DEVELOPMENT OF ECONOMICAL, HIGH-PERFORMANCE, LOW-POLLUTING FEEDS AND FEEDING STRATEGIES

REPORTING PERIOD	4/01/99 - 3/31/00		
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FUNDING LEVEL	$76,000		
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PROJECT OBJECTIVES

1. To determine accurate indices of phosphorus status, and to use these indicators to determine the phosphorus requirement of grow-out sized rainbow trout.
2. To determine the effects of the following on fish growth performance, feed efficiency ratios, nutrient retention, and product quality:
   *a. Modified fish meals, other alternate protein sources, and alternate dietary oil sources as protein and energy sources in salmonid diets;
   *b. High energy diets (25–30% lipid);
   c. Feed manufacturing technology (cooking extrusion and steam pelleting) on high nutrient density diets and in standard trout production diets;
   d. Development of phase-feeding programs to optimize the benefits of high nutrient diets, manufacturing processes, and alternate feed ingredients.
4. To continue development and validation of the *in vitro* feed digestibility assay, which is intended to predict nutritional value of a feed ingredient or diet.
5. To determine the optimum feed ingredient particle size for trout feeds with respect to apparent digestibility and proportion of settled solids and soluble material in fecal wastes.
6. To draft a RAC Results publication, "*New Biochemical Test Measures Protein Digestibility of Feed Ingredients for Fish.*"

ANTICIPATED BENEFITS

This project will assist and benefit the aquaculture industry by providing information from which wise decisions can be made by both industry and environmental regulatory agencies. These regulatory agencies are concerned with reducing the levels of phosphorus and other nutrients in hatchery effluents. The information generated in this project will provide multiple strategies related to feed formulation and manufacture, feeding practices, and the development of practical *in vitro* digestibility tests that will help contribute to reducing pollution in hatchery effluents. Relationships between the efficiency of utiliza-

tion of the diet, growth rates of the fish, health indices of the fish, and digestible phosphorus and protein levels in the feed will be determined and adjusted to meet the dietary requirement for the grow-out stages. Information on bioavailability of various key feed ingredients will be used to properly formulate low-polluting feeds for salmonids.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. To evaluate the effects of feeding high energy diets (30% lipid) during the grower and finishing phases of production on fish growth performance, feed efficiency rations, nutrient retention, and product quality

Last year, we reported the results of feeding five extruded diets (EXT 10%, EXT 15%, EXT 20%, EXT 25%, EXT 30% lipid), and a commercial floating trout feed (COM) to rainbow trout (starting weight ~120 g/fish) in a freshwater system for 16 weeks. Indices of performance and food fish product quality were measured. The results indicated that among the six dietary groups, there were no significant differences in final body weights, weight gain, FCRs, feed intake, and drawn weights. The EXT 25% and EXT 30% treatments had significantly higher visceral-somatic indices than the EXT 10% group. This year, apparent digestibility coefficients, protein retention, phosphorus retention, and feed and fish mineral analyses were completed for that trial. In a new trial, the diets EXT 15% and EXT 30% were fed to rainbow trout for 16 weeks to confirm the previous results.

Compared to a 15% extruded lipid diet, feeding rainbow trout a 30% extruded lipid diet does not have a significant effect on body weight, feed intake, other nutritional indices (FCR, digestibility coefficients, SGR, P and N retention). A higher feeding level, which was achieved in the Pelleting Method Trial, resulted in a significantly higher lipid deposition in the fillet and viscera of the EXT 30% group compared to the EXT 15% treatment. In both trials, the VSI was significantly higher in the EXT 30% group than in the EXT 15% group, which means more by-product was formed when the higher lipid diet was fed. Because there are reports from industry that higher lipid feeds (e.g., 30% lipid) result in bigger, fatter fish, this experiment was repeated at the U.S. Fish and Wildlife hatchery in Ennis, MT. The goal was to determine the effects, if any, of feeding 15%, 20%, 25%, or 30% lipid feeds to rainbow trout at a higher feeding rate (resulting in a higher FCR).

Objective 2. To compare the effect of feed manufacturing technology (cooking-extrusion, expansion, and steam pelleting) on high nutrient density and in standard trout production diets on fish growth performance, feed efficiency ratios, nutrient retention, and product quality

The feed formulation for this study was the same as the EXT 15% diet used in the previous High Energy Diet Trial. The mash for the diets was mixed at Nelson and Sons, Inc. then portions were sent to Dr. Rick Barrows at USFWS (Bozeman, MT) [*extruded* (**EXT 15%**; cooked and pellets formed under high pressure) and *steam pelleted* (**STM**; compression pellets)]; to Wenger, Inc. (Sabetha, KS) [*Universal Pellet Cooker* (**UPC**), which is a combination of some aspects of both compressed pelleting and extrusion]; and to Pro-form Feeds (Chilliwac, BC) [*expanded* or annular gap (**EXP)**, where frictional and steam energy partially cook ingredients which are then steam pelleted without pressure]. These four diets (extruded, steam pelleted, UPC, and expanded), a 30% extruded diet (referred to as **EXT 30%**), and a commercial trout feed (Silver Cup Feeds, Murray, UT; referred to as **COM**) were fed to three replicate tanks per treatment, for a total of eighteen tanks. The EXT 30% diet was compared to the EXT 15% diet, repeating treatments from the first trial (see report of results in the previous section).

Given the feed formulation that was used in this study, the method of pelleting did not appear to have a significant effect on fish performance (e.g., weight gain, feed intake, feed conversions, etc.). Pellet stability was not altered by the pelleting method, although the UPC method resulted in a significantly higher percentage float and a higher sinking rate than the STM, EXP, or EXT methods. The degree of gelatinization was not significantly altered. Thus, in terms of maximizing fish performance and feed pellet stability, it appears that all four pelleting methods are equally suitable.

Objective 3. Evaluate the potential of modified fish meals, other alternate protein sources, and alternate dietary oil sources as protein and energy sources in salmonid diets during the grower and finishing phases of production on fish growth performance, feed efficiency ratios, nutrient retention, and product quality

Because fish oil, like fish meal, is not considered to be a sustainable ingredient in fish feeds, a pilot study was designed to determine the effect on fish performance and feed palatability of maximum substitution of fish oil with either soybean lecithin or poultry fat. Rainbow trout were fed diets formulated to contain a total of 20% lipid, and 15% of the lipid was from either fish oil, soybean lecithin, or poultry fat. The fish were fed for 9 weeks. Data indicated no significant differences in body weight, feed intake, or FCR. Colorimeter analyses of the raw fillets indicated that the lecithin fillets were more yellow than either the fish oil or poultry fat fillets. Overall, it appears that lecithin and poultry fat included in fish diets at a maximum substitution level in a 20% lipid feed do not affect feed intake or fish performance; however, the color of the fillets caused by the inclusion of lecithin in the feed could affect consumer acceptability; this will be tested in a larger, longer term feeding trial..

Objective 4. Continue development and validation of the in vitro feed digestibility assay, which is intended to predict nutritional value of a feed ingredient or feed

An improved method for measuring *in vitro* protein hydrolysis was developed. This method used an o-phthaldialdehyde (OPA) reagent to measure the amount of amino groups released. Feeds from the High Energy Diet Trial were tested using this method. The best correlation with apparent digestibility coefficients obtained *in vivo* was with the 4-enzyme system (enzymes derived from mammalian and microbial sources) without the EXT 10% treatment data. Improvements to the assay included the use of Tricine buffer (pH 8). Procedures for purifying rainbow trout pepsinogens were established and their pH optima, pH stability, and activity in the presence of specific inhibitors and activators were characterized. Rainbow trout pepsin samples from fish of different age and environments were characterized and appeared to have the same activity.

Objective 5. Determine the optimum feed ingredient particle size for trout feeds with respect to apparent digestibility and proportion of settleable solids and soluble material in fecal wastes

The impact of ingredient particle size on the digestibility of trout feed pellets and fish growth performance was investigated. Ingredient particle size in the range tested in this study had no significant effect on trout growth. When coarse particle diets were fed, solid particles in the water were more coarse than when either the fine or medium particle diets were fed. Because the coarse particles can be easily collected by sedimentation and/or with screen filtration, this information will be useful for determining to how best remove solids from aquaculture systems, such as recirculating facilities and raceways. Ultimately, determining the optimal grinding size will contribute to reducing the effluent load, reducing feed production costs, and maintaining a better feed conversion.

WORK PLANNED FOR NEXT YEAR

The timeline for 2000-01 (Year 4) will be:

1. A trial testing the effects of 10-30% lipid diets fed to rainbow trout for 4-5 months was run in Ennis, MT. The fish were harvested this month (September 2000) and sent to the UW for analyses. The results will be presented in the final report next year.
2. A trial testing the effects of substituting soybean lecithin or poultry fat for fish oil was run for 4-5 months at the Hagerman Fish Culture Center, and the fish were shipped to the UW in August 2000 for analyses. Fish performance and product quality will be evaluated. The results will be presented in the final report next year.
3. The effects of adequate levels of vitamin E (300 mg/kg feed) and high levels (1500 mg/kg) will be tested in diets containing either 15% or 30% lipid in extruded diets in Hagerman in Oct.-Jan. 2001. Fish performance, feed digestibility, food fish product quality (in particular the oxidative stability),

and *in vitro* tests of immune response will be measured. The results will be presented in the final report next year.

4. Additional feed samples, with known *in vivo* digestibility, will be analyzed using the modified OPA method with Tricine buffer to obtain a standard curve. Gastric proteases from rainbow trout and steelhead trout acclimated to seawater will be further purified and characterized. The zymogen distribution of steelhead trout, a sea-going strain of rainbow trout, will be studied, and the behavior of the zymogens in the presence of salts will be investigated.
5. Complete analyses of the feed particle size excretion study.

IMPACTS

1. With the diet formulations and feeding levels achieved in two feeding trials, it appears that feeding either 15% or 30% lipid diets to 100 g rainbow trout has little effect on the performance of the fish. However, the viscera and fillet accumulate more fat when a higher lipid diet is fed, although the whole body weights may not different. One disadvantage of feeding a high fat feed is the production of more by-product in the form of viscera.
2. Feeds made with four different pelleting technologies (extruded, steam pelleted, expanded and the Universal Pellet Cooker) supported similar growth and performance of market-sized rainbow trout. Tests measuring pellet stability and characteristics [water activity, % float, sink rate, % cook (degree of gelatinization)] did not reveal any major differences among the four feeds. Therefore, from a fish husbandry and effluent management perspective, all four pelleting technologies appear to be equally suitable.
3. Poultry fat and soybean lecithin as major lipid ingredients in rainbow trout feeds appear to support fish performance; however, soybean lecithin may impart a visible yellow color to the fillets.
4. The *in vitro* method for protein digestibility requires less time and operating expense than *in vivo* methods.
5. Ingredient particle size up to 1.68 mm (in our standard WRAC formulation, extruded feeds) had no significant effect on rainbow trout growth (starting weight ca. 90 g/fish). The large particles in the solid waste from the coarse particle diets can be easily collected by sedimentation and/or with screen filtration from aquaculture systems, such as recirculating facilities and raceways. Ultimately, determining the optimal grinding size will contribute to reducing the effluent load, reducing feed production costs, and maintaining a better feed conversion.

SUPPORT

FISCAL YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
99	76,000	35,000	14,200	34,000	7,000	110,200	186,200

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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