NUTRITION[9]

Project ***Termination Report*** for the Period
September 1, 2004 to May 31, 2007

NCRAC FUNDING: $200,000 (September 1, 2004 to May 31, 2007)

PARTICIPANTS:

Paul B. Brown	Purdue University	Indiana
Donald L. Garling	Michigan State University	Michigan
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Industry Advisory Council Liaison:		
Curtis Harrison	Harrison Fish Farm, Hurdland	Missouri
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Donald L. Garling	Michigan State University	Michigan
Non-Funded Collaborators:		
Mark E. Griffin	Land O-Lakes/Purina Feeds, St. Louis	Missouri

REASON FOR TERMINATION
The project objectives were completed.

PROJECT OBJECTIVES
(1) Develop cost-effective fish meal-free diets for grow out of hybrid striped bass with an initial minimum weight of 100 g (3.5 oz).

(2) Develop cost-effective fish meal-free diets for grow out of yellow perch with an initial weight of 10 g (0.35 oz).

[9]This is a 2-year project that was chaired by Paul B. Brown and began September 1, 2004.

PRINCIPAL ACCOMPLISHMENTS

OBJECTIVE 1

Researchers at Purdue University (Purdue) were delayed in starting the Nutrition project because a fire completely destroyed the Aquaculture Research Laboratory in October 2004 as the project was beginning. Temporary wet lab space was occupied from January 2005 until June 2006, but that space was inadequate for conducting the proposed studies. The Aquaculture Research Laboratory was reconstructed and was occupied in June 2006 at which time the proposed studies with hybrid striped bass and yellow perch commenced. Feedstuffs acquired and analyzed for both studies include distillers dried grains with solubles, sunflower meal, canola meal, soybean meal, corn gluten meal, brewer's yeast, poultry by-product meal/feather meal (1:1 w:w), meat and bone meal, fish meal, and whole ground wheat. Bone meal, fish meal, and whole ground wheat.

In the study with hybrid striped bass, numerous dietary formulations were attempted. Dietary crude protein concentrations were maintained at 36% of the diet and the essential amino acid concentrations met the established requirements assuming 80% availability from practical ingredients. Dietary lipid concentration was maintained at 10% of the diet using fish oil with ethoxyquin. Use of plant-based ingredients resulted in dietary formulations that would not meet the requirements in diets containing 36% dietary crude protein. All possible combinations were attempted, including formulations in which all plant-based ingredients were provided. Using the nutrient limitations established above, dietary crude protein must be in the range of 30–34% of the diet. However, combinations of animal-based ingredients and plant-based feedstuffs met the established nutrient restrictions. Two series of diets were established, one series contained meat and bone meal, and the other contained the 1:1 mixture of poultry by- product meal and feather meal in combination with whole wheat and one of the plant-based ingredients. For example, meat and bone meal/soybean meal, meat and bone meal/canola meal, etc. Whole wheat was used as the carbohydrate source, which will be required for extrusion processing of diets. A positive control diet containing only fish meal was also formulated and fed to fish. Initial weight of fish was 110.0 g (3.9 oz), the experimental system was a recirculating system and all fish were fed to satiation twice daily.

Significant differences were detected in mean feed consumption, weight gain, feed conversion ratio (FCR) and specific growth rate (SGR). Statistical analysis revealed significant differences in mean feed consumption across treatments, but the multiple ranking test imposed was unable to differentiate mean values. Mean weight gain of fish fed the positive control diet and the series containing poultry by-product meal was significantly greater than fish fed the series of diets containing meat and bone meal. Similarly, FCR and SGR of fish fed the positive control diet and those fed the poultry by-product series was significantly improved compared to fish fed the meat and bone meal diets. Mean FCR of fish fed the positive control diet was 2.0, mean FCR of fish fed the poultry by-product series of diets was 1.5–1.7, while mean FCR of fish fed the meat and bone meal diets was 2.9–3.0. Mean SGR of fish fed the positive control diet was 0.96, while mean SGR of fish fed the poultry by-product series of diets was 0.98–1.06. Mean SGR of fish fed the meat and bone meal series of diets was 0.67–0.72.

Based on these results, and operating under the formulation assumptions outlined above, fish meal-free diets using blends of animal and plant-based ingredients result in feed acceptance and production parameters that are not different from fish fed fish meal- based diets. Lower dietary crude protein concentrations will allow increased use of plant-based ingredients. The relatively lower results in fish fed the meat and bone meal series of diets was surprising as that ingredient was proven beneficial in a separate series of studies conducted at Purdue. Differences between the previous and current studies may be the result of the uncertainties in source material for meat and bone meal, as well as processing conditions and overall quality.

Research at Southern Illinois University- Carbondale (SIUC) has been conducted to determine the maximum percentage of corn gluten meal that could be used as a substitution for fish meal in hybrid striped bass diets without adversely affecting growth. Two 2-month feeding trials were conducted in a recirculating system with associated mechanical and biological filtration. Isonitrogenous, isocaloric diets containing 40% crude protein and 12% crude lipid were fed twice daily to satiation throughout both trials. During the first trial, ten ~40.0 g (1.41 oz) fish were stocked into each tank and fed five diets ranging from 0– 30% fish meal. Based on the results from this study, a second trial was conducted feeding seven diets containing 0–24% fish

meal using ten ~18.0 g (0.63 oz) fish per tank. All practical diets included fish meal, corn gluten meal, soybean meal, wheat middlings, fish and canola oils (50:50), sodium phosphate, dicalcium phosphate, vitamin and mineral mixes, choline, and carboxymethylcellulose.

After the first trial, SIUC researchers observed no significant differences ($P < 0.05$) in growth between the 30 and 22.5% fish meal dietary treatments. At the conclusion of the second feed trial SIUC researchers found that hybrid striped bass fed less than 20% fish meal demonstrated significantly lower ($P < 0.05$) weight gain; however, SGR and FCR were maintained in treatments containing 12 and 16% fish meal, respectively.

SIUC researchers found partially substituting fish meal with corn gluten meal in hybrid striped bass diets is possible without adversely affecting growth. Long- term benefits from this study include an improvement of the efficiency of aquaculture feeds for hybrid striped bass and a reduced reliance on the fish meal industry.

In 2006, SIUC researchers conducted a 10- week feed trial in a 28 tank recirculating system stocked with 10 sunshine bass (9.3 ± 6 g, [0.33 ± 0.21 oz] mean individual weight) per tank. Seven isonitrogenous, isocaloric (40% crude protein and 15% crude lipid) diets containing graded levels (0, 20, 40, 60, 80, or 100%) of menhaden to canola oils with 20% menhaden meal, or 100% canola oil with 20% lipid-extracted menhaden meal (LEMM), were fed twice daily to apparent satiation throughout the trial.

Replacing menhaden oil with canola oil resulted in significant differences ($P < 0.05$) in production parameters. Weight gain, SGR , and FCR were not significantly different in diets containing 60% or less canola oil as a replacement for menhaden oil. The fatty acid (FA) profile of the fillet was highly responsive to dietary FA changes; significant differences were apparent for almost every FA between dietary treatments. Saturated, total n-3, and highly unsaturated FA were highest in fillets from fish fed diets rich in menhaden oil and

monounsaturated and total n-6 FA were highest in the fillets of fish fed diets high in canola oil. Liposomatic indices were highest for fish fed 0% menhaden oil with LEMM (5.60 ± 0.27%) compared to fish fed diets containing menhaden oil (range: 3.2– 4.4%). Oxidative stability of both liver and fillet tissue decreased in response to dietary menhaden oil inclusion.

SIUC researchers were able to reduce dietary intake of marine oils to 40% without negatively impacting growth of hybrid striped bass fingerlings. Highly unsaturated fatty acid (HUFA) content of the fillet was comparable to wild striped bass when feeding at least 80% menhaden oil with 20% menhaden meal. Data from this study suggest a 40% menhaden oil/20% menhaden meal diet can be used during grow out of sunshine bass fingerlings without altering production. Prior to harvest using an 80% menhaden oil/20% menhaden meal diet may be suitable as a finishing diet to re-establish HUFA levels in the fillet.

In 2007, SIUC conducted a 10-week feed trial in a 28 tank recirculating system stocked with 10 sunshine bass *Morone chrysops* × *M. saxatilis* (5.2 ± 0.22 g [0.18 ± 0.01 oz]; individual mean weight ± standard deviation) per tank. Fish within replicate tanks ($N = 4$) were fed one of seven isonitrogenous, isolipidic (40% crude protein and 15% crude lipid) experimental diets containing graded levels (0, 10, or 20%) of menhaden meal with a feed attractant (1% dry matter) or a control diet containing 30% menhaden meal without a feed attractant. Two feed attractants were evaluated in this study as well, commercially available Finnstim S (Danisco Animal Nutrition, Wiltshire, England) and a plant-based experimental product soluble canola protein concentrate (SCPC; MCN BioProducts, Saskatoon, Saskatchewan, Canada). Corn gluten meal was the alternative dietary protein source used to replace menhaden meal in all experimental feeds containing feed attractants. Fish were fed twice daily to apparent satiation throughout the trial.

Plant-based alternative protein sources replaced up to 67% of menhaden meal fed to sunshine bass without negatively impacting production parameters when Finnstim S or SCPC were added to the diets. FCR of fish fed reduced menhaden meal were not statistically different from control fish ($P < 0.05$). Additionally, no differences in weight gain, feed intake, or FCR were attributed to feed attractant type. This suggests differences in weight gain observed when fish were fed 0% menhaden meal diets with either attractant are attributable to reductions in feed intake observed in the same treatments.

Previously, Lewis and Kohler found 20% dietary menhaden meal was needed to maintain sunshine bass production performance when corn gluten meal was used as the alternative protein source. However, dietary inclusion of a feeding attractant (Finnstim S or SCPC; 1% dry matter) in this study-maintained growth rates and feed conversion ratios when feeding as little as 10% fish meal to sunshine bass. Reducing dietary fish meal by 10% with the addition of soluble canola protein concentrate reduced dietary protein expenditures by 11%.

OBJECTIVE 2

University of Wisconsin-Madison (UW- Madison) investigators conducted a grow- out trial on yellow perch comparing four diets. All diets were formulated to be 41% crude protein and 10.5% crude fat and meet or exceed the nutritional requirements for rainbow trout. The control diet was a commercial trout grower containing a high percentage of fish meal. The experimental diets were similar to the control diet, except that the fish meal was replaced with animal and plant meal mixes in the following ratios: 75% animal meal mix/25% plant meal mix, 55% animal meal mix/45% plant meal mix, and 35% animal meal mix/65% plant meal mix. Each of the experimental diets contained 5% shrimp meal to enhance palatability.

In April 2005, Mark Griffin at Land O'Lakes/Purina Feeds had approximately 31.8 kg (70.0 lb) of each of the experimental diets made into 2.0 mm (0.08 in) sinking pellets. The diets were subsequently shipped to the UW-Madison's facilities at the Lake Mills State Fish Hatchery, Lake Mills, Wisconsin, where they were kept in frozen storage.

In mid-May 2005, UW-Madison investigators set up 12, 220.0-L (58.1-gal) flow through tanks as described in the original proposal. Each tank was stocked with approximately 60 yellow perch having a mean weight of 15.0 g (0.53 oz). The fish in each tank had been fed a sinking commercial trout food (Silver Cup, Murray, Utah). Beginning in early June, the fish were transitioned to the new experimental diets (3 tanks per diet) over a two-week period. After the transitional period, UW-Madison investigators observed that the feeding behavior of all of the perch in the four treatment groups was extremely poor. After an additional 3-week period all of the fish were weighed and measured. Extremely poor growth rates were noted in all of the groups. Because of the poor feeding response, UW-Madison investigators terminated the experiment, and in July 2005, a new experiment was set up with different fish (mean weight = 28.0 g [1.0 oz]). For this experiment the transition of the fish onto the experimental feeds was altered by mixing equal amounts of Silver Cup™ trout food and the experimental diets and then approximately 5% of freeze-dried krill flakes was added to each mixture. For one month the fish were fed this mixture, and all fish ate well. After one month the Silver Cup diet was eliminated from the mixture, and the fish were fed the experimental feeds for an additional month with a declining amount of krill. After this time, all of the fish were showing a good feeding response to the experimental diets alone. UW- Madison researchers then conducted the grow-out phase of the study as originally proposed. Shortly after the beginning of the grow-out phase, fish that were being fed any of the three new experimental diets began to show a reduced feeding response. The mean weight gains, feed/gain ratios, and survivals (%) of the different groups of fish for the grow-out study were as follows: trout food: 41.0 g (1.45 oz), 1.34, and 91%; 75% animal meal: 22.0 g (0.78 oz), 1.65, and 80%; 55% animal meal: 17.0 g (0.60 oz), 2.8, and 61%; and 35% animal meal: 24.0 g (0.85 oz), 2.5, and 61%.

Clearly, the experimental fish meal-free diets proved sub-optimal for yellow perch growth, survival, and performance. UW- Madison investigators believe that the poor performance of the experimental diets may have been due, at least in part, to low palatability rather than inadequate nutritional properties. This belief is driven by the fact that the fish seemed to readily feed and consume the experimental diets as long as a small amount of krill was mixed into the food. As soon as the krill was eliminated, the feeding responses of the fish declined

markedly. This finding was surprising, given that all of the diets contained 5% shrimp meal to enhance palatability. Fillets from all treatment groups were subjected to sensory analysis comparisons by the UW-Madison Department of Food Science, and no differences were found among the four fish groups. The studies on reproduction showed no negative effects of the experimental diets, as fish from each treatment group that were overwintered showed normal egg and sperm development.

Michigan State University (MSU) researchers conducted two experimental studies to examine specific effects of trypsin inhibitors (TIs) on the growth and performance in formulated fish feeds for yellow perch. These studies consisted of a Phase I Growth Study and Phase II Extended Study, and were designed to assess if TIs in soybean meal (SBM) limit its inclusion level in diets for fingerling yellow perch.

A semi-purified control and four experimental diets containing graded levels of TI were used to study effects of TIs associated with SBM on yellow perch fingerlings. Test diets were manufactured by collaborators at Purdue and formulated to be 34% crude protein and 12% crude fiber. TI (Soybean Trypsin Inhibitor CAS #9035- 81-8, USB Corporation) inclusion rates were 0, 0.975, 1.95, 2.925, and 3.9 g TI/kg (ppm) feed representing estimated SBM equivalencies of 0, 15, 30, 45, and 60%SBM (diets TI0, TI15, TI30, TI45, and TI60, respectively). TI inclusion rate SBM equivalencies were based on the average value of 6.5 mg TI/g (ppt) SBM from the range of 5.0–8.0 mg TI/g (ppt) SBM (Dr. Craig Russet, Director of Agri Business with Central Soya).

Young-of-the-year yellow perch were obtained from the Ohio State University Center for Aquaculture Development. The fish were transported to MSU's Aquaculture Research Laboratory and acclimated to water conditions in a 225.0-L (59.4-gal) flow tank system over a 30-day period. Fish were fed a commercial trout diet over the acclimation period. A total of 270 fish were randomly distributed in 15, 225.0-L (59.4- gal) tanks, 18 fish per tank, and acclimated to conditions of a partial recirculating aquaculture system to be used during the feed trial. Fish were fed the experimental control diet over this additional 10-day acclimation period.

The partial recirculating aquaculture system consisted of the fish rearing units, settling basin, rotating biological contactor, and aeration column. Flow rates were maintained between 3.7–5.6 Lpm (1.0–1.5 gpm) based on target exchange rates of 1.0–1.5 water exchanges per hour. Fresh water continual flow to the system varied between 0.5–1.0 Lpm (0.13–0.26 gpm). Water temperature for the Phase I growth study remained between 17.4–19.3°C (63.3– 66.7°F), with a mean temperature value of 18.5°C (65.3°F). Water temperature for the Phase II extended study remained between 18.7–22.8°C (65.6–73.0°F), with a mean temperature value of 19.9°C (67.8°F). Dissolved oxygen remained near constant at 95% saturation; total ammonia nitrogen concentrations remained below 1.0 mg/L (ppm) (0.006 ppm unionized ammonia); nitrate concentrations remained below Hach nitrate test kit (colorimetric) detection levels. All other water quality parameters fell within acceptable limits for yellow perch.

For both Phase I and II studies, fish were fed in triplicate, either the control diet, or one of five treatment diets, two-times daily (8:00– 9:00 am and 4:30–5:30 pm). Total weight samples were conducted on day-1 of each trial and repeated every 2–4 weeks.

The Phase I Growth Study examined effects of TIs on growth and body composition of yellow perch fingerlings over an initial feed trial period of 85 days. Average initial weight of fish from all tanks was measured to be 4.11 ± 0.36 g (0.14 ± 0.01 oz). Feeding levels were calculated on a constant % body weight (%BW) basis and adjusted every two weeks according to the theoretical optimal feed levels for salmonids at a FCR of 1.0. Feed levels fell both above and below satiation levels of the fish across feeding times based on observations of excess feed in tank bottoms at various times through the feed trial.

At the end of the Phase I study, total weights were taken. Three fish were randomly selected for weight and length measurements and were euthanized in tricaine methane sulfonate (MS-222) at a concentration of 500 mg/L (ppm). The 3 fish were ground, pooled, frozen, and held at-20°C (-4°F) for subsequent whole-body composition analysis.

Three mortalities occurred over the 85 day Phase I feed trial: one each for the control, TI15, and TI30 diets. Results show that the TI60 diet resulted in the lowest values for k (condition factor), SGR, and protein efficiency ratio (PER). TI60 body composition samples had the highest composition of ash and lowest composition of lipids. Body compositions showed an increasing linear trend in ash, $y = 0.4029\times + 16.563$ ($R^2 = 0.90$), with increased TIs in the diet. Body ash composition was statistically different between TI0 and TI60. No other parameters tested were statistically different ($P = 0.10$). FCRs ranged from a minimum of 1.43 (TI45) to 1.67 (TI60).

The Phase II study examined long term effects of TIs on yellow perch growth, body composition, and intestinal morphology characteristics. Phase II was conducted over a period of 56 days as an extension to the Phase I trial. Together, these studies combine to examine the affects of SBM TIs for 5 months of continual TI ingestion.Average initial weight of fish from all tanks was measured to be 11.81 ± 1.99 g (0.42 ±0.07 oz).

Feed levels in Phase II were slightly different than that of Phase I in that %BW was calculated individually for each tank based on a constant k as determined from the Phase I study. This adjustment reduced feed level variations between tanks. Feed levels were adjusted bi-weekly based on total weight samples or FCR rates determined from the last weight sample taken. Feed levels fell both above and below satiation levels of the fish across feeding times based on observations of excess feed in tank bottoms at various times through the feed trial.

At the end of the Phase II study, total weights were taken and all fish were euthanized in tricaine methane sulfonate (MS-222) at a concentration of 500 mg/L (ppm). Ten fish were randomly selected for weight and length measurements, and excision of whole livers which were weighed for hepatosomatic index (HSI) determination. Small intestines were excised from the first three fish samples and fixed in 10% neutral buffered formalin for subsequent intestinal histological examination. Three whole fish subjects, pooled within tank, were frozen at -20°C (-4°F) for proximate body composition analysis.

There were no mortalities observed over the 56-day feed trial. Results of the extended study indicate that there were no significant differences in k, SGR, PER, FCR, or body proximate analysis among diets. The only significant difference observed was for HSI. Yellow perch on the TI15 diet showed significantly lower HSI values than perch fed TI30, TI45, or TI60 diets. No dietary effects were observed on intestinal histopathology samples between 0% SBM and 60% SBM equivalency diets.

Researchers at Purdue attempted to formulate diets for yellow perch with only plant-based ingredients using similar dietary restrictions used in studies with hybrid striped bass (36% dietary crude protein, 8% lipid, quantified methionine, arginine and lysine requirements and predicted requirements for the remaining essential amino acids).

As experienced with hybrid striped bass, dietary formulations with only plant-based proteins could not meet the requirements of yellow perch in diets containing 36% dietary crude protein without substantial supplementation with feed grade amino acids. Thus, a similar series of diets was developed using meat and bone meal or poultry by-product meal blended with plant- based ingredients. Those diets were fed to juvenile all-female yellow perch (mean initial weight 11.2 g; 0.4 oz) held in recirculating systems. All diets were fed to satiation twice daily.

Perch fed the diets containing poultry by- product meal in combination with canola meal and sunflower meal, and perch fed the diet containing meat and bone meal in combination with canola meal had significantly lower weight gain, SGR and higher feed conversion ratios than in fish fed other diets. There were no clear distinctions in perch fed the experimental diets as seen in similar diets fed to hybrid striped bass. It appears perch may be sensitive to certain combinations of protein-supplying ingredients and selected ones should be evaluated individually.

IMPACTS

The development, testing, and use of fish meal-free diets are critical to the aquaculture industry for two primary reasons. First, some critics of aquaculture have expressed the opinion that wild fish populations are hurt by the growth of aquaculture because of the industry's dependence on fish meal.

Second, fish meal is an expensive dietary ingredient that raises the cost of food, and thereby increases overall fish production costs. This project should provide the key information needed by commercial feed producers so that they can begin providing a quality fish meal-free or fish meal-reduced diet to producers.

Work completed by SIUC researchers has demonstrated that plant-based protein and lipid sources can partially replace marine feedstuffs in the diets fed to sunshine bass without negatively impacting production and fillet quality. The results suggest feeding a 40% menhaden oil/20% menhaden meal diet during grow out is sufficient to maintain production. However, it appears menhaden meal can be reduced to 10% with the inclusion of a suitable feed attractant. Regardless, higher concentrations of menhaden oil (80% of the dietary lipid) are needed in the diet to maintain fillet HUFA content. Partial replacement of marine

feedstuffs in sunshine bass diets enables producers to utilize fish meal and oil supplies more efficiently, leading to a more cost-effective diet formulation for this industry.

Work completed at UW-Madison clearly indicated that the experimental fish meal- free diets tested proved sub-optimal for yellow perch growth, survival, and performance. The investigators believe that the poor performance of the experimental diets may have been due, at least in part, to low palatability rather than inadequate nutritional properties.

The TI studies conducted at MSU suggest that negative effects of SBM in plant-based feeds may be more of a culmination of anti- nutritional properties, including combined effects of TIs, lectins, phytate, saponins, etc. Based on results with yellow perch, these effects could be more severe than those observed in salmonids. At this time MSU researchers caution the use of SBM for yellow perch diets, and recommend additional research in the area of developing commercial SBM-based feeds and effects of carbohydrates on yellow perch.
Studies conducted at Purdue indicate there is a sufficient body of knowledge for formulating alternative diets for both species. Ingredient choice needs to be carefully considered in new formulations and perch may have sensitivities to certain ingredient combinations.

RECOMMENDED FOLLOW-UP ACTIVITIES

Data for hybrid striped bass indicate alternative dietary formulations can be developed using the available nutrient requirements. Additional nutritional requirements would be beneficial, particularly relating to health status of fish. Data for yellow perch are also promising, indicating alternative formulations can be developed. However, there appear to be ingredient limitations in perch that should be explored, perhaps in conjunction with ingredient suppliers. Relatively low consumption of feeds by perch remains a significant problem, limiting growth and time to market. Understanding the controlling mechanisms would help alleviate this fundamental problem.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for this NCRAC-funded Nutrition project.

SUPPORT

YEAR	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2004-05	$99,250		$1,000			$1,000	$100,250
2005-06	$100,750						$100,750
TOTAL	$200,000		$1,000			$1,000	$201,000