SALMONIDS[(10)]

Project Termination Report for the Period
September 1, 1997 to August 31, 2001

NCRAC FUNDING LEVEL: $160,000 (September 1, 1997 to August 31, 2001)

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REASON FOR TERMINATION

The project objectives were completed.

PROJECT OBJECTIVES

(1) Develop and evaluate practical and economically viable diets that are fish meal free or as fish meal free as practical:

- using soy, or other oil-seed products that are regionally available, and
- using Shasta, Donaldson, and Kamloop strains of rainbow trout and/or Arctic charr for the evaluation.

(2) Evaluate the effects of water temperature on the growth/stress response in salmonid strains or species (as listed in Objective 1) under outdoor commercial culture conditions in the upper and lower portions of the North Central Region.

(3) Investigate the effects of trace mineral supplementation on the growth and stress response of rainbow trout in high density culture, as evaluated by plasma cortisol levels and fin nipping behavior.

PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Researchers at Purdue University (Purdue) developed a new series of 10 experimental diets and fed those to Shasta strain rainbow trout of 52 g (1.8 oz) initial weight. Weight gain, feed intake, feed conversion ratio, and specific growth rate of fish fed two of the formulations free of fish meal were not significantly different from fish fed a control diet. However, both diets contained fish oil as the lipid source. Another study was completed using rainbow trout that compared a variety of lipid sources in practical diets. The lipid sources evaluated included fish oil, canola oil, solvent-extracted soybean oil, cold-pressed soybean oil, and flax oil. In addition, each oil was evaluated as a 1:1 mixture with all possible combinations of lipid sources. Weight gain, feed consumption, and feed conversion ratios were not significantly different among dietary treatments. Tissues were analyzed for their fatty acid concentrations. This project was designed to continue research on new dietary formulations for rainbow trout. Appropriate vitamin and mineral premixes and several lipid sources were identified that can be used in practical diets.

Purdue also conducted two separate studies in which a fish meal free basal diet formulation for trout continued to be modified. In the first experiment, the focus was on vitamin and mineral premixes as these had been identified as problem areas in previous studies. In the second experiment, the focus was on lipid sources. In both experiments, Purdue researchers acquired Shasta strain rainbow trout and fed triplicate groups of them a variety of diets, 10 in each experiment. All fish were greater than 50 g (1.8 oz) starting weight so both experiments represented the grow-out phase of production. In the first experiment, fish were fed several different types of vitamin and mineral premixes. Based on weight gain, tissue mineral concentrations, and mineral retention, a supplemental mineral premix was best. A nutritionally complete mineral premix resulted in decreased weight gain. There were no differences with the vitamin premixes used. In the second experiment, Purdue researchers identified several lipid sources that contained sufficient n-3 fatty acids to meet the requirement of trout. Those were fed alone or in combination; no significant difference in weight gain of fish was observed. Further, tissue fatty acid concentrations did not indicate essential fatty acid deficiencies. Lipid sources used were flax oil, canola oil, cold-pressed soybean oil, and various combinations. These data are significant advances in the ability to formulate fish meal free diets for rainbow trout. In many species, as fish meal is reduced, mineral supplementation must be increased. This is not the case in trout. Further, they are very tolerant of a wide range of vitamin premixes.

Alternative lipid sources appear to be appropriate for meeting the energy and essential fatty acid needs of rainbow trout.

Two feeding experiments were carried out at Ohio State University (OSU). First, a 16-week feeding trial was conducted to examine the feasibility of using a fish meal analog (FMA) as a fish meal replacement in diets for juvenile rainbow trout. Fish weighing 0.96 ± 0.07 g (0.034 ± 0.002 oz) were divided into 15 groups and three groups were fed one of five isonitrogenous diets containing 0, 20, 40, 60, or 100% of FMA protein (control, FMA20, FMA40, FMA60, and FMA100, respectively). The FMA consisted of 25% of meat and bone meal, 24.5% of leather meal, 20% of squid liver powder, 15% of feather meal, 7.5% of blood meal, 7.5% of poultry by-product meal, and 0.25% each of methionine and lysine. Fish began to show differences in growth rate from the eighth week followed by significant differences at the 12^{th} and 16^{th} weeks. At the final 16^{th} week weighing, fish fed diets FMA40, FMA60, and FMA100 exhibited significantly lower weight gain, feed efficiency, protein efficiency ratio, and specific growth rate than fish fed FMA20 and control diet. Hematocrits of fish fed FMA-containing diets were significantly lower than that of fish fed a control diet. FMA inclusion up to 60% in diets did not show differences in apparent protein digestibility compared to the control (85.0 ± 1.9%), whereas the FMA100 group was significantly lower (77.7 ± 4.4%). These results suggest that a FMA could be used up to 20% as fish meal protein substitution in diets for juvenile rainbow trout without adverse effect on growth rate and hematological indicators.

In the second experiment at OSU, fish meal protein was replaced partially or entirely with a mixture of animal by-products and/or plant protein mixtures (soybean and cottonseed meals, SM and CM, respectively). Fish averaging 0.96 g (0.034 oz) were divided into 18 groups (three replicates per diet). Six diets were formulated as follows (expressed as % protein): (1) control diet = 100FM; (2) APM 50 = 50 APM + 50 FM; (3) APM100 = 100 APM; (4) CM-CA = 25 CM (California product) + 25 SM + 50 APM; (5) CM-TN = 25 CM (Tennessee product) + 25 SM 50 APM; and (6) CM-AR = 25 CM (Arkansas product) + 25 SM + 50 APM. The results of the weight gain and feed efficiency showed that fish meal can be entirely replaced by a mixture of animal by-products and either of two cottonseed meal products (1,370 ± 17, 1,330 ± 16, and 1,350 ± 10% body weight gain for control, CM-TN and CM-AR diets, respectively). Significantly lower hematocrit levels were found in fish fed five test diets compared to those fish fed the control diet. Higher concentrations of total gossypol were found in feces of fish fed CM-TN and CM-AR diets than those fed CM-CA diet. The percentage of dietary gossypol accumulated in the whole body indicated that the majority of this substance is excreted in feces. The gossypol isomer selectively accumulated in liver and bile is the (+) isomer, whereas equal proportions of (+) and (-) isomers were found in diet, whole body, and feces. The findings suggest that a fish meal free diet could be used without adverse effects for 16 weeks on growth performance and histopathological changes in liver in juvenile rainbow trout.

At Michigan State University (MSU) tanks were stocked with rainbow trout and experimental or reference diets were randomly assigned. Dietary treatments consisted of:

(1) negative reference (sub-optimal protein), (2) positive reference (fish meal based), (3) soybean meal substituted-untreated diet, and (4) soybean meal substituted-pretreated with phytase. Experimental diets were formulated with 35% crude protein, protein to energy ratio of 100, and vitamin and mineral premixes added to meet the requirements of the fish. The fish were subjected to a two-week acclimation period prior to the start of the study to allow them to adjust to their new surroundings and feeding regime. Diets were fed three times a day to three replicates of 16 fish per treatment for a period of 10 weeks. Feed rates were calculated on a percent body weight basis, which was determined during the two-week acclimation period by feeding the fish to satiation. Fish were weighed every two-weeks and feed rates were adjusted according to weight gain. Initial findings showed that phytase pretreatment of soybean meal: (1) significantly increased weight gain and protein deposition above the fish meal based diet and (2) significantly improved feed conversion ratios. Results from this study were presented at the World Aquaculture Society meeting in Sydney, Australia in April-May 1999. The data showed that phytase pretreatment of plant products in diets increased growth significantly above fish meal diets. The data also showed that without phytase pretreatment diets could be formulated that were not significantly different from fish meal diets. The Insulin-Like Growth Factor (IGF-I) isolation procedure was also refined to work on rainbow trout.

A study on dietary iron retention in rainbow trout was also completed. Blood serum levels were tested for IGF-I and re-tested a second time for accuracy. On the treatment level for the eight-week study the fish meal based diet was significantly higher from all other dietary treatments. When IGF-I levels were analyzed by week the fish meal based diet was significantly higher compared to all other dietary treatments for the first six weeks. In the eighth week both the fish meal and the soybean meal diets treated with phytase were significantly higher than all other dietary treatments, but not each other. These data suggest that IGF-I serum levels can be used as a rapid indicator of nutritional status in rainbow trout as early as two weeks. Statistical analysis of the data by week in the first study demonstrated that the fish meal treatment was significantly different from all others at weeks two, four, and six. This research also shows that there may be a long term effect of phytase treatment on circulating IGF-I levels resulting in increased IGF-I levels in the blood, as shown in week eight where there were no differences between the fish meal based diets and the soybean meal diet treated with phytase.

Tanks at MSU were stocked with rainbow trout and experimental or reference diets were randomly assigned. Dietary treatments consisted of: (1) positive reference (fish meal based), (2) soybean meal substituted-phytic acid added, (3) soybean meal substituted-pretreated with phytase, and (4) commercial diet. Experimental diets were formulated with a 35% crude protein, protein to energy ratio of 100, and vitamin and mineral premixes added to meet the requirements of the fish. The fish were subjected to a two-week acclimation period prior to the start of the study to allow them to adjust to their new surroundings and feeding regime. Diets were fed three times a day to three replicates of 12 fish per treatment for a period of at least six weeks. Feed rates were calculated on a percent body weight basis, which was determined during the two-week acclimation period by feeding the fish to satiation. Fish were weighed every two weeks and feed rates were adjusted according to weight gain. There were no significant differences in growth

for all dietary treatments. The IGF-I isolation procedure was refined to work on rainbow trout. Blood serum samples for both studies were tested by RIA (radioimmuno assay) for IGF-I serum levels. Because of a material shortage, blood samples were pooled by week and treatment for IGF-I analysis.

On the treatment level there were no significant differences among the commercial, fish meal based, and soybean meal phytase treated dietary treatments in both IGF-I blood serum levels and in growth. The soybean meal diet with phytic acid added treatment was significantly lower then all other dietary treatments in both IGF-I levels and growth. These data suggest that IGF-I can be used as a rapid indicator of nutritional status and that phytic acid may have a effect on circulating IGF-I levels in the blood.

OBJECTIVE 2

The plan of work in the initial proposal was to conduct studies on Arctic charr and Donaldson strain rainbow trout at Rushing Waters Fisheries, Inc., Palmyra, Wisconsin. By the time of initiation of the project, however, Rushing Waters personnel determined that they would be unable to conduct the study. Accordingly, as per the backup plan detailed in the original proposal, the Arctic charr study was conducted at the University of Wisconsin-Madison (UW-Madison) campus.

In March 1998, approximately 120 Arctic charr (average total length 160 mm [6.3 in], weight 31 g [1.1 oz]) and 100 rainbow trout (average total length 150 mm [5.9 in], weight 34 g [1.2 oz]) were obtained from Rushing Waters Fisheries, Inc., Palmyra, Wisconsin, and Trout Haven, Bryant, Wisconsin, respectively. The fish were held in separate 750-L (198-gal) flow-through tanks at a water temperature of 12.5°C (54.5°F). In April 1998, the fish were weighed, measured, and 25 fish were transferred into each of four 120-L (32-gal) flow-through tanks (two tanks of rainbow trout, two of Arctic charr). Over the next two weeks, the water temperature was gradually raised to 15°C (59.0°F) in two tanks (one rainbow trout tank, one charr tank) and lowered to 10°C (50.0°F) in two tanks. After three weeks acclimation, six fish from each tank were quickly removed, anesthetized, and bled via the caudal vasculature. The remaining fish were given an acute stress challenge test by holding them out of the water for 1 min, then randomly placing them into separate tanks. Groups of six fish were then netted, anesthetized, and bled at 1, 3, and 24 h following the stressor.

In October 1998 UW-Madison researchers validated the cortisol enzyme linked immnosorbent assay (ELISA) for use with Arctic charr serum and analyzed the samples in November. Results indicate a significant difference in cortisol concentrations over time between fish held at 10°C (50.0°F) and 15°C (59.0°F) (mostly due to differences in baseline [time 0] cortisol concentrations), but no significant difference in stress responsiveness between rainbow trout and Arctic charr.

In spring 1999, Freshwater Farms of Ohio, Inc. (Urbana, Ohio) stocked Shasta and Donaldson strain rainbow trout in an outdoor raceway and monitored growth, survival, and incidence of disease. The experiment was completed in October 1999 when the

cortisol stress response was measured in fish subjected to an acute stress challenge test. The Donaldson trout had a significantly lower cortisol stress response compared to the Shasta trout. At both time 0 (baseline, resting) and 3 h post-stress, cortisol levels in the Donaldson strain were lower than in the Shasta strain. It is concluded from these data that Donaldson trout may be more resistant to the harmful effects of stress than the Shasta strain fish.

OBJECTIVE 3

A questionnaire was designed by MSU researchers to evaluate the extent and impact of trout fin nipping/erosion in the North Central Region (NCR). The questionnaire was reviewed by two MSU social scientists with expertise in survey methods and by three North Central Regional Aquaculture Center (NCRAC) extension contacts. Minor revisions were made prior to pre-testing the questionnaire. The questionnaire was pre-tested in Michigan by sending it to 15 trout producers.

NCRAC extension contacts in eight states were asked to provide the names and addresses of up to 15 trout producers in their state. One state contact and the State Aquaculture Coordinator chose not to cooperate in the survey, three states had too few producers (1 or 2) to participate, and one state specialist (Nebraska) chose to survey his producers by phone interview using the questions provided. Responses were received from 34 of 42 producers surveyed in Michigan, Missouri, and Wisconsin which represented an 87% response rate. Fin nipping/erosion was rated as not a problem with significant economic impact by 30 producers (88%) who responded by mail and by those who participated in phone interviews. Two producers each indicated a moderate or severe fin nipping/erosion problem. Because so few producers indicated that fin nipping/erosion was a problem, causes of their problems could not be identified.

IMPACTS

OBJECTIVE 1

Grow-out diets that are free of fish meal have been developed and tested in Shasta strain rainbow trout at Purdue. Fish fed two formulations exhibited responses that were not significantly different from fish fed a control diet. Results from this research are being used in the NCR as the basis for new dietary formulations using regional feed ingredients that are manufactured in this region. The advantages of this approach should be to reduce cost of feeds and improve profitability of aquaculture operations. These formulations can be used by producers in the NCR and provide the impetus for regional dietary manufacturing using local ingredients. Further, a new feed mill has been established in Ohio that focuses on manufacturing fish diets that are free of fish meal. These impacts should ensure profitable trout production in the NCR. There is significant interest in adding other salmonid species to the overall regional production. These new diets, or modifications of them, should be evaluated in additional strains of trout and alternative species suitable for culture in the NCR.

Research at OSU has provided strong evidence that a diet with 15% cottonseed meal, 15% soybean meal, and 20% animal by-product meal can be used to produce grow-out diets for rainbow trout without compromising growth rate or health indicators (liver histology). Taking into account current prices for fish meal ($560/ton) and cottonseed meal ($140/ton), this replacement should make considerable difference in feed costs. It is recommended, however, that cottonseed meals should be used with caution for rainbow trout because of the phytoestrogens and gossypol content.

MSU research has shown that improved utilization of dietary phosphorus and nitrogen will reduce the impact of aquaculture on the water quality of streams receiving water discharge from fish farms. This research demonstrated that phytase can be used to improve utilization of phosphorus and nitrogen and that IGF-I can be used as a rapid indicator of nutritional status in rainbow trout. Circulating IGF-I levels in the blood can be used as a rapid indicator of nutritional status in rainbow trout and that phytase treatment may have a long term effect on circulating IGF-I serum levels. This research also showed that pretreatment of plant feedstuffs with phytase can improve growth in rainbow trout all plant diets that makes them comparable to fish meal based diets, and that phytic acid does have a negative effect on growth.

OBJECTIVE 2

The identification of additional trout strains or species which can be reared under sub-optimal thermal conditions in the NCR will maximize productivity and profitability of aquaculture facilities in the region. In addition, the availability of rainbow trout strains or species with improved growth rate, feed conversion, and disease resistance will greatly improve the production efficiency of private and public fish hatcheries throughout the region.

UW-Madison data showed a lower stress response in Donaldson strain trout which suggests that this trout strain might be used to improve the production efficiency of private and public rainbow trout hatcheries in the NCR. Such stress-resistant fish are likely to show improved growth rates, feed conversion, and disease resistance compared to more stress-prone strains. It is recommended that farmers further evaluate this strain.

Data did not support the hypothesis that Artic charr might be used in place of rainbow trout to maximize productivity and profitability of aquaculture facilities in the region, particularly in the cold winter months. Both rainbow trout and Arctic charr performed well under the culture conditions evaluated in the UW-Madison study and no advantage of charr was detected. Indeed, one regional producer has recently abandoned charr production because of the high cost of charr eggs, and because charr sales had a negative impact on their rainbow trout sales.

RECOMMENDED FOLLOW-UP ACTIVITIES

- • Although much is known about general salmonid nutrition there is still a need for additional information on the requirements of these fishes raised under the conditions that are common in the NCR;
- • there is also a need for continued efforts into finding ways of lowering the cost of fish meal replacements using locally available ingredients; and
- • additional work is needed in regard to shortening the time needed for diet evaluations and strain comparisons using techniques such as IGF.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Salmonid activities.

SUPPORT

YEAR	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
1997-98	$80,403	$92,640	$23,500[a]		$23,750[b]	$139,890	$220,293
1999-00	$79,597	$134,145	$23,500[a]		$23,750[b]	$181,395	$260,992
TOTAL	$160,000	$226,785	$47,000		$47,500	$321,285	$481,285

[a]Ohio State University Korean Project

[b]National Cottonsead Products Association

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Publications in Print

Adelizi, P.D., R.R. Rosati, K. Warner, Y.V. Wu, T.R. Muench, M.R. White, and P.B. Brown. 1998. Evaluation of fish meal-free diets for rainbow trout, *Oncorhynchus mykiss*. Aquaculture Nutrition 4:255-262.

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Manuscripts

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Papers Presented

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