Report of the Plant Products in Aquafeed Strategic Planning Workshop: An Integrated, Interdisciplinary Research Roadmap for Increasing Utilization of Plant Feedstuffs in Diets for Carnivorous Fish

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INTRODUCTION

On November 21–22, 2005, approximately 30 aquaculture and agronomy researchers and representatives from federal technical agencies met at the USDA National Center for Cool and Coldwater Aquaculture in Leetown, West Virginia. The goal was to develop an integrated (cross-disciplinary), strategic approach to improve understanding of the factors limiting inclusion of plant feedstuffs in the diet of carnivorous fishes. The participants drafted a roadmap of the research required to reach that goal and how that research would not only benefit the aquaculture, grain and oil-seed producers, and feed industries, but also improve supply and affordability of nutritious seafood to the consumer. The specific objectives of the conference were to define goals, performance measures, present status, and 2- and 4-year targets.

This workshop was the next step in a process that began with a meeting of international experts in Twin Falls, Idaho, in June 2005. At that meeting, the group organized itself into the...

RATIONALE FOR DEVELOPING PLANT-BASED AQUACULTURE FEEDS

Demand for seafood continues to increase at a rate that is predicted to result in a significant global increase in farmed fish production over the next 15 years. To meet that demand, the aquaculture industry needs to identify feedstuffs that can be used to produce economically viable and sustainable aquafeeds. Alternatives to fish meal and fish oil, on which many present aquafeeds are largely based, are required to continue the increase in aquaculture production. The aquafeeds industry has recognized for many years that increased utilization of plant protein in formulated diets is essential, but many limits to increasing the use of plant-based ingredients have been identified. These alternative ingredients must be cost effective, support optimal fish performance, have minimal environmental impact, and result in a product that is appealing and nutritious.

While domestication of terrestrial livestock species occurred over thousands of years, we remain largely hunter-gatherers in the sea. Yet, given recurring reports of health benefits from eating fish, and dire predictions about the state of marine fish stocks and diversity, aquaculture is likely to continue to be the fastest growing agricultural production system.

Concurrent to the increase in aquaculture production, the demand for agricultural products for biofuels and other bio-based industrial products is expected to increase the supply of co-products. Co-products such as soybean meal and distillers dried grains will become increasingly economical for the feed industry as demand for soybean oil for biodiesel and corn for ethanol increases.

Fish meal has been the protein source of choice in aquafeeds for many reasons, including its high-protein content, excellent amino acid profile, high-nutrient digestibility, general lack of anti-nutrients, relative low price, and its wide availability. However, demand for fish meal is expected to exceed the annual world supply in the next decade, and this increased demand will change the economic and nutritional paradigms that up to now have resulted in high use levels of fish meal in many aquafeeds, especially those for carnivorous species.

Despite considerable research efforts, there are still unknown factors that limit the amount of plant feedstuffs that can be included in diets of carnivorous species, including most marine species of present and potential commercial importance. As an example, for reasons that are not clear, carnivorous species have limited tolerance to soybean meal that ranges from none in Chinook salmon, *Oncorhynchus tshawytscha*, to somewhat higher in diets for rainbow trout, *Oncorhynchus mykiss* (Olli and Krogdahl, 1994), and Atlantic salmon, *Salmo salar* (Olli et al., 1994; Bæverfjord and Krogdahl, 1996; Krogdahl et al., 2003). Yet, the salmon industry is clamoring for a stable source of protein in terms of cost and supply, and such a supply would benefit other established and emerging sectors of the aquaculture industry as well.

It is likely that a combination of plant-derived feed ingredients will be required to replace fish meal, and that supplements will be needed to produce aquafeeds without fish meal that support growth rates necessary for economic production of farmed fish. While the supply of fish meal and oil is arguably sustainable, the anticipated growth internationally in aquaculture is expected to exceed the supply of these items in the next decade. In addition to concerns about the sustainability of fisheries resources, other issues including the potential presence of organic and inorganic contaminants in fish meal and the net effect of supply and demand economics in the global market require enhanced efforts to thoroughly evaluate reasonable alternatives.

Unlike earlier in human history when trial-and-error experimentation and manipulation of species led to the domestication of plants and animals, we are now poised to domesticate aquatic species thoughtfully and sustainably. The tools of genomics, including nutritional genomics, provide the opportunity not only to obtain comprehensive metabolic information about the farmed fish and their responses to various diets, but about the human nutritional value of the product. The tools are available to develop diets that are highly digestible, resulting in minimal waste and minimal negative impact on aquatic ecosystems. Developing more digestible, plant-based feeds need not take thousands of years or even tens of years if industry continues to work collaboratively with researchers and funding programs, using all available technologies, to target the strategic objectives outlined here.

STANDARDIZE RESEARCH APPROACHES AND PROTOCOLS

Significant resources are currently used to evaluate the nutritional quality and possible health implications of feedstuffs with the potential of replacing fish meal as a protein source in aquaculture. Moreover, the aquaculture industry continues to request 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 increasing. Studies to determine nutrient requirements and nutritional quality of ingredients are resource demanding and time consuming.

It is apparent in published results that variability is great concerning the nutritional quality of a given feedstuff, even when tested within one fish species. The reasons for these apparently contradictory results are often difficult to interpret as ingredient sources, physiological stage of the fish, and other experimental conditions differ greatly between experiments. If more standardized protocols were used, the quality of the data would improve, and limited human and funding resources would be saved. In
addition, higher quality data are needed for the development of useful models across ingredients and species, models that may reduce the need for nutritional experiments.

Standardization is needed for reference feed ingredients as well as the experimental ingredients under evaluation. Detailed information should be given regarding processing conditions, chemical composition, and appropriate quality parameters, including levels of anti-nutrients, storage, and processing conditions. Several of the required analyses are complicated and expensive, calling for standardization and perhaps centralization. Conditions such as dietary inclusion levels, feeding regime, duration of feeding trial, diet palatability and feed intake, method of feces collection, marker level, etc., should be selected to maximize quality and usefulness of the results. Nutrient digestibility, growth, body composition, fish function and health, as well as flesh quality should be investigated employing acknowledged methods as appropriate for the feedstuff and species under investigation. Design of nutritional studies as well as statistical testing tools should be developed to save resources and increase the quality and usefulness of the output. For appreciation of the accuracy of experiments, some measure of variability should be given for all results. Possibilities for the establishment of a network of databases holding information on characteristics of feed ingredients as well as results of experiments on the individual or tank level should be explored. Such a network would greatly improve possibilities for model development and universal use of data from nutritional studies in fish.

**Goal 1:** Establish standardized research approaches and protocols for systematic evaluation of plant feedstuffs across carnivorous fish species.

**Objective:** Define standard response measurements and standardization of feed input compounds.

**Performance Measures:**
- Develop protocols and methods for the characterization of strains/fractions of plant materials for aquafeeds.
- Evaluate innovative protocols and feeding regimes.
- Develop standardized methodologies for determining feed intake, palatability, flesh quality, and other factors to be used in fish genetics screening programs.
- Improve estimates of nutrient requirements in cold- and warm-water species, based on available nutrients under variable physiological and environmental conditions, employing biochemical and physiological response parameters, and adopting modeling approaches.
- Develop standardized physiological methods to determine the impact of diet on fish physiology (gut, liver, and muscle) and health.

**ENHANCE FISH GERMPLASM AND DISCOVER GENES**

Evaluation of genotype by diet interactions in aquaculture species for specific dietary components has only recently begun on a limited basis. Commercial diets for carnivorous fish species have traditionally contained high levels of fish meal and fish oil as protein and energy sources due to their cost effectiveness. Omnivorous fish species such as tilapia and catfish have demonstrated a greater proclivity for utilizing plant feedstuffs and carbohydrate for energy, but comparative studies among the different species have been limited to date. Furthermore, a large number of aquaculture species have only recently become domesticated, and, as such, most of the initial research with these species has involved general determination of their dietary requirements.

Given the great diversity of fish species that may be cultured, the physiology and metabolism of these organisms varies substantially. In aquaculture there have been very few studies attempting to discern the effects of genotype on nutrient utilization. However, in other animal systems, the effects of dietary changes on specific physiological parameters have been studied and have been linked to individual genes. Several vertebrate genomes have been completely sequenced, which allows for the retrieval of homologous sequences. With this information, the correlations of biochemical information with genetic and molecular data will be very useful in providing improved insight into the functions of unknown genes or systems responses to nutrient changes.

The important next step for aquaculture is to determine whether carnivorous fish that have been evolutionarily selected to utilize animal protein as their main nutrient source can be selected for improved utilization of plant material. In the past, diet studies were roughly correlated by relating growth indices, such as weight gain, feed efficiency, growth rate, digestibility, and proximate analysis of whole fillets, to variable changes of dietary components. With the recent impetus of genomics and the increasing amount of sequence information available, an improved understanding of cellular signaling and the role of genes and proteins in pathways can be combined with what is occurring in the animal, not only physiologically. This will revolutionize how nutritionists evaluate diets in that now they can relate precise dietary changes with specific physiological pathways that are being modulated by nutritional differences.

**Goal 2:** Enhance fish germplasm and discover genes.

**Subgoal 2.1:** Determine the basis for bioactivity of plant products and ability to utilize plant products in fish.

**Objective 2.1:** Develop and use genomic tools in fish that improve plant product utilization.

**Performance Measures:**
- Increase current genome sequence information for major fish species.
- Use genomic methods to develop markers for identification of quantitative trait loci (QTL) for improved plant product use.
- Use transcriptomics to identify candidate genes involved in plant product use.
- Use proteomics to identify candidate genes involved in plant product use.
Enhance fish genetic resources to optimize their use in aquafeeds.

**Objective 2.2:** Develop novel high-performance germplasm able to utilize high levels of plant products.

**Subgoal 2.2:** Develop fish strains representing the available breadth of genetic variability to screen response to plant products in diets and establish agreements to conduct testing.

**Subgoal 2.2:** Develop standard comparison lines of fish to characterize utilization of plant products.

**ENHANCE PLANT FEEDSTOCKS**

Currently, all non-modified, plant-derived feedstuffs that might replace fishmeal have characteristics that place them at a disadvantage in terms of their suitability for use in aquafeeds. Biologically active compounds in plant feedstocks, some of which belong to a rather ill-defined group known as anti-nutritions, have been described to some extent. However, a wide range of the compounds found in plants and their effect in fishes are unknown. While it is widely accepted that the feed industry will draw from various plant feedstocks when creating aquafeeds, soybean is the most widely studied and may have a role as a model for other plant sources.

Developments in instrumentation and chemical approaches over the last few years have greatly improved, and such approaches provide the means to describe and predict the nutritional value of soybeans and other plant feedstuffs on the one hand, and assay adverse anti-nutrient effects on the other hand through detailed chemical analyses. Information profiling and the identification of single volatile, semi-volatile, and flavor compounds may lead to the identification of unknown compounds that impact digestibility of these products and influence the physiology, growth, and health of farmed fish interactively, thus allowing the evaluation of these feedstuffs and fractions with differential levels of anti-nutrients and to identify specific anti-nutrients. Plant feedstuffs can be fractionated and tested both for anti-nutrient activity (in collaboration with fishery biologists), metabolic composition, and protein components. Statistical analysis will be used to identify metabolic correlates of anti-feedant activity.

**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.

**Subgoal 3.1:** Define the components of plants that result in adverse and positive growth and nutritional functions.

**Objective 3.1:** To elucidate the physiological reasons for intolerance of various plant feedstuffs in carnivorous fish.

**Performance Measures:**

- Characterize the compounds and actions of small molecules in soybean and other plant feedstuffs that result in reduced palatability and anti-nutritional actions using metabolomic and other molecular approaches (e.g., lipidomics).
- Determine the role of protein and fiber content on growth and nutrition.
- Characterize immunological response to soybean meal and other plant proteins and the role of anti-nutritional activity of the proteins as well as synergistic effects of co-consumption of multiple proteins that induce adverse feed response.
- Characterize positive factors in plants that enhance growth and nutrition.
- Improve understanding of the genetic and environmental influences on variation in quantity and quality of carbohydrates, proteins, and oils in potential plant sources.

**Subgoal 3.2:** Selection and/or creation of plant varieties optimized for use in fish diets.

**Objective 3.2:** To develop strategies to mitigate the feed intolerance and/or improve the digestibility, nutritional value, and feed efficiency of plant ingredients in aquafeed formulations.

**Performance Measures:**

- Screen plant germplasm resources to eliminate adverse growth and nutritional substances and to enhance positive factors producing optimized conventional lines.
- Produce genetically modified plant varieties that synthesize beneficial compounds that could not otherwise be produced, such as altered fatty acids, pigments, vitamins, and cofactors.
- Solve the production and processing problems related to segregating plant varieties (whether conventional or genetically modified) optimized for fish feed through industry and user conferences and agreements.

**INTERACTIONS BETWEEN GASTROINTESTINAL MICROBIOTA AND PLANT TOLERANCE IN FISH**

It has been documented in a number of food animals that their gastrointestinal microbiota play important roles affecting the nutrition and health of the host organism. Thus, various means of altering the intestinal microbiota to achieve favorable effects, such as enhancing growth, digestion, immunity, and disease resistance of the host organism, have been investigated in various terrestrial livestock species as well as in humans. Dietary supplementation of prebiotics, which are classified as non-digestible food ingredients that beneficially affect the host by stimulating
growth and/or activity of a limited number of health-promoting bacteria such as *Lactobacillus* and *Bifidobacter* spp. in the intestine, while limiting potentially pathogenic bacteria such as *Salmonella*, *Listeria*, and *Escherichia coli*, have been reported to favorably affect various terrestrial species. However, such information is extremely limited for aquatic organisms. Effects of probiotics, defined as live microbial feed supplements, on gastrointestinal microbiota have been studied in some fishes, but the primary application of microbial manipulations in aquaculture has been to alter the composition of the aquatic medium. In general, the gastrointestinal microbiota of fishes, including those produced in aquaculture, have been poorly characterized, especially the anaerobic microbiota. Therefore, more detailed studies of the microbial community of cultured fish are needed to potentially enhance the effectiveness of prebiotic and probiotic supplementation.

**Goal 4:** Improve understanding of the interactions between plant-based diets and intestinal microbiota and their effects on the intestinal morphology, nutrient transport, gut-associated immune responses, and disease resistance in carnivorous fish species.

**Objective 4:** Determine the effects of intestinal microbiota (both native and dietary probiotic supplements) on growth, immune responses, disease resistance, and feeding efficiency of carnivorous fish species fed plant-based diets.

**Performance Measures:**

- Develop methods to characterize the native populations of gastrointestinal microbiota in fish.
- Characterize native gut microbiota in model species of plant-tolerant and plant-sensitive fish.
- Determine if intestinal microbiota play a role in maintaining intestinal morphology of plant-tolerant and plant-sensitive fish.
- Determine if intestinal microbiota play a role in nutrient transport of plant-tolerant and plant-sensitive fish.
- Determine if intestinal microbiota play a role in immune-mediated responses of plant-tolerant and plant-sensitive fish.
- Develop practical approaches to exploit positive interactions between intestinal microbiota and plant-based diets.
- Develop an interactive database for immuno-nutrition of fish.

**PROCESSING TO OPTIMIZE PLANT-BASED AQUAFEEDS**

Feed processing methods have been used for many years to improve the physical characteristics and nutritional value of aquafeeds. The effects of manufacturing methods on some known compounds are well known. However, as compounds in plants with activity in fish continue to be identified, the effects of processing will need to be characterized. In addition, new processing technologies are likely to increase the nutritional value of plant-derived ingredients for use in aquafeeds.

The new technologies include biological enhancement, which involves the use of microorganisms to modify the chemical composition of the feed ingredient. Yeast and bacterial and fungal fermentations have been investigated, and current results demonstrate great potential for removing anti-nutrients and adding essential nutrients such as protein and amino acids. Other methods, such as mechanical modification, may also be used to increase nutrients and decrease anti-nutrients. The commercial utility of any processing or pre-processing technology depends not only on the nutritional quality of the product, but on the economics of its application. Chemical modification, or extraction technologies, have also been used for many years to produce high-protein, high-quality plant ingredients such as wheat gluten meal and soy protein concentrate. These products have been too expensive for significant inclusion in aquafeeds in the past, but market factors (i.e., increasing fish meal prices) along with modifications in technologies are increasing the use of these products in aquafeeds. Removing anti-nutrients and increasing protein content of plant products through processing technology may be the fastest and most cost-effective way to increase the use of plant products in aquafeeds.

**Goal 5:** Improve and optimize ingredient processing, feed manufacturing technology, and feed formulations to increase inclusion of plant-derived ingredients in the diet of carnivorous fish.

**Objective 5:** Develop plant-based feed formulations that support rapid, efficient, and cost-effective growth of carnivorous fish.

**Performance Measures:**

- Compile a list of enhanced products and manufacturing technologies.
- Evaluate the economics, composition, processing specificity, values of by-products, and interaction with processing methods for raw plant materials, including but not limited to grains, oilseeds, and specialty grains.
- Develop and evaluate mechanical, physical, chemical, enzymatic, and biological processing methods for improving quality of plant-based ingredients.
- Determine the effects of processing technology on bioavailability of required nutrients and other biologically active compounds that have a positive or negative activity.
- Determine the effect of enhanced processing methods on feed texture, ingredient palatability/feed consumption, and *in vivo* digestibility of nutrients.
- Evaluate effect of feeding improved ingredients on fish growth, product quality, and nutrient retention.
- Develop and evaluate plant-based feeds for cold- and warm-water monogastric species that maximize growth, minimize costs, and limit environmental impact of aquaculture.
- Evaluate improved feed formulations for cold- and warm-water carnivorous species in production systems.
STORAGE, NUTRITIONAL, AND SENSORY QUALITY
OF FARMED FISH RAISED ON PLANT-BASED FEEDS

Success or failure of any commercial aquaculture endeavor ultimately depends upon repeat purchasing by consumers, whether at the retail, wholesale, or food service level. Aquatic foods market research consistently indicates that product quality is the single most important attribute affecting fish purchasing behavior. Given the recent onslaught of negative media attention on farm-raised fish, consumers are also focused on the potential health effects associated with fish consumption. As we move forward toward increasing the use of plant products in aquafeeds, the effects of these newly formulated diets on fish product quality and consumer health must be evaluated.

Although quality is a complex issue, for the purposes of this article, quality refers primarily to the color, flavor, texture, and nutrient composition of the fish product. Numerous studies have reported that all of the previous attributes, as well as shelf-life stability of fish fillets, can be affected by diet composition.

Consumers are increasingly interested in the health benefits of eating seafood. The same group of consumers is likely to be among the most environmentally aware, so there are opportunities for the industry to provide a highly nutritious source of protein raised on sustainable, renewable crops (not contaminated with PCBs or other organo-chlorides), and that are farmed with minimum negative impact on the environment.

Researchers and feed companies are at present exploring techniques for replacing fish oil with plant-based oils and then “finishing” with fish oil and/or algal oil in order to ensure that the fish oil composition is optimal for human health.

Goal 6: Optimize the storage, nutritional, and sensory quality of aquaculture species for human consumption.

Objective 6: Determine the effect of plant ingredients on the stability, safety, sensory quality, and nutritional value of farm-raised carnivorous fish species.

Performance Measures:
- Characterize the stability and storage characteristics of aquacultured carnivorous species raised on diets that optimize the inclusion of plant products.
- Characterize the nutritional quality to humans of aquacultured carnivorous species raised on feeds that optimize the inclusion of plant products.
- Using trained taste panels, characterize the sensory characteristics of aquacultured carnivorous species raised on diets that optimize the inclusion of plant products.

COMMUNICATION AND OUTREACH

Mechanisms will be established to coordinate research across the goals outlined in this plan. Coordination will involve researchers, funding agencies, the aquaculture industry, farmers, and other stakeholders, and will ensure that interaction among international experts from diverse disciplines is facilitated and that data are widely available and usable. It will include means for expanding the network of researchers with relevant expertise.

This goal also includes coordination and supply of the standard and non-standard plant materials, aquafeed, and fish used among the various research projects, including the provision of defined materials and conditions across research projects. This will enable cross-comparisons and maximize information retrieval.

A virtual “meeting place” will be provided for integrating the research results, processing technologies, chemical analyses, and chemical profiles in the aquafeed with bioactivities and physiology. These data will facilitate determining which compounds are responsible for which physiological responses.

In addition, the established network will provide a conduit for the results of the research implemented under this strategic plan to the public-at-large as science-based information on which to base decisions about the impacts of aquaculture on human health and the environment.

Goal 7: Establish an international communications network for research on optimizing plant products in aquafeeds.

Objective 7: Provide a framework for effectively coordinating the research, exchange of data, and establishment of standards for research performed under this umbrella.

Performance Measures:
- Build a matrix/database of compounds in plants on which the effect on fish has been studied.
- Bioinformatics: Have mechanisms in place so that genomic data can be integrated with physiological and molecular responses and analyzed.
- Develop mechanisms for data prospecting from the database and for assessing quality of data and updating information in the database.
- Develop and maintain a website, list-serve, or other technologies for posting information; sharing information such as models and techniques; and hosting discussion groups.
- Coordinate plant materials, aquafeeds, and fish used among the various research projects, including providing defined materials and conditions across research projects. Supply researchers with “standard” samples suitable for comparison of methodology and species.
- Develop and implement an organizational structure to guide the implementation of the PPA Strategic Research and Action Plans and convene follow-up meetings as needed.
- Develop and implement an outreach plan that promotes the dissemination of science-based information on use of plant-based diets in aquaculture.

CONCLUSION AND FUTURE PLANS

This strategic plan marks a major departure from the historic domestication of livestock species and their diets, and outlines
a collaboration and cooperation not previously seen in aquaculture nutrition research. The approach endorsed by PPA workshop participants and described in this article represents a coordinated, interdisciplinary, cross-species effort to improve understanding, not only of aquacultured fish species, but the plant feedstuffs in their diets—the plant products currently in use and co-products from a bio-based economy.

The participants are all aware that optimal levels of plant products in the diet of carnivorous fish species have not been attained. However, all are optimistic that through collaborative efforts utilizing modern investigative tools and the roadmap outline in this article, more fish meal can be replaced by plant-based ingredients.

Future workshops will be held to refine the performance measures described in the PPA Strategic Plan estimating time to complete, producing a budget, and identifying resources, including expertise available to tackle the problems. It is the intention of the PPA Working Group that any researcher with an interest in, and expertise relevant to any aspect of the Strategic Plan, is welcome to participate in the PPA Working Group and workshops.

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