PART I: SUMMARY

PROJECT TITLE: Cost-effective, alternative protein diets for rainbow trout that support optimal growth, health and product quality

REPORT GIVEN IN YEAR: 2010

REPORTING PERIOD: 04/16/2010-09/01/2010

AUTHOR: Wendy M. Sealey

FUNDING LEVEL: $119,864

PARTICIPANTS:

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246 Third Ave. E.
Twin Falls, ID 83301
208/734-9590

Technical Advisor: Chris Nelson, Nelson and Sons, Inc.

PROJECT OBJECTIVES:

Objective 1: Identify commercially available alternate ingredient combinations that can meet the production needs of rainbow trout.

Objective 2: Refining alternative feedstuff blends and examining the benefits of amino acid supplementation, CSU, USFWS-Gaylord and USDA – year 2.

Objective 3: Examining the effects of alternative feedstuffs on product quality and fish health.

Objective 4: On-farm trial of alternative feedstuff formulations at Magic Springs Farm, SeaPac of Idaho, year 3.

Objectives 5-8: (Outreach, U of I, Fornshell, years 1-3)

5. Develop a project website on the WRAC homepage and update regularly.
6. Present research results in cooperation with field day and meetings.
7. Develop at least one WRAC Extension publication,"*Alternative ingredient utilization in trout diets*"
8. Conduct direct site visits to Western region feed manufacturers' plants for presentation of projects results.

ANTICIPATED BENEFITS: The aquaculture industry will benefit from the results obtained from objective 1a and 1b through an ability to formulate trout diets utilizing the ingredients tested on an equal digestible nutrient basis. The benefits from Objective 1a to determine the compositional analysis of alternative ingredients for inclusion into trout feeds and Objective 1b to determine the digestibility and availability of those nutrients in extruded feedstuffs to the trout being fed those ingredients will allow for formulation of nutritionally balanced diets with the alternative ingredients. The data will provide an increased understanding of the amino acid needs of trout and the amino acid content and availability of commercially available alternative feed ingredients can be utilized to identify appropriate ingredient combinations, thus maintaining the ability to produce cost-effective feeds while maintaining fish growth, health and product quality.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS: To address year one's objectives, the methods of Cho et al. (1982) and Bureau et al. (1999) were used to estimate apparent digestibility coefficients (ADCs). Yttrium oxide served as the inert maker. A complete reference diet meeting or exceeding all known nutritional requirements of trout was blended with the test ingredients in a 70:30 ratio (dry-weight basis) to form test diets. Diets were manufactured by cooking extrusion (DNDL-44, Buhler AG, Uzwil, Switzerland) and top-coating with fish oil using a vacuum coater (A.J. Mixing, Ontario, Canada). Digestibility trials for rainbow trout were conducted at the Bozeman Fish Technology Center. Each diet was fed to three different tanks of fish for 14 days prior to fecal collection. Fecal samples were obtained in three collections by manual stripping, 16-18 h post-feeding for trout. Fecal samples for a given tank were freeze dried and stored at -20 °C until chemical analyses were performed.

Over 25 commercially available and novel ingredients have been sourced. Analysis of ingredient quality was conducted through standard procedures (AOAC 1995) to determine the proximate composition (moisture, ash, crude protein, starch, lipid and gross energy) of the feed ingredients as well as the mineral and amino acid content. Apparent digestibilities of nutrients and energy and amino acid availability from the ingredients in compounded, extruded diets have also been determined.

USEFULNESS OF FINDINGS: With the rapid rise in feed ingredient costs likely to continue for the foreseeable future and the finite source of fish meal, alternative aquafeed ingredients are necessary to minimize feed cost. An improved understanding of a wider variety of ingredients also improves formulation security and can help buffer feed price fluxes by providing nutritionists a variety of ingredients to choose from while still meeting nutrient demands when competition for high protein ingredient occurs and/or a currently utilized ingredient becomes unavailable. Limited data on the nutritive value of an ingredient, or synthetic amino acids, may be reasons that alternative ingredients have generally yielded sub-optimal performance. The data generated by these objectives can be utilized by researchers to improve study designs for assessing performance of alternative ingredients.

WORK PLANNED FOR NEXT YEAR: Objective 1c: The objective to determine the growth response to alternative ingredient blends and effects on palatability and potential alterations in oxygen demands will be addressed in year two utilizing ingredients chosen from year 1 screening for nutrient composition and digestibility.

Objective 2: Refinement of feedstuff blends will progress based on results from year 1 and Objective 1c. Feeding trials will be performed at Colorado State University and Bozeman Fish Technology Center to determine an optimized blend of ingredients and amino acid supplementation to maximize nutrient utilization and growth. Effects of to these blends on nutrient assimilation and metabolic demands will be measured as previously described.

Objectives 5-8: During the second year of the project, work to address the extension and outreach objectives will also begin. Specifically, a project website will be started.

IMPACTS: None to date.

SUPPORT:

YEAR	WRAC USDA Funding	OTHER SUPPORT					Total Support
		University	Industry	Other Federal	Other	Total	
2010	119,864						
TOTAL	**119,864**						

SUBMITTED BY: Wendy M Sealey 9/09/2010
Title: (Wendy M Sealey, Work Group Chair) Date

APPROVED: Chris Nelson 9/10/2010
Technical Advisor (Chris Nelson) Date

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

Papers presented:
Wendy M. Sealey, T. Gibson Gaylord, Frederic T. Barrows, Carolyn Ross, Chris Myrick and Gary Fornshell, "Cost-effective, alternative protein diets for rainbow trout that support optimal growth, health and product quality", USTFA session of the World Aquaculture Society Meeting in San Diego, CA in March 2010.

PART I: DETAILS

PROJECT TITLE: Cost-effective, alternative protein diets for rainbow trout that support optimal growth, health and product quality

REPORT GIVEN IN YEAR: 2010

REPORTING PERIOD: 04/16/2010-09/01/2010

AUTHOR: Wendy M. Sealey

FUNDING LEVEL: $119,864

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Technical Advisor: Chris Nelson, Nelson and Sons, Inc.

PROJECT OBJECTIVES:

Objective 1: Identify commercially available alternate ingredient combinations that can meet the production needs of rainbow trout.

Objective 1a (Chemical analyses of alternative ingredients, USFWS-Gaylord– year 1)

Objective 1b (Digestibility of alternative ingredients in extruded diets, USFWS-Gaylord and Sealey and USDA – year 1)

Objective 1c: (Preliminary growth and respirometry trials with blended alternative feedstuffs, CSU and USDA - year 1).

Objective 2: Refining alternative feedstuff blends and examining the benefits of amino acid supplementation, CSU, USFWS-Gaylord and USDA – year 2.

Objective 3: Examining the effects of alternative feedstuffs on product quality and fish health.

Objective 3a (Product quality, WSU-year 2 and 3)

Objective 3b (Fish health, USFWS-Sealey-years 1-3)

Objective 4: On-farm trial of alternative feedstuff formulations at Magic Springs Farm, SeaPac of Idaho, year 3.

Objectives 5-8: (Outreach, U of I, Fornshell, years 1-3)

5. Develop a project website on the WRAC homepage and update regularly.
6. Present research results in cooperation with field day and meetings.
7. Develop at least one WRAC Extension publication,"*Alternative ingredient utilization in trout diets*"
8. Conduct direct site visits to Western region feed manufacturers' plants for presentation of projects results.

ANTICIPATED BENEFITS: The aquaculture industry will benefit from the results obtained from objective 1a and 1b through an ability to formulate trout diets utilizing the ingredients tested on an equal digestible nutrient basis. The benefits from Objective 1a to determine the compositional analysis of alternative ingredients for inclusion into trout feeds and Objective 1b to determine the digestibility and availability of those nutrients in extruded feedstuffs to the trout being fed those ingredients will allow for formulation of nutritionally balanced diets with the alternative ingredients. The data will provide an increased understanding of the amino acid needs of trout and the amino acid content and availability of commercially available alternative feed ingredients can be utilized to identify appropriate ingredient combinations, thus maintaining the ability to produce cost-effective feeds while maintaining fish growth, health and product quality.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS: Year 1: To address year one's objectives, the methodologies employed included standard procedures that are well established in the investigators' laboratories (e.g., Gaylord et al. 2004). The methods of Cho et al. (1982) and Bureau et al. (1999) were used to estimate apparent digestibility coefficients (ADCs). Yttrium oxide served as the inert maker. A complete reference diet meeting or exceeding all known nutritional requirements of trout was blended with the test ingredients in a 70:30 ratio (dry-weight basis) to form test diets. Diets were manufactured by cooking extrusion (DNDL-44, Buhler AG, Uzwil, Switzerland) with an 18-s exposure to an average of 127 °C in the sixth extruder barrel section. The die plate was cooled to an average temperature of 60 °C. Pressure at the die head varied from 200 to 320 psi, depending on test diet. Pellets of 3-mm were produced then dried in a pulse-bed drier (Buhler AG, Uzwil, Switzerland) for 25 minutes at 102 °C with a 10-minute cooling period. Final moisture levels were less than 10%. The final step was top-coating the dried and cooled feed with fish oil using a vacuum coater (A.J. Mixing, Ontario, Canada). For the digestibility trials, rainbow trout were cultured at the Bozeman Fish Technology Center. Fish weighing approximately 200-g each were stocked at a rate 30 per 140-L poly tank. Water temperature was maintained at 14 °C. Lighting was maintained on a 13:11 h diurnal cycle. Each diet was fed to three different tanks of fish. Each diet was randomly assigned to a tank of fish and fed to apparent satiation twice daily for 14 days prior to fecal collection. Fecal samples were obtained in three collections by manual stripping, 16-18 h post-feeding for trout. Manual stripping of all fish in each tank was accomplished by netting and anesthetizing the fish, followed by gently drying and then applying pressure to the lower abdominal region to express fecal matter into a plastic weighing pan. Care was taken to exclude urinary excretions from the collection. Fecal samples for a given tank were freeze dried and stored at -20 °C until chemical analyses were performed.

Over 25 commercially available and novel ingredients have been sourced (Table 1). Analysis of ingredient quality was conducted through standard procedures (AOAC 1995) to determine the proximate composition (moisture, ash, crude protein, starch, lipid and gross energy) of the feed ingredients as well as the mineral and amino acid content (Table 2). Apparent digestibility of nutrients and energy (Table 3) and amino acid availability (Table 4) from the ingredients in compounded, extruded diets is ongoing.

Table 1. Dry matter, fat, protein, energy and phosphorus composition of ingredients assessed for nutrient digestibility.						
Ingredient		DM	Fat	Protein	Energy	Phosphorus
		%	%	%	(cal/g)	%
			Dry weight basis			
Soybean meal, solvent extracted dehulled		94.12	1.74	51.9	4685	0.8
Soy Protein Concentrate, Profine VF, Solae		96.06	0.03	72.2	4710	1.0
Barley Protein Concentrate, Montana Microbial		92.74	5.89	56.9	5577	1.0
Corn Protein Concentrate, Empyreal 75, Cargill		94.18	4.16	81.3	5896	0.3
Spirulina, Carbon Capture Corporation		93.01	2.19	61.5	4818	1.4
Spirulina, Earthrise		95.05	0.51	72.7	5252	1.3
Menhaden fish meal - Special Select, Omega Protein		93.34	7.58	69.4	5719	3.3
Krill meal,		94.30	22.17	56.3	4659	1.5
NuPro, Alltech		94.99	0.52	48.1	4605	1.7
Blood meal, poultry 13		94.19	0.93	98.8	5201	0.3
Blood meal, poultry 8521		90.50	0.68	102.3	6171	0.2
Soy product modified, Hamlet Protein		92.82	1.26	59.1	4851	0.8
Chicken concentrate, American Dehydrated Food		93.96	17.08	78.1	6102	0.9
Soy protein, fermented, Omnitek		97.39	1.13	60.0	4515	0.9
ADM Biomass, Archer Daniels Midland		96.18	6.59	82.0	5731	1.1
Distillers Dried Grains With Solubles, HiProtein						
Distillers Dried Grains With Solubles, Wentworth						
Distillers Dried Grains With Solubles, Valero						
Soybean meal, triple null						
Soybean meal, Williams unheated						
Soybean meal, Williams heated						
Soy Protein Concentrate, Selecta 60, Solae						
Distillers Dried Grains With Solubles Protein Isolate						
Distillers Dried Grains With Solubles Mineral concentrate						
Algae Meal, Kent Bio Energy						
Earthworm meal						

Table 2. Amino acid composition of ingredients assessed for nutrient digestibility.

		Sum AA	Ala	Arg	Asp	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser
		%	%	%	%	%	%	%	%	%	%	%	%	%	%
		Dry weight basis													
Soybean meal, solvent extracted dehulled		52.2	2.4	4.4	6.1	9.4	2.2	1.3	2.3	4.5	2.7	0.7	2.9	2.8	3.0
Soy Protein Concentrate, Profine VF, Solae		75.8	3.3	6.3	9.0	14.3	3.3	1.9	3.4	6.0	3.9	1.0	4.0	4.4	4.5
Barley Protein Concentrate, Montana Microbial		60.0	2.5	3.4	3.5	15.4	2.2	1.2	2.3	4.6	1.8	0.9	3.7	7.4	3.0
Corn Protein Concentrate, Empyreal 75, Cargil		95.6	7.9	2.9	5.4	20.0	2.4	1.8	3.4	15.1	1.5	2.1	5.8	9.3	5.4
Spirulina, Carbon Capture Corporation		59.8	4.4	4.7	6.1	9.7	3.0	0.9	3.1	5.3	2.3	1.3	2.9	2.5	3.3
Spirulina, Earthrise		70.2	5.2	5.8	7.4	11.1	3.5	1.1	3.7	6.1	2.8	1.6	3.3	2.9	3.9
Menhaden fish meal - Special Select, Omega Protein		66.3	4.7	4.8	6.5	9.7	5.6	1.4	2.8	5.1	4.4	2.1	2.9	3.9	3.2
Krill meal,		55.2	3.1	3.7	6.0	7.4	2.7	1.2	2.9	4.7	3.5	1.7	4.1	2.8	2.8
NuPro, Alltech		44.7	3.3	2.7	5.1	6.5	2.1	1.0	2.2	3.5	3.1	0.7	2.2	1.8	2.8
Blood meal, poultry 13															
Blood meal, poultry 8521															
Soy product modified, Hamlet Protein															
Chicken concentrate, American Dehydrated Food															
Soy protein, fermented, Omnitek															
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Soybean meal, Williams unheated															
Soybean meal, Williams heated															
Soy Protein Concentrate, Selecta 60, Solae															
Distillers Dried Grains With Solubles Protein Isolate															
Distillers Dried Grains With Solubles Mineral concentrate															
Algae Meal, Kent Bio Energy															
Earthworm meal															

Table 3. Apparent digestibility coefficients for dry matter, fat, protein, energy and phosphorus from ingredients fed to rainbow trout.						
		DM	Fat	Protein	Energy	P
Soybean meal, solvent extracted dehulled		75.3	85.8	95.9	80.8	10.1
Soy Protein Concentrate, Profine VF, Solae		94.3	187.3	103.1	98.0	35.0
Barley Protein Concentrate, Montana Microbial		97.3	102.9	99.7	96.9	82.4
Corn Protein Concentrate, Empyreal 75, Cargill		95.0	91.7	89.5	91.3	58.2
Spirulina, Carbon Capture Corporation		77.5	85.4	80.5	75.4	83.5
Spirulina, Earthrise		83.9	69.7	80.3	83.1	91.7
Menhaden fish meal - Special Select, Omega Protein		98.3	101.9	91.7	104.5	57.3
Krill meal,		85.0	99.1	84.4	92.0	59.9
NuPro, Alltech		19.9	5.8	36.7	24.8	62.7
Blood meal, poultry 13		76.3	98.7	86.1	82.7	60.7
Blood meal, poultry 8521		77.9	-39.1	79.2	73.9	174.2
Soy product modified, Hamlet Protein		79.1	36.9	73.4	73.3	108.2
Chicken concentrate, American Dehydrated Food		67.6	89.6	89.6	72.7	44.8
Soy protein, fermented, Omnitek		70.3	7.1	85.1	90.6	10.1
ADM Biomass, Archer Daniels Midland		53.9	78.0	90.1	68.0	-4.6
Distillers Dried Grains With Solubles, HiProtein						
Distillers Dried Grains With Solubles, Wentworth						
Distillers Dried Grains With Solubles, Valero						
Soybean meal, triple null						
Soybean meal, Williams unheated						
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Soy Protein Concentrate, Selecta 60, Solae						
Distillers Dried Grains With Solubles Protein Isolate						
Distillers Dried Grains With Solubles Mineral concentrate						
Algae Meal, Kent Bio Energy						
Earthworm meal						

Table 4. Apparent digestibility coefficients for amino acids from ingredients fed to rainbow trout.

	Sum AA	Ala	Arg	Asp	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
Soybean meal, solvent extracted dehulled	96	97	99	95	95	95	97	97	97	97	97	91	96	94	92	98	96
Soy Protein Concentrate, Profine VF, Solae	102	104	102	99	101	101	103	102	104	101	105	102	103	102	100	104	102
Barley Protein Concentrate, Montana Microbial	98	96	99	96	98	96	96	100	99	94	99	100	99	97	96	100	97
Corn Protein Concentrate, Empyreal 75, Cargil	90	91	92	87	90	85	87	88	91	88	92	91	91	91	86	92	88
Spirulina, Carbon Capture Corporation	82	79	78	81	85	84	82	84	80	88	91	78	87	79	87	86	75
Spirulina, Earthrise	84	82	74	83	82	88	88	87	86	90	95	87	95	85	90	89	76
Menhaden fish meal - Special Select, Omega Protein	94	93	95	89	95	85	94	98	99	94	95	96	92	94	95	99	96
Krill meal,	82	93	93	79	86	83	82	92	91	89	88	39	85	85	83	45	90
NuPro, Alltech	46	54	59	68	47	36	35	46	41	53	47	26	33	36	31	51	37
Blood meal, poultry 13																	
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Algae Meal, Kent Bio Energy																	
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USEFULNESS OF FINDINGS: With the rapid rise in feed ingredient costs likely to continue for the foreseeable future and the finite source of fish meal, alternative aquafeed ingredients are necessary to minimize feed cost. An improved understanding of a wider variety of ingredients also improves formulation security and can help buffer feed price fluxes by providing nutritionists a variety of ingredients to choose from while still meeting nutrient demands when competition for high protein ingredient occurs and/or a currently utilized ingredient becomes unavailable. Limited data on the nutritive value of an ingredient, or synthetic amino acids, may be reasons that alternative ingredients have generally yielded sub-optimal performance. The data generated by these objectives can be utilized by researchers to improve study designs for assessing performance of alternative ingredients. An improved understanding of a wider variety of ingredients also improves formulation security and can help buffer feed price fluxes by providing nutritionists a variety of ingredients to choose from while still meeting nutrient demands when competition for high protein ingredient occurs and/or a currently utilized ingredient becomes unavailable.

WORK PLANNED FOR NEXT YEAR: *Objective 1c:* **(Preliminary growth and respirometry trials with blended alternative feedstuffs, CSU and USFWS-Gaylord- year 1).** In order to assess ingredient/diet palatability and growth potential of fish fed the test ingredients identified and analyzed in objectives 1a and 1b, a series of feeding trials will be conducted using the plant-based feedstuffs and animal-based feed ingredients described above by blending to optimize nutritional profile and substitute up to 100% of the fish meal in diets for rainbow trout.

Animal Husbandry: Procedures for conducting the feeding trials are well established in each respective investigator's laboratory (e.g., Gaylord et al. 2006; Myrick and Cech 2000). All diets will be fed under standardized conditions to triplicate lots of juvenile (~20 g) fish for an appropriate length of time (~12 weeks) to assess treatment effects. Feeding will be performed twice daily to apparent satiation. In brief, experimental diets will be offered to each tank of fish over a 30-minute period until feeding activity ceases with minimal residual diet remaining in the tank at which time feed offered will be quantified.

Diet Formulation and Experimental Design: Diets will be formulated on a digestible-energy and available-amino-acid basis. Amino acid balance will be maintained to meet the known amino acid requirements or rainbow trout. All diets will be formulated to meet or exceed other known nutritional requirements of trout (NRC 1993). The control diet will be formulated to contain 45% crude protein for trout to mimic commercial formulations. Six test diets will be formulated on an available-amino-acid-basis utilizing a mixture of protein feedstuffs defined in objective 1b for which amino acid availabilities will be known. Three diets will be strictly of plant protein origin (1. a blend of commonly utilized plant proteins, 2. a blend of ingredients available commercially but limited by cost and/or availability and 3. a blend of novel produced plant proteins with future potential) and three others will be combinations of plant and animal proteins.

All diets will be manufactured at BFTC laboratory using a twin-screw cooking extruder as previously described and dried to a final moisture level of less than 7%. Each diet will be randomly assigned to a minimum of three tanks of fish. Fish will be fed by hand to apparent satiation three times each day, 6 days per week for a total of 12 weeks. The amount of feed consumed will be determined as previously described under objective 1. All fish within a tank will be counted and weighed as a group every 3 weeks. Feed consumption, weight gain, and feed conversion ratio (FCR) will be calculated according to the following formulas:

$$\text{Feed consumption} = \frac{g\ \ dry\ \ feed\ \ consumed \times 100}{100\ \ g\ \ body\ \ mass/day}$$

$$\text{Weight gain} = \frac{\left(final\ \ weight(g) - initial\ \ weight(g)\right) \times 100}{initial\ \ weight}$$

$$\text{FCR} = \frac{g\ \ dry\ \ feed\ \ fed}{g\ \ wet\ \ weight\ \ gain}$$

Whole-tank Respirometry: One variable of interest to both researchers and trout producers is the oxygen consumption rate of fish fed diets composed of alternative protein sources, because such data can provide insight into the specific dynamic action associated with processing the diets (Jobling and Spencer-Davies 1980).We will measure the oxygen consumption rate of the tanks in each treatment every 2 weeks using either a modified static respirometry approach or a modified open (flow-through) respirometry approach. In both cases the oxygen concentrations in the tanks will be measured with a Strathkelvin model 928 6-channel oxygen analyzer.

In the static respirometer approach, the inflow to the tank is temporarily stopped and the initial oxygen concentration measured. After a fixed amount of time (e.g., 15 minutes), the final oxygen concentration is measured. The oxygen consumption rate (MO_2) is then calculated using the following equation:

$$\dot{MO}_2 = \frac{\left(initial\ O_2\ concentration - final\ O_2\ concentration\right) \times Volume}{Time} \qquad \text{(Cech 1990)}$$

This value can be adjusted for atmospheric oxygen exchange at the tank's surface by depleting the oxygen level in a fishless ("blank") tank and then measuring the rate at which the concentration returns to equilibrium with the atmosphere.

In the flow-through respirometry approach, the oxygen concentrations at the tank inlets and outlets are measured simultaneously with the water flow rate (Q). The oxygen consumption rate is then calculated using the following equation.

$$MO_2 = \left(C_{O_2-in} - C_{O_2-out}\right) \times Q \quad \text{(Cech 1990)}$$

In both cases, the final oxygen consumption rate (mg O_2/h) would be divided by the biomass of fish in the tank to provide the mass-specific oxygen consumption rate (Mg O_2/h/g).

Sample Collection and Analyses: At the termination of the 12-week experiment, all fish will be counted and weighed. Three fish per tank will be bled, euthanized and dissected to determine body condition indices including:

$$\text{Muscle Ratio (MR)} = \frac{\left(\text{fillet mass with ribs (g)} * 100\right)}{\text{fish mass (g)}}$$

$$\text{Hepatosomatic Index (HSI)} = \frac{\left(\text{liver mass (g)} * 100\right)}{\text{fish mass (g)}}$$

$$\text{Intraperitoneal Fat Ratio (IPF)} = \frac{\left(\text{peritoneal fat mass (g)} * 100\right)}{\text{fish mass (g)}}$$

Analysis of proximate composition and energy in ingredients, diets and fish tissues will be performed according to standard methods as previously described. Plasma and dietary amino acids will be

quantified according to Fleming et al. (1992) utilizing an Agilent 1100 series HPLC. To determine dietary amino acids, diets will be capped with nitrogen and hydrolyzed in 6 M HCl at 110°C for 16 h (AOAC 1995). For plasma amino acids, plasma proteins will be precipitated with 1.5M perchloric acid, followed by centrifugation at 17,500 x *g* for 5 minutes. All samples will be derivatized with o-phthaldialdehyde (P0532, Sigma-Aldrich Co., St. Louis, MO) immediately prior to injection on a 5 µm Agilent Hypersil AA ODS column (part number 79916AA-572, Agilent Technologies, Palo Alto, CA) using an automated injection sequence.

For the determination of whole-body protein, individual amino acid and energy retention values, ten fish will be sampled at the time of stocking and three additional fish per tank will be sampled per replicate at the end of each feeding trial. Protein retention, amino acid, and energy retention efficiency values will be calculated as follows:

$$\text{Protein retention efficiency (PRE)} = \frac{(\text{protein gain (g)} * 100)}{\text{protein fed (g)}}$$

$$\text{Amino acid retention efficiency (AARE)} = \frac{(\text{amino acid gain (g)} * 100)}{\text{amino acid fed (g)}}$$

$$\text{Energy retention efficiency (ERE)} = \frac{(\text{energy gain (kJ)} * 100)}{\text{energy fed (kJ)}}$$

Statistics: Differences among response variables will be evaluated by ANOVA and deemed significant at $P<0.05$ (SAS software program Proc GLM, Version 7, SAS Institute, Cary, NC). Where significant effects are detected comparisons will be performed using Tukey's means separation (Tukey 1953; Kramer 1956).

Objective 2: (**Refining alternative feedstuff blends and examining the benefits of amino acid supplementation, CSU and USFWS-Gaylord – year 2**) Optimization of the amino acid balance of the alternative protein diets will be performed through blending of ingredients and supplementation of amino acids. The amino acid composition of fillet muscle in rainbow trout, and relative levels, will be used as a basis for formulation of diets on an available-amino-acid basis. Growth studies will be conducted with a fish meal control diet and test diets formulated to meet an ideal amino acid profile equalized on available amino acid content.

Diet Formulation and Experimental Design: Plant-based diets will be tested against commercial-type formulations containing fish meal. Diets will be formulated on a digestible-energy and available-amino-acid basis. Amino acid balance will be maintained equivalent to the ideal amino acid profile of rainbow trout. All diets will be formulated to meet or exceed other known nutritional requirements of these species (Webster and Lim 2002). Protein sources will be blended in order to meet the ideal amino acid profile when possible and supplemental synthetic amino acids will be utilized to augment the profile when necessary. The control diet will be formulated to contain 45% crude protein for trout to mimic commercial formulations. Test diets will be formulated on an available-amino-acid-basis utilizing a mixture of protein feedstuffs defined in objective 1 for which amino acid availabilities will be known. Supplementation of diets with methionine, lysine, threonine and/or tryptophan (all are commercial available in feed grade quantities) will be adjusted as needed to meet the ideal amino acid profile determined from fillet protein. Negative control diets will be identical to the test diets but without amino acid supplementation. Each diet series will be performed in a high nutrient density diet of 45% digestible protein and 20% lipid and a low nutrient density diet of 40% digestible protein and 15% lipid with and without amino acid supplementation. Therefore nine diets will be tested: 1)

commercial control at 45 DP and 20% lipid, 2) plant 45/20, 3) plant 45/20 +AA, 4) animal 45/20, 5) animal 45/20+AA, 6) plant 40/15, 7) plant 40/15+AA, 8) animal 40/15 and 9) animal 40/15+AA. Animal husbandry, respirometry, and analytical procedures will be identical to procedures utilized in objective 1c.

During year two work on the extension and outreach objectives will also begin.

Objectives 5-8 (Outreach, U of I, Fornshell, years 1-3)

Objectives 1&2:

Target Audience: Feed manufacturers and trout growers

Intended Learning Outcomes: New information will be provided to feed manufacturers to increase precision in diet formulation for cost-effective utilization of alternative ingredients in trout feeds.

Intended Management and/or Behavioral Outcomes: Manufacture of cost-effective low fish meal diets based on project results.

Inputs: Specific inputs are formulation limits and data regarding potential of alternative ingredients produced by research PIs. See budget for funding of individual PIs. PI for Outreach knows all the trout feed manufacturers in the west and will contact them personally. Trout growers will be contacted through their respective feed suppliers, current mailing lists, Extension contacts, and the US Trout Farmers Association.

Outputs: Project webpage, popular press articles in relevant publications, and presentations at relevant state, regional and national aquaculture meetings, and WRAC Extension publication, "*Alternative ingredient utilization in trout diets*" and direct consultation of projects PI's with regional feed manufacturers. The project webpage, popular press articles, and presentations at aquaculture meetings will inform stakeholders of the project's progress and provide a mechanism to rapidly distribute relevant information during the life of the project. The WRAC Extension publication will provide the scientific rationale (based on project results) for the use of alternative ingredients in reduced fish-meal trout feed formulations.

Evaluation Plan: Interviews and survey of feed manufacturers to determine if their formulations are incorporating research results.

Objective 3:

Target Audience: Trout growers and feed manufacturers

Intended Learning Outcomes: Knowledge of changes to fillet quality and consumer preference due to alternative ingredients and reduction of fish meal in the feeds.

Intended Management and/or Behavioral Outcomes: Willingness to use new feeds if there are no negative impacts to fillet quality.

Inputs: Results of trained taste panel and fillet composition results. PI for Outreach has regular mail and email lists for all trout growers in Idaho and knows how to contact growers in other states. In addition, trout growers will have the opportunity to taste test trout fed the different feeds (see objective 4 below).

Outputs: Same as above.

Evaluation Plan: Present the results of the trained taste panel and fillet composition to trout growers at state or regional trout aquaculture meetings, and survey them as to whether or not they would use the new feeds based on the results.

Objective 4:

Target Audience: Trout growers and feed manufacturers

Intended Learning Outcomes: Knowledge of how alternative feeds compare to conventional trout feed and the effect on taste and fillet composition.

Intended Management and/or Behavioral Outcomes: Adoption of the alternative feeds by growers and the manufacture of the feeds by feed companies based on research results.
Inputs: Trout production facility for field trial.
Outputs: Same as above, with the addition of a field day at the end of the demonstration trial where growers and feed manufacturers can see the results of the trial as they compare the fish fed the different feeds and sample them for potential differences in taste due to the feeds. With the assistance of Carolyn Ross, design a statistically valid triangle taste test to determine if the field day participants can differentiate the trout fed different feeds. Posters, handouts, and presentations will be part of the field day.

IMPACTS: None to date.

SUPPORT:

YEAR	WRAC USDA Funding	OTHER SUPPORT					Total Support
		University	Industry	Other Federal	Other	Total	
2010	119,864						
TOTAL	119,864						

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:
Papers presented:
Wendy M. Sealey, T. Gibson Gaylord, Frederic T. Barrows, Carolyn Ross, Chris Myrick and Gary Fornshell, "Cost-effective, alternative protein diets for rainbow trout that support optimal growth, health and product quality", USTFA session of the World Aquaculture Society Meeting in San Diego, CA in March 2010.

SUBMITTED BY: Wendy M Sealey 09/09/2010
Title: (Wendy M. Sealey, Work Group Chair) Date

APPROVED: Chris Nelson 09/10/2010
Technical Advisor (Chris Nelson) Date

INSTITUTION: University of Idaho, Extension

PRINCIPAL INVESTIGATOR: Gary Fornshell

Year 2 Budget FY2010

SALARIES:		**$0**
BENEFITS:		**$0**
	$0	
	$0	
	$0	
TRAVEL:		**$1,000**
Meeting: room (3 days x $100)	$300	
Per Diem	$200	
Airfare	$500	
SUPPLIES:		**$0**
EQUIPMENT:		**$0**
OTHER DIRECT COSTS:		**$200**
Web Page maintenance	$200	
TOTAL:		**$1,200**

INSTITUTION: Washington State University

PRINCIPAL INVESTIGATOR: Dr. Carolyn Ross

			Year 2 Budget FY2010
SALARIES:			**$9,750**
	Research Scientist(12 mts @ 0.8%)	$1,714	
	Trained Panel (12 months @1.4%)	$4,161	
	Untrained Panel (12 months @1.4%)	$3,875	
BENEFITS:			**$0**
		$0	
		$0	
		$0	
TRAVEL:			**$2,000**
	Meeting: room (4 days x $120)	$480	
	Per Diem	$350	
	Airfare	$500	
	Work Group Meeting-Idaho (3 days x 100)	$300	
	Per Diem (3 days)	$120	
	Airfare	$250	
SUPPLIES:			**$0**
EQUIPMENT:			**$0**
OTHER DIRECT COSTS:			**$0**
TOTAL:			**$11,750**

INSTITUTION: USDA, ARS

PRINCIPAL INVESTIGATOR: Dr. Rick Barrows

Year 2 Budget FY2010

SALARIES:		**$0**
BENEFITS:		**$0**
TRAVEL:		**$0**
SUPPLIES:		**$4,500**
Extruder supplies	$4,000	
Ingredients & vitamins for feeds	$500	
EQUIPMENT:		**$0**
OTHER DIRECT COSTS:		**$1,500**
Shipment of feed	$1,500	
TOTAL:		**$6,000**

INSTITUTION: Colorado State University

PRINCIPAL INVESTIGATOR: Dr. Chris Myrick

		Year 2 Budget FY2010
SALARIES:		**$24,582**
Graduate student(9 mts @100%)	$18,848	
Undergraduate technician(546 hrs@10.50/hr)	$5,734	
BENEFITS:		**$1,245**
Graduate Student (6.30% [Y2])	$1,187	
Undergraduate Technician (1.01%)	$58	
TRAVEL:		**$2,600**
Travel (specify destination and purpose)		
Project Workgroup Meetings - ID	$780	
Colorado Wyoming AFS Meeting - present project results	$520	
USA Aquaculture Meeting - present project results	$780	
National AFS Meeting - present project results	$520	
SUPPLIES:	$7,800	**$7,800**
EQUIPMENT:	0	**$0**
OTHER DIRECT COSTS:		**$1,000**
Publication costs	$1,000	
TOTAL:		**$37,227**

INSTITUTION:		US Fish and Widlife Service, Bozeman Fish Technology Center	
PRINCIPAL INVESTIGATOR:		Dr. Gibson Gaylord	
		Year 2 Budget FY2010	
SALARIES:			**$9,832**
	Student (12 mths @ 25%)	$9,832	
BENEFITS:			**$2,950**
	Student (@ 30%)	$2,950	
TRAVEL:			**$2,000**
	WAS Meeting: room (3 days x $100)	$300	
	Per Diem	$350	
	Airfare	$650	
	Work Group Meeting-Idaho (3 days x 100)	$300	
	Per Diem (3 days)	$150	
	Airfare	$250	
SUPPLIES:			**$4,058**
	Chemicals	$2,000	
	Fish Feeds	$1,000	
	Reagents & vitamins for feeds	$1,058	
EQUIPMENT:			**$0**
OTHER DIRECT COSTS:			**$0**
TOTAL:			**$18,840**

INSTITUTION:	USFWS Bozeman Fish Technology Center		
PRINCIPAL INVESTIGATOR:	Dr. Wendy M. Sealey		
		Year 2 Budget FY2010	
SALARIES:			**$33,403**
	Scientific aide (12 mts @ 5%)	$3,863	
	Graduate Student (12 mths @ 50%)	$24,720	
	Weekend feeder (12 mths @ 15%)	$4,820	
BENEFITS:			**$2,605**
	Scientific aide (@ __37__%)	$1,429	
	Graduate Student (@3%)	$742	
	Hourly Weekend Feeder (@ __9__%)	$434	
TRAVEL:			**$2,500**
	WAS Meeting: room (4 days x $120)	$480	
	Per Diem	$350	
	Airfare	$1,000	
	Work Group Meeting-Idaho (3 days x 100)	$300	
	Per Diem (3 days)	$120	
	Airfare	$250	
SUPPLIES:			**$6,550**
	Chemicals	$2,500	
	Fish Feeds	$600	
	Reagents & vitamins for feeds	$450	
	Histology supplies, slide processing	$3,000	
EQUIPMENT:			**$0**
OTHER DIRECT COSTS:			**$0**
TOTAL:			**$45,058**