EFFECTS OF THE STEROID, METHANDROSTENOLONE, ON GROWTH AND GROSS PATHOLOGY OF CHANNEL CATFISH

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Abstract: Methandrostenolone was not effective in promoting growth in channel catfish (*Ictalurus punctatus*) at the 2.5 mg/kg of body weight/day dosage level. There were no significant differences (.05 level) in treated and untreated fish with respect to total weight gain, percent crude protein, and moisture content of the flesh. No differences were observed in gross morphology, liver to body weight ratios, or microscopic examination of liver sections. However, after approximately 3 to 4 wks in a distilled water bath, the hemotoxylin/eosin stain had cleared only from liver sections of the catfish receiving the steroid.

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The effects of temperature have long been recognized as a limiting factor in fish culture (Lagler 1956). It has been determined that channel catfish will feed less and therefore less growth will occur in localities having cooler average water temperatures (Lovell 1975a). The average time required to grow channel catfish from fingerling to a marketable size (0.45 to 0.57 kg) is approximately 210 days of temperatures of at least 15.5 C (Montfort et al 1969; Brown et al 1969). However, in east Tennessee, there are only approximately 150-160 days of temperatures above the 15.5 C level (Potter 1975).

To overcome the problem of a shortened growing period, local catfish growers have alternative measures for producing marketable sized fish. One method involves the stocking of larger fingerlings, which is initially a more expensive procedure. Another way to produce marketable sized fish is to retain the catfish through a second growing season, delaying investment returns and increasing costs from over-wintering. The utilization of heated water is a possible alternative measure; however, it is usually not economically feasible since large amounts of heated water are necessary.

The desired growth-stimulating results might be obtained by the incorporation of a steroid into the diet of the channel catfish. The feasibility of such a venture is encouraged by the effects which these compounds have had in promoting growth in other organisms such as dogs and humans (Kochakion and Murlin 1935, Foss 1960, Bayer and Bailey 1963). One of the first studies dealing with the effect of synthetic steroids on fish was conducted by Hoar et al (1955). Their findings indicated that a significant increase in the locomotor activity of young Pacific salmon (*Oncorhynchus nerka* Walbaum) and goldfish (*Carassius auratus* Linnaeus) was achieved when thyroxine sodium, methyltestosterone, stilbestrol, dienestrol or ethinyl estradiol were put into an immersion with the fish. The concentration of the immersions in which the fish were treated ranged from stilbestrol at 1:1,600,000 for salmon to methyltestosterone at 1:2,800,000 for the goldfish. An increased growth response was reported for goldfish.

Bulkley (1972) incorporated diethylstilbestrol (DES) into the diet of channel catfish by spraying DES on fish chow and then regrinding to insure a homogeneous mixture. Three dosage levels were utilized: 6.7, 67, and 670 mg of DES/kg of body weight/day. It was noted that since some of the food was not ingested by the fish, the true dosage level was unknown. The results indicated that renal hypertrophy and appetite suppression increased as the dosage level increased. At the highest dosage level (670 mg), weight loss, gross edema, and urine retention occurred.

Bulkley and Swihart (1973) investigated the possibility of promoting growth through steroid action. The effect of the synthetic steroid, stanozolol, was tested on goldfish and channel catfish. Stanozolol is an anabolic steroid (Kruskemper 1968) and its androgenic level is similar to that of methandrostenolone (Arnold et al 1963). Four treatment levels were used by Bulkley and Swihart (1973); 0.0, 0.25, 2.50 ,and 25 mg of stanozolol/kg of body weight/day. There was no significant growth difference between the treatment levels for the catfish. However, a significant initial increase in growth was noted with the goldfish although this difference did not continue through the 28 days of the study. Renal hypertrophy occurred at the 25 mg level although the liver was apparently not affected.

An exhaustive search of the literature indicates that no work has been done concerning the effects of methandrostenolone on fish. However, it has been analyzed as an anabolic agent for the promotion of growth in a variety of situations with most of this research being conducted with mammals, ranging from rats to humans. There is an apparent lack of work done with this steroid and its effects on poikilothermic vertebrates.

This study was designed to evaluate the effects of the methandrostenolone on growth and gross pathology of channel catfish. The authors would like to express their appreciation to J. J. Chart of CIBA-GEIGY Pharmaceuticals for providing the methandrostenolone used in this study and H. Shewmake of Fin Fair Inc. for donating the styrofoam holding boxes. In addition, special recognition is due K. Cottrell, D. Harned, C. Knauth, S. Nifong, B. Smith, J. Taylor, H. Waddle, and M. Warren for their assistance during the course of the study.

MATERIALS AND METHODS

Fifty-four channel catfish were used in this study; they ranged in weight from 22 to 43 g and from 153 to 187 mm in total length. The catfish were randomly divided into 3 groups (A,B,C) of 18 fish each and individually assigned to holding boxes in a recirculating system (Perry 1977). The flow-through design was used because: (1) it allowed the individualization of fish without tagging, (2) the temperature could be kept at the same level for all fish, and (3) the system was relatively inexpensive to construct.

All of the fish were fed once a day, 6 days/week. Four days they received Purina #5 trout chow at a rate of 1.5 percent of the body weight for each individual fish. This sinking ration was used after it was found that floating chow tended to clog the overflow tubes in the boxes. The rate of 1.5 percent body weight was used because the average water temperature of 23.5 C was somewhat lower than the optimal feeding level temperature (Calhoun 1966). Also, the 1.5 percent level was used to reduce the possibility of fungal diseases which may be associated with any excesses of uneaten food (Amlacher 1970). The fish had been living and feeding in this experimental system for several months prior to the initiation of the research.

Finding a method for administering the anabolic steroid presented a problem. Methandrostenolone is a white crystalline powder which does not readily dissolve in water (Chiapuzio 1971). Therefore, the inethod used by Hoar et al. (1955) of placing the steroid into an immersion with water in which the fish were also kept would probably not be as efficacious with methandrostenolone because it may involve an active uptake response by the channel catfish. The method used by Bulkley (1972) is practical for culturing operations; however, the situation surrounding the problem of uneaten food makes the actual dosage level values questionable. One possible technique for administering a known dosage to the channel catfish is the injection method. The findings from Chiapuzio (1971) with injected rats in comparison to the results of MacMaster and Alamin (1971) with the orally administered rats indicate that, as stated before, the anabolic properties of methandrostenolone may be more effective when the steroid is administered orally. Also, the increased handling of the fish during the injections may affect their feeding. The solution we chose was to incorporate the steroid into the diet by injecting a known dosage level.

There is no established dosage level for this steroid for channel catfish. The dosage rate of 2.5 mg of methandrostenolone/kg body weight/day was chosen for comparative purposes because the same rate was used by Bulkley and Swihart (1973) with stanozolol.

Each catfish in group A received 0.875 mg of methandrostenolone/day. The crystalline steroid readily dissolved into a solution with ethanol.

Injected earthworms were utilized to introduce the solution to the channel catfish. The worms averaged 30 mm in length and weighed an average of 0.8 g. Each earthworm fed to group A received an injection of 0.1 cc of the methandrostenolone/ethyl alcohol solution; the earthworms fed to group B were injected with 0.1 cc of ethanol only. Group C received earthworms with no injectate. One earthworm was fed to each channel catfish per treatment day.

The solution was injected subcutaneously into the head region of the earthworm. They were fed to the catfish as quickly as possible following injection to eliminate any loss of injectate due to leakage; in addition, the potential metabolism of the steroid by the earthworm was reduced.

Channel catfish weight and length measurements were taken on days 0, 21, 42, and 63. Precautions were undertaken throughout the measuring procedures to eliminate

undue stress and fungal disease outbreaks that might result from rough handling of the fish.

After final weighing, all fish were killed and examined for gross pathological changes. The livers were removed intact from the catfish after weighing, coated with talc for quick freezing, and stored frozen in a cylinder of liquid nitrogen. The dressed catfish were wrapped in aluminum foil, frozen, and held for later analysis.

Thirty-six of the 54 catfish livers were randomly chosen from the 3 groups (12 from each treatment group) and inspected microscopically following the methods outlined by Humason (1962). A minimum of 6 sections were taken from each liver and placed on microscope slides.

When each liver sectioning was completed, each slide was labeled and placed in a hemotoxylin/eosin (H & E) stain, using Delafield's hemotoxylin and eosin Y. These stains were chosen from their fast staining ability, and their effectiveness with the staining of internal organs (Humason 1962; Melton, personal communication). The slides were immersed in the Delafield's hemotoxylin stain for 3 sec and then into the eosin Y for 10 sec. Staining time was less than recommended by Humason (1962), but the effectiveness of the longer staining times were not observable on the slides in question. After staining, the slides were rinsed in distilled water and stored in a distilled water bath.

Liver sections were inspected microscopically to determine any differences in color with respect to pigment or intensity, any changes between tissues, or any other abnormalities. Comparisons were made within and between the treatment groups.

Weight may be affected by cellular growth through the uptake of proteins and by water retention within the tissues (Kruskemper 1968). The amount of crude protein present was determined by Kjeldahl analysis of the flesh. The dressed catfish carcasses were thawed and individually ground into fine species with a meat grinder. One g samples were used for each of the 36 randomly chosen fish. To measure the amount of water retained within the tissues, 1 g samples were weighed before and after drying in a vacuum oven. The Kjeldahl and dry weight analyses followed the Association of Official Analytical Chemists specifications (1975) and the recommendations of Lovell (1975b).

The evaluation of growth data involved 3 analytical models. The raw data were initially analyzed with a crossed covariance model to determine if any differences existed within the groups. To investigate the differences found among the treatments, a two-way analysis of covariance test was conducted. A t-test was also utilized to compare the slopes of the regressions of data from the 3 treatment groups when the data within the groups was combined.

The percentage of crude protein on a fresh basis was derived from the Kjeldahl analysis and was analyzed using a two-way analysis of covariance. To determine the percentage of crude protein, the following formula was used:

$$\% \text{ Crude Protein} = \frac{\text{ml. HCL used} \times \text{N (HCL)} \times \text{Meg. wt. of nit.} \times 100 \times 6.25}{\text{fresh sample weight}}$$

The percent crude protein values underwent arc sin transformations (Sokal and Rolf 1969) to normalize the distribution of the percentage values prior to the statistical analysis.

The moisture content of the catfish flesh was determined when the percentage of dry matter was calculated. The following formula was used for this calculation:

$$\% \text{ Dry Matter} = \frac{\text{Dry sample weight} \times 100}{\text{Fresh sample weight}}$$

Moisture content was calculated in the following manner:

$$\% \text{ Moisture Content} = 100 - \% \text{ Dry Matter}$$

These values also underwent an arc sin transformation prior to the statistical analysis with the two-way analysis of covariance.

RESULTS AND DISCUSSION

Statistical analyses of the growth data revealed there were no significant differences at the .05 level among the 3 treatment groups over time. However, the t-test indicated that although the 3 treatment groups had significantly different initial and final weights, the slopes of lines between these points did not differ significantly. The significant

initial increase noted by Bulkley and Swihart (1973) did not occur at the dosage level used. The average weight and length values for the 3 treatment groups over the 4 data periods are shown in Table 1.

Table 1. Average weight and length values of channel catfish in 3 treatment groups.

Treatment Group[a]		*Days following initiation of treatment*			
		0	21	42	63
A	Weight (g)	32.9	33.0	34.3	35.0
	Length (mm)	169	169	169	170
B	Weight (g)	30.7	30.9	31.9	32.6
	Length (mm)	166	167	167	167
C	Weight (g)	31.2	31.9	33.2	33.8
	Length (mm)	167	167	167	168

[a]Treatment Groups: A - methandrostenolone, ethyl alcohol, earthworms and chow.
B - ethyl alcohol, earthworms and chow.
C - earthworms and chow.

Macroscopic investigation of external and internal morphology indicated there were no obvious differences among the treatment groups. One fish from the control group developed an apparent blockage in the intestine which may have caused its loss of weight throughout the study. All of the other fish appeared to be healthy and no mortality occurred during the study. The gross pathological differences found by Bulkley and Swihart (1973) seems to have been a factor of the high dosage level; no differences were noted by them when lower dosages were used.

The statistical analyses comparing the ratios of liver to body weights among the 3 groups indicated there were no significant differences ($P > 0.05$). Livers were examined because of adverse effects this steroid has had upon the organ in other studies (Kaupp and Preston 1962). In addition, a cursory examination of the kidneys revealed no observable differences among the 3 groups.

Microscopic investigation of the liver sections indicated there were no observable differences within or among the treatment groups. However, after approximately 3 to 4 wks in the distilled water bath, the H & E stain cleared from those sections of the fish receiving methandrostenolone, while those sections from the other 2 groups remained darkly stained. The mechanism involved with the loss of stain was undetermined.

Analysis of the test conducted on catfish flesh (Kjeldahl analysis) indicated there were no significant differences ($P > 0.05$) in the percentage of crude protein among the 3 groups. No significant differences were noted among the treatment groups for the percentage of moisture content of the flesh ($P > 0.05$).

The values for the percentage crude protein (Table 2) varied from those reported by Deyoe and Skoch (1970). Their study found the crude protein levels of the heads, skin, and viscera of channel catfish ranged from 11.5 to 14.2 percent. In this study, the Kjeldahl analysis was conducted on tissues of dressed catfish. The values for the crude protein levels ranged from 18.3 to 29.1 percent. The ratio of tissue to bone content for the portions of the catfish analyzed may offer a possible explanation for differences among reported values.

Hoar and Randall (1969) determined the average moisture content of freshwater teleosts to be 71.4 $\pm$ 0.60 percent. This value is higher than the range (57.8 to 59.0%) found in the study by Deyoe and Skoch (1970) who again used moisture levels obtained from the heads, skin, and viscera of channel catfish. Tissue analyzed in this study (73.6 to 80.0% moisture) more closely approximated the average value given by Hoar and Randall (1969).

Table 2. Average percent crude protein and percent moisutre content levels for 3 treatment groups.

Treatment Group[a]	*Percent Crude Protein*	*Percent Moisture Content*
A	20.41	78.74
B	21.71	77.43
C	19.96	78.04

[a]Treatment Groups: A - methandrostenolone, ethyl alcohol, earthworms and chow.
B - ethyl alcohol, earthworms and chow.
C - earthworms and chow.

It was assumed that if catfish ingested the earthworm injected with methandrostenolone, it would assimilate the steroid into its system. The lack of steroid effect (indicated by growth results and supported by evidence from the Kjeldahl and moisture content analyses) did not necessarily support that assumption. However, loss of stain by the liver sections from fish receiving the steroid may indicate some utilization of it. Methandrostenolone or by-products of its metabolism may have been stored in the liver; this could possibly have caused the lack of stain retention by those liver sections.

The lack of uniform criteria upon which to base research involving steroids was a problem that confronted this study, as well as many others (Idler 1972). As stated before, unpredictability of results makes application of steroids for the promoting of growth little more than trial and error. To test each steroid for its effect on various types of fish would be both costly and time consuming. In addition, if investigations heretofore are any indication, the possibility of promoting growth in fish may be very slight.

The insolubility of methandrostenolone makes comparisons between this study and the work by Hoar et al. (1955) difficult. Although similar dosage levels of different steroids were used, comparisons between this study and the study by Bulkley and Swihart (1973) were also limited. The problem also arises as to which method of application is most effective, i.e., incorporating the steroid into the chow diet, or injecting the solution containing the steroid into the fish or a food item for the fish. Perhaps more study concerning dosage rates and means of administering the steroid would provide a solution to this problem.

Even if progress is made in steroid improvement of channel catfish growth, more problems may develop. There is a possibility of legal restrictions imposed by the Food and Drug Administration (FDA) on the use of the steroid with human food items. It would appear that even if a steroid were found to improve growth in channel catfish, the prospects of FDA approval are unlikely in the very near future.

It was concluded from this study that methandrostenolone was not effective in promoting growth in channel catfish at the 2.5 mg/kg body weight/day dosage level. No abnormal pathological effects were associated with the steroid at the dosage level used.

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