

THE IDENTIFICATION OF THE SEX STEROID, TESTOSTERONE, IN VARIOUS COMMERCIAL SALMON DIETS

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ABSTRACT

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The sex steroid hormone, testosterone, was identified in seven salmon commercial diets at levels from 0.4 ng/g feed to 7.0 ng/g feed. The biological ramifications of steroids in fish diets on the salmon's development, fitness and ultimate seawater survival are discussed.

INTRODUCTION

Steroid hormones are secreted from endocrine organs and are found in all vertebrates. Adrenocortical and gonadal steroid hormones are responsible for a multitude of significant biological activities in vertebrates. Androgens are steroids which, in general, mediate the reproductive functions which include gametogenesis and differentiation and maintenance of somatic tissue. Other functions of androgens include the development of secondary sex characteristics and modulation of sexual behavior. In salmon, steroids are known to influence, in part, the various processes associated with development, smoltification, survival, and reproduction.

It has been suggested that commercial fish diets may contain androgens, the male sex steroids. Meal and oil in fish diets, in many cases, are derived from reproductively mature teleost fish which often have high circulating plasma levels of androgens including testosterone and 11-ketotestosterone (Fostier et al., 1983) with probable additional high concentrations in the testes. Exogenously administered naturally occurring androgens or synthetic androgens are known to alter gonadal development by accelerating spermatogenesis in salmonids (Higgs et al., 1982). One or more androgens in fish diets may be linked, therefore, to an apparent increase in the incidence of male sexual precocity in state, federal, and private salmon hatcheries. Thus, our objective was to determine if various commercial fish diets do contain the sex steroid, testosterone.

MATERIALS AND METHODS

Samples of seven commercial salmon diets (mash to 1/4" pellet) were obtained and labelled A, B, C, D, E, F, or G. Samples were stored at -20°C until assayed.

Other methods for determination of testosterone in the fish feed were attempted. However, fish feeds contain a large percentage of oils which interfered in the separation techniques and/or radioimmunoassay. The following method proved to be reliable on the basis of reasonable recovery and replicability.

Radioinert (range from 100 pg to 1000 pg) or radioactive (1, 2, 6, 7 ³H(N); 2000 dpm) testosterone was added to separate 0.25 g homogenized samples and incubated for 1 h at 22°C prior to extraction. After two ether extractions and 70% methanol/hexane partition, the fractions were dried in a speedvac (Savant Instruments). The extracts were then subjected to partition chromatography on Celite columns. Estradiol was eluted with 40% ethyl acetate in isooctane. Testosterone and dihydrotestosterone were collected after eluting with 20 and 10% ethyl acetate, respectively. Percent recovery was determined with the radioactive testosterone and ranged between 38% and 65% (N=10). Extracts were reconstituted in 0.25 ml of phosphate-buffered saline-gelatin (PG). Levels of testosterone were measured by radioimmunoassay as described by Sower and Schreck (1982). Serial dilutions of radioinert testosterone that had been previously added to the feed sample did not vary significantly between the observed and expected values.

Following the determination of recovery and accuracy, samples (0.25 g) from the various fish diets were analyzed using the same procedures. Five or 10 samples from each diet were assayed.

RESULTS

Six of the seven diets contained measurable quantities (0.4–7.0 ng/g feed) of testosterone (Table 1). These quantities were determined from several samples from one or two batches of diets. A typical commercial

TABLE 1

Quantity of testosterone identified in various commercial fish diets

	Testosterone (ng/g feed)
A	0.4 ± 0.1
B	1.6 ± 0.2
C	1.1 ± 0.1
D	1.7 ± 0.1
E	2.7 ± 0.1
F	7.0 ± 0.2

diet consists of fish (30%), fish meal, cottonseed meal, whey, wheat germ meal, shrimp meal (one or a combination of these meals, 63%), oil (6%), vitamin premix (1.5%) and choline chloride (0.5%). It is suspected that testosterone levels would change throughout the year depending on the firm's source of fish product which would change due to availability and cost. The fish component can vary considerably; typical fish used include herring, tuna viscera, turbot, salmon viscera, hake, and/or shrimp or crab-meal. The quantity of testosterone in the diet also appears to be related to some degree to the method of preparing the diet. The F diet which had the highest level of testosterone (7.0 ng/g feed) was vacuum dried compared to "flame dried" in all other diets except the A diet which is acid treated and had the lowest level of testosterone (0.4 ng/g feed). These various processes attempt to insure quality and consistency, yet are not apparently adequate to degrade the steroid, testosterone, in their diets.

DISCUSSION

This study demonstrates that various commercial diets contain one of the natural occurring androgen hormones, testosterone. Juvenile salmon exposed to relatively low constant levels of testosterone during their entire freshwater phase from the critical fry stage until release at smoltification could be adversely affected by this steroid in development, fitness, and ultimately seawater survival. Normally, juvenile salmon in the wild would not be exposed to steroids in their diet since they are unable to eat reproductively active fish. Wild coho salmon as early fry eat drifting organic material such as chironomid larvae, ephemeropteran exuviae and pupal cases and some copepods (Mundie, 1969). Steroids have not been identified in these particular invertebrates. Steroidogenesis has been shown to be present in vertebrates and some representatives of the mollusca and echinoderm groups (Sandor et al., 1975). Most invertebrate groups have sterols (hormones of steroidal nature) such as ecdysone, but not the usual vertebrate steroids (Sandor et al., 1975). Thus, one would not suspect that insects or crustaceans in the larval or juvenile stage would contain steroids such as testosterone.

Testosterone administered at doses of 0.2 to 200 $\mu\text{g}/\text{day}$ for 1–3 months has been demonstrated to induce the onset of precocious sexual development in male juvenile rainbow trout (Crim et al., 1982; Crim and Evans, 1983; Magri et al., 1985). The physiological basis for male precocious sexual development in salmonids has not been well understood and only the above mentioned recent studies have elucidated some of the endocrine factors that are involved in this process. The pituitary gland has been shown in these studies to respond to testosterone as determined by increased secretions of gonadotropin(s) indicating a functional steroid feedback system in the hypothalamus–pituitary axis (Gielen et al., 1982; Crim and Evans, 1983; Magri et al., 1985). The response of the fish to testosterone

does depend on the dose, time of initiation, and duration of treatment. It has been demonstrated that long-term administration of 17 α -methyl-testosterone started at the onset of exogenous feeding in steelhead trout fry compared to long-term administration of this steroid started 1–2 months after the onset of exogenous feeding induced greater gonadal changes, including a greater percentage of male sexual precocity (Sower et al., 1983). Even though the testosterone levels noted in the commercial diets in the present study are lower than that used in the above studies, a low constant dose of testosterone may be partly responsible for increased precociousness. For instance, unexplained high percentages of male sexual precocity have been reported in untreated salmon: over 50% in juvenile Atlantic salmon (Saunders et al., 1982); and 38% in steelhead trout (Sower et al., 1983). Additionally, other studies have shown the unusual appearance of intersex in juvenile chum salmon (Nakamura, 1984) and coho salmon (Sower and Fawcett, unpublished data, 1984). These observed high percentages of precocity and intersex salmon could be in part due to the steroid, testosterone, in the diet.

The potential effects of these steroids on the salmon's various physiological and reproductive functions have not been determined. However, it has been shown that 93.1% of 5 μ g/g feed of ³H-testosterone fed to coho salmon was absorbed by the fish, as determined by the amount of total radioactivity in various tissues with 6.1% of total radioactivity concentrated in soft tissues (Fagerlund and McBride, 1978). If a daily food intake of 3% of body weight is assumed, a daily intake of about 0.26 ng of testosterone per gram of fish could occur, calculated from the F diet. Depending on the type of feeding regime (percent body weight or satiation feeding), juvenile salmon could potentially ingest doses of testosterone that are only 10–100 fold less than the physiological range of androgens in reproductively active adult male salmon (Schreck et al., 1972a, b; Sower and Schreck, 1982; Fostier et al., 1983). One or a combination of several factors — genetic, environmental, nutritional, and endocrine — have been implicated in the initiation of reproductive development of salmon (Crim and Evans, 1978; Dodd et al., 1978; Glebe et al., 1980; Thorpe et al., 1980). Thus, testosterone in low doses fed daily to juvenile salmon could be a potential factor in determining the onset of maturity. The levels of testosterone and other steroids in the diets during the year and from year to year, and the physiological effects during critical developmental processes, have yet to be determined.

As the increase of number of smolts released has not significantly enhanced returns, as evident by the release and return data of coho salmon over a 40-year period in Oregon (Gunsolus, 1978), recent efforts by fisheries' scientists and agencies have focused on the release of smolts "better fitted for marine survival" (Mahnken, 1982). These efforts include the understanding, definition, and optimization of juvenile freshwater development, in particular, the smoltification process in hatchery reared salmon. One

of the many areas of research on juveniles has been the potential use of steroids as growth promoting agents which in theory, would enhance the fish's ultimate seawater survival due to its increased size (Fagerlund and McBride, 1975; Yu et al., 1979; Fagerlund et al., 1980; Schreck and Fowler, 1982; Sower et al., 1983). Androgens, the male sex steroid hormones, have been the most intensively studied in various fish and have been, in many cases, shown to be anabolic resulting in increased growth. Androgens include the naturally occurring steroids, testosterone, 11-ketotestosterone, and dihydrotestosterone and various potent synthetic androgens such as 17 α -methyltestosterone and testosterone propionate. However, even with the obvious benefits of such agents, problems and deleterious side effects have been noted, limiting their usefulness (see review by Higgs et al., 1982).

Many of the noted side effects of exogenous androgens administered to juvenile salmon depend on the dose, length and timing of treatment, and rearing conditions (temperature and photoperiod). The dosages generally used in these studies are at least 10 \times higher (ranging from 0.01 μ g/g to 10 μ g/g diet) than the levels of testosterone found in the diet (0.4–7.0 ng/g) of the present study. There have been no reported studies of the use of lower doses of administered testosterone over a long period of treatment in terms of effects on gametogenesis and other physiological processes. However, low or high doses of androgens alter gonadal development by accelerating spermatogenesis, causing the degeneration of testicular tissue or inducing intersex gonads which contain both male and female elements (Yamazaki, 1972; Yu et al., 1979; Schreck and Fowler, 1982; Sower et al., 1983). Androgens also increase the epidermal thickness which causes a change in pigmentation (Yamazaki, 1972; McBride and Fagerlund, 1973, 1976; Sower et al., 1983), thereby potentially modifying the silvering process of smoltification. Steroids can induce hypertrophy or degeneration in the liver and/or kidney of fish (Ashby, 1957; Hirose and Hibiya, 1968; McBride and Van Overbeeke, 1971; Fagerlund and McBride, 1975; Simpson, 1976). Androgens can affect thyroid function (Van Overbeeke and McBride, 1971; Higgs et al., 1977; Hunt and Eales, 1979; Fagerlund et al., 1980). High doses of anabolic steroids can reduce normal seawater readiness and survival (Fagerlund and McBride, 1975; Fagerlund et al., 1980). Exogenous doses of steroids may thus diminish the relative fitness of fish which may reduce the salmon's potential of survival to adulthood.

The biological ramifications of testosterone or potentially other steroids in fish diets on development, fitness, and ultimate seawater survival are significant. Confounding effects of these dietary steroids on previous experimental data using juvenile salmon are implicated and need to be addressed.

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