshavanath (1990) and Shobhana and Nandeesha (1994). On the contrary, Yu *et al.* (1979) obtained increased ash content in coho salmon treated with 2.5 ppm testosterone.

It is well established that hormones administered at anabolic levels are metabolized and eliminated from the body of fish (Lone and Matty 1981; Johnstone *et al.* 1983; Rothbard *et al.* 1990; Satyanarayan Rao *et al.* 1990; Dash *et al.* 1995), making the fish safe for human consumption after an appropriate withdrawal period which would vary according to the species and the culture temperature.

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