Alternative Feedstuffs for Aquaculture:

A Joint Technical Session of NOAA, USDA, and the
Plant Products in Aquafeed Working Group

Held at the 2010 Aquaculture America Conference
San Diego, California

Wednesday, March 03 8:30 - 4:30
Thursday, March 04 8:30 - 12:15

Co-Chairs:
Dr. Frederic Barrows, Dr. Diane Bellis, Dr. Delbert Gatlin,
Dr. Michael Rust, Dr. Jeffrey Silverstein

Agricultural Research Service

USDA
United States Department of Agriculture
National Institute of Food and Agriculture

Dear Participants:

On behalf of the National Oceanic and Atmospheric Administration (NOAA), the U.S. Department of Agriculture (USDA), and the Plant Products in Aquafeed (PPA) Working Group, we welcome you to the second Joint Technical Session on Alternative Feedstuffs for Aquaculture. The first Alternative Feeds technical session was organized by PPA in 2007 at a meeting in San Antonio. This joint session was convened for the first time last year at Aquaculture America in Seattle. Through these dialogues, we continue to make progress toward identifying economical alternative feeds for use in aquaculture production that reduce pressure on wild fisheries and maintain the important human health benefits of seafood consumption.

Fish meal and fish oil are important components in the feeds of many farm-raised species, from pigs and poultry to farmed fish. For many cultured species (such as carp, salmon, tilapia, trout, catfish, and shrimp) fish meal and fish oil supply the essential amino acids and fatty acids required for normal growth. A growing body of scientific evidence shows that fish meal and fish oil have significant human health benefits as well. However, increasing demand for these products as well as mounting pressure on the wild capture fisheries that supply them are combining to make alternative feeds a top issue for global aquaculture development and growth.

The Plant Products in Aquafeed Working Group is an informal group of experts working on a parallel track to identify research needed to optimize the use of plant feedstuffs in the diets of cultured carnivorous fish. The group first met in 2005 to identify approaches to most effectively use all available technologies to develop highly digestible, sustainable, and economical alternatives to fish meal-based aquaculture diets. PPA developed the 'PPA Working Group Strategic Research Plan' as a guide to coordinate and enhance development of novel research.

In 2007, following on and broadening PPA's efforts, NOAA and USDA's Agricultural Research Service and National Institute of Food and Agriculture launched the Alternative Feeds Initiative, the primary purpose of which is to identify alternative dietary ingredients for aquafeeds that will reduce the amount of fish meal and fish oil while maintaining the important human health benefits of farmed seafood.

NOAA, USDA, and PPA look forward to collaborating with all of you on this critical research. We hope you enjoy the session and appreciate your participation.

Michael Rubino
NOAA Aquaculture

Jeff Silverstein
USDA/ARS

Gary Jensen
USDA/NIFA

Delbert Gatlin
Plant Products in Aquafeed Workgroup

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Richard Nelson, Pres. Nelson and Sons - Silvercup Fish Feed	What the Feed Industry Needs from Laboratory Studies on New Ingredients
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12:00 **Dave Jirsa**, Rick Barrows, Mark Drawbridge, Ron Hardy
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Advancing the U.S. Initiative on Alternative Feeds for Aquaculture

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On a global scale, significant improvements have been made in reducing the reliance on fish meal and fish oil for feeds for many cultured species. The National Oceanic and Atmospheric Administration (NOAA) and other federal agencies play a vital role in that research and the transfer of that technology to industry. Since 2007, NOAA, in partnership with the U.S. Department of Agriculture (USDA) launched the Alternative Feeds Initiative to accelerate the development of alternative feeds for aquaculture. This presentation will address the major milestones of the joint initiative on aquafeeds and identify new priority areas for research.

The overall purpose of the NOAA-USDA Alternative Feeds Initiative is to identify alternative dietary ingredients that will reduce the amount of fishmeal and fish oil contained in aquaculture feeds while maintaining the important human health benefits of farmed seafood. Ultimately, the initiative will lead to the commercialization of alternatives for some species which will result in reduced dependence on marine fish resources by feed manufacturers and seafood farmers worldwide. The relatively high cost of fish meal and fish oil – and growing pressure on the wild fisheries that supply the fish meal and fish oil – are adding up to make alternative feeds one of the top issues facing the global aquaculture industry, fueling research on suitable alternative feed ingredients. Under the initiative, NOAA is partnering with the USDA's Agricultural Research Service and National Institute of Food and Agriculture to build on ongoing federal research to identify alternative protein and oil sources for aquaculture feeds.

Moving Toward Sustainable Fish Feeds

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Aquaculture is set to reach a landmark in 2009, supplying half of the total fish and shellfish for human consumption. With the production of farmed fish eclipsing that of wild fish, another major transition is also underway: aquaculture's share of global fishmeal and fish oil consumption more than doubled over the past decade to 68% and 88%, respectively. This trend reflects rapid growth in aquaculture production and decreased use of fishmeal in the livestock sector in response to higher prices, but it belies significant improvements in aquaculture feed efficiencies that have occurred simultaneously. Impressive gains have been achieved in reducing feed conversion ratios (FCRs) for piscivorous fish and in substituting nonfish ingredients into formulated feeds. The volume of omnivorous species production has also risen. The ratio of wild fish input via industrial feeds to total farmed fish output (excluding filter feeders) has fallen by more than one-third from 1.04 in 1995 to 0.63 in 2007, a decline that underscores the expanding volume of omnivorous fish produced on farms and market pressures to reduce fishmeal and fish oil levels in aquafeeds. Nonetheless, serious challenges remain for lowering the aggregate level of fishmeal and fish oil inputs in feeds and alleviating pressure on reduction fisheries over time. In this session, we examine advances being made in the use of fishmeal and fish oil in industrial aquafeeds and alternative nonforage fish ingredients.

Plant Products in Aquaculture: Beans, Bugs, and Fish Guts – Coordinating Research on Alternative Diets

Diane Bellis, Delbert Gatlin III, and Frederic T. Barrows

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Given the confluence of interest of the environmental community, aquaculturists, and the aquaculture feed industries in finding alternatives to fish meal and fish oil, and given the scientifically interesting and complex research issues surrounding understanding the wide spectrum of response to all alternatives to fish meal, an international group of researchers came together informally in 2005 to form the Plant Products in Aquafeed (PPA) Working Group. In 2007, PPA published the Strategic Research Plan, a research roadmap. The PPA Strategic Plan was based on a "white paper" (2007) that reviewed current knowledge. The PPA Strategic Plan identifies specific performance measures with associated timelines, and points to resources outside of the fish nutrition research community (e.g., plant genomicists, the model fish research community, and bioinformaticists) likely to help break the impasse in understanding the interaction between terrestrially grown feedstuffs and marine consumers.

At the outset, the PPA Working Group recognized that mechanisms would need to be established to coordinate research across the goals outlined in the PPA Strategic Research Plan. PPA has been successful at creating opportunities for dialogue among researchers which has lead to proposals and critical projects and programs. Two examples are: cooperation between PPA, NOAA, and USDA to host this session, and; the collaboration between USDA/ARS, Solae, Bunge, and ADM to make standard, well-characterized feedstocks available to fish nutritionists. We have attracted and maintained industry (e.g. United Soybean Board, Silvercup, Solae) and technical agency (notably USDA/ARS, DOC/NOAA) support. Nevertheless, several challenges persist.

We would like to be more effective at bringing together teams of experts to focus research on the goals and performance measures outlined in the PPA Strategic Research Plan. This will not only expedite breakthroughs in understanding the limitations on using alternatives but should result in more competitive research proposals to USDA/AFRI and expand potential funding sources to the well funded agencies such as NSF and NIH. In addition, we have not had the resources (primarily time and expertise) necessary to develop a vision of how to use the tools of computational biology to integrate existing data, identify gaps, and develop models that are user-friendly and globally accessible.

We will describe options for more effectively addressing these challenges.

Taurine: An Essential Nutrient in Fish

Shin-Kwon Kim*, Kyoung-Duck Kim, Kang-Woong Kim, Hae-Young Lee, Maeng-Hyun Son, Masahito Yokoyama, and Toshio Takeuchi

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Fish meal is known to be the best source of protein in aquaculture of finfish. However, the nutrients of fish meal have yet to be determined precisely. Although taurine is not contained in alternative protein sources such as soy been meal, cottonseed meal and corn meal. Taurine is the most abundant amino acid among the free amino acids profiled in fish meal. Taurine is one of the candidate nutrients that have potential as a growth promoter. This study was conducted to investigate the taurine as an essential nutrient of fish.

Different taurine level diets were prepared by the supplementation of taurine to the basal composition. Fish meal that was washed with 70% ethanol to remove taurine was used as the sole protein source. At the end of the experiments, fish were weighed and analyzed for amino acids free in the body and the composition of the conjugated bile acids. The feeding behavior of fish was observed throughout the experimental period.

The body weight and feed efficiency of juvenile flounder were improved by taurine supplementation in the experimental diets. The taurine contents of the whole body and tissues increased with in increase of the taurine supplementation. The conjugated bile acids in the gall bladder consisted of taurocholic acid and taurochenodeoxycholic acid, which increased with the increase of the dietary taurine level. Abnormal feeding behavior such as multiple feeding while swimming in the water column was observed in the taurine low level group. These findings indicate that taurine is essential nutrient for normal growth and development of flounder.

Pilot Program to Characterize Feed Ingredients for the Development of Alternative Aquaculture Feeds

Frederic T. Barrows, Wendy M. Sealey and Diane Bellis

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Numerous studies, using a wide variety of species, have been conducted to reduce/eliminate fish meal from aquaculture feeds. However, variability in sources and inadequate chemical characterization of dietary ingredients has been cited as primary limitations when comparing results from these various studies. Recently, the Plant Products in Aquafeeds Working Group (PPA) developed a Strategic Plan to advance the development of fish meal free diets through improved research coordination. A primary objective of Goal #1 of that Strategic Plan is the standardization of feed input compounds used in research studies. Standardization of feed ingredients is complicated by the fact that the small ingredient quantities needed by most research groups limits their ability to obtain consistent commercial products. Additionally, it is generally cost prohibitive for individual researchers to fully characterize both the nutrient and anti-nutrient profiles of each ingredient used in experimental feeds. For these reasons, a pilot program is being developed to address these needs and provide this service for the research community.

Through this new program, small quantities (~25 kg/ ingredient/shipment) of characterized ingredients will be made available to interested researchers. Each ingredient will be supplied with an analysis of nutrients (proximate composition, and amino acids) and anti-nutrients (specific anti-nutrients dependant on ingredient). All ingredients will be characterized using consistent protocols at contracted analytical laboratories. Possible ingredients in the program include; soy protein concentrate, soy bean meal, corn protein concentrate, poultry by-product meal, barley flour, and barley protein concentrate. The only obligations of receiving the various ingredients will be the acknowledgement of the sponsors as the ingredient source and providing all sponsors with a reprint of any manuscripts published using these ingredients. The level of interest in participation in this program is currently unknown and may affect the number of ingredients available and the longevity of the program, though anecdotal evidence suggests a high interest. We welcome comments and statements of interest.

Certain Nutrients in Animal Proteins May Play Critical Roles in the Diets of Aquacultured Animals

Peng Li and Delbert M. Gatlin III

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Many scientific reports have identified nutrients and other biochemicals in rendered protein ingredients that positively influence animal growth, development, reproduction, and health. Often defined as dietary "non-essential," nutrients such as taurine, proline, hydroxyproline, glycine, nucleotides, carnitine, and cholesterol have been shown to possess physiological significance and potential applications in the production of various terrestrial and aquatic species at different developmental stages. In recent years, several of these biochemicals have been demonstrated as being conditionally essential in the diet of aquacultured species, especially when higher levels of plant feedstuffs were included in the diet at the expense of marine feedstuffs. It is expected that proper dietary inclusion of rendered protein ingredients can provide farmed animals with balanced amino acids and minerals, reduce energy costs for metabolic transformation during growth and development, as well as increase feed utilization efficiency and profitability.

Use of animal protein ingredients in aquaculture feed, land animal feed, and pet food has dramatically declined after the outbreak of Bovine Spongiform Encephalopathy (BSE) in 2003. Although the prevalence of this disease has been strictly controlled in countries such as the United States, the ban on import of ruminant protein ingredients is unlikely to be lifted by many countries in the near future. However, increased prices of feedstuffs in commodity markets, competitive use of feed ingredients for biodiesel production, and uncertainty about the impact of global climate change on agriculture are having dramatic effects on profitability and sustainability of global animal agriculture, and even food safety. This presentation will highlight advancements in enhancing the nutritional value and biosecurity of rendered animal protein ingredients, and their potential importance in aquafeeds.

Soymeal Composition: Variation, Measurability and Value Capture Opportunities

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The aquaculture industry continues to request sustainable diets for additional fish species, each of which has a unique set of nutrient requirements and interactions between feed and health. Hence, the need for requirement studies and evaluation of nutritional quality is ongoing. From the literature, it is apparent that evaluation of nutritional quality of a feedstuff may give quite different results on different occasions, even when tested within one fish species. The reasons for apparently contradictory results are often difficult to interpret. One variable in assessing nutrient requirements and evaluating diets is variability in the feedstock. This is true for all sources of protein including fishmeal. In order to be assured that minimum feed specifications will be achieved, animal feeders factor in a "cushion" to ensure that minimum requirements for growth are met. This "cushion" reflects a less than optimum utilization of ingredient resources, and an increase to the cost of production. It is likely to be greater when there is less statistical confidence in a particular measurement. Identifying and better understanding measurement error supports the efforts of measurement providers to improve capability as well as measurement users to identify business opportunities and best applications.

The study had two primary objectives. The first was to determine the variation in nutritional value in samples of soymeal, closely representing commercial production, that were submitted by U.S. soybean processors in early and mid season blind surveys. Each sample was analyzed by wet chemistry methods to determine crude protein (combustion and Kjeldahl methods), fat, fiber, amino acid composition, and solubility factors (KOH and PDI). The second objective was to evaluate components of measurement error in SBM in both NIR and wet chemistry methods. 60 SBM samples were submitted by U.S. processors in May 2009. Each sample was split into 2 blind replicates. Wet chemistry analyses were performed by the University of *Missouri* Agricultural Experiment Station Chemical Laboratories. Samples were also analyzed by near infrared spectroscopy (NIR) using various equipment at the University of Minnesota.

The data suggests that the degree of intra-group variation in soymeal attribute composition is large enough to question the confidence in predictions based on generalized results between groups or anecdotal evidence, in comparison to direct measurement. The data also demonstrates how the impact of random error can be reduced; hence, confidence can be improved in applications involving increased measurement frequency such as in-line NIR. The following data will be reported and discussed: (1) range and distribution of attribute values (identified by sample codes); (2) range, distribution and comparison of solubility measurements; (3) comparison of attribute values to prior sample sets (similar populations); (4) relationship of amino acid values to crude protein; (5) comparison of crude protein methods (combustion vs. kjeldahl); (6) comparison of solubility methods (KOH vs. PDI); and (7) results of NIR scans, along with comprehensive analysis of measurement repeatability.

A Meta-Analysis of Essential Amino Acid Requirements of Fish

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The formulation of cost-effective feeds supporting optimal growth, health, and product quality in a given species requires a comprehensive understanding of the nutritional requirements of the animal and accurate characterization of the nutritive value of feed ingredients.

A number of reviews have identified significant gaps in our understanding of essential amino acid (EAA) requirements of fish and highlighted the great variability in estimates of EAA requirements amongst and within species. Differences in methodological approach and analysis of data appear to be at the source of some of the differences in the estimates of requirements. Specifically, the experimental design (e.g. number of diets), response parameter (e.g., weight gain, growth rate, protein deposition), mathematical and statistical models (broken-line, asymptotic, ANOVA, etc.) used for response analysis, and mode of expression of requirements (% diet, % protein, g/MJ digestible energy) are all factors that appear to contribute to this great variability in estimates of requirement.

The project involved a systematic, statistically sound, meta-analysis on EAA requirements of different aquatic species based on published scientific studies. A survey of the literature identified well over 200 articles dealing with EAA requirements of fish and shrimp. A sub-set of studies, limited to important species (salmonidae, cyprinidae, cichlidae, and some marine species) and meeting a certain set of criteria was created and used for the meta-analysis. These criteria included sufficient number of test diets, disclosure of the amino acid composition of the diets, satisfactory experimental conditions (adequate husbandry practices, feed delivery, water quality, etc.) and growth and feed utilization data, and information on proximate composition (crude protein, lipid) of the fish.

Various independent and dependent parameters (e.g. dietary EAA levels, dietary digestible energy, temperature-corrected growth rate, feed efficiency, protein and energy retentions, etc.) were standardized or calculated to allow meaningful integration and comparison of results from different studies. These parameters were then analyzed using different mathematical and statistical models by species or groups of species. Results of the meta-analysis will be presented.

Physiological Consequences of Fish Meal Replacement with Plant-Derived Proteins: Alterations in Stress Response and Tolerance

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The need to identify suitable alternative proteins for aquafeeds is clear. However, fish meal (FM) replacement may alter the responses of cultured fishes to changing environmental conditions and result in undesirable changes in physiological status. Anecdotal observations of mortality following handling among hybrid striped bass (*Morone chrysops* x *M. saxatilis)* (HSB) fed reduced FM feeds led us to investigate the response of HSB to a controlled stress challenge (net-chasing) after being reared on reduced or FM-free feeds. The results of this study confirmed a significant increase in stress-related mortality among fish fed increasing amounts of alternative protein (Figure 1). As a result of this observation, we launched a series of stress challenge trials in conjunction with standard FM replacement studies, investigating the influence of various plant-derived proteins on stress tolerance and robustness of carnivorous fishes.

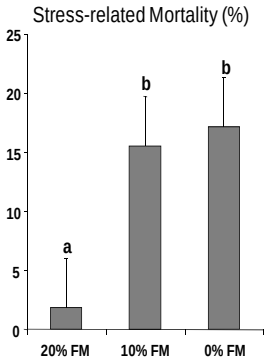

Figure 1. Stress-related mortality of HSB fed diets (~40% crude protein) containing graded levels of FM. Replicate tanks of fish were raised on diets indicated for 10 weeks, challenged with a net-chasing stressor, and monitored for 24 hours post-challenge. Bars with common letter labels are not statistically different ($p > 0.05$).

Results to-date indicate that replacing FM with plant protein sources such as corn gluten meal, soybean meal, or soy protein concentrate affects the stress response of HSB. For example, post-challenge levels of plasma glucose showed a trend of increasing with dietary inclusion of soybean meal at the expense of FM (Figure 2). Although no statistically significant differences were resolved for plasma cortisol, the values illustrated a similar pattern of heightened stress response among HSB raised on reduced-FM feeds (Figure 2). These data, coupled with the previous observations of mortality, suggest that the robustness of HSB may be compromised by feeding alternative proteins, even in the absence of gross production performance impairment.

In addition to reviewing the implications of physiological alterations observed in these and other studies with HSB and largemouth bass (*Micropterus salmoides)* fed plant protein-based feeds, potential mechanisms and corrective approaches will be discussed.

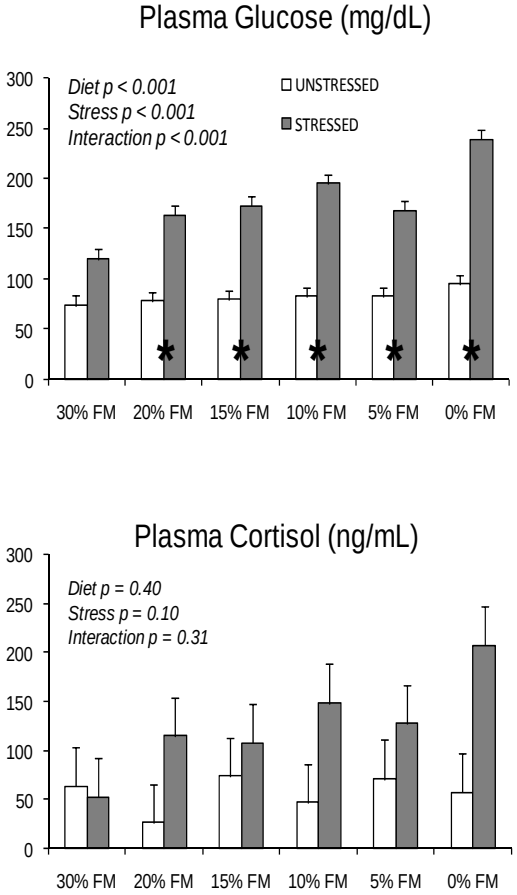

Figure 2. Plasma glucose and cortisol levels observed in HSB fed diets (~40% crude protein) containing graded levels of FM. Replicate tanks of fish were raised on the diets indicated for 8 weeks, challenged with a low-water stressor, and then monitored for 24 hours post-challenge. Paired bars with asterisks are statistically different ($p < 0.05$).

Soy Protein Concentrate in Practical Diets for Yellow Perch (Perca flavescens); Growth and Expression of Stress Related Genes

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Due to elevated prices of fish meal (FM), soy protein concentrate (SPC) has become a viable option for use in practical fish diets. SPC contains crude protein and essential amino acid (EAA) concentrations similar to those found in fish meal (FM), but has not been formally evaluated in diets fed to yellow perch (YP, *Perca flavescens*). Based upon computer-based formulations and results from studies using other species of fish, it appears that SPC may be able to replace up to 100% of the fish meal currently used in YP diets. This study was an evaluation of SPC in diets for juvenile yellow perch and the affects of the protein source on stress related genes.

Diets contained 38% crude protein, 10% lipid and the appropriate levels of essential amino acids (EAA). Diets were formulated to meet or exceed the known dietary EAA requirements or those predicted from whole bodies. Experimental diets contained SPC at increasing concentrations (25, 50, 75, and 100%) in place of fish meal, whereas the control diet contained no SPC. All diets were formulated to be isonitrogenous, isolipidic and isocaloric.

Twelve juvenile all-female YP were randomly stocked into each of the 15 110-L tanks. Each diet was fed to triplicate groups of fish. Due to the feeding nature of yellow perch, fish were fed a restricted ration (2.0% body-weight / day). Weights of feed were adjusted every two weeks during the 8-week trial. At the conclusion of the study, three fish will be collected from each tank for proximate analysis. Standard nutritional indices like feed consumption, weight gain, feed efficiency, proximate composition, and nutrient retention will be presented. Additionally, liver samples will be collected from five fish per tank. Total RNA will be individually extracted from these liver samples and transcript abundance of genes associated with stress response quantified using quantitative real-time PCR. Genes analyzed include metallothionein, heat shock proteins 70 and 90, heat shock protein transcription factor 1, glutathione synthase, glutathione S-transferase, and calnexin.

Catfish Ghrelin Physiology: Implications in Fish Carbohydrate Metabolism

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It is accepted that omnivorous fish species such as channel catfish have a greater proclivity for utilizing plant feedstuffs and carbohydrate for energy; however, the basis of this inclination is relatively unknown. Evaluation of metabolic and endocrine pathways and genes with regulatory implications in carbohydrate utilization is important for understanding the basis for bioactivity of plant feedstuffs and the ability of fish to utilize plant feedstuffs. Ghrelin is a gut hormone and was identified as the natural ligand of the growth hormone (GH) secreatgogue-receptor (GHS-R). As such, ghrelin is thought to function primarily as a GH-releasing hormone. In general, gut hormones have been shown to regulate a multitude of physiological functions including appetite and energy balance. Ghrelin is the only known orexigenic (appetite stimulating) gut hormone and has been recently implicated in several other biological actions in mammals, including insulin regulation and carbohydrate metabolism.

Recently, the ghrelin peptide and cDNA were isolated from the stomach of channel catfish. In addition to the amidated ghrelin peptide (common among fish), a Gly-extended, non-amidated ghrelin peptide (ghrelin-Gly) was also isolated. Furthermore, two ghrelin receptor cDNAs were isolated from channel catfish pituitary and partially characterized. Tissue distribution, ontogeny, and effects of endocrine and dietary manipulations have been examined. Observed meal-related changes in catfish ghrelin expression are indicative of ghrelin's accepted involvement in feeding regulation in fish. Other observations suggest greater GH-releasing potency of the Gly-extended ghrelin (ghrelin-Gly) in channel catfish and a putative role for the amidated catfish ghrelin as a regulator of pancreatic endocrine function and glucose metabolism. These findings have not been reported for other fish species for which ghrelin has been partially characterized, the majority of which are carnivorous. Ghrelin's suggested importance in feeding, growth hormone regulation, and carbohydrate metabolism make potential differences in ghrelin physiology between fish species with different dietary proclivity a fertile area of research. Understanding these differences may provide an important next step in determining whether carnivorous fish can be selected for improved utilization of plant feedstuffs.

Sexual Dimorphism in the Response of the Hepatic Transcriptome to Dietary Carbohydrate Manipulation in the Zebrafish (Danio Rerio)

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We examined the effect of long term manipulation of dietary carbohydrate on the hepatic transcriptome of both male and female zebrafish (*Danio rerio*). Zebrafish were fed diets comprised of 0%, 15%, 25%, or 35% carbohydrate from the larval stage through sexual maturity, then sampled for hepatic tissue, growth, proximate body composition, and retention efficiencies. Using Affymetrix microarrays and qRT-PCR, we observed substantial sexual dimorphism in the hepatic transcriptome. Males up-regulated genes associated with oxidative metabolism, carbohydrate metabolism, energy production, and amelioration of oxidative stress, while females had higher expression levels of genes associated with translation. Restriction of dietary carbohydrate (0% diet) significantly affected hepatic gene expression, growth performance, retention efficiencies of protein and energy, and percentages of moisture, lipid, and ash. The response of some genes to dietary manipulation varied by sex; with increased dietary carbohydrate, males up-regulated genes associated with oxidative metabolism (e.g. *hadhβ*) while females up-regulated genes associated with glucose phosphorylation (e.g. glucokinase). Our data support the use of the zebrafish model for the study of fish nutritional genomics, but highlight the importance of accounting for sexual dimorphism in these studies.

Made-to-Order Meals: Re-Designing Soybean Seed Composition to Meet Specific Aquafeed Demands

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Soybean meal is a promising plant-based protein source for use in the aquaculture industry. With the release of the soybean genome sequence, identifying genes underlying specific seed composition traits has become much easier. Coupled with traditional soybean breeding strategies and marker assisted selection, Schillinger Genetics is creating specialized soybean meals to meet the demand for a plant-based protein source with significantly modified anti-nutritional content for use in the aquaculture industry.

We have already developed a number of lines with altered seed composition traits, such as increased protein (15-20% increase), producing defatted meals with 58% protein, reduced raffinosaccharides (raffinose + stachyose below 0.5%), and reduced trypsin inhibitor content (60-70% reduction). We anticipate that important improvements in soybean meal for use in aquafeeds will be made by modifying these traits. Furthermore, by utilizing increased protein content as a foundation and adding the other altered soymeal composition traits to include lectin and P-34 allergen nulls, the prospects for new "select aqua specie" soymeals becomes a reality.

As more is known about fish nutritional and digestion requirements, we can continue to utilize genetics tools to efficiently breed new seed composition traits to make soybean meal further specialized. In addition, as soybean seed composition is adjusted to meet the nutritional demands of specific aqua species, its inclusion in aquafeed rations will no longer be limited since the anti-nutritional content has been significantly modified.

This work will contribute to the NOAA-USDA Plant Products in Aquaculture Working Group's Strategic Plant Goal 3: "Enhance the inherent composition of crops to provide a beneficial balance of bioactive compounds in order to optimize their use in aquafeeds for carnivorous fish."

Update on Engineering Optimal Grain and Legume Seed Phosphorus

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The quality of cereal grain and legume seed products for use in aquaculture feeds could be enhanced by engineering both the chemistry and amount of seed phosphorus (P). Adequate amounts of nutritionally-available P must be provided for optimal fish production, but high levels of P in aquaculture effluents represents an environmental hazard. Protein products obtained from seeds are relatively high in P (perhaps ranging from 5 to 10 gm P kg^{-1}) but at least 75% of this P is found as phytic acid P (*myo*-inositol 1,2,3,4,5,6 hexa*kis*phosphate), a form of P most fish do not efficiently utilize, and thus excrete.

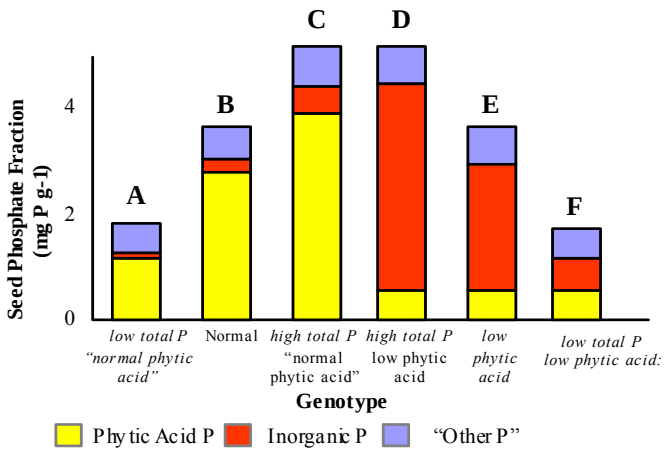

Figure 1. Seed phosphorus fractions in normal, low phytic acid, high total P and low total P crop genotypes.

"Low phytate/high available P" genotypes have now been developed in numerous crops including maize (*Zea mays* L.) and soybean (*Glycine max* L. (Merr.). In normal seeds (Fig. 1B), about 75% of seed total P is found as phytic acid P, less than 10% as inorganic P, and the remainder in "other" forms of P. In most low phytate types (Fig. 1E), phytic acid reductions are matched by increases in inorganic P, so that seed total P remains largely unchanged. Since most non-phytic acid P is nutritionally "available" to fish, available P is greatly increased in low phytic acid types.

We are currently also screening for "high" and "low" seed total P genotypes. The genotype illustrated in Fig. 1C produces seed that is high total P but the proportion of total P found as phytic acid P is normal. In contrast, Fig. 1D illustrates a high seed total P genotype that is also low phytic acid. This seed would be very high in inorganic P and would have very high "available P." This probably would represent the ideal seed P amount and chemistry for use in aquaculture feeds. However, a "low total P/low phytic acid P" type (Fig. 1F), in which seed total P is reduced but seed available P is increased, might also prove useful in certain cases. The status of this work will be reviewed. This work will contribute to the NOAA-USDA Plant Products in Aquaculture Working Group's Strategic Research Plan Goal 3: "Enhance the inherent composition of crops to provide a beneficial balance of bioactive compounds in order to optimize their use in aquafeeds for carnivorous fish."

Effects of Low Levels of Naturally Occurring Fusarium Mycotoxins on the Performance and Health of Rainbow Trout

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Deoxynivalenol (DON), a *Fusarium* mycotoxin, is a major contaminant of cereal grains worldwide which has previously been shown to adversely affect many livestock species. Increased use of plant protein sources in aquaculture feeds due to the rising cost of fish meal may result in an increased risk of exposing aquaculture species to DON and other mycotoxins. In a recent growth trial, we investigated the effects of low levels of DON from naturally contaminated sources of corn on the performance and health of rainbow trout. Seven experimental diets were formulated to contain graded levels of DON in a low realistic range (i.e. concentrations that could be encountered in practice). Diets 1 through 5 contained 0.3 (control), 0.8, 1.4, 2.0 and 2.6 ppm DON respectively. Diet 6 contained 1.9 ppm DON from a different source of corn. Diet 7 was formulated to be identical to Diet 1 (control) and represented a pair-feeding treatment with Diet 5 (2.6 ppm DON). The experimental diets were fed to rainbow trout (initial average weight = 24.3 g/fish) for 8 weeks. Weight gain, feed intake and feed efficiency (FE, gain:feed) of the fish decreased significantly ($p<0.05$) with increasing levels of DON in the diets. Performance of fish fed Diets 4 and 6 was not significantly different. Fish in the pair-feeding treatment had significantly higher FE ($p<0.05$) compared to their counterparts fed the diet containing 2.6 ppm DON (Diet 5). Significant histopathological changes in liver and intestine were observed with increasing dietary levels of DON. Together, these results suggest that rainbow trout are extremely sensitive to low levels of DON from naturally contaminated plant ingredients. Consequently, mycotoxin risk management strategies could become critical as the use of alternative plant ingredients in salmonid species feeds increases.

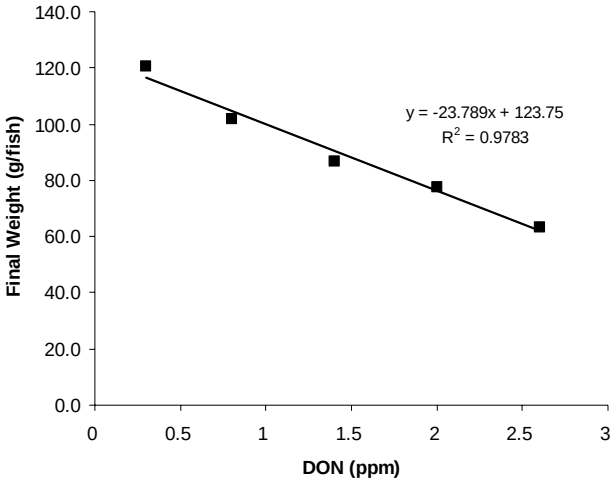

Figure 1. Final body weight (g/fish) of rainbow trout fed experimental diets containing low levels of DON from naturally contaminated corn.

Effects of Dietary Prebiotics on Growth Performance, Immune Responses, and Intestinal Morphology of Red Drum (Sciaenops ocellatus)

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A study was conducted with juvenile red drum to evaluate four different prebiotic compounds: fructooligosaccharide (FOS) in the form of inulin, galactooligosaccharide (GOS), Bio-MOS®, and Previda™. The latter two contain mannanoligosaccharides (MOS) derived from yeast and hemicellulose extract, respectively. The prebiotics were each added at a level of 1% at the expense of cellulose to the basal diet which was formulated to contain 41% protein equally from fish meal and soybean meal, and 10% lipid. Each diet was fed to groups of 12 fish, initially averaging approximately 7 g in triplicate 38-L aquaria twice daily to apparent satiation for 8 weeks.

Red drum fed the diet containing Previda™ had significantly ($P \leq 0.05$) greater weight gain than fish fed the basal diet and the one supplemented with Bio-MOS® (table 1). Fish fed the basal diet had significantly lower weight gain than fish fed the diets containing Previda™, GOS, and FOS. Feed efficiency (FER) and protein efficiency ratio (PER) of fish fed the various diets were not significantly different although fish fed the basal diet had the lowest values. Fish fed the Bio-MOS® diet had lower survival than fish fed the other diets, although no significant difference in survival was observed between fish fed the basal and experimental diets. The hepatosomatic index (HSI), muscle ratio and condition factor were not significantly affected by the dietary treatments.

The immunological responses of fish fed the diet supplemented with FOS exhibited significantly lower neutrophil oxidative radical production than fish fed the other diets. In addition, serum lysozyme activity was significantly lower in the fish fed the basal diet compared to those fed the diets supplemented with prebiotics. Quantitative changes in the ultra-structural characteristics of the gastrointestinal tract of red drum fed the various diets were evaluated using histological methods. Enterocyte fold height of proximal-, mid-, and distal-intestine as well as the height of pyloric caeca were not significantly affected by dietary prebiotics. However, the height of microvilli in the pyloric caeca, proximal- and mid-intestine was significantly increased by these compounds.

Results from this study indicate that Previda™ supplementation produced the most consistent responses in terms of enhanced growth performance, immunostimulation and gut histology compared to all the other prebiotics and basal diet. In contrast, the two different prebiotics containing MOS produced mixed responses in red drum.

TABLE 1. Growth, feed utilization and survival of red drum

Diet	Initial weight (g)	Weight gain (%)	Survival (%)	FER	PER
Basal	7.1±0.13	329.0±52.89a	83.1±17.35	0.58±0.04	1.4±0.08
FOS	7.1±0.20	424.9±50.05bc	80.6±12.73	0.63±0.04	1.6±0.10
Bio-MOS	7.2±0.15	354.9±18.06ab	58.3±22.09	0.63±0.05	1.4±0.15
Previda	7.2±0.06	430.9±9.14c	86.1±4.82	0.63±0.02	1.5±0.03
GOS	7.0±0.11	408.4±33.26bc	80.6±9.62	0.61±0.02	1.4±0.05

Data represent mean ± SD. Values in the same column with different superscripts are significantly different ($P < 0.05$).

Effects of Seafood-Processing Wastes Used in Aquafeeds on Biological Indices and Proximate Composition of Juvenile Red Drum (Sciaenops ocellatus)

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There is considerable potential for increased efficiency of US aquaculture through development of nutritious, cost-effective alternatives to traditional marine protein feedstuffs such as fishmeal (FM) whose global supply continues to increase in demand and price. Therefore, development of protein concentrates from seafood processing wastes and co-products such as soybean meal represents a valuable opportunity to enhance the efficiency of seafood production through aquaculture, while limiting negative impacts of underutilized wastes produced during seafood processing and also sparing other marine proteins. In this study, seafood processing by-products from channel catfish, shrimp and marine fish processing plants were blended with soybean meal and subjected to dry extrusion to produce three distinct protein concentrates. Each product was analyzed for nutrient composition and further characterized for incorporation into aquafeeds as FM substitutes.

A reference diet with all protein from menhaden FM and six experimental diets with 25 and 50% of FM protein replaced with each of the three seafood by-products were compared. All diets had 40% crude protein and 10% lipid. Groups of twelve juvenile red drum with an initial weight of 4.7 ± 0.2 g/fish were collectively weighed and placed into individual 110-L aquaria which were part of a closed, re-circulating system equipped with sand and biological filters. Brackish water (6 ± 1 ppt) circulated through aquaria at 1 L/min and was kept at 26 ± 1 C, with ~ 100% oxygen saturation and < 0.5 mg/L unionized ammonia. After stocking, fish were fed for one week a diet consisting of a 50:50 substitution of FM with soybean meal which was also used as the control diet after this conditioning period. During the feeding trial, fish in triplicate aquaria were fed each diet at a fixed percentage of body weight/day (BWD) divided into two equal daily allotments (am/pm). Feeding rate was progressively reduced from 6 to 3% BWD with weight gain (WG), so that a feed rate close to satiation was maintained without overfeeding. The feeding trial conducted for 8 weeks after which WG, feed efficiency (FE), hepatosomatic index (HSI), intraperitoneal fat (IPF), muscle ratio (MR), and survival data, as well as carcass proximate composition values for ash, moisture, lipid and crude protein were obtained and subjected to factorial analysis of variance (ANOVA, TABLE 1). Also, a complete analysis of mineral concentrations was conducted on menhaden FM and the seafood processing byproducts.

Generally, WG, survival, HSI, IPF and MS were not significantly different ($P > 0.05$) for fish fed any of the diets. However, FE was significantly increased by the inclusion of the shrimp by-product meal, with the 50% substitution of this ingredient resulting in the highest FE value (0.8 g gain/g feed). Whole-body samples for proximate composition are under analytical review. Preliminary results indicate that high FM substitution levels (50% of dietary protein) with the co-extruded seafood by-products are feasible without detrimental effects on any performance indicator. Further feeding trials substituting higher levels of FM in diets for juvenile red drum appear warranted.

TABLE 1 Biological indices and proximate composition of juvenile red drum fed diets with different seafood byproducts[1]

Experimental diet	Weight gain[2] %	Feed efficiency g gain/g fed	Survival %	HSI[3]	IPF[4]	MR[5]
1. Menhaden fishmeal	602.8	0.65[bc]	80.6	3.7	0.6	65.6
2. 50:50 FM:SBM	532.7	0.68[abc]	88.9	3.1	0.6	53.9
3. 25% shrimp	654.9	0.72[ab]	83.3	3.2	0.8	55.5
4. 50% shrimp	643.5	0.76[a]	91.9	2.7	0.6	51.2
5. 25% black drum	537.4	0.72[ab]	94.5	3.3	0.9	53.1
6. 50% black drum	641.2	0.70[abc]	80.6	2.3	0.4	43.1
7. 25% catfish	657.9	0.74[ab]	91.7	3.3	1.2	53.1
8. 50% catfish	621.1	0.61[c]	88.9	3.4	0.7	52.4
ANOVA						
P > F[6]	0.085	0.032	0.649	0.270	0.201	0.281
Pooled SE	33.17	0.03	6.29	0.37	0.19	5.26

[1] Means of three individual fish from each of three replicate groups. Values in a row that do not have the same superscript letters are significantly different at $P \leq 0.05$ based on Duncan's multiple range test.
[2] Percent of initial weight.
[3] Hepatosomatic index (HSI) = liver weight * 100/total fish weight.
[4] Intraperitoneal fat (IPF) ratio = total IPF weight * 100/total fish weight.
[5] Muscle ratio (MR) = total muscle (fillet) weight * 100/total fish weight.
[6] Significance probability associated with the F statistic.

Replacement Of Fish Meal In Sunshine Bass (Morone chrysops ♀ x Morone Saxatilis ♂) Diets With Proteinaceous Fermentation Biomass

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Increasing demand and rising costs of fish meal (FM) coupled with static landings of reduction fisheries have made continued use of FM in aquafeeds environmentally and economically unsustainable. Animal and plant-derived proteins have been investigated as alternatives to FM with variable success. Observed limitations of traditional alternative proteins have led nutritionists to investigate new alternatives to FM. Yeast-based, proteinaceous fermentation biomass (PFB), a co-product of bio-ethanol production, is a promising novel alternative because of its composition and increasing availability. We evaluated production performance of juvenile sunshine bass (~ 16g) reared on diets (38% crude protein, 15% crude lipid) containing increasing amounts of PFB (Table 1).

Table 1. Experimental feed formulation

Ingredient	Control (30% FM)	22.5% FM	15% FM	7.5% FM	0% FM
PFB	0.00	13.46	27.00	41.33	50.69
Menhaden FM	30.00	22.50	15.00	7.50	0.00
Soybean Meal	30.00	30.00	30.00	30.00	30.00
Wheat Bran	23.31	17.34	11.28	4.47	0.35
Soy Protein Concentrate	0.00	0.00	0.00	0.00	1.00
Soy Protein Isolate	0.00	0.00	0.00	0.00	1.00
Canola Oil	5.34	5.34	5.35	5.34	5.45
Menhaden Fish Oil	5.34	5.34	5.35	5.34	5.45
Carboxymethylcellulose	2.00	2.00	2.00	2.00	2.00
Choline Chloride	0.60	0.60	0.60	0.60	0.60
Vitamin/Mineral Premixes	3.42	3.42	3.42	3.42	3.42
L-Methionine	0.00	0.00	0.00	0.00	0.04

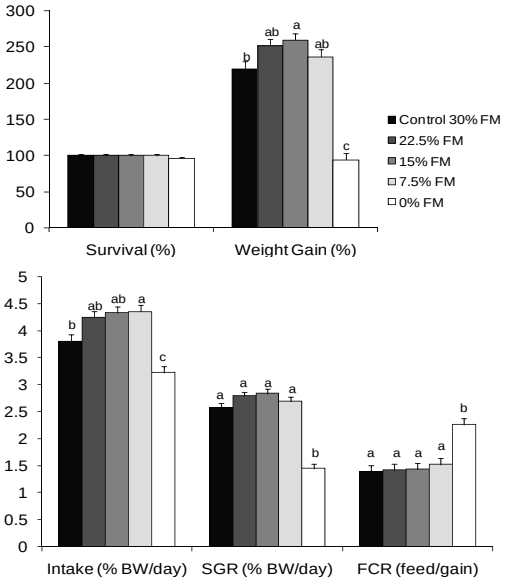

Figure 1. Production performance by dietary treatment (means ± standard error). Bars with common superscripts are not significantly different (*P > 0.05*); absence of superscripts indicates lack of significant treatment effect.

Although complete FM replacement resulted in significantly reduced performance, partial substitution of FM with PFB resulted in equivalent, or in some cases, enhanced intake and growth after 45 days (Figure 1). However, significantly reduced feed intake and specific growth rate (SGR), in the 0% FM group suggests the palatability of PFB may be a limiting factor impacting production performance. Whole carcass composition did not vary with respect to percent moisture, ash or protein, though significantly higher crude lipid content in the 7.5% FM treatment suggests protein conversion efficiency may have been affected in this group. Our data indicates that PFB can partially replace FM without negatively affecting production performance. However, performance may be negatively affected by diets in which PFB replaces all protein provided by FM.

Soy Protein Concentrate as a Replacement for Fishmeal in the Fingerling Diet for Grass Carp Production in China

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Grass carp (*Ctenopharyngodon idella*) is the No. 1 freshwater fed species cultured in China. The ASA-IM aquaculture program in China conducted a pond feeding trial in 2008 with grass carp to evaluate its growth performance and feed conversion efficiency from the advanced fry to fingerling stage with a newly formulated all-plant protein fingerling diet. The test diet was formulated to be nutritionally equivalent to the ASA-IM standard fingerling diet, with 36% crude protein and 7% crude lipid. The standard fingerling feed (36/7 std.) contained 20% fishmeal; while the all-plant protein variant of the diet (36/7 SPC) completely replaced fishmeal with soy protein concentrate (65% crude protein), and included supplemental methionine to make it methionine equivalent to the 36/7 standard diet.

The feeding trial was conducted in three 5.0-mu (0.33 ha) ponds at the demonstration farm of the Heilongjiang Provincial Fishery Technology Extension Center in Harbin, the northern part of China. Grass carp fry of size 0.17 g were stocked in early July at a density of 4,000 fish/mu (60,000 fish/ha), together with 1,000 silver carp fry per mu (15,000 fish/ha). Grass carp fry in the three trial ponds were first fed the ASA-IM formulated 41/11 crumble fry feed from stocking size to an average size of 3.0 g, at which time the grass carp were weaned to the ASA-IM newly formulated 36/7 SPC test diet. The feeding trial lasted 82 days starting from July 3 to September 23, 2008. The grass carp grew from 0.17 g to an average weight of 150.4 g with an average FCR of 0.98:1. The average gross production of grass carp and silver carp was 476 kg/mu (7,140 kg/ha) and 50 kg/mu (750 kg/ha) respectively. The grass carp fingerling trial with 36/7 SPC diet yielded a net economic return of RMB 35,145 from the 15 mu of ponds, and represented an average net return of RMB 2,343 per mu ($5,246/ha). Harvested fish had average market prices of RMB 12/kg ($1.79/g) for grass carp and RMB 4/kg ($0.60/kg) for silver carp. The average return on investment (ROI) for the three ponds was 65.6%.

The feeding trial results indicated that grass carp fingerlings do not have a requirement for fishmeal in their diet and that an all-plant protein feed, in which fishmeal is replaced with SPC, can be used to efficiently and economically produce grass carp fingerlings in ponds at the northern part and elsewhere in China.

Alternative Feeding Strategies to Maximize Marine Feedstuff Replacement in Largemouth Bass Culture While Maintaining Production Performance

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High demand for fish meal (FM) and fish oil (FO) as well a reduced harvest from capture fisheries has led to increasingly higher prices of FM and FO. Land based feedstuffs are a possible alternative to the high economical and ecological costs of marine feedstuffs. Poultry byproduct meal (PBM) is a possible alternative to FM because of a similar amino acid profile and acceptance by a variety of carnivorous taxa. Similarly, poultry fat (PF) could possibly replace FO in largemouth bass (LMB) diets without affecting production performance while reducing cost. Accordingly, we evaluated production performance of LMB after feeding diets containing increasing amounts of PBM and PF. Diets (40% protein, 10% lipid) containing graded levels of FM to PBM (15, 7.5, 0% FM) as well as graded levels of FO to PF (6, 3, 0% FO) were fed to juvenile (~25 g) fish in a 3 x 3 factorial design. After 12 weeks, fish were harvested and data were analyzed by a two-way ANOVA (α=0.05).

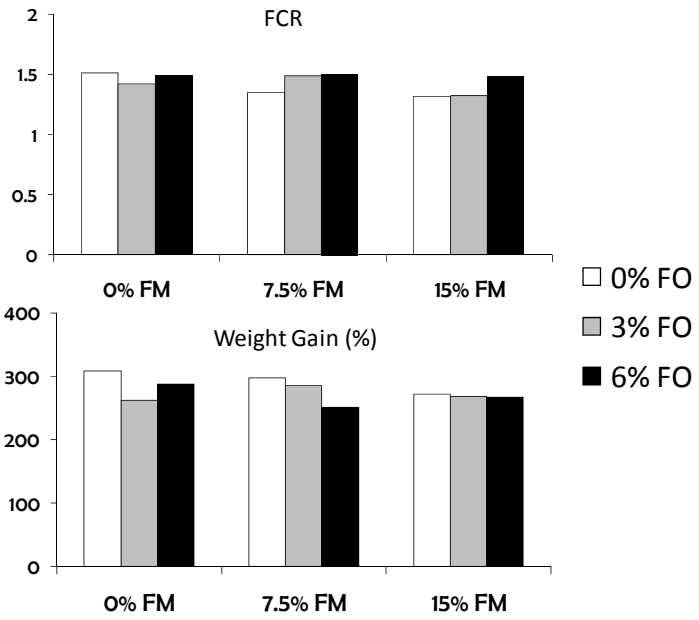

Figure 1. FCR and weight gain of LMB fed increasing amounts of PBM and PF at the expense of FM and FO. No significant difference were observed.

Feed conversion ratio (FCR), weight gain, and consumption were not significantly affected by dietary treatment. Increasing amounts of poultry by products, especially PF, led to lowered long-chain polyunsaturated fatty acid and increased mono-unsaturated fatty acid content in fillet lipid. Production performance was not affected by complete replacement of marine feedstuffs. PBM and PF are possible alternatives to FM and FO, although fillet fatty acid profile will be affected by implementation of poultry byproduct in LMB feeds.

Development of Practical Soy-Based Diets for White Seabass (Atractoscion Nobilis) and California Yellowtail (Seriola Lalandi)

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White seabass (WSB), *Atractoscion nobilis* and California yellowtail (YT), *Seriola lalandi* are two top candidate species for ocean farming in southern California. Both species are carnivorous and are currently reared on standard commercial diets whose nutrient profile and ingredient composition have not yet been "optimized" relative to long-term sustainability issues associated with fish meal and oil. This project is a continuation of research conducted in 2008 to develop and validate open plant-based feed formulations for the WSB and YT. The current goals of this research are to (1) determine optimum protein levels in a soy-based diet and (2) further optimize soy-based diets by manipulating alternative ingredients and supplements.

The first trial for WSB tested a series of diets formulated to contain 46, 43, 40, 37, 34, 31% protein and 12, 10.8, 9.5, 8.25, 7, 5.75% lipid, respectively with 20-30% fish meal. At the conclusion of this trial weight gain ranged from 278-346% and FCR from 1.3-1.4. The results indicate that 40% protein is a reasonable level to support maximum growth under these conditions. The second trial utilized a 40% protein 10% lipid test diet formulated with 18% fish meal and the remainder of the protein from plants (primarily soy). Supplement of methionine and taurine were evaluated (in the 18% fish meal diet) and the results compared to a diet containing 36% fish meal as well as a fishmeal free diet using poultry by-product meal with methionine and taurine supplements.

Results indicate that diets supplemented with 1x and 2x taurine gave a significant improvement in performance over un-supplemented diets containing 18 or 36% FM (Table 1). The 2x methionine diet performed better than the 18% FM diet. The poultry diet performed better than the 18 and 36% FM diets. It would appear that methionine may be marginally deficient and the taurine had a positive effect on growth.

Table 1. WSB trial 2 results

Diet	% gain	FCR
FM36	192.0^{d}	1.35^{c}
FM38	134.5^{f}	1.74^{a}
1xMe	146.9^{ef}	1.64^{b}
2xMe	159.0^{e}	1.55^{b}
1xTa	233.6^{b}	1.16^{d}
2xTa	290.4^{a}	0.98^{e}
Poult	240.4^{b}	1.04^{e}

The first trial for YT tested a series of diets formulated to contain 52, 48, 44, 40% protein and 16, 14, 12, 10% lipid, respectively with 41-54% fish meal. At the conclusion of this trial % weight gain ranged from 578-932% and FCR from 0.77-1.0. Results indicate that 48% protein is a reasonable level to support maximum growth under these conditions. The second trial is currently underway utilizing a 48% protein 14% lipid test diet formulated with 20% fish meal and the remainder of the protein from plants (primarily soy) to evaluate the necessity of dietary supplements for methionine, lysine, and taurine.

Effects of Canola Protein Concentrate on Growth of Atlantic Salmon (Salmo salar)

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Atlantic salmon (*Salmo salar*) is an important aquaculture species with 9400 MT produced in 2005. Atlantic salmon is a carnivorous species that does not tolerate high levels of plant feed ingredients in the diet. New sources of protein must be found for the continued growth of the salmon industry. A 38-week feeding trial was conducted at the National Cold Water Marine Aquaculture Center (Franklin, ME) with juvenile Atlantic salmon to evaluate canola protein concentrate. A commercial diet (Signature Salmon 3.5 mm, Northeast Nutrition, Truro, Nova Scotia) was used and modified by the manufacturer to contain 10 or 20% canola protein concentrate replacing fishmeal and poultry by-product meal. The three diets were formulated to contain 48% protein, and 21% lipid. Diets were manufactured and analyzed by the commercial feed producer. Each experimental diet was randomly assigned to triplicate tanks containing 20 Atlantic salmon with an initial weight of 133.0 ± 7.8 g per fish. The fish were stocked into nine 0.265 m^3 tanks connected to a common drum filter and biological filter to maintain optimal water quality. Water in the system was brackish water (3 ‰) from a ground source, and temperature (7.5 to 14°C) and dissolved oxygen were monitored continuously and ammonia, nitrite, nitrate, carbon dioxide, and pH were monitored weekly. Fish fed 20% canola protein concentrate had significantly lower growth compared with the fishmeal diet (p=0.0385). There was not any significant difference in feed efficiency (p=0.2170) or protein efficiency ratio (p=0.2081). Canola protein concentrate significantly depressed growth when included in the diet at 20%.

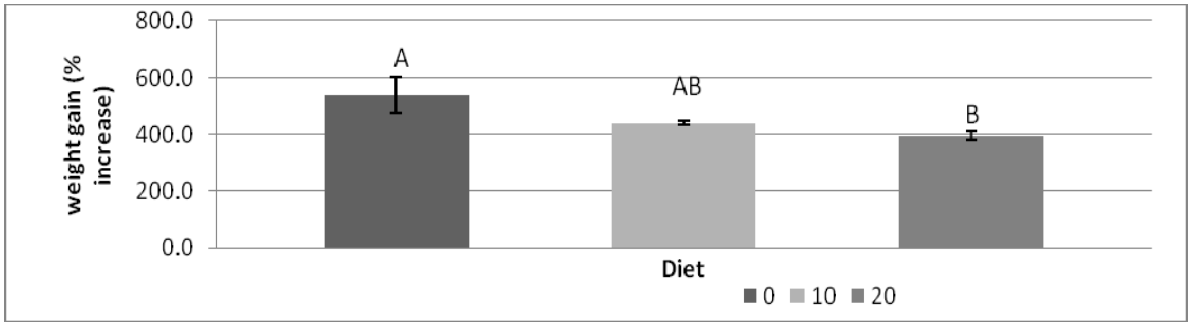

Figure 1. Weight gain (% increase) of Atlantic salmon fed increasing amounts of canola protein concentrate. B. FCR of Atlantic salmon fed increasing amounts of canola protein concentrate. Error bar represent 1 SEM.

Minimizing The Use Of Fishmeal In Diets For White Seabass (Atractoscion nobilis) And California Yellowtail (Seriola lalandi)

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This research addresses Sea Grant's Strategic Plan research goal of sustainable resource use. Specifically to minimize the use of fish meal and fish oil in the diet of two of California's top candidates for offshore aquaculture – the California yellowtail (YT), *Seriola lalandi* and white seabass (WSB), *Atractoscion nobilis*. It also addresses recent California legislation (SB201) which states "To reduce adverse effects on global ocean ecosystems, the use of fish meal and fish oil shall be minimized. Where feasible, alternatives to fish meal and fish oil, or fish meal and fish oil made from seafood harvesting byproducts, shall be utilized . . ." Limited information is available on acceptability and utilization of alternative low fish meal diets by either California yellowtail or white seabass. These studies used the "blended protein" substitution approach, which allows for the determination of interactive and additive effects of multiple ingredients.

In the first 8 week trial with WSB we evaluated 5 diets (40-44% protein) including a 52% fishmeal (FM) control diet, three diets with decreasing levels of FM from 20-0% replaced with a protein blend (MB1) consisting of soy protein concentrate (SPC), corn gluten meal (CGM), soybean meal (SBM), poultry by product meal (PBM) and blood meal (BM). A fifth 10% FM diet was also evaluated replacing FM with a protein blend (MB4) consisting of CGM, PBM, SBM and BM. WSB were stocked into 300L tanks supplied with 20°C recirculated seawater at a size of 5.6g and fed 4-7% body weight daily. Fish were weighed every two to three weeks.

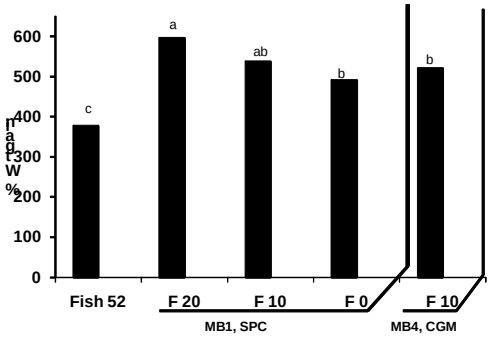

Figure 1. Differences in weight gain of WSB after 8 weeks of feeding on various diets.

After 8 weeks fish weighed 26.5-38.4g (375-595% gain) (Figure 1). Results indicate significantly better gain in the 20% FM diet (595%) with the poorest growth in the 52% FM control diet (377%). However, survival (%) was highest in the 52% FM control diet (99.0%) and the 10% FM diet containing MB4 (94.5%) and decreased significantly in the remaining three diets with increasing level of MB1 (91.0-61.0%). FCR was significantly poorest in the 52% FM control diet (1.25) followed by the 10% FM diet with MB4 (0.98). The remaining three diets (20-0%FM with MB1) had the best FCR's (0.90-0.92). A trial is currently underway testing similar diets (47% protein) on YT.

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