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The Effects of Naturally Occurring Androgens in Practical Diets Fed to Normal-Sired and Jack-Sired Progeny of Coho Salmon (*Oncorhynchus kisutch*)*

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ABSTRACT

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Three batches of vacuum-dried meal from spawned salmon were made. In the first batch, the testes from the males were removed before drying. In the second, the testes were not removed. In the third batch, the testes were not removed and the gonads removed from the first batch were added. Analysis of the meals showed levels of 0.2, 0.7, and 1.5 ng testosterone/g of dry meal for batch 1 (0× gonad), batch 2 (1× gonad), and batch 3 (2× gonad), respectively. The meals were used to make three test diets similar to the Oregon Moist Pellet, OP-4. Each diet was fed to two groups of juvenile coho salmon (*Oncorhynchus kisutch*), one normal-sired and one jack-sired, for 119 days. Fish fed the 2× gonad diet weighed more ($P<0.05$) at the end of the study than fish fed the 0× gonad diet. Jack-sired progeny were larger ($P<0.05$) than normal-sired progeny fed 1× and 2× gonad diets. Fish length, feed conversion ratio, and protein efficiency ratio were similarly affected by dietary treatment. The study demonstrated that low levels of naturally occurring androgens can cause a physiological response in juvenile coho salmon.

INTRODUCTION

The size of coho salmon (*Oncorhynchus kisutch*) smolts at seawater entry is critical in determining survival, percentage return as adults, and the percentage of fish returning as precocious males, that is, as jacks (Hager and

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Nobel, 1976; Fryer and Bern, 1979; Bilton, 1980; Brannon et al., 1982). Both environmental and genetic factors influence freshwater growth rates of salmonids. Environmental factors include water temperature, feeding rate, photoperiod, and diet composition (Donaldson and Brannon, 1975; Novotny, 1975; Iwamoto, 1982; Reinitz, 1983; Plotnikoff et al., 1984). Genetic factors include age of the male at spawning and stock of both the male and female (Roley, 1973; Iwamoto, 1982; Iwamoto et al., 1984).

Diet is one of the easiest to manipulate of the environmental factors that influence growth rate in juvenile coho salmon in practical salmon culture. Aside from increasing feeding rate, using high quality ingredients, and formulating a balanced diet containing levels of essential nutrients in excess of the requirements of the fish, addition of anabolic steroids to salmon diets is the most direct way to increase growth rate. Coho salmon fed practical diets supplemented with 1–10 ppm 17-α-methyltestosterone grew more rapidly than fish fed control diets, sometimes weighing 121% more than control diet-fed fish (McBride and Fagerlund, 1973; Fagerlund and McBride, 1975; McBride and Fagerlund, 1976; Fagerlund et al., 1979; Yu et al., 1979). Higher dietary androgen levels (>10 mg/kg fed) appear to inhibit growth in coho salmon (McBride et al., 1982). In other species of salmonids, the growth response to androgen supplementation of the diet is less dramatic or absent (Ashby, 1957; Sower et al., 1983).

Dietary androgens may affect gonadal development. The appearance of undesirable secondary sexual characteristics (McBride et al., 1982) and degenerative changes in the testes (McBride and Fagerlund, 1973, 1976; Fagerlund and McBride, 1975; Fagerlund et al., 1979; Sower et al., 1983) have been documented. The relationship between growth rate and gonadal development may also be reflected in the incidence of sexual precocity. While the exact mechanism for the production of precocious males ("jack", "grilse") is uncertain, genetics, culture conditions, and growth rate appear to be involved. Roley (1973) and Iwamoto et al. (1984) have shown that precocious sires tend to increase the incidence of precocity in the progeny. Furthermore, larger smolts return more frequently as precocious males (Hager and Nobel, 1976; Bilton, 1980; Brannon et al., 1982) indicating the relationship to growth rate.

Recently, Sower and Iwamoto (1985) reported that commercial salmon diets may contain levels of naturally occurring testosterone sufficient to affect growth rates. Fish meals made from mature fish, such as herring, are the most probable source of testosterone in practical diets, and the switch from fishing for herring for reduction to fishing for roe has likely resulted in an increase in the concentration of testosterone in herring meals. As a result, previous studies in which androgens were added to practical diets may have had actual levels of androgens much higher than the researchers intended. Thus, this study was designed to determine if naturally occurring androgens in a practical salmon diet influenced growth, feed conversion ratios, and protein efficiency ratios of juvenile

coho salmon reared at an elevated water temperature similar to that used to produce underyearling coho salmon smolts. In addition, genotype × diet interactions were examined by feeding diets containing different levels of naturally occurring androgens to the progeny of 1-year and 2-year males.

METHODS

Design of experiment

Gametes from normally maturing coho salmon females and normally and precociously maturing males (Table 1) of the School of Fisheries 1984 returns were combined to form two discrete genotypic groups of progeny: "normal-sired" and "jack-sired" depending on the source of the milt. Fertilized eggs were incubated in vertical flow incubator trays (Heath-Techna, Kent, WA)[1] and later transferred to rearing troughs after hatch. At or near 100% yolk absorption (72 days; 1384.4 degree-days post-fertilization), 400 fry of each progeny groups were randomly assigned to eighteen 200-l circular tanks. A combination of ambient lake and heated lake water provided 4–8 l/min rearing water at a mean temperature of 13.5°C (range 10.5–16.4°C). Feces and uneaten food were removed daily from each tank. A natural photoperiod was used.

The diet was formulated following the Oregon Moist Pellet (OP-4) formulation (Table 2). Androgen level was controlled by vacuum drying equal weights of male and female salmon carcasses and then varying the amounts of testes included with the carcasses. Diet 1 contained no testes, producing a zero gonad meal (0× gonad). Diet 2 contained the normal amount of testes (6.79% of body weight) (Gunstrom, 1968; R.N. Iwamoto and J.R. Borghetti, unpublished data, 1984), producing a single gonad meal (1× gonad). In diet 3, an equal amount of male gonad was added to a gonad intact batch, producing the double gonad meal (2× gonad).

TABLE 1

Mean weight and lengths of the adult female and male coho salmon used to produce the test fish

	N	Fork length (cm) $X \pm SD$	Weight (kg) $X \pm SD$
Females	24	51.9±3.6	1.71±0.41
Males			
Normal	15	55.4±5.8	1.96±0.65
Jacks	16	27.1±2.4	0.23±0.07

[1]Use of trade names in this publication does not imply endorsement by the National Marine Fisheries Service.

TABLE 2

Composition of experimental diets

Ingredient	Percent in diet
Vacuum-dried salmon meal	50.0
Sodium bentonite	3.0
Wheat germ meal	3.9
Dried whey product	4.0
Vitamin premix[1]	1.5
Mineral premix[2]	0.1
Wet fish hydrolysate	30.0
Herring oil	7.0
Choline chloride (70% liquid)	0.5
Total	100.0

[1]Vitamin premix supplied the following in mg/kg diet: *d*-biotin, 0.6; pyridoxine-HCl, 37.5; vitamin B_{12}, 0.06; ascorbic acid, 891; α-tocopheryl acetate, 503; folic acid, 16.5; myo-inositol, 132; menadione sodium bisulfite complex, 18; niacin, 188; *d*-calcium pantothenate, 115; riboflavin, 53; thiamin mononitrate, 46.

[2]Mineral premix supplied the following in mg/kg diet: zinc sulfate, 75; manganous sulfate, 75; ferrous sulfate, 10; copper sulfate, 1.5; potassium iodate, 0.5; cobalt sulfate, 5.

Replicate samples of the three diets (0×, 1×, and 2× gonad) were dried in an oven at 105°C for 24 h and then subjected to proximate analysis. Protein was measured by the macro-Kjeldahl method (AOAC, 1975), moisture and ash by the AOAC (1975) procedures, and fat by the Goldfisch method (Joselyn, 1970), using methylene chloride in place of ether. Gross energy was determined by bomb calorimetry (Parr Instrument Co., Moline, IL). Dietary androgen levels were determined by the procedures described by Sower and Iwamoto (1985).

This study consisted of the two genotypes (normal-sired and jack-sired progeny) and three treatments (0×, 1×, and 2× gonad diet), with their replicates per treatment. The fish were fed at the rate of 10% of body weight for the first 24 days and at the rate of 7% of body weight for the remainder of the experiment. In the first 2 weeks of the feeding trial, fish were fed every 30 min. Feeding frequency was decreased to four and three times a day in the following weeks until the end of the experiment.

Total weight of the fish in each tank was determined at six times (30, 57, 73, 91, 105, and 119 days post-incubation). At each of the six times, 50 fish per tank were also randomly sampled for individual weight and fork length measurements. Weight was measured to the nearest 0.01 g and fork length to the nearest 1.0 mm for the first four sampling dates, and to the nearest 0.1 g and 1.0 mm for the remaining sampling dates. Growth rate was estimated by calculating instantaneous growth rates (IGR), where

IGR = 100 [ln (final wet weight) − ln (initial wet weight)]/time in days

At 91 days post-incubation, several tanks had exceeded a static loading density of 18.1 kg/m^3. Excess fish were randomly removed to equalize static loading densities of 18.1 kg/m^3 among tanks.

Statistical analysis

Analysis of variance was used to detect significant differences among genotype and treatment groups. Genetic background and diets were analyzed as cross-classified factors and were considered as fixed effects. Replications (i.e., tanks) were considered as random effects. The following model was used for all analyses.

$$V_{ijkl} = \mu + G_i + D_j + GD_{ij} + R_k + GR_{ik} + DR_{jk} + GDR_{ijk} + E_{ijkl}$$

where μ = overall mean; G_i = effect of the ith genetic background group; D_j = effect of the jth diet; GD_{ij} = interaction of genetic background and diet; R_k = effect of the kth replicate; GR_{ik} = interaction of genetic background and replicate; DR_{jk} = interaction of diet and replicate; GDR_{ijk} = interaction of genetic background, diet, and replicate; and E_{ijkl} = residual effects.

All analysis were performed using the ANOVA subprogram of the SPSS statistical package (Nie et al., 1975). Variance component estimates were also performed to determine the relative contribution of each main effect and interaction term to the total variation. The Student–Newman–Keuls test was used to find significant differences ($P < 0.05$) among treatment means.

RESULTS

The experimental diets were equivalent in proximate composition and gross energy content. The percentage moisture was 29.7%, while percentage protein, fat, and ash were 58.3%, 21.1%, and 12.4% on a dry weight basis, respectively. The gross energy content of the diets was 5078 kcal/kg dry diet. The salmon meal, which constituted 50% of the experimental diets, had levels of testosterone proportional to the amount of gonad added to each batch of meal. The levels of testosterone were 0.2 ng/g meal for the 0× gonad meal, 0.7 ng/g for the 1× gonad meal, and 1.5 ng/g for the 2× gonad meal. The testosterone levels of Diets 1, 2, and 3 were 0.087, 0.308, and 0.808 ng/g, respectively.

The average final weight and length of the juvenile coho salmon after 119 days of feeding were significantly ($P < 0.05$) influenced by both genotype and dietary treatment (Tables 3 and 4). The average weight and length increased with increased levels of testosterone in the diet and jack-sired progeny were significantly ($P < 0.001$) larger than normal-sired progeny at 119 days. Genotype × diet interaction, although statistically significant, was not a major con-

TABLE 3

Average weight, average fork length, condition factor, feed conversion ratios, and protein efficiency ratios of coho salmon fed the experimental diets for 119 days[1]

	Normal-sired progeny			Jack-sired progeny		
	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3
Average weight (g ± SD)	13.9 ±0.8[a]	15.2 ±1.6[a]	21.5 ±2.1[b]	16.8 ±1.1[a]	22.5 ±1.9[b]	26.9 ±1.3[c]
Average fork length (cm ± SD)	10.3 ±0.1[a]	10.7 ±0.3[ab]	12.1 ±0.5[c]	11.0 ±0.2[b]	12.1 ±0.2[c]	12.9 ±0.2[d]
Condition factor (±SD)[2]	1.19±0.11	1.18±0.07	1.21±0.07	1.21±0.08	1.24±0.07	1.23±0.07
Feed conversion ratio[3]	1.32	1.34	1.14	1.20	1.17	1.05
Protein efficiency ratio[4]	1.38	1.27	1.47	1.51	1.46	1.63

[1]Initial weight = 0.24 g. Initial fork length = 3.2 cm. Mean values in rows followed by the same letter are not significantly different ($P < 0.05$).
[2]Condition factor = (weight (g) × 100)/length (cm^3).
[3]Feed conversion ratio = dry weight fed/wet weight gain.
[4]Protein efficiency ratio = wet weight gain/dry weight protein fed.

TABLE 4

Mean squares and percentage of total variation (in parentheses) for weight, length, and condition factor at 119 days post-incubation

Source of variation	Trait					
	Weight		Length		Condition factor	
Genotype, G	5927.648***	(18.4)	199.505***	(15.0)	0.233***	(7.5)
Diet, D	5487.662***	(25.5)	229.404***	(26.0)	0.019	(0.6)
GD	286.408***	(2.3)	6.813*	(1.2)	0.033**	(2.7)
Replicate, R	52.832	(0.1)	1.006	(0.1)	0.017	(0.6)
GR	99.549	(0.1)	3.205	(0.4)	0.004	(−0.2)
DR	169.361***	(1.9)	7.186***	(1.9)	0.010	(0.6)
GDR	59.410	(0.5)	2.762	(0.8)	0.000	(−1.8)
Residual	36.509	(51.2)	1.598	(54.7)	0.006	(90.0)

***$P < 0.01$; **$P < 0.01$; *$P < 0.05$.

tribution to the total variation. Differences among dietary treatment groups began to emerge after 57 days of feeding in both the normal-sired and jack-sired fish (Figs. 1 and 2). For normal-sired fish, the groups fed the 2× gonad diet grew more rapidly from 30 days of feeding until the end of the experiment compared to the groups fed the 0× or 1× gonad diets. With jack-sired fish, the groups fed the 2× and 1× gonad diets grew more rapidly than the group fed the 0× gonad diet from 30 days of feeding onward. Jack-sired progeny had higher average weights and lengths than normal-sired progeny at the end of the feeding trial.

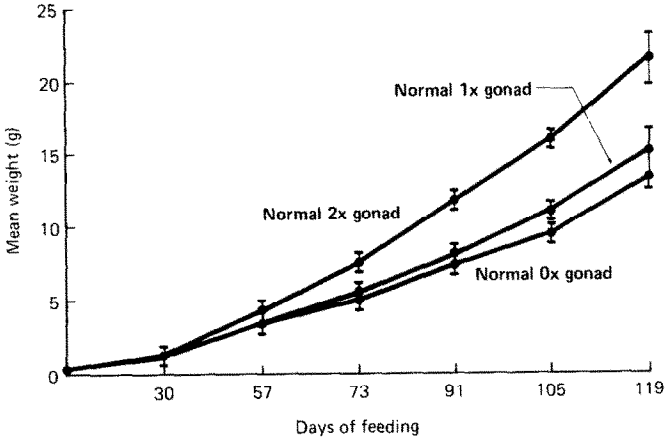

Fig. 1. Mean weights for normal-sired coho salmon progeny fed the three experimental diets. Vertical bars are 95% confidence interval of the mean of data from three tanks.

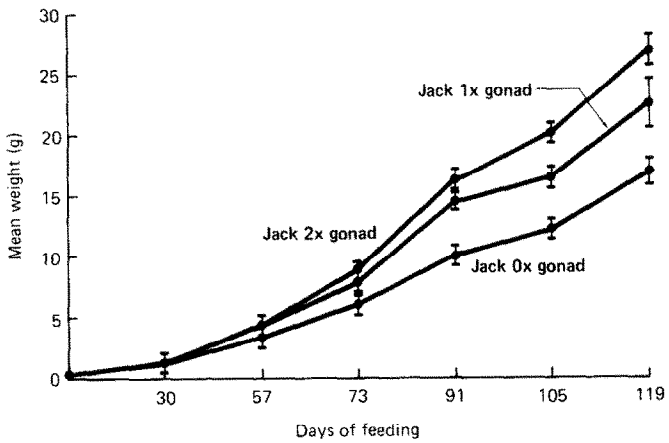

Fig. 2. Mean weights of jack-sired coho salmon progeny fed the three experimental diets. Vertical bars are 95% confidence interval of the mean of data from three tanks, except for the 91-, 105-, 119-day data where only two replicate tanks were available for the 2× gonad diets.

Instantaneous growth rates showed that growth was increased by dietary gonad level up to 91 days of feeding in both the normal-sired and jack-sired progeny (Figs. 3 and 4). By the last period (105–119 days), fish fed the 0× gonad diet had higher instantaneous growth rates than fish fed the 1× or 2× gonad diets. Condition factor of the fish was unaffected by diet.

Feed conversion and protein efficiency were also influenced by dietary treatment (Table 3). Although no significant differences were detected ($P > 0.05$), fish fed the diets containing the highest level of testosterone had the lowest feed conversion ratios.

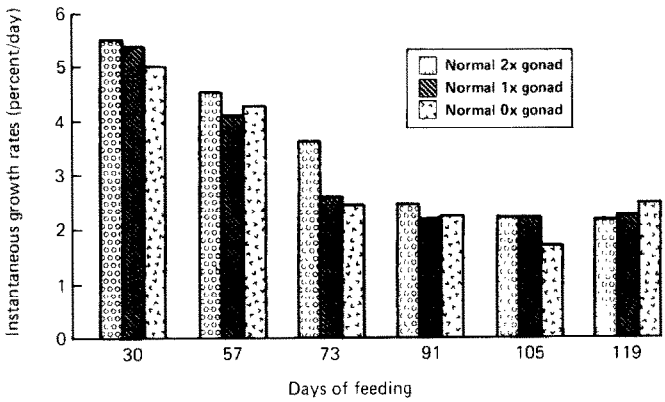

Fig. 3. Instantaneous growth rates of normal-sired coho salmon progeny fed the three diets.

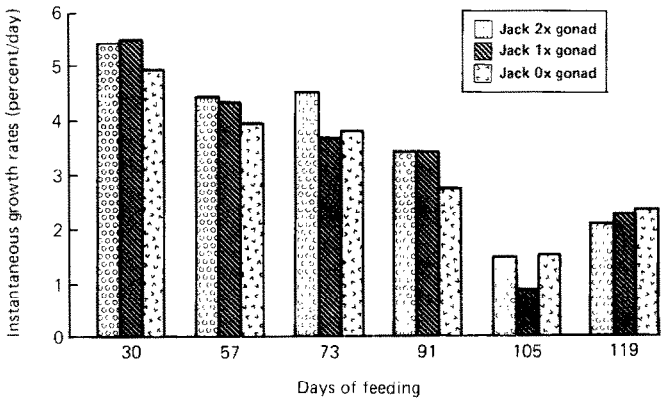

Fig. 4. Instantaneous growth rates of jack-sired coho salmon progeny fed the three diets.

DISCUSSION

Growth of juvenile coho salmon was significantly influenced by the presence of naturally occurring androgens in the diet. Growth was highest for fish fed the diet containing the highest level of androgens. In a previous study, a growth response was observed in juvenile coho salmon fed diets containing 0.2 μg 17-α-methyltestosterone/g diet for 97 days and for 269 days at either 11.5 or 16.5 °C (Fagerlund and McBride, 1975). A higher dose (1 μg/g) did not cause a further increase in relative weight gain in fish reared at 11.5 °C, but a marked increase was observed in fish reared at 16.5 °C. The duration of feeding, feeding rate, rearing temperature, and photoperiod all influence the response of juvenile coho salmon to 17-α-methyltestosterone (Higgs et al., 1982). The results of

this study also indicated that genetic background (i.e., normal male vs. jack male sires) may also be a contributing factor.

The dietary levels of androgens in this study were much lower than the levels of steroid hormones added to diets in previous work by others. The level of androgen supplementation to the diet of salmonids in various studies has ranged from 0.2 to 100 μg/g diet (Donaldson et al., 1979). In the present study, the level of dietary testosterone influencing growth was in the range 0.09–0.81 ng/g diet. In contrast to earlier work in which the diets of salmonids were supplemented with a single steroid hormone, the diets in the present study contained a mixture of steroids which naturally occur in fish meal, including testosterone, 11-ketotestosterone, and dihydrotestosterone (Sower and Iwamoto, 1985). Thus, the total levels of steroid hormones in the diets used in this study were likely higher than just the levels of testosterone. In addition, the growth response observed in this study may be the result of the mixture as well as the level of steroid hormones present in the various salmon meals.

The demonstration of a growth response to low levels of steroid hormones in practical salmonid diets has several important ramifications. First, with the exception of Yu et al. (1979), previous studies involved the addition of steroid hormones to practical diets. As noted by Sower and Iwamoto (1985), practical diets contain naturally present testosterone as well as other steroid hormones. Thus, previous results may have been confounded by the presence of steroid hormones which can cause a physiological response at low levels. Second, naturally occurring steroid hormones may provide a means of influencing growth in juvenile Pacific salmon without the problems of using steroid hormones of synthetic origin. Finally, the demonstration of a growth response to low levels of naturally occurring steroid hormones suggests a mechanism that may be involved in the increased incidence of precocious males among hatchery-raised salmon. Further research to elucidate the connection between precocity and the level of naturally occurring androgens is recommended.

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