

Feed Ingredients and the Reduction of Dependence on Marine Harvested Products for  
Aquaculture Feeds

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Funding for the development of this document was provided by the Tides Canada Salmon Aquaculture Innovation Fund. The mention of trade names or commercial products in this article is solely for the purpose of providing specific information and product examples and does not imply recommendation or endorsement by the U.S. Department of Agriculture or the U.S. Department of Interior.

## **Executive Summary**

Fishmeal has been harvested from the oceans for centuries as an animal feed, and for many years was included at low levels in poultry and swine diets due to the unidentified growth factors it contained. The first compounded fish feeds consisted primarily of fishmeal and fish oil with some wheat, vitamins and minerals. Fishmeal is believed to contain all the nutrients required by fish and by its inclusion in fish feeds historically made formulation of diets easy and economical. As aquaculture expanded in the last three decades the amount of fishmeal used by aquaculture has grown from 10 to over 70% by some estimates, with a decreased proportion consumed by terrestrial animals. This increased consumption of marine-harvested products by aquaculture has occurred even though the percentage of fishmeal in feeds for many fish species has decreased dramatically. Fishmeal production, however, is static at best and the demand will continue to increase. The need to search for alternatives to fishmeal has been apparent and active for decades, but the intensity of the search has increased on a global scale in the last decade. The first step in coping with these changing circumstances was to determine what nutrients are provided by fishmeal that are not found in alternative ingredients. Many minerals, amino acids and vitamins have been identified and are now commonly supplemented in low or fishmeal free feeds. Nutritionally complete fishmeal-free diets are a reality today for many species including trout and marine species, but on a commercial scale some fishmeal may still be needed in salmon diets due to the lack of availability of feed-grade nutrient supplements and/or effective alternatives for some yet to be identified nutrients present in fishmeal.

The most pressing issue is not just finding replacement ingredients for fishmeal but identifying a consistent supply of high quality ingredients with a suitable available nutrient content. The production of both terrestrial and aquatic animals will continue to increase as the world population continues to increase creating competition among the sectors for feed ingredients. Ingredients produced from residuals not able to be used as human food will be valuable, but the scale of production may be more for regional use. Traditional ingredients such as the co-products from soy oil production for food (soybean meal and feed grade soy protein concentrates) will continue to play an important role, along with other major commodities such as corn and wheat. Because of the increasing need for human food and thus an increasing need for animal feed ingredients, there is a flurry of commercial activity in the development of new types of feed ingredients.

Newly developed ingredients are originating from a variety of places including existing large scale industries such as ethanol and amino acid production and fishery processing, to smaller scale products like uncertified tree nuts. Some ingredients are being produced de-novo using waste streams from other industries such as single cell proteins and insects. The first step in evaluating these ingredients is to determine the nutritional quality through a series of feeding studies. Many laboratories have extensive programs to evaluate the nutritional quality of potential ingredients and important factors include: 1) palatability, the effect on feed intake 2) nutrient digestibility, the nutrients available to the animal or do they pass through the digestive tract 3) the presence of anti-nutrients (i.e. trypsin inhibitor, gossypol, glucosinolates) or other deleterious compounds. This information can then be coupled with additional criteria including availability, consistency of quality, consistency of supply, cost relative to active ingredients and competing uses such as food or fuel to aid in determination of commercial potential. The available nutrient profile is a primary criterion when evaluating nutritional quality. The availability, scalability or volumes of products will determine if the product can be regional or

global in nature, and both sources of product will be needed in the future for various markets. If the production of the ingredient is unpredictable or undependable due to uncontrollable factors the commercial potential of the product is limited. The consistency of quality of the product is very important for commercial viability. If the nutrient or anti-nutrient profile is changing with each shipment, then buyers will soon not depend on that ingredient. Aquatic animal production is similar to any other business in that input costs determine the profitability of the operation. Protein sources are often compared on a price per unit of crude protein, and even more effectively as price per unit of digestible protein.

Additional factors that affect the economic value of the potential ingredients must also be considered. The effect the ingredient has on the feed manufacturing process is critical. Ingredients can affect the amount of energy required to produce a ton of feed by being very viscous or slippery. Ingredients also affect the amount of expansion of the pellet which affects oil absorption capabilities and the quality of the final feed. Pellet durability is an essential factor because feeds are transported either pneumatically or mechanically and if the pellets break the result is loss of product which can in turn degrade water quality. The effect of an ingredient on waste management is becoming increasingly important. It has been determined that some ingredients cause a diarrhea effect in salmonids making removal of the fecal waste from the rearing water difficult. Lastly, an important aspect of potential ingredients is its effect on the final product quality, including but not limited to, changes in fish texture and flavor and whether the ingredient alters or confers additional human health benefits (i.e. omega 3 fatty acids).

At present, ingredients for both animal and fish feed include fishmeal, fish oil, soybeans and several other grains. As our knowledge of fish nutrient needs and of novel ingredient quality increases, however, the future composition of aquafeeds will likely be much different. Combining this knowledge is necessary to facilitate the controlled expansion of aquaculture and provide a safe sustainable food supply for consumers in the future.

## Introduction

Aquaculture is the fastest growing sector of agriculture and production is expected to continue to increase. The State of World Fisheries and Aquaculture 2012 (FAO 2012) projects that total fisheries output will increase 15% to 172 million tonnes in 2020, with the expansion largely accounted for by growing aquaculture share. Along with this growth is an increasing trend for aquaculture to be dependent on compounded feeds. The types of species/species groups dominating fed aquaculture production and the recent focus to increase and intensify production of crustaceans, marine finfish, and diadromous fishes, reflects a tendency for increasing reliance on aquafeeds for their production (Rana et al. 2009). In 2008, about 31.5 million tons or 46.1% of total global aquaculture production were dependent upon the direct use of feed, either as a single ingredient, as farm-made aquafeeds or by the use of industrially manufactured compound aquafeeds (FAO 2009). Total industrial compound aquafeed production increased almost four fold from 1995 to 2008, with production growing at an average rate of 10.9 percent per year (Tacon et al. 2010). More recent industry data projects the Aquafeeds market to grow at an annual growth rate of 11.7% from 2013 to 2018 and reach 82,390.5 KMT by 2018 (Feedmachinery.com; June 2014).

The continued success of responsible aquaculture and the recognized contribution it makes to global food security relies on providing responsibly-sourced feed. With this increasing demand for compounded feeds comes an increasing need for nutritious, sustainable and cost-effective feed ingredients. Specifically, as the demand for aquafeeds grows the need for additional sources of protein and lipids increases. Although competition for marine-harvested products from other feed sectors and the limited supply have resulted in an intensive rise in feed costs (Tacon and Metian 2008), these increases are not limited to marine-harvested products. Many of the key ingredients traditionally used in commercial aquaculture feeds are internationally traded commodities. Therefore, aquafeed production is also subjected to any common global market shocks and volatility. Since 2005, the commodity price index rose by about 50% and the prices of soybean meal, fishmeal, corn and wheat rose by 67, 55, 125 and 130%, respectively and the cost of major oils used in the aquafeed industry has increased by up to 250%. (Rana et al. 2009).

Inconsistencies in marine-harvested product prices and vulnerability to sharp supply shifts have resulted in numerous global initiatives undertaken to reduce the dependence of aquaculture on fishmeal and fish oil. This has resulted in a global search for alternative feed ingredients that may have a more consistent supply and are less expensive. For example, the French National Institute of Agriculture has proposed that dietary fishmeal inclusion levels for rainbow trout should be reduced from current levels of 25-30% down to 5% (Rana et al. 2009). Similarly in the United States, the Agricultural Research Service (USDA) in cooperation with other Federal Agencies, including NOAA and USFWS, and Universities, has conducted research for over a decade to identify the essential nutrients in fishmeal that limit growth and reduce performance when feeding fish-meal free feeds to fish. These initiatives and others have resulted in considerable knowledge that has led to substantial reductions in dietary fishmeal inclusion rates for many species as compared to the levels used 10 years ago. These initiatives also have led to expanded research efforts to identify strengths and limitations of other traditional and novel aquafeed ingredients.

## **Factors Affecting Nutritional Quality of an Ingredient**

While it sounds simple enough to just add specific nutrients to the diet that fishmeal provides it is in fact quite complex. Different sources of nutrients are digested, absorbed, transported and metabolized at different rates. It is important for any fishmeal-free feed to be formulated taking this into consideration for the most efficient use of the diet and the highest performance of the fish. The quantity and quality of nutrients present in an ingredient determine the ability to provide nourishment and affects the nutritive and metabolic processes of the body including growth, reproduction, and maintenance of health and is thus a primary when evaluating nutritional quality. However simple chemical analyses, while important characteristics, are not the best indicators of nutrient quality and must be validated with numerous other factors including palatability, nutrient availability and the presence of anti-nutrients.

### **Palatability**

Palatability is defined as the combination of attractiveness and ingestion of a diet and is essential to feed development. Palatability is important because, no matter how digestible and available the nutrients and energy from an ingredient may be, if the ingredient reduces feed intake then it will slow animal growth and have limited value as a feed ingredient. It is crucial to differentiate effects on feed intake from effects on utilization of nutrients from ingredients. Feed intake is the key performance criteria in palatability assessments for fish. Reduced growth and perhaps other problems can be the result of including a low palatability ingredient in the diet unless palatability enhancers are added along with the ingredient. Substances such as betaine and specific amino acids have been shown to increase feed intake in fish.

### **Nutrient availability**

Nutrients in feeds cannot be used until they are absorbed into the body and transported to cells where metabolism takes place, thus digestibility is an important assessment of a potential aquafeed ingredient's value. Determining nutrient digestibility in specific ingredients and diets for fish has been an active area of research for decades. Apparent digestibility coefficients (ADCs) can be determined for each macro-nutrient or nutrient category (crude protein, crude lipid, etc.). This value represents the percentage of the nutrient or nutritional category that is absorbed by the fish. This information is needed by researchers and feed manufacturers to formulate feeds to precisely meet the dietary requirements of fish without over-formulating the target value. ADCs are also necessary for determining the nutritional and economic value of alternative ingredients. This information is often developed by major feed companies or can be found in National Research Council publication (NRC 2011) and from specific laboratories (<http://www.ars.usda.gov/Main/docs.htm?docid=21897>).

It is essential to know the nutrient digestibility of feed ingredients in order to formulate diets that maximize the potential of the ingredients and the growth and feed efficiency of the fish. The digestibility of nutrient such as protein, fats, amino acids and minerals is determined through feeding known amounts of a nutrient and an indigestible marker to determine the Apparent Digestibility Coefficients (ADC). ADC's are the percentage of that nutrient that is actually digested and absorbed by the fish. This step in the ingredient evaluation process does

necessarily produce a positive or negative result, but helps when determining the economic value of an ingredient.

### **Anti-nutritional compounds**

Anti-nutritional compounds in plants evolved as a protective mechanism for seeds to avoid consumption by birds and other animals, or to pass intact through the gut by affecting digestive processes. Two classic examples of an anti-nutritional compounds in plants includes the trypsin inhibitors present in soybean meal that reduce the activity of digestive enzymes in the gastrointestinal (GI) tract and phytic acid (found in most plant ingredients) that reduces the bioavailability of phosphorus, zinc and other minerals. In animal products, compounds associated with decaying tissue such as putresine and cadaverine are naturally present at low concentrations but at high concentrations can be toxic. Identifying the presence of these compounds in any novel ingredient is absolutely essential in determining appropriate inclusion levels.

### **Other Factors That Affect the Economic Value of the Potential Ingredients**

Nutritional quality must also be balanced against how the potential ingredient alters manufacturing, and waste management. Supply chain affects including ingredient consistency and delivery consistency characteristics must also be considered. Finally, once all other factors have been addressed the cost per functional unit value can be calculated.

### **Feed manufacturing related factors**

Evaluation of how an ingredient affects the feed manufacturing process and the effect of the ingredient on final pellet quality is an important characteristic to feed manufacturers. An ingredient can increase feed production costs by becoming very viscous or sticky thus increase electrical energy required or decrease product quality by reducing durability of the pellet or reducing oil absorption capacity. Regardless of the nutritional value of an ingredient, if it cannot be practically included in a feed with the physical properties required to optimize its delivery to a given fish species, then its value as an ingredient is significantly reduced. These properties can be simultaneously addressed during the production of diets for digestibility studies conducted with extruded diets. In those studies, the reference diet is produced and energy requirements, temperatures, pressures, torque and specific mechanical energy are recorded. The test diet generally contains 70% reference diet and 30% test ingredient. By comparing the processing parameters between the two diets, the effect of high inclusion levels of the test ingredient can be determined. Pellet quality can be accessed through the determination of the Pellet Durability Index (PDI). There are a variety of devices available that simulate either mechanical or pneumatic conveying and a sample of feed is placed into the device such that conveying is simulated over a given period. The percentage of intact pellets surviving the test is determined by sieving. PDI is an indicator of what percentage of the feed might become unusable before the feed gets to the fish, making it a primary consideration of both feed manufacturers and fish farmers. Large fish farms receive feed in bulk, not bags, and feed is distributed to a raceway,

tank or pen through distribution systems. By assessing the effects of an ingredient on pellet quality and properties, the ability to manage pellet quality through ingredient selection is improved. The interaction between the feed manufacturing process and the mixture of ingredients used in a feed formulation has a significant and varying effect on the final cost, stability, consistency and acceptability of the feed.

### **Effect of an ingredient on waste management**

All waste in aquaculture operations originally comes from the feed, and both excess/uneaten feed and feces must be removed as much as possible before either releasing the water from the facility or recycling the water. Several challenges must be overcome to deliver nutrients via manufactured feeds in an aquatic environment. Conventional feeds in water can rapidly deteriorate, lose nutrients and pollute the culture system. The manufacturing of aquatic feeds has continually evolved over the years to meet these challenges by packaging a mixture of dietary ingredients into a form that can be delivered, consumed and effectively utilized by the fish. The exact formulation of the feed will affect both the amount of feces produced and the amount of soluble nutrients released into the water (i.e. ammonia, phosphorus). Feeding extruded, high-energy feeds is one management tool that can be used to reduce waste, since these are highly digestible nutrient dense feeds. In a properly managed farm, approximately 30% of the feed used will become fecal waste. However, not only the amount but consistency of the waste is important. The consistency of that waste often causes the greatest challenges for aquaculture engineers and feed manufacturers as diet formulas become more dependent on plant-based ingredients.

When trout are fed fishmeal-based feeds a solid fecal pellet is produced and production systems (i.e. raceway quiescent zones, filtration systems in recirculating systems) were designed around the characteristics of the feces. Trout fed plant-based diets, however, exhibit a condition similar to diarrhea in terrestrial species. This condition causes the feces to consist of very fine particles that do not settle in the quiescent zone of raceways and are difficult to remove in recirculating aquaculture systems. Aquaculture engineers had been investigating factors that affect fecal particle size in flow-through production systems with commercial diets, and recognized the importance of fecal particle size on waste management efficiencies (Brinker and Rosch 2005). A wide variety of compounds thought to increase the size and durability of fecal particles were evaluated using this technique including: lignin sulphonate, algae meal (alginbind), modified starch (non-gelatinized), sodium-alginate, fish gelatin, guar gum, resistant starch and powdered cellulose (Brinker et al. 2005). Guar gum and alginate showed the greatest potential for increasing fecal stability so that factors such as feeding activity, distance to the filters and water flow rate and pattern would not decrease particle size beyond the ability of filters or settling devices to remove them. Working with rainbow trout, the optimal level of guar gum in the diet was determined to be 0.3% of the diet (Brinker 2007). There is always a concern of possible adverse effects on fish performance when inert ingredients are added to the diet. Inclusion levels of guar gum at 0.3% did not affect rainbow trout growth or feed conversion ratio when added to either a fishmeal-based or plant protein-based diet (Brinker and Rieter 2011). The digestibility of crude protein, lipids, starch and phosphorus were also unaffected by the addition of guar gum to the diet. The use of dietary guar gum also has a greater effect for larger rainbow trout (642 g/fish) than medium (162 g/fish) or smaller trout (43 g/fish) by making the feces larger, but does not remedy the underlying cause of diarrhea (Brinker 2009).

Fecal particle size is considered a direct measure of the mechanical stability of feces in self-cleaning tank systems (Brinker 2007). It is known that diet composition affects fecal particle size, but the effect of individual ingredient changes in a formulation on fecal particle size was recently described in a series of short-term studies (Barrows et al. 2014). In that study, diets that contained 52% of the test ingredients were manufactured and fed to juvenile trout. Trout fed a diet that contained either menhaden meal, sardine meal, poultry by-product meal or corn protein concentrate had significantly larger fecal particles (1.5 to 3.5 mm) and less fecal fines (<0.3 mm) than fish fed any of five different soy products or spirulina. Guar gum was effective at reducing the percentage of small fecal particles in diets containing 30% soy or less. There was a positive correlation ( $R^2 = 0.84$ ) between the percentage of fines and the total carbohydrate content of the feed. Similar correlations were observed for crude fiber ( $R^2 = 0.83$ ) and non-starch carbohydrate ( $R^2 = 0.86$ ).

These data demonstrate the importance of screening potential ingredients for their effect on waste management. Similarly, methods to modify and improve fecal stability such as modifications in gut microflora through the use of pre or probiotics and/or the development of complimentary ingredient combinations that can improve fecal stability is also an area of ingredient development where substantial improvements can be made.

### **Ingredient availability, consistency of quality and supply**

The best growth and feed efficiency of fish is obtained when the feed composition is kept reasonably constant without drastic ingredient shifts, so feed manufacturers rely on ingredient suppliers to provide a consistent product on a regular basis. This can be difficult for start-up companies due to the difficulties of scaling up the production process, but must be considered when planning the development of a product. If the production of the ingredient is unpredictable or undependable due to uncontrollable factors the commercial potential of that product is limited. The consistency of product quality is similarly important for commercial viability. In the future as there is more and more competition for feed ingredients more leeway may be given to ingredient suppliers, but the suppliers that can produce large volumes, consistent quality, and on a regular basis will always be preferred.

### **Cost relative to active ingredients**

Feed manufacturers can withstand some fluctuations in price, but price stability is valuable in itself. Aquaculture production like any animal feeding operation is sensitive to the cost of feeds since feeds can often represent more than 50% of the production costs. A potential ingredient may have fantastic nutritional characteristics but it can easily be priced out of formulations when more ingredients becoming available. As an example, protein sources are often compared on a price per unit of crude protein, and even more effectively as price per unit of digestible protein. If two ingredients contain 50% crude protein and ingredient A has 95% ADC for protein and ingredient B has 75% ADC for protein, ingredient A has a higher economic value. Diets can still be formulated with these two ingredients that when fed will result in equal growth, but more of ingredient B will have to be included and more nitrogen will be lost in the feces decreasing protein retention efficiency.



## **Traditional Ingredients in Aquafeeds**

Traditional terrestrial plant product ingredients such as the co-products from soy oil production for food, e.g. soybean meal and feed grade soy protein concentrate, will continue to play an important role, along with other major commodities such as corn and wheat. Ingredients produced from terrestrial animal residuals not able to be used as human food will be valuable, but the scale of production for many products may be more appropriate for regional use.

### **Terrestrial plant products**

Recently, there has been an increased emphasis on using plant products in aquafeeds (Gatlin et al. 2007). Although plants are generally investigated based on their regional abundance, those with wide ranging distribution and abundance have received the most attention for aquafeeds. These include cereal grains such as corn and wheat, legumes like soybean meal and oilseeds, such as canola.

Processed soybeans are the world's largest source of protein for animal feed and the second largest source of vegetable oil. The soy industry is based upon oil production for human foods with the meal considered a co-product for animal feed. Soybean meal is the most common source of plant proteins used in aquafeeds, with feeds for some species containing from 15 to 30% soybean meal, with a mean of 25% in 2008. In global usage terms, and based on a total compound aquafeed production of 27.1 million tons in 2007, it is estimated that the aquaculture feed sector consumed about 6.8 million tons of soybean meal (25.1 % of total compound aquafeeds by weight; FAO 2011). Although, feeding high levels of soybean meal can result in a condition similar to diarrhea for rainbow trout, the recent development of feed-grade soy protein concentrates that do not cause this condition are attractive fish feed ingredients due to their relative abundance, high protein levels and low levels of anti-nutrients. Soybeans selectively bred to contain lower levels of specific antinutrients including oligosaccharides and allergic proteins also have been developed and commercialized which offer increased utilization for this ingredient in salmonid diets.

Corn products are attractive fish feed ingredients due to their abundance, and corn protein concentrates with as high as 75% crude protein are commercially available (Cargill Corn Division, Empyreal 75). Ground corn provides starch to a diet to act as a binder during cooking extrusion. Corn products, however, contain several pigments that can be deposited in the flesh of salmonids resulting in an undesirable color of the fillet in some species.

Wheat is most often used in aquafeeds as whole ground wheat or wheat flour primarily as a starch source for binding extruded feeds as mentioned above for ground corn. Wheat gluten meal, although historically too expensive for inclusion in animal feeds is now being used more often due to its high protein content (~77-80%) and its feed manufacturing related functional properties. When heated, wheat gluten has binding capacity that provides water stability to the pellets, which although valuable can also limit the inclusion level in the diets. When wheat gluten is included at 4 to 6% of the diet water stability will be increased; however, with diet inclusion levels above 15%, the pellets become so hard feed consumption by even the aggressive rainbow trout decreases.

Similarly, global canola production has grown rapidly over the past 40 years, rising from the sixth largest oil crop to the second largest (USDA, ERS). Canola meal as a by-product of

human oil consumption has potential as protein source for fish diets similar to that of soybean meal. Canola meal has a favorable amino acid balance, and through fractionation, canola meal protein levels can be increased to levels comparable to other vegetable protein concentrates.

### **Terrestrial animal products**

In general, terrestrial livestock by-products are good sources of dietary protein, lipids and minerals. Of the different by-products, liver meal and poultry by-product meal have a well-balanced overall amino acid profile, with other by-product meals usually having specific essential amino acid imbalances, including blood meal (isoleucine and methionine deficiency), hydrolysed feather meal (methionine and lysine deficiency) and to a lesser extent meat and bone meal (methionine and tyrosine deficiency). The major land animal protein meals and lipids available in the marketplace include meat by-product meals and fats that are produced from slaughtered farmed livestock (cattle, pig, sheep, etc.). These products include meat and bone meal, meat meal, meat solubles and lard/tallow. Poultry by-product meals and fats produced from the processing of farmed poultry are also available and include poultry by-product meal, turkey meal, feather meal, chick hatchery waste and poultry fat. Blood by-product meals are co-products of the processing of farmed livestock (ruminant and monogastric) and include blood meal, hemoglobin meal and dried plasma products. In common with fishery by-products, terrestrial livestock products are also rich dietary sources of cholesterol, minerals and trace elements, fat soluble and water soluble vitamins and other important nutrients, including arachidonic acid, taurine, nucleotides and hydroxyproline.

### **Emerging Sources of Ingredients**

An important category of emerging feed ingredients are products that are developed from existing industries using underutilized or waste streams. Valuable nutrients can be reclaimed from what was once considered waste streams using traditional or modified processing methods. Numerous products are already on the market or in the pilot production phase. Included below are some examples of products that we have evaluated in our laboratory (Table 1). This section is not intended to be all inclusive as many others are currently being investigated around the world. The newly developed ingredients to be discussed include products from existing large scale industries such as ethanol and amino acid production and fishery processing, to smaller scale products like tree nuts. Some examined ingredients are being produced de-novo using waste streams from other industries such as bacterial bio-flocs and insects.

### **Fuel industry co-products**

The utilization of by-products from the alternative fuels industries has been of increasing interest by the animal feed industry due to potential for increased volumes of these products. Ethanol production increased more than thirteen-fold from 2000 to 2013 (Lim and Yildrom-Askoy, 2008; RFA, 2014). Subsequently, the utilization of these co-products as aquaculture feed ingredients has been of increasing interest as well (Welker et al. 2014). Specifically, two feed grade ethanol industry by-products have been investigated in our laboratories, the grain-based

by-product Distiller's Dried Grains with Solubles (DDGS) and the spent yeast fraction, Grain Distiller's Dried Yeast (GDDY).

Distillers dried grains with solubles (DDGS) is a co-product of the fuel ethanol industry and is most often produced from maize, at least in the U.S. Proximate composition of DDGS differs among maize varieties, production plants and changes in ethanol production technology. While variation exists, most DDGS contains approximately 25-30% protein (Wu et al. 1997) and although DDGS contain lower amounts of lysine and methionine than fishmeal they are not known to contain certain anti-nutritional factors (e.g. trypsin inhibitors and gossypol) although some concerns about potential mycotoxin contamination have been expressed. Utilizing available amino acid values for a novel high protein DDG product (HPDDG) in our laboratory allowed for formulation of diets where fishmeal levels were reduced from approximately 25% to 13% and HPDDG was included at 23% of the diet without compromising growth or production efficiency of rainbow trout. Lysine, methionine and threonine supplementation was required and negative effects on pellet quality required alteration in extrusion parameters to ensure adequate pellet durability (Sealey et al. 2014a submitted).

Grain distillers dried yeast (GDDY) is a single-cell protein source produced as a co-product during the wet mill fermentation of ethanol from corn. It is the yeast fraction suspended in the distilled fermentation media and separated from corn gluten meal. Research on the effects of yeast products in the diets of rainbow trout has historically focused on their role as immune-stimulants at inclusion rates that were predominantly less than 5% of diet rather than macro-nutrient sources (Gatesoupe 2007). However, yeast proteins also have potential as dietary protein sources. The protein and amino acid content of yeast make it conducive to inclusion in high protein aquafeeds and potentially as a partial replacement for the protein and amino acids from fishmeal. Recent research in our laboratories has defined relevant inclusion rates of GDDY as a protein source in commercial and plant-based diets for rainbow trout. Based on these studies, GDDY can be included in both commercial-type, fishmeal-based diets and plant-based diets for rainbow trout up to 18 or 15%, respectively (Hauptman et al. 2014ab), without decreasing growth performance or negatively impacting fillet color (Sealey et al. 2014b submitted).

## **Fishery processing products**

Commercial processing of fish for human consumption, whether from wild fisheries or aquaculture, results in a residual mass of various fresh-fish cuttings. These cuttings are composed of differing proportions of unrecovered skeletal meat, heads, fins, viscera, and bones, have excellent freshness, and can become raw material for further processing. Several commercial companies are currently reclaiming proteins, lipids, and bones from fresh fish cuttings by converting this raw material into high quality fishmeals, fish oils, fish bone meals, and liquid fish hydrolyzates. BioOregon Protein, Warrenton, Oregon (Ron Anderson, 503-861-2256) is a subsidiary of Pacific Seafood (Clackamas, Oregon) and has been utilizing fresh fish cuttings from fish originally harvested and processed for human consumption to produce a high protein, low-ash fishmeal (SeaPro 75) for the past eight years. More recently, this company has expanded its processing so that in addition to SeaPro 75 it now recovers fish oils, fish bone meals, and a unique soluble protein meal (ProMega 55) from fresh fish cuttings. ProMega 55 contains minimum levels of 55% CP and 24% fat, and has been shown to increase weight gain in

yellowtail (*Seriola lalandi*) during weaning (Stuart et al, 2015). The protein and oil containing materials can also be stabilized with acid to create a hydrozylate and are available in both Norway (Scanbio AS) and Chile (Blue Wave).

### **Barley protein concentrate**

Barley grain is low in protein like wheat and corn (12-14%), and its starch content provides binding capacity similar to wheat and corn in extruded feeds. Although soy and corn protein concentrates have been available and used in fish feeds for many years, commercially available barley protein concentrates have only recently been developed. The primary use of barley is currently for malting in the brewing of beer and while some barley is also grown for animal feed, demand is low and production is falling. Brewing companies have strict standards for malting barley regarding plumpness and protein content. If a harvest of barley is too high in protein >13% CP, then it is rejected and sold as feed barley. Thus, higher protein barley is a perfect starting material for a non-traditional concentrating method which produces a concentrate with approximately 55-62% CP and ethanol as a co-product. The process was jointly developed and patented by ARS and Montana Microbial Products (Missoula, Montana, Cliff Bradley, 406-542-1176) which has built a pilot plant and is producing product for trout feeds.

### **Nut products**

The nut industry (almond, pistachios, and walnuts) in the U.S. is centered in California. A standard for color, size, insect damage, etc. has been developed for each nut to assure that only the finest nuts enter the human market. Nuts that are not certified for human consumption must be sold as animal feed. The nutrient profile of the non-certified product is not useful for fish feeds, but Adaptive Bio-Resources (ABR, Escalon, California, John Hamilton, 970-396-7258) has developed a process that results in a product that contains approximately 55% CP and 10% fat. That nutrient profile allows for the addition of the ingredient in diets of many species. Trout feeding studies have found the products to be highly digestible and able to either replace all the fishmeal or all the soy protein concentrate in rainbow trout diets (Barrows and Frost 2014). A study also has recently been completed with Atlantic salmon showing similar results at the Freshwater Institute in Shepherdstown, West Virginia. A pilot plant is currently operational and ABR is selling products for trout and tilapia feeds at this time.

### **Single-cell proteins**

Single cell proteins (SCP) have been available for the animal feeding industries for decades and have focused primarily of yeasts. A new series of SCP products have been developed from the waste streams of other industries. Bioflocs which are a mix of particulate organic material with associated bacteria, algae, protozoa, rotifers, copepods, nematodes and other micro-organisms assimilate waste nutrients (or pollutants) from the water column, cleaning the water and producing new proteins. The proteins produced in bioflocs by bacteria are single cell proteins and are similar in composition to proteins originating from yeast and algae. Manipulating the type and age of the organisms in a bio-reactor allows the producer to control the protein content and other nutrients in the product. Some commercial products are almost entirely bacteria while others will use a mixture of organisms.

Three different SCP products are currently being developed as fish feed ingredients and have been preliminarily characterized including MrFeed™, ProFloc™, and Knip-Bio™. The concentration of protein and fat in the final products depends both on feedstock, the organism and the processing conditions. Thus differences between products and the potential for designing products for a variety of applications exist but all have a generally favorable amino acid profile for formulating diets for a variety of fish species.

A highly versatile process has been developed by Menon Industries (San Diego, California, Dr. Suresh Menon, 858-675-9990 Ext. 111, <http://menon.us>) that can utilize a variety of feedstocks including spent grains, green wastes, c-molasses, food wastes, etc. These waste streams are converted into a protein product called MrFeed™ that also contains prebiotics to promote animal health. Both high and low protein and fat products can be produced with a typical analysis of 46% CP and 5% fat, and some products contain long chain fatty acids as well.

Another SCP product called ProFloc™ utilizes byproduct streams from the beverage and food industries clarifying their processing water, and also can use effluents from palm oil milling, potato processing, citrate production, and wet corn milling (Nutrinsic Corp, Glendale, Colorado, Andy Logan, 720-744-3605 <http://nutrinsic.com>). This product contains a minimum of 60% CP and 4% fat.

Knip-Bio (Harvard, Massachusetts, Larry Feinberg, 413-627-8149) produces a SCP product that contains 62% CP and 1% fat. Utilizing a unique waste stream, methanol, this product looks, smells and handles differently than the other two SCP products and is just entering the market.

### **Algal/aquatic plant products**

There is tremendous enthusiasm and potential for the development of algal products for aquafeeds. Microalgae such as spirulina, chlorella and dunaliella can be produced by lower-cost open-pond technologies and are marketed as dry powders, and their nutritional profiles are well-documented. Only spirulina is currently commercially available in the U.S., but its inclusion is cost-prohibited for production diets for most species, but seems to provide some yet to be defined nutrients in fishmeal-free diets for marine fish such as white sea bass (*Atractoscion nobilis*) and California yellowtail (*Seriola lalandi*). Spirulina is high in CP (~66%) but low in fat (~2%) where whole chlorella has 27% CP and 27% fat. Chlorella is a species that has been evaluated as a potential source of bio-diesel so significant research has been done on evaluating the extracted biomass. Spirulina has a protein ADC of 76% compared to only 13% for chlorella, but both algae's have high fat digestibility with ADC's of 100% and 71%, respectively. Many other algal species are being evaluated for productivity and cost to produce, and it is expected that several new products will be introduced into the market place in the next 5 years.

Macroalgae such as the 'kelps' laminaria, undaria, and durvillea, and the brown rockweed ascophyllum, occur in dense stands that can be harvested economically, and they have a long history of use as sources of iodine, as soil amendments, and animal feed additives to supply trace elements (<http://www.thefishsite.com/>). These macro-algae can be grown around net-pen farms effectively reclaiming nutrients released by the fish farming operations. Macroalgae are dependent on dissolved nutrients to grow, and in addition to being grown around net pen operations can be grown on shore as part of what is termed Integrated Multi-

Trophic Aquaculture Systems (IMTA). A variety of organisms, both plant and animal have been proposed for such systems, again in attempt to reclaim lost nutrients and convert them into valuable products.

There is a large market for macro-algae's in the human food market. Maine Coast Sea Vegetables (<https://www.seaveg.com/shop/>) sells a variety of products directly to consumers. Until this market is satisfied, or unless the macro-algae is grown for on farm use, introduction of the macro-algae into the fish feed ingredients market is not imminent. Although, this source of ingredients does offer great potential since it can be grown with little input costs and space is plentiful, but it has yet to reach commercialization in the U.S.

Algae also have been recognized as an obvious alternative source of 'fish oil' fatty acids for use in fish feeds, especially eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (ARA). There are very few alternatives to fish oil that provide these polyunsaturated fatty acids (PUFA) needed to provide essential nutrients and produce fillet fatty acid profiles that make farmed fish the heart and brain healthy products the consumers expect. Algae products for this purpose are more often grown in the more expensive, yet effective, bio-reactors with heterotrophic (no sunlight needed) species of algae. DHAgold™ (DSM Nutritional Products, <http://www.dsm.com>) is an algal product that has been shown to increase the levels of DHA in rainbow trout fillets and maintain consumer acceptance (Betiku et al. 2014). This product contains 16% CP and 56% lipid, of which 42% is DHA. Alltech (Winchester, Kentucky, <http://www.alltech.com>) also produces a heterotrophic algae in fermentors, but to date this product has not been evaluated by our laboratory.

Aquatic plants also have been shown to have great potential as fish feed ingredients. Parabel™ produces both a protein concentrate and a lower protein meal from aquatic plants (<http://www.parabel.com/products1>). The Parabel protein concentrate typically contains 61-65% CP and 6-8% fat. The concentrate also has been found to be highly digestible in rainbow trout relative to micro-algae with ADC's for protein and fat of 72% and 82%, respectively. The material also provides some feed manufacturing benefits including some expansion during extrusion and increased oil absorption capacity.

## **Insect meals**

There have been numerous stories in the press recently on the use of insects in both animal feeds and human foods. As an example, recently an "*Insects feed the world*" conference was held in the Netherlands (<https://www.youtube.com/watch?v=2gqYvq5qBmE>) that discussed among other things the benefits of consuming lower on the food chain. In the U.S., however, insects are most likely to become animal feeds before entering the human markets. Insects are natural food sources for many fish. In a recent FAO report, "*Edible insects: Future prospects for food and feed security*", the potential of using insects as a source of protein and other nutrients in diets for poultry and farmed fish is explored and coupled with the ability of these animals to scavenge nutrients from a variety of waste stream sources. Preliminary research in rainbow trout shows that some insects such as the black soldier fly larvae (BSF) can effectively replace fishmeal (St. Hilare et al. 2007a). Further, when these insects are fed fishery processing waste they can recycle the LC PUFA from the offal (St. Hilare et al. 2007b) reducing the need for supplemental fish oil while maintaining fillet fatty acid profiles and no negative effects on taste, texture and flavor thus consumer acceptance (Sealey et al. 2011).

Increased automatization of culture, harvesting and processing of insect production is leading to increased commercialization. Recently a South African company announced \$11 million in funding to build a commercial-scale farm for its Magmeal product. The Vancouver-based Enterra Feed Corporation (<http://www.enterrafeed.com/>) has reported that it plans to triple production of BSF products for pet food and eventually for aquaculture feed by summer 2015. EnviroFlight, based in Ohio in the U.S. (<http://www.enviroflight.net/>), also produces BSF supplying zoo's and pet bird feed and its products have performed very well in feeding studies with rainbow trout in our laboratory. We found an Enviroflight product to contain 35% CP and 32% fat and had ADC's for protein and fat of 77% and 96%, respectively. Most notably was the very high ADC's for phosphorus which was 89% compared to an average of 46% for fishmeal. Another company, Ynsect, hopes to be farming mealworms and BSF near Paris on a large scale by 2016. Protix Biosystems in the Netherlands is now planning to expand its BSF farming operation, selling larvae lipids for use in animal feed, and protein to pet food manufacturers. Laws that define insects as animals, however, increase processing requirements and prevent their inclusion in feeds in some regions where feeding terrestrial animals to livestock is not allowed.

### **Nutrients Not Ingredients; Replacement of the Nutrients from Marine Harvested Products**

The jobs of the aquaculture nutritionist and the feed production personnel are to ensure that each pellet offered to captive fish contains every essential nutrient required. Feeding a high fishmeal diet makes this job easy, but as described previously this luxury is no longer available. Researchers have needed to determine what essential nutrients fishmeal provides that plant and other ingredients do not contain. Fishmeal contains typically 60% to 72% protein, 10% to 15% ash and 5% to 12% fat. Fishmeal is a well-balanced source of high-quality protein and fatty acids. Fish oil provides substantial amounts of highly available energy. As ingredients in aquaculture feed, fishmeal and fish oil supply essential amino acids and fatty acids reflected in the normal diet of fish. Fish oil is a major natural source of the healthy omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids are not synthesized by the fish, but become concentrated in fish further up the food chain from the marine phytoplankton (microscopic marine algae and microbes) that do synthesize them.

Fishmeal also contains significantly more ash (minerals) than plant based ingredients and the minerals in plant products often have decreased availability since the storage form (phytate) is unavailable to mono-gastric animals like fish. Phosphorus is an essential element involved in many biological reactions including skeleton formation. A lack of available dietary sources of this mineral necessitates supplementation even in some fishmeal-based diets, but supplementation is particularly important in plant-based diets that do not contain fishmeal or animal products. Supplementing plant-based diets with sodium, magnesium and potassium to the levels found in fishmeal improved the health of rainbow trout fed plant-based diets (Barrows et al. 2010). Inositol is a vitamin like substance that was not included in vitamin mixes in fishmeal diets, but been shown to be beneficial when trout were fed plant-based diets (Barrows et al. 2008a).

The amino acid profile, or the relationship of one to another, is much different between fishmeal and plant derived ingredients. Often these ingredients are limiting in lysine, methionine and possibly threonine. These three amino acids are available commercially, affordable and routinely used in terrestrial animal feeds, pet foods, human supplements as well as aquafeeds.

Feeding diets balanced on an available (digestible) amino acid basis results in fast growth and a minimization of expensive protein and amino acids. This in turn results in more efficient use of limited nutrients and a reduction in release of nitrogen from the fish farm. The amino acid pattern of the fillet or whole body composition has been used as a target for balancing amino acids and increases the efficiency of protein utilization (Gaylord and Barrows 2009). The amino acid taurine, which is not found in plant meals in significant levels (NRC 2011), has also been shown to be needed when feeding fishmeal-free diets to variety of species including rainbow trout (Gaylord et al. 2006; Gaylord et al. 2007), yellowtail *Seriola quinqueradiata* (Takagi et al. 2008), flounder *Paralichthys dentatus* (Enterria et al. 2011), parrot fish *Oplegnathus fasciatus* (Lim et al. 2013), white seabass *Atractoscion nobilis* (Jirsa et al. 2014). Taurine is not found as part of fish muscle protein but is circulated throughout the body, and has many other biological functions. These include metamorphosis of larval fish, digestion, and improved feed efficiency and growth (Pinto et al. 2010; Hawkyard et al. 2014; Kim et al. 2007; Matsunari et al. 2008). The biological mechanisms are present in fish to synthesize taurine, but many fish have evolved consuming an abundant supply of taurine so this ability is often not adequate when fed fishmeal-free diets, and this ability is species-dependent. Taurine is found, and approved for use, in many human supplements for body builders and energy drinks, but is not currently approved for use in aquaculture feeds in United States. The Soy Aquaculture Alliance, however, has supported several research projects to address data gaps in the needs and safety of taurine supplementation for fish and the approval process is now underway.

As described above, fish nutritionists today have an ever increasing body of knowledge to formulate feeds that provide essential nutrients to fish from a variety of ingredient sources. The independence of aquaculture from marine harvested products can only be done through a deep understanding of the nutrition and economics of the ingredients. Economics not only plays a role in the cost of the ingredients, but also what the consumer is willing to spend for a product fed a specific diet.

### **Other Important Considerations**

When developing novel ingredients, regulatory approval, consumer acceptance and final product quality in terms of human health impacts also must be evaluated. Although independent of nutritional quality and cost-effectives, each of these areas can be crucial bottlenecks to commercialization and prevent wide-spread industry adoption.

### **Regulatory approval**

Potential feed ingredients must undergo substantial scrutiny prior to be allowed for use in animal feeds. Materials should be consistent from batch to batch, cannot be a combination of other ingredients and should provide nutrition, color, taste or aroma for the animal. New feed ingredients for aquaculture feeds also must be demonstrated to be safe and effective for the intended use prior to marketing the ingredient. However, there are differences in the approach to the safety assessment and what ingredients must be evaluated using this process. Each regulatory jurisdiction outlines its process in the regulations specific for that jurisdiction.

In the United States, feed ingredients are accepted for market by obtaining a new Association of American Feed Control Officials (AAFCO) ingredient definition, gaining



approval as a food additive through the FDA or by determining they can be classified as the Generally Recognized As Safe (GRAS) status for the intended use. To obtain an ingredient definition under AAFCO, an ingredient manufacturer provides a proposed definition, name and description of the ingredient along with the purpose, ingredient limitations and supporting data and safety assessments from controlled trials for review by AAFCO. AAFCO will then determine whether the ingredient warrants a new definition, or fits into an existing ingredient definition with or without definition modifications. Once a preliminary determination is made by AAFCO, FDA will evaluate the safety and efficacy of the ingredient. The FDA requires that all feeds follow the Federal Food, Drug, and Cosmetic Act of 1958 (FFDCA). This act requires all foods to 1) be pure and wholesome, 2) be produced under sanitary conditions, 3) contain no harmful substances, and 4) be truthfully labeled. Upon completion of the FDA evaluation, FDA will return their findings to AAFCO to support or deny the ingredient as defined. Ingredient definitions that make it through this process are then published in the AAFCO Official Publication. Ingredients that may have animal or human health concerns are not considered appropriate for review by AAFCO and are directly submitted for approval through the FDA Food additive process. Examples of ingredients that would fall under this category would include those that are marketed to mitigate, treat, or prevent disease or those with human safety concerns. Once food additive approval from FDA is obtained, these ingredients can then be considered by AAFCO for definition. Although the U.S. food and drug laws and regulations also permit self-determination of Generally Recognized As Safe as a legal process, liability issues and unwillingness by individual states to register a feed ingredient that does not have an FDA or AAFCO listing limit application of the GRAS process. In Canada, all feeds for use in livestock must be approved for market by the Canadian Food Inspection Agency (CFIA) and listed in Schedules IV and V of the Feeds Regulation (1983). Although AAFCO shares all proposed ingredient definitions with the CFIA ingredient, manufacturer's wanting approval in Canada must still submit a formal application to CFIA.

## **Human health benefits**

Changes in dietary ingredient composition not only affect growth and health of the fish, but also can alter product quality in farmed fishes (Tocher et al. 2004; Karalazos et al. 2011; Turchini et al. 2013 ab). Product quality of farmed fishes is defined by customer's needs and expectations, and often includes the long chain n-3 PUFA content of the fillet (Liu et al. 2004; Torstensen et al. 2005). For most fish species studied to date, the fatty acid profile of the fish generally reflects that of the diet. Dietary fatty acid profile is of utmost importance to final product quality for its flavour and beneficial effect on human health. A variety of studies have demonstrated that as long as essential fatty acid requirements are met by feeds during rapid growth, immune challenge or stress events, alternative lipids can be fed as replacement for fish oil. To ensure product quality when using alternative lipids, various strategies have been developed to "finish" a production cycle with a diet high in these heart and brain healthy fatty acids. These finishing diets have been effective in maintain the healthfulness of farm raised fish when supplies of fish oil, or other sources of essential fatty acids are limited.

Fish oil had been the primary source of energy in fish feeds of the past, as discussed above, and with it came large amounts of essential fatty acids. These fatty acids are deposited in the fish fillet and visceral fat. There is a great deal of variability among species in the amount of fat held in the edible portion of the fish. Salmon can contain up to 17-20% where tilapia often

contain only 3-7% (NRC 2011). There is considerable discussion among consumer groups that farmed raised fish are not as healthy as wild reared fish and the comparison is often also made across species. This is misleading because even wild harvested tilapia will not have the quantities of the heart healthy and brain healthy fatty acids (known as long chain poly-unsaturated fatty acids, PUFAs) as a wild Coho salmon for example. Feeding rainbow trout held in freshwater in the hatchery a diet supplemented with menhaden fish oil will result in a fillet with levels of the important PUFAs 4 to 5 times greater than found in wild rainbow trout raised in freshwater. Another consideration of the healthfulness of a fish product concerns the length of the fatty acids. Flax oil and camelina oil contain high levels (~50%) of omega-3 fatty acids. However, these short chain fatty acids do not provide the health benefits of longer chain PUFA and the consumer can often be misled by confusing longer chain PUFAs (EPA and DHA) with total omega-3 fatty acid levels. Healthfulness of a fish fillet for humans is thereby not only determined by what it has eaten or been fed, but also on the species that one consumes.

### **Consumer acceptance**

Consumers are becoming more aware and educated about the manner in which their food is produced including, for animal products, what their food has been fed. This trend is evident when certain major food retailers that sell farmed fish developed guidelines for production of aquatic species that prohibit the use of any land animal derived ingredients based purportedly on consumer input. The composition of the feed fed also affects the sustainability rating given by the Monterey Bay Aquarium to a particular species, but currently allows the use of land animal products. Another consumer group demands that fish feed contain no GMO ingredients. Still others utilize carbon footprint calculations to define their preferred ingredients. This divergence in opinions on the best composition of feeds has resulted in a variety of niche markets for aquaculture products and specially formulated feeds. Often times these specifications can be met and provide for good growth and health of the fish, but the feeds will be more expensive than those without similar constraints on ingredient composition. These formulations also indicate value-added propositions for smaller ingredient suppliers will the flexibility to fill the nutritional of these formulations.

### **Conclusions**

The composition of aquafeeds has changed tremendously in the last 10 years with decreasing quantities of fishmeal and fish oil being used, and changes will continue in the future. The research community has developed the knowledge to completely remove fishmeal from aquafeeds for many species if needed. Marine-harvested materials at reduced levels remain cost effective and nutritionally complete ingredients for aquaculture feeds, but an increased number of suitable alternatives continue to be developed should consumer groups limit their inclusion for some species in the future. Currently fishmeal-free diets are effective but are still more expensive than traditional diets so a premium must be obtained by the producer to remain profitable. For example, rainbow trout fed fishmeal and fish oil free diets are available commercially and sold at a premium to selective high-end restaurants. The real challenge in the future for the aquafeed producers, and thus fish farmers, is the identification of abundant sources of high quality ingredients. This provides a great economic opportunity to ingredient suppliers.

### **Disclaimer**

The mention of trade names or commercial products in this article is solely for the purpose of providing specific information and product examples and does not imply recommendation or endorsement by the U.S. Department of Agriculture or the U.S. Department of Interior.

## References

- Barrows, F.T., Gaylord, T.G., Sealey, W.M., Haas, M.J. and Stroup, R.L. 2008a. Processing soybean meal for biodiesel production; effect of a new processing method on growth performance of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 283, 143-147.
- Barrows, F.T., Gaylord, T.G., Sealey, W.M., Porter, L., Smith, C.E. 2008b. The effect of vitamin premix in extruded plant based and fish meal based diets on growth efficiency and health of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 283, 148-155.
- Barrows, F.T., Gaylord, T.G., Sealey, W.M., Porter, L., and Smith, C.E. 2010. Supplementation of plant-based diets for rainbow trout, *Oncorhynchus mykiss* with macro-minerals and inositol. *Aquaculture Nutrition* 16(6), 654-661.
- Barrows, F.T., Frost, J. 2014. Evaluation of modified processing waste from the nut industry, algae and an invertebrate meal for rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 434, 315-324.
- Barrows, F.T., Gaylord, T.G., Sealey, W., Rawles, S.D. 2010. Database of nutrient digestibility's of traditional and novel feed ingredients for trout and hybrid striped bass. <http://www.ars.usda.gov/Main/docs.htm?docid=21897>
- Barrows, F.T., Overturf, K., Welker, T., Liu, K. 2014. The effect of feed ingredients and feeding regimen on fecal output of rainbow trout and impact of soy products in a serial reuse system. Final report to Soy Aquaculture Alliance, Indianapolis, IN.
- Betiku, O.C., F.T. Barrows, C. Ross and W.M. Sealey (2014). The effect of total replacement of fish oil with DHA-Gold<sup>®</sup> and plant oils on growth and fillet quality of rainbow trout (*Oncorhynchus mykiss*) fed a plant-based diet, *Oncorhynchus mykiss*. *Aquaculture Nutrition*, In Press.
- Brinker, A., Rosch, R. 2005. Factors determining the size of suspended solids in flow-through fish farms. *Aquaculture Engineer* 32, 1-19.
- Brinker, A., Schroder, H.G., Rosch, R. 2005. A high resolution technique to size suspended solids in flow-through fish farms. *Aquaculture Engineer* 32, 325-341.
- Brinker, A. 2007. Guar gum in rainbow trout (*Oncorhynchus mykiss*) feed: The influence of quality and dose on stabilization of faecal solids. *Aquaculture* 267, 315-327.
- Brinker, A. 2009. Improving the mechanical characteristics of faecal waste in rainbow trout: the influence of fish size and treatment with non-starch polysaccharide (guar gum). *Aquaculture Nutrition* 15, 229-240.
- Brinker, A. Reiter, R. 2011. Fish meal replacement by plant proteins substitution and guar gum addition in trout feed, Part 1: Effects on feed utilization and fish quality. *Aquaculture* 310, 350-360.
- Enterria A., M. Slocum, D.A. Bengston, P.D. Karayannakidis and C.M. Lee. 2011. Partial Replacement of Fish Meal with Plant Protein Sources Singly and in Combination in Diets for Summer Flounder, *Paralichthys dentatus*. *Journal of World Aquaculture Society*. 42, 26-31.
- FAO 2009. *The State of World Aquaculture and Fisheries 2009*. FAO. Rome, Italy. 176 pp.

- FAO 2011. FAOSTAT. Food and Agriculture Organization of the United Nations, Rome, Italy
- FAO 2012. *The State of World Aquaculture and Fisheries 2012*. FAO, Rome, Italy. 230 pp.
- FAO. 2013. *Edible Insects: Future Prospects for Food and Security*. FAO, Rome, Italy. 187pp
- Gatlin, D. M. III., Barrows, F.T., D. Bellis, P Brown, J. Campen, K. Dabrowski, T.G. Gaylord, R. W. Hardy, E. Herman, G. Hu, Å Krogdahl, R. Nelson, K. Overturf, M. Rust, W. Sealey, D. Skonberg, E. Souza, D. Stone, R. Wilson, Wurtele, E. 2007. Expanding the Utilization of Sustainable Plant Products in Aquafeeds – A Review, *Aquaculture Research*. 38, 551-579.
- Gaylord, T.G., Teague, A.M. and Barrows, F.T. 2006. Taurine supplementation of all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *Journal of the World Aquaculture Society* 37(4), 509-517.
- Gaylord, T.G., Barrows, F.T., Teague, A.M., Johansen, K.A., Overturf, K.E. and Shepherd, B. 2007. Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 269, 514-524.
- Gatesoupe, F.J. 2007. Live yeasts in the gut: natural occurrence, dietary introduction, and their effects on fish health and development. *Aquaculture* 267, 20-30.
- Hawkyard M, B. Laurel, C. Langdon. 2014. Rotifers enriched with taurine by microparticulate and dissolved enrichment methods influence the growth and metamorphic development of northern rock sole (*Lepidopsetta polyxystra*) larvae. *Aquaculture* 424–425, 151–157.
- Jirsa D., A.L. Davis, G.P. Salze, M. Rhodes, M. Drawbridge. 2014. Taurine requirement for juvenile white seabass (*Atractoscion nobilis*) fed soy-based diets. *Aquaculture* 422–423, 36–41.
- Karalazos, V., Bendiksen, E. A. & Bell, J.G. (2011) Interactive effects of dietary protein/lipid level and oil source on growth, feed utilization and nutrient and fatty acid digestibility of Atlantic salmon. *Aquaculture* 311, 193-200.
- Kim, S.K., Matsunari, H., Takeuchi, T., Yokoyama, M., Murata, Y., Ishihara, K., 2007. Effect of different dietary taurine levels on the conjugated bile acid composition and growth performance of juvenile and fingerling Japanese flounder *Paralichthys olivaceus*. *Aquaculture* 273, 595–601.
- Lim, C., and M. Yildirim-Aksoy., 2008. Distillers dried grain with solubles as an alternative protein source in fish feeds. Pages 67–92 in H. Elghobashy, K. Fitzsimmons, and A. S. Dinah, editors. *Proceedings of the 8<sup>th</sup> international symposium on tilapia in aquaculture*. Agriculture Press Unit, Agriculture Research Center, Cairo, Egypt.
- Lim S.J., D.H. Oh, S. Khosravi, J.H. Cha, S.H. Park, K.W. Kim, K.J. Lee. 2013. Taurine is an essential nutrient for juvenile parrot fish *Oplegnathus fasciatus*. *Aquaculture* 414–415, 274–279.
- Liu, K.K.M., Barrows, F.T., Hardy, R.W. & Dong, F.M. 2004. Body composition, growth performance and product quality of rainbow trout (*Oncorhynchus mykiss*) fed diets containing poultry fat, soybean/corn lecithin or menhaden oil. *Aquaculture* 238, 309-328.

- Matsunari, H., Furuita, H., Yamamoto, T., Kim, S.K., Sakakura, Y., Takeuchi, T. 2008. Effect of dietary taurine and cystine on growth performance of juvenile red sea bream *Pagrus major*. *Aquaculture* 274, 142–147.
- National Research Council (NRC). 1993. Nutrient Requirements of Fish. National Academy Press, Washington.
- National Research Council (NRC). 2011. Nutrient Requirements of Fish. National Academy Press, Washington.
- Pinto, W., Figueira, L., Ribeiro, L., Yúfera, M., Dinis, M.T., Aragão, C. 2010. Dietary taurine supplementation enhances metamorphosis and growth potential of *Solea senegalensis* larvae. *Aquaculture* 309, 159–164.
- Rana, K.J.; Siriwardena, S.; Hasan, M.R. 2009. Impact of rising feed ingredient prices on aquafeeds and aquaculture production. *FAO Fisheries and Aquaculture Technical Paper*. No. 541. Rome, FAO. 63p.
- RFA (Renewable Fuels Association) 2014. Industry Resources: Co-products. Renewable Fuels Association, Washington, D.C., (available at <http://www.ethanolrfa.org/pages/industry-resources-coproducts>).
- Sealey, W.M., Hooley, C.G., Rosenstrater, K.A., Gaylord, T.G., Barrows, F.T. Examination of the Ability of a Mycotoxin Deactivation Product to Improve Growth and Nutrient Utilization in Juvenile Rainbow Trout (*Oncorhynchus mykiss*) Fed Dried Distiller's Grains. *Animal Feed Science and Technology* submitted 8/29/2014.
- Sealey, W.M., O'Neill, T.J., Peach, J.T., Gaylord, T.G., Barrows, F.T. Block, S.S. Refining Inclusion Levels Of Grain Distillers Dried Yeast In Commercial-Type And Plant-Based Diets For Juvenile Rainbow Trout (*Oncorhynchus mykiss*). *Journal of the World Aquaculture Society* submitted 09/04/2014.
- Sealey, W.M., Gaylord, T.G., Barrows, F.T., Tomberlin, J.K., McGuire, M.A., Ross, C., St-Hilaire, S. 2011. Sensory analysis of rainbow trout (*Oncorhynchus mykiss*) fed enriched black soldier fly prepupae. *Journal of the World Aquaculture Society* 42, 34-45.
- St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W., Sealey, W.M. 2007a. Fly prepupae as a feedstuff for rainbow trout *Oncorhynchus mykiss*. *Journal of the World Aquaculture Society*. 38, 309-313.
- St-Hilaire, S., McGuire, M.A., Tomberlin, J.K., Cranfill, K., Mosley, E.E., Newton, L., Sealey, W.M., Irving, S., Sheppard, C. 2007b. Fish offal recycling by the black soldier fly produces a feedstuff high in Omega-3 fatty acids. *Journal of the World Aquaculture Society*. 38, 413-417.
- Stuart, K., Barrows, F.T., Rust, M.B., Johnson, R.B., Hawkyard, M., Langdon, C., Drawbridge, M. The use of experimental microdiets on two marine finfish species. Abstract submitted to *Aquaculture America* 2015.
- Tacon, A. G. J. and Metian, M. (2008), *Aquaculture Feed and Food Safety*. *Annals of the New York Academy of Sciences*, 1140: 50–59. doi: 10.1196/annals.1454.003
- Tacon A.G.J., Metian M., Turchini G.M., De Silva S.S., 2010, Responsible aquaculture and trophic level implications to global fish supply. *Rev. Fish. Sci.* 18, 94–105.

- Takagi, S., Murata, H., Goto, T., Endo, M., Yamashita, H., Ukawa, M., 2008. Taurine is an essential nutrient for yellowtail *Seriola quinqueradiata* fed non-fish meal diets based on soy protein concentrate. *Aquaculture* 280, 198–205.
- Tocher, D.R., Fonseca-Madrigal, J., Dick, J.R., Ng, W., Bell, J.G., Campbell, P. 2004. Effects of water temperature and diets containing palm oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes of rainbow trout (*Onchorhynchus mykiss*). *Comp. Biochem. Physiol. B-Physiol.*, 137, 49-63.
- Torstensen, B.E., Bell, J.G., Rosenlund, G., Henderson, R.J., Graff, I.E., Tocher, D.R., Lie, Ø. & Sargent, J.R. 2005. Tailoring of Atlantic salmon (*Salmo salar* L.) flesh lipid composition and sensory quality by replacing fish oil with a vegetable oil blend. *J. Agric. Food Chem.*, 53, 10166-10178.
- Turchini, G.M., Hermon, K., Cleveland, B.J., Emery, J.A., Rankin, T. & Francis, D.S. (2013a). Seven fish oil substitutes over a rainbow trout grow-out cycle: I) Effects on performance and fatty acid metabolism. *Aquaculture Nutrition* 19, 82-94.
- Turchini, G.M., Hermon, K., Moretti, V.M, Caprino, F., Busetto, M.L., Bellagamba, F., Rankin, T., Francis, D.S. (2013b). Seven fish oil substitutes over a rainbow trout grow-out cycle: II) Effects on final eating quality and a tentative estimation of feed related production costs. *Aquaculture Nutrition* 19, 95-109.
- Welker, T., Lim C., Barrows, F.T., Liu, K. 2014. Use of distiller dried grains with soluble (DDGS) in rainbow trout diets. *Animal Feed Sci. & Tech.* 195,47-57.
- Wu, Y. V., Rosati R. R., Brown, P.B., 1997. Use of corn-derived ethanol products and synthetic lysine and tryptophan for growth of tilapia (*Oreochromis niloticus*) fry. *Journal of Agricultural and Food Chemistry* 45, 2174-2177.

## WEB LINKS

Insects Feed the world: <https://www.youtube.com/watch?v=2gqYbq5qBmE>

Edible Insects FAO report: <http://www.fao.org/docrep/018/i3253e/i3253e00.htm>

Aquafeed market growth: <http://www.feedmachinery.com/news/world/aquafeeds-market-is-estimated-to-grow-at-a-cagr-of-117-from-2013-to-2018-2617/>

Canola: USDA, ERS. <http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx>

Digestibility Database: <http://www.ars.usda.gov/Main/docs.htm?docid=21897>

Algae as alternatives to fishmeal: <http://www.thefishsite.com/articles/1767/the-use-of-algae-in-fish-feeds-as-alternatives-to-fishmeal>

Maine Coast Sea Vegetables: <https://www.seaveg.com/shop/>

Algae Products, DSM Nutritional Products: <https://www.dsm.com>

Algae Products, Alltech: <http://www.alltech.com>

Algae Products, Parabel: <http://www.parabel.com/products1>

AAFCO, A Guide to Submitting New Ingredient Definitions to AAFCO:

[http://www.aafco.org/Portals/0/AAFCO/idc/definition\\_request\\_guidelines\\_020112.pdf](http://www.aafco.org/Portals/0/AAFCO/idc/definition_request_guidelines_020112.pdf)

CFIA: Feed Ingredient Assessment and Authorization - Regulatory Framework Proposal:

<http://www.inspection.gc.ca/animals/feeds/consultations/feed-ingredient-assessment-and-authorization/eng/1383769140408/1383769681366>

FDA, Food Additive Petitions for Animal Food:

<http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/ucm056809.htm>



**Table 1.** Examples of Emerging Ingredients for Aquafeeds and Their Characteristics.<sup>1</sup>

<b>Examples of Emerging Ingredients</b>							
<b>Ingredient</b>	<b>Crude Protein</b>	<b>Crude Lipid</b>	<b>Feed Intake</b>	<b>Effect on Extrusion</b>	<b>Special Traits</b>	<b>Supplier</b>	<b>Contact</b>
Fresh Fish Cuttings meal	75%	8%	Good	Neutral	Low ash, phosphorus	BioOregon Proteins	Ron Anderson
ProOmega 55	55%	24%	Excellent	Unknown	High in LCPUFA		503-861-2256
Non-GMO Soymeal	56%	1%	Neutral	Beneficial	High protein, low oligosaccharides, low trypsin inhibitors	SchillingerGenetics, Navita Premium Feed Ingredients	Corey Nikkel 515-225-1166
Barley Protein Conc.	59%	5%	Neutral	Neutral	Potentially large volumes	Montana Microbial Products	Cliff Bradley 406-544-1177
Mixed Nut Meal	55%	10%	Excellent	Expansion		Adaptive BioResources	John Hamilton 970-396-7258
MrFeed™, SCP	46%	5%	Unknown	unknown	Reclaims nutrients	Menon Industries	858-675-9990
ProFloc™, SCP	60%	4%	Unknown	Unknown	Reclaims nutrients	Nutrinsic Corp	Andy Logan 720-744-3605
Knip-Bio™, SCP	62%	1%	Unknown	Unknown	Non-food feed stock	Knip-Bio	Larry Fienberg 413-627-8149
Spirulina	72%	1%	Neutral	Beneficial	Good source of trace minerals	Earthrise Nutritionals	800-949-7473
DHAGold™	15%	53%	Neutral	Can cover starch	Can replace fish oil	DSM Nutritional	Jeffrey Alix 973-257-8505
Parabel™ protein conc.	64%	6.2%	Good	Expands well	Has pigment with unknown effect	Parabel	321-409-7436
Insect meal	Variable 34%	Variable 32%	Excellent	Lipid content a concern	High phosphorus digestibility, reclaims nutrients	Enterra	Andrew Vickerson 604-639-1628
Insect meal	Variable 34%	Variable 35%	Excellent	Lipid content a concern	High phosphorus Digestibility, reclaims nutrients	Enviroflight	Glen Courtright 937-767-1988

<sup>1</sup> The mention of trade names or commercial products in this table is solely for the purpose of providing specific information and product examples and does not imply recommendation or endorsement by the U.S. Department of Agriculture or the U.S. Department of Interior.