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

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REPORTING PERIOD **4/01/97 - 3/31/98**

FUNDING LEVEL **$70,000 FOR 1997-98**

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PROJECT OBJECTIVES

(OBJECTIVES WITH * WERE ADDRESSED IN FY '98)

*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 tein 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. 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. 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 the environmental regulatory agencies concerned with reducing the levels of phosphorus and other pollutants in hatchery effluents. The information generated in this project will provide multiple strategies, such as options to select ingredients, formulations, processing techniques, to reduce pollutant levels in hatchery effluents for the three major life history stages of salmonids. Relationships between the efficiency of utilization of the diet, growth rates of the fish, health indices of the fish, and digestible phosphorus level in the feed will be determined and adjusted to meet the dietary requirement for each life history stage. Information on bioavailability of various key feed ingredients will be used to properly formulate low-polluting feeds. Finally, development of low-ash fish meals will provide the aquaculture industry with more feed ingredient choices from which to formulate feeds and will involve cooperation of the fish meal industry directly with the aquaculture industry.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. To determine accurate indices of phosphorus status, and to use these indicators to determine the phosphorus requirement of grow-out sized rainbow trout.

A series of experiments were designed to determine the minimum dietary requirement of P for large rainbow trout based on accurate and sensitive response criteria. Experiments were conducted to improve new approaches and to evaluate various physiological parameters. Results indicated that urinary P concentration was by far the most sensitive indicator among other response criteria. Fish responded to a different dietary P intake within 1 day by regulating urinary excretion, which was near zero within 5 days on low P diets (0.10 and 0.32g P/100g diet). In the groups of fish fed 0.52g P, 0.76g P or 1.07g P/100g diet, fish continually excreted a certain amount of P in the urine during the 24 day feeding period, and no trend was observed of either a consistent decrease or increase in urinary P levels over time. The dietary P requirement estimated from urinary P concentrations is between 0.32 and 0.52g P/100g dry diet, which is lower than the requirement estimations determined previously by other researchers with small rainbow trout.

It is concluded that the renal response for dietary P is a far more sensitive and rapid indicator than any other response criteria for P. By closely monitoring P concentrations in urine, the requirement level for P (and possibly for some other nutrients) can be estimated within a few days. This rapidity and sensitivity will be particularly useful to research nutrient requirements for large fish at various physiological stages and under various environmental conditions.

Objective 2. To 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.

Approximately two-thirds of total phosphorus in various grains is present as phytate, which is not well-utilized by fish and other monogastric species. Besides its low availability of phosphorus, phytate is reported to reduce the availability of other dietary nutrients to the animals. Single-gene, non-lethal *low phytic acid (lpa)* mutations in corn and barley cause the seed to store most of the phosphorus as inorganic phosphorus instead of as phytate phosphorus. Theoretically, using these mutant grains containing lower levels of phytate in animal feeds should reduce phosphorus excretion by the animals, providing that available phosphorus levels in feeds containing these grains are appropriately adjusted downward.

This study was conducted as a first step to determine if the biological availability of phosphorus in the low-phytate mutants of barley, dent corn and flint corn differed significantly from that in ordinary grains for fish. Six ingredients (grains) were evaluated: three ordinary grains—barley , dent corn, and flint corn—and three low-phytate mutant grains of the same variety—low-phytate barley, low-phytate dent corn, and low-phytate flint corn. These grains were each incorporated into either casein or fish meal based diets, or into a low ash diet.

Results indicated that in low ash diets, low-phytate grains markedly increased the apparent availability of phosphorus. Fecal phytate-P and total-P were significantly lower in fish fed low-phytate grains than ordinary grains in the low-ash test diet. Fecal phosphorus content decreased ca. 50% in average (in phytate-phosphorus) or 43% in average (in total phosphorus) when ordinary grains were replaced with low-phytate grains in the low-ash diet. In contrast, with the higher ash diets, inclusion of the low-phytate grains had little effect on apparent availability of phosphorus or of other minerals.

This work suggests that allelic variation at a single gene of a feed grain species can potentially have a major impact on reducing phosphorus flow through fish farms, and likely other forms of livestock production. Feeding trials, both at the laboratory and farm-scale, are needed to confirm the potential of low-phytate grains to achieve this reduction and support production expectations. Further work is also needed to demonstrate that the low-phytate grains will be successful in commercial crop production; however, with dent corn, large-scale production is already occurring. Even if the economics of production of low-phytate grains are less favorable than ordinary grains, the use of low-phytate grains in fish and animal feeds may remain advantageous because reducing phosphorus into the environment is rapidly increasing in priority as a part of global environmental awareness.

Objective 3. Evaluate the effects of feeding high energy diets (25-30% lipid) during the grower and finishing phases of production on fish growth performance, feed efficiency ratios, nutrient retention, and product quality.

The purpose of this trial is to compare the effects of feeding high-energy versus low-energy diets on the growth, performance and product quality of rainbow trout *(Oncorhynchus mykiss)*. Diets with lipid concentrations of 10, 15, 20, 25, 30% and a commercial control diet (Silver Cup) are being fed to 100 g post-juvenile trout until they reach 400-500 g; growth, performance and fillet quality of the fish will be measured. All diets were formulated to contain low-ash protein ingredients with a total protein level of ca 43% and a total phosphorus level of ca 1%. The diets were based on practical, production type diet formulations, contained low-ash protein ingredients, and had a total protein level of ca 43% and a total phosphorus level of ca 1%. The primary ingredients were fish meal, wheat products, soybean meal, and fish oil. The levels of wheat gluten and soybean meal were held constant in all diets. To standardize the production process, all of the fish oil in each feed was included in the mix prior to extrusion, and no fish oil was top-dressed on the feeds. Approximately 200 kg of each diet was processed using a Buhler Twin-Screw Cooking Extruder (Model DNDL-44). After several BFTC Screw Configurations were tested, the final diets were produced using a Screw Configuration W98D and a 3 mm die. After extrusion, the diets were dried in a pulse-type, fluidized bed dryer (Buhler, OTW) at 250°F until they contained less than 10% moisture.

In addition to the 5 dietary treatments, a commercial diet (Silver Cup) is being fed as a control to post-juvenile rainbow trout (starting weight ~100 g). The trout were randomly distributed into 3 tanks for each treatment for a total of 24 tanks, 35 per tank. After the trial is completed, measurements will be made of visceral weight and gain, liver weight, and determination of the whole body and fillet composition and will be used to calculate fish weight and gain, fish length and gain, fish girth, feed efficiency ratio (weight gain/feed fed), carcass proximate carcass composition, fillet proximate composition, phosphorus retention ratio, visceral-somatic index (viscera weight/carcass weight), hepatosomatic index (liver weight/carcass weight), protein efficiency ratio, and percentage energy retention (gain in energy by fish/energy consumed). At the completion of the study, sensory analysis (color, flavor, aroma) and chemical analysis [thiobarbituric acid reactive substances (TBARS) and peroxide values] will be performed on freshly harvested fillets, and on fillets stored for 5, 10, and 20 days at refrigeration temperatures (0-5°C) and for 1, 3, and 6 months at freezing temperatures (-20 and –35°C).

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 diet.*

Twelve feed ingredients (meals: menhaden premium, herring, anchovy, feather, poultry by-product, de-boned fish, soybean; gluten meals: wheat, corn; wheat midds; casein) were analyzed for phosphorus

by the AOAC methods for animal feeds with some modifications. Total phosphorus was analyzed using the vanadomolybdophosphoric acid colorimetric method. With the exception of wheat midds, all the plant feed sources had a low phosphorous content compared to feed from animal sources.

Soluble phosphorus was analyzed after digestion at 37°C by either: (a) simulated gastric digestion at pH 3 for 18 h; (b) simulated intestinal digestion at pH 9 for 1 h; and (c) two-step digestion, simulated gastric digestion for 18 h at pH 3 followed by simulated intestinal digestion for 1 h at pH 9. Each digestion condition was performed using either commercially available enzymes or trout enzymes with similar enzyme activity.

Treatment with porcine pepsin increased the phosphorous solubility over that for incubation without enzymes in all ingredients except anchovy meal and wheat midds. Treatment with trout pepsin increased phosphorous solubility for all ingredients except anchovy meal, poultry by-product meal and wheat midds. In addition to the three ingredients that exhibited a large increase in phosphorous solubility with porcine pepsin, wheat gluten digest also had a relatively large increase in phosphorous solubility (37%) after digestion with trout pepsin. Wheat gluten phosphorous solubility was 57% with porcine pepsin and 86% with trout pepsin.

Overall, the best correlation between *in vivo* and *in vitro* phosphorous digestibility for plant and animal ingredient sources was observed when a two step digestion with trout enzymes, corrected for non-enzymatic digestion, was employed. *In vitro* results for animal feeds correlated better than those for plant feed ingredients when conditions simulated intestinal digestion. It is concluded that phosphorous solubility after a two-step trout enzyme digestion is a good measure of phosphorous digestibility for plant and animal sources of ingredients.

Objective 5. 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.

The digestibility studies were conducted at the University of Idaho Fish Culture Experiment Station at Hagerman, Idaho. Three typical feed ingredients including soybean, wheat and fish meal were tested. The materials were ground on a hammer mill with three different screen sizes (3 mm, 1.6 mm, 0.6 mm). Particle size distribution of the ground material was characterized before pelleting using a series of sieves, No. 10 (2.0 mm), No. 14(1.4 mm), No. 18 (1.0 mm), No. 30 (0.6 mm) and No. 40 (0.3mm). For each ingredient, the three products were combined with a casein-gelatin, semi-purified diet(basal diet) containing 1% chromic oxide so that the ingredient constituted 30% by weight of the diet mixture. The mixture was then cold-pelleted and frozen until use.

These feeds were fed to ca. 100 g rainbow trout to determine Apparent Digestibility Coefficients (ADCs) of dry matter, protein and phosphorus in the three test ingredients. Results of the ADC studies indicated no differences in ADC protein among particle sizes for fish meal and wheat, but differences were detected among particle sizes for soybean meal, as one might expect. To confirm these results, a new study is being conducted in which three ingredients are being ground and tested as before. These ingredients are fish meal, meat and bone meal, and soybean meal. The meat and bone meal was obtained from a commercial producer before it had been ground, providing us with the opportunity to grind it particle sizes less fine that commercial products. In addition to measuring ADC for protein, we will measure ADC for phosphorus to determine if bone particle size affects phosphorus availability.

A system has been setup in the Aquaculture Research and Teaching Laboratory at Washington State University on the Pullman campus to house fish for growth studies. This system consists of ten 125-gallon tanks, a 10 ft^3 plastic beads biofilter and a sump. Operated in a recirculating mode, this system allows three replicates of 3 treatments. The system was acclimated and stocked with fingerling rainbow trout for evaluating growth performance of different feed with different ingredient particle sizes.

Objective 6. To draft a RAC Results publication, "New Biochemical Test Measures Protein Digestibility of Feed Ingredients for Fish".

A draft of this publication has been written and reviewed by the Director of WRAC. Currently, photographs are being taken for inclusion in the publication.

USEFULNESS OF FINDINGS

1. *Accurate determination of the phosphorus requirement for large rainbow trout.* The research this year has more closely defined the phosphorous requirement in market sized rainbow trout. Since the requirement for large rainbow trout was found to be lower than the requirement reported by other researchers for smaller fish, feed manufacturers will be able to produce lower phosphorus feeds for the grow out period without the risk of inducing a phosphorus deficiency. Major reductions in effluent phosphorus levels can be realized if lower phosphorus feeds are used.

2. *Urinary P concentrations were found to be the most sensitive and rapid indicator than any other response criteria tested for monitoring dietary P and estimating the dietary P requirement.* Although expressing the requirement on a dry diet basis (~ 0.32-0.52 g/100 g dry diet) is useful for feed manufacturers, it is suggested that expressing the requirement on the basis of gain in body protein is more accurate.

3. *Potential of low-phytic acid varieties of corn and wheat to be successfully incorporated into low phosphorus feeds with no additional costs or effort.* The ordinary varieties and low-phytic acid varieties of barley, dent corn, and flint corn were fed to fish to determine phosphorus availability in several different formulated feeds. Compared to the ordinary grains, the low-phytate grains (barley, dent corn, and flint corn) in low-ash test diets increased the apparent availability of phosphorus, reduced fecal phytate-P (~50%) and total-P (~43%), and potentially can reduce phosphorus flow through fish farms.

4. *Phosphorous solubility after a two-step trout enzyme digestion is a good measure of phosphorous digestibility for plant and animal sources of ingredients.* The best correlation between *in vivo* and *in vitro* phosphorous digestibility for plant and animal ingredient sources was observed when a two step digestion with trout enzymes, corrected for non-enzymatic digestion, was employed.

5. *The effect of particle size on digestibility depends on the ingredient.* There were no differences in ADC protein among different particle sizes for fish meal and wheat, but differences were detected among particle sizes for soybean meal.

WORK PLANNED FOR NEXT YEAR

The timeline for 1998-99 (Year 2) will be:

1. Complete the study examining the effects of high energy diets (25-30% lipid) fed during the grower and finishing phases of production on fish growth performance, feed efficiency ratios, nutrient retention, and product quality of fresh, refrigerated stored, and frozen stored fish fillets will be completed.
2. 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.
3. Compare the effect of feed manufacturing technology ("extruded" = cooked and pellets formed under high pressure; "expanded" = steam pelleted without pressure; "pelleted" = compaction pelleted without added steam or pressure) using a high lipid formulation (confirmed in the current feeding trial) on fish growth performance, feed efficiency ratios, nutrient retention, and product quality. (While the different feed manufacturing technologies were supposed to have been a part of our current feeding trial, more time was needed to test different formulations on the different pelleting machines, and to arrange for commercial locations to produce 1 or 2 of the diets. Hence, this study will be run this coming year as a separate trial.)
4. Continue development and validation of the *in vitro* feed digestibility assay which is intended to predict nutritional value of a feed ingredient or diet. Additional feed samples with known *in vivo* P

digestibilites will be tested to validate this method.

5. Determine if differences in *in vivo* digestibility of specific protein ingredients ground to specific particle size will result in differences in fish performance when fed to rainbow trout; develop a protocol for a feed efficiency index system.
6. Complete and distribute the RAC Results publication, *"New Biochemical Test Measures Protein Digestibility of Feed Ingredients for Fish."*

IMPACTS

1. Lower phosphorus feeds can be formulated for grow-out size rainbow trout.
2. Low phytate varieties of grains incorporated into low ash feeds can help to reduce phosphorus excretion by fish.
3. The *in vitro* methods for phosphorous bioavailability requires less time and operating expense than *in vivo* methods.
4. Feed digestibility can be improved by grinding the ingredients to the optimal particle size, increasing feed utilization efficiency and reducing waste excretions. By defining the relationship between digestibility and ingredient particle size, the optimal grinding size can be determined that will result in lower feed production costs.

SUPPORT

FISCAL YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
97–98	70,000	36,000	14,200	30,000	7,000	38,200	157,200
TOTAL							

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Arndt, R.E., Hardy, R.W., and Dong, F. M. 1998. Effects of heat treatment and substitution level on palatability and nutritional value of soybean meal in feeds for coho salmon (*Oncorhynchus kisutch*). Aquaculture (submitted).

Haard, N.F. and Weerasinghe, V. 1998. *In Vitro* Digestibility of Salmonid Feed to Assess Phosphorus Bioavailability. Presented at 43rd Atlantic Fisheries Technological Conference, July 25-29, Newfoundland, Canada.

Haard, N.F. 1997. Metodos in vitro para evaluar la calidad de proteinas alternativas para la alimentacion de salmones. Biotecnologia Marina: La Quimica en el Aprovechamiento de los Recursos Pesqueros, Montevideo, Uruguay, Dec. 12. Invited speaker.

Skonberg, D.I. 1997. A Nutritional Approach to Reduce Phosphorus Pollution in Hatchery Effluent. Ph.D. Dissertation, University of Washington, Seattle, WA. 176 pp.

Skonberg, D.I., Hardy, R.W., Barrows, F.T., and Dong, F.M. 1998. Color and flavor analyses of fillets from farm-raised rainbow trout fed low-polluting feeds. Aquaculture (in press).

Sugiura, S.H. 1998. Development of Low Pollution Feeds for Sustainable Aquaculture. Ph.D. Dissertation, University of Washington, Seattle, WA. 254 pp.

Sugiura, S.H., Dong, F.M., and Hardy, R.W. 1998. Requirement of rainbow trout for dietary phosphorus. J. Nutrition (submitted).

Sugiura, S.H., Raboy, V., Young, K.A., Dong, F.M., and Hardy, R.W. 1998. Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). Aquaculture (in press).

Sugiura, S.H., Dong, F.M., and Hardy, R.W. 1998. Effects of dietary supplements on the availability of minerals in fish meal. Aquaculture 160: 283-303.

Sugiura, S.H., Dong, F.M., Rathbone, C.K., and Hardy, R.W. 1998. Apparent digestibility coefficients of macro- and microelements and protein in various feed materials determined using coho salmon (*Oncorhynchus kisutch*). Aquaculture 159: 177-202.

Weede, N.E. 1997. Low Phophorus Plant Protein Ingredients in Finishing Diets for Rainbow Trout (*Oncorhynchus mykiss*). M.S. Thesis, University of Washington, Seattle, WA. 150 pp.

Weerasinghe, V. and Haard, N.F. 1998. *In vitro* method for phosphorous digestibility of trout *(Oncorhynchus mykiss*) feed. Aquaculture Nutrition, (submitted).

Weerasinghe, V. and Haard, N.F. 1998. *In Vitro* Digestibility of Fish Feed Using Digestive Enzymes From The Test Organism. In: Proceedings of 43rd Atlantic Fisheries Technology Conference, F. Shahidi, ed. (submitted).