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

REPORTING PERIOD	4/01/98 - 3/31/99		
AUTHOR	Faye M. Dong, Work Group Chair		
FUNDING LEVEL	$70,000		
PARTICIPANTS	Frederic T. (Rick) Barrows*	US Fish and Wildlife Service	Montana
	Shulin Chen*	Washington State University	Washington
	Gary Fornshell *(Extension Rep.)*	University of Idaho	Idaho
	Norman F. Haard*	University of California	California
	Faye M. Dong*	University of Washington	Washington
	Ronald W. Hardy*	University of Idaho	Idaho
TECHNICAL ADVISOR	Chris Nelson	Murray Elevator	Utah

* funded participant

PROJECT OBJECTIVES

1. Complete the study comparing the effects of low and high energy diets (10-30% lipid) fed during the grower and finishing phases of production on fish performance and product quality of fish fillets.
2. Compare the effect of feed manufacturing technology on fish growth performance, feed efficiency ratios, nutrient retention, and product quality.
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 performance and product quality.
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 impact of ingredient particle size upon the digestibility of trout feed pellets and fish growth performance.
6. Complete and distribute the RAC Results publication, *"New Test Measures Protein Digestibility."*

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 and with using sustainable and cost-effective ingredients in fish feeds. The information generated in this project will provide multiple strategies, such as options to select ingredients, formulations, and feed 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, digestible phosphorus level in the feed, quality and shelf life of the food fish product, and the yield of both fish processing byproduct and utilizable food fish product will be determined.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. Complete the study comparing the effects of low and high energy diets (10-30% lipid) fed during the grower and finishing phases of production on fish performance and product quality of fish fillets.

Although salmonid feeds now contain 30-40% lipid compared to 12-14% in the past due to the perceived economical advantage of raising faster growing fish with improved feed conversion ratios (FCR), and the opportunity to reduce the amount of expensive fish meal in feeds, the potential effects on fish growth, feed utilization, fillet product quality and shelf life, and percentage of non-utilizable fish processing waste have not been investigated in rainbow trout. This study compared the suitability of fish meal-based feeds containing 10-30% lipid on performance and product quality of post-juvenile rainbow trout (*Oncorhynchus mykiss*). Six diets (10, 15, 20, 25, 30% lipid, and a commercial trout diet) were each fed (apparent satiation) to 3 replicate tanks of rainbow trout (starting weight ~120 g/fish) in a freshwater system for 16 weeks. Indices of performance and food fish product quality were measured.

Results indicated that among the 6 dietary groups, there were no significant differences in: final body weights (400 g/fish); weight gains (280 g/fish); FCRs (average = 1.1); feed intake; and dress-out weights. The 25% and 30% lipid treatments had significantly higher ratios of viscera weight to body weight than the 10% group ($P<0.05$). At day 0, concentrations of fillet thiobarbituric acid reactive substances (TBARS) were not significantly different, but after storage for 5 days at 0 to 5°C, the 30% treatment had significantly higher TBARS than any of the 10-25% groups (4.3 vs. 1.4 mm malonaldehyde/kg fillet); after 12 and 20 days, the 30% was significantly higher than the 15% group. Storage at either –20°C or –30°C for up to 7 months resulted in the 30% lipid group and sometimes the 25% lipid group having significantly higher TBARS than the lower lipid groups. Sensory triangle tests indicated that increases in fishy odor or flavor of the 30% group occurred mainly after the longer storage periods. The reasons for the observed increase in TBARS, the lighter color, and the fishier aroma/flavor of the 30% group compared to the lower dietary lipid groups are being investigated.

Although in industry the lipid levels in rainbow trout feeds are increasing, the suitability of this practice should be examined because of the potential increase in the amount of non-utilizable product (visceral fat) and decrease in the shelf life of the food fish. While wet weight gain may be higher or more economical in trout fed high-fat diets, there may not be a commensurate gain in dressed and or fillet weight. The results of this study are contrary to reports of the beneficial effects of high-lipid feeds in Atlantic salmon farming. Further research is necessary to confirm the results of the present study and if possible to determine the reasons for differences in response to high-lipid feeds between Atlantic salmon and rainbow trout.

Objective 2. Compare the effect of feed manufacturing technology on fish growth performance, feed efficiency ratios, nutrient retention, and product quality.

Since the previous trial indicated no apparent advantage to feeding high lipid diets to market-sized rainbow trout, the next trial is using a the same formulation and a 15% lipid level. The choice of 15% lipid also allowed us to include steam pellets, which are difficult to produce if dietary lipid levels exceed 16-18%. The mash for the diets was mixed at Nelson and Sons, Inc. then portions were sent to Dr. Rick Barrows at USFWS (Bozeman, MT) [1-*extruded* (cooked and pellets formed under high pressure); 2-*steam pelleted* (compression pellets)], to Wenger, Inc. (Sabetha, KS) [3-*Universal Pellet Cooker (UPC)* which is a combination of some aspects of both compressed pelleting and extrusion], and to Pro-form Feeds (Chilliwac, BC) (4-*expanded* or annular gap, 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 “extruded 30%L”), and a commercial trout feed (Silver Cup; Nelson and Sons, Murray, UT) are each being fed to 3 replicate tanks per treatment, for a total of 18 tanks. The extruded 30%L diet will be compared to the extruded15% lipid diet, repeating treatments from the first trial.

After three months of feeding, the following groups have significantly different whole body weights ($P < 0.05$): commercial > steam pelleted, extruded, UPC, and expanded; and extruded 30%L > expanded. After three months, there are some differences in feed intake (steam pelleted > extruded, extruded 30%L, UPC; and expanded > extruded 30%L) and in FCR (commercial, extruded 30%L are better than steam pelleted, expanded; extruded, UPC are better than expanded). In comparing the extruded (15% lipid) to the extruded 30%L diets using the Students t-test, there are no significant differences in whole body weight, feed intake, or FCR after three months.

Other tests will be performed using the feeds. An *in vivo* digestibility trial will be performed in fecal sedimentation tanks at the Hagerman Laboratory. The feeds as-is will be fed to rainbow trout and feces will be collected. Acid-insoluble ash or some other endogenous inert marker will be measured, rather than re-grinding and re-pelleting the feeds with an exogenous marker such as chromic oxide or yttrium oxide. Feeding the original pellets will allow us to test for potential effects of the pelleting technologies on particle size, especially of the wheat starch, that may affect the digestibility coefficients. Several tests will be performed on the pelleted feeds in addition to the routine proximate analyses: (1) pellet buoyancy; (2) pellet stability in water; (3) degree of gelatinization of the starch; and (4) TBARS. Low pellet buoyancy can reduce the rate of feed intake, low pellet stability in water can lower feed intake and increase pollution through feed wastage, a high degree of gelatinization may increase the digestibility of the starch, and a high level of TBARS may indicate detrimental effects of specific time/temperature heat treatments of the pelleting technologies.

The effects of feeding 15% and 30% lipid extruded feed are being determined again in this trial, and the appropriate tests previously done in the first trial will be performed in this one. At the end of the trial (4 months), the drawn weight of the fish and TBARS of the fresh and 2 month stored (–20°C) fillets will be determined. In addition, the fatty acid profiles in the fillets and viscera will be measured at the Hagerman Laboratory. In comparing the 15% and 30% steam extruded dietary treatments by a Students t-test instead of by ANOVA, there is still no significant difference in whole body weight between the two treatments after the first, second, and third month of feeding.

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 performance and product quality.

Because fish oil, like fish meal, is not considered to be a sustainable ingredient in fish feeds, part of this project will determine the suitability of two alternate sources of lipid: poultry fat and soybean lecithin. We are currently obtaining samples of two grades of poultry fat from Tyson, Inc. These samples will used by Dr. Rick Barrows to determine the range of substitution levels possible in fish meal based, steam extruded feeds. In separate feeds, Dr. Barrows will also be testing liquid soy lecithin to determine substitution levels. We predict that it will be easier to have higher substitution levels with poultry fat than with soy lecithin due to the natural emulsifying action of the latter. Feed formulations containing one or two substitution levels each of poultry fat and lecithin will have more than adequate levels of essential fatty acids from fish meal and added fish oil.

The suitability of these feeds compared to a control feed containing only added fish oil and to a commercial trout feed will be tested in rainbow trout, starting weight ~100 g. Diets will be fed for 16 weeks, and the usual nutritional indices will be measured, and tests for product yield, quality, and shelf stability will be determined. The *in vivo* digestibility coefficients of the diets will be measured in the fecal sedimentation tanks. Levels of TBARS in the feeds and fillets will be analyzed to see if the lipid ingredients affected oxidative stability. In addition, the fatty acid profiles of the diets, fillets, and viscera will be measured at the Hagerman Laboratory to determine the retention rate and distribution of the dietary fatty acids. Fatty acid analysis of the fillets will also be used to determine oxidative stability in fillets stored at refrigerated and freezing temperatures.

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.*

A. *Analysis of degree of hydrolysis using o-phthaldialdehyde (OPA)*

A protocol for determining *in vitro* degree of hydrolysis (DH) using OPA was developed which require less time than the previously mentioned *in vitro* method. This method also allows for analysis of many samples at one time. Each fish feed is digested either with 4-enzyme mixture (described by Satterlee et al. 1988) or with a similar method using trout ceca enzymes (described by Dimes and Haard, 1994). The amount of free amino groups produced due to the hydrolysis of peptide bonds in the protein, which is measured using OPA reagent, is used in order to measure the DH%.

B. *Standard curve*

The procedure in part (A) was used to prepare a standard curve for determining *in vivo* %DH from *in vitro* %DH. Twelve feed ingredients, including seven animal products and five plant products, were used in determining the standard curve. The ingredients were menhaden premium meal, herring meal, feather meal, anchovy meal, poultry by-product meal, de-boned fish meal, soybean meal, wheat flour, wheat gluten, corn gluten, wheat middling, and casein as the control. Digestibility with both the 4-enzyme mixture and trout ceca enzyme preparation was used.

The nitrogen content of the twelve ingredients was analyzed by the DNAR analytical lab facility on the University of California, Davis campus. Nitrogen content ranged from 2.5% to 13.5%. Apparent DH% (ingredients) ranged from 85.9% for feather meal to 100% for casein, wheat flour, and wheat gluten. Percent degree of protein hydrolysis by OPA method (%DH) with the 4-enzyme system ranged from 12.87% for feather meal to 36.51% for casein. With the trout pyloric ceca enzymes, the %DH ranged from 9.55% for feather meal to 27.63% for casein.

Correlation between apparent degree of hydrolysis and *in vitro* degree of hydrolysis and the respective standard curves were calculated. In addition to correlation analysis of twelve feeds, analysis was carried out separating plant feeds and animal feeds. Goodness of fit for all the feeds: $r^2=0.45$ and $r^2=0.38$ for 4-enzyme and trout enzymes, respectively. A better and significant correlation was observed when considering only the animal feeds ($r^2=0.91$, 4-enzymes; $r^2=0.76$, trout; $p<0.05$). In all cases, 4-enzymes showed a better correlation than trout enzymes.

In addition to the above twelve feeds, two more sets of feed (from two different studies) were analyzed for degree of protein digestibility using OPA method. First were five animal feeds including Chilean Noruega, Chilean Negativo, Chilean Positivo, Norsea Mink, and herring meal. The other set is from a soybean study with 3 diets: Diet 1 (No soy protein), Diet 3 (50% soy protein) and Diet 5 (100% soy protein).

C. Purification of trout pepsinogen

Attempts have been made to establish a purification procedure for gastric proteases from rainbow trout. For this purpose, stomachs from three years old rainbow trout (fresh water) raised in the aquaculture facility on campus were used. Extraction and purification of gastric zymogens was performed using a neutral buffer. A protease assay with acid denatured hemoglobin and electrophoresis were used as protease detection methods. Preliminary work has shown that there are two major zymogens and possibly one minor zymogen present in the rainbow trout gastric mucosa. More work is necessary to refine the methods for native electrophoresis.

Several purification techniques were followed: ammonium sulfate precipitation, chromatofocusing, gel filtration, and ion-exchange chromatography. The purification sequence of alkaline extract, ammonium sulfate precipitation, gel filtration, and anion exchange chromatography will be evaluated next.

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.

In vivo digestibility study

This study was conducted at the University of Idaho Fish Culture Experiment Station at Hagerman, Idaho under the direction of Dr. Ron Hardy. Three feed ingredients, including fish meal, des moines and bone meal, were tested. The materials were ground using a hammer mill with three different screen sizes (0.6, 1.6, and 3.0 mm, referred as Fine, Medium and Coarse, respectively) for fish meal and des moines, and two screen sizes (Fine and Coarse) for bone meal. The 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 (0.3 mm). The particle size distributions of ground fish meal for three screen sizes were determined. When the feed pellets were made for the tests, 30 parts of each ingredient was combined with 70 parts of the casein-gelatin, semi-purified diet (basal diet) by weight. The mash contained chromic oxide as the inert marker and the pellets were cold extruded.

The particle size of the test ingredients had no significant effect ($P>0.05$) on the digestibility of dry matter and crude protein. The digestibility of phosphorus in des moines group was significantly ($p=0.0064$) lower in the coarse particle treatment than the other treatments of smaller particle sizes. This pattern, however, was not observed in the bone meal group and the fish meal group, where no significant difference was observed among treatments. The reason for the lower digestibility of phosphorus in the coarsely ground des moines was not clear. It may have been due to an experimental error, such as if the large bone particles in the ingredient separated either from the dough whenthe ingredients were mixed or from the feed pellets.

Growth and excretion study

This study was conducted using a recirculating system in the Aquaculture Research and Teaching Laboratory at Washington State University on Pullman campus. The system consisted of ten 125-gallon tanks, a PBF-10 plastic beads biofilter (Aquaculture Systems Technologies, LLC., New Orleans, LA), a 40 W UV light (Aqua Ultraviolet, Temecula, CA), a sump, a chiller, and an ADC-1 data acquisition and control board system (Remote Measurement Systems, Inc., WA). One hundred eighty rainbow trout were randomly distributed into six tanks of the system, each tank containing 30 fish.

Feed pellets, with the same experimental diets as the research group used, were made at Bozeman Fish Technology Center of the US Fish and Wildlife Service under the direction of Dr. Rick Barrows. The main ingredients, including fish meal, wheat flour and soybean meal, were mixed, and then ground using a CSCREW FE-401 super fine mill (Jacobson Machine Works, Inc., Minneapolis, MN), and a 66-B hammer mill (Jacobson Machine Works, Inc., Minneapolis, MN) with two different screen sizes (1.0, and 3.0 mm). The super fine mill produced the particle size referred as Fine, and the hammer mill produced Medium and Coarse particles corresponding to the two screen sizes, respectively. After minor diet ingredients were mixed in, the materials were pelleted into sinking pellets (3 mm in diameter) using a DNDL-44 pellet machine (Buhler, Switzerland). At the end of the trial, there were no significant difference in fish weight gain and feed conversion ratio between the test groups fed with the tested pellets of different particle sizes. The results of the excretion study have not been completely analyzed.

Objective 6. To draft a RAC Results publication, **"New Test Measures Protein Digestibility"**. This publication was printed in August 1999 and is included with this report (Attachment 1).

USEFULNESS OF FINDINGS

1. *There does not appear to be an advantage to feeding 30% rather than 15% lipid diets; on the contrary, there may be disadvantages.*

While there were no significant differences in body weight gain, feed intake, FCR, SGR, or lipid concentration in the fillets, there were significantly higher visceral somatic indices (higher visceral weight compared to total body weight) in fish fed the 25% and 30% lipid diets, and a higher percentage of visceral lipid in the 30% group than in the 10% group. This means that when the higher lipid diets are fed, more of the dietary calories may be deposited as lipid in the viscera, increasing the weight of this waste product.

The data also indicate that even though the fillets from fish fed the different diets had the same concentration of lipid, the fillets from the fish fed the higher lipid diets tended to have higher TBARS, to be paler in color, and to have a stronger aroma than the fillets from the 10% dietary treatment.

2. *The manufacturing technique for pelleting fish feeds does not appear to significantly affect body weight gain, but there are significant differences among some of the groups in feed intake and FCR.* The reason for these differences will be tested further by measuring digestibility coefficients, TBARS, and other tests on the feeds.
3. *It may be possible to partially substitute fish oil in feeds with other lipid ingredients that are less expensive, such as poultry fat.*
   The pilot study will begin in Autumn 1999.
4. *A protocol for determining in vitro degree of hydrolysis using o-phthaldialdehyde (OPA) was developed which requires less time than the previously developed in vitro method.*
   This method also allows for analysis of many samples at one time.
5. *Ingredient particle size in the range tested in this study had no significant effect on trout growth and the digestibility of dry matter, crude protein and phosphorus.*
   These results, upon further verification, will be useful for determining the optimal grinding size, reducing feed production costs, and maintaining higher feed conversion efficiency.

WORK PLANNED FOR NEXT YEAR

The timeline for 1999-00 (Year 3) will be:

1. Retest the effects of feeding 30% versus 15% lipid in extruded feeds.
2. Determine the effect of feed manufacturing techniques on nutritional indices and feed and food fish product quality.
3. Determine the maximum levels of poultry fat and lecithin that can be incorporated in extruded diets.
4. Determine the effects of feeds containing poultry fat or lecithin on fish performance and food fish product quality.
5. Improve the accuracy and further validate the OPA method of in vitro digestibility. Further verify that particle size has no significant effect on trout growth and on the digestibility of dry matter, crude protein and phosphorus.
6. Futher verify that particle size has no significant effect on trout growth and on the digestibility of dry matter, crude protein and phosphorus.
7. Start to develop phase-feeding programs to optimize the benefits of high nutrient diets, manufacturing processes, and alternate feed ingredients on fish growth, performance, feed effciency ratios, nutrient retention, and product quality.

IMPACTS

1. The optimum lipid level in post-juvenile rainbow trout diets appears to be between15% and 25% lipid.
2. Different pelleting technologies may affect fish performance.
3. It may be possible to significantly substitute part of the added fish oil in feeds with other lipid ingredients.

4. An *in vitro* method for protein digestibility requires less time and operating expense than *in vivo* methods.
5. Determining the optimal particle size of feed ingredients will help to reduce feed costs, to reduce pollution by increasing digestion and absorption, and to maintain high conversions of feed to body weight gain.

SUPPORT

FISCAL YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
98	70,000	41,000	14,200	30,000	7,000	92,200	162,200

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

Arndt, R.E., Hardy, R.W., Sugiura, S.H., and Dong, F.M. 1999. Effects of heat treatment and substitution level on palatability and nutritional value of soy defatted flour in feeds for coho salmon (*Oncorhynchus kisutch*). Aquaculture 180: 129-145.

Gildberg, A., Simpson, B.K., and Haard, N.F. 1999. Uses of Enzymes from Marine Organisms. Chapter 22. In: Seafood Enzymes. N.F. Haard and B.K. Simpson, eds. Marcel Dekker Inc., N.Y. (in press).

Haard, N.F. 1999. The Role of Adaptation and Other Intraspecific Factors. Chapter 1, In: Seafood Enzymes. N.F. Haard and B.K. Simpson, eds. Marcel Dekker, Inc., N.Y. (in press).

Hardy, R.W. October, 1998: US Trout Farmers Association, Annual Meeting, Twin Falls, Idaho, panel discussion on high-energy feeds for trout.

Hardy, R.W. October, 1998: American Feed Industry Assoc., Aquaculture Committee, Twin Falls, Idaho. Presentation on alternative feed ingredients and high-energy feeds for farmed fish.

Schwertner, M.A., Hardy, R.W., Barrows, F.T. and Dong, F.M. Effects of high energy feeds on performance and product quality of post-juvenile rainbow trout (*Oncorhynchus mykiss*). Poster presented at the Institute of Food Technologists annual meeting, Chicago, IL, July 1999.

Sugiura, S.H., Raboy, V., Young, K.A., Dong, F.M., and Hardy, R.W. 1999. Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 170: 285-296.

Sugiura, S.H., Dong, F.M., and Hardy, R.W. 1999. Rapid response of rainbow trout to dietary phosphorus concentrations. Aquaculture Nutrition (submitted).

Sugiura, S.H., Dong, F.M., and Hardy, R.W. 1999. Requirement of rainbow trout for dietary phosphorus. J. Nutrition (submitted).

Weerasinghe, V. and Haard, N.F. 1999. *In vitro* method for phosphorous digestibility of trout *(Oncorhynchus mykiss*) feed. Aquaculture Nutrition (submitted).

Weerasinghe, V. and Haard, N.F. 1999. *In vitro* digestibility of fish feed using digestive enzymes from the test organism. In: Proceedings of 43rd Atlantic Fisheries Technology Conference (F. Shahidi, ed.) (in press).