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Effects of Anabolic Hormone Treatment
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on Growth and Protein Utilization
of Juvenile Rainbow Trout (Salmo gairdneri)

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EFFECTS OF ANABOLIC HORMONE TREATMENT AND DIETARY PROTEIN TO ENERGY RATIO ON GROWTH AND PROTEIN UTILIZATION OF JUVENILE RAINBOW TROUT (SALMO GAIRDNERI)

By

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ABSTRACT

EFFECTS OF ANABOLIC HORMONE TREATMENT
AND DIETARY PROTEIN TO ENERGY RATIO
ON GROWTH AND PROTEIN UTILIZATION
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A four phase feeding study was used to evaluate the potential of anabolic steroid hormones to increase growth rates and alter dietary protein to metabolizable energy (P:ME) relationships for optimum growth of juvenile rainbow trout (Salmo gairdneri). Treatment with 2.0 mg 17α - methyltestosterone (MT)/kg of diet produced weight gains in fish ranging between 10-30% greater than controls over treatment periods of 8 to 10 weeks. Treatment with 1.0 or 2.0 mg estradiol-17β/kg of diet decreased gain, and reduced the anabolic effectiveness of MT when in combination with this androgen.

A diet containing 340 kcal ME/100 g of dry ingredients with a P:ME ratio equal to 100 mg/kcal ME produced maximum efficient protein deposition in fish treated with 2.0 mg MT/kg of diet. The optimum P:ME for growth of fish fed diets containing 390 kcal ME was reduced from 120 in controls to 100 in hormone treated fish. A minimum dietary ME level of greater than 320 kcal was required to produce

advantages in growth and efficiency measurements of fish due to hormone treatment at the P:ME ratio of 100. The extent of protein anabolism due to hormone treatment in fish fed diets with marginal ME levels was dependent upon the extent of use of protein as an energy source and the maintenance of a minimum functional P:ME ratio.

MT treatment increased the condition factor (wt/l^3) of fish by lowering the rate of increase in linear carcass bone growth in relation to whole body weight gain. Whole body fat content was not changed with treatment, but was redistributed from visceral to carcass components. Hormone withdrawal reduced the growth of fish to below the level of controls and negated the advantages in protein deposition and efficiency due to treatment.

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INTRODUCTION

The use of anabolic steroid hormones in domestic livestock is an accepted practice for increasing growth rates and feeding efficiencies of treated animals (Lu and Rendel 1976, Trenkle and Burroughs 1978, Galbraith and Topps 1981). At present, these agents are not approved for use in aquaculture in the United States. Interest in improving the growth rates and feed efficiencies of rainbow trout (Salmo gairdneri) is high due to normally slow growth rates and high feed costs (Dash 1982) associated with raising these fish. However, application of anabolic steroids in salmonid culture has only received limited attention.

Previous work has established that anabolic-androgenic steroids can increase the growth rates and feed efficiencies of juvenile salmonids fed standard diets (Higgs et al. 1982). In general, studies directed at establishing optimum treatment regimens have focused on the manipulation of treatment dose or duration with little regard for diet composition. Since standard diets are empirically formulated based on optimum growth responses of non-hormone-treated fish, they may be inadequate to support optimum growth of hormone treated fish. Changes in the growth metabolism of fish treated with steroids may produce changes

in optimum nutritive balances essential for optimum growth. Optimum nutritive balances in steroid-supplemented diets may produce further improvements in growth and feed efficiency beyond steroid supplementation alone.

The purpose of this work was to examine the interaction between anabolic hormone treatment and dietary protein to metabolizable energy (P:ME) ratio in promoting maximum efficient rates of protein depostion in juvenile rainbow trout. In mammals, anabolic hormone treatment appears to enhance dietary protein utilization for growth (Kochakian 1976, Vander Wal 1976) which suggests that reduced protein levels in diets supplemented with hormones may be warranted. Protein levels have been reduced in diets supplemented with 17 α -methyltestosterone for rainbow trout (Ince et al. 1982) and coho salmon (Fagerlund et al. 1983) without loss of the steroid growth promoting activity. However, studies directed at examining changes in optimum protein or P:ME relationships for hormone treated fish have not been examined. The potential for more efficient protein utilization with hormones is particularly important in salmonid culture since protein, the most expensive component in feeds, comprises as much as 50% of the dry ingredients in some salmonid diets (NRC 1981).

In addition to examining absolute growth, the relative growth of muscle to other body tissues as a function of hormone treatment and dietary P:ME ratio was examined.

Steroid hormone treatment has produced changes in condition factor (McBride and Fagerlund 1973, 1976; Fagerlund and McBride 1975,1977; Simpson 1976; Saunders et al. 1977; Schreck and Fowler 1982), body conformation (McBride and Fagerlund 1976, Schreck and Fowler 1982), and somatic indicies of a variety of non-muscle tissues (Yamazaki 1972,1976; McBride and Fagerlund 1973,1976; Fagerlund and McBride 1975; Sower 1978; Matty and Cheema 1978) of salmonids. These changes suggest alterations in relative tissue growth, especially between the growth of muscle and bone. Although body weight gain is an index of the effectiveness of an anabolic agent, the efficiency of treatment should be based on the relative anabolic response of muscle to the response of other tissues. Muscle tissue is the desired end product of production; all other body components essentially are considered waste. Gonadal hormones (Brannang 1971) and varying dietary P:ME ratio (Jackson 1976) affect the relative growth of muscle and bone in cattle. These factors also may affect relative muscle growth in salmonids. Understanding dietary protein to energy interactions with hormone treatments in salmonids may suggest further treatment strategies to enhance not only absolute but also relative muscle growth.

The ultimate goal of this work was to provide trout feed formulators with information on how to maximize dietary

protein utilization for muscle growth through steroid supplementation. A primary goal of fish nutrition research is to limit dietary protein costs without limiting growth. Maximized protein utilization and subsequent growth enhancement will reduce production times and costs to commercial aquaculturists, and state and federal agencies involved in hatchery production of salmonids. It was the intent of this project to provide such information to further assess the use of anabolic hormones is fish culture.

This project was conducted in four consecutive with the following respective objectives:

1. To evaluate the relative growth responses of fingerling rainbow trout fed semipurified diets supplemented with various combinations and single preparations of the androgens 17α - methyltestosterone (17 α-methyl-4-androst-17β -ol-3-one) and 5α -dihydrotestosterone (5α - androstan-17β -ol-3-one), and the estrogen estradiol 17 β (1,3,5[10]-estratriene-3,17β -diol) at 2.0 mg/kg of dry diet.
2. To determine possible interactions between dietary P:ME intake and steroid supplementation in generating maximum efficient rates of protein deposition in trout fed experimental semipurified diets and a selected hormone preparation.

3. To determine possible interactions between dietary ME intake at a constant P:ME ratio and steroid supplementation in generating maximum efficient rates of protein deposition in trout fed experimental diets and a selected hormone preparation.
4. To evaluate the potential of using selected dietary P:ME and total ME levels in steroid supplemented practical diets fed to trout based on maximum efficient rates of protein deposition.

This dissertation includes four chapters which contain the major results and conclusions drawn from the project. Chapter 1 contains the results on weight gain, feed conversions, and body composition of fish fed eight diets supplemented with various combinations of the androgens and estrogens screened for use in subsequent studies. 17α - methyltestosterone at a level of 2.0 mg/kg of dry diet was chosen as the most anabolically effective hormone preparation. Chapter 2 contains the effects on growth of various body components when fish were fed diets containing 17 α -methyltestosterone and varying dietary P:ME ratios. Special emphasis was placed on the changes in weight gain of muscle and bone and the resultant effects on condition factor (wt/l^3; Fulton, 1911) of fish. Hormone treatment and mid-range dietary protein to energy levels (120 - 140)

promoted higher relative rates of soft tissue to hard tissue gain. Absolute muscle gain was directly related to whole body gain, although muscle gain/l^3 of fish was increased with treatment. In Chapter 3, changes in dietary P:ME ratios and total ME levels that occurred with 17 - methyltestosterone treatment to promote maximum efficient rates of protein deposition are presented. It was estimated that a reduction in dietary P:ME from 120 to 100 mg of protein/kcal ME of diet and a total ME level of 340 kcal was optimum for growth of steroid treated fish. In addition, changes in fat composition of the whole body and empty carcass (eviscerated whole body) in relation to hormone activity are discussed. Hormone treatment redistributed fat from the viscera to the carcass without loss of whole body fat. Chapter 4 includes the growth and efficiency responses of hormone-treated and non-hormone-treated trout fed practical diets with marginal ME levels at a high and the optimum P:ME ratio for growth of hormone-treated fish recommended in the semipurified diet studies. Fish were examined over a ten week hormone treatment period and a subsequent eight week hormone withdrawal period. The results in growth and efficiency factors obtained with practical formulations confirmed the observations obtained with semipurified diets: a minimum dietary ME level of greater than 320 kcal was required to produce advantages of

hormone treatment at the P:ME ratio of 100. It appeared that at marginal dietary ME levels more protein was utilized to supply the energy for enhanced growth thus lowering the apparent P:ME ratio of the diet. The extent of growth enhancement due to hormone treatment was dependent upon the extent of use of protein as an energy source and the maintenance of a minimum functional P:ME ratio. Hormone withdrawal from the diet drastically reversed all advantages gained with treatment.

REVIEW

I. Introduction

The "anabolic steroids" most commonly referred to in animal production are natural analogues or synthetic derivatives of the male and female sex hormones. Interest in using steroids and steroid-like compounds to enhance protein deposition in domestic animals was generated from the early studies of Kochakian and Murlin (1935) on the effects of sex steroids on increasing nitrogen balance in castrate dogs. Androgenic and estrogenic agents have been used in the domestic livestock industry since the 1950's to increase growth rates and feed efficiences of treated animals and have yielded substantial savings in production costs (Lu and Rendel 1976, Trenkle and Burroughs 1978, Galbraith and Topps 1981).

Initial interest in the use of anabolic agents in fish culture arose from the success obtained in the domestic livestock industry. Both androgenic and estrogenic compounds have been tested for growth promoting activity in a number of commercially important fishes. The relative success of each type of compound tested has been dependent upon the species examined. For instance, androgenic agents

were more effective growth promoters of juvenile salmonids than similarly used estrogenic compounds (Higgs et al. 1982). Estrogens, however, were more effective than androgens at similar treatment levels in promoting the growth of yellow perch (Mailson et al. 1985). No androgenic (Bulkley and Swihart 1973) or estrogenic (Bulkley 1972) agent tested to date has increased the growth rate of channel catfish. Androgens have promoted the growth of a number of Tilapia species (Guerrero 1975,1976) and the common carp (Lone and Matty 1980, 1981, 1982). Studies with estrogenic compounds in these two species have not been conducted.

The mechanisms of action of anabolic hormone treatment in fish are poorly understood. As a result, it is of interest to examine the metabolic effects of hormone use in domestic livestock to lend perspective on possible modes of action in fish. However, besides a vast species difference, comparisons of activity between fish and livestock is difficult due to dissimilarities in hormone treatment regimens. In domestic livestock, animals are treated with hormones during the last phase of growth prior to slaughter (Trenkle 1976, Trenkle and Burroughs 1978, Heitzman 1979, 1980). Most hormone treatments of fish have been conducted during early growth phases and are usually discontinued 1-2 years before slaughter. In addition, androgens and

estrogens are generally applied differentially to sexes of livestock or to castrate animals. Fish stocks contain intact males and females. Despite these differences, however, basic mechanisms may be similar.

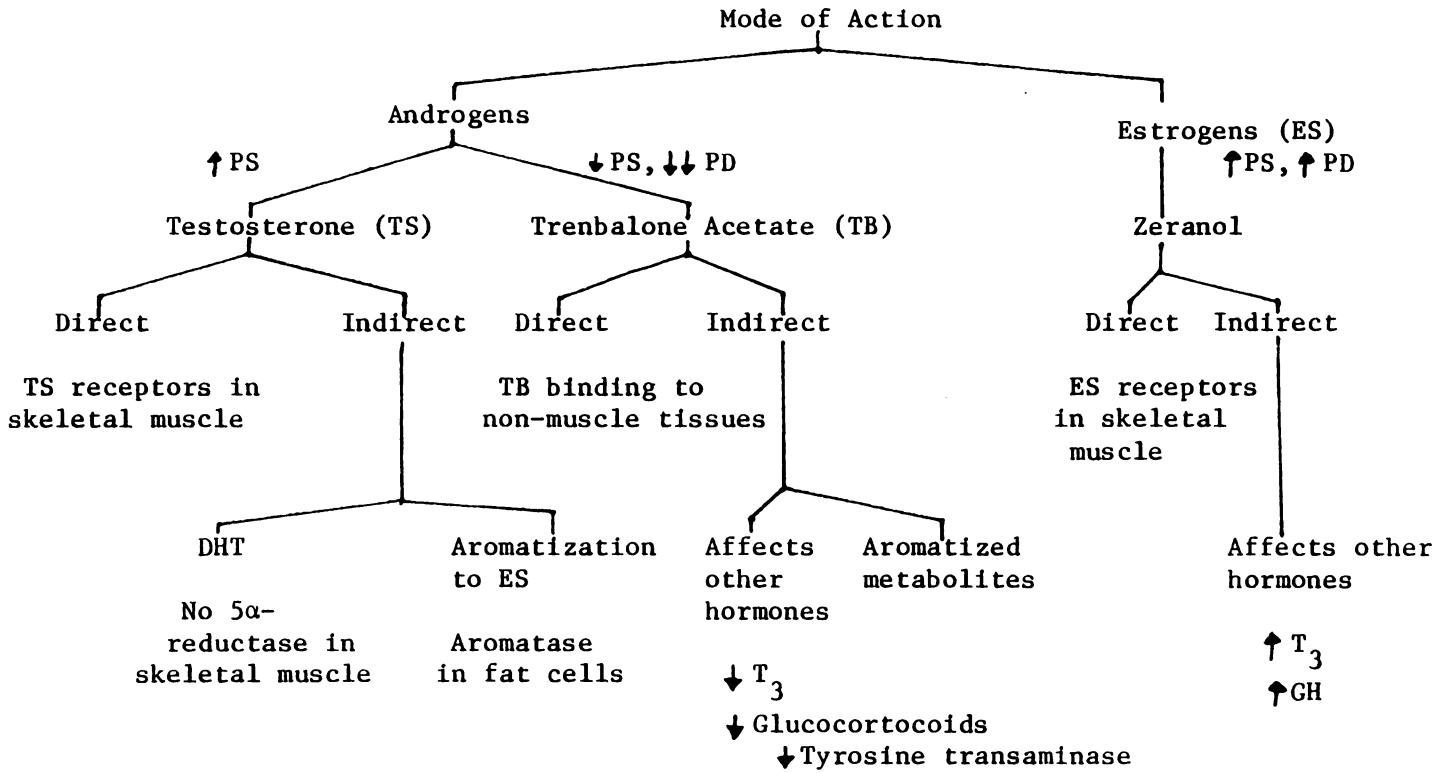
The intent of this review is to concentrate on the known metabolic effects of anabolic steroid treatment in animals that could potentially affect dietary protein and energy utilization and subsequently dietary protein and energy requirements for growth. While it is still not clear how steroids enhance growth and feed efficiency in livestock, much evidence has been accumulated to suggest possible modes of action. The review of steroid treatment studies with fish is restricted to the salmonid family, the most widely studied fish species with respect to hormone treatment to date.

II. Domestic Livestock

A. Modes of Steroid Action

The effect of anabolic hormones on protein metabolism in domestic livestock was reviewed by Buttery and Sinnett - Smith (1984). An adaptation of their review and speculations is presented in Figure 1 which diagrams proposed direct and indirect mechanisms of exogenous hormone treatments on altering protein synthesis and degradation with evidence avaliable to support these proposed mechanisms. It is apparant that the effects of androgen and estrogen treatment are not specific and that similar

Figure 1. Possible modes of action of anabolic agents used in the domestic livestock industry adapted from review and speculation of Buttery and Sinnett - Smith (1984).(PS = protein synthesis, PD = protein degradation, DHT = 5α-dihydrotestosterone, GH = growth hormone, T_3 = triiodothyronine).



mechanisms of action may exist. Heitzman (1979, 1980) and Heitzman et al. (1981), however, have indicated that anabolic responses are maximized in bulls treated with estrogens, heifers treated with androgens, and castrates with a combination of androgenic and estrogenic compounds, and suggested that the maximum anabolic response at the cellular level may be dependent on an optimum circulating level of both androgens and estrogens in the blood. The overall effects of androgens and estrogens may be independent and additive, although considerable overlap probably exists in the mechanisms involved. It is generally believed, however, that androgens act more through some direct mechanism to affect protein synthesis while estrogens act more by indirectly affecting the metabolism of other endogenous anabolic hormones.

The most probable mode of action of estrogenic anabolic compounds in steers is through increased circulating levels of growth hormone. High circulating levels of growth hormone are positively correlated with high growth rates of cattle (Purchas et al. 1970, Ohlson et al. 1981) and administration of diethylstilbestrol and Synovex-S (estradiol + progesterone) has increased growth hormone plasma levels (Trenkle 1976) and secretion rates (Gopinath and Kitts 1984a). Gopinath and Kitts (1984b) suggested that a concomitant increase in thyroid activity and rise in plasma thyroxine (T_4) levels potentiates the anabolic

effects of growth hormone. An increased thyroid activity may also explain decreases in tissue fat content and improvements in feeding efficiency normally observed with estrogen treatment (Lu and Rendel 1976) by increasing the metabolic rate of the animal (Grodsky 1981). In contrast, the anabolic effect of the androgen, trenbolone acetate, in cattle is not related to a change in growth hormone levels (Heitzman et al. 1977, 1980; Galbraith and Watson 1978), and T_4 levels are reduced with treatment (Heitzman et al. 1977, 1980).

Maurice et al. (1985) examined possible changes in metabolizable energy values of feeds of female turkeys treated with an anabolic dosage of trenbolone acetate. These researchers found that the decreased feed intake and improved efficiency due to treatment was not regulated through an increased yield of metabolizable energy from the feed. Although not measured, they speculated that body fat content was not changed based on previous observations and suggested that trenbalone acetate dampened basal metabolism by inhibiting thyroid activity thus partitioning more protein and energy into growth. It is noteworthy to add that the anabolic action of estrogens in cattle also do not appear to be associated with enhanced nutrient absorption (Trenkle 1976), although this mechanism is involved in the anabolic action of antimicrobial agents in both ruminant

(Broome 1980) and monogastric (O'Connor 1980) animals. Effects of steroids appear to be regulated more through changes after absorption.

Clancy et al. (1986) suggested that changes in metabolic efficiency of steers implanted with androgen-estrogen combinations can be partly explained by a shift in muscle fiber proportions from more anaerobic to more aerobic types. These authors speculated that since aerobic metabolism is more efficient than anaerobic metabolism in producing ATP from glucose residues, increased proportions of aerobic type fibers due to treatment can facilitate protein synthesis in muscle and result in more efficient protein production.

B. Diet x Hormone Interactions

Since protein is the building block of growth, changes in metabolic efficiency and growth rates due to steroid treatments may alter dietary protein and protein to energy needs. However, potential interactions between protein level and hormone treatment in domestic livestock have not been adequately examined.

A minimum dietary protein (Jones and Hogue 1960) and energy concentration (Preston and Burroughs 1958) was necessary to obtain an anabolic response in hormone-treated lambs. Above minimum requirements, however, the type of

ration, level of energy, or level of protein did not appear to be sigificant factors in the effect of anabolic agents in lambs (Preston and Burroughs 1958, Jones and Hogue 1960), cattle (Klosterman et al. 1954,Fowler et al. 1970, Vander Wal 1976), or turkeys (Castaldo et al. 1983). Higher growth responses were obtained in these animals with higher protein densities in accordance with responses observed in control animals. Steroid-treated pigs, however, grew faster when fed higher dietary protein densities while untreated animals grew faster on lower protein level diets (Baker et al. 1967, Binder et al. 1972).

III. Salmonids

The salmonids are an important commercial and recreational fish species. Much evidence has been accumulated on the effects of endogeneous sex hormones on the reproductive cycle in various species and on the effects of exogeneous treatment in juveniles. Coincidental evidence from studies with reproductively mature salmon suggests a role of the sex hormones in growth of adult fish. Immature Atlantic salmon which have reduced levels of androgens grow slower than mature fish (Hunt et al. 1982). Adult salmonids sterilized by high dose androgen treatment during the alevin stage grow slower than intact adults (Donaldson and Hunter 1985). The examination of the role of endogeneous sex

hormones in adults may suggest possible modes of action in treated juveniles.

A. Endogeneous Hormone Action

1. Adults

Endogenous sex hormones are involved in the control of the reproductive cycle and timing of gamete production in adult salmonids (Scott and Sumpter 1983). The rise in hormone levels prior to spawning is thought to activate processes which promote sperm and egg maturation. During non- or preovulatory peroids, plasma levels of testosterone in rainbow trout males (Scott et al. 1980) and females (Scott et al. 1983), and estradiol-17β in females (Scott et al.1983) are below 100 ng/ml. The levels of testosterone in the male rise to between 100 - 150 ng/ml with spermiation. Testosterone in the female rises to above 300 ng/ml with vitellogenesis. This rise in testosterone with vitellogenesis in the female occurs independent of the time of year for spawning (Scott et al. 1983). Levels of estradiol in the female also rise with vitellogenesis, but do not exceed 50 ng/ml of plasma. At least part of the circulating estradiol levels in females is related to aromatase conversion of testosterone in the granulosa layer of the ovarian follicle (Kagawa et al. 1985).

Despite their quantitative importance, testosterone and estradiol may not be the most qualitatively important hormones involved in the spawning process. For instance, a concomitant rise in 11-ketotestosterone with testosterone levels in the male is thought to play a more important role than testosterone in spermiation (Scott et al. 1980). Precocious sexual development of males associated with high circulating testosterone levels (Sower et al. 1983) appears dependent upon an appropriate level of gonadotropin (Magri et al. 1985).

The surge of hormone flow prior to spawning of Pacific salmonids, which die soon after spawning, is thought to contribute to the degenerative changes associated with the spawning process including the development of the kype in the male (Idler et al. 1961), darkening of the skin, loss of flesh quality (Idler et al. 1961, Van Overbeeke and McBride 1971), and changes in muscle composition (Ando et al. 1986).

2. Juveniles

Steroid hormones are important for sexual differentiation of rainbow trout. However, it appears that androstenedione and its derivatives are more important in the process than testosterone (van den Hurk and van Oordt 1985). Androstenedione derivatives arise from gonadal conversions of corticosteroid secretions from interrenal tissues during the first 60 - 100 days of life (van den Hurk

and Lambert 1982, van den Hurk et al. 1982). In contrast, testosterone secretions are not detected until around day 200 of life (van den Hurk and van Oordt 1982) past the period of early differentiation. However, methyltestosterone treatment of trout fry during first feeding (40 - 60 days of life) alters the sex ratio towards more males (Yamazaki 1983).

B. Exogenous Hormone Action

1. Steroid Effectiveness

Estrogens have not been effective growth promoters of juvenile salmonids at dose rates used. Growth rates were decreased in rainbow trout fed diethylstilbestrol at 50 to 500 mg/kg diet (Ghittino 1970) or 1.2 mg/kg diet (Matty and Cheema 1978), and estradiol 17-β at 20 mg/kg diet (Johnstone et al. 1978). In the latter study, significant differences were not observed between the growth of control or treated fish approximately 5 months after cessation of treatment indicating a compensatory response to inhibited growth caused by the estrogen. In contrast, estradiol 17- fed at 2.5 mg/kg diet increased the weight gains of coho salmon (Yu et al. 1979); however, methyltestosterone, an androgen, was more effective at the same dose.

Most androgens examined have improved growth rates and weight gains of juvenile salmonids. Chlorotestosterone

acetate was an ineffective growth promoter of coho salmon fingerlings (McBride and Fagerlund 1976), but increased weight gains of one year old rainbow trout (Hirose and Hybia 1968). The natural circulating androgens, testosterone (McBride and Fagerlund 1976, Yu et al. 1979) and 11-ketotestosterone, (McBride and Fagerlund 1976)increased the growth rates of coho salmon parr. The growth rates of juvenile rainbow trout were increased with the synthetic androgen ethylestrenol (Simpson 1976). Norethandrolone, another synthetic androgen, enhanced the growth of trout in one study (Matty and Cheema 1978), but not in another (Ostrowski 1982). The most widely studied androgen to date, 17 α -methyltestosterone, has increased the growth rates of Atlantic salmon (Simpson 1976), coho salmon (McBride and Fagerlund 1973, 1976; Higgs et al. 1977; Yu et al. 1979, Fagerlund et al. 1980, 1983) chinook salmon (McBride and Fagerlund 1973), kokanee (Yamazaki 1976), pink salmon, steelhead trout (Fagerlund and McBride 1977), and rainbow trout (Ince et al. 1982).

B. Dose Effect

The magnitude of the growth responses obtained with 17 -methyltestosterone are dependent upon treatment dose. In comparative studies lasting between 10 and 57 weeks, growth rates of coho (McBride and Fagerlund 1973, 1976; Fagerlund and McBride 1975) and pink salmon (Fagerlund and McBride

1977) fed 10 mg/kg diet were greater than fish fed 1 mg/kg diet. No difference was observed in weight gain of steelhead trout fed levels between 1 and 5 mg/kg diet (Fagerlund and McBride 1977). A 0.2 mg/kg diet dose produced smaller percentage gains over untreated fish than a 1.0 mg/kg dose in coho (Fagerlund and McBride 1975) and pink (Fagerlund and McBride 1977) salmon. Doses of 30 mg/kg (Johnstone et al. 1978) or 50 mg/kg diet (Yamazaki 1976) decreased gain of rainbow trout. Similar trends of weight gain response to dose in coho salmon were observed with 11-ketotestosterone, testosterone and oxymethalone (McBride and Fagerlund 1976).

In addition, the magnitude of androgenic side effects has increased with hormone dose. Numerous side effects have been noted with 10 mg/kg treatments of 17 α-methyltestosterone, 11-ketotestosterone, testosterone, and oxymethalone. External alterations of salmonids have included a marked thickening (McBride and Fagerlund 1973) and dulling of the skin (Fagerlund and McBride 1975), a yellow tinting of the fins (Fagerludn and McBride 1975) and ventral surfaces (McBride and Fagerlund 1976), and a general thickening and widening of the body (Fagerlund and McBride 1975) with an increased condition factor (Fagerlund and McBride 1975, 1977). Testicular degeneration occurred (Fagerlund and McBride 1975; McBride and Fagerlund 1973,

1976) although ovaries were not affected (Fagerlund and McBride 1975).

Androgenic changes have been slight or insignificant in fish fed levels of hormone between 0.2 to 5.0 mg/kg diet. Some alterations in head shape and skin color have been noted in a few fish (Fagerlund and McBride 1977). Skin thickness has either increased (Sower 1978) or not changed (McBride and Fagerlund 1973). Condition factor was increased (Saunders et al. 1977, Schreck and Fowler 1982). No significant changes were observed in exocrine pancreas (Higgs et al. 1977), liver, heart, and kidney (Yu et al. 1979) histology; however, interrenal and endocrine pancreas tissue size were increased (Higgs et al. 1977). Thyroid tissue size was either increased (Higgs et al. 1977) or not changed (Miline and Leatherland 1980). Limited testicular degeneration was noted (Fagerlund and McBride 1975, 1977; Higgs et al. 1977, Fagerlund et al. 1980). More commonly, spermatogenesis (McBride and Fagerlund 1976,Fagerlund and McBride 1977, Yu et al. 1979) or no testicular change was observed (Fagerlund et al. 1979). Ovaries were only slightly altered (McBride and Fagerlund 1973, Fagerlund and McBride 1977, Higgs et al. 1977) or not affected (McBride and Fagerlund 1973, 1976; Fagerlund and McBride 1977; Yu et al. 1979; Fagerlund et al. 1979; Fagerlund et al. 1980). Generally, for both high and low androgenic hormone dosage ranges, the intensity of side effects has increased with

increased duration of treatment.

Dosage level has not altered depletion characteristics of administered anabolic androgenic treatments. Similar rates and patterns of tissue uptake, retention, and depletion have occurred in coho salmon fed 17 α - methyltestosterone- 1,2 -^{3}H at 1 mg/kg diet (Fagerlund and Dye 1979), or 40 mg/kg diet (Johnstone et al. 1983) and ^{3}H-testosterone fed at 5.0 mg/kg diet (Fagerlund and McBride 1978). In all studies, tissue levels were generally reduced to less than 1.0 ppb in all edible tissues (skin and muscle) 10 days after steroid withdrawal from the diet. The highest concentrations of radioactivity were observed in the liver. In Atlantic salmon, the primary route of excretion of an ingested dose of ^{3}H-testosterone and its metabolites was through the bile quantitatively conjugated with glucoronic acid (Truscott 1983).

C. Dietary Protein Level Effect

Weight gain responses obtained with hormone treatment are dependent to some extent on dietary protein content. Ince et al. (1982) found that a 3.5 mg/kg dietary treatment of ethylestrenol increased weight gains of juvenile rainbow trout fed dietary protein levels of 32%, 43%, and 53% for 60 days; however, the percentage gains over control fish were highest for fish fed 32% protein. The advantages in weight

gain due to treatment persisted through a 30 day hormone withdrawal period and no interaction between dietary protein content and gain of previously treated fish through this period was evident. Fagerlund et al. (1983) found a seasonal effect of dietary protein content on hormone activity of juvenile coho salmon fed 1.0 mg/kg diet of methyltestosterone for 36 weeks. Reductions in dietary protein content produced less advantages in percentage gain during colder months when growth rates of fish were normally slow. Higher temperatures have increased the growth promoting response of methyltestosterone in coho salmon (Fagerlund and McBride 1977).

D. Feed Utilization

Feed conversions (Simpson 1976, Fagerlund et al. 1979a, Schreck and Fowler 1982, Ince et al. 1982) and protein efficiency ratios (Fagerlund et al. 1983) of juvenile salmonids have been improved with anabolic hormone treatments. Various theories have been suggested to explain the improvements. Ince et al. (1982) suggested that hormone treatment promotes dietary protein absorption from the gut with a concomitant decrease in fat absorption. These researchers found that treatment with ethylestrenol decreased protein excretion and increased fat excretion in juvenile rainbow trout. Yamazaki (1976) found changes in pancreatic and intestinal gut histology of coho salmon

treated with methyltestosterone suggesting the possibility of enhanced digestive and/or absorptive ability. In contrast, Simpson (1976) suggested that treatment with methyltestosterone and ethylestrenol promotes a change in metabolic rate of Atlantic salmon. This researcher found that treatment decreased visceral fat content and suggested that the energy was channeled into growth. This observation was corraborated by the observations of Fagerlund et al. (1979b).

Fagerlund et al. (1979a, 1980, 1983) have noted an increased appetite of hormone treated coho salmon. It has not been determined if appetite increases were a consequence or a cause of the increased weight gain.

E. Metabolic Effects

Kochakian (1976) has shown that the anabolic response to hormone treatment in mammals was reflected by changes in rates of amino acid incorporation into tissues and by changes in nucleic acid to protein (P) ratios. Anabolic hormone treatment has increased the rate of ^{14}C - leucine incorporation into skeletal muscle tissue of juvenile rainbow trout (Matty and Cheema 1978). Moisture and fat-free whole body DNA-P, RNA-P, and RNA-P/DNA-P ratios were not changed in steelhead trout treated with methyltestosterone (Sower et al. 1983); however, growth was not increased in treated fish. In contrast, a growth

promoting dose of ethylestrenol increased total RNA in muscle of rainbow trout (Lone and Ince 1983); but, the P/RNA ratios, an indicator for comparison of RNA concentration to fractional protein synthetic rate (Millward and Waterloo 1978), was not changed.

Higgs et al. (1977) suggested that exogeneous hormone treatment alters thyroid activity in juvenile coho salmon which resulted in increased growth. These researchers found that the responses in thyroid histology and growth of fish fed triiodothyronine (T_3) and 17^{α} -methyltestosterone together were greater than the sum of the two hormones administered singly. However, Fagerlund et al. (1980) found that the response of the thyroid to a combined T_3 and methyltestosterone treatment dose was related to the physiological state of the thyroid associated with the time of year, while growth responses were independent of time of year. Methyltestosterone did not affect plasma thyroid hormone levels or thyroid histology in rainbow trout (Miline and Leatherland 1980).

GENERAL METHODOLOGY

A detailed description of the specific methods and materials used in each study is presented in each chapter of this dissertation. This section contains explianations of general diet preparation methods, general fish culture conditions, feeding regimen, fish weighing techniques, and statistical models used throughout the project.

I. Experimental Diet Preparation

A. Steroid stock solution

Steroid stock solutions were prepared at a 1 mg/kg concentration by dissolving 0.200 grams of steroid in 200.00 grams of soybean oil. Stirring and mild heating was applied to facilitate mixing. All solutions were refrigerated at 10^{o}C and were remixed before use.

All steroids used in Study 1 were initially dissolved in 15 ml ethanol and then dissolved in the oil. Ethanol facilitated dissolution of estradiol - 17β. Ethanol was not needed to dissolve methyltestosterone and was not used in subsequent semipurified diet test studies. Ethanol was used as a carrier to add methyltestosterone to practical diets in Study 4.

B. Diet preparation

All feeds were mixed, dried, and stored as outlined by Garling and Wilson (1976). Dry dietary ingredients were mixed in an industrial food mixer (Univex Model M-12B) for 20 minutes. A mixture of soybean oil, cod liver oil, and steroid stock solution (if included) was added slowly to ensure complete homogeneity and mixed for 15 minutes. Warm water (50 - 60^{o}C) was then slowly added with mixing until the diet clumped to a dough-like consistency.

The dough-like material was passed through a meat grinder attached to the mixer forming a spagehetti-like product. This product was dried in a forced air drying oven (without heat) for 24 hours, cut in a Waring Blender, and passed through standard U.S. Sieves numbers 6 and 10 yielding pellets from 1.18 to 3.35 millimeters in size.

The pellets were graded and fed to fish according to fish size (1.18 - 2.00 mm pellets for 0.5 - 3.5 gm fish; 2.00 - 2.40 mm pellets for 3.5 - 8.0 gm fish; 2.40 -3.35 mm pellets for 8.0 gm or greater sized fish). Equivalent sized pellets were placed in labeled plastic food containers and refrigerated at 10^{o}C until used. Dry matter composition was determined on all diets; water content ranged between 8 and 12%.

C. Vitamin and mineral premixes

Vitamin and mineral supplements were added to semipurified rations as a percentage of prepared premixes (Tables 1,2). Each premix was formulated based on NRC (1978) vitamin and mineral recommendations for coldwater fishes. The premixes were prepared by ICN Biochemicals® using purified ingredients and alphacellulose as a carrier.

II. General Fish Culture Conditions

Fish were maintained in 110 liter flow-through aquaria with supplemental aeration and a continuous well water supply. Water temperature was a constant 11.5°C and flow was set at approximately 1.0 - 1.5 liter/minute. Lighting was supplied by overhead fluorescent lamps set on a 14:10, light:dark regimen.

III. Feeding Regimen

Appropriate feeding regimens are difficult to define in fish nutrition studies. Standard "ad libitum" regimens typically used with domestic livestock are not feasible for use with fish due to the instability of feeds in water. "Demand" - type feeders with a feeding bar that extends into the water and placed above fish tanks have been used to mimic ad libitum feeding. Fish learn to hit the bar which moves a plate that allows feed to fall from the feeder into

Table 1. Vitamin mixture for use in purified fish diets (NRC 1978).

Vitamin	mg/g premix[a]	Vitamin	IU/g
Choline Cl	450.0	Vitamin A	500
Niacin	100.0	Vitamin D_3	200
Inositol	20.0	Vitamin E	5
Ascorbic acid	15.0		
Vitamin K[b]	12.0		
Calcium pantothenate	6.0		
Pyridoxine	1.5		
Riboflavin	1.5		
Thiamin HCl	1.5		
Antioxidant[c]	1.0		
Folacin (folic acid)	0.5		
Biotin	0.15		
Vitamin B_{12}	0.003		

[a] These quantities added to alphacellulose to make 1 gram.

[b] Menadione dimethylpyrimidinol bisulfite.

[c] Butylated hydroxytoluene (BHT) and/or ethoxyquin.

Table 2. Mineral mixture for use in purified fish diets (NRC 1978).

Mineral	g/kg premix
$CaHPO_4 \cdot 2H_2O$	366.046
$CaCO_3$	261.714
KH_2PO_4	176.834
NaCl	106.100
$MgSO_4$	53.050
KCl	17.683
$FeSO_4 \cdot 7H_2O$	8.842
$MnSO_4 \cdot H_2O$	6.189
$ZnCO_3$	2.653
$CuSO_4 \cdot 5H_2O$	0.531
KIO_3	0.177
$NaMoO_4 \cdot 2H_2O$	0.147
$CoCl_2$	0.030
Na_2SeO_3	0.004

the water. However, it has been the experience in this lab that fish either constantly hit the feeding bar or produce excessive wave action during feeding which promotes excessive feed wastage. Feeding fish a number of times per day on a "satiation" basis has also been used; however, this method appears subject to experimentor bias since the definition of "satiation" and the number of times to feed fish per day is subjective. "Fixed" feeding rates (i.e.% body weight/day) have been criticized in that they do not allow for possible adjustments in feeding level for fish if growth is altered by the experimental conditions.

In this project, fish were fed a diet dry matter amount equivalent to 3.5% (except Study 1 where fish were fed 4.0 % per day) of their wet body weight per day divided equally into a morning and evening feeding. This fixed rate regimen was chosen based on an experiment conducted in this lab which was extended from work of Grayton and Beamish (1977).

Grayton and Beamish (1977) found that maximum growth rates of rainbow trout fingerlings (10 gram initial weight) obtained by feeding satiation amounts of a standard salmonid feed 2 - 6 times per day amounted to feeding fish 3.5% of their wet body weight per day. In our lab experiment, we examined the contribution of experimentor bias on the level of "satiation" by feeding trout fingerlings (4.5 gram initial weight) at satiation levels defined independently by

4 different experimentors and at a standard 3.5% body weight per day basis. This experiment revealed that the 3.5% feeding rate produced rates of gain of fish comparable to those of fish fed on a satiation basis with less variablitiy in feed conversion measurements over time. Experimentors fed fish at different mean rates (4.9, 4.7, 4.5, 3.9% per day) and constantly changed these rates over time. These adjustments in feeding level, however, did not affect weight gain or body composition of fish. It was concluded that the 3.5% body weight per day feeding basis was sufficient to promote rapid growth of 4 - 10 gram trout fingerlings in lieu of satiation feeding and should be used instead of satiation feeding when efficiency values are also of interest.

Feeding levels were adjusted according to the total weight of fish in a tank measured at 14 day intervals. Fish were fed the first 13 days of each period; they were weighed and not fed on day 14 in order to eliminate false weight readings due to gastrointestinal fill. The next feeding period began the following day. Feeding levels were adjusted when death occurred within a feeding period by subtracting an expected average dry matter amount of feed eaten/dead fish from the initial calculated feeding level for that particular group of fish. Fish were not replaced in tanks when deaths occurred since differences in growth rates of replacement fish and of remaining fish in a tank

would bias the overall growth rate of the unit.

IV. Weighing

Fish were dip-netted from aquaria and placed into a perforated plastic basket immersed in a water-filled plastic tray for transport to the weighing area. The basket, with fish, was then lifted out, tipped to one corner, and drained until drops of water fell slowly from the edge of the basket. The basket with fish was placed in a water-filled container located on a weighing scale (Fisher Counter Scale). The weight of container, fish and basket was recorded. The basket with fish was then lifted from the container, water drained into the container as above, and fish were returned to their tank. The basket was drained again, placed back in the container, and reweighed. The total wet weight of the fish was determined as the wet weight of the fish, basket plus container (first weighing) minus the wet weight of the basket plus container (second weighing). All tanks were treated with 2.0 ppm Acrifavine@ immediately after all fish were weighed to reduce the chance of bacterial infection from handling.

V. Statistical Models

Standard one-way, nested, and two-way ANOVA models were used throughout the project (Gill 1978). A unique two

factor split plot model was designed to test the effects of hormone treatment level and diet fed as a function of time on growth and efficiency factors. The model is represented as:

$$Y_{ijkl}=\mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + D_{(ij)_l} + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + (D\gamma)_{(ij)_{kl}} + E_{(ijkl)}$$

where

μ = true mean of the response

α_i = hormone treatment level

β_j = dietary treatment fed

$(\alpha\beta)_{ij}$ = the interaction of hormone treatment level with diet fed

$D_{(ij)_l}$ = residual error associated with the interaction

γ_k = period of growth (time)

$(\alpha\gamma)_{ik}$ = the interaction of hormone treatment level with time

$(\beta\gamma)_{jk}$ = the interaction of dietary treatment fed with time

$(\alpha\beta\gamma)_{ijk}$ = the interaction of hormone treatment level, dietary treatment fed, and time

$(D\gamma)_{(ij)_{kl}}$ = residual error associated with time

$E_{(ijkl)}$ = residual error associated within subjects (replicates)

n = total number of observations

All effects except the residuals were regarded as fixed. The degrees of freedom and sums of squares for each source factor are presented in Table 3.

Table 3. Degrees of freedom (df) and sum of square (ss) estimations for the 2 factor split plot model.

Source	df	SS
Hormone Trt. Level (A)	(a-1)	$SS_A = \sum_{i=1}^{a} y^2 i.../bcr - (y..../n)^2$
Dietary Trt. Fed (B)	(b-1)	$SS_B = \sum_{j=1}^{b} y^2 .j../acr - (y..../n)^2$
(AB)	(a-1)(b-1)	$SS_{AB} = \sum_{i=1}^{a} \sum_{j=1}^{b} y^2 ij../rc - (y.../n)^2 - SS_A - SS_B$
Subject/AB	ab(r-1)	$SS_{Subj/AB} = \sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{l=1}^{r} y^2 ijk./c - \sum_{i=1}^{a} \sum_{j=1}^{b} y^2 ij../rc$
Period of Growth (C)	(c-1)	$SS_C = \sum_{k=1}^{c} y^2 ..k./abr - (y..../n)^2$
(AC)	(a-1)(c-1)	$SS_{AC} = \sum_{i=1}^{a} \sum_{k=1}^{c} y^2 i.k./br - (y..../n)^2 - SS_A - SS_C$
(BC)	(b-1)(c-1)	$SS_{BC} = \sum_{j=1}^{b} \sum_{k=1}^{c} y^2 .jk./ar - (y..../n)^2 - SS_B - SS_C$
(ABC)	(a-1)(b-1)(c-1)	$SS_{ABC} = \sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{c} y^2 ijk./r - (y..../n)^2 - SS_A - SS_B - SS_{AB} - SS_{AC} - SS_{BC}$
Error	ab(r-1)(c-1)	$SS_{Error_c} = SS_Y - SS_A - SS_B - SS_{AB} - SS_{Subj/AB} - SS_C - SS_{AC} - SS_{BC} - SS_{ABC}$

where $SS_Y = \sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{c} \sum_{l=1}^{r} y^2 ijkl - (y..../n)^2$; n=abcr

The assumption of a homogeneous variance - covariance matrix for the model was tested using multivariate analysis. The validity of assuming equal variance - covariance structure from treatment to treatment and period to period was ensured by using conservative critical values (Gill 1978) for each test conducted.

CHAPTER 1

Dietary androgen-estrogen combinations in growth promotion of fingerling rainbow trout.

OBJECTIVE

This study was designed to examine the growth responses of fingerling rainbow trout fed semipurified diets containing various combinations of the androgens, 17 α-methyltestosterone (MT) and 5 α-dihydrotestosterone (DHT), and the estrogen, estradiol-17 β (ES). MT has been shown to be an effective growth promoter when fed to juvenile salmonids (Donaldson et al. 1979, Higgs et al. 1982) and has produced gains in rainbow trout fed levels between 1.0 and 12.5 mg/kg diet (from Simpson 1976, Fagerlund and McBride 1977). ES was moderately anabolic when fed to coho salmon at 2.5 mg/kg diet (Yu et al. 1979); but retarded the normal growth of trout when fed at 5.0 mg/kg diet (Sower et al. 1983) and 20.0 mg/kg diet (Johnstone et al. 1978). DHT is a nonaromatizable androgen (Mainwaring 1976) that is not considered important in the proliferation (Powers and Florini 1975) or growth (Mainwaring 1976) of skeletal muscle. Potential anabolic effects of this steroid had not

been examined in salmonids.

MATERIALS AND METHODS

Eight semipurified diets (Table 4) were prepared containing various combinations of MT, ES, and DHT at 2.0 mg/kg diet and a control diet without steroid supplementation. Diets containing a single steroid preparation received 2.0 mg steroid/kg diet while combination diets were formulated on a 1.0:1.0 mg/kg basis. The MT:DHT:ES diet contained 0.5:0.5:1.0 mg steroid/kg diet, respectively, to maintain a 1:1 androgen:estrogen regimen throughout the combinations. Respective steroid-soybean oil stock solutions (1 mg/gm) were initially prepared by dissolving each steroid in 10 ml ethanol and then adding the ethanol to the oil. Appropriate amounts of each steroid stock solution replaced a similar amount of the soybean oil component used in formulating each diet. All diets were mixed, dried, and stored as outlined by Garling and Wilson (1976).

Twenty fish (five months old, 0.7 gms. initial weight) were placed in each 110 l flow-through aquarium supplied with well-water at a flow rate of 1 lpm with continuous aeration. Water temperature was a constant 11.5^{o}C. Overhead fluorescent lighting was set on a 14:10 light:dark regimen. Four tank replicates were used per treatment. Mean fish

Table 4. Semipurified diet formulation receiving various combinations of 17α-methyltestosterone, 5α-dihydrotestosterone, or estradiol-17β at 2.0 mg/kg of dry ingredients.

Component	Percent Dry Diet
Protein[1]	40.00
Casein	32.79
Gelatin	14.13
Dextrin	28.15
Soybean oil	6.45
Cod Liver oil	2.00
α-Cellulose	11.48
VMP[2]	5.00

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[1]Based on 90% protein in a 70:30 mixture of casein:gelatin (NRC 1981).

[2]VMP = vitamin and mineral premix according to NRC (1973) recommendations (Tables 1,2).

weight of each unit was obtained by dividing the total weight of fish in each tank by the number of fish in each tank.

Tanks of fish were fed at 4.0% of the total wet body weight of fish per unit per day on a dry matter basis divided into two equal feedings (1000-1100 and 1600-1700 hrs.) for ten weeks. The 4.0% rate appeared to be in moderate excess of the immediate needs of the fish since a minimum of uneaten feed collected in all tanks after each feeding. Feeding rates were adjusted every two weeks and when mortalities occurred. Fish were not replaced in the units when death occurred. Mortalities were less than 1.0% and independent of type of diet fed.

A split-plot analysis of variance was used to examine the effects of treatments through time on average weight gain (AWG) and feed conversion (FC). The validity of assuming equal variance-covariance structure from treatment to treatment and period to period was ensured by using conservative critical values (Gill 1978). Tukey and Dunnett's comparisons were applied where appropriate.

Body composition was analyzed by standard AOAC (1980) methods. Five fish from each replicate group were pooled for analysis. Values obtained represented the mean of two determinations from each replicated group. The effects of treatment on body composition were analyzed with a nested ANOVA and Tukey-type and Scheffe comparisons.

RESULTS AND DISCUSSION

In mammals, the advantage of using androgen-estrogen combinations to stimulate growth is based upon finding appropriate treatment levels which maximize the growth promoting potential of each steroid (Heitzman 1976, 1979). Since separate growth promoting mechanisms are affected by each hormone (Heitzman 1979), the additive effects enhance growth beyond that which can be obtained by a single hormone preparation. The levels of ES used to treat the trout in our study either alone or in combination with DHT depressed ($P < 0.05$) growth (Table 5) and did not permit adequate examination of combinations to promote growth. Combining ES at levels that depressed growth with MT at levels that stimulated growth resulted in suppression ($P < 0.05$) of the anabolic response that occurred with MT alone. Fish fed MT and/or DHT with ES gained the same weight as controls. Estrogenic compounds are generally thought to decrease the growth of salmonids when administered even at low doses (eg. 1 mg/kg diet) (Higgs et al. 1982), although Yu et al. (1979) reported that a 2.5 mg/kg diet dose of ES was anabolic in coho salmon. The results of this study indicate that growth depressing levels of ES also negate the growth stimulating effects of MT when administered in combination with this androgen.

Table 5. Mean average weight gain/fish (grams) of replicate groups (n = 4) of Rainbow Trout fingerlings (initial weight 0.7 gm.) fed steroid supplemented diets. Values having different superscripts are significantly different ($P < .05$) estimated by Tukey comparisons of means, (standard error of means = 0.1 grams).

Dietary Steroid[1] Composition	Respective MG Steroid Contribution/KG Diet	Average Weight Gain/ Fish
MT	2.0	7.1^{a}
MT:DHT	1.0:1.0	7.0^{a}
MT:DHT:ES	0.5:0.5:1.0	5.9^{b}
MT:ES	1.0:1.0	5.8^{b}
C	0.0	$5.6^{b,c}$
DHT	2.0	$5.5^{b,c,d}$
DHT:ES	1.0:1.0	$5.0^{d,e}$
ES	2.0	4.6^{e}

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1 Supplemented as 2.0 mg/KG diet (MT = methyltestosterone; DHT = 5 -dihydrotestosterone; ES = estradiol-17).

2 Initial average weight/fish = 0.7 grams.

MT fed at 2.0 mg/kg diet enhanced the average weight gain (AWG) of treated fish above controls by 27.3% ($P < .05$) after the ten week feeding period (Table 5). This percentage gain above controls is comparable with findings of other low level MT treatments in various salmonids (Higgs et al. 1977; Fagerlund and McBride 1975b, 1977; McBride and Fagerlund 1973, 1976; Fagerlund 1973, 1976; Fagerlund et al. 1979a,b; and Fagerlund et al. 1980). A similar gain was observed in fish fed the MT:DHT combination diet ($P < .05$); however, due to the ineffectiveness of DHT fed alone ($P > .2$), the response was probably a result of the MT alone. DHT also did not alter the growth depressing effects of ES, but the percentage whole body protein was increased ($P < 0.05$) in fish fed DHT combined with MT or ES (Table 6).

The ineffectiveness of DHT alone to promote growth resulted, in effect, in feeding graded levels of MT to the rainbow trout (MT alone = 2.0 and MT:DHT = 1.0 mg MT/kg diet). Similarity in growth rates of fish fed 2.0 and 1.0 mg MT/kg may have resulted from an overlap in biologically significant dosages. Fagerlund and McBride (1977) also observed no significant differences in the growth promoting effects of graded feeding levels of MT between 1.0 and 5.0 mg/kg diet in steelhead trout.

The increases in AWG ($P < .01$) obtained with MT (or MT:DHT) treatment over controls first noted during weeks 6-8 were not associated with significant changes in FC within

Table 6. Influence of experimental diets on whole body (percent wet weight) moisture, protein, lipid, and ash content. Values[1] within columns having different superscripts are significantly different ($P < .05$) estimated by Tukey comparisons of means.

Dietary Steroid Composition[2]	Moisture	Protein	Lipid	Ash
C	78.25^{a}	11.69^{a,b}	7.00^{a}	1.90^{a}
DHT	79.18^{a}	10.87^{a}	7.12^{a}	1.40^{a}
MT	79.12^{a}	11.16^{a,b}	7.28^{a}	1.83^{a}
ES	78.96^{a}	11.96^{b}	6.91^{a}	1.27^{a}
MT:ES	79.10^{a}	11.54^{a,b}	6.54^{a}	1.60^{a}
MT:DHT	77.29^{a}	12.07^{b}	7.57^{a}	2.00^{a}
DHT:ES	78.68^{a}	12.11^{b}	6.45^{a}	1.75^{a}
MT:DHT:ES	79.12^{a}	11.40^{a,b}	6.91^{a}	1.64^{a}
SEM[3]	0.61	0.23	0.29	0.16

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1 Values represent the mean of 8 determinations per treatment, 2 samples from each of 4 replicate groups (5 fish per group).

2 Supplemented as 2.0 mg/kg diet (MT = methyltestosterone; DHT = 5 α -dihydrotestosterone; ES = estradiol -17 β; C = control).

3 Standard error of the means.

these periods (Tables 7 and 8). This indicated that an improvement in nutrient utilization was not the primary impetus for the increased weight gain observed in MT treated fish after eight weeks. A combination of small, insignificant ($P > 0.20$) gains in weight and increased amounts of feed presented designated by the percentage body weight per day feeding schedule over eight weeks was more attributable to the greater gain. MT treated fish again gained more body weight ($P < 0.01$) than controls during weeks 8-10 because they were larger and were fed more and not because their FC was significantly ($P > 0.20$) improved. Previous authors (Fagerlund et al. 1979a, Fagerlund et al. 1980, Fagerlund et al. 1979b) have reported improvements in FC after long term, low level MT treatment; however, these changes were also associated with increased feed intake of fish fed on a satiation basis. This raises the question of whether the increased gain observed in these studies was due primarily to increased feed utilization, increased intake, or both. The results of this study indicate that the short term (2 and 10 weeks) increase in weight gain of MT treated fish is not caused by a significant improvement in feed utilization.

In contrast, decreased ($P < 0.01$) gain of ES treated fish (weeks 8-10) was associated with increased ($P < 0.01$) FC. This was due to a decreased intake of presented feed

Table 7. Average weight gain/fish (AWG) in grams of controls and gram deviations (+ or -) in AWG of treated fish relative to controls over bi-monthly periods.

	Periods (weeks):				
Diet	0-2	2-4	4-6	6-8	8-10
Controls AWG	0.6	0.6	1.1	1.3	2.0
MT	0.1	0.1	0.2	0.5**[1]	0.6**
MT:DHT	-0.1	0.1	0.2	0.6**	0.6**
MT:DHT:ES	0.1	0.1	0.1	0.3*	-0.3*
DHT	-0.1	0.1	0.0	0.1	-0.2
MT:ES	-0.1	0.1	0.1	0.3*	-0.2
DHT:ES	0.0	0.1	0.0	-0.2	-0.5**
ES	-0.1	0.1	-0.1	-0.2	-0.7**

1
Dunnetts test: **P < .01 and *P < 0.05.
Mean Squared Error of AWG and Period Interaction:
(Subject/A) X B(MSe) = 0.02. SE (of the difference between 2 treatment means) = 0.1.

Table 8. Average feed conversions (FC), in grams dry wt feed/grams wet weight gain of controls and deviations (+ or -) in feed conversion of treated fish relative to controls (+ or -) over bi-monthly periods.

	Periods (weeks):				
Diet	0-2	2-4	4-6	6-8	8-10
Control FC	0.59	0.99	0.91	1.15	1.19
MT	-0.04	-0.08	-0.10	-0.16	-0.12
MT:DHT	0.03	-0.14	-0.13	-0.28*[1]	-0.11
MT:DHT:ES	-0.06	-0.16	-0.01	-0.03	0.35**
DHT	-0.01	-0.19	-0.10	-0.11	-0.01
MT:ES	0.03	-0.09	-0.08	-0.08	0.17
DHT:ES	0.05	0.01	0.00	0.34**	0.24*
ES	0.06	-0.07	0.05	0.23*	0.41**

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[1] Dunnetts test: **P < 0.01 and *P < 0.05.
Mean Squared Error of FC and Period Interaction:
(Subject/A) X B(MSe) = 0.0125. SE (of the difference between 2 treatment means) = 0.08.

and/or a decreased tissue use of absorbed nutrients which slowed growth. Decreases in feed intake of ES treated ovariectomized rats result from an increase in triglyceride avaliability for metabolic fuel (Roy and Wade 1977) caused by a hormone induced mobilization of body fat stores (Watkins et al. 1972, Kim and Kalkhoff 1975, Roy and Wade 1977, Ferreri and Naito 1978). Since fat content was not changed ($P > 0.20$) in trout of this study (Table 6), metabolic control of feed intake was not suggested. The levels of ES used were probably toxic to the fish and depressed their appetite. Growth depressing levels of androgens and estrogens have decreased appetite in treated salmon (Fagerlund and McBride 1975) and carp (Yamazaki 1976). Appetite depressing effects of high doses of gonadal steroids have also been observed in mammals (Wade and Gray 1979).

SUMMARY AND CONCLUSIONS

The results of this study confirm the anabolic effectiveness of MT in rainbow trout, enhancing gain by 27.3% after ten weeks. ES, however, depressed growth at the levels used and appeared to inhibit the anabolic action of MT when in combination with this androgen. The effects of MT and ES on growth differed when examined over time intervals; MT enhanced growth without improving feed conversion while ES depressed feed utilization and/or

acceptance (i.e., appetite). DHT had no effect on body weight gain.

It is concluded that a 1.0 or a 2.0 mg/kg dietary treatment of 17α -methyltestosterone is anabolic in rainbow trout fingerlings over a ten week period.

CHAPTER 2

Effect of 17 α-methyltestosterone treatment and varying dietary protein to energy ratios on relative tissue growth in juvenile rainbow trout with special emphasis on the growth of muscle and bone.

OBJECTIVE

This study was designed to examine the effects of anabolic hormone treatment and varying dietary protein to energy level on muscle weight gain relative to the gain of other body tissues in juvenile rainbow trout. Emphasis was placed on the gain of muscle relative to carcass bone and the effects on condition factor of fish. A 2.0 ppm dietary treatment of 17 α-methyltestosterone was used. The results of this study indicate that both hormone treatment and dietary protein to energy ratio affect relative rates of tissue weight gain, and that the individual effects are additive to some degree when certain combinations of hormone and diet protein to energy level are fed to rainbow trout.

MATERIALS AND METHODS

Five semipurified isocaloric diets (390 kcal ME/kg of dry diet) varying in P:ME level from 80 to 160 mg protein/kcal ME (Smith 1984) of dry diet were prepared with and without 2.0 mg 17 α-methyltestosterone (MT)/kg dry diet (Table 9). Fat and dextrin levels were adjusted for changes in protein content. Dextrin levels did not exceed 30 % of the total dry diet ME to avoid potential adverse effects of high carbohydrate levels on rainbow trout (Hilton et al. 1982). Dietary fat did not exceed levels considered practical for commercial fish feed formulations (12% of the dry diet). The soy and cod liver oil feed components were adjusted in all diets to maintain a constant percentage of fatty acid contribution from each component. Two grams of a steroid-soybean oil stock solution (1 mg/gm) replaced an equivalent amount of the soybean oil in the feeds to establish the hormone treatment level. All diets were mixed, dried, and stored as outlined by Garling and Wilson (1976).

Fish were maintained in 110 l flow-through aquaria using a well water supply at 1.5 lpm and a constant temperature of 11.5^{o} C with supplemental aeration. Overhead fluorescent lighting was maintained on a 14:10, light:dark regimen.

Table 9. Constant energy-varying protein level diets with or without 2.0 mg/kg 17α -methyltestosterone/kg dry diet fed to juvenile rainbow trout.

	PERCENT DRY DIET				
PROTEIN[1]	31.20	39.00	46.80	54.60	62.40
Casein	24.27	30.33	36.40	42.47	48.53
Gelatin	10.40	13.00	15.60	18.20	20.80
Dextrin	41.25	29.07	16.88	12.65	0.47
Soybean oil	9.35	9.35	9.35	7.00	7.00
Cod liver oil	2.65	2.65	2.65	2.00	2.00
α-Cellulose	7.08	10.60	14.12	12.68	16.20
VMP[2]	5.00	5.00	5.00	5.00	5.00
Metabolizable Energy[3,4]	390				
P:ME Level[5]	80.0	100.0	120.0	140.0	160.0

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1 Based on 90% protein in a 70:30 mixture of casein:gelatin (NRC 1981).

2 VMP = vitamin and mineral premix according to NRC (1973) recommendations (Tables 2,3).

3 Based on metabolizable energy (ME) values of 4500 kcal/kg for a 70:30 mixture of casein:gelatin, 3200 kcal/kg dextrin, and 8500 kcal/kg oil (Smith 1984, personal communication).

4 Total ME calculated for all diets.

5 mg protein/kcal ME in 100 grams of dry diet.

Four replicate tanks of 20 fish, each, were used for each feed treatment. The initial weight of each fish was approximately 5 grams with a total weight per tank of 100 7 grams. Each tank of fish was fed a dry matter amount of diet equal to 3.5% of the total wet body weight of the unit per day divided into two equal feedings (0900-1000 and 1800-1900 hr.) for ten weeks. This feeding rate is sufficient to promote the rapid growth of rainbow trout fingerlings in lieu of satiation feeding (Grayton and Beamish 1977). The amount fed per tank was adjusted every two weeks and when mortalities occurred. The average weight of each fish/unit was calculated based on the total weight of fish/each unit divided by the number of fish at the end of each two week weighing period. Average weight gain/fish (AWG) was calculated from period to period.

At the end of the experiment, two fish from each replicate tank were weighed (gm) and measured (total length, cm) prior to evisceration, decapitation, and skinning. In addition, all fins, and the entire rib cage with muscle were removed and weighed. The remaining carcass was then weighed wet and placed in a forced air drying oven at 40^{o} C for 20 hours and reweighed. This low level heating cooked and flaked the muscle which facilitated its removal from the bones. After separation, the cooked muscle and remaining bone were weighed.

The wet weight of muscle and wet weight of bone were

used to calculate muscle to bone (M:B) ratios. The recorded weights of cooked muscle were readjusted based on loss of water from each carcass. The recorded weights of bone were not readjusted since water loss from bone was assumed to be minimal. The cooking process was not considered adequate to evaporate water from bone surrounded by muscle since an average of 25% water remained in each carcass after cooking.

Bone ash content in the whole body (WB) and empty carcass (EC) of fish was measured following standard A.O.A.C. (1980) methods. The EC was an eviscerated WB including head, skin, and fins. Values obtained represented the mean of two samples from each of four replicate groups for each hormone x diet treatment combination.

The condition factor (CF)(Fulton 1911) of fish was calculated as follows:

$$CF = \text{total fish weight (gms)/total fish length (cm)}^3 \times 100$$

A two-way analysis of variance was used to examine the effects of steroid treatment and dietary P:ME level on AWG, M:B ratio, percent weight distribution of tissue components, bone ash content in the WB and EC, and CF. In addition, the effect of the various steroid and P:ME treatments on M:B ratios and CF were examined using a covariance analysis with carcass weight as a nusiance variable between replicates.

Tukey and Dunnett comparisons were applied where appropriate. Simple linear regression was applied to various factors in attempts to account for relationships between the random variables. Plots of residual values were used to test for non-linear trends.

RESULTS AND DISCUSSION

A. Hormone Effects.

MT enhanced ($P < 0.01$) average weight gain (AWG) by 17.8% (Table 10), but did not ($P > 0.25$) alter the percentage muscle weight distribution (Table 11) of treated fish. A single linear regression equation ($y = 0.45x - 0.77$, $P < 0.001$) was valid ($P > 0.20$) to describe the muscle growth of both treated and untreated fish (Figure 2). Very little scatter of points occurred along the regression line indicating a strict relationship ($r^2 = 0.97$) of muscle weight to body weight irrespective of MT treatment. An increase in AWG of treated fish was predictive of an increase in muscle gain. Fagerlund and McBride (1975) observed that the percentage flesh weight of coho salmon treated with 1.0 ppm MT was not affected, but did note that the percentage was decreased in fish treated with 10 ppm MT. The severity of hormone side effects is often related to dose (Higgs et al. 1982). It is possible that doses of MT higher than the 2.0 ppm dose used in this study could have also reduced the percentage muscle in our trout.

Table 10. Effects of 2.0 mg 17 α-methyltestosterone(MT)/kg dry diet treatment for eight weeks on various body component characteristics of juvenile rainbow trout.

CHARACTER	Measurement of fish fed hormone concentration (mg MT/kg dry diet) of: 0.0	2.0	SEM[1]
Muscle:Bone[2]	19.06[3]	20.43	------
Condition Factor[4]	1.11	1.22*[5]	0.0132
- Muscle/l^3	0.464	0.507*	0.00721
- Head/l^3	0.121	0.139*	0.00387
- Skin/l^3	0.089	0.106**	0.00175
- Fin/l^3	0.0131	0.0142*	0.000357
- Viscera/l^3	0.256	0.274	0.00529
- Carcass bone/l^3	0.0260	0.0257	0.00173
- Rib/l^3	0.145	0.153	0.00393
Carcass Weight/l^3	0.488	0.530*	0.00734
Average Weight Gain (gms)	16.3	19.2**	0.570
Mean Fish Length (cm)	11.9	12.6**	0.214

1 SEM = standard error of the mean determined based on homogeneous variance. Heterogeneous variance was found for M:B ratios. Individual standard deviations were 0.922 for controls and 0.774 for treated fish values.

2 Expressed as wet weight muscle/wet weight bone.

3 Values represent the mean of 2 determinations each of 4 replicates and 5 pooled dietary treatments.

4 Condition Factor = total fish weight(gms)/total fish length(cm)^3 x 100. Individual body components were weighed separately and expressed as weight of component (gm)/total fish length (cm) x 100.

5 Superscript denote significant (*P<0.05,**P<0.01) differences from control values determined using Dunnett comparisons.

Table 11. Percentage weight distribution of various body components examined in 17^{α} - methyltestosterone (MT) treated and non-treated juvenile rainbow trout.

Body Component	Percent of total body weight at hormone concentration (mg MT/kg diet) of: 0.0	2.0	SEM[1]
Head	11.06[2]	11.34	0.232
Skin	8.23	8.81*[3]	0.161
Fin	1.19	1.16	0.033
Muscle	41.58	41.66	0.450
Carcass bone	2.35	2.16*	0.080
Rib section	13.26	13.60	0.238
Viscera	22.33	21.27	0.321

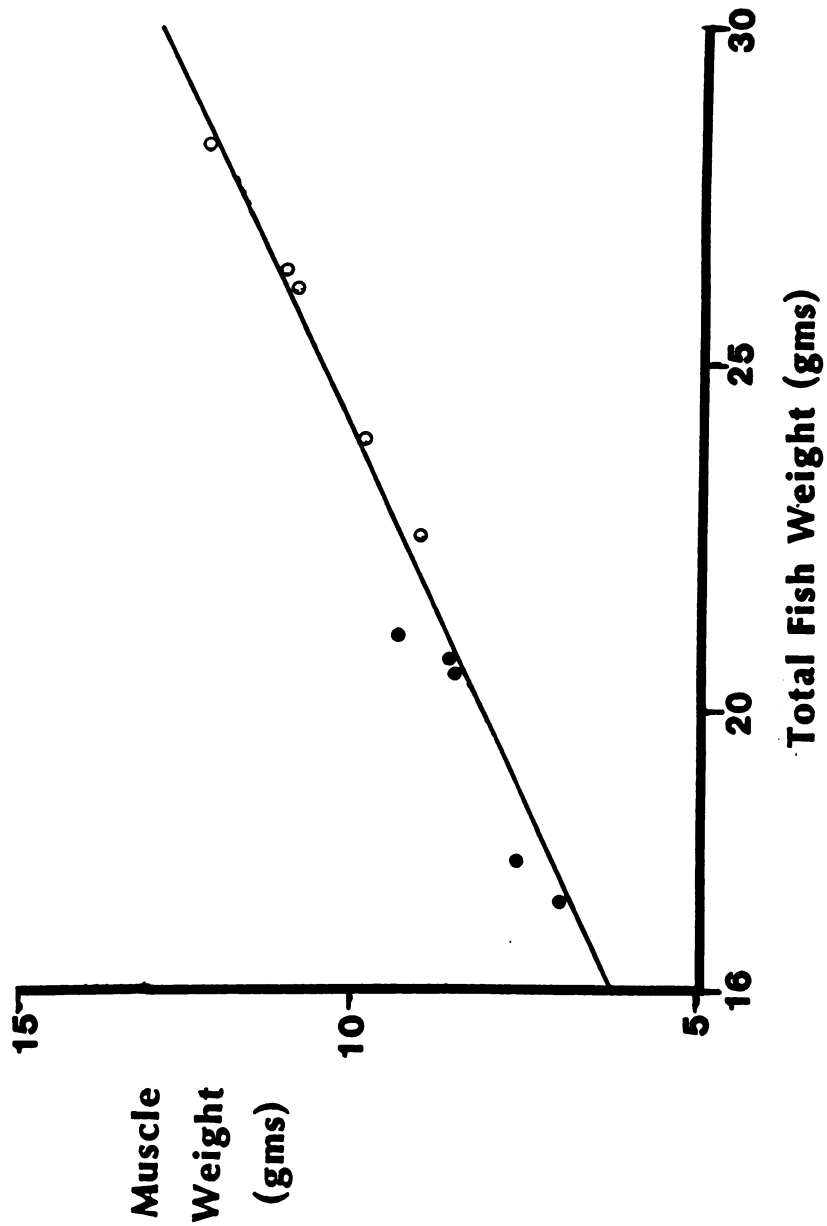
---

[1] SEM = standard error of the mean determined based on homogeneous variance.

[2] Values represent the mean of 2 determinations per each of 4 replicate groups and 5 dietary P:ME level treatments.

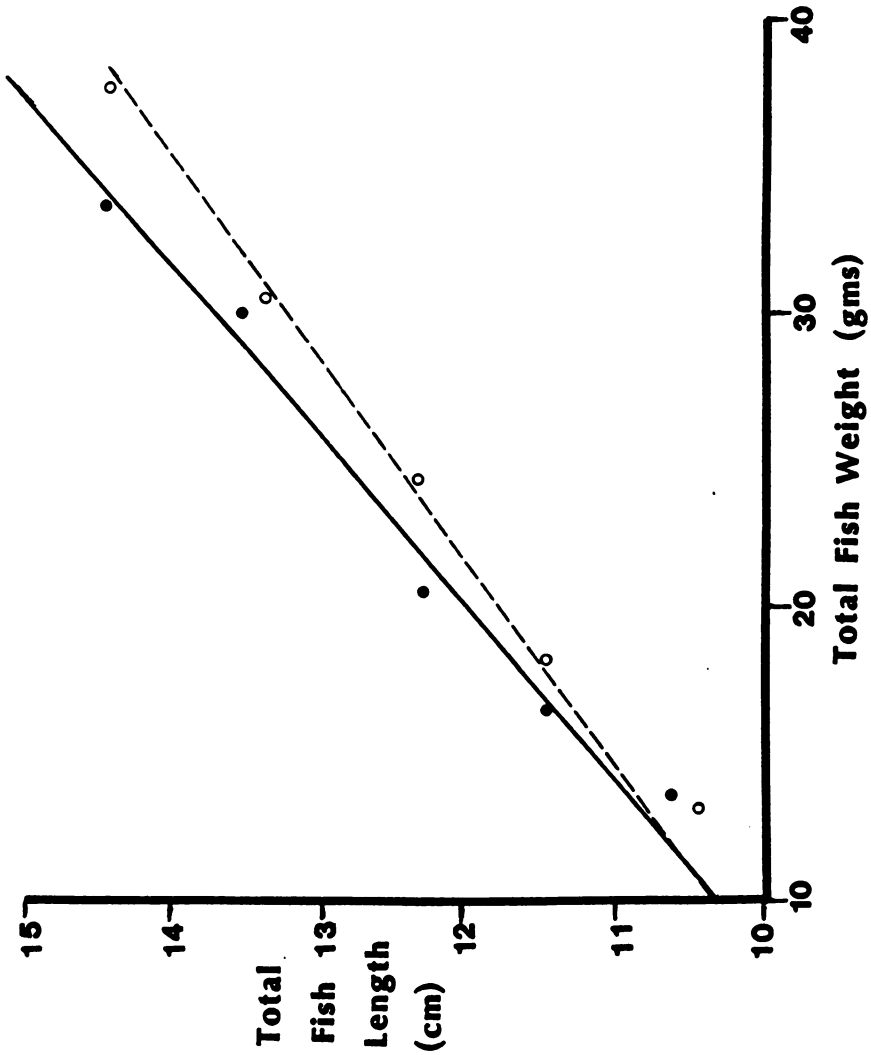
[3] Superscript denotes significant($P<0.05$) difference from control values determined by Dunnett comparisons.

Figure 2. Plot of mean values of 2 samples from each of 4 replicate groups at each dietary treatment fed (n=80) of wet muscle weight to total fish weight of 17^{α}-methyltestosterone(MT) treated (^{O}) and non-treated (•) fish fed varying dietary protein to energy (P:E)levels. Individual regression lines of non-treated ($y = 0.47x - 1.01, r^2=0.99$, $P < 0.001$) and treated ($y = 0.45x - 0.92, r^2 =0.97$, $P < 0.001$) fish were not different ($P > 0.20$ for b_o and b_1) resulting in a single linear regression expression for both groups ($y = 0.45x - 0.77$, $r^2= 0.97$, $P<0.001$). Standard error for b_o=0.267 and for b_1=0.008.


Muscle
Weight
(gms)
15
10
5
16
20
25
30
Total Fish Weight (gms)

MT treatment increased($P < 0.01$) the percentage skin weight and decreased ($P < 0.05$) the percentage carcass bone weight of fish (Table 11). An increase in skin thickness has been observed with low to high level treatments of hormones in salmonids (Sower 1978, Schreck and Fowler 1982) and may have accounted for the increased weight of this tissue observed in our fish. Conversely, the decreased percentage weight of bone in treated fish did not appear to be related to a decreased weight accumulation of this tissue. The absence of any change in bone/ln^3 due to treatment ($P>0.25$) indicated that bone weight was being accrued in a normal way in relation to linear bone growth, although percentage bone ash may have been increased. Since neither EC nor WB ash content of trout changed ($P>0.25$) correspondingly with the lowered percentage bone weight of MT treated fish, a compensatory increase in bone ash probably occurred. Takashai et al. (1983) found that treatment with sex hormones increases the bone mineralization process and bone ash content in immature male quail. Furthermore, the decreased bone percentage was not related to an inhibition of linear bone growth since MT treated fish were longer ($P<0.001$) than controls. The rate of increase in linear bone growth of treated fish was lowered ($P<0.08$) by 12 % (Figure 3) and a disproportionate effect of treatment on linear bone and weight growth was evident. The lower rate of increase in bone length gain to whole body gain resulted

Figure 3. Plot of mean values (r=4,u=2) of total fish length to total fish weight of 17α -methyltestosterone (MT) treated (0--0) and untreated (●—●) juvenile rainbow trout. Plotted values represent the mean total length and total weight of fish measured in the length classes associated with Figure 3: 10.0-10.9,11.0-11.9,12.0-12.9,13.0-13.9,14.0-14.9. Individual linear regression lines for treated ($y = 0.17x + 8.50$, $r^2=0.92$, $P<0.001$) and untreated ($y = 0.15x + 8.89$) fish had similar ($P>.40$) origins (b_o), but different ($P<0.08$) slopes (b_1). Standard error for $b_o=0.345$ for control fish and 0.383 for MT treated fish, and for $b_1=0.008$ for control fish and 0.007 for MT treated fish.


Total Fish Length (cm)
15
14
13
12
11
10
10
20
30
40
Total Fish Weight (gms)

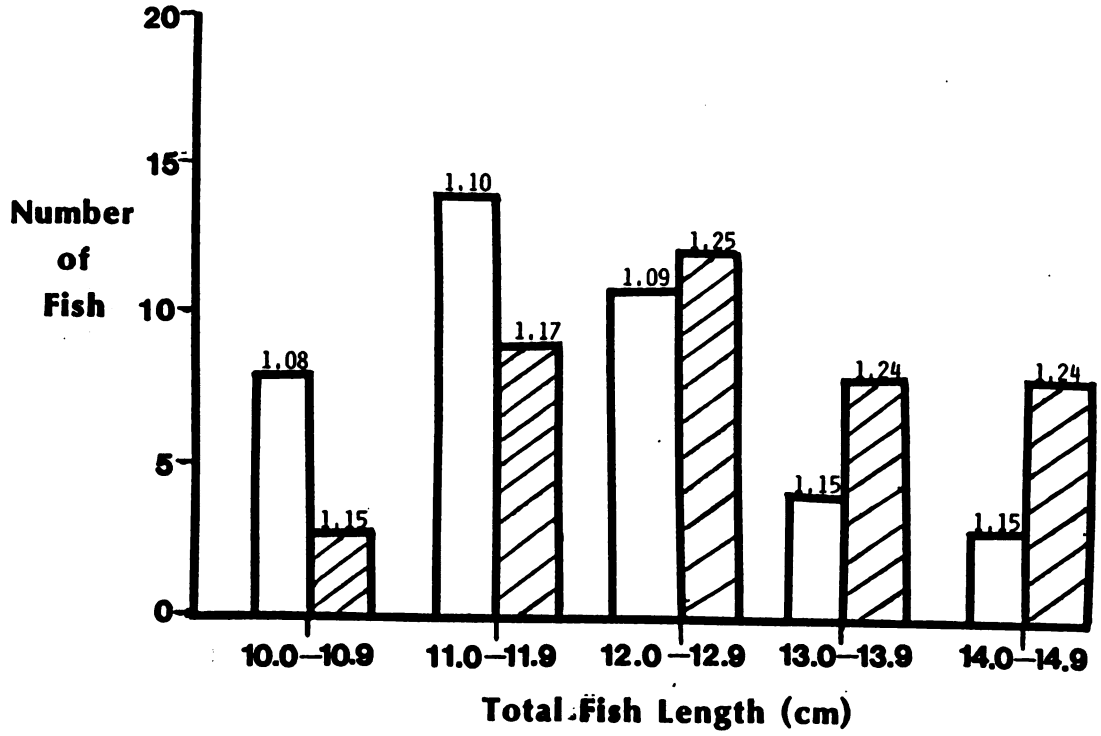
in a lower bone weight accumulation and lower percentage contribution of bone to the percentage total body weight.

Simpson (1976), Saunders et al. (1977) and Fagerlund and McBride (1977) have observed a disproportionate effect of hormone treatment on weight and length increase that results in an increased condition factor (CF) of salmonids. However, it is not clear whether this increase is due to an accelerated weight gain, a decreased length gain, a combination of both these effects, or a disproportionate increase in both weight and length. In this study, the decreased percentage rate of increase in linear bone growth that occurred with MT treatment (12%) could not fully explain the percentage change in CF that occurred in fish, since the CF increased ($P<0.01$) by only 9.9% (Table 10). At least part of this discrepancy was related to a disproportionate increase in the percentage weight gain of body components making up the CF function. For example, skin weight/ln^3increased ($P<0.01$) by 19.1% while fin weight/ln^3 increased ($P<0.05$) by only 8.5% due to treatment. Furthermore, viscera and rib section components increased in weight gain in relation to length gain in a similar ($P>0.25$) fashion as controls. The increase in CF due to MT treatment was a combination of a disproportionate effect of the hormone on the weight gains of the various body components measured and a decreased rate of increase in linear bone increase.

Changes in the CF value have been used to indicate changes in nutritional status of fish by relating the degree of body weight gain to body length gain (Ricker 1975). The higher the CF value, the better the "condition" of fish and, supposedly, the greater muscle percentage in the carcass; the lower the CF value, the lower the condition and muscle percentage. Fagerlund and McBride (1977) suggested that an increase in CF of hormone treated salmon produced fish with a greater percentage muscle in the carcass. Although muscle/l^3 of our trout increased ($P<0.05$) with MT treatment, the value increased in direct proportion to the increase in CF value (i.e. muscle/l^3 increased by 9.3%). Consequently, the change in CF value of MT treated fish was not indicative of a change in percentage muscle weight to whole body weight (Table 11). However, the percentage change in CF due to MT treatment was indicative of a change in the absolute amount of muscle gained per length of fish.

Changes in CF of both treated and non-treated fish were related to size of fish measured (Figure 4). The histogram in Figure 4 indicates that there was a tendency for longer fish to have a higher mean CF. Further, the shift in bar representations to the right for MT treated fish indicated that there were more long MT fish than controls measured. If the sampling procedure was representative, then the increase in CF due to MT treatment was related, at least in part, to the ability of treated fish to reach a larger

Figure 4. Frequency histogram of the number of 17 -methyltestosterone (MT) treated (▨) and nontreated (□) juvenile rainbow trout occurring in a size range associated with a mean condition factor (CF = total fish weight (gms)/total fish length^3 x 100) value. Mean CF values for treated and untreated fish in each length class are presented at the top of each bar representation.


20
15
10
5
0
Number of Fish
1.08
1.15
1.10
1.17
1.09
1.25
1.15
1.24
1.15
1.24
10.0–10.9
11.0–11.9
12.0–12.9
13.0–13.9
14.0–14.9
Total Fish Length (cm)

size more quickly than controls. Within each size range measured, however, treated fish had a higher mean CF than control fish and an effect of the hormone was evident.

There was no difference($P > 0.25$) in M:B ratios between hormone-treated and untreated fish (Table 10). This was an unexpected result considering that MT decreased the percentage weight distribution of bone. A poor correlation of muscle to bone growth in both control ($r^2=0.48$) and treated ($r^2 =0.69$) fish, however, accounted for extreme variability in M:B values (MSe = 50.146) and the insignificance of any apparent difference in M:B ratios. Higher correlations of muscle to bone growth resulted when individual P:ME levels were examined (Table 12); however, the variability in the range of these values also suggests that muscle and bone growth were more dependent on hormonal or dietary effects than on each other. The higher correlations with treatment may reflect the disproportionate influence of the hormone on the growth of muscle and bone and not a more concerted growth effort between these tissues caused by MT.

In cattle muscle and bone growth are highly correlated and bone stretch on muscle is thought to be a primary impetus for muscle growth in these animals (Berg and Butterfield 1976). In fish, a similar growth correlation would seem less likely since bone does not construct a solid

Table 12. Correlation values (r^2) for muscle weight as a function of carcass bone weight in 17α - methyltestosterone (MT) treated and untreated rainbow trout fed varying dietary protein to energy (P:ME) levels.

Diet P:ME Level[1]	Correlation (r^2) Values for fish fed diets containing: 0.0 mg MT	2.0 mg MT
80	0.55	0.93
100	0.80	0.77
120	0.46	0.79
140	0.62	0.96
160	0.84	0.39

[1] Expressed as mg protein/kcal ME in 100 grams of dry diet.

base for stretch in these animals. The bone of fish is structured by apposition in accordance with the evolution of this animal in a bouyant environment. In contrast, a more solid construction of bone is required by terrestrial animals to support the body against the force of gravity. Indeed, the M:B ratios of the fish in this study were 4-5 times the magnitude of that observed in cattle (Kempster 1978) suggesting that bone has a limited role in fish as a support structure against the weight of muscle and thus would be a questionable stimulus for muscle growth.

B. Dietary Effects.

Dietary P:ME ratio affected length ($P < .10$) and the percentage bone ($P<.05$), head ($P<.10$), skin ($P<.10$), and viscera ($P<0.05$) weight of fish(Table 13). In addition, muscle/ln^3 ($P<0.05$), skin/ln^3 ($P<0.01$), rib/ln^3($P < .05$), M:B ratio ($P<.10$), and CF ($P<.10$) were altered (Table 14). In general, a pattern of lower relative rates of hard tissue (bone, head) to soft tissue (muscle, skin) growth occurred when fish fed P:ME ratios of 120 and 140 are compared to fish fed the 80 and 100 ratios. However, a linear trend of increasing soft to hard tissue growth with increasing dietary P:ME ratio was not evident since the 160 ratio diet most often produced results in fish similar to the 80 and 100 ratios. Jackson (1976) found in cattle that higher protein to energy levels promoted more bone growth and

Table 13. Effect of dietary protein to energy (P:ME) level on various body component characteristics of juvenile rainbow trout.

Character	Measurement of fish fed dietary P:ME level of: 80	100	120	140	160	SEM[2]
* Muscle:Bone[3]	a,b[4] 20.72	a 16.60	b 23.35	a,b 19.08	a,b 18.99	-------
* Condition Factor[5]	1.14a[6]	1.15a,b	1.20b,c	1.21b	1.13a	0.0195
Muscle/l^3	0.474a	0.461a	0.496a,b	0.516b	0.482a,b	0.0114
Head/l^3	0.129	0.136	0.130	0.133	0.122	0.00612
Skin/l^3	0.091a	0.097a,b	0.106b	0.098a,b	0.096a	0.00276
Fin/l^3	0.0129	0.0145	0.0130	0.0143	0.0134	0.000565
Viscera/l^3	0.272	0.258	0.263	0.260	0.266	0.00837
Carcass bone/l^3	0.0238	0.0284	0.0227	0.0284	0.0259	0.00273
Rib/l^3	0.135a	0.180b	0.161b,c	0.155a,c	0.137a	0.00622
Carcass/l^3	0.496a	0.488a	0.514a,b	0.538b	0.504a,b	0.0116
Average Weight Gain(gms)	17.5	18.6	18.7	17.2	17.0	0.901
* Mean Fish Length (cm)	12.4a	11.8b	12.4a	12.5a	12.4a	0.338

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1 Expressed as mg of protein/kcal ME in 100 grams of dry diet.

2 SEM = standard error of the mean determined based on homogeneous variance. Heterogeneous variance was found for M:B ratios and individual standard deviations calculated for each P:ME level are not represented.

3 Expressed as wet weight of muscle/wet weight of bone.

4 Values represent the mean of 2 determination per each of 4 replicates for each dietary treatment from both hormone and non-hormone treated groups.

5 CF = total body wet weight(gms)/total fish length(cm)^3 x 100. Individual body components experessed as compoment wt/l x 100.

6 Values having different lettered superscripts are significantly ($P < 0.05$,* $P < 0.10$) different determined by Tukey-type comparisons.

Table 14. Effect of dietary protein to energy (P:ME) level[1] on percent weight distribution of various body components.

Body Components	Percent of total body weight of fish fed dietary P:ME level of: 80	100	120	140	160	SEM[2]
	3 4					
* Head	11.33a,b	12.17a	10.89b	10.83b	10.80b	0.367
* Skin	8.02a	8.72a,b	8.98b	8.20a,b	8.69a,b	0.255
Fin	1.12	1.29	1.10	1.19	1.19	0.052
Muscle	41.17	40.05	41.56	41.99	42.09	0.712
Carcass bone	2.10a,b	2.49b	1.91a	2.35a,b	2.32a,b	0.127
Rib section	12.35a,c	12.77b,c	13.48a,b	13.80b	11.65c	0.377
Viscera	23.91a	22.51a,b	22.08a,b	21.64b	23.36a,b	0.507

1 Expressed as mg of protein/kcal ME in 100 grams of dry diet

2 SEM = standard error of the mean determined based on homogeneous variance.

3 Values represent the mean of 2 determinations per each of 4 replicates for both hormone and non-hormone treated groups.

4 Values having different lettered superscripts are significantly ($P < 0.05$, $*P < 0.10$) determined by Tukey - type comparisons.

lower muscle growth. The results with trout suggest that there is an optimum dietary range of maximum soft to hard tissue growth in these animals.

C. Hormone x Diet Interaction.

The CF was the only characteristic measured that exhibited ($P<0.05$) a diet x hormone interaction. The changes with diet P:ME ratio or hormone treatment that occurred with each individual body component measurement were similar ($P>0.25$) irrespective of the combination of treatment factors. The CF, however, represented a combination of all body components and was indicative of the average effect of all individual body component measurements.

Those fish fed the diet with a P:ME of 140 and treated with MT had the highest CF ($P<0.10$) of all fish except MT treated fish fed the diet with a P:ME equal to 120 (Table 15). This response suggests the occurrence of an additive effect of diet and hormone treatment consistent with the individual effects of P:ME level and MT treatment observed. Since it was found that changes in fish length can effect the relative weight contribution of bone to total body weight we compared the CF to a measure of relative soft to hard tissue growth. A high dietary P:ME level which promoted relatively lower rates of bone growth coupled with steroid treatment which also minimized linear bone growth should

Table 15. Mean (r=4, u=2) Condition Factor (CF) of 17α - methyltestosterone (MT) treated and non-treated fish fed varying dietary P:ME levels. Values with different[1] lettered superscripts are significantly (P<0.05) different[2] determined by Tukey-type comparisons.

Dietary P:ME Level[3]	CF of fish fed MT concentrations (mg/kg diet) of: 0.0	2.0
	[4,5]	
80	1.11a,b	1.18a,c
100	1.09a,b	1.20a,c
120	1.19a	1.22a,c,d
140	1.12a,b	1.31d
160	1.06b	1.20a,c

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1 Wet weight of fish(gms)/total fish length(cm)^{3}x 100.

2 The interaction was significant at P<0.05; minimum significant difference at this P level equals 0.12.

3 As mg protein/Kcal ME in 100 grams of dry diet.

4 Values represent the mean of 2 determination per each of 4 replicates for each treatment combination.

5 Standard error of treatment combination means = 0.0256 calculated based on homogeneous variance.

produce fish with a higher CF. These observations are consistent with those of Ince et al.(1982) who fed varying dietary P:ME ratios similar to those used in this study to ethylestrenol treated trout fingerlings (27 gram initial weight). These researchers noted that increasing dietary protein levels exerted a preferential effect on weight as opposed to length gain of hormone treated fish. Furthermore, a similar response with protein level did not occur in controls in this study consistent with their observations.

SUMMARY AND CONCLUSIONS

In summary, both low level MT treatment and varying dietary P:ME ratio affected relative tissue growth of juvenile rainbow trout in this study. A tendency towards higher rates of soft tissue growth and lower rates of hard tissue growth was evident with MT treatment and dietary P:ME ratios of 120 and 140. These effects on relative muscle to bone growth were additive to some degree with combinations of hormone treatment and P:ME ratio and results manifested in CF values. A decreased rate of linear bone growth resulted in an increase in CF of MT treated fish. Muscle growth was related to whole body growth, but not bone growth in hormone treated and untreated fish.

It is concluded that the effects of MT treatment on percentage muscle weight of the trout body are minimal within the confines of the time and dosage of treatment used

in this study. Increases in total body weight of treated fish is a good indicator of increases in muscle gain. Combinations of hormone treatment and dietary protein to energy ratio may be used to maximize relative muscle growth to bone growth in fish; however, these combinations do not necessarily increase the absolute amount of muscle gained in fish.

CHAPTER 3

Optimum dietary protein to energy and total energy levels of 17 α -methyltestosterone treated juvenile rainbow trout.

OBJECTIVE

The present study was designed to establish optimum dietary P:ME ratio and total protein and metabolizable energy levels for juvenile rainbow trout fed semipurified diets supplemented with 2.0 mg MT/ kg of diet. This optimum reflected maximum-efficient protein deposition in the whole body of fish per unit of diet fed based on compromises between protein intake and efficiency of protein gain. This chapter presents observations of changes in dietary protein needs of MT treated fish and establishes an optimum dietary P:ME ratio and ME level within the ranges used and level of hormone supplemented in this study. In addition, changes in fat composition of the whole body and empty carcass of fish due to MT treatment are discussed.

MATERIALS AND METHODS

Two consecutive semipurified diet feeding trials were used to determine the optimum diet formulation for steroid mediated growth. The first trial was designed to establish an optimum P:ME ratio for growth of MT treated fish fed a single dietary energy level. The second trial was designed to estimate optimum total protein and metabolizable energy levels for MT treated fish fed a constant P:ME level. The manipulations of dietary protein and energy in trial 2 were based on the optimum P:ME ratio determined for MT treated fish in trial 1. The mean initial weights of fish used in trial 1 (5.4 $\pm$ 1.1 gms) and trial 2 (3.7 $\pm$ 0.7 gms) were kept relatively constant to avoid any size related differences in nutritional requirements of rainbow trout (NRC 1981).

Five basal semipurified test diets containing 80, 100, 120, 140, and 160 mg protein/kcal metabolizable energy (ME) of dry diet were prepared for use in trial 1 with 390 kcal ME/100 grams of dry diet (Table 16). The energy level chosen was considered adequate to determine optimum P:ME relationships for growth. Recalculated data from the work of Lee and Putnam (1973) indicate that maximum growth responses of fish can be supported within total energy ranges between 370 and 440 kcal ME of dry diet. The ME values were calculated and adjustments in dietary components

Table 16. Constant energy-varying protein level diets with or without 2.0 mg 17α-methyltestosterone/kg of dry diet fed to juvenile rainbow trout.

	PERCENT DRY DIET				
PROTEIN[1]	31.20	39.00	46.80	54.60	62.40
Casein	24.27	30.33	36.40	42.47	48.53
Gelatin	10.40	13.00	15.60	18.20	20.80
Dextrin	41.25	29.07	16.88	12.65	0.47
Soybean oil	9.35	9.35	9.35	7.00	7.00
Cod liver oil	2.65	2.65	2.65	2.00	2.00
α-Cellulose	7.08	10.60	14.12	12.68	16.20
VMP[2]	5.00	5.00	5.00	5.00	5.00
Metabolizable Energy[3]	390				
P:ME Level[4]	80.0	100.0	120.0	140.0	160.0

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1 Based on 90% protein in a 70;30 mixture of casein:gelatin (NRC 1981).

2 VMP = vitamin and mineral premix according to NRC (1973) recommendations (Tables 1,2).

3 Total metabolizable energy (ME) in 100 grams of dry diet calculated for all diets. Based on (ME) values of 4500 kcal/kg for a 70:30 mixture of casein:gelatin, 3200 kcal/kg dextrin, and 8500 kcal/kg oil (Smith 1984, personal communication).

4 mg protein /kcal ME of dry diet.

were based on values determined for rainbow trout by Smith (1984). Fat and dextrin levels were adjusted for changes in protein content. Dextrin levels did not exceed 30 % of the total dry diet ME to avoid potential adverse effects of high carbohydrate levels on rainbow trout (Hilton et al. 1982). Dietary fat did not exceed levels considered practical for commercial fish feed formulations (12% of the dry diet). The soy and cod liver oil feed components were adjusted to maintain respective $^{n-3}$ fatty acid profiles throughout all diets.

Four basal semipurified test diets containing total energy levels of 300, 340, 370, and 390 kcal ME/100 gms of dry diet were prepared for use in trial 2 with a constant P:ME level of 100 mg protein/kcal ME (Table 17). Maximum levels of fat and dextrin were regulated as described in trial 1. Dietary fat was reduced to a minimum of 6% in the 300 kcal diet; however, the contribution of soy and cod liver oil components were sufficient to meet minimum fatty acid requirements of trout (NRC 1981). No attempts were made to maintain similar fatty acid profiles from the oil components throughout these diets.

All basal diets were prepared with or without 2.0 mg MT/kg of dry diet. Two grams of a steroid-soybean oil stock solution (1 mg MT/gm) per kilogram of dry diet replaced an equivalent amount of the soybean oil component in the feeds to establish the hormonal treatment level. All diets were

mixed, dried, and stored as outlined by Garling and Wilson (1976).

Fish were maintained in 110 l flow-through aquaria using a well water supply at 1.5 lpm and a constant temperature of 11.5^{o} C with supplemental aeration. Overhead florescent lighting was maintained on a 14:10, light:dark regimen.

Four replicate tanks of 20 fish (100$\pm$ 5 gms) (trial 1) and 25 fish (100$\pm$ 3 gms) were used for feed treatments in trials 1 and 2, respectively. Each tank of fish was fed a dry matter amount of diet equal to 3.5% of the total wet body weight of the unit per day divided into two equal feedings (0900 - 1000 and 1800 - 1900 hrs.) for eight weeks. This feeding rate was sufficient to promote the rapid growth of rainbow trout fingerlings in lieu of satiation feeding (Grayton and Beamish 1977). The amount fed per tank was adjusted every two weeks and when mortalities occurred. Fish were not replaced in the units when death occurred. The average weight gain of each fish/unit (AWG) was calculated at the end of each two week weighing period based on the total weight of each unit and the number of fish in each unit. The fish were not fed on the day of weighing to avoid incorporating stomach contents into the weight gain results.

Table 17. Constant protein to energy varying total protein and energy level diets with or without 2.0 mg 17α - methyltestosterone/kg of dry diet fed to juvenile rainbow trout.

	PERCENT DRY DIET			
Protein[1]	39.00	37.00	34.00	30.00
Casein	30.00	28.78	26.45	23.33
Gelatin	13.00	12.33	11.33	10.00
Dextrin	29.07	33.91	33.21	30.95
Soybean oil	9.35	7.00	4.50	2.00
Cod liver oil	2.65	2.00	3.00	4.00
α -Cellulose	10.60	10.98	16.51	24.72
VMP[2]	5.00	5.00	5.00	5.00
P:ME level[3]	100.0			
Metabolizable Energy[4]	390.0	370.0	340.0	300.0

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1 Based on 90 % protein in a 70:30 mixture of casein:gelatin (NRC 1981).

2 VMP = vitamin and mineral premix according to NRC (1973) recommendations.

3 mg protein/kcal metabolizable energy (ME) of dry diet calculated for all diets.

4 Total ME in 100 grams of dry diet. Based on ME values of 4500 kcal/kg for a 70:30 mixture or casein:gelatin, 3200 kcal/kg dextrin, and 8500 kcal/kg oil (Smith, personal communication).

The relative daily gain (RDG) of fish was determined from period to period and calculated on a percent per day per fish basis as:

$$RDG\ (\%) = ((WT_f - WT_i)/WT_i)/14\ days \times 100$$

where WT_f = the final mean weight of a fish in a unit at the end of a 14 day feeding period, and WT_i = the initial mean weight of a fish in a unit at the beginning of a 14 day feeding period.

The protein gain (PG), productive protein value (PPV), and maximum-efficient protein gain/100 grams of diet fed (MEPG) were determined at the end of each feeding trial and calculated on a per fish basis as :

$$PG\ (gm) = P_f - P_i$$

$$PPV\ (\%) = PG \times 100/\text{total gms protein fed}$$

$$MEPG\ (gm) = PPV \times \text{gms protein in 100 grams of diet fed}$$

where P_f = the final mean whole body protein content of a fish in a unit at the end of the 8 week feeding trial, and P_i = the initial mean whole body protein content of a fish in a unit at the beginning of the 8 week feeding trial.

Whole and empty carcass body composition were analyzed by standard A.O.A.C (1980) methods. The empty carcass was an eviscerated whole fish body. Five fish from each replicated group were pooled for both whole and empty carcass analysis. Values obtained represented the mean of

two determinations from each replicated group.

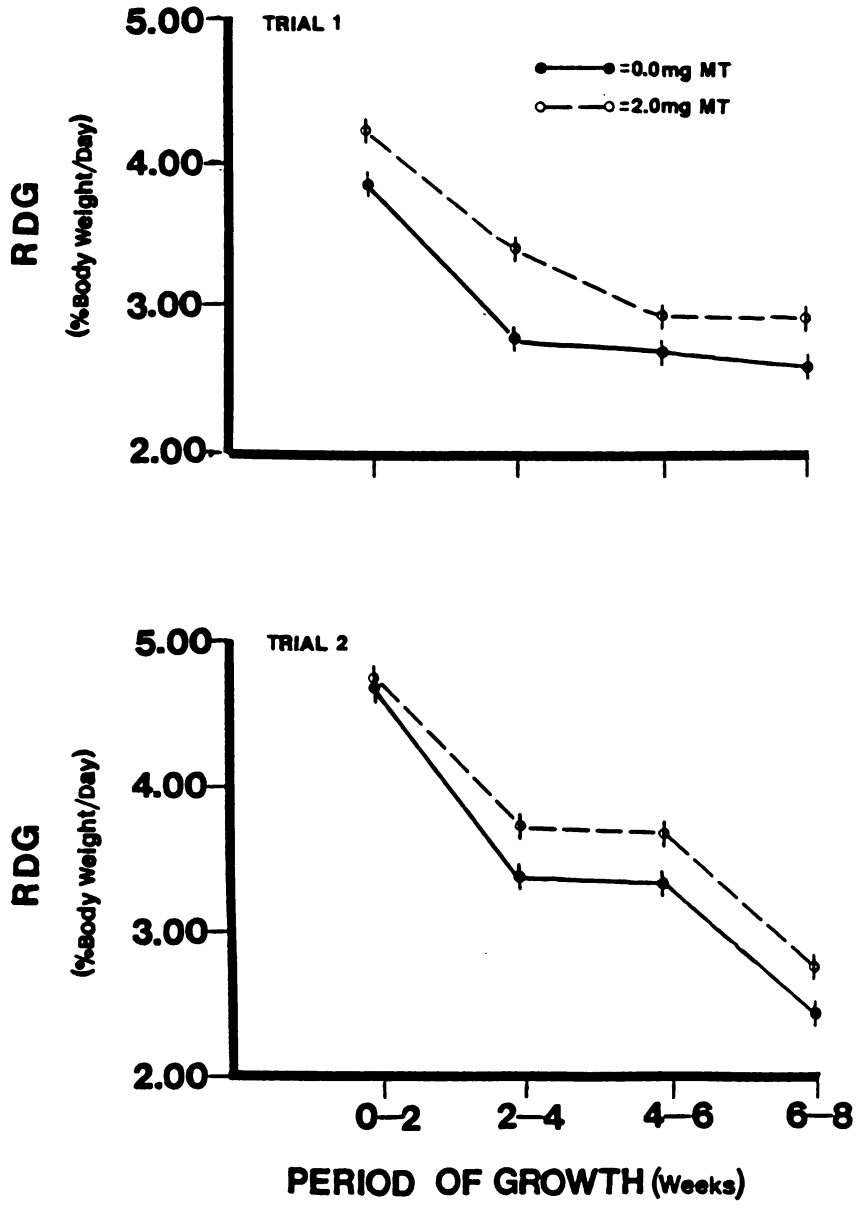
Livers obtained from fish viscera in each hormone x diet treatment combination replicate were pooled according to dietary treatment and analyzed for lipid and protein content following A.O.A.C. (1980) methods. Values obtained represented the mean of two determinations from each dietary treatment. Each dietary treatment represented a replicate to test the effects of hormone treatment on changes in liver composition.

A split-plot analysis of variance (ANOVA) was used to examine the effects of hormone and nutritive treatment through time on RDG. The validity of assuming equal variance-covariance structure from treatment to treatment and period to period was ensured by using conservative critical values (Gill 1978). The effects of nutritive and hormone treatment on PG, PPV, MEGP, and body compositions were analyzed using a two-way nested ANOVA. A simple two-way ANOVA was used to test effects on AWG. A one-way ANOVA was used to test the effects of hormone treatment on liver lipid and protein content. Tukey, Dunnett, Schiffe, and non-orthogonal comparisons were applied where appropriate in each ANOVA design.

RESULTS AND DISCUSSION

Low level 17α -methyltestosterone (MT) treatment has increased the weight gains of juvenile salmonids over periods of 10 to 57 weeks (for review see Higgs et al. 1982). In general, the percentage gains over controls recorded increased with time as the hormone was continously applied; however, it is not clear whether the final gains observed resulted from a continual influence of the hormone over the treatment period or from initial improvements in gain that were carried over to a period of decreased hormonal influence on growth. The difference has important implications on the relative effective treatment period and, more importantly for the present context, on the ability to determine optimum nutritive requirements based on steroid mediated alterations of growth. Estimations should be made over the period of hormonal influence. In this study 8 week treatment periods were employed. Increases in average weight gain (AWG) of 18% in trial 1 ($P < 0.001$) and 14% in trial 2 ($P<0.01$) were obtained due to MT. These increases resulted from relative daily gains (RDG's) of treated fish that equalled or, more commonly, exceeded ($P<0.05$) that of control fish at each period measured (Figure 5). The persistence of above normal rates of gain was maintained with time despite the increasing size of treated fish throughout each trial and the effect ($P<0.001$) of increasing

Figure 5. Effect of time on relative daily gain (RDG = $((Wt_f - Wt_i))/14$ days x 100) of 17αmethyltestosterone (MT) treated and untreated juvenile rainbow trout. Standard error of combination means (AC) for level of MT treatment (A) sharing the same sampling point in time (C) = 0.084 for trial 1, and = 0.086 for trial 2 based on homogeneous variance for both variance - covariance matrices.



time on decreasing RDG that occurred. These observations confirm that the estimations of optimum dietary conditions for steroid mediated growth that occurred were based over a period of hormonal influence.

Estimations of optimum dietary conditions for growth were based on the expected level of protein deposition in fish per 100 grams of dry diet fed and represented by MEPG. These values were considered a reflection of protein efficiency, but on an equal diet fed basis rather than on an equal protein fed basis as indicated by PPV. In this respect, the performance of fish was predicted by accounting for limitations in growth caused by limitations in dietary protein intake. High efficiency values estimated by PPV's may be achieved with diets that contain levels of other nutrients that limit protein intake of fish at a feeding. Growth is limited, subsequently, and the apparent value of the diet reduced since longer times and more feedings are required to produce comparable rates of gain. The MEPG, however, evaluates gain as a function of protein efficiency (ie. PPV) and diet protein intake, and is a more realistic perspective of diet performance since fish are commonly fed a particular level of diet.

The MEPG values of MT treated fish were correspondingly higher ($P < 0.05$) than the values obtained for controls at all dietary P:ME ratios fed except for fish fed a P:ME of 80 (Table 18). A similar effect of MT treatment with PPV

Table[18]. Effect of 17α -methyltestosterone and varying dietary protein to energy level content on growth characteristics measured in juvenile rainbow trout after eight weeks. Values within columns not sharing the same superscript letter are significantly ($P < 0.05$) different. Mean values for steroid effect not sharing the same superscript symbol are significantly (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$) different.

Steroid Level (mg/kg diet)	Dietary P:E Level[1]	Dietary Protein[2] (%)	AWG[3] (g)	Whole Body Protein[4] (%)	PG[5] (g)	Protein Fed[6] (g)	PPV[7] (%)	MEPG[8] (g)
0.0	80	31.13	16.6 a,b,c[9]	13.14 a	2.17 a	6.3 a	34.4 f	10.78 a,b
	100	38.95	17.2 a,b,c,d,e	13.76 a,b	2.37 a,b,c	8.1 a,b	28.3 d	11.04 a,b
	120	46.60	17.0 a,b,c,d	14.24 a,b	2.43 a,b,c	9.3 b,c,d	25.9 c	12.07 c,d
	140	54.47	16.0 a,b	13.91 a,b	2.23 a,b	11.2 d,e,f	20.0 b	10.87 a,b
	160	62.27	14.7 a	14.00 a,b	2.06 a	12.6 f,g	16.4 a	10.18 a
		Means	16.3	13.81	2.25	9.5	25.1	10.99
2.0	80	31.13	18.3 b,c,d,e	13.30 a	2.44 b,c	6.6 a,b	36.9 g	11.49 b,c
	100	38.95	20.0 d,e	14.04 a,b	2.81 c	8.3 a,b,c,d	33.8 f	13.17 e
	120	46.60	20.3 e	14.03 a,b	2.79 c	9.9 c,d,e	28.2 d	13.14 e
	140	54.47	18.4 b,c,d,e	14.89 b	2.74 c	11.6 e,f	23.8 c	12.96 d,e
	160	62.27	19.3 c,d,e	14.06 a,b	2.71 c	14.7 g	18.9 b	11.74 b,c
		Means	19.3 ***	14.06	2.70 ***	10.2 *	28.3 ***	12.49 ***

[1]P:E = mg protein/ kcal metabolizable energy (ME) in 100 grams of dry diet.
[2]Presented on a percent dry weight basis.
[3]Average wet weight gain per fish.
[4]Determined on a percent wet weight basis.
[5]Protein gain = final whole body protein content - initial whole body protein content calculated on a per fish basis.
[6]Total dry weight grams of protein fed per fish.
[7]Productive protein value = PG x 100/total dry weight grams of protein fed per fish.
[8]Maximum-efficient protein gain = PPV x grams protein in 100 grams of diet fed calculated on a per fish basis.
[9]SEM values for each treatment combination based on homogeneous variance are as follows: AWG = 0.637, % whole body protein = 0.295, PG = 0.103, gms protein fed = 0.622, PPV = 0.465, MEPG = 0.212.

determinations was obtained except that the PPV's decreased linearly with increasing protein level while MEPG values reached an optimum level. Diets containing P:ME ratios of 100, 120, and 140 produced higher ($P<0.05$) MEPG values than diets containing 80 and 160 in MT treated fish. Control fish reached a maximum MEPG value($P<0.05$) when fed the P:ME equal to 120. The obtainment of a maximum MEPG at a lower P:ME ratio in hormone fed fish indicated a reduction in P:ME needs from 120 to 100 with MT treatment.

A reduction in optimum dietary P:ME needs of 20 mg/kcal metabolizable energy (ME) in MT treated fish is consistent with a theory of increased protein utilization, however, the mechanism of the hormonal influence in not clear. Ince et al. (1982) suggested that the improvements in dietary protein utilization with hormone treatment are regulated through changes in gastrointestinal absorptive function. They found an inverse relationship between alterations in dietary protein and dietary fat assimilation induced by a 3.5 ppm treatment of ethylestrenol in rainbow trout fingerlings fed diets containing 43% and 53% protein for 60 days. Protein assimilation increased and fat assimilation decreased from the diets at a rate of 25 and 29 mg of protein/kcal ME of fat, respectively, based on the 8.5 kcal/gm ME calculation for fat used in our study. This altertion in absorption is consistent with the reduction in optimum dietary P:ME needs for maximum protein deposition

observed in our study corroborating an effect of hormone treatment at the level of digestion. However, no evidence for changes in metabolic efficiency due to hormone treatment are available for fish. The possibility still remains that the decrease in P:ME needs are due to a reduced dependence of absorbed protein for energy needs. Changes in gut histology of trout with MT treatment have been noted (Yamazaki 1976) suggesting the possibility of altered absorptive ability.

Similar ($P>0.25$) MEPG values were obtained in MT treated fish fed a dietary P:ME ratio equal to 80 as untreated fish fed a P:ME ratio equal to 120 (Table 18). This result indicated that normal growth rates of trout may be maintained with a reduction in dietary protein content and maintenance of metabolizable energy level with MT treatment. The decrease in P:ME level corresponded to a reduction in dietary protein content by 1/3 (Table 16). This prediction in deposition, based on the per diet fed criterion used in this study, agrees closely with growth responses of hormone treated trout fed varying dietary protein levels observed in other studies. Ince et al. (1982) obtained the same growth of trout fed semipurified diets containing 32% protein and treated with ethylestrenol as untreated fish fed a diet containing 43% protein. This represented a reduction in protein content by 26%.

Fagerlund et al. (1983) were able to maintain above normal gains with a 1 ppm treatment of MT in juvenile coho salmon fed practical diets reduced in protein level by 31%. In both these studies, the reductions of P:mE were from approximately 125 to 90 mg protein/kcal ME.

The diet containing 340 kcal ME was considered optimum for growth of MT treated fish fed a P:ME level of 100 (Table 19). This dietary energy level was chosen since it was the minimum level that produced higher ($P<0.05$) MEPG values in fish than all control diets. The diet containing 300 kcal ME and supplemented with MT produced similar ($P>0.25$) MEPG values in fish as treated diets containing higher energy levels, but higher ($P<0.05$) values thant unsupplemented diets containing 300 and 370 kcal ME only. A protein level of 30% or less at a P:ME ratio of 100 maybe too low too promote gains in protein deposition due to MT treatment. Indeed, the inability of MT in trial 1 to increase the MEPG value of fish fed 31% protein with sufficient dietary energy (i.e. P:ME = 80) may have indicated the requirement for a minimum dietary protein level to induce a response to anabolic hormone treatment.

MEPG values obtained by MT fed fish exceeding 13 grams were significantly ($P<0.05$) higher than any value obtained by control fish (Tables 18,19). MEPG values of control fish averaged less than 12 grams in trials. However, it is tenuous to suggest that this MEPG level was maximum for

Table 19. Effect of 17α-methyltestosterone and varying total dietary protein and energy level content on growth characteristics measured in juvenile rainbow trout. Values within columns not sharing the same superscript letter are significantly (P<0.05) different. Mean values for the steroid effect not sharing the same superscript symbol are significantly (*** P < 0.001, ** P < 0.01, * P < 0.05) different.

Steroid Level (mg/kg diet)	Dietary Energy Level[1]	Dietary Protein[2] (%)	AWG[3] (g)	Whole Body Protein[4] (%)	PG[5] (g)	Protein Fed[6] (g)	PPV[7] (%)	MEPG[8] (g)
0.0	300	30.87	13.2 a[9]	13.22 a	1.75 a	4.6 a	37.5 f	11.58 a
	340	34.26	13.1 a	13.84 a	1.81 a,b	5.2 a,b	35.3 c,d	12.10 a,b
	370	36.27	13.8 a,b	13.59 a	1.88 a,b,c	5.8 a,b	32.3 a,b	11.70 a
	390	38.46	14.2 a,b	13.85 a	1.97 a,b,c	6.2 a,b	31.7 a,e	12.19 a,b
		Mean	13.6	13.63	1.84	5.5	34.2	11.89
2.0	300	30.87	14.6 a,b	13.81 a	2.02 a,b,c	4.8 a	41.5 g	12.81 b,c
	340	34.26	15.9 a,b	13.72 a	2.17 b,c	5.5 a,b	39.0 f	13.35 c
	370	36.27	15.3 a,b	13.94 a	2.13 b,c	5.8 a,b	36.8 d,e	13.35 c
	390	38.46	16.3 b	13.58 a	2.21 c	6.5 b	34.0 b,c	13.09 c
		Mean	15.5 **	13.78	2.13 *	5.7	37.8 ***	13.15 ***

[1]kcal metabolizable energy in 100 grams of dry diet based on values determined by Smith (1984).
[2]Presented on a dry weight basis.
[3]Average wet weight gain per fish.
[4]Determined on a percent wet weight basis.
[5]Protein gain = final whole body protein content - initial whole body protein content calculated on a per fish basis.
[6]Total dry weight grams of protein fed per fish.
[7]Productive protein value = PG x 100/total dry weight grams of protein fed per fish.
[8]Maximum-efficient protein gain = PPV x grams protein in 100 grams of diet fed calculated on a per fish basis.
[9]SEM value for each treatment combination based on homogeneous variance are as follows: AWG = 0.645, % whole body protein = 0.284, PG = 0.080, gms protein fed = 0.358, PPV = 0.416, MEPG = 0.189.

these fish since adjustments in protein and energy in diets of controls in trial 2 were not based on their determined optimum P:ME ratio of 120. Rates of efficient deposition might have been increased further with ME level manipulations above or below 390 kcal at this P:ME ratio. An optimum response trend would have been indicated. No trend in MEPG values with increasing total energy was apparent for control fish fed diets containing a P:ME of 100. Although an optimum response trend with dietary ME level did not ($P>0.25$) occur in MT treated fish fed the P:ME of 100, these fish seemed to reach a maximum MEPG value of 13.35 grams when fed diets containing 340 or 370 kcal ME. Increasing the total dietary ME level to 390 kcal reduced this value to 13.09 grams. This agreed closely with the value obtained in trial 1 (13.17) in fish fed the same diet.

The pattern of changes in whole body (WB) and empty carcass (EC) fat content due to diet nutritive state were similar ($P>0.25$) (Table 20). In general, WB and EC fat levels were decreased by increasing dietary P:ME ratio and increased with increasing total protein and energy. These responses in fat deposition to P:ME state are consistent with responses seen previously in WB determinations of rainbow trout fed similar energy levels as this study (Lee and Putnam 1973), and with channel catfish fed varying dietary protein and energy levels (Garling and Wilson 1976).

Table 20. Effect of 17 -methyltestosterone (MT)[1] and varying nutritive state on percentage whole body[2] and empty carcass[3] fat content of juvenile rainbow trout. Values are presented on percentage wet weight basis and represent the mean of 2 determinations each of 4 replicate groups of 5 pooled fish.

Nutritive Variable	Percent Fat[3]				
P:ME Level[4]	Whole Body		Empty Carcass		Percentage Increase in Empty Carcass Fat Due to MT Treatment
	MT[5] 0.0	MT[5] 2.0	MT[5] 0.0	MT[5] 2.0	
80	10.74c,d[6]	10.73a,c	7.31a	9.18c	25.6c
100	11.21d,e	11.46e	8.05a,b,c	8.91b,e	10.7b
120	10.25b,c	10.50b,c	7.82a,b	8.30a,b,c	6.2a,b
140	9.99b	8.78a	7.12a	7.39a	3.8a
160	8.96a	8.53a	7.34a	7.57a	2.7a
Mean	10.11	10.00	7.53	8.27 **	
Metabolizable Energy					
300	7.99a	8.24a,b	6.13a	6.73a,b	9.8a
340	8.91b,c	8.70a,b	6.45a	7.98b,c	23.7b
370	8.68a,b	9.65c,d	7.01a,b	8.64c,d	23.3b
390	10.43e	10.28d,e	8.01b,c	9.52d	18.9b
Mean	9.00	9.22	6.90	8.22 *	

[1] Entire body including viscera.
[2] Eviscerated whole body.
[3] Calculated as percent fat in empty carcass(EC) of control fish -percent fat in EC of MT treated fish/ percent fat in EC of control fish.
[4] mg protein/kcal metabolizable energy in 100 grams of dry diet.
[5] Dietary steroid concentration as mg/kg of dry diet.
[6] Standard errors of the means in P:ME level effect for steroid level = 0.112 in the EC, 0.093 in the WB;and steroid x diet interaction = 0.251 in the EC, 0.147 in the WB; and in total energy level effect for steroid level = 0.151 in the EC, 0.080 in the WB; steroid x diet interaction = 0.302 in the EC, 0.160 in the WB; for % increase in EC fat = 1.47 for P:ME effect, and 1.89 for energy effect.

Moreover, since the difference between WB and EC measurements was visceral contents, the parallel of changes in fat deposition between the two compartments suggested parallel patterns of fat deposition into visceral and carcass components. This pattern is similar to one seen in chickens (Becker et al. 1979) where visceral fat is highly correlated with body fat content (Hood 1982,1984). Patterns of lipogenesis in chickens (Hood 1984) and trout (Lin et al. 1977a, 1977b; Henderson and Sargent 1981) are also similar, occurring primarily in the liver rather than in the adipose.

MT treatment increased EC fat of fish in both trial 1 ($P<0.01$) and trial 2 ($P<0.001$), but did not ($P>0.25$) alter WB fat of fish in either trial. This results indicated a degree a fat redistribution within the body of treated fish from visceral to carcass compnents. Moreover, diet composition altered the degree of fat redistributed by the hormone. A decreasing trend ($P <0.05$) of percent fat in the EC with increasing P:E level was obtained in treated fish, but not ($P>0.25$) in controls (Table 20). Correspondingly, the percentage fat redistributed to the EC decreased with increasing P:E level and MT treatment. Higher protein levels apparently prevented more dramatic alterations of fat redistribution due to treatment. A low dietary energy level (300 kcal ME) also limited the percentage fat moved. This redistribution was not related to the fat content of fish

since a similar ($P>0.25$) percentage increase in EC fat content was obtained in fish fed the diets containing 340 ns 390 kcal ME although these diets produced differences ($P<0.05$) in WB fat content. A similar lack of correlation between percent EC fat increase and WB fat content was obtained in fish fed dietary P:ME ratios between 80 and 120.

Simpson (1976) had noted a direct decrease in the percentage fat content in the viscera of ethylestrenol treated Atlantic salmon and suggested that the loss was probably associated with a need to supply higher energy requirements due to steroid treatment. It was not possible to examine changes in energy needs of the trout used in the present study since changes in energy needs of controls were not based on their optimum P:ME ratio of 120. However, since fat was only redistributed and not removed from the body it appears that no additional dietary energy was required for the hormone treated trout beyond the scope of their increased growth activity.

The site of the increased fat deposition in the EC of MT treated fish was not determined. The skin and muscle of fish were not analyzed separately. Fagerlund and McBride (1977) found that the percentage fat in muscle of steelhead trout and pink salmon increased with MT treatment. Increases in skin thickness has also been reported in hormone treated trout (Sower 1978), but this was associated

with hypertrophy of stratium germinatium cells of the skin and not due to an increase in fat content. Changes in subcutaneous fat deposition in salmonids due to hormone treatment have not been investigated.

It has been suggested that estrogens are important in liver metabolism during spawning periods of oviparous fishes for the production and transport of vitellogenin, a lipophosphoglycoprotein involved in egg maturation. Increases in liver protein, RNA (Medda et al. 1980), and lipid (Dasmahapatra and Medda 1982) content of Singi fish (Heteropneustes fossilis, Bloch) treated with estradiol dipropionate have been related to enhanced lipogenic and protein synthetic activity in the liver (Dasmahaparta and Medda 1982) in addition to the effects of estradiol on regulating the absorption of synthesized protein by the ovaries (Medda et al. 1980). Observations of increased liver protein synthetic activity have suggested the involvement of estradiol 17-β in hepatic vitellogenesis of coho salmon (Bhattacharya et al. 1985). Androgens, however, have had little effect on liver lipid or protein metabolism of fish (Medda et al. 1980, Dasmahapatra and Medda 1982, Lone and Matty 1982) including juvenile rainbow trout (Lone and Ince 1983), although reductions in liver lipid deposition in some salmonids has been observed (Simpson 1976, Fagerlund et al. 1983). In the present study, MT increased liver protein content of fish in both trial 1

($P<0.001$) and trial 2 ($P<0.05$), and decreased ($P<0.05$) liver lipid content of fish in trial 1 (Table 21). These changes correlate with the redistribution of fat from visceral components into the carcass of treated fish, and suggest that the liver may have been directly stimulated by MT to cause visceral fat movement. The impetus for fat movement with MT treatment in juveniles may be similar to that observed for estrogen and vitellogenin transport in adults.

SUMMARY AND CONCLUSIONS

The results of this study indicate that a 2 ppm treatment of MT enhances dietary protein utilization for growth of juvenile rainbow trout. Although the mechanism for the enhanced utilization is not clear, it is evident that treatment lowers the optimum dietary P:ME needs for maximum efficient protein deposition on a per diet fed basis. MEPG values above 13 grams/100 grams of diet fed were obtained for treated fish fed optimum rations, and a diet containing a P:ME ratio of 100 mg of protein/kcal of metabolizable energy in the dry diet and a total ME level of 340 kcal was minimal to produce these gains. MT treated fish fed the diet containing a P:ME ratio equal to 80 produced rates of maximum efficient protein deposition equal to that of untreated fish fed a diet containing a ratio of 120. The effect of diet nutritive content on whole body and

Table 21. Effect of 17α -methyltestosterone (MT) treatment on percent dry weight liver protein[1] and lipid[2] content of juvenile rainbow trout. Treatment values[3] containing different superscripts are significantly (*** P < 0.001, ** P < 0.01, * ,P < 0.05) different from control values.

		Percent Dry Weight			
		Protein	SEM[4]	Lipid	SEM
	Mg MT/kg diet				
Trial 1	0.0	39.68		34.16	
	2.0	47.14 ***		26.34 *	
			1.01		2.54
Trial 2	0.0	42.47		33.40	
	2.0	47.70 *		31.24	
			1.44		1.74

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1 Protein percent calculated from Kjeldahl nitrogen analysis.

2 Lipid percent calculated from total ether-methanol extract.

3 Values represent the mean of two samples from pooled dietary replicates.

4 SEM = Standard error of the mean based on homogeneous variance.

empty carcass composition of steroid treated and non-steroid treated fish were similar. Steroid treatment, however, redistributed visceral fat to the empty carcass. The liver is suspected to have been directly stimulated by MT to cause fat movement.

It is concluded that the recommendations of dietary P:ME ratio and ME level obtained in this study may be used to formulate diets that will maximize dietary protein utilization for growth of MT treated fish over a period of anabolic influence. This includes maximizing utilization for strategies of accelerated growth or maintenance of normal growth rates. Furthermore, changes in fat content of the fish body due to MT treatment do not appear to be related to an increase in metabolic needs of fish, but to a redistribution of fat within the body.

CHAPTER 4

Effect of 17 α-methyltestosterone treatment and withdrawal on growth and dietary protein utilization of juvenile rainbow trout fed practical diets with varying protein to metabolizable energy ratios and limited total metabolizable energy level.

OBJECTIVE

This study was designed to examine the interaction of hormone treatment and protein to metabolizable energy (P:ME) ratio on growth and dietary protein utilization of trout fed practical diets limiting in total ME level. In the previous semipurified diet tests it was noted that a total dietary ME level below 340 kcal (ie. 300 kcal) did not produce significant advantages in fish due to methyltestosterone treatment at the determined optimum P:ME of 100. This suggested that enhanced growth activity was probably limited through some limitation in effective dietary protein level or functional dietary P:ME ratio since it is assumed that with limiting energy more protein would be required to supplement limiting non-protein energy needs for the

additional growth activity. As a result, higher P:ME levels may be required to elicit a response when dietary energy is limiting. Practical diets were used as a test for similar hormone activity and P:ME relationships in fish fed formulations actually used in common fish culture practice as the responses obtained in fish fed the semipurified rations. In addition, the effects of hormone withdrawal from the diet on subsequent growth and protein utilization of fish were examined. This chapter presents results which confirm observations across practical and semipurified studies and suggests that the obtainment of enhanced growth activity in trout fingerlings fed methyltestosterone treated diets containing marginal total energy levels is related to the extent of use of avaliable dietary protein to supply the additional energy required by the activity and the maintenance of a minimum functional P:ME ratio. Hormone withdrawal drastically reversed any improvements in growth or protein utilization of fish to levels below that of controls. The severity of these withdrawal effects at the low dietary ME level used was inversely related to dietary P:ME ratio.

MATERIALS AND METHODS

Two diets containing 48% and 32% protein with and without 2.0 mg 17 α -methyltestosterone (MT)/ kg of dry ingredients were used in this study (Table 22). The lower protein level diet was formulated specifically to contain the same metabolizable energy (ME) but 1/3 the total dietary protein content as the higher protein level diet. The diets were also formulated to contain total ME levels below 340 kcal. Estimates of the dry matter composition and available ME of the feeds were obtained from the open formula analyses. The percent dry matter composition of the GR6-30 feed was not changed and was used as a basis to formulate the lower protein level experimental diet. The lower protein level diet was formulated from the GR7-30 feed. Since this feed contained a higher dry matter protein level than desired (38%), the dietary protein level of the experimental diet was established by dilution of the feed with additions of semipurified ingredients. Dextrin and codliver oil were used to adjust total protein, ME, and protein to ME (P:ME) ratio of the diet based on the ME contributions by each ingredient. The ME values for these ingredients were based on values estimated by Smith (1984, personnel communication).

Both commercial feeds were ground with a Waring Blender. The semipurified ingredients were added in the

Table 22. Gross component composition of experimental diets formulated from practical feeds GR6-30 and GR7-30 [1] and semipurifed ingredient additions [2]. Diets were prepared with and without 2.0 mg 17$^{\alpha}$ -methyltestosteron (MT)/kg of dry diet and fed to juvenile rainbow trout.

Component	Percent Dry Diet[3]	
Protein Level[4]	48%	32%
Protein	47.99	32.00
Fat[5]	14.71	14.75
Fiber	2.84	3.25
Minerals	3.39	3.39
Vitamins	0.25	0.25
NEM[6]	30.82	32.63
Dextrin	-----	13.73
Metabolizable energy[7]	319.95	315.89
P:ME ratio[8]	150.00	100.00
Proximate Composition:		
Protein	46.33	30.05
Fat	13.29	13.45
Ash	9.65	7.14

[1] Glencoe Mills, Minn. GR6-30 used as is to make 48% protein level diet. GR7-30 used to make 32% protein level diet.

[2] Dextrin, codliver oil, NRC (1978) vitamin and mineral premixes added only to GR7-30 feed to make 32% protein level diet.

[3] Based on component percentages and metabolizable energy (ME) values estimated from open formula analyses and/or available ME values for semi-purified ingredients estimated by Smith (1984, personal communication) of 3200 kcal/kg of dextrin and 8500 kcal/kg of oil.

[4] GR6-30 feed contained 22.28% fish protein, 16.24% vegetable protein, and 9.47% non-fish animal protein. GR7-30 feed contained 14.47% fish protein, 14.75% vegetable protein, and 9.46% non-fish animal protein.

[5] Fish fat was 93.18% of the total fat in GR6-30 feed and 87.21% of total fat in GR7-30 feed.

[6] NEM = non extractable materials estimated to be contained in GR6-30 and GR7-30 feeds.

[7] Calculated total ME in 100 grams of dry diet.

[8] P:E = mg protein/kcal ME of dry diet.

appropriate amounts to the GR7-30 feed. Hormone treated diets were prepared by adding a solution containing 2.0 mg MT/20 ml ethanol to each kilogram of dry ingredients. Ethanol was added at 20 ml/kg of dry ingredients to each control diet. All diets were reconstituted with distilled water, dried, pelleted, and stored as outlined by Garling and Wilson (1976).

The experiment was conducted over a continuous 18 week period divided into an initial 10 week hormone treatment phase (Phase 1) and a subsequent 8 week hormone withdrawal phase (Phase 2). Fish receiving the hormone treated diets were immediately switched to their respective control diets upon completion of the 10 week hormone treatment phase.

Fish were maintained in 110 l flow-through aquaria using a well water supply (1.5 lpm) and constant temperature (11.5^{o}C) with supplemental aeration. Overhead florescent lighting was maintained on a 14:10, light:dark regimen.

Four replicate tanks of fish were used for each feed treatment throughout the experiment. Each tank contained twenty fish (initial weight = 3.8 $\pm$ 0.4 grams each) during the first 10 weeks. The number was reduced to ten fish each during the last 8 weeks to accommodate the increasing loading factor (kg/lpm) in tanks. This reduction process was conducted at random. The initial weight in Phase 2 of fish previously fed hormone treated diets was 17.2 $\pm$ 2.5 gms compared to the initial weight of controls of 15.3 $\pm$ 1.6

gms. The initial weight of fish receiving the 48% protein level diet was 16.9 $\pm$ 2.4 gms, and 15.5 $\pm$ 2.1 gms for fish fed the 32% protein level diet.

Tanks of fish were fed 3.5% of the total wet body weight per day on a dry matter basis divided into two equal feedings (0900 - 1000 and 1800 - 1900 hrs.) for the entire 18 weeks. This feeding rate is sufficient to promote the rapid growth of rainbow trout fingerlings in lieu of satiation feeding (Grayton and Beamish 1977). The amount fed per tank was adjusted every two weeks and when mortalities occurred. Fish were not replaced in the units when death occurred. Mortalities were less than 1% and independent of type of diet fed. The average weight gain of each fish/unit was calculated at the end of each two week weighing period based on the total weight and number of fish in each unit. The fish were not fed on the day of weighing to avoid incorporating stomach contents into the weight gain results.

The relative daily gain (RDG) of fish per tank was determined from period to period and calculated on a percent per day per fish basis as:

$$RDG\ (\%) = ((WT_f - WT_i)/\ WT_i)/14\ \text{days} \times 100$$

where WT_f = the final mean weight of a fish in a unit at the end of a 14 day feeding period, and WT_i = the initial mean weight of a fish in a unit at the beginning of a 14 day feeding period.

Whole and empty carcass body composition were analyzed by standard A.O.A.C. (1972) methods. The empty carcass was an eviscerated whole fish body including head, skin, and fins. Five fish from each replicated group (20 fish/treatment combination) were pooled for both whole and empty carcass analysis. Values obtained represented the mean of two determinations from each replicated group.

The protein gain (PG), productive protein value (PPV), and maximum efficient protein gain/100 grams of dry diet fed (MEPG) were determined at the end of each feeding phase and calculated on a per fish basis as:

$$PG\ (gm) = P_f - P_i$$

$$PPV\ (\%) = PG/\text{total gms protein fed}$$

$$MEPG\ (gm) = PPV \times \text{gms protein in 100 gms of dry diet fed}$$

where P_f = the final mean whole body protein content of a fish in a unit at the end of a treatment phase, and P_i = the initial mean whole body protein content of a fish in a unit at the beginning of a treatment phase.

A split-plot analysis of variance was used to examine the effects of hormone and nutritive treatment through time an bimonthly changes in RDG and on phase changes in PG, PPV, and MEPG. The validity of assuming equal variance-covariance structure from treatment to treatment and period

to period was ensured by using conservative critical values (Gill 1978). Tukey, Dunnett, Schiffe, and non-orthogonal comparisons were applied where appropriate in the design.

RESULTS AND DISCUSSION

I. GROWTH AND PROTEIN EFFICIENCY

A. Hormone Effects.

Fish fed 17 α -methyltestosterone (MT) over the 10 week treatment period had a higher relative daily gain (RDG) ($P<0.05$), protein gain (PG) ($P<0.10$), productive protein value (PPV) ($P<0.01$), and maximum efficient protein gain (MEPG) ($P<0.05$) than untreated controls (Table 23, Phase 1). This increase in all growth and efficiency factors was in agreement with that observed in the previous studies where trout were fed semipurified diets supplemented with MT, although the absolute values were somewhat lower than those obtained with semipurified diets. The higher overall values obtained with semipurified diets might be expected since semipurified diets generally contain more highly utilizable ingredients than practical feeds.

Hormone withdrawal drastically reversed the improvements in growth and efficiency values due to MT treatment to below the level of controls (Table 23, Phase 2). A lower RDG ($P<0.05$), PG($P<0.01$), PPV ($P<0.01$), and

Table 23. Effect of 17α-methyltestosterone treatment for 10 weeks (Phase 1) and subsequent withdrawal for 8 weeks (Phase 2) on growth and protein efficiency values of juvenile rainbow trout. Values across columns containing different lettered superscripts are significantly different at P<0.05 and those containing different symboled superscripts are different at P<0.10.

	Phase 1 Response in fish fed diets containing:		Phase 2 Response in fish fed diets containing:		
Factor[1]	0.0 mg MT	2.0 mg MT	0.0 mg MT	2.0 mg MT	SEM[2]
W_i[3]	3.8^{a}	3.9^{a}	15.3^{b}	17.2^{c}	-----
W_f[4]	13.7^{a}	15.8^{b}	38.4^{c}	36.4^{c}	-----
RDG[5](%)	2.12^{a}	2.43^{b}	1.84^{c}	1.44^{d}	0.102
PG[6](gm)	$1.30^{\alpha,a}$	$1.63^{\beta,a}$	3.28^{b}	2.53^{c}	0.120
PPV[7](%)	22.2^{a}	25.6^{b}	26.7^{b}	15.7^{c}	0.602
MEPG[8](GM)	8.12^{a}	9.26^{b}	7.79^{a}	5.82^{c}	0.234

[1] Denotes diet fed in Phase 1. All fish in Phase 2 were fed respective control diets.

[2] Standard Error of the Mean (SEM) = $(MSe/br)^{1/2}$ for hormone treatment level (a) x period (c) interaction based on homogeneous variance for the variance-covariance matrix. Calculations for W_i and W_f based on standard two way ANOVA: SEM for W_i Phase 1 = 0.086, Phase 2 = 0.753, and for W_f Phase 1 = 0.543, Phase 2 = 2.680.

[3] W_i = initial weight/fish at the beginning of a phase.

[4] W_f = final weight/fish at the end of a phase.

[5] RDG = relative daily gain calculated on a percent per day per fish basis as, ((WTi - WTf)/WTi)/14 days x 100 where WTf= the final mean weight of a fish in a tank at the end of a 14 day feeding period, and WTi = the final mean weight of a fish in a tank at the beginning of a 14 day feeding period.

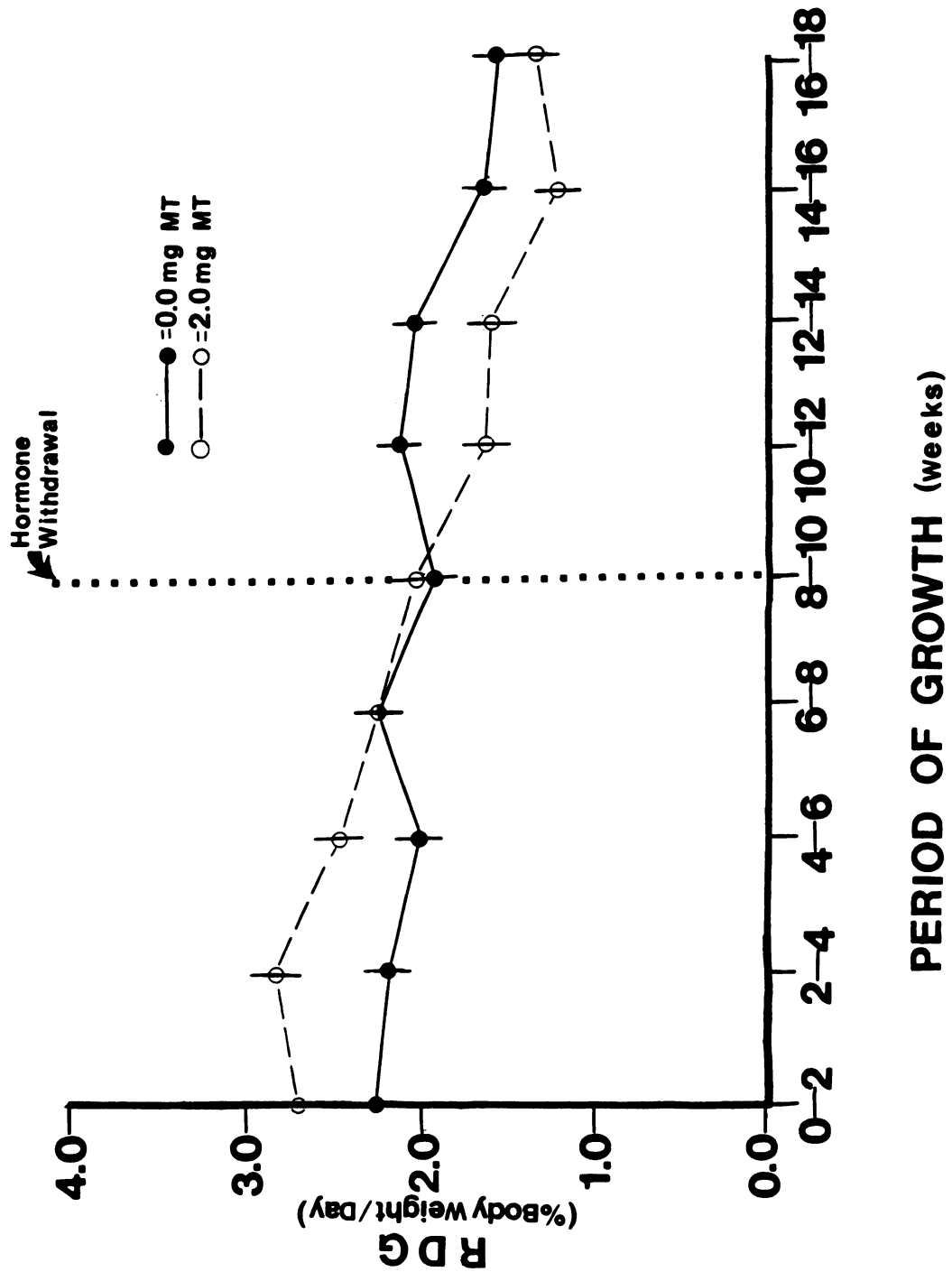
[6] PG = protein gain calculated as Pf - Pi where Pf = the final mean whole body protein content of a fish in a tank at the end of a treatment phase, and Pi = the final mean whole body protein content of a fish in a tank at the beginning of a treatment phase.

[7] PPV = productive protein value calculated as PG/total grams of protein fed per fish x 100.

[8] MEPG = maximum efficient protein gain calculated as PPV x grams protein in 100 grams of diet fed per fish.

MEPG ($P<0.01$) was recorded in fish previously fed MT treated diets during withdrawal. These changes represented an accumulation of effects over time as evidenced by the consistent drop ($P<0.05$) in RDG at all periods of Phase 2 (Figure 6) as opposed to a drop in these characteristics due to a drop in RDG at any one period. The precise reason for the decline in RDG is not clear since an appropriate control on withdrawal (ie. MT treated group for 18 weeks) was not used. The response could have been related to the absence of the hormone or to carry over effects of the hormone from the treatment phase. In mammals, continuous treatment with an anabolic steroid over a long period of time produces a pharmocologic "wearing off" of the treatment effectiveness and gradual decline of growth rates to below levels of control animals (Kochakian 1976). A more severe and immediate decline in growth occurs upon hormone withdrawal and is designated the "rebound " effect. Fagerlund and McBride (1977) observed similar responses in juvenile steelhead trout (Salmo gairdneri) fed 1.0 ppm of MT in a 57 week experiment. Declines in specific growth rates (%body weight/day) to below levels of controls in fish fed the hormone continuously did not occur until between weeks 41 and 57 of the experiment. Hormone withdrawal after 16 weeks, however, produced declines in periods measured after 25 weeks of the experiment. Percentage gains of the withdrawal group also were reduced to below the level of

Figure 6. Changes in relative daily gain (RDG = ((WT_f - WT_i)/WT_i)/14 days x 100 where WT_f= the final mean weight of a fish in a tank at the end of a 14 day feeding period and WT_i = the initial mean weight of a fish ia a tank at the beginning of a 14 day feeding period.) with time of juvenile rainbow trout fed 17 α-methyltestosterone (MT) treated diets for 10 weeks and hormone free diets for 8 weeks. Hormone treated fish were fed 2.0 mg MT/kg of dry diet for the first 10 weeks and were switched to receiving respective hormone free diets for the final 8 weeks of the experiment. Standard error of the mean for hormone level (a) x period (c) interaction (MSe/br) 1/2 is 0.102 based on homogeneous variance for the variance-covariance matrix.


Hormone Withdrawal
=0.0 mg MT
=2.0 mg MT
RDG
(%Body Weight/Day)
4.0
3.0
2.0
1.0
0.0
0–2
2–4
4–6
6–8
8–10
10–12
12–14
14–16
16–18
PERIOD OF GROWTH (weeks)

controls in these periods. The residual effects of MT treatment on the trout in the present study were immediate and dramatic. At the end of the entire 18 week experimental period there was no difference observed in the final mean values of W_f ($P>0.25$), RDG ($P>0.25$), PG ($P>0.10$), and MEPG ($P>0.10$) of fish due to the initial hormone treatment. Indeed, the PPV of control fish was higher ($P<0.001$) than that of treated fish averaged for the entire study.

B. Dietary Protein Level Effects

Fish fed the 48% protein level diet had a higher RDG ($P<0.05$), PG ($P<0.001$), and MEPG ($P<0.001$) at the end of the experiment than those fish fed the 32% protein level diet. PPV, however, was higher ($P<0.001$) for fish fed 32% protein. The responses obtained at the end of the experiment were similar to the responses obtained at each phase. Increasing time, however, decreased ($P<0.001$) the relative values for each characteristic measured except PG which was increased ($P<0.001$) in the second phase of the study. This effect on PG was a reflection of the larger size of fish during Phase 2.

C. Hormone x Dietary Protein Level Interactions.

Hormone withdrawal produced a greater decline growth and efficiency values of MT treated fish fed 32% protein than fish fed 48% protein. During Phase 1, MT treated

fish fed the diet containing 32% protein had a higher ($P<0.01$) PPV value and thus were more efficient than fish fed diets containing 48% protein (Figure 7). However, during the withdrawal phase PPV values for both groups were the same ($P>0.20$). In contrast, there was no change ($P>0.20$) through time in efficiency values of control fish fed either 32% or 48% protein, and those fish fed 32% protein were always more efficient ($P<0.01$). The greater drop in efficiency of fish fed MT treated diets with 32% protein produced greater declines in MEPG values of these fish (45% decrease) than those fish fed the diet with 48% protein (31% decline), although a non-parallel response trend with time did not ($P>0.20$) exist (Figure 8). Rainbow trout fed lower protein levels appaer more sensitive to the negative effects of MT withdrawal.

In contrast, Ince et al.(1982) obtained possible evidence for a preventative effecr of a low dietary protein level to the growth depressing effects of ethylestrenol withdrawal on juvenile rainbow trout. They found that treated fish fed 32% protein had the same percentage increase in mean body weight over controls after 60 days of hormone treatment as after 30 days of withdrawal. However, the weight of treated fish fed 43% protein was not significantly different than controls after the withdrawal period although they were heavier than controls after the treatment period. Although a longer withdrawal period as

Figure 7. Change in productive protein value (PPV = PG x 100 /total grams protein fed to fish where PG = $P_f - P_i$, P_f = the final mean whole body protein content of a fish in a tank at the end of a treatment phase, and P_i = the initial mean whole body protein content of a fish in a tank at the beginning of a treatment phase) of juvenile rainbow trout fed varying dietary protein levels after 10 weeks of treatment with 17α -methyltestosterone (MT) (Phase 1) and 8 weeks of hormone free diets (Phase 2). Hormone treated fish were fed 2.0 mg Mt/kg of dry diet for the first 10 weeks and switched to receiving respective hormone free diets for the final 8 weeks of the experiment. Standard error of the mean for each hormone (a) x dietary protein (b) level in a period (c) $(MSe/r)^{1/2}$ is 0.851 based on homogeneous variance for the variance-covariance matrix.

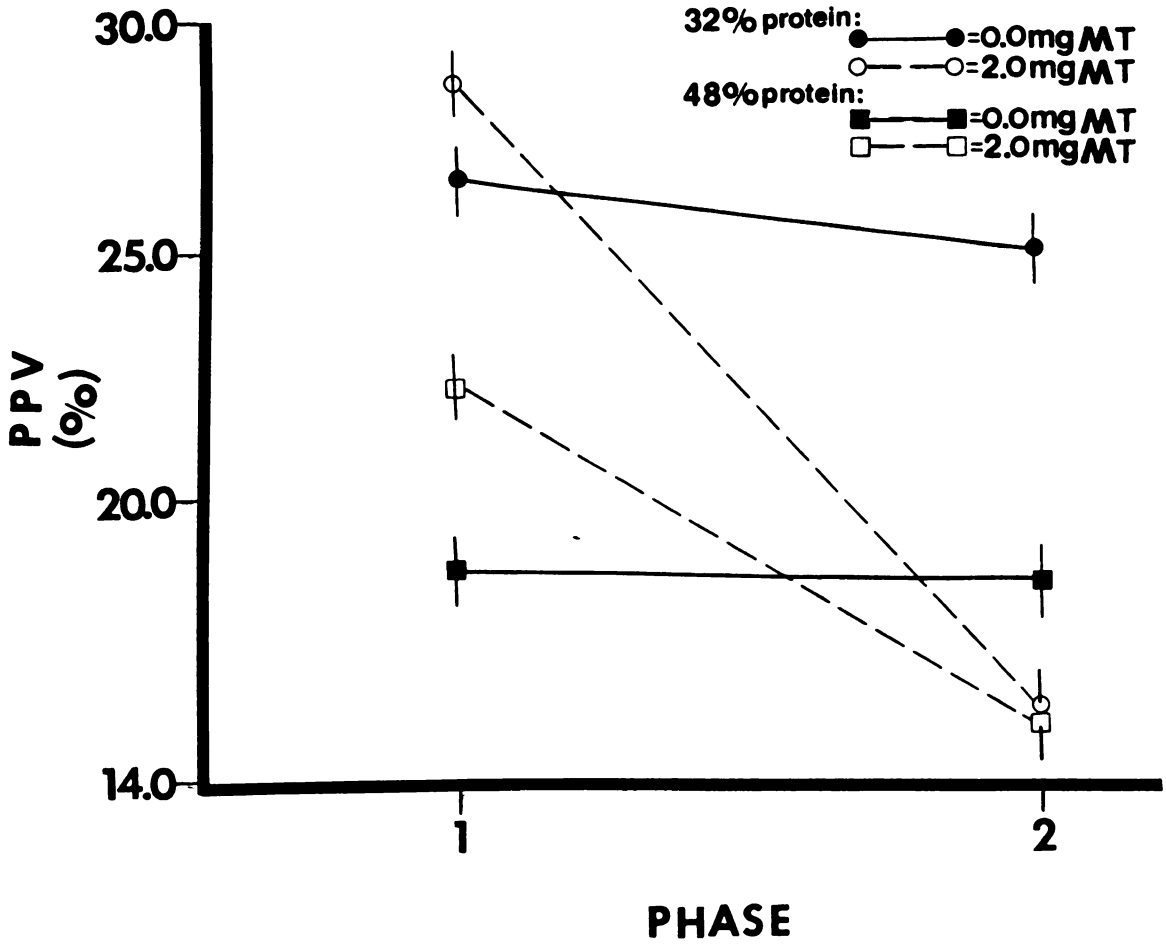
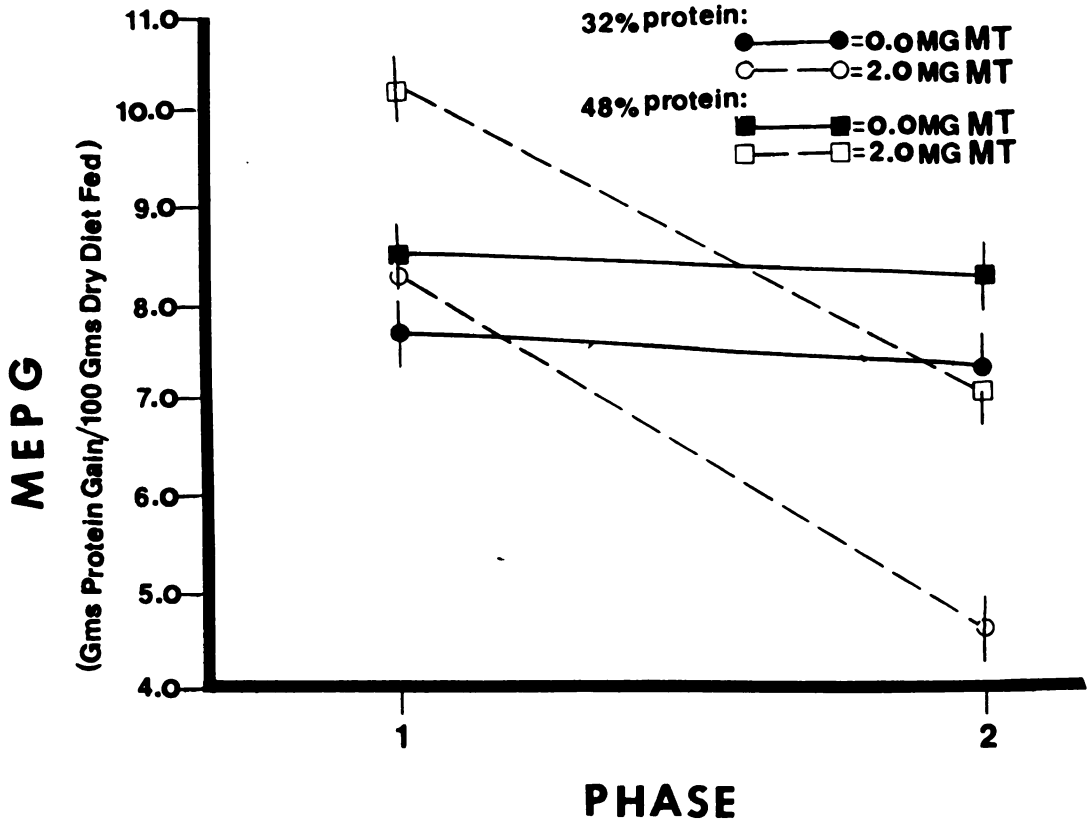

32% protein:
=0.0mg MT
=2.0mg MT
48% protein:
=0.0mg MT
=2.0mg MT
30.0
25.0
20.0
14.0
PPV (%)
1
2
PHASE

Figure 8. Change in maximum efficient protein gain (MEPG = PPV x grams in 100 grams of dry diet fed where PPV = PG/total grams protein x 100 actually fed to fish, PG = $P_f - P_i$, P_f = the final mean whole body protein content of a fish in a tank at the end of a treatment phase, and P_i = the initial mean whole body protein content of a fish in a tank at the beginning of a treatment phase) values of juvenile rainbow trout fed varying dietary protein levels after 10 weeks of treatment with 17^{α}-methyltestosterone (MT) (Phase 1) and 8 weeks of hormone free diets (Phase 2). Hormone treated fish were fed 2.0 mg MT/kg of dry diet for the first 10 weeks and were switched to receiving respective hormone free diets for the final 8 weeks of the experiment. Standard error of the mean for each hormone (a) x dietary protein (b) level in a period (c) $(MSe/r)^{1/2}$ is 0.331 based on homogeneous variance for the variance-covariance matrix.


32% protein:
=0.0 MG MT
=2.0 MG MT
48% protein:
=0.0 MG MT
=2.0 MG MT
MEPG
(Gms Protein Gain/100 Gms Dry Diet Fed)
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
1
2
PHASE

that used in our study could have resulted in decreased percentage weight gains over controls in their fish fed a diet with 32% protein, the difference in the growth response with hormone withdrawal and dietary protein level was different than that which occurred in our study. The possibility exists that the response of trout to anabolic hormone withdrawal is specific to the agent used for promoting growth.

The dramatic drop in efficiency during withdrawal affected the PG of MT treated fish fed 32% protein. These fish gained the same ($P>0.20$) amount of protein in Phase 1 as in Phase 2, while all other fish gained more ($P<0.01$) protein during Phase 2 including MT treated fish fed 48% protein. Initial improvements in gain of MT treated fish fed 32% protein during the treatment phase, however, were enough to prevent ($P>0.10$) any apparent loss in protein gain compared to other fish at the end of the entire 18 weeks.

II. BODY COMPOSITION

A. Hormone Treatment Phase

Steroid treatment promoted an increase in empty carcass (EC) ($P<0.01$) and whole body (WB) ($P<0.10$) fat, WB protein ($P<0.05$) and EC ash ($P<0.10$) contents, and decreased WB ($P<0.05$) and EC ($P<0.01$) water contents of fish (Table 24). In general, the patterns of response to MT treatment in the present study are similar to those observed in fish fed

Table 24. Percent wet weight whole body (WB) and empty carcass (EC) composition of juvenile rainbow trout due to concentration of 17 $^{\alpha}$ -methyltestosterone (MT) and dry matter protein level in the diet during the phase of hormone treatment. Values with different superscripts are significantly different (****P<0.001, ***P<0.01, **P<0.05, *P<0.10) from respective dietary variable values.

		Dietary Variable			
		Mg MT/kg of Diet		% Dietary Protein	
		0.0	2.0	32	48
% WB			**		***
of:	Water	72.66	71.88	71.53	73.01
			**		****
	Protein	13.96	14.51	13.80	14.67
			*		***
	Fat	10.61	11.26	11.88	9.99
	Ash	2.43	2.56	2.48	2.51
% EC			***		*
of:	Water	74.75	73.80	74.00	74.56
					***
	Protein	14.63	14.86	14.39	15.09
			***		***
	Fat	7.23	8.26	8.23	7.27
			*		
	Ash	2.64	2.81	2.67	2.78

1 The empty carcass represents an eviscerated whole body.

2 Standard error of the means for hormone and protein level effects in the whole body are 0.295 for water, 0.200 for protein, 0.335 for fat, and 0.080 for ash, and in the empty carcass are 0.214 for water, 0.228 for protein, 0.145 for fat, and 0.081 for ash based on homogeneous variance.

semipurified diets with exceptions due to the particular P:ME ratio of the diets used. In the semipurified diets tests varying patterns of response due to MT treatment in WB and EC fat and protein composition of trout fed varying P:ME ratio and total ME levels. Despite these patterns, however, it was found that MT treatment only increased EC fat content with a concomitant decrease in EC water content, and was suggested that the increase was related to a movement of visceral fat and not related to an increase or decrease in WB fat content. Indeed, most studies with salmonids have suggested that WB fat content of fish treated with low levels of hormone (0.2 - 5.0 ppm) is decreased (McBride and Fagerlund 1976, Fagerlund and McBride 1977, Fagerlund et al. 1979, Fagerlund et al. 1980) or not changed (Fagerlund and McBride 1975), although high hormone treatment levels (10 ppm) increase WB fat content (Fagerlund and McBride 1975,1977). The present association of increase EC fat with WB fat due to a 2.0 ppm treatment of MT is not in full agreement with previous general observations, although it can be argued that the effects on the WB are minimal based on the level of significance use in the statistical test.

The increase in WB protein can be explained partly from previous observations as a response to a particularly high dietary P:ME ratio. With semipurified rations the WB protein content of MT treated trout fed a dietary P:ME equal to 140 was higher than control fish fed 140 and MT treated

fish fed 100. The increase in WB protein of trout in the present study due to MT treatment was higher ($P<0.05$) for fish fed the diet with 48% protein (P:ME =150) than fish fed the diet with 32% protein (P:ME = 100). The interaction in this study was more important than the effect of the hormone treatment alone; however, it does not explain why MT treated fish fed the lower, 32% protein level diet, also had a higher WB protein content than their respective control group. Fagerlund et al. (1983) found that coho salmon treated with a 1.0 ppm dose of MT and fed a low protein, low lipid diet had higher WB protein levels; but, in general, WB protein (wet weight) of salmonids was not affected by low doses of this steroid (Yu et al. 1979, Fagerlund et al. 1980). Fagerlund et al. (1979) noted an increase in the percentage dry weight of protein content of coho salmon treated with 1.0 ppm MT.

The ash content of an animal carcass is considered to primarily reflect the mineral content of bone. Anabolic steroids have been shown to increase bone mineralization with a concomitant increase in matrix formation in immature male Japanese quail (Takashai et al. 1983). Fagerlund et al. (1983) found an increase in body ash content of coho salmon treated with 1.0 ppm MT and fed a low protein, low lipid diet. In the sempurified test studies it was found that MT treatment promoted a decreased rate of linear bone

growth that accounted for a decreased bone weight percentage in the trout body; but, it was also speculated that the mineral content of carcass bone was actually increased since no difference occurred in WB or EC ash content between control or MT treated fish. The ash content in the semipurified diets used in the previous studies was 4%, while the practical diets used by Fagerlund et al. (1983) averaged over 10% and in the present study averaged over 8% (Table 20). There may be an interaction of MT treatment and dietary ash content on increasing bone mineralization in trout.

An increase in dietary protein content increased the percentage water ($P<0.01$, $P<0.10$) and protein ($P<0.001$, $P<0.01$), and decreased ($P<0.01$) the percentage fat in both WB and EC of fish (Table 25). The changes in WB composition with increasing dietary protein content are in agreement with the observations of others (Lee and Putnam 1973) as well as the parallel of changes in the WB and EC compartments observed in the semipurified diets test studies.

B. Hormone Withdrawal Phase.

Hormone withdrawal negated all positive changes that occurred with treatment except for the effects on EC ash content (Table 25). EC ash content remained high ($P<0.01$) in MT treated fish through the withdrawal phase. It is

Table 25. Percent wet weight whole body (WB) and empty carcass (EC) composition of juvenile rainbow trout due to withdrawal of 17$^{\alpha}$ -methyltestosterone from the diet after 10 weeks of treatment and dry matter protein level in the diet during the phase of withdrawal. Values with different superscripts are significantly different (***P<0.01, *P<0.10) from respective dietary variable values.

		Dietary Variable			
		Mg MT/kg of Diet		% Dietary Protein	
		0.0	2.0	32	48
% WB of:	Water	71.24	70.91	70.74	71.40
	Protein	14.19	14.41	14.21	14.30
	Fat	10.64	10.53	10.92	10.25
	Ash	2.52	2.54	2.58	2.48
% EC of:	Water	73.11	72.84	72.92	73.03
	Protein	15.71	15.45	15.38	15.77 *
	Fat	7.11	7.21 ***	7.42	6.91 ***
	Ash	2.34	2.60	2.65	2.29

1 The empty carcass represents an eviscerated whole body.

2 Standard error of the means for hormone and protein level effects in the whole body are 0.527 for water, 0.251 for protein, 0.593 for fat, and 0.067 for ash, and in the empty carcass are 0.229 for water, 0.292 for protein, 0.308 for fat, and 0.050 for ash.

3 Represents MT treatment level of fish previously fed the hormone. All fish were fed respective control diets during the withdrawal phase.

again not clear whether this effect was due to the absence of the hormone in the diet or to carry over effects of the treatment phase; however, it is apparant that hormone withdrawal has no influence on bone mineral resorption.

Dietary protein level had little effect on WB or EC composition of fish during Phase 2. Only EC fat ($P<0.10$) and ash ($P<0.01$) content were reduced in fish fed diets containing 48% protein. The decrease in fat content is consistent with the results obtained in Phase 1, although the results with ash content were unique. The reason for the general inconsistency in body composition results between phases in not clear.

SUMMARY AND CONCLUSIONS

Limiting dietary ME level limited the anabolic response to hormone treatment of fish fed a dietary P:ME ratio equal to 100 mg protein/kcal ME. The response was enhanced with an increased protein density of the diet. A mechanism for this phenomena was suggested whereby the apparant P:ME ratio of the diet fed to treated fish was functionally reduced as more protein was used to supply additional energy needs to supply the enhanced growth activity stimulated by MT. The extent of this use and subsequently the extent of anabolic response was dependent upon the manitenance of some minimum protein or functional

P:ME ratio. Hormone withdrawal from the diet reversed the improvements in growth and efficiency factors due to treatment to below the level of controls until the advantages gained by treatment were negated. Steroid treatment also increased empty carcass fat content of fish in agreement with earlier observations. Hormone withdrawal, however, also reversed this effect, and fish previously fed the hormone had the same empty carcass fat content as fish continuously fed a hormone free diet.

From these results and results obtained in the optimum diet studies, it is clear that the use of MT treatment to promote maximum efficient rates of protein deposition in juvenile rainbow trout is valid only if minimum dietary P:ME and total ME relationships exist. Dietary ME energy levels below 340 kcal and P:ME ratio below 100 mg protein/kcal ME were probably too limited to support enhanced growth activity. The effects of MT withdrawal from the diet are drastic, especially in fish fed reduced dietary protein levels, and deserve further attention.

FINAL CONCLUSIONS AND SPECULATIONS

The responses in maximum efficient protein deposition obtained in this project suggest two different strategies of trout production that can be obtained through anabolic hormone treatment and dietary protein to energy level manipulations. First, increased growth rates of fish can be obtained with hormone treatment and a small reduction in dietary protein to energy level of presently formulated standard-type diets. Increased growth with a more efficient diet formulation can reduce turnover times of fish and feed costs at commercial aquaculture facilities thus reducing overall production costs and increasing profits. State or federal trout producers interested in the recreational aspects of trout production can benefit from increased growth rates of fish since the survival of a stocked fish is dependent to a large extent upon the size of the fish at stocking. Second, normal growth rates of fish can be maintained with hormone treatment by substantially lowering dietary protein levels. The 1/3 reduction in dietary protein content suggested in this project can substantially reduce feed costs since protein, the most expensive component in feeds, comprises as much as 50% of the total dry ingredients in some salmonid diets. Both commercial and

recreational fish producers interested in maintaining current levels of production can benefit from reduced feed costs. Dietary protein to metabolizable energy (P:ME) manipulations with or without hormone treatment may be used to increase the ratio of muscle to non-muscle growth. This strategy may be important only to the commercial producer; however, it is emphasized that such strategies may not necessarily produce maximum rates of whole body growth or dietary protein utilization.

The reduction in P:ME ratio for optimum growth enhancement or maximum protein efficiency are only valid for diets that contain sufficient ME levels. Diets containing ME levels below 340 kcal appear too limited to produce advantages due to hormone treatment at a dietary P:ME ratio of 100. Protein deposition is limited to the extent that protein is utilized for the additional energy needs of growth and the apparent P:ME of the diet is functionally reduced to a level not conducive to producing advantages of methyltestosterone treatment. Higher dietary P:ME ratios which normally limit the anabolic response produce advantages in gain when energy is limiting presumably by the ability to supply more protein for the energy of the enhanced growth activity before a minimum functional P:ME ratio is reached.

The potential advantages that can be gained with hormone treatment are tempered by the drastic drop in growth

and efficiency values of fish observed with hormone withdrawal. The advantages in growth and efficiency due to treatment are quickly lost. However, the extent of the residual effects are not known since it was not possible to extend the hormone withdrawal study past 18 weeks due to the limitations of our facility. Stress from increased loading factors in our experimental aquaria would have reduced growth rates independent of treatment effects due to deteriorating water quality if the experiment had been continued. The continued negative effects of hormone withdrawal on fish growth must be determined before recommendations on hormone use can be made. A withdrawal phase or extended treatment period is inherent to the strategy of hormone use in juvenile trout since these fish are typically raised for 1 - 3 years at commercial grow-out facilities prior to slaughter. The questions arise as to whether the advantages of hormone treatment last only during the period of treatment and, if so, whether fish can be fed hormones for an extended period of time. If the production goal is to produce fish past a certain critical stage of development (eg. minimum size for maximum stocking survival), then a decrease in growth rate after withdrawal may not be too important unless fish grow at a slower rate for a significant period of time. However, if continuous growth is important and is dependent upon a period of

hormone withdrawal or extended hormone treatment, then it is essential that these factors are examined. Fagerlund et al. (1979b) found that an experimental group of coho salmon previously fed an anabolic dostage of 17$^{\alpha}$ - methyltestosterone (1mg/kg diet) for 7 months as juveniles returned to streams to spawn as 3-year-old adults almost as abundantly as fish in the control group and at a greater rate than hatchery produced fish. The rate of return of precocious males was unchanged. Egg viability and gonad development of progeny of hormone treated fish was normal, although the male to female ratio was decreased from 1.25 to 1.00.

It was apparent that some improvement in dietary protein utilization for growth was at least partly responsible for the increased growth activity in methyltestosterone treated trout. The agreement of the decrease in P:ME needs for optimum growth of trout in this project and the shift in amount of protein and fat (energy ?) absorption with ethylestrenol treatment observed by Ince et al. (1982) is argument for a digestive effect of hormone treatment in trout. The changes noted would, in effect, allow treated fish to absorb protein and energy from a less protein dense diet at a rate similar to untreated fish fed a more protein dense diet. However, such a shift as observed in this project would predict that treated fish fed the less protein dense diet would deposit a similar amount of protein

as untreated fish fed the more protein dense diet. This was not the case as treated fish fed the diet with a P:ME ratio of 100 still deposited more protein than control fish fed the diet containing a P:ME ratio of 120. Hormone mediated effects after absorption apparently occurred. Indeed, these effects may have precluded the digestive role of treatment, and the correlation of changes in optimum protein requirements and shifts in protein and fat absorption may have been coincidental. Since the interaction between hormone treatment and diet composition failed to alter whole body composition of fish, a strictly digestive affecting role of hormone treatment would not fully explain the anabolic activiy of methyltestosterone on trout. Changes in body composition should have occurred in accordance with the altered patterns of absorption. The potential changes in protein utilization after absorption in treated trout do not appear to be related to an improved metabolic efficiency that utilizes body energy stores since fat only redistributed within the body and not removed as a source of energy expenditure to spare protein for growth. This contention is further supported by the observation that appetite is increased in methyltestosterone treated fish (Fagerlund et al. 1979a, Fagerlund et al. 1980, Fagerlund et al. 1983). Improvements in postabsorptive protein utilization of steroid treated trout are probably regulated

in much the same manner as in other steroid treated animals through changes in basal metabolic rates and protein metabolism.

The question of steroid growth promoting activiy, feed intake, and feed efficiency was partially addressed in the initial steroid screening study. In contrast to subsequent dietary studies, gains in growth of fish with methyltestosterone treatment in the screening study were obtained without significant changes in feed conversions which suggests that increased feed intake plays a more important role in enhancing growth of treated trout than increased feed efficiency. A discrepancy in hormone activity between studies was apparent. It is possible that the difference in growth promotion and efficiency due to treatment between the screening and diet studies may be related to the size or age of fish treated with the hormone. In the screening study, growth was not altered in steroid-fed fish until after 6 weeks of treatment when the fish weighed approximately 3 grams (the initial weight of these fish was 0.7 grams). The growth of fish in later studies increased typically after the first 2 weeks of hormone feeding. Fish in the subsequent studies were approximately 4 grams initial weight. Mailson et al. (1985) found a size related effectiveness to anabolic estrogen treatments in perch and suggested that a response to treatment was probably related to the development of appropriate hormone

receptors. A 3 - 5 gram rainbow trout raised near optimum temperatures (12°C) in our lab is approximately 6 - 7 months of age post-egg fertilization (180 - 210 days old). Testosterone secretion does not begin in trout until around 200 days of age (van den Hurk et al. 1982). A correlation between testosterone secretion and development of receptors probably exists. The effectiveness of anabolic methyltestosterone treatment in altering growth on any level is also probably related to the development of these receptors in target tissues. The development of appropriate receptors at the time the trout were 3 - 5 grams would have accounted for the delay in growth promotion observed in the screening study. The effects of methyltestosterone on feeding behavior may be an initial consequence of treatment once the appropriate receptors are developed. Indeed, it is conceivable that methyltestosterone treatment in intact juvenile rainbow trout improves protein depositon and dietary efficiency on three levels: first, on a preabsorptive level by increasing appetite; second, on an absorptive level by increasing protein and decreasing energy assimilation; third, on a postabsorptive level by altering protein and whole body metabolism.

A final speculation on effective hormone treatment is concerned with the response obtained with estradiol-17 in the screening study. Estradiol may be anabolic in trout, although this estrogen decreased the weight gain of fish

when applied alone and decreased the anabolic effectiveness of methyltestosterone when in combinations with this androgen. If attempts to maximize growth in fish by optimizing circulating androgen and estrogen levels is valid as it appears to be in domestic livestock, then the levels of estrogen used in the screening study were probably in excess of growth pormoting levels. Natural, circulating levels of testosterone in adult male and female trout are 10 - 20 times the magnitude of estradiol during spawning and non-spawning periods (Scott and Sumpter 1983); however, the treatment levels of estradiol used in the screening study were equivalent to the levels of androgens used. Indeed, levels of methyltestosterone 10 - 20 times the magnitude of that which promotes growth also decrease growth rates of salmonids (Yamazaki 1976, Johnstone et al. 1978). Lower levels of estradiol may be more appropriate to use than those used in this study.

In an undergraduate research project conducted by Mike Harris (B.S. 1986) the growth rates of juvenile rainbow trout (4 grams initial weight) fed levels of 0.0, 0.05, 0.10, and 0.50 mg estradiol/ kg diet were examined for 8 weeks. Despite the lack of statistically significant differences in growth ($P>0.25$), an approximate trend of increasing average weight gain and relative daily gain with increasing steroid dose appeared evident. The relative

daily gain increased, respectively, from 2.85 to 2.83, 3.01 and 3.11% per day. Average weight gain increased, respectively, from 3.3 to 3.4, 3.4 and 4.2 grams. This trend may suggest that estradiol treatment levels of between 0.50 and 1.0 mg/kg diet may be anabolic in rainbow trout. Low treatment levels of estradiol relative to levels of testosterone are more in accordance with potential anabolic effectiveness of estrogens as it related to circulating levels in the adult trout. If such levels are anabolic, then combinations of androgens and estrogens to promote maximum growth should be reconsidered. Reports of estradiol treatment levels below 1.0 ppm in fish have not been published.

The potential for use of anabolic hormones in salmonid culture is emphasized by the practical application of steroids to large numbers of fish as low level diet supplements, the limited cost of application, and the favorable effects of treatment on growth rates and feed conversions. The results of this project indicate that further advantages of steroid use through reduced dietary protein levels for optimum growth responses can be obtained. However, the effects of hormone withdrawal and extended treatment are necessary to determine if hormones are to be used with juvenile fish. The examination of these factors requires longer length studies necessitating the use of larger rearing units than those used in this project to

compensate for increasing fish size. In addition, the use of other anabolic hormones which have less severe effects upon withdrawal should be examined. Rainbow trout fed a diet containing an anabolic dosage of ethylestrenol for 60 days and then a hormone-free diet for 30 days maintained the growth advantages due to treatment throughout the withdrawal phase (Ince et al. 1983). The mechanisms of hormone growth promoting action in fish are poorly understood and require additional attention.

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