EFFECTS OF GENISTEIN ON GROWTH, DEVELOPMENT AND REPRODUCTION OF RAINBOWN TROUT *Onchorynchus mykiss* AND ATLANTIC SALMON *Salmo salar*

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Executive Summary

Increasingly, plant protein sources including soybeans are being evaluated as replacements for fish meal in the diets of farmed carnivorous fish. Many plant protein sources contain factors that can exert nutritional and antinutritional effects in fish, and alter other developmental processes including reproduction.

Genistein is an isoflavone that is found in significant concentrations in soybeans. Genistein can have both estrogenic and antiestrogenic effects, either of which, in turn, can have stimulatory or suppressive actions on growth and reproduction in fish. The purpose of this study was to assess the effects of genistein on the growth, development and reproduction of rainbow trout and Atlantic salmon, to clearly elucidate the potential positive and negative effects of this isoflavone before large-scale attempts are made to incorporate increasing amounts of soybean products into commercial salmonid diets.

In rainbow trout, for a one year period fish were fed diets containing genistein at four concentrations: 0 (control), 500, 1,000 and 3,000 ppm. At all doses tested, genistein had no effect on growth or feed conversion. Serum levels of vitellogenin, measured at four times during the treatment period, were higher in genistein-treated fish (all doses) than controls. We found no effects of genistein on serum levels of reproductive hormones, morphology and histology of the gonads (both sexes), egg production, fertility rates, fry size nor viability. Accordingly, we suggest that the genistein content of soy products should not be a factor in determining their incorporation into the diets of rainbow trout.

In Atlantic salmon parr, for a six-month period fish were fed diets containing genistein at four concentrations: 0 (control), 500, 1,000 and 3,000 ppm, and a fifth group of fish were fed estradiol-17β (E_2) at 20 ppm as a positive control. At all doses tested, genistein had no effect on growth or feed conversion. At the end of the treatment period, serum levels of vitellogenin were higher in the E_2 and genistein-treated fish than in controls. E_2 and genistein at all doses tested inhibited the process of smoltification, as measured by 24-hr seawater challenge tests. Accordingly, the incorporation of genistein into the diets of Atlantic salmon parr may be contraindicated.

We suggest that further studies be conducted to evaluate the effect of genistein on Atlantic salmon smolts, to determine the appropriateness of high soybean meal content on Atlantic salmon grow-out diets.

Progress 3/4/03 – 6/1/03

In early April, 2003, the juvenile rainbow trout were randomly assigned to 12 tanks at 35-37 fish per tank. The tanks are being supplied with flow-through water at 15° C, and the photoperiod is adjusted to 16 L:8 D. Diets containing genistein at 0, 500, 1,000, and 3,000 ppm were made as described in our initial proposal, using Silver Cup trout feed (Nelson and Sons, Inc., Murray, Utah) as a base diet. Each diet is now being fed to three tanks of fish.

The fish are currently being fed twice daily to satiation. The fish in all four treatment groups are feeding vigorously and growing rapidly. To date the fish have been weighed and measured three times. The fish currently weigh between 7 and 10 g. At this point in time we have measured no statistical differences in growth or feed conversion between the four treatment groups. During the next quarter we will begin sampling fish for analysis of reproductive development, using the measures described in our original proposal.

Progress 6/1/03 – 8/31/03

During this time period the fish in all treatment groups have continued to feed vigorously and grow rapidly. The majority of the fish now weigh 50-100g. We now have the first indication of a differential effect due to treatment. The trout being fed the control diet are gaining weight at approximately the same rate as the fish being fed the diet containing genistein at 3,000 ppm. The fish being fed diets containing genistein at 500 and 1,000 ppm are gaining weight faster than the other two groups. This increase in weight gain is due to increased food consumption, because we have not measured any difference in food conversion between the different treatment groups.

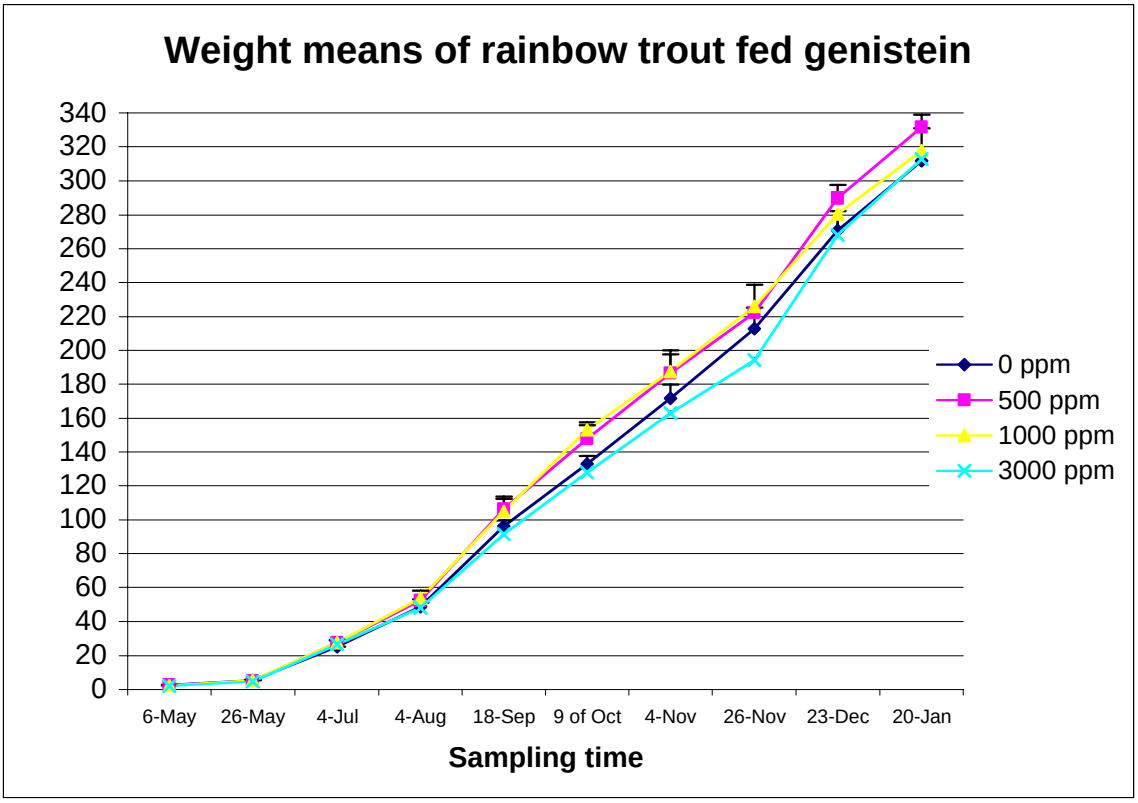
We have also begun to sample fish for analyzing reproductive development. Also, in early November, we will sample fish of each treatment group and send them to the laboratory of Denise Skonberg (University of Maine) for her studies on the flesh characteristics of the different treatment groups.

Progress 9/1/03 – 11/17/03

As of early November the majority of the fish now weigh 150-200g. The fish that are being fed diets containing genistein at 500 and 1,000 ppm remain heavier than the fish in the other two groups. This difference is due to increased food consumption, because we have not measured any difference in food conversion between the different treatment groups.

We have collected numerous samples from fish for analyzing reproductive development. We recently sent samples of fish fillets from each treatment group to the laboratory of Denise Skonberg (University of Maine) for her studies on flesh characteristics.

Year 1 final report 1/15/04



The funding period for year 1 of this project has ended, however, because of biological constraints in the nature of reproduction in rainbow trout, our study will continue through April, 2004. The rapid growth phase of our experiment has now been completed. At no dose level did genistein have a negative effect on the growth or feed conversion of rainbow trout. In fact, trout fed genistein at 500 ppm grew slightly but not significantly faster than trout in the other 3 treatment groups.

Photoperiod and temperature conditions were recently changed, in order to stimulate reproductive maturation in the fish. We have collected several sample sets of blood and gonads for analyses of reproductive development. These samples will be analyzed at the end of our study (April). To date we have observed normal reproductive development in males in all of the treatment groups.

In anticipation of our year 2 studies, we have obtained a group of atlantic salmon juveniles, and have begun acclimating them to our laboratory.

Progress 1/15/04 – 5/31/04

In rainbow trout, we collected numerous blood and tissue samples to evaluate reproductive development. Assays of these samples are nearing completion, and we should be able to report on these data in the next progress report. Female trout were too small or young to mature. Accordingly, we are continuing to raise fish from each of the treatment groups under ambient temperature/photoperiod conditions, and will attempt to spawn these fish next autumn/winter.

In atlantic salmon, we are conducting one study to evaluate the effects of genistein on the process of smoltification, because compounds with sex steroid activity are known to affect this important physiological process. For this experiment, in early December, 2004, juvenile atlantic salmon were assigned to 15 tanks with 30 fish per tank. The water temperature was set up to 15 C, and the photoperiod is adjusted to 14L:10D. This condition was set up to maximize the fish growth rate. Diets containing 0 ppm, 500 ppm, 1000 ppm, 3000 ppm of genistein and 20 ppm of estrogen were prepared. Each diet is being fed to three tanks of fish, and the fish are being weighed and measured monthly. On April 21, the water temperature was dropped to 4-7 C and the photoperiod was adjusted to 8L:14D. These conditions were set up to induce the fish to smolt. We anticipate that the fish should undergo smoltification sometime this month. To date we have not measured any affect of genistein on the growth or feed conversion of atlantic salmon.

Progress 6/1/04 – 8/31/04

Rainbow trout. Our analyses of blood and tissue samples to determine the effect of genistein on reproductive development have been delayed, but should be completed within the next month. To assess the effect of genistein on reproductive function, we are currently adjusting the temperature/photoperiod of trout being raised as brood fish. We anticipate that these fish will spawn this winter.

Atlantic salmon. Our studies on the effects of genistein on growth and smoltification of atlantic salmon are continuing. Unexpectedly, the fish in our study have not yet undergone smoltification. To date we have not measured any affect of genistein on the growth or feed conversion of atlantic salmon. We have also begun tissue culture studies to determine the effect of genistein on steroid hormone metabolism.

Progress 9/1/04 – 11/30/04

Rainbow trout. We have found that all doses of genistein stimulated the production of vitellogenin, but no other obvious effects on reproduction have been measured to date. Males are currently spermiating, and females are approaching spawning.

Atlantic salmon. We have found that genistein inhibits smoltification, suggesting that soy-based foods with significant genistein content are contra-indicated for Atlantic salmon parr. No effect on growth was measured, and we have no evidence to suggest that soy-based foods are contraindicated for Atlantic salmon grow-out.

Progress 12/1/04-2/28/05

Rainbow trout. We found that fish from all treatment groups spawned normally and produced normal fry. We conclude that genistein in the diets at the tested concentrations resulted in no adverse effects on rainbow trout reproduction.