EFFECTS OF STEROIDS AND STEROID ANTAGONISTS ON GROWTH, GONADAL DEVELOPMENT, AND RNA/DNA RATIOS IN JUVENILE STEELEFT HEAD TROUT

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ABSTRACT


Steroids incorporated into Oregon Moist Pellets were fed to steelhead trout (*Salmo gairdneri*) to determine if growth could be enhanced. In 1976, steelhead trout were treated with 17α-methyltestosterone, diethylstilbesterol, diethylstilbesterol plus clomiphene citrate, 17α-methyltestosterone plus flutamide (4’-nitro-3’ trifluoromethylisobutranilide), or flutamide from the onset of exogenous feeding. In 1977, steelhead trout were started on treated diets 1 month after the onset of exogenous feeding and received testosterone propionate, testolactone, methylandrostenediol, 17α-methyltestosterone, flutamide, estradiol, or estradiol plus progesterone.

Fish treated with flutamide (20 μg/g food) or diethylstilbesterol (5 μg/g) plus clomiphene citrate (15 μg/g) from the onset of exogenous feeding showed an increase in weight compared with controls. The androgens and other estrogens tested did not enhance the growth of steelhead trout. All compounds (except flutamide) fed to the fish from the onset of feeding caused abnormal sex ratios. Androgens induced more males while estrogens induced more females. 17α-methyltestosterone (1, 5, 15, or 35 μg/g), 17α-methyltestosterone (5 μg/g) plus flutamide (20 μg/g), testolactone (10 μg/g), and methylandrostenolone (10 μg/g) advanced spermatogenesis. 17α-methyltestosterone (2 or 35 μg/g), testolactone (10 μg/g), methylandrostenolone (10 μg/g), or flutamide (20 μg/g) caused hypertrophied granulosa cells or atretic oocytes in some ovaries. Intersexes containing both testicular and ovarian tissue in the gonads were found in some fish treated with 17α-methyltestosterone (5 μg/g), 17α-methyltestosterone (5 μg/g) plus diethylstilbesterol (5 μg/g), 17α-methyltestosterone (5 μg/g) plus flutamide (20 μg/g), or diethylstilbesterol (5 μg/g) plus clomiphene citrate (5 μg/g). 17α-methyltestosterone at 1, 5, 15, or 35 μg/g caused significant linear dose-dependent increases in epidermal thickness. There were no differences in the RNA/DNA ratio between treatments.

We conclude from these studies that steelhead trout do not respond positively in terms of growth enhancement from steroid treatment as has been shown in other salmonids.
However, the androgen antagonist, flutamide, may be a potential growth enhancer in steelhead trout and may also be used as an antiandrogenic compound when administered with an androgen.

INTRODUCTION

Culture of anadromous salmonids could be facilitated if the time needed for rearing to release size in hatcheries with exceptionally cold water could be shortened. Genetic and environmental factors, nutrition, and hormones all influence the growth process. Growth rates in fish have been shown to be increased by certain hormones, including pituitary growth hormones, anabolic steroid hormones, and thyroid hormones (Donaldson et al., 1978). In salmonids, hormones, particularly the steroid hormones, have been shown to be most effective in enhancing growth as has been excellently reviewed by Donaldson et al. (1978). Treatment of steelhead trout (Salmo gairdneri) has resulted in variable growth responses; however, there is some indication that 17α-methyltestosterone, a synthetic steroid, may promote growth at least during certain stages of development (Fagerlund and McBride, 1977).

The mechanism of action of these hormones on growth is not fully known. It has been assumed that the anabolic action of androgenic, and to a lesser extent estrogenic, hormones increases nitrogen retention in the form of proteinaceous tissue. Nitrogen retention is indicative of an increase in the rate of protein synthesis (concomitant with an increase in RNA concentration) and a decrease in the rate of protein catabolism which results in a progressive weight gain (Kochakian, 1976). This increase in protein synthesis may be reflected by the RNA-P/DNA-P ratio. Basically the RNA/DNA ratio is an index of the growth per cell (Sable, 1974). This ratio could be correlated with the anabolic effect of the steroids and also could be indicative of short-term growth.

Anabolic steroids may have limited usefulness if androgenic or estrogenic properties are expressed. Both high doses and prolonged administration of 17α-methyltestosterone (methyltestosterone) in salmon (Oncorhynchus) have induced structural changes in testes (McBride and Fagerlund, 1973, 1976) and discoloration and thickening of the epidermis (Yamazaki, 1972). Administration of a synthetic antiandrogen with an anabolic steroid may block the androgenic effects without affecting growth. Schreck (1973) showed that the antiandrogen cyproterone acetate may inhibit androgen uptake in testes of rainbow trout. Flutamide (4'-nitro-3'-trifluoromethylisobutyranilide) is considered a potent antiandrogen in mammals and is thought to interfere with the binding of testosterone at the target tissues (Hellman et al., 1977). No antiandrogenic effects of flutamide have been reported in fish. We thus evaluated the effects of flutamide when administered alone or in combination with methyltestosterone in an attempt to reduce the known side effects of methyltestosterone.

The main objectives of this study were to determine if steroids can promote
growth of steelhead trout, to determine if the RNA/DNA ratio could be correlated with the anabolic effect of the steroids, and to evaluate possible side effects from the administration of the steroids and steroid antagonists.

METHODS AND MATERIALS

1976 Studies

Summer steelhead trout from the Oregon Department of Fish and Wildlife’s Cole Rivers Hatchery were transferred as eyed eggs to Oregon State University’s Smith Farm facility, Corvallis, Oregon, in April 1976. Three hundred fry were randomly placed in each of 12 340-liter circular fiberglass tanks in May 1976, and maintained in 11°C well water under natural photoperiod. The fish were fed Oregon Moist Pellets (OMP) (Bioproducts, Inc., Warrenton, OR) several times daily at a certain percentage ranging from 2.5 to 6.0% of body weight. The steelhead trout were started on the treated diets immediately after the yolk sac was completely absorbed. The treatments were as follows: (1) control; (2) 1 µg/g methyltestosterone; (3) 5 µg/g methyltestosterone; (4) 15 µg/g methyltestosterone; (5) 35 µg/g methyltestosterone; (6) 5 µg/g methyltestosterone plus 5 µg/g diethylstilbestrol; (7) 10 µg/g diethylstilbestrol; (8) 5 µg/g diethylstilbestrol plus 15 µg/g clomiphene citrate; (9) 5 µg/g methyltestosterone plus 20 µg/g flutamide; and (10) 20 µg/g flutamide. Clomiphene citrate (1-p (β-diethylamino ethoxy)-phenyl-1, 2-diphenyl, -2-chloroethylene) is a non-steroidal estrogen antagonist.

The fish were fed treated diets for 220 days, after which they were fed untreated control feed for 71 days. Lengths and weights of 50 individual fish from each treatment were recorded at 34, 62, 105, 156, 203, and 291 days after the start of treatment. Condition factors \( f = (\text{weight (g)}/\text{length (cm)})^{1.25} \times 1000 \) were calculated.

1977 Studies

Summer steelhead trout from Cole Rivers Hatchery were transferred as 1-month-old fry to Smith Farm facility on 3 July 1977 and similarly another group was retained at Cole Rivers Hatchery. Two hundred and fifty fry were randomly placed in each of 12 340-liter fiberglass tanks and started on the treated diets at each of the facilities on 18 July 1977, one month after exogenous feeding. On 2 December 1977, numbers of fish in each tank were reduced to 150 at Smith Farm facility and to 137 at Cole Rivers Hatchery to standardize densities to account for mortalities that had occurred at each of the facilities. The fish were fed OMP several times daily for the first 2 months of the test period and then fed once a day to satiation for the duration of the test period. Treatments at each of the facilities were as follows: (1) control; (2) 5 µg/g testosterone propionate; (3) 10 µg/g testolactone; (4) 10 µg/g methylandrostenedione; (5) 2 µg/g methyltestosterone; (6) 20 µg/g flutamide;
(7) 5 μg/g estradiol; and (8) 5 μg/g estradiol plus 5 μg/g progesterone.

The fish were fed the treated diets for 175 days, after which they were fed control feed for 78 days. Group weights were recorded at 25 and 67 days after the start of treatment, and lengths and weights of 50 individual fish from each treatment were recorded at 137, 175, and 253 days.

Diet preparations

The treated diets were prepared by adding the test compounds to the oil fraction of the OMP before mixing with the other ingredients. The pellets were made at Oregon State University Seafoods Laboratory in Astoria.

Histology

Gonads, liver, and skin from five fish from each treatment were preserved in Bouin’s solution, imbedded in paraffin, sectioned at 10 μm, and stained with Harris’s hematoxylin and eosin for histological evaluation. The thickness of the epidermis was measured, stages of gametogenesis were evaluated, and liver structure was noted.

RNA/DNA ratios

Six fish from each treatment from the 1976 studies were frozen at 156 and 220 days after the onset of treatment for determination of RNA-P/DNA-P ratios. Muscle and bone tissues of these fish were dehydrated and defatted in a chloroform-methanol solution (2 : 1 for 4 h) followed by ether (100% for 4h). Both extractions were done in a Goldfish Extraction Apparatus. The tissues were pulverized with a mortar and pestle and stored in capped bottles. DNA and RNA were extracted together from the ground tissue with hot trichloroacetic acid as described by Sable (1974). RNA-P content was determined using the Burton (1956) and Bulow (1970) modification of the diphenylamine reaction. Analysis of nucleic acid content (μg phosphate per 100 μg dry fat-free tissue) of samples was done colorimetrically using a Beckman DB-G grating spectrophotometer.

Statistics

One way analysis of variance (ANOVA) was used to analyze treated fish compared with control fish for the weight data, condition factors, RNA/DNA ratios, and skin thickness samples. Least Significant Difference (LSD) was used in cases found to be significant. Chi-square analysis was used to analyze sex ratio data of the treatment groups (observed) and the control group (expected).
RESULTS

1976 Studies

Steelhead trout treated with flutamide weighed 23.3\% more than control fish \((P<0.01)\) at the end of 203 days and maintained a significant difference until the end of the test period (Fig. 1). Since the mean weights of the two control groups did not differ, data for these groups were pooled for the comparisons with treated fish. All of the methyltestosterone-treated fish (1, 5, 15, or 35 \(\mu g/g\)) had considerably lower growth rates \((P<0.01)\) compared with the weights of the control fish at the end of study (Fig. 1). Fish treated with diethylstilbestrol plus clomiphene citrate \((5 : 15 \mu g/g)\) weighed 15.0\% more than the controls \((P<0.01)\) (Fig. 1). However, there were 50\% fewer fish in this group during the last 120 days of the treatment period because of a disease outbreak in this tank. Fish treated with diethylstilbestrol were deleted from the study because they had severe mortality due to disease. Mortality in all other treatments did not exceed five fish per group during the test period. Methyltestosterone plus flutamide and methyltestosterone plus diethylstilbestrol groups did not have any significant difference in weight compared to the control. Condition factors of the fish were not affected \((P<0.01)\) by the treated diets.

RNA-P/DNA-P ratios from treated fish in 1976 sampled in October and in January were not different \((P<0.05)\) from the control RNA-P/DNA-P ratio at each of those times (Fig. 2).

The testes of the control fish sampled on day 203 consisted of small, defined tubules containing spermatogonia, and ovaries had oogonia and early
Fig. 2. Mean RNA/DNA ratios (µg phosphate per 100 µg dry fat-free tissue) and 95% confidence limits of muscle and bone tissue of steelhead trout treated with methyltestosterone (MT), methyltestosterone plus diethylstilbestrol (MT plus DES), diethylstilbestrol plus clomiphene citrate (DES plus CC), methyltestosterone plus flutamide (MT plus FL), or flutamide (FL). Dosage (µg/g food) given under each compound. No significant difference noted among treated fish compared to controls in October or January at P<0.05, in the 1976 studies.

stages of oocyte I according to the classification of Beams and Kessel (1973). All of the steroids except flutamide affected gonadal development. Intersexes containing both testicular and ovarian tissue in the gonads were found in some fish treated with methyltestosterone (5 µg/g), methyltestosterone plus diethylstilbestrol (5 : 5 µg/g), methyltestosterone plus diethylstilbestrol (5 : 20 µg/g) and diethylstilbestrol plus clomiphene citrate. Advanced stages of spermatogenesis were present in fish treated with methyltestosterone (1, 5, 15, or 35 µg/g), and methyltestosterone plus flutamide. Methyltestosterone at 5, 15, and 35 µg/g induced degeneration of some gonads (no germ cells present or hypertrophy of the germinal epithelium without differentiation). The few affected ovaries of fish treated with methyltestosterone at 35 µg/g were degenerate. Fish treated with the highest level (35 µg/g) of methyltestosterone showed extreme hyperplasia or poorly developed gonads. The altered gonads apparently did not revert to normal after the fish had been on control feed for 71 days.

There were 61% males and 39% females in the control group (Table I). The ratio was not significantly different from a 1 : 1 expected ratio. All of the compounds except flutamide caused a significant deviation from the control ratio (P<0.01, not including intersexes). Flutamide-treated fish, however, had sex ratios similar to that of the controls. Methyltestosterone treatments gave significantly higher proportions of males whereas diethylstilbestrol plus clomiphene citrate gave a higher proportion of females. Methyltestosterone plus flutamide treatment resulted in 89% males and 11% intersex with no females.

All methyltestosterone-treated fish showed highly significant increases (P<0.01) in the thickness of the epidermis (Fig. 3). Fish treated with methyltestosterone plus flutamide had a significant decrease in the thickness of the
TABLE I

Percent of male, female and intersex of steelhead trout from the 1976 studies with various compounds. All treatments except flutamide are significantly different from control at $P<0.05$

<table>
<thead>
<tr>
<th>Treatment (µg/g feed)</th>
<th>Male</th>
<th>Female</th>
<th>Intersex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>61</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td>Methyltestosterone (5)</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Methyltestosterone (15)</td>
<td>93</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Methyltestosterone (35)</td>
<td>77</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Methyltestosterone (5) plus diethylstilbestrol (15)</td>
<td>15</td>
<td>44</td>
<td>41</td>
</tr>
<tr>
<td>Diethylstilbestrol (5) plus clomiphene citrate (15)</td>
<td>12</td>
<td>73</td>
<td>15</td>
</tr>
<tr>
<td>Methyltestosterone (5) plus flutamide</td>
<td>89</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Flutamide</td>
<td>58</td>
<td>42</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 3. Mean epidermis thickness (mm) of steelhead trout treated with methyltestosterone (MT), diethylstilbestrol plus methyltestosterone (DES plus MT), diethylstilbestrol plus clomiphene citrate (DES plus CC), diethylstilbestrol (DES), methyltestosterone plus flutamide (MT plus FL), or flutamide (FL). Dosages (µg/g food) given under each compound. ** Significantly different from control at $P<0.01$.

epidermis. As the dosage of methyltestosterone increased (1, 5, 15 to 35), the epidermis thickness increased showing a direct, linear dose-dependent response ($r^2 = 0.998$, $t_{calc} = 32.36$). The stratum germinativum cells appeared hypertrophied in the epidermis of methyltestosterone-treated fish. The diethylstilbestrol plus clomiphene citrate or flutamide-treated fish showed no increase in epidermis thickness. None of the treatments appeared to affect liver.


1977 Studies

The treatment groups at Cole Rivers Hatchery were reared at different densities, due to an epizootic which appears to have obscured any conclusions concerning growth-promoting action of the test compounds. Large differences between mean weights of fish in replicate tanks support this contention. The treatment regimes employed did not appear to affect sex differentiation. Initiation of treatment at least 1 month after the onset of exogenous feeding appeared to prevent differentiation of sex by the test compounds.

At Cole Rivers Hatchery, there was a low incidence of precocious reproductive development by the males (3.3–16.7%). This appears atypical for the stock and is contrary to preliminary studies, where 38% of the males were precociously ripe. This enigma is difficult to resolve. It can possibly be based in part on the fact that this study involved the summer run of Rogue River steelhead trout, while the preliminary study used the winter run of these fish. At Smith Farm facility in 1976 and 1977, winter-run steelhead trout were used and were found to have a low incidence of precocious development.

At Smith Farm facility no significant differences (P<0.05) in weight were noted between treated and control fish at day 175. Steelhead trout treated with flutamide, estradiol, and estradiol plus progesterone had significantly (P<0.05) smaller weight gains than control fish at the end of 253 days (Table II). Mortality was high during the last month of the test period, apparently due to nitrogen saturation of the water supply.

At Smith Farm facility, gonads of the control fish on days 175 and 253

**TABLE II**

Mean weights (±SE) of steelhead trout treated with various compounds in the 1977 Cole Rivers Hatchery at the end of the experiment, day 253. Total mortalities from day 0 to day 253 are shown for all treatment groups

<table>
<thead>
<tr>
<th>Treatment (µg/g food)</th>
<th>Mean weight (g)</th>
<th>Total mortality (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.66 ± 1.26</td>
<td>22</td>
</tr>
<tr>
<td>Control</td>
<td>11.21 ± 1.16</td>
<td>6</td>
</tr>
<tr>
<td>Testosterone propionate (5)</td>
<td>11.44 ± 1.42</td>
<td>26</td>
</tr>
<tr>
<td>Testosterone propionate (5)</td>
<td>8.99 ± 0.88</td>
<td>59</td>
</tr>
<tr>
<td>Testolactone (10)</td>
<td>13.56 ± 1.36</td>
<td>9</td>
</tr>
<tr>
<td>Methyltestosterone (10)</td>
<td>11.56 ± 0.89</td>
<td>30</td>
</tr>
<tr>
<td>Flutamide (20)</td>
<td>* 7.61 ± 0.89</td>
<td>5</td>
</tr>
<tr>
<td>Flutamide (20)</td>
<td>8.56 ± 0.63</td>
<td>3</td>
</tr>
<tr>
<td>Estradiol (5)</td>
<td>* 7.86 ± 1.02</td>
<td>8</td>
</tr>
<tr>
<td>Estradiol (5) plus progesterone (5)</td>
<td>* 8.20 ± 0.84</td>
<td>42</td>
</tr>
<tr>
<td>Estradiol (5) plus progesterone (5)</td>
<td>9.39 ± 1.10</td>
<td>13</td>
</tr>
<tr>
<td>Methyltestosterone (2)</td>
<td>11.59 ± 0.89</td>
<td>3</td>
</tr>
</tbody>
</table>

* Significantly different from control at P<0.05.
were at the same stages as found in control fish of the 1976 studies. Some fish treated with testosterone, methyltestosterone, methyltestosterone and flutamide had altered gonads. Methyltestosterone, methyltestosterone, and flutamide-treated fish had testes that showed advanced stages of spermatogenesis. The few affected ovaries of fish treated with testolactone, methyltestosterone, and methyltestosterone, and flutamide showed oocytes surrounded by what appeared to be hypertrophied granulosa cells. Gonads from fish treated with testosterone propionate, estradiol, and estradiol plus progesterone did not differ from those of the controls. No intersexes were observed in this study.

DISCUSSION

The present study provides the first evidence that an androgen antagonist has growth promoting effects in steelhead trout. However, flutamide enhanced growth in this study only when the fish were started on treatments at the onset of feeding. When flutamide treatment was initiated 1 month after feeding began, the growth rate tended to be less than that of the control fish. The underlying cause of enhanced growth by flutamide is unknown. Flutamide's suggested mode of action is inhibition of the androgens on target cells (Neri et al., 1972). In our study, flutamide when administered with methyltestosterone appeared to inhibit the effects of methyltestosterone by preventing decreased growth rates and by decreasing skin thickness; methyltestosterone alone increased skin thickness and showed decreased growth rates. Flutamide thus appeared to antagonize the effects of an administered androgen but showed androgenic effects when administered alone.

Androgens did not enhance the growth of steelhead trout. Fish treated with methyltestosterone at the onset of feeding weighed less than did the control fish. Spermatogenesis was advanced. However, in fish where treatment was started 1 month after feeding, there was an increased growth rate during the first 175 days, followed by a decreased growth rate so that by the termination date (day 253), methyltestosterone-treated fish weighed the same as the controls. Fagerlund and McBride (1977) noted a similar trend in steelhead trout treated with methyltestosterone (1 µg/g) from 1 month after the yolk sac was absorbed. Methyltestosterone has been shown to be an effective growth promoter for coho salmon (Oncorhynchus kisutch) and chinook salmon (O. tshawytscha) (Fagerlund and McBride, 1975; Fagerlund et al., 1979; Schreck and Fowler, 1982). Methyltestosterone effectiveness as a growth promoter varies among the Salmonidae.

The lack of growth enhancement by the other androgenic hormones may be due to the dosage of hormone used. Furthermore, the possibility that the androgens may be more effective at different stages of maturity cannot be excluded. Treatment with hormones at the onset of feeding may be more effective in promoting growth. However, since methyltestosterone and testolactone induced changes in the gonads, lower dosages may be more effective for growth enhancement.
These data present clear evidence that methyltestosterone affects the skin thickness; increasing doses of methyltestosterone caused a dose-dependent increase in skin thickness. The doses of methyltestosterone used are much lower (1, 5, 15 or 35 µg/g) than doses of methyltestosterone (50 or 100 µg/g) which caused significant increases in skin thickness in pink salmon (O. gorbuscha) (Yamazaki, 1972). Skin thickening is a phenomenon associated with maturing salmon which have high levels of testosterone and/or 11-ketotestosterone (Yamazaki, 1972; Campbell et al., 1980).

Diethylstilbestrol plus clomiphene citrate promoted growth in steelhead trout in these studies, while the other estrogenic hormones failed to do so. Diethylstilbestrol has been shown to have a strong anabolic action in livestock. Diethylstilbestrol at a low dosage (0.6 µg/g) enhanced growth in plaic (Pleuronectes platessa), but the highest dosage (2.6 µg/g) failed to enhance growth in these fish (Cowey et al., 1973). Diethylstilbestrol alone may be an effective growth promoter for steelhead trout, but effective dosages need to be determined. Clomiphene citrate, an antiestrogen, alone or in combination with diethylstilbestrol, may also be an effective growth promoter in fish. Clomiphene citrate has strong ovulatory actions in fish (Kapur and Toor, 1979), but other possible estrogenic responses in fish have not been reported. Clomiphene citrate has been shown to increase uterine weight in rats (Katzenellenbogen et al., 1979; Adashi et al., 1980). Clomiphene citrate is considered to act on the uterus and pituitary in rats as a long-acting estrogen (Adashi et al., 1980). Further studies are needed to determine the effects of clomiphene citrate alone or in combination with other hormones as a potential growth promoter in fish.

Estradiol or estradiol plus progesterone-treated fish did not grow as rapidly as the controls. Only in coho salmon has estradiol been shown to accelerate growth (Yu et al., 1979). The differences in growth were not apparent until the end of the test period, previous to which time all the groups had high mortality. Estradiol-treated fish (20 µg/g) have also been shown to have higher mortalities than controls in brook trout (Salvelinus fontinalis) (Johnstone et al., 1979) and in Atlantic salmon (Salmo salar) (Sower, unpublished observations). Estrogens may thus have some unknown toxic effects in fish and may not be feasible for use in experimental studies at high concentrations.

There were no differences in the RNA/DNA ratio or RNA-P content between treatments. In young fish, growth occurs largely by cell division (excluding muscle), and Sable (1974) suggested that RNA may be a better measurement of short-term growth. Since the samples tested were mostly muscle tissue, the RNA content may not reflect growth in these young fish.

All compounds except flutamide fed to the fish from the onset of feeding caused abnormal sex ratios, indicating that the sex differentiation process was affected. Sexual differentiation of gonads in rainbow trout (S. gairdneri) has been shown to occur within 6 weeks after hatching (Lebrun, 1977). Other sexual alterations that were observed in methyltestosterone (15 µg/g), methyltestosterone plus diethylstilbestrol, diethylstilbestrol, and methyltesto-
sterone plus flutamide-treated fish were the intersexes, which may or may not be functional. Jalabert et al. (1975) produced functional hermaphroditic trout (self-fertilizable by artificial insemination) by feeding estrone or methyltestosterone. Diethylstilbestrol induced higher percentages of intersexes than methyltestosterone. The same trend was noted in Jalabert et al.'s (1975) study where estrone induced more hermaphrodites than methyltestosterone. Curiously, in the methyltestosterone plus diethylstilbestrol group, there was a higher percentage of females. Apparently, diethylstilbestrol, a synthetic compound, exerted a stronger influence on sexual differentiation when fed fish during the sex determination process than did methyltestosterone at the concentrations tested.

In conclusion, steelhead trout do not respond to anabolic steroids as has been shown in other salmonids. This may indicate species specificity. We have demonstrated that, even with low doses of androgens and different feeding regimes, growth is not enhanced and gonadal tissue and skin thickness are affected. As Donaldson et al. (1978) stated, the steroids which showed the greatest increase in growth in salmonids also induced marked side effects. Thus we suggest that treatment with steroids may not be feasible in enhancement of growth in steelhead trout. Future studies with flutamide, the antiandrogen, may show potential uses for this compound in growth enhancement or as an antiandrogenic agent when administered with an anabolic steroid.

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