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: 2012

REPORTING PERIOD: 09/01/2011-09/08/2012

AUTHOR: Wendy M. Sealey

FUNDING LEVEL: $118,317 (year 3)

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Project Monitor: 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 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 from year 2 feeding trials furthered that knowledge by increasing understanding of the amino acid needs of trout and identifying appropriate ingredient combinations that can meet those needs, thus maintaining the ability to produce cost-effective feeds while maintaining fish growth, health and product quality.

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

Analysis of the nutrient composition of commercially available and novel ingredients evaluated in digestibility trials in year one were completed. Proposed digestibility trails have been completed; evaluation of other potential ingredients (including feed grade plant concentrates and improved soy ingredients) continue as funding allows on a cost-sharing basis by USDA, ARS and USFWS. Results from these completed WRAC-funded evaluations and co-supported analyses have been compiled into a database entitled: *"Nutrient Digestibility of Fish Feed Ingredients*" available through rick.barrows@ars.usda.gov.

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

DDGS Trial at BFTC (Objective 1c -completed)

The protein ADCs and amino acid AACs of HPDDG coupled with its higher protein content relative to other DDG products suggested an increased potential to replace fishmeal in rainbow trout diets. Further, when diets were balanced for digestible protein, lysine, methionine and threonine, growth performance was comparable. These data indicate that 50% of dietary fishmeal can be successfully replaced by a high quality DDG product without compromising growth or necessitating mycotoxin deactivator inclusion.

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

Results BFTC: Significant effects of ingredient blends, nutrient concentration target and interactions were observed. At 12 wks post-feeding, adjusting the nutrient targets for the fish meal-based diets nor the animal product diets appear to affect fish performance. Adjustment of nutrient targets does improve performance of trout fed the three diet combinations based on plant ingredients. The improvements in growth when amino acids are supplemented to an ideal protein basis make fish performance when fed the PPD and PFP diets equivalent to FMD. Supplementing amino acids up to the ideal protein targets improved all plant based diets but the NPD treatment fish failed to attain final fish weights equivalent to the other treatments.

Results CSU: Final wet weights ranged from 382 g (PPD) to 440 g (FMD+). Feed group was found to be a statistically significant predictor variable ($F=4.70$, $P=0.0010$), with fish fed the FMD+ having the highest mean weight, followed by the PFP+ (426.6) and FMD (424.2). It should be noted that many of the means were not significantly different from each other suggesting that the performance of fish consuming these feeds is similar among many of the diets. Feed conversion ratios among feeds were generally low, ranging from 0.93 (FMD+) to 1.24 (NPD+). Feed group ($F=7.02$, $P=0.0011$) was statistically significant, while protein:lipid ratio ($F=0.0042$, $P=0.9489$) and the feed group and protein:lipid ratio interaction ($F=0.27$, $P=0.8938$) were not statistically significant at the $\alpha= 0.05$ level.

In contrast, no diet group or protein level effects on respirometry was observed in regards to SDA max or SDA duration. Respirometry data are *noisy*, likely because of whole-tank approach (fish are probably never all quiescent); however, it is a realistic simulation of a culture tank/raceway.

Objective 3: Examining the effects of alternative feedstuffs on product quality-(ongoing). Effect of diet on sensory properties of trout fed different protein blends at CSU (completed)

Fish fed the different dietary protein blends for 15 weeks at CSU as described above in Objective 2 were filleted and analyzed for consumer preference at WSU. For each protein:lipid ratio, diet treatments compared to respective FMD control. Subsequently, the different protein:lipid ratios were compared for each diet.

Panelists perceived the FMD control fillet as significantly more firm than PPD. However, the PPD^{+} fillet was perceived as possessing a more firm texture ($P\leq0.001$).

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 information exists on how alternative blends alter performance and product quality. The data generated by this year's objectives has addressed that void and can be utilized by formulators to identify appropriate ingredient combinations that maintain growth and product quality.

WORK PLANNED FOR NEXT YEAR:
Objective 2: Refinement of feedstuff blends will continue based on results from years 1 and 2. Feeding trials will be performed at Colorado State University and Bozeman Fish Technology Center to determine the minimum necessary level of lysine supplementation to a plant-based diet, evaluate how low protein can be reduced utilizing supplemental amino acids and how these changes alter nutrient utilization, growth, respirometry and product quality. The performance of these blends on nutrient assimilation and metabolic demands will also be measured in an on-farm pilot scale trial.

Objectives 5-8: During the third year of the project, work to address the extension and outreach objectives will also continue. Specifically, an outreach event in combination with the pilot scale trial will be conducted.

IMPACTS: None to date.

SUPPORT:

YEAR	WRAC USDA Funding	OTHER SUPPORT					Total Support
		University	Industry	Other Federal	Other	Total	
2012	**$118,317 $33,446[1]**	**$16,482**	**Troutlodge fish for studies ($1,000)** **Ingredient donations ($4,000)** **PilotStudy ($20,000**	**$46, 380**			
TOTAL	**$151,763**	**$16,482**	**$25,000**	**$46,380**			**$239,625**

SUBMITTED BY:_____ _____
Title: (Wendy M. Sealey, Work Group Chair) Date

APPROVED:

_____
Technical Advisor (Chris Nelson) Date

[1] CSU requested an additional $34,446 in support from WRAC for the 2012 FY to allow continued support of the graduate student and research activities.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

Year 1:

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.

Year 2:

Outreach publication:

Gary Fornshell "Evaluating Ingredients for Aquafeeds" submitted WRAC Office.

Papers presented:

Wendy M. Sealey, T. Gibson Gaylord, Frederic T. Barrows, Carolyn Ross, Chris Myrick and Gary Fornshell, "Alternative protein research in rainbow trout", USTFA Meeting in Twin Falls, ID in September 2011.

Chris G. Hooley, Kurt A. Rosenstrater, T. Gibson Gaylord, Frederic T. Barrows and Wendy M. Sealey
"Examination of the effect of a mycotoxin deactivation product to improve growth and nutrient utilization in juvenile rainbow trout *Oncorhynchus mykiss* fed high protein distiller's dried grains", fish feed nutrition workshop, Pine Bluff, AR in September 2011.

Year 3:

Outreach publications:

Gary Fornshell, Colorado Aquaculture Association, The Fish Line
"More than the fish farmer wants to know about feeds", July 2012

Gary Fornshell, Twin Falls County Extension Newsletter
"Understanding fish feeds", August 2012

Papers presented:

Chris G. Hooley, Kurt A. Rosenstrater, T. Gibson Gaylord, Frederic T. Barrows and Wendy M. Sealey
"Examination of the effect of a mycotoxin deactivation product to improve growth and nutrient utilization in juvenile rainbow trout *Oncorhynchus mykiss* fed high protein distiller's dried grains", USAS, Las Vegas, NV in February 2012.

T.Gibson Gaylord, W.M. Sealey, and F.T. Barrows
"Evaluation of ingredient combinations from differing origins (fishmeal, terrestrial animal protein and plants) and two different formulated nutrient targets on rainbow trout growth and production efficiency", USAS, Las Vegas, NV in February 2012.

Christopher D. Craft, C. A. Myrick, T. G. Gaylord, W. M. Sealey, and F. T. Barrows
"Evaluation of alternative protein sources for use in rainbow trout feeds", 10th International Congress on the Biology of Fishes, Madison, WI in July 2012

Christopher D. Craft, C. A. Myrick, T. G. Gaylord, W. M. Sealey, and F. T. Barrows
"Performance of rainbow trout fed on alternative protein diets-growth, oxygen consumption and sensory characteristics" US Trout Farmers Annual Meeting, Denver, CO in September 2012

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: 2012

REPORTING PERIOD: 09/01/2011-09/08/2012

AUTHOR: Wendy M. Sealey

FUNDING LEVEL: $119,864 (year 1); $118,645 (year 2); **$118,317 (year 3)**

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Project Monitor: 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 – Barrows, year 1)

Objective 1c: (Preliminary growth and respirometry trials with blended alternative feedstuffs, CSU, USFWS, 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 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 from year 2 feeding trials furthered that knowledge by increasing understanding of the amino acid needs of trout and identifying appropriate ingredient combinations that can meet those needs, thus maintaining the ability to produce cost-effective feeds while maintaining fish growth, health and product quality.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS: Year 2:

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

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

Analysis of the nutrient composition of commercially available and novel ingredients evaluated in digestibility trials in year one were completed. Proposed digestibility trails have been completed; evaluation of other potential ingredients (including feed grade plant concentrates and improved soy ingredients) continue as funding allows on a cost-sharing basis by USDA, ARS and USFWS. Results from these completed WRAC-funded evaluations and co-supported analyses have been compiled into a database entitled: *"Nutrient Digestibility of Fish Feed Ingredients*" available through rick.barrows@ars.usda.gov.

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

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 preliminary feeding trial was conducted using a plant-based feedstuff (HPDDG) and two concurrent feeding trials at BFTC and CSU using plant-based and animal-based feed ingredients were conducted in year 2 to address Objectives 1c and 2 simultaneously.

DDGS Trial at BFTC (Objective 1c -completed)

Experimental Design: A 2 X 2 factorial feeding trial that examined protein source (menhaden fish meal, MFM or HPDDG) and mycofix supplementation (yes or no) was conducted where a control diet (40% digestible protein, 20% crude lipid) was compared to a test diet where HPDDG replaced half of the MFM (25% inclusion) on a digestible protein basis. Diets were balanced for available lysine, methionine, threonine and total phosphorus. Biofix plus was supplemented (0.2%) to subsamples of each protein base diet via vacuum assisted top coating in the dietary oil portion. All four diets (Table 1) were then fed to four replicate tanks per treatment of juvenile rainbow trout, initial weight (39.2g ± 1.0g) for nine weeks in a 15C recirculating system. Bulk fish weight and feed intake were recorded every three weeks, At nine weeks, three fish per tank were sampled for proximate composition. However, because a significant amount of fines were observed during the first feeding trial, diets were ground and re-pelleted, and a second feeding trial that utilized the same methods and controlled for pellet quality was performed.

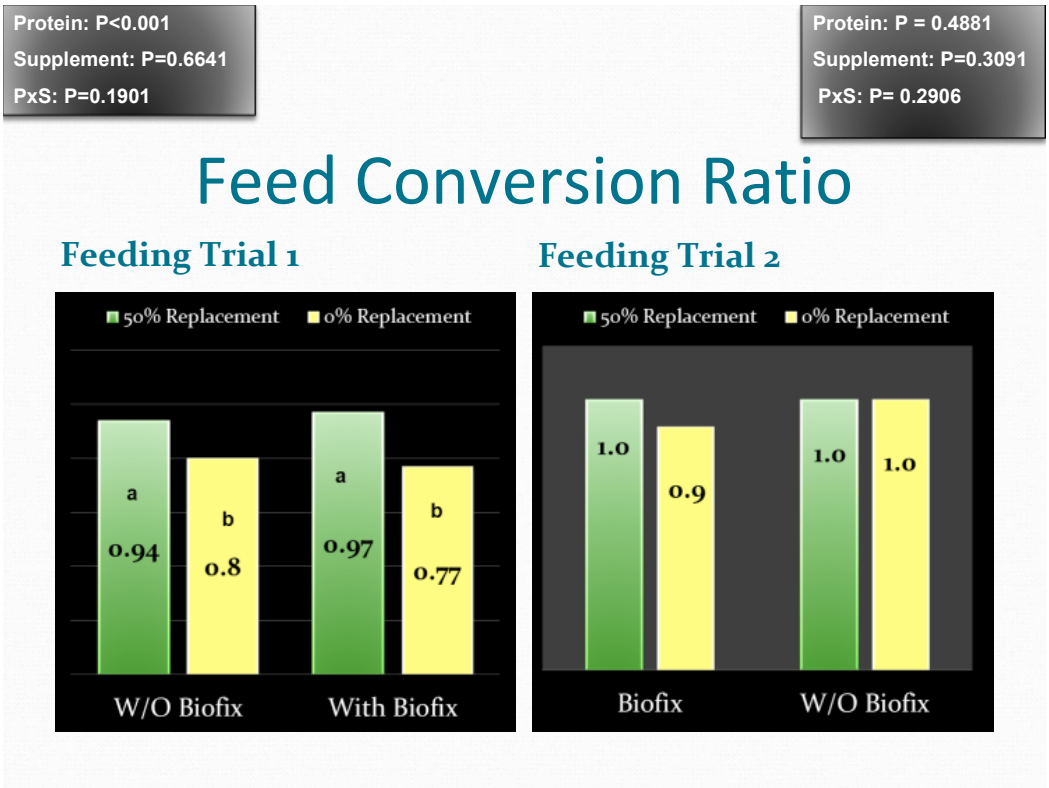
Table 1. Diets for HPDDG feeding trials

Ingredient (%DM)	FM	HPDDG
HPDDG	0.0	23.8
FM Average	24.9	13.5
Wheat flour	17.7	3.0
Corn Protein	5.0	5.0
Poultry blood meal	3.1	3.2
Soybean meal	14.9	15.1
Chicken concentrate	14.2	14.4
Menhaden fish oil	13.7	13.5
Lecithin	0.9	0.9
Stay-C 35	0.2	0.2
Vitamin premix ARS	0.9	0.9
TM ARS 640	0.1	0.1
NaCl	0.3	0.3
Magnesium Oxide	0.1	0.1
Potassium chloride	0.5	0.5
Choline Cl 50%	0.9	0.9
Taurine	0.5	0.5
Ytrium	0.1	0.1
Dical Phosphate	0.0	1.2
DL-Methionine	0.4	0.5
Lysine HCl	1.5	1.9
Threonine	0.1	0.2

Results: Calculated protein ADCs were 81, 88, and 83% for Wentworth, Valero and HPDDG, respectively. However, rainbow trout growth performance results demonstrated significant negative effects of fish meal replacement by HPDDG on growth (P=<0.0002; Figure 1) and FCR (P=<0.0001; Figure 2) in the first trial but no significant effects were observed in the second. In contrast, no significant benefit of Biofix plus supplementation or significant interaction between protein source and Biofix plus supplementation was observed in either feeding trial.

Figure 1. HPDDG Studies Growth Results

Figure 2. HPDDG Studies FCR Results



Conclusions: The protein ADCs and amino acid AACs of HPDDG coupled with its higher protein content relative to other DDG products suggested an increased potential to replace fishmeal in rainbow trout diets. Further, when diets were balanced for digestible protein, lysine, methionine and threonine, growth performance was comparable. These data indicate that 50% of dietary fishmeal can be successfully replaced by a high quality DDG product without compromising growth or necessitating mycotoxin deactivator inclusion.

Objective 2: Refining alternative feedstuff blends and examining the benefits of amino acid supplementation, CSU, USFWS-Gaylord and USDA – year 2. (Blends study completed, three other studies, ongoing)

Blends with supplemental AA Trials at BFTC and CSU (completed)

Experimental Design: For the experiment, fish from a common lot were obtained as in-kind industry support from Trout Lodge in December 2010 and divided between and cultured at BFTC and CSU, respectively. A defined starter diet was then formulated and produced by BFTC staff to ensure common dietary history until the study could be initiated. Ten test diets (Tables 2 & 3) were formulated and manufactured in adequate quantities to support the concurrent feeding trials at BFTC and CSU. At conclusion of the feeding trials (mid-September), fillet samples were obtained for assessment of fillet quality (Objective3a).

Diet Formulation and Manufacture: Diets were formulated on a digestible-energy and available-amino-acid basis. Diets were formulated in as a 5X2 factorial experiment (Tables 2 & 3).

Five ingredients combinations were utilized consisting of:

1) (Fishmeal Diet, FMD) Menhaden fishmeal special select, Soybean Meal 48%CP, Corn Protein Concentrate, Poultry by-product meal, pet food grade, and Blood meal

2) (Animal Product Diet, APD) Poultry by-product meal - pet food grade, Soybean Meal 48%CP, Corn Protein Concentrate, Feather meal, Blood meal

3) (Plant Product Diet, PPD) Soy Protein Concentrate, Corn Protein Concentrate, and Soybean Meal 48%CP

4) (Novel Plant Protein Diet-NPD) Soy Protein Concentrate-Hamlet Protein, Corn Protein Concentrate, and High Protein Distillers Dried Grains

5) (Plant Products with Future Potential-PFP) Ultralow oligosaccharide defatted soybeans, Spirulina, Corn Protein Concentrate, Barley Protein Concentrate

Two nutrient concentrations were targeted:

1) To meet amino acid targets of Rainbow trout (Hardy 2002) utilizing approximately 45% crude protein (40-42% digestible protein)

2) To meet the ideal amino acid balance of rainbow trout muscle for Lys, Met and Thr utilizing approximately 40% crude protein (37-38% digestible protein)

All diets were formulated to meet or exceed other known nutritional requirements of trout (NRC 1993). Diets (Tables 2 and 3) were formulated on an available-amino-acid-basis utilizing a mixture of protein feedstuffs defined in objective 1b for which amino acid availabilities are known. Diets were manufactured at BFTC laboratory using a twin-screw cooking extruder and dried to a final moisture level of less than 7%.

Table 2. Traditional plant, novel plant and animal protein series for BFTC and CSU Blend study. (% dry matter basis)

Traditional Plant			Novel Plants			Animal Proteins		
Ingredient	**PPD**	**PPD +**	**Ingredient**	**NPD**	**NPD +**	**Ingredient**	**APD**	**APD +**
Corn Protein Concentrate	23.00	17.00	Corn Protein Concentrate	22.00	9.60	Corn Protein Concentrate	9.00	7.03
Soybean Meal 48%CP	15.00	15.00	Soybean Meal 48%CP	0.00	12.00	Soybean Meal 48%CP	15.00	11.71
Soy Protein Concentrate	23.00	17.00	HPDDG DDGS	15.00	12.00	Feather meal	5.00	3.00
Wheat flour	11.99	19.18	Hamlet Protein	35.00	28.00	Poultry by-product, pet food	27.00	25.00
Stay-C 35	0.15	0.15	Wheat flour	1.71	7.26	SC Blood 13	5.00	3.00
Vit.premix	1.00	1.00	Stay-C 35	0.15	0.15	Wheat flour	17.65	23.90
TM ARS 640	0.10	0.10	Vit.premix	1.00	1.00	Stay-C 35	0.15	0.15
NaCl	0.28	0.28	TM ARS 640	0.10	0.10	Vit. premix	1.00	1.00
Magnesium Oxide	0.06	0.06	NaCl	0.28	0.28	TM ARS 640	0.10	0.10
Potassium chloride	0.56	0.56	Magnesium Oxide	0.06	0.06	NaCl	0.28	0.28
Monocalcium Phosphate	3.40	3.80	Potassium chloride	0.56	0.56	Magnesium Oxide	0.06	0.06
Choline Cl 50%	1.00	1.00	Mono-cal Phosphate	3.10	3.55	Potassium chloride	0.56	0.56
DL-Methionine	0.37	0.70	Choline Cl 50%	1.00	1.00	Mono-cal Phosphate	1.10	1.50
Lysine HCl	0.19	3.16	DL-Methionine	0.39	0.81	Choline Cl 50%	1.00	1.00
Threonine	0.00	0.82	Lysine HCl	0.54	3.20	DL-Methionine	0.44	0.74
Tryptophan	0.00	0.00	Threonine	0.00	0.78	Lysine HCl	0.00	2.95
Taurine	0.50	0.50	Tryptophan	0.00	0.00	Threonine	0.00	0.91
Astaxanthin	0.08	0.08	Taurine	0.50	0.50	Tryptophan	0.00	0.00
Lecithin	1.00	1.00	Astaxanthin	0.08	0.08	Taurine	0.50	0.50
Fish oil	18.12	18.41	Lecithin	1.00	1.00	Astaxanthin	0.08	0.08
Biofix Plus	0.20	0.20	Fish oil	17.33	17.89	Lecithin	1.00	1.00
			Biofix Plus	0.20	0.20	Fish oil	14.88	15.33
						Biofix Plus	0.20	0.20

Table 3. Plants with future potential and fish meal protein series for BFTC and CSU Blend study. (% dry matter basis)

	Plants with Future Potential			Fishmeal	
Ingredient	**PFP**	**PFP +**	**Ingredient**	**FMD**	**FMD +**
Barley Protein Concentrate	12.00	9.60	MFM SS Mean	22.00	17.60
Corn Protein Concentrate	12.00	9.60	Soybean Meal 48%CP	15.00	12.00
EARTHRISE SPIRULINA	15.00	12.00	Corn Protein Concentrate	15.00	12.00
Soy full fat, Schillinger Gen., Ultra Low Oligo, defatted 3010ULO	30.00	24.00	Poultry by-product, pet food	6.00	5.30
Wheat flour	5.42	14.40	SC Blood 13	4.00	3.20
Stay-C 35	0.15	0.15	Wheat flour	16.36	22.81
Vitamin premix ARS 702	1.00	1.00	Stay-C 35	0.15	0.15
TM ARS 640	0.10	0.10	Vitamin premix ARS 702	1.00	1.00
NaCl	0.28	0.28	TM ARS 640	0.10	0.10
Magnesium Oxide	0.06	0.06	NaCl	0.28	0.28
Potassium chloride	0.56	0.56	Magnesium Oxide	0.06	0.06
Monocalcium Phosphate	2.30	2.80	Potassium chloride	0.56	0.56
Choline Cl 50%	1.00	1.00	Monocalcium Phosphate	0.50	1.40
DL-Methionine	0.31	0.66	Choline Cl 50%	1.00	1.00
Lysine HCl	0.00	2.94	DL-Methionine	0.22	0.58
Threonine	0.00	0.74	Lysine HCl	0.00	2.79
Tryptophan	0.00	0.00	Threonine	0.00	0.77
Taurine	0.50	0.50	Tryptophan	0.00	0.00
Astaxanthin	0.08	0.08	Taurine	0.50	0.50
Lecithin	1.00	1.00	Astaxanthin	0.08	0.08
Menhaden fish oil	18.04	18.33	Lecithin	1.00	1.00
Biofix Plus	0.20	0.20	Menhaden fish oil	15.99	16.62
			Biofix Plus	0.20	0.20

BFTC Fish feeding and sampling: Each diet was randomly assigned to three tanks of fish. Fish were fed by hand to apparent satiation three times each day, 6 days per week for a total of 12 weeks. All fish within a tank are being counted and weighed as a group every 3 weeks.

CSU Fish feeding and sampling: At CSU, each diet was likewise assigned randomly to three tanks containing 15 fish. Fish were individually marked using a visual implant elastomer tag with a unique 3 digit alphanumeric code, implanted in the eyelid adipose tissue. Fish were fed by hand to apparent satiation twice each day, 7 days per week, for a total of 12 weeks. All fish within each tank were individually identified, measured, and weighed every three weeks. This feeding trial concluded in Oct. 2011, with samples collected for consumer evaluation obtained and shipped to WSU (see methods and results below) while respirometry trials were conducted at 6 weeks post-feeding and 12 weeks post-feeding.

Results BFTC: Significant effects of ingredient blends, nutrient concentration target and interactions were observed (Figure 3). At 12 wks post-feeding, adjusting the nutrient targets for the fish meal-based diets nor the animal product diets appear to affect fish performance. Adjustment of nutrient targets does improve performance of trout fed the three diet combinations based on plant ingredients. The improvements in growth when amino acids are supplemented to an ideal protein basis make fish performance when fed the PPD and PFP diets equivalent to FMD. Supplementing amino acids up to the ideal protein targets improved all plant based diets but the NPD treatment fish failed to attain final fish weights equivalent to the other treatments. Interactive effects also were noted for feed conversion ratios with supplementing amino acids to the ideal protein targets improving FCRs across all treatments but to a greater extent for the PFP treatment (Table 4). Once again the NPD diet supplemented to an ideal protein target failed to improve FCRs to the level of the other diets. Protein and energy retention efficiencies were improved when amino acids were supplemented to the ideal protein target with an average improvement of 30% for PRE and 13% for ERE. Ingredient blend had effects on PRE and ERE with the NPD treatment being lowest (Table 4). Whole body and fillet compositional analysis was relatively unaffected by dietary treatment except for whole body protein and fillet protein which were higher due to amino acid supplementation to ideal protein targets (Table 5). Fillet yield was increased by 4% when diets were supplemented to ideal protein targets (Table 6). Relative liver size (HSI) was affected by ingredient blend with interactive effects due to nutrient target. Relative viscera mass was decreased in fish fed diets supplemented to ideal protein amino acid targets, but condition factor was unaffected by diet.

Figure 3. Bozeman Blends Study Weight Gain Results

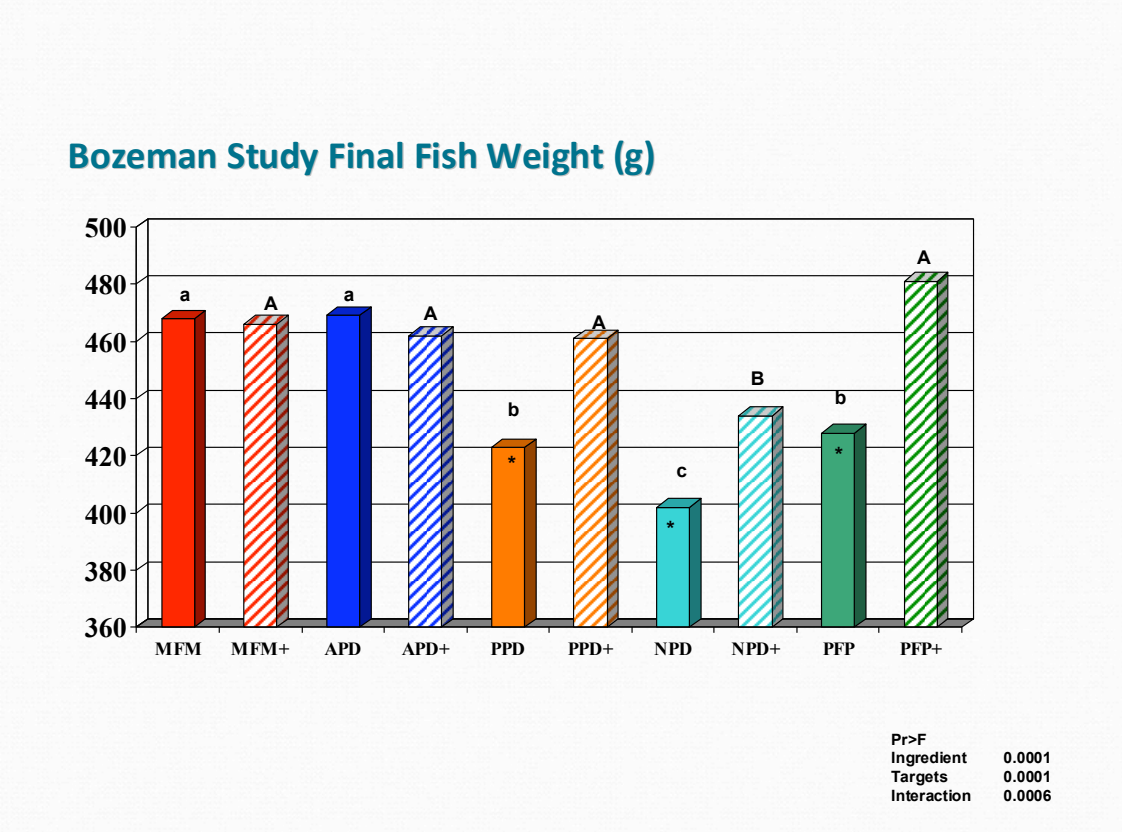


Figure 3.

[1] Probability associated with the F statistic.

[2] When interactive effects were detected common lower case and upper case letters indicate significant effects at P<0.05 within a supplement target.

[3]When interactive effects were detected asterisk indicates significant effects at P<0.05 due to supplement target with a protein source.

Table 4. Growth and production performance indices of rainbow trout with an initial weight of 72 g fed alternative protein source diets supplemented to two targeted supplement levels for 12wks.

		Final Fish Weight[2,3,4]	Weight gain	Thermal Growth Coefficient	Feed Conversion Ratio	Feed Intake	PRE	ERE
Diet		g	g		g feed / g gain	g /100g bw/day	%	%
FMD	1	468a	396a	2.86a	0.88c	1.92d*	39.2x	56.2xy
APD	2	469a	396a	2.84a	0.90c	2.07c	33.8xyz	61.2x
PPD	3	423b*	352b*	2.66b*	0.89c	1.86d	37.8xy	57.4x
PFP	4	428b*	356b*	2.68b*	1.02b*	2.19b*	31.0yz	55.0x
NPD	5	402c*	330c*	2.56b*	1.18a	2.45a	27.9z	40.4y
FMD+	6	466A	395A	2.86A	0.86B	1.85B	47.3	58.7
APD+	7	462A	390A	2.84A	0.85B	1.93AB	44.6	60.3
PPD+	8	461A	390A	2.84A	0.82B	1.85A	45.4	70.6
PFP+	9	481A	409A	2.92A	0.88AB	1.97A	42.0	65.6
NPD+	10	434B	361B	2.69B	0.93A	2.00A	41.2	52.0
Pr>F[1]								
Pooled S.E.M.		6.66	6.50	0.029	0.024	0.027	3.26	7.39
Ingredient		0.0001	0.0001	0.0001	0.0001	0.0001	0.0010	0.0050
Supplement		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001[4]	0.0127[4]
IngXSuppl		0.0006	0.0006	0.0009	0.0010	0.0001	0.5581	0.4048

[1] Probability associated with the F statistic.
[2] When interactive effects were detected common lower case and upper case letters indicate significant effects at P<0.05 within a supplement target, NRC vs Ideal Protein respectively.
[3]When interactive effects were detected asterisk indicates significant effects at P<0.05 due to supplement target with a protein source.
[4] When no interactive effects were noted the superscripts x,y,z indicate significant differences between ingredient blends pooled by nutrient target.
[4] Supplemented>unsupplemented
[5] Unsupplemetned>supplemented

Table 5: Whole body and fillet moisture, protein and fat content (wet weight basis) of rainbow trout fed alternative protein source diets supplemented to two targeted supplement levels for 12wks.

Diet	WB Moisture %	WB Protein %	WB Fat %	Fillet Moisture %	Fillet Protein %	Fillet Fat %
FMD	62.4	15.7	18.3	75.4	19.2	4.1
APD	63.5	14.2	17.7	75.2	18.1	4.6
PPD	61.8	15.2	20.1	75.1	18.0	4.7
PFP	61.2	14.5	20.2	73.9	18.2	6.3
NPD	62.7	15.1	18.5	74.7	18.3	5.5
FMD+	62.4	16.6	17.5	75.1	20.0	5.5
APD+	63.2	16.0	17.4	74.3	19.6	4.9
PPD+	63.5	16.3	17.2	73.2	19.7	3.9
PFP+	62.8	15.9	18.1	74.2	20.2	4.6
NPD+	64.6	15.7	15.5	74.9	19.8	3.4
Pr>F[1]						
Pooled S.E.M.	1.13	0.51	1.16	0.92	0.44	1.08
Ingredient	0.6505	0.2544	0.3947	0.6586	0.4245	0.6992
Supplement	0.1230	0.0019[4]	0.0223[5]	0.3550	0.0001[4]	0.4134
IngXSuppl	0.88017	0.8331	0.7178	0.7415	0.7555	0.6043

PRE = protein retention efficiency, ERE=energy retention efficiency, WB= wholebody

[1] Probability associated with the F statistic.

[2] When interactive effects were detected common lower case and upper case letters indicate significant effects at $P<0.05$ within a supplement target.

[3]When interactive effects were detected asterisk indicates significant effects at $P<0.05$ due to supplement target with a protein source.

[4] Supplemented>unsupplemented

[5] Unsupplemetned>supplemented

Table 6: Condition indices of rainbow trout fed alternative protein source diets supplemented to two targeted supplement levels for 12wks.

	Fillet yield	HSI	VSI	CF
Diet	%	%	%	%
FMD	56.7	1.16a	12.7	1.64
APD	54.1	1.27ab	13.5	1.64
PPD	54.1	1.23a	13.5	1.63
PFP	56.2	1.18ab	12.3	1.57
NPD	55.8	1.06b	12.4	1.53
FMD+	56.7	1.24AB	12.0	1.59
APD+	56.2	1.31A	12.9	1.69
PPD+	58.5	1.11BC	12.1	1.61
PFP+	58.1	1.18AB	12.4	1.67
NPD+	57.7	0.97C	11.8	1.63
Pr>F[1]				
Pooled S.E.M.	1.066	0.031	0.406	0.046
Ingredient	0.2899	0.0001	0.0950	0.4741
Supplement	0.0023 idp>nrc	0.3410	0.0248 idp<nrc	0.2064
IngXSuppl	0.6933	0.0250	0.4643	0.3907

Fillet yield with rib and pin bones, HSI=hepatosomatic index, VSI = viscerosomatic index, CF=condition factor

[1] Probability associated with the F statistic.

[2] When interactive effects were detected common lower case and upper case letters indicate significant effects at P<0.05 within a supplement target.

[3]When interactive effects were detected asterisk indicates significant effects at P<0.05 due to supplement target with a protein source.

[4] Supplemented > unsupplemented

[5] Unsupplemented > supplemented

Results CSU:

Feed Consumption

All tanks of fish responded aggressively when feed was offered for the duration of our trial, with estimated feed consumption rates between 4.47 (PFP) and 3.80 g/fish/d (PPD) (Table 7). It should be noted, however, that the feed consumption rates of individual fish were not directly measured. As these consumption rates were only approximations, no statistical analyses were performed on this metric.

Wet Weight

Final wet weights ranged from 382 g (PPD) to 440 g (FMD+) as shown in Table 7 and Figure 4. Feed group was found to be a statistically significant predictor variable (F=4.70, P=0.0010), with fish fed the FMD+ having the highest mean weight, followed by the PFP+ (426.6) and FMD (424.2). It should be noted that many of the means were not significantly different from each other (Table 7) suggesting that the performance of fish consuming these feeds is similar among many of the diets. Protein:lipid ratio, conversely, was not statistically significant (F=2.16, P=0.1425). The interaction between these two terms was found to be a statistically significant predictor variable (F=2.71, P=0.0297). Table 7 lists the mean final wet weights for individual fish fed the 10 different experimental diets.

FCR

Feed conversion ratios among feeds were generally low, ranging from 0.93 (FMD+) to 1.24 (NPD+) (Table 7). Feed group (F=7.02, P=0.0011) was statistically significant, while protein:lipid ratio (F=0.0042, P=0.9489) and the feed group and protein:lipid ratio interaction (F=0.27, P=0.8938) were not statistically significant at the $\alpha = 0.05$ level. Fish fed the FMD+ and APD+ (0.95) had the lowest FCR values, with many mean FCR values having no statistical difference from one another. Mean FCRs for the 10 treatments are summarized in Table 7.

SGR

Specific growth rates were significantly affected by feed group (F=10.37, P<0.0001), with the highest SGR seen for FMD+ (1.91) and FMD (1.90) (Table 7), while the PPD (1.74) and NPD (1.75) feeds had the lowest SGR. The protein:lipid ratio (F=0.08 , P= 0.7750), and the interaction between feed group and protein:lipid ratio (F=1.07, P=0.3686) were not significant.

In contrast, no diet group or protein level effects on respirometry was observed in regards to SDA max or SDA duration. Respirometry data are *noisy*, likely because of whole-tank approach (fish are probably never all quiescent); however, it is a realistic simulation of a culture tank/raceway.

Figure 4. CSU Blends Study Weight Gain Results