

Effects of dietary testosterone on growth and sex ratio in juvenile Atlantic salmon (*Salmo salar*)

Kim M. Lewis and Stacia A. Sower

Department of Zoology, University of New Hampshire, Durham, New Hampshire, 03824, U.S.A.

Accepted: August 1, 1991

Keywords: dietary testosterone, diets, reproduction, growth, juvenile atlantic salmon

Abstract

Diets to which testosterone (1 or 10 ng/g diet) had been added were fed to juvenile Atlantic salmon (*Salmo salar*) for nine months beginning for months after hatching (Experiment 1) and upon completion of yolk absorption (Experiment 2) to determine the effects on growth, gonadal development, and sex ratio. Dietary testosterone at 10 ng/g fed to juvenile salmon at four months after hatching (Exp. 1) induced significant changes in condition factor (0.69 ± 0.01) compared to controls (0.79 ± 0.01) at the end of the test period. In both experiments, salmon treated with 10 ng/g diet induced a significantly higher percentage of male fish compared to controls. Dietary testosterone at 1 ng/g fed to juvenile salmon beginning four months after hatching induced significant increases in weight (18.95 ± 0.99) and length (13.58 ± 0.23) compared to controls (14.55 ± 1.50 and 11.94 ± 0.43 , respectively). In Experiment 1 or 2, there was no apparent influence of dietary testosterone on precocious male sexual development. Dietary testosterone at 1 or 10 ng/g fed to juvenile salmon upon completion of yolk absorption (Exp. 2) induced no consistent changes in growth in juvenile Atlantic salmon. These studies indicate that low levels of dietary testosterone may influence physiological responses in juvenile Atlantic salmon dependent upon timing of treatment.

Introduction

In earlier studies, Sower and Iwamoto (1985) demonstrated that commercial salmon diets contained testosterone at levels from 0.4–7.0 ng/g feed. From these studies, the authors suggested that low levels of dietary androgens may influence various physiological responses during critical developmental processes. Normal function of androgens in salmon includes, in part, mediating gametogenesis, differentiation, development of secondary sex characteristics and modulation of sexual be-

havior. Exogenously administered naturally occurring androgens or synthetic androgens are known to alter gonadal development by accelerating spermatogenesis in salmonids (Higgs *et al.* 1982). A two- to three-fold increase in occurrence of male sexual precocity was observed in chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) treated with diets containing added testosterone (nanogram levels) compared to controls (Iwamoto, Hershberger, and Sower, unpublished data). Advances in spermatogenesis were reported after testosterone administration in At-

lantic salmon (*Salmo salar*) (Crim and Peter 1978). The physiological basis of precocious development is not well understood; however, naturally occurring dietary androgens may accelerate male gonadal development in juvenile salmon.

Low levels of dietary androgens have been demonstrated to influence physiological responses in juvenile coho salmon (Borghetti *et al.* 1989). Using feed containing reproductively mature fish, these authors demonstrated that low levels of naturally occurring androgens in the diet induced increased growth response in juvenile coho salmon. Fish weight, length, feed conversion ratio, and protein efficiency ratio were affected by such diet.

There are no reported studies on the use of low doses of dietary testosterone over a long period of treatment in terms of effects on gametogenesis and growth in Atlantic salmon. The objective of this study was to determine the effects of the presence of testosterone at levels that may be found in commercially prepared diets on the growth and reproductive development of juvenile Atlantic salmon during their first year of development. Many of the noted effects of exogenous androgens depend on dose, length and timing of treatment. Nanogram quantities of testosterone, comparable to concentrations detected in commercial feeds, were administered to Atlantic salmon parr four months after hatching (Experiment 1) and to Atlantic salmon in the fry stage at the onset of feeding (Experiment 2).

Materials and methods

Experiment 1 (1987–1988)

Four thousand Atlantic salmon (*Salmo salar*) were reared from eggs to juveniles at New Hampshire Fish and Game Powder Mill fish hatchery (New Durham, NH) and transported to the Anadromous Fish and Aquatic Invertebrate Research laboratory (AFAIR laboratory) at the University of New Hampshire in September, 1987. The juvenile Atlantic salmon were randomly divided into three replicated treatment groups and maintained in two-meter circular fiberglass tanks, supplied with reser-

voir water at ambient water temperature under natural photoperiod. Water temperatures ranged from 1–13°C. Diets were prepared from base feed obtained from Stinson Canning Co. (Bath, ME). Testosterone was added to oil which was mixed into the feed mixture. The control diet contained prepared feed without the addition of testosterone and the experimental diets contained 1 ng testosterone/g feed or 10 ng testosterone/g feed. The juveniles were fed these diets twice daily at a total ration of 0.5 to 3.1% of body weight per day.

Twenty fish from each treatment were sampled eight times (Sept., Dec. 1987; Jan., Feb., March, Apr. 5, Apr. 19, May 1988) for total length, wet weight, sex, and gonads. Condition factor was calculated as $w(g)/l^3 (cm^3) \times 100$.

Experiment 2 (1988–1989)

In 1988, 900 Atlantic salmon alevins were reared at Powder Mill fish hatchery (New Durham, NH), supplied with gradient fed lake water at ambient water temperature and under natural photoperiod. Water temperatures ranged from 1 to 17°C during the experimental period. The fish were randomly divided into three treatments (as in Experiment 1) and fed experimental diets at first feeding following yolk absorption (May, 1988) and continued through May, 1989. Fish were transferred to the AFAIR laboratory in September, 1988. Fifteen fish from each treatment were sampled nine times (Sept., Nov., Dec. 1988; Jan., Feb., March, Apr. 7, Apr. 21, May 1989) for total length, wet weight, sex, and gonads.

Sampling and histology

All fish were sexed macroscopically at the time of sampling. Sex was also verified by histological examination. Gonadal tissues were preserved in Bouin's solution, dehydrated in a series of alcohols, and embedded in paraffin. Gonad samples were sectioned at 10 μ m, stained with Harris' hematoxylin and eosin, and analyzed for degree of gonadal development.

Table 1. Final mean weight, total length, and condition factor, calculated as $CF = W(g)/l(cm)^3 \times 100$, of juvenile Atlantic salmon

	Control	Dietary testosterone (ng/g)	
		1	10
<i>Weight (g)</i>			
May (Exp 1)	14.55 ± 1.50	18.95 ± 0.99*	15.75 ± 1.21
May (Exp 2)	19.20 ± 1.30	20.66 ± 1.10	21.80 ± 0.75
<i>Length (cm)</i>			
May (Exp 1)	11.94 ± 0.43	13.58 ± 0.23*	12.95 ± 0.38*
May (Exp 2)	13.28 ± 0.22	13.44 ± 0.20	13.71 ± 0.16
<i>Condition Factor</i>			
May (Exp 1)	0.79 ± 0.01	0.73 ± 0.01	0.69 ± 0.01*
May (Exp 2)	0.82 ± 0.03	0.83 ± 0.01	0.83 ± 0.01

Data are shown as mean ± SEM; Experiment 1, n = 20; Experiment 2, n = 15; * indicates significant variation from controls at $p < 0.05$.

Table 2. Percent of male and female fish within each treatment group

	Dietary testosterone (ng/g)					
	Control		1		10	
	Male	Female	Male	Female	Male	Female
Experiment 1	54	46	50	50	66	33*
Experiment 2	53	47	44	56	62	38*

Experiment 1, n = 160; Experiment 2, n = 135; * significant variation from 1:1 sex ratio at $p < 0.05$.

Testosterone concentration was determined in the feed of the various treatments. Testosterone was extracted three times from feed samples using a Dounce homogenizer with hexane:benzene (2:1) in 1 ml volumes. Samples were centrifuged at $2200 \times g$ for 5 minutes and the supernatant decanted. The supernatants were lyophilized and reconstituted in 1 ml phosphate-buffered saline-gelatin (PG). Each sample was filtered (0.45 μm filters) and eluted on a C-18 Sep-pak with 5 ml methanol. The peak fractions were pooled, dried, and frozen ($-20^\circ C$) until was measured by radioimmunoassay using methods described by Sower and Schreck (1982).

Statistics

Sex ratios were analyzed by chi square, and data on weights, lengths, and condition factors were tested for each sampling date by a preliminary analysis of variance followed by Student-Newman-Keuls mean separation test.

Results

Experiment 1

At the end of the test period, fish treated with testosterone at 1 ng/g diet (18.95 ± 0.99 g, $\bar{x} \pm SEM$) weighed significantly more than controls (14.53 ± 1.50 g) (Table 1). Fish treated with testosterone at 1 or 10 ng/g were significantly longer (13.58 ± 0.24 and 12.95 ± 0.33 cm, respectively) than controls (11.94 ± 0.44 cm). Fish treated with 10 ng/g testosterone (0.69 ± 0.01) had a significantly lower condition factor than controls (0.79 ± 0.01).

There were significant differences in sex ratio from the 1:1 expected ratio in fish treated with 10 ng/g testosterone at the end of the test period (Table 2). There were no significant differences in sex ratio in fish treated with 1 ng/g testosterone.

Histological examination revealed slight changes in gonadal maturation throughout the study period. All females had oocytes in the early perinucleolar stage of development and the majority of testicular development was confined to the spermatogonial stage. An exception of seven males in advanced stages of spermatogenesis were noted over the experimental period. One fish in each treated diet including controls in September and May, and one fish treated with 1 ng/g testosterone in January had testes with primary spermatocytes. In September, two fish (one from each treatment group) had gonads containing both testicular and ovarian tissue referred to as intersex fish. The gonad of one intersex fish exposed to 1 ng/g testosterone treatment also contained of primary spermatocytes.

The actual concentration of testosterone calculated in the feed averaged 0.49 ± 0.10 ng/g feed (control), 0.85 ± 0.14 ng/g feed (1 ng/g diet), or 3.88 ± 0.13 ng/g feed (10 ng/g diet).

Experiment 2

At the end of the test period, there were no significant differences in weight, length or condition factor of fish treated with 1 or 10 ng/g testosterone compared to controls (Table 1). There were significant differences in sex ratio from the 1:1 expected ratio in fish treated with 10 ng/g testosterone at the end of the test period (Table 2). There were no significant differences in sex ratio in fish treated with 1 ng/g testosterone.

There was little evidence of advanced gametogenesis in any fish in treated or control groups. One fish from control in each of September, December, and January had intersex gonadal tissue. Two fish from each treated group in September had intersex tissue which also contained lobules of primary spermatocytes. All other males had testes in the spermatogonia stage while females had ovaries with cells in the early perinucleolar stage of oocyte development.

Discussion

Androgens stimulate growth and regulate reproductive functions such as development of secondary sex characteristics, gametogenesis, and sexual behavior. Testosterone and 11-ketotestosterone are believed to be the primary sex steroids involved in salmonid reproductive development. In the present study, low levels of dietary testosterone fed to juvenile Atlantic salmon for nine months beginning four months after hatching (Exp. 1) induced physiological responses while low levels of testosterone fed beginning at the time of yolk absorption (Exp. 2) induced no consistent physiological changes. In both experiments, dietary testosterone at 10 ng/g apparently induced a greater percentage of males, indicating that the sex differentiation process may have been affected to a certain degree.

The concentration of testosterone used in this study (1 ng/g diet or 10 ng/g diet) were comparable to levels present in commercial feeds (0.4 ng/g feed–7.0 ng/g feed) (Sower and Iwamoto 1985), and approximately 1,000–10,000-fold less than concentrations used in previous studies (reviewed

by Donaldson *et al.* 1979; Higgs *et al.* 1982; Donaldson and Hunter 1982). In the present study, dietary testosterone at 1 or 10 ng/g fed at four months after hatching induced either significant increases in length and weight or condition factor compared to controls in the present study. However, dietary testosterone at these same doses did not apparently influence growth when fed at the time of yolk absorption. Borghetti *et al.* (1989), using feed partially composed of reproductively mature fish, demonstrated that growth in coho salmon was significantly influenced by the presence of naturally occurring androgens in the diet fed from the time of yolk absorption at levels of 0.2–1.5 ng/g. In contrast to the present study, Borghetti's fish diet contained a mixture of steroids which naturally occur in fish meal and the fish were reared at higher water temperatures (10.5 to 16.4°C). The noted growth of the juvenile coho salmon may be due to the combination of androgens and rearing temperature. Fagerlund and McBride (1975) demonstrated a marked increase in growth in coho salmon treated with 17 α -methyltestosterone in salmon reared at 16.5°C compared to 11.5°C. In addition, the total concentration of steroid hormones in diets used in Borghetti's study is likely to be higher compared to one steroid. Species differences may also account for the differences in growth response. In chinook salmon, a 25% maximum weight increase compared to controls has been demonstrated in chinook salmon in response to various concentrations (ranging from 1 to 50 μ g/g diet) of methyltestosterone (Schreck and Fowler 1982; McBride and Fagerlund 1973) in contrast with a much more pronounced 92% increase in growth in coho salmon to methyltestosterone (Fagerlund *et al.* 1980). Rearing temperature, dose, length and timing of treatment can all influence the response of juvenile salmon to exogenous treatment of steroids.

Normal sex differentiation in rainbow trout appears between day 45 and day 55 after fertilization (van den Hurk and Slof 1981). This coincides developmentally with the completion of yolk sac absorption and the salmon's first acceptance of food. The stage of gonadal differentiation most sensitive to exogenous steroid manipulation occurs one to

two weeks after first feeding (reviewed by Donaldson and Hunter 1982). The results of the present study are surprising and unexplained since treatment with 10 ng/g testosterone four months after hatching appeared to induce a greater percentage of male compared to female fish. As expected fish fed at the onset of exogenous feeding with 10 ng/g testosterone did have significant differences in sex ratios, with a greater percentage of male fish. As reviewed by Donaldson and Hunter (1982), sexual differentiation occurs more rapidly in *Oncorhynchus* species compared to Atlantic salmon with females differentiating before the males.

Dietary androgens ranging from μg to mg/diet may accelerate gonadal development and induce sexual precocity in males (Fagerlund and McBride 1975; Magri *et al.* 1985; Sower *et al.* 1983). In the present study, 1 or 10 ng of testosterone/g diet induced no apparent degenerative effects and may have induced only slight advances in spermatogenesis.

In summary, these studies indicate that low concentrations of dietary testosterone at levels that may be found in commercially prepared diets may induce physiological responses that depends on timing of treatment.

Acknowledgement

We thank Jane Calvin, Thom Bolduc, Doug Adams, Caleb Slater, Craig Schmidt and Linda Penney for their help during sampling of fish. We thank New Hampshire Fish and Game for providing fish and facilities. This work was supported by NOAA New Hampshire/Maine Sea Grant (Project R/ICD-96).

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