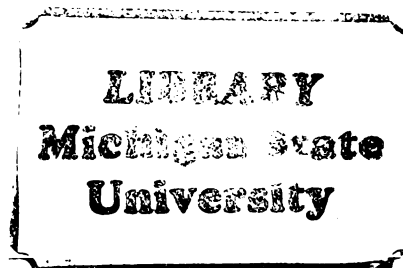




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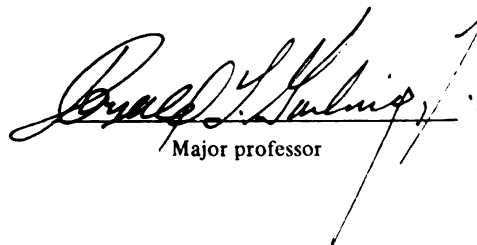


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presented by

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ANABOLIC STEROID SUPPLEMENTATION LEVELS IN
GROWTH PROMOTION OF FINGERLING RAINBOW TROUT

By

Anthony Charles Ostrowski

A THESIS

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ABSTRACT

INTERACTIONS BETWEEN VARYING DIETARY PROTEIN AND ANABOLIC STEROID SUPPLEMENTATION LEVELS IN GROWTH PROMOTION OF FINGERLING RAINBOW TROUT

By

Anthony Charles Ostrowski

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Isocaloric semipurified diets containing varying levels of protein (35, 40, 45%) and norethandrolone (0, 2.5, 5.0, 10.0 mg/kg diet) were fed to fingerling rainbow trout in two separate experiments to examine subsequent dietary interactions in growth promotion. Average weight was not significantly increased ($P > .05$) in fish fed any dietary formulation at the end of Experiment I (13 g trout fed for 10 weeks) or Experiment II (5 g fish fed for 6 weeks). Some decreases ($P < .05$) in average weight and instantaneous daily gain occurred in fish fed the highest dietary protein level in association with the two highest steroid supplement levels during both experiments. Possible explanations for the limited steroid effect are related to low anabolic effectiveness of norethandrolone, dietary inadequacies, or highly potent steroid activity. Considerations for future experiments are discussed.

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A most loving appreciation goes to my parents for their undaunted support and understanding.

I would especially like to dedicate this work to Uncle Tony.

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INTRODUCTION

Rising production costs in intensive salmonid culture have prompted a search for more economical ways to produce fish. In 1981, approximately 60% of the total costs of trout production in the United States were for the purchase of commercial feeds (Dash 1982). These costs can be reduced by promoting more efficient feed and protein utilization. Optimization of dietary protein is essential, since protein is the primary and most expensive component in typical salmonid feeds (NRC 1981).

Enhanced growth rates also can create production advantages, although the primary objectives of rapid growth might differ between commercial and recreational salmonid culture. Commercial production could be improved by reducing turnover time which would increase overall profits. Recreational stock production, especially advanced fingerling production, could be improved by producing larger fish in the same time frame which would increase survival potential. Additionally, growth enhancement could reduce the risk of death or disease normally associated with prolonged holding times of fish raised where seasonal water temperature extremes metabolically lower growth rates (Cowey 1980).

Growth rates and feed efficiencies of domestic livestock are enhanced with two general types of growth promoting agents: 1) those

agents that increase nutrient availability by altering feed digestion and assimilation, and 2) anabolic agents that increase tissue efficiency in the utilization of already absorbed nutrients by altering physiological processes associated with growth (Broome 1980, O'Conner 1980). Their use in terrestrial animal production systems is a generally accepted practice, yet has received limited attention in aquaculture. Of the types examined, the anabolic agents appear particularly promising for use with salmonids (Donaldson et al. 1979).

Research has centered primarily on the use of anabolic steroids because of their practical application to large numbers of fish as low level diet supplements, their limited cost of application (Fagerlund et al. 1980). Steroid treatment also has been proposed as a means to indirectly enhance growth by promoting sex reversal or sterility (Yamazaki 1976, Johnstone et al. 1978) which subsequently channels dietary energy away from maturation and formation of reproductive products and into growth. In general, previous attempts to enhance the growth of salmonids with steroids have focused on determining optimum hormone supplementation of standard diets. Such studies, however, do not indicate the full benefits possible with steroid use.

Standard diet formulations, based on normal optimum growth responses of fish (NRC 1981), might be inadequate in meeting the protein demands for optimum steroid activity. Since effective steroid treatment in mammals appears to alter normal protein metabolism (for reviews see Kruskemper 1968; Kochakian 1976a; Lu and Rendel 1976), similar alterations in protein metabolism can be

expected in steroid treatment of salmonids. Thus, normal dietary requirements for protein might be changed in fish fed steroid-supplemented diets.

Proper manipulation of dietary protein and anabolic steroid supplementation levels might produce additional economic benefits. Improved protein utilization induced by steroid treatment can lower the dietary protein requirements for optimum growth and thus reduce the amount of protein needed in supplemented diets. Higher dietary protein levels coupled with steroid supplementation might increase growth rates and shorten fish turnover time at aquaculture facilities further, and thus outweigh the cost incurred by increasing the dietary protein content. No studies, to date, have observed such dietary protein and steroid interactions in salmonids.

The objectives of this study were:

- 1) To examine the relative effectiveness of varying steroid supplement levels on growth rates and feed efficiencies of juvenile rainbow trout fed experimental semipurified diets supplemented with the synthetic androgen, norethandrolone (17α -ethyl- 17β -hydroxy-19-norandrost-4-en-3-one).
- 2) To examine the interactions between protein intake and norethandrolone supplement levels on the growth, protein utilization, and feed conversions of rainbow trout fingerlings fed experimental diets.

The synthetic androgen, norethandrolone, was chosen for this study since Potts et al. (1976) has shown that norethandrolone

produced greater gains in skeletal muscle growth with fewer deleterious side effects than methyltestosterone (17α -methyl- 17β -hydroxandrost-4-en-3-one), the more widely used steroid in salmonid studies. Norethandrolone has been shown to positively affect the growth of juvenile rainbow trout (Cheema and Matty 1977; Matty and Cheema 1978).

The results gained from this study could ultimately provide trout feed formulators with information on how to maximize protein utilization through steroid supplementation. Maximized protein utilization and subsequent increases in growth rate will reduce production time and costs to commercial aquaculturists and state and federal agencies involved in hatchery production of salmonids.

REVIEW

Use of steroid sex hormones has been proposed to promote the growth of juvenile salmonids both directly by altering physiological processes associated with growth and indirectly by affecting sexual development. The direct stimulation of growth has been observed; but, the advantages of the indirect method are based on supposition. Investigations of indirect methods have not proceeded beyond the inducement of sex reversal or sterility. However, the postponement or elimination of energy needs for gonadal maturation and formation of reproductive products might channel dietary energy normally used for gonadal development into growth. Consequently, reductions in growth rates, feed efficiencies, flesh quality, and increased susceptibility to infections normally associated with the maturation process (Johnstone et al. 1978) might be avoided.

Androgens and estrogens have preferentially affected the sexual development of salmonids when administered during the period of gonadal differentiation. This period normally occurs sometime shortly after yolk sac absorption is complete and during the first acceptance of feed (Padoa 1939; Ashby 1957). Post-gonadogenic treatments of young salmonids have largely affected muscle growth processes; this strictly myotropic response has been loosely termed as the "anabolic"

nature of these compounds. Thus, the growth promoting method investigated has been dependent upon the timing of steroid treatment.

All female stocks resulted when rainbow trout and Atlantic salmon were fed 20 mg/kg diet 17 β -estradiol for minimum periods of 30 and 21 days after first feeding, respectively (Johnstone et al. 1978). Conversely, the sex ratio of rainbow trout was shifted towards an all male population when fed 1 mg/kg diet 17 α -methyltestosterone from 2-25 days after hatching (Yamazaki 1976). Increasing the level of 17 α -methyltestosterone to 3 mg/kg diet fed from first feeding for 90 days produced all male stocks of rainbow trout and Atlantic salmon (Johnstone et al. 1978). Similar results were observed for brook trout (Johnstone et al. 1979a). Johnstone and co-workers (1979b) have developed a technique to produce monosex populations of rainbow trout using the progeny of sex reversed rainbow trout.

High doses of either androgens or estrogens have produced sterile salmonid populations. Methyltestosterone inhibited gonadal development of both sexes when fed at 30 mg/kg diet for 120 days to first feeding Atlantic salmon (Johnstone et al. 1978) and 50 mg/kg diet for five months to newly hatched rainbow trout (Yamazaki 1976). Gametogenic rainbow trout also became sterile after one week when fed 50 or 500 ug/kg methyltestosterone or estradiol at 1% body weight per day (Billard et al. 1982).

Although the production of sex reversed or sterile populations is feasible in culture systems, the advantages of these techniques are unclear. Initial losses in fish growth and elevated death rates due to treatment might outweigh the longterm prospects of added

body growth. Billard et al. (1982) concluded that substantial mortalities would prohibit the high dose treatment of adult fish. Significant losses of young fish also were noted during the production of sterile populations (Johnstone et al. 1978; Yamazaki 1976). Similarly, the process of sex reversal made rainbow trout and Atlantic salmon fry more susceptible to adverse conditions and depressed their growth somewhat (Johnstone et al. 1978). These methods would not benefit fingerling stock production since fish are sold prior to the onset of maturation and the occurrence of any deleterious effects associated with the processes. Indeed, monosex or sterile populations are obviously not desirable if any future reproduction is expected.

In contrast, the use of steroids to directly affect growth processes appears to be a more promising growth promoting technique. Sex steroids and compounds with similar activity have been used extensively in beef production since the 1950s (for reviews see Bird 1976, Lu and Rendel 1976, Scott 1978, Trenkle and Burroughs 1978). However, the anabolic nature of these compounds (Kruskemper 1968, Kochakian 1976, Heitzman 1980) has not been tissue specific, and the proliferation of particular side effects (Trenkle 1969, Signoret 1976, Aschbacher 1978, Trenkle and Burroughs 1978) has been considered detrimental to the health of the animal and quality of the product (Heitzman 1979). Thus androgenic, estrogenic, and progestagenic compounds have been administered at relatively low levels in the final growth phase of animals in attempts to optimize carcass muscle growth and limit the side effects of use (Aschbacher et al.

1975, Trenkle 1976, Aschbacher 1978, Trenkle and Burroughs 1978, Heitzman 1976, 1979, 1980).

Few advantages have been gained with anabolic steroids in the production of most terrestrial monogastric animals (Fowler 1976, Trenkle and Burroughs 1978). The limited, if any increases in muscle deposition or feed efficiencies have not justified changes in present or more effective growth promoting techniques (Fowler 1976, O'Connor 1980).

Similarly, estrogens have not been effective growth promoters of juvenile salmonids. 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 1975, 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 controls and treated fish approximately 5 months after cessation of treatment; this suggested a compensatory response to inhibited growth caused by the estrogen. In contrast, estradiol 17- β 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. Indeed, observed rates of muscle protein synthesis in rainbow trout after exogenous steroid treatment (Cheema and Matty 1977, Matty and Cheema 1978) implies that only androgens are myotropic in salmonids.

Most androgens examined have improved the growth rates and weight gains of juvenile salmonids. Only methalone and chlorotestosterone acetate proved ineffective in rainbow trout (Matty 1975) and coho salmon (McBride and Fagerlund 1976), respectively. However, chlorotestosterone acetate increased weight gains of one year old

rainbow trout (Hirose and Hybia 1968). Norethandrolone (Cheema and Matty 1977, Matty and Cheema 1978), ethylestrenol (Simpson 1976), and dimethazine (Matty 1975, Cheema and Matty 1977, Matty and Cheema 1978) produced favorable growth responses of rainbow trout. The growth rates of coho salmon parr were increased with testosterone (McBride and Fagerlund 1976, Yu et al. 1979) and 11-ketotestosterone (McBride and Fagerlund 1976), two naturally occurring salmonid androgens (Ozon 1972), and oxymethalone (McBride and Fagerlund 1976). The most widely studied androgen, 17- α methyltestosterone, significantly increased weight gains of coho salmon (McBride and Fagerlund, 1973, 1976; Fagerlund et al. 1980, Higgs et al. 1977, Yu et al. 1979), chinook salmon (McBride and Fagerlund 1973), kokanee (Yamazaki 1976), steelhead trout and pink salmon (Fagerlund and McBride 1977). Comparative studies with 17- α methyltestosterone have indicated that relative growth increases are dependent upon treatment dose.

In general, increased doses of 17- α methyltestosterone produced progressively greater growth responses in treated fish. However, additional advantages in growth were not observed with treatment levels exceeding 10 mg/kg diet in coho salmon (McBride and Fagerlund 1973) or levels between 1 and 5 mg/kg diet in steelhead trout (Fagerlund and McBride 1977). Progressive percent weight increases over controls were 22.8, 24.1, and 32.9 for pink (Fagerlund and McBride 1977) and 4.1, 71.0, and 125.0 for coho salmon (Fagerlund and McBride 1975) when fed levels of 0.2, 1.0, and 10.0 ppm for 44 days and 57 weeks, respectively. Diet treatments of 10 mg/kg also produced significantly greater growth responses than 1 mg/kg diet

treatments in coho salmon after 42 days (McBride and Fagerlund 1973) and 32-34 weeks (McBride and Fagerlund 1976). Similar results were observed for 11-ketotestosterone, testosterone, and oxymethalone after 32-34 weeks (McBride and Fagerlund 1976). Although relatively greater growth responses have been obtained, the occurrence of associated androgenic side effects also have increased with higher treatment doses.

Numerous side effects have been noted with 10 mg/kg treatments of 17- α methyltestosterone, 11-ketotestosterone, testosterone, and oxymethalone. External alterations of fish included a marked thickening (McBride and Fagerlund 1973) and dulling (Fagerlund and McBride 1975) of the skin, a yellow tinting of the fins (Fagerlund 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).

In contrast, androgenic changes have been slight or not significant at levels ranging between 0.2 and 5.0 mg/kg diet. Some alterations in head shape and skin color have been noted in a few fish (Fagerlund and McBride 1977) although skin thickness was not affected (McBride and Fagerlund 1973). No significant changes were observed in exocrine pancreas (Higgs et al. 1977), liver, heart, and kidney (Yu et al. 1979); however, interrenal, thyroid, and endocrine pancreas tissue size were increased (Higgs et al. 1977). 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 dose ranges, the intensity of these side effects has increased with increased duration of treatment.

As in domestic livestock production the presence of side effects has fostered concern for steroid use. Changes in overall body composition might be detrimental to the quality of fish produced for food; the appearance, survival, and reproductive success of stock fish might be affected adversely (Fagerlund and McBride 1977). Permissible levels of residual steroid metabolite levels in edible tissues also have received attention (Fagerlund and McBride 1978, Fagerlund and Dye 1979). The limitation of steroidal side effects has been a goal in salmonid research, however, a dilemma persists in simultaneously attempting to obtain the full anabolic benefit of steroid use.

Few studies have investigated possibilities of limiting androgenic side effects beyond the manipulation of dose or duration of steroid treatment. Steroid withdrawal after 10 mg/kg diet treatment was effective in slowing down subsequent side effects in coho salmon (Fagerlund and McBride 1975). However, growth rates also declined until there was no significant difference in weight between treated

fish and fish maintained on a control diet for the entire test period. Fagerlund and McBride (1977) noted that the growth rates of coho salmon maintained at 16.5°C treated with 0.2 and 1.0 mg/kg diet methyltestosterone, respectively, were correspondingly increased; but after 269 days of treatment, the group which received 0.2 mg/kg diet at 16.5°C weighed 231% more than the 11.5°C control group. Only minor testicular changes were noted.

Other studies (Matty 1975, McBride and Fagerlund 1976, Cheema and Matty 1977, Matty and Cheema 1978) have obtained positive growth responses with other synthetically produced androgens possessing myotropic/androgenic ratios (Herschberger et al. 1953) more favorable than methyltestosterone (Potts et al. 1976). More myotropically active compounds have produced greater muscle growth responses in relation to their side effects in mammals (Potts et al. 1976, Trenkle and Burroughs 1978). Their use in salmonid culture would appear promising but has not received wide attention.

Manipulation of dietary protein levels in steroid-supplemented diets should be desirable. Protein appears to be an important dietary component mediating the anabolic response, since effective androgen treatment has stimulated enhanced nitrogen retention from the diet in mammals (Kruskemper 1968, Kochakian 1976a, Heitzman 1980). In steroid-treated rats, this nitrogen retaining effect has increased with increasing dietary protein content, however, only until an "adequate" level for growth was reached (Kochkian 1950, Kochakian

and Van der Mark 1952, Wright and Kochakian 1953). Defining adequate dietary protein levels for steroid mediated growth of salmonids is feasible, since standard diets are empirically formulated based on normal optimum growth responses (NRC 1981).

Vander Wal (1976) fed estrogen treated and untreated rations of 16, 18, and 20% crude protein to respective groups of 11 week old calves. After seven weeks, weight gains of the treated groups progressed with increasing protein content of the diet and all treated groups had higher live weight gains than all untreated groups. The best overall feed efficiency was obtained with the steroid supplemented diet containing the lowest protein level. He concluded that weight gains comparable to untreated animals fed optimal protein levels could be obtained in animals fed steroid supplemented diets at levels 25% below those protein levels considered optimum.

Evidence of further enhanced steroid mediated growth with increases in dietary protein level have been inconclusive. In lambs, growth has been enhanced through protein-energy interactions in some studies (Preston and Burroughs 1985), but not in others (Jones and Hogue 1960). Significant interactions were not observed between protein, energy, and stilbestrol treatment in steer calves (Klosterman et al. 1954). Baker et al. (1967) indicated a sex dependent increase in the rate of gain with increased protein level of treated swine rations; however, similar results were not obtained in the studies of Binder and co-workers (1972). Inconsistencies among type and level of dietary protein used, and differences in protein/energy ratios of

diets between studies could have accounted for the discrepancies. However, in general, higher dietary protein levels without increases in energy levels have resulted in increased carcass tissue leanness.

MATERIALS AND METHODS

I. General

Two laboratory feeding experiments were conducted. In Experiment I, semi-purified diets (Tables 1, 2, 3) containing either 35, 40, or 45% protein supplemented with 0.0, 2.5, 5.0 or 10.0 milligrams norethandrolone per kilogram of diet (mg/kg diet) were fed to triplicate groups of fingerling rainbow trout (15 ± 2 , total group weight approximately 200 gm) for a 10 week period. In Experiment II, triplicate groups of smaller fingerlings (20 ± 2 , total group weight approximately 100 gms) were fed either the 35 or 45% protein semi-purified diet supplemented with norethandrolone at 0.0 and 10.0 mg/kg diet for 6 weeks.

Fish were maintained in 110 liter flow-through aquaria with supplemental aeration and a continuous well water supply. Water temperature was a constant 12°C and flow was set at approximately 1 liter/minute. Lighting was supplied by overhead fluorescent lamps set on a 14:10, light:dark regime.

II. Experimental Diet Preparation

A. Steroid Stock Solution

A 1 mg/gm steroid stock solution was prepared by dissolving 0.2 grams norethandrolone in 200.0 grams soybean oil. Stirring and mild

Table 1. Percent composition and P/E ratio of experimental diets on a dry weight basis.

Protein level (%)	35	40	45
Ingredient (% of diet):			
Casein	28.78	32.79	36.88
Gelatin	12.40	14.13	15.90
Dextrin	33.89	28.15	22.29
Alpha-cellulose	11.48	11.48	11.48
Vitamin mix ¹	0.55	0.55	0.55
Mineral mix ²	4.00	4.00	4.00
Choline chloride	0.45	0.45	0.45
Soybean oil	6.45	6.45	6.45
Cod liver oil	2.00	2.00	2.00
P/E ratio ³	109	125	140

¹For composition see Table 2.

²For composition see Table 3.

³ $\frac{\text{mg protein}}{\text{kcal of diet}}$; kcal of diet determined empirically using physiological fuel values of 4, 4, and 9 for protein, carbohydrate and lipid, respectively.

Table 2. 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		

^aThese quantities added to alphacellulose to make 1 gram

^bMenadione dimethylpyrimidinol bisulfite

^cButylated hydroxytoluene (BHT) and/or ethoxyquin

Table 3. Mineral mixture for use in purified fish diets (NRC 1978).

Mineral	g/kg premix
$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	366.046
CaCO_3	261.714
KH_2PO_4	176.834
NaCl	106.100
MgSO_4	53.050
KCl	17.683
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	8.842
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	6.189
ZnCO_3	2.653
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.531
KIO_3	0.177
$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	0.147
CoCl_2	0.030
Na_2SeO_3	0.004

heating was applied to facilitate mixing. This solution was refrigerated at 10°C and was remixed before use.

B. Diet Preparation

Twelve isocaloric semi-purified test diets were prepared varying in protein and steroid content (Tables 1, 2, 3). Percent composition of dietary ingredients was based on the standard NRC (1978) test diet for use with coldwater fishes. Physiological fuel values of 4, 4, 9 for protein, carbohydrate, and fat, respectively, were used to estimate metabolizable energy content (Pike and Brown 1967). As protein levels were increased, equivalent amounts of dextrin were decreased. The steroid stock solution also replaced an equivalent amount of the soy oil component to establish levels of 2.5, 5.0, or 10.0 milligrams norethandrolone per kilogram diet.

All dry ingredients were mixed in an industrial food mixer (Univex Model M-12B) for twenty minutes. A mixture of soybean oil, cod liver oil, and steroid stock solution (if needed) was added slowly to ensure complete homogeneity and mixed for 15 minutes. Finally, warm water (50-60°C) was slowly added with mixing until the diet clumped to a dough-like consistency.

The dough-like material was passed through a hand driven meat grinder forming a spaghetti-like product which was dried in a forced air drying oven (without supplemental heating) for 24 hours, cut in a Waring blender, and passed through a U.S. Number 6 sieve yielding 3.35-2.80 millimeter pellets. The pellets were then placed in labeled plastic food containers and refrigerated at 10°C until used. Dry matter composition was determined on all diets; water content averaged $12.9 \pm 2.4\%$.

III. Experimental Procedure

A. Initial Conditioning Period

An initial two week conditioning-training period was conducted prior to each experiment in order to curb any spurious responses to steroid treatment due to varying body energy reserves (Kochakian 1953) and to acclimate fish to the experimental environment and feed. During the first week, all fish were maintained in a 1200 ℓ fiberglass holding tank and fed a commercial diet (Martin Feeds) on a wet matter basis equalling 3.0% wet body weight/day separated into two equal feedings per day. After this conditioning period, 25 randomly chosen fish were transferred to each experimental tank.

The second week was designed to train fingerlings to accept the experimental diets. Fish were to be fed a decreasing ratio of commercial: semi-purified (35% protein reference diet) feed; beginning with a 100:0 ratio, the commercial feed content was to be decreased and semipurified diet correspondingly increased by 25% every two days. However, in Experiment I, fish accepted the semi-purified diet so readily that all tanks were switched to feeding 100% semi-purified diet by the third day of training. Subsequently, in Experiment II, fish were fed 100% semi-purified diet on the first day of training. The training period in both experiments was maintained for the full week to fully acclimate fish to the environment. After this period the number of fish was reduced in each tank to obtain day 0 weight of fish.

B. Weighing and Feeding Level Adjustment

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, drained into the container as above, and the 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 then treated with 2 parts per million Acriflavine (immediately after all fish were weighed) to reduce the chance of bacterial infection.

Fish were fed a dry weight of experimental feed equalling 3.0% of the total wet body weight per day per tank equally divided into a morning (1000-1100 hrs.) and evening (1600-1700 hrs.) feeding.

Feeding levels in both experiments were adjusted every 2 weeks (14 days). Fish were fed the first 13 days; they were weighed and not fed on day 14 in order to eliminate false readings due to gastrointestinal fill (Meyer and Garrett 1967). The following day began the next feeding period. Rates were also adjusted when deaths occurred by subtracting an expected average dry matter amount of feed eaten/dead fish from the initial calculated feeding rate for that

particular group during the remaining feeding period. Fish were not replaced when deaths occurred.

The first feeding period in Experiment I lasted 17 days. Fish were initially fed at 4.5% wet body weight per day for the first three days and at an adjusted 3.0% rate for the following 13 days since fish failed to consume all feed when fed at the higher rate. Fish were weighed and not fed on day 17. Subsequent feeding periods lasted the normal 14 day period.

IV. Data

Total live wet weight of fish per tank was recorded. The average wet weight/individual/tank was calculated (i.e. total live wet weight per tank/total number of live fish). All weights were determined to the nearest 0.5 grams, and used to adjust daily feeding rates for the following feeding period (i.e. total live wet weight x .03 x $\frac{\text{wet weight diet}}{\text{dry weight diet}}$). Feeding rates were readjusted for mortalities within a period by assuming the prior calculated average weight/individual for each death.

Instantaneous Daily Gain (IDG) was calculated after each feeding period using the average weight/individual/tank:

$$\text{IDG} = \frac{(\log e (\text{final weight}/\text{initial weight}))}{\text{number of days in feeding period}} \times 100$$

(Dean 1982).

Feed conversions (total dry matter fed/total wet weight gain) were calculated only for live fish at the end of each experiment. An assumed dry matter amount of feed eaten by fish which died during a feeding period (i.e., wet weight dead fish x .03 x number of days alive in the feeding period x $\frac{\text{dry weight diet fed}}{\text{wet weight diet fed}}$) was subtracted from the

total dry matter amount eaten and not calculated into the feed conversion ratio. Protein efficiency ratio (grams wet weight gain/total dry matter protein fed) was similarly calculated.

V. Statistical Analysis

A two-way analysis of variance was performed to test for interactions (between all combinations of protein level and steroid supplement levels) and treatment effects. A Type I probability of error of 0.05 or less ($P \leq 0.05$) was considered statistically significant. When differences occurred, comparisons were made using Duncan's New Multiple Range Test (Duncan 1955).

RESULTS

I. General

The combined effect of dietary protein and steroid supplement level produced no significant interactions ($P > 0.05$) between average weight/fish, protein efficiency ratio (PER), or feed conversion (FC). Similarly, this effect did not alter instantaneous daily gain (IDG) of fingerlings in Experiment I, but produced significant differences in this variable in the final growth phase (day 28-42) of fish in Experiment II. Significant differences ($P < 0.05$) were observed between growth characteristics and dietary protein levels containing equivalent steroid supplement levels, and between characteristics and steroid supplement levels of diets containing equivalent protein levels.

Fish health and good environmental tank conditions were maintained throughout each experiment. Any uneaten feed or feces that accumulated were removed weekly and had no detrimental effect on water clarity. Fish readily accepted feed, appeared lively, and exhibited no external signs of disease or infection, nor any change in body condition or color associated with steroid diet supplements (Fagerlund and McBride 1975, 1977; McBride and Fagerlund 1973, 1976) throughout the experiments. Total mortalities of fish were low in Experiment I

(< 1.0%) and Experiment II (7.7%). Fish deaths were independent of type of diet eaten in both experiments.

II. Experiment I

A. Average Weight/Fish and Instantaneous Daily Gain

Significant differences ($P < .05$) in IDG and average weight/fish were observed only between dietary protein levels supplemented with 5.0 and 10.0 mg/kg diet norethandrolone (Tables 4, 5, 6, 7). The IDG of fingerlings decreased during the initial growth phase (day 0-17) as dietary protein levels increased from 35 to 45% at both supplement levels (Tables 6, 7), and as protein levels increased from 40 to 45% at only the 5.0 mg/kg level (Table 6). A compensatory increase in IDG was observed in fish fed the 45% protein level diet containing 10.0 mg/kg diet norethandrolone (Table 7) during the subsequent period (day 17-31). The final average weight/fish decreased only as protein levels were increased from 40 to 45% at the 5.0 mg/kg diet supplement level (Table 6).

An increased steroid level (10.0 mg/kg diet) produced a significantly lower ($P < 0.05$) IDG at day 45-59 of fish fed the 40% protein level diet (Table 7). No other growth differences due solely to steroid treatment were observed.

B. Feed Conversion (FC) and Protein Efficiency Ratio (PER)

The FC of fingerlings differed ($P < .05$) between dietary protein level only among diets supplemented with 5.0 mg/kg diet norethandrolone (Table 8). FC was lowered in fish fed diets containing 40% protein while no differences existed between diets containing 35% or 45% protein.

Table 4. Mean average weight/fish¹ and instantaneous daily gain² of replicate groups (n=3) of rainbow trout fingerlings fed semipurified reference diets (without steroid supplementation) used in Experiment I.

Protein level (%)		35		40		45	
Day	0	$\bar{W} \pm (SD)$	$\frac{IDG \pm (SD)}{\bar{W} \pm (SD)}$	$\bar{W} \pm (SD)$	$\frac{IDG \pm (SD)}{\bar{W} \pm (SD)}$	$\bar{W} \pm (SD)$	$\frac{IDG \pm (SD)}{\bar{W} \pm (SD)}$
	0	13.2 (0.2)		13.2 (0.3)		13.4 (0.8)	
	17	20.5 (1.5)	2.58 (0.43)	21.2 (0.6)	2.77 (0.24)	17.4 (3.2)	1.47 (0.88)
	31	23.1 (2.2)	0.84 (0.37)	24.1 (1.3)	0.94 (0.29)	20.9 (3.7)	1.34 (0.34)
	45	29.0 (3.2)	1.62 (0.11)	30.1 (0.4)	1.59 (0.47)	26.7 (4.3)	1.75 (0.15)
	59	37.4 (5.1)	1.82 (0.20)	37.9 (0.8)	1.65 (0.20)	33.1 (3.3)	1.51 (0.29)
	73	44.4 (5.8)	1.22 (0.27)	46.7 (4.1)	1.47 (0.48)	38.7 (9.3)	1.06 (0.49)

¹ Average weight/fish ($\bar{W}$) = $\frac{\text{total live wet weight gain (gms)}}{\text{total number of live fish}}$

² Instantaneous daily gain (IDG) = $\frac{(\log e (\text{final weight}/\text{initial weight}))}{\text{number of days in feeding period}} \times 100$

Table 5. Mean average weight/fish¹ and instantaneous daily gain² of replicate groups (n=3) of rainbow trout fingerlings fed semipurified diets supplemented with 2.5 mg/kg diet norethandrolone used in Experiment I.

Protein level (%)		35	40	45
Day	0	$\overline{W} \pm (SD)$	$\overline{W} \pm (SD)$	$\overline{W} \pm (SD)$
	0	13.7 (0.5)	13.5 (0.9)	14.2 (0.7)
	17	20.1 (0.4)	20.5 (1.7)	20.3 (0.7)
	31	24.7 (0.7)	24.2 (2.7)	25.5 (1.7)
	45	31.0 (0.6)	30.3 (3.7)	31.1 (0.3)
	59	37.4 (2.0)	38.6 (5.4)	39.5 (2.0)
	73	45.0 (4.3)	47.1 (7.1)	48.4 (3.1)
			</	

¹Average weight/fish ($\bar{W}$) = $\frac{\text{total live wet weight gain (gms)}}{\text{total number of live fish}}$

²Instantaneous daily gain (IDG) = $\frac{(\log e (\text{final weight/initial weight}))}{\text{number of days in feeding period}} \times 100$

Table 6. Mean average weight/fish¹ and instantaneous daily gain² of replicate groups (n=3) of rainbow trout fingerlings fed semipurified diets supplemented with 5.0 mg/kg diet norethandrolone used in Experiment I.

Protein level (%)		35		40		45	
Day ³		<u>$\bar{W} \pm (SD)$</u>	<u>IDG $\pm$ (SD)</u>	<u>$\bar{W} \pm (SD)$</u>	<u>IDG $\pm$ (SD)</u>	<u>$\bar{W} \pm (SD)$</u>	<u>IDG $\pm$ (SD)</u>
Day ³	0	13.0 (0.4)		13.2 (0.1)		13.5 (0.6)	
	17	20.2 (1.2)	2.59 (0.36) ^a	20.1 (0.6)	2.48 (0.23) ^a	17.8 (1.9)	1.63 (0.41) ^b
	31	24.4 (0.2)	1.36 (0.37)	23.6 (1.0)	1.13 (0.30)	21.9 (3.2)	1.45 (0.26)
	45	30.4 (0.6)	1.59 (0.07)	28.4 (0.5)	1.31 (0.27)	27.6 (3.7)	1.65 (0.33)
	59	37.4 (2.0)	1.19 (0.24)	35.6 (0.7)	1.61 (0.24)	32.4 (4.1)	1.16 (0.41)
	73	40.1 (2.6) ^{a,b}	0.77 (0.38)	44.3 (1.7) ^a	1.56 (0.17)	36.6 (2.0) ^b	0.90 (0.79)

¹Average weight/fish ($\bar{W}$) = $\frac{\text{total live wet weight gain (gms)}}{\text{total number of live fish}}$

²Instantaneous daily gain (IDG) = $\frac{(\log e (\text{final weight/initial weight}))}{\text{number of days in feeding period}} \times 100$

³Significant differences (P < .05) between values across columns denoted by differing superscript letters.

Table 7. Mean average weight/fish¹ and instantaneous daily gain² of replicate groups (n=3) of rainbow trout fingerlings fed semipurified diets supplemented with 10.0 mg/kg diet norethandrolone used in Experiment I.

Protein level (%)	35		40		45	
	$\bar{W} \pm (SD)$	IDG $\pm$ (SD)	$\bar{W} \pm (SD)$	IDG $\pm$ (SD)	$\bar{W} \pm (SD)$	IDG $\pm$ (SD)
Day						
0	13.6 (0.5)		13.6 (0.3)		13.6 (0.9)	
17	20.7 (0.7)	2.46 (0.08) ^{a3}	19.9 (0.2)	2.26 (0.09) ^{a,b}	19.4 (0.9)	2.10 (0.01) ^b
31	24.6 (1.3)	1.25 (0.22) ^a	23.5 (1.1)	1.18 (0.29) ^a	24.7 (0.8)	1.71 (0.12) ^b
45	30.5 (1.7)	1.52 (0.12)	28.5 (2.7)	1.34 (0.36)	31.2 (1.4)	1.68 (0.11)
59	37.2 (3.8)	1.42 (0.36)	33.1 (3.3)	1.08 (0.21) ^{*4}	37.4 (2.2)	1.29 (0.15)
73	44.4 (5.1)	1.25 (0.13)	38.4 (4.6)	1.04 (0.17)	43.2 (2.8)	1.02 (0.26)

¹ Average weight/fish ($\bar{W}$) = $\frac{\text{total live wet weight gain (gms)}}{\text{total number of live fish}}$

² Instantaneous daily gain (IDG) = $\frac{(\log e (\text{final weight/initial weight}))}{\text{number of days in feeding period}} \times 100$

³ Significant differences (P < .05) between values across columns denoted by differing superscript letters.

⁴ Asterick (*) denotes significant difference (P < 0.5) due to steroid treatment between values at the 40% protein level in Tables (4,5,6,7,) representing this feeding period.

Table 8. Mean feed conversions¹ of replicate groups (n=3) of rainbow trout fingerlings fed semipurified diets used in Experiment I.

Protein level (%)	35	40	45
Steroid level (mg/kg)	<u>FC ± (SD)</u>	<u>FC ± (SD)</u>	<u>FC ± (SD)</u>
0.0	1.61 (0.15)	1.53 (0.20)	1.88 (0.43)
2.5	1.66 (0.21)	1.53 (0.13)	1.54 (0.09)
5.0	1.87 (0.09) ^{a2}	1.58 (0.08) ^b	1.98 (0.15) ^a
10.0	1.67 (0.16)	1.98 (0.25)	1.72 (0.07)

¹Feed conversion (FC) = $\frac{\text{total wet weight gain}}{\text{total dry weight feed fed}}$

²Significant differences (P < .05) between values across columns denoted by differing superscript letters.

The PER of fish fed diets containing 35% protein was greater ($P < .05$) than fish fed diets containing 45% protein at all steroid supplement levels except 2.5 mg/kg diet (Table 9). No significant differences in PER were observed in fish fed all diets supplemented with 2.5 mg/kg diet norethandrolone.

Significant differences ($P < .05$) in PER of fish fed diets containing 40% protein were variable. The PER of the reference group (fish fed at 0.0 mg/kg diet) was not different from fish fed either the 35 or 45% protein level reference diets. At 5.0 mg/kg diets, however, the PER was higher than those fish fed 45% protein, yet still no different from those fish fed 35% protein. As the supplement level reached 10.0 mg/kg diet, the PER of fish fed the 40% protein diet was similar to those fish fed 45% protein, but was now significantly lower than fish fed 35% protein.

A difference ($P < .05$) between dietary steroid concentration and PER was observed only in diets containing 40% protein. The 10.0 mg/kg diet level significantly lowered the PER of fish; all other steroid supplement levels did not produce significant differences in PER within this diet.

III. Experiment II

A. Average Weight/Fish and Instantaneous Daily Growth

IDG decreased ($P < .05$) in the final growth phase of fish (days 28-42, Table 10) due to the combined effect of increased dietary protein and steroid concentration to 45% and 10.0 mg/kg diet respectively. However, this decrease did not result in differences in average weight/fish at the end of the experimental period (Table 11).

Table 9. Mean protein efficiency ratios¹ of replicate groups (n=3) of rainbow trout fingerlings fed semipurified diets used in Experiment I.

Protein level (%)	35	40	45
Steroid level (mg/kg)	<u>PER ± (SD)</u>	<u>PER ± (SD)</u>	<u>PER ± (SD)</u>
0.0	1.79 (0.17) ^a	1.64 (0.20) ^{a,b}	1.23 (0.26) ^b
2.5	1.74 (0.22)	1.63 (0.13)	1.45 (0.09)
5.0	1.53 (0.07) ^a	1.59 (0.09) ^a	1.13 (0.09) ^b
10.0	1.75 (0.18) ^a	1.28 (0.15) ^{b*} ³	1.29 (0.05) ^b

¹Protein efficiency ratio (PER) = $\frac{\text{total wet weight gain}}{\text{total dry weight protein fed}}$

²Significant differences (P < .05) between values across columns denoted by differing superscript letters.

³Asterisk denotes significant difference (P < .05) due to steroid treatment between values down column.

Table 10. Mean instantaneous daily gain¹ of replicate groups (n=3) of rainbow trout fingerlings fed semipurified diets used in Experiment II.

Day	Diet Composition ²			
	35:0.0	35:10.0	45:0.0	45:10.0
	<u>IDG ± (SD)</u>	<u>IDG ± (SD)</u>	<u>IDG ± (SD)</u>	<u>IDG ± (SD)</u>
0				
17	1.24 (0.32)	1.24 (0.18)	1.23 (0.28)	1.26 (0.14)
28	1.53 (0.21)	2.13 (0.49)	2.04 (0.07)	2.28 (0.60)
42	1.83 (0.08) ^{a,b} ³	2.05 (0.12) ^a	1.99 (0.14) ^{a,b}	1.68 (0.12) ^b

¹Instantaneous daily gain (IDG) = $\frac{(\log e \text{ (final weight/initial weight)})}{\text{number of days in feeding period}} \times 100$

²Protein level (%):steroid supplement level (mg/kg)

³Significant differences (P < .05) between values across columns denoted by differing superscript letters.

Table 11. Mean average weight/fish¹ of replicate groups (n=3) of rainbow trout fingerlings fed semi-purified diets used in Experiment II.

Diet Composition ²					
	35:0.0	35:10.0	45:0.0	45:10.0	
	<u>$\bar{W} \pm (SD)$</u>	<u>$\bar{W} \pm (SD)$</u>	<u>$\bar{W} \pm (SD)$</u>	<u>$\bar{W} \pm (SD)$</u>	
Day	0	5.4 (0.3)	5.6 (0.3)	5.5 (0.5)	5.4 (0.3)
	14	6.4 (0.6)	6.7 (0.4)	6.6 (0.4)	6.4 (0.3)
	28	8.1 (0.7)	9.1 (1.1)	8.7 (0.5)	8.8 (1.0)
	42	10.3 (1.0)	12.1 (1.7)	11.5 (0.6)	11.2 (1.2)

¹Average weight/fish ($\bar{W}$) = $\frac{\text{total live wet weight gain (gms)}}{\text{total number of live fish}}$

²Protein level (%):steroid supplement level (mg/kg)

B. Feed Conversion (FC) and Protein Efficiency Ratio (PER)

No significant interactions ($P < .05$) were observed in FC (Table 12).

The PER of fish fed the 35% protein diet was significantly greater ($P < .05$) than those fed the 45% protein diet at the 10.0 mg/kg diet steroid supplement level (Table 12).

Table 12. Mean feed conversions¹ and protein efficiency ratios² of replicate groups (n=3) of rainbow trout fingerlings fed semipurified diets used in Experiment II.

Diet Composition	Feed Conversion	Protein Efficiency ratio
	<u>FC ± (SD)</u>	<u>PER ± (SD)</u>
35: 0.0 ³	1.79 (0.30)	1.63 (0.30) ^{a,b} ⁴
35:10.0	1.44 (0.20)	2.02 (0.29) ^a
45: 0.0	1.59 (0.20)	1.51 (0.09) ^{a,b}
45:10.0	1.61 (0.17)	1.39 (0.15) ^b

¹Feed conversion (FC) = $\frac{\text{total wet weight gain}}{\text{total dry weight feed fed}}$

²Protein efficiency ratio (PER) = $\frac{\text{total wet weight gain}}{\text{total dry weight protein fed}}$

³Protein level (%):steroid supplement level (mg/kg)

⁴Significant differences (P < .05) between values down column denoted by differing superscript letters.

DISCUSSION

The absence of any apparent steroid anabolic activity in either experiment was unexpected. Previous studies (Cheema and Matty 1977, Matty and Cheema 1978) have shown that dietary norethandrolone supplementation, at similar levels used in this study, increased the growth rates of fingerling rainbow trout. Failure to confirm this activity lead to a reevaluation of the results presented by Cheema and Matty (1977) and Matty and Cheema (1978). Recalculation of their data revealed that percent weight increases over controls were much lower than reported. Consequently, the statistical significance of their findings and the anabolic effectiveness of norethandrolone in salmonids is questionable. Kochakian (1976b) has indicated that growth responses to androgen treatment can vary depending upon the species in question. A limited response in rainbow trout might be expected with norethandrolone treatment, since the anabolic effectiveness of this steroid was based on growth studies with mammals (Potts et al. 1976).

A comparison of the results of this study and those of Cheema and Matty (1977) and Matty and Cheema (1978) revealed that norethandrolone has similar, yet limited, growth effecting qualities in fingerling rainbow trout. A slight, but insignificant ($P > .05$) trend was observed in the growth of fish as steroid supplement levels

increased in all diets varying in protein level in this study (Figure 1). The average weight/fish was increased somewhat at the 2.5 mg/kg supplement level; subsequent levels generally depressed growth.

Cheema and Matty (1977) and Matty and Cheema (1978) noted a similar "wearing off" of anabolic activity in juveniles at the end of their 60 day experimental periods as norethandrolone supplement levels increased from 2.5 to 5.0 mg/kg diet. Considering the questionable significance of their results ($P = .05$), the results of this study thus appear indicative of the true effect of norethandrolone in fingerling rainbow trout.

However, the result implied interactions between dietary formulations and steroid activity. Although such an interaction is significant ($P < .05$) only in Experiment II (Table 10), a similar association of significant decreases in growth characteristics of fish with diets containing the higher dietary protein level (45%) supplemented with the higher steroid levels (5.0 and 10.0 mg/kg diet) was evident in Experiment I (Tables 6, 7). This suggested that either increases in dietary protein level increased steroid catabolic activity, or increased steroid activity due to high steroid supplementation caused compositional inadequacies in this dietary formulation which resulted in depressed growth of fish.

Growth decreases have been observed in salmonids fed diets high in protein accompanied by low or inappropriate non-protein energy sources (Lee and Putnam 1973, Lee and Wales 1973). A high demand for non-protein energy could have been created at high steroid supplement levels which were necessary to promote norethandrolone anabolic activity. However, the inability of the diet to satiate this demand caused the

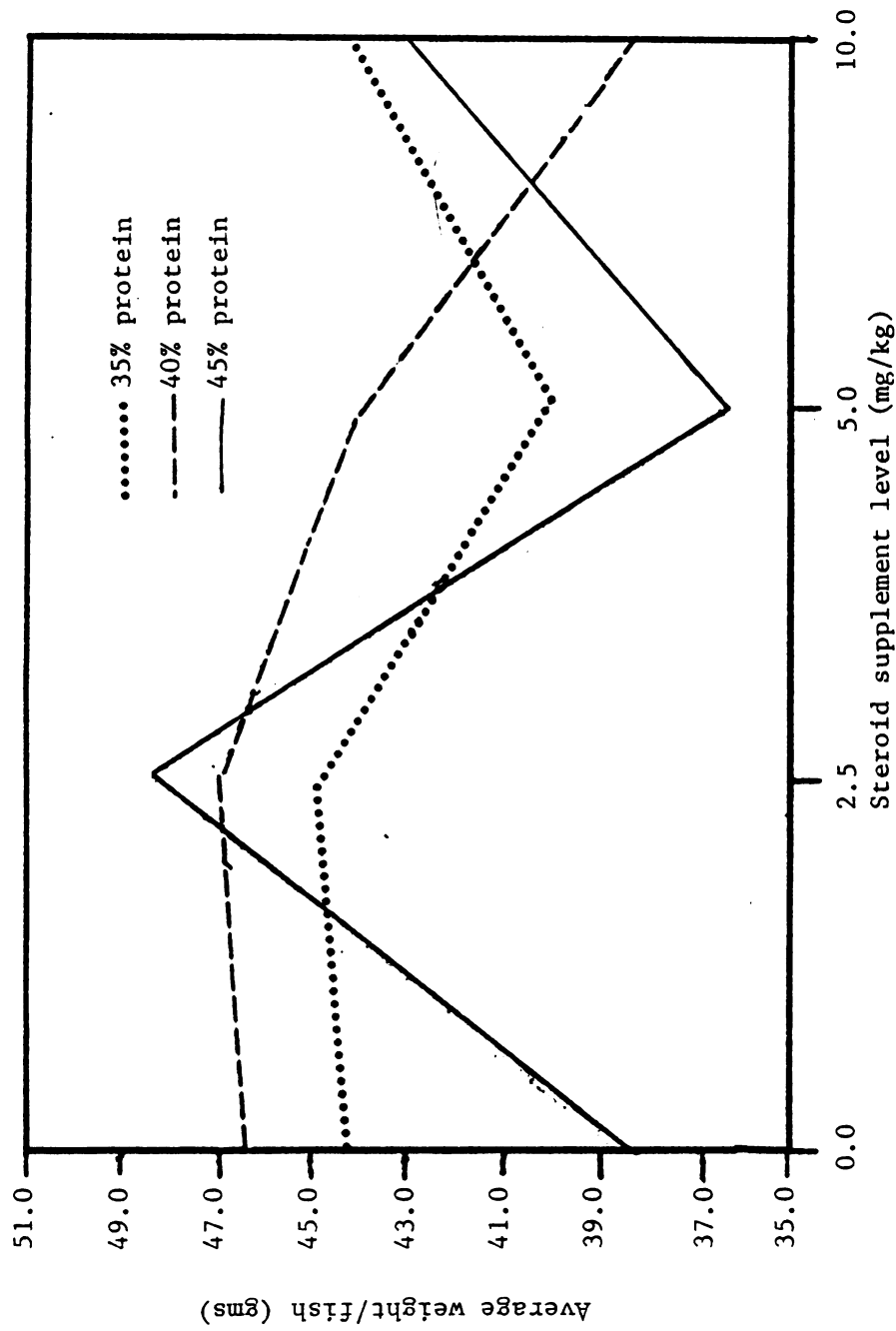


Figure 1. Final mean average weight/fish¹ (day 73) of replicate groups (n=3) of rainbow trout fingerlings fed semipurified diets containing varying levels of protein and norethandrolone supplements used in Experiment I. Significant differences ($P < .05$) occurred only between the 40 and 45% protein level diets at the 5.0 mg/kg supplement level.

$$^1 \text{Average weight/fish} = \frac{\text{Total live wet weight gain (gms)}}{\text{Total number live fish}}$$

decreases in growth. Indirect evidence has suggested that the energy requirements of steroid treated salmonids appear to be increased, since these fish eat to meet their energy requirements (Lee and Putnam 1973); steroid treatment has increased the appetite of fish fed to satiation (Yu et al. 1979, Fagerlund et al. 1979, Fagerlund et al. 1980) and decreased the body fat stores of fish fed on a limited feeding schedule (Simpson 1976). The presence of a marginal dietary protein: energy (P/E) ratio could explain the lack of any anabolic activity due to increased dietary protein level. Since the maintenance of adequate P/E ratios in diets fed to steroid treated mammals was required to promote additional growth advantages with increases in dietary protein level (Preston and Burroughs 1958, Landau 1976).

The interplay between dietary P/E ratios and steroid activity was also implied as protein and P/E ratios decreased in the formulations. Decreases in these dietary components have typically resulted in reduced growth of salmonids (Ringrose 1971, Lee and Putnam 1973) fed similar dietary lipid levels and P/E ratios within the range used in this study (8.45 g/kg and 140-190 mg P/kcal E, respectively) (Table 1). However, the relatively high levels of protein and dextrin and, thus, total dietary energy in diets used in this study may account for the reduced magnitude of expected growth differences. Varying growth responses have been obtained in channel catfish fed diets that contained similar P/E ratios, but differed in total dietary protein and energy (Garling and Wilson 1976); once optimum total dietary energy levels were reached, increases in the P/E ratio of these diets (beyond a considered optimum) did not significantly

($P = .05$) enhance the growth of fingerlings further. However, although sufficient energy appeared present in these diets for anabolic activity, growth increases did not occur at higher steroid supplement levels since a corresponding increase in protein level was also necessary to promote anabolic activity.

In contrast, the limited steroid effect on the growth of treated fish could have been similar to effects observed in mammals (Kochakian 1950, Kochakian et al. 1950, Kochakian and Van der Mark 1952, Kochakian and Webster 1958, Kochakian and Endahl 1959, Edgren 1963), where even low steroid treatment levels were too potent for anabolic activity. Steroid activity was characterized by an anabolic response which correspondingly increased with increasing dose up to a level which then depressed growth. More anabolically active androgens tended to shift the maximum anabolic and, thus, minimum catabolic dose to a lower level which higher dietary protein levels reduced the time for the anabolic/catabolic response. Contingent on this supposition, the advanced anabolic nature of norethandrolone would have required lower dietary supplement levels for effective anabolic treatment. This effect was unlikely unless another complicating factor was involved, since no prevalent or significant ($P = .05$) anabolic activity was observed at any time during the experiments that could have been offset by a catabolic response. Subsequent changes in body weight would have occurred only after anabolic activity was observed due to a continual depletion of body fat stores, in partial response to a steroid-induced appetite suppression. The wide variations in the final weight of fish (Table 13) suggested that significant, yet simultaneous,

Table 13. Ranges in the final dry patted weight in grams (smallest-largest) of individual fish in each replicate group (n=3) of Experiment I.

Protein level (%)	35	40	45
Steriod level (mg/kg)			
0.0	18.6-100.3	17.2-73.8	15.9-53.1
	24.0-66.5	28.7-77.4	23.2-73.5
	28.9-76.8	37.6-79.4	27.5-81.7
2.5	13.7-79.1	24.1-80.0	31.6-70.7
	21.6-79.1	25.3-72.4	33.8-62.8
	27.5-102.7	37.1-90.6	39.3-79.4
5.0	24.3-53.3	17.5-69.0	18.2-69.4
	26.9-89.3	22.1-76.6	20.7-47.1
	31.2-74.3	39.2-66.3	27.4-62.6
10.0	24.2-54.3	15.4-45.7	20.4-62.3
	29.3-98.2	18.1-62.8	20.9-81.9
	35.9-68.5	25.3-60.6	21.9-69.0

steroid growth promoting and growth depressing activities could have occurred between individuals which were masked when examined together. However, this response was probably not steroid related since the range in growth of reference groups (i.e. those fish fed unsupplemented diets) was similar.

Varying growth responses have been obtained between normal, immature male (Rubenstein 1941, Selye 1941, Shay et al. 1941, Turner et al. 1941, Meyer 1949) and female (Shay et al. 1941, Joss et al. 1963) rats treated with similar androgen doses in response to the additive nature exogenous treatment has upon endogenous androgen levels (Kochakian and Endahl 1959). Preestablished endogenous levels reduced the effective exogenous growth promoting dose that could be used with males. Growth depressing treatment levels of males promoted the growth of females. However, once circulating maximum anabolic levels were reached in both sexes, additional treatment eventually reversed the initial weight gains. This effect has not been considered in dietary steroid supplement studies with salmonids.

Mechanisms of norethandrolone activity were inconclusive in this study. Although anabolic activity was apparently absent in fingerling rainbow trout caused, in part, by dietary inadequacies, the presence of significant ($P < .05$) catabolic activity at only the highest protein and steroid supplement levels alludes to the possibility of a limited anabolic response which was insensitive to statistical analysis ($P = .05$). Protein efficiency ratios and feed conversions could not be used to examine mechanisms since the values could have been artifacts of uneaten feed. These values were only intended to be

used for practical evaluation (Meyer and Garrett 1967) of feed utilization. The results did, however, indicate some considerations for future studies that should be desirable.

Proper P/E ratios for steroid anabolic activity should be established. Normal protein and energy interactions would conceivably be altered with steroid treatment (Munro 1964) since it promotes changes in normal protein metabolism (Kruskemper 1968, Kochakian 1971, Young 1980). Differences in optimum P/E ratios with different minimal total protein and energy requirements might be indicated if treatment induced more efficient protein utilization and/or enhanced the energy requirements for growth. The cost/benefit of altering dietary protein levels can then be examined adequately to gain the full economic benefit of steroid treatment. Indeed, the results of this study indicated that the standard diet formulation used (45% protein level) (NRC 1978) was economically inefficient (Zeitoun et al. 1976) in protein recommendations since similar growth was obtained in fish fed lower dietary protein levels.

Dosage levels used should reflect the anabolic activity of the steroid. A relatively high dosage level for the anabolic effect could have explained why other highly anabolically active steroids were ineffective in promoting body weight gains of treated salmonids in previous studies (Matty 1975, McBride and Fagerlund 1976). Weight gains could have occurred with very low steroid doses not examined in such studies.

Complexing steroid treatments might promote the growth of both males and females to obtain the full benefit of anabolic steroid treatment. In practice, both sexes are raised together. Diets supplemented with an androgen-estrogen mixture might produce a better

over-all response than a low dose of a single steroid that would only effectively increase the growth of one sex, or a high dose that might create some catabolic activity. Heitzman (1976, 1979) has stated that although different sexes of cattle respond differently to androgen and estrogen treatment, a mixture of both compounds produces a positive and maximum response in both sexes. The mechanisms of action (Perry 1976, Trenkle and Burroughs 1978), however, might not be the same in fish. Alternatively, the effect of a single type steroid can be examined in populations whose sex ratios have been altered by previous steroid treatment (Yamazaki 1976, Johnstone et al. 1978) or in the monosex progeny of sex-reversed fish (Johnstone et al. 1979b).

Controlled intake feeding studies should be used to correctly assess steroid potential in production situations. Feeding fish to satiation was not considered a valid technique for practical investigation in this study since feeding rates are only periodically adjusted based on body weight in typical salmonid culture (Leitritz and Lewis 1980). Additionally, greater gains in body weight of steroid treated fish fed to satiation (Yu et al. 1979, Fagerlund et al. 1979, 1980) may have simply been a consequence of the increased feed intake (Peter 1979). If steroid activity only produced appetite increase in treated fish, then compensation would not occur for fish fed on a controlled schedule. Steroid treatment might then be ineffective or detrimental in promoting the growth of fish; growth and feed efficiency increases noted in previous studies might not occur.

Examining the efficacy of steroid treatment in salmonid culture requires not only the knowledge of which agent promotes the optimum

growth response, but also the understanding of physiological and nutritional interrelationships that can be used to promote the maximum and most economical response. Hopefully, this study has indicated the importance of such interrelationships.

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