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An Overview of Progress toward Developing an All Plant-based Diet for Rainbow Trout

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Abstract：Feeds for rainbow trout have historically contained high quantities of fish meal and fish oil as cost effective sources of both essential amino acids and fatty acids. Research results from around the world have identified ingredients to replace fish meal and fish oils, but limits still exist in completely removing these ingredients from the diet. The USDA-ARS Trout-Grains Project, officially titled "The integration of nutritional, genetic and physiological approaches to improve production efficiency of rainbow trout (*Oncorhynchus mykiss*)" was initiated with a primary goal to identify cost effective replacements for fish meal. The project team consists of two fish nutritionists, a fish geneticist, a grain chemist and a plant geneticist. This group of scientists addresses a range of issues, including selection/development of cereal grains with improved nutritional profiles, development of methods to fractionate valuable components of grains, and development of families of trout that are better able to utilize nutrients from plant-based feeds. The nutrition component of the project focuses on evaluation of new and traditional ingredients, nutrient requirements for specific life stages, and identification of nutrients in fish meal not present in plant meals. Finally we are developing a database of nutrient availability from plant-based ingredients that will have potential to supply the nutritional needs of rainbow trout in aquaculture. Some highlights of our research findings have been: 1) Taurine may be conditionally indispensable when only plant-derived protein sources are utilized. 2) Vitamin premix formulations benefit from modification when fish meal is replaced by plant derived ingredients, or when poor quality fish meals are used in extruded trout feeds. 3) Starch amylose/amylopectin ratios in energy feed ingredients will affect carbohydrate and energy digestibility. This article will present the results of laboratory and pilot scale studies of feeding fish meal-free diets to rainbow trout.

Key words：rainbow trout, feeds, alternative ingredients, plant protein

Background

The primary reason fish meal and fish oils have been used routinely in rainbow trout and many other carnivorous fish feeds is that they have been affordable sources of amino acids and fatty acids that support optimal growth, or near optimal growth, and product quality. Although these ingredients have historically been more expensive than other sources of protein or fats/oils, the high growth rates and feed efficiencies observed when fish meal and fish oil are used makes them cost effective. With increasing demands for fish meals and fish oils, along with fluctuating supplies, price volatility has increased interest in identifying and characterizing alternative sources of protein and fats. Although many

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animal derived protein and fats are excellent feed ingredients for rainbow trout, consumer preferences and concerns limit their inclusion in some fish feeds. Therefore, plant-derived proteins and oils have been the focus of research performed by the Trout-Grains Project team of the USDA Agricultural Research Service in Aberdeen and Hagerman, Idaho.

Approach

One aspect of evaluating the potential of plant-derived ingredients as a source of nutrients and energy has been to thoroughly define the nutrient composition of those ingredients. The digestibility and availability of nutrients and energy from an ingredient is determined by *in vivo* testing. A database of apparent digestibility coefficients for protein and energy has been produced for multiple ingredients used in fish feeds or that may have potential as substitutes to fish meals in extruded feeds (Gaylord *et al.,* 2008a). The next step is to determine the amino acid availability from these ingredients for rainbow trout (Gaylord *et al.,* 2009). Although this database covers a variety of ingredients, new ingredients and new processing techniques are continually becoming available and provide other potential sources of dietary nutrients and energy. Barrows *et al.* (2008a) demonstrated that new processing technologies developed to remove the oil from soybeans for bio-diesel production may result in a meal that can be used in trout feeds. However, differences in the availability of amino acids in this product may differ slightly from that of traditional hexane-extracted soybean meal and careful attention to available amino acid balance should be undertaken when formulating fish diets with soybean meal from bio-diesel production.

The information garnered from amino acid availability studies of individual ingredients is leading to improved diets that not only meet the available amino acid requirements of rainbow trout but also could reduce total dietary protein concentrations and improve net protein balance. Gaylord and Barrows (2009) determined that rainbow trout fed plant-protein based diets balanced on available amino acid grew equally well when crude protein levels were reduced and limiting essential amino acids were supplemented. In fact, results in our laboratory show that protein retention efficiencies were improved by reducing dietary crude protein while balancing essential amino acids without reducing growth or feed conversion efficiencies.

Increased inclusion of plant-proteins in rainbow trout feeds may require greater attention to levels of other nutrients that are abundant in fish meal. One such instance was demonstrated when Barrows *et al.* (2008b) determined that vitamin premixes could be optimized for both plant-based as opposed to fish meal-based diets and for extrusion and storage losses of vitamin availability. These refinements in vitamin levels may equate to considerable savings in feed costs while maintaining optimal health of the fish. A second consideration when using all plant-based ingredients in fish feeds is that compounds not normally deemed necessary, and previously included in the diet due high levels of fish meal, may be left out. One such compound is taurine. Taurine is an amino sulfonic acid derived from methionine via cysteine. This bioconversion has been demonstrated in rainbow trout but may be suboptimal depending on methionine supply. Gaylord *et al.* (2006) demonstrated that taurine supplementation in a plant-based diet improved trout growth and feed conversion ratios; whereas, there was no benefit from taurine supplementation of a fish meal-based diet. These benefits were observed even though the total sulfur amino acid contents of the diet were theoretically adequate. In a follow up trial, Gaylord *et al.* (2007) observed marginal gains with taurine supplementation to a plant-based diet and detrimental effects of methionine supplementation. The mechanism whereby taurine supplementation benefits rainbow trout is still unclear but its incorporation into bile salts for fat digestion may be one factor (Kim *et al.,* 2008). Potentially, other compounds found in fish meal and other animal meals may also be absent from plant-derived ingredients. Not only have these compounds not been clearly defined, but their potential benefits as constituents of feeds for trout or other carnivorous fish have not been adequately investigated. Another such compound, hydroxyproline, has been shown to be beneficial in Atlantic salmon (Aksnes *et al.*, 2008);

however, additional research is needed in this area.

In order to include cereal grains and legumes as primary protein sources for carnivorous fish, nutritional components need to be fractionated in order to both increase protein concentrations as well as remove anti-nutritional factors. Currently, protein concentrates are available from cereal grains and include wheat gluten meal, corn gluten meal, rice protein concentrate and barley protein concentrate. The digestibility of protein and energy from these products is generally high for rainbow trout although other factors may limit inclusion. In rainbow trout feeds, xanthophylls from corn gluten meal when included at levels over 10% of the diet will impart an undesirable yellow color to the fillets. The use of wheat gluten meal also may be limited in carnivorous fish diets due to its undesirable effects at high levels on pellet quality after extrusion. Legume meals such as soybean have detrimental effects when fed to trout. These effects include intestinal enteritis that limits dietary inclusion to less than 20% in trout feeds. Protein concentrates from soybeans (soy protein concentrates and soy protein isolates) do not have these limitations since the anti-nutritional factors have been removed. These products also have produced high digestibility coefficients in rainbow trout. Hence, the use of fractionation technology to remove undesirable substances in the raw materials has resulted in several nutrient-dense, feedstuffs of plant origin.

Determining the nutrient content of various fractions of cereal grains and by-products is one of the first steps in developing technologies that may lead to refined ingredients for aquafeeds. Liu *et al.* (2007) determined the phosphorus and mineral concentrations that would influence the nutritional value of ingredients from mechanical fractionation of barley. Liu (2007a, 2007b) also developed laboratory methods for abrading various layers of barley to identify layers with potentially improved nutrient profiles. Using the methods developed, Liu *et al.* (2009) further showed that dry fractionation is the method of choice for separating barleys into fractions with varying levels of protein, beta-glucan and/or starch. However, selection of a specific single or combined method is needed for achieving maximum shifts of a particular nutrient. Determination of functional lipids in differing barley fractions may also lead to improved nutritional value for fractionated cereal grains for fish feeds (Liu and Moreau, 2008).

Identification of grain by-products that may be refined for utilization as feed ingredients for fish is also progressing. Liu (2008) determined the potential of size fractionation technologies for further concentrating nutrients of interest/value in corn distillers dry grains with solubles (DDGS). Further evaluations of DDGS fractionation technologies and compositional analyses of the developed fractions will be necessary to evaluate DDGS as an ingredient for aquafeeds.

The development of plant cultivars with improved nutritional value is also underway. The successful development of low phytic acid (LPA) lines of barley to improve phosphorus availability is one example. Dorsch *et al.* (2003) identified potential low phytic acid genotypes that have been further assessed as feed ingredients for rainbow trout. Sugiura *et al.* (1999) and Overturf *et al.* (2003) quantified increased phosphorus availability from the low phytic acid varieties of barley compared to wild type barleys, and recently the 'Clearwater' variety, a hull-less low phytic acid barley, has been registered (Bregitzer *et al.*, 2008). Another nutritional trait that has been successfully screened in barley varieties is starch type (waxy vs. normal starch). The nutritional value of waxy lines of barley has been analyzed with respect to their ratio of amylose to amylopectin, and the digestibility of these starch fractions has been determined in rainbow trout (Gaylord *et al.*, 2008b). Further development of cereal grain varieties for improved nutritional value has been on-going. Hu *et al.* (2007) has been surveying genome-derived SSR markers as potential tools to aide in oat genetic research. Hu and Burton (2008) modified an enzymatic beta-glucan measurement method into a high throughput and low cost assay format. This tool will help to speed up the improvement of starch related grain qualities of cereal for better use in fish feed.

Conclusion

Fish meal is a good source of nutrients in

aquaculture feeds, but is not required by trout for optimal growth. Many opportunities exist for improvements in the nutritional value of crops for aquafeeds. Supplementing plant derived ingredients with essential nutrients allows for the complete replacement of fish meal in the diet of rainbow trout. The USDA-ARS Trout-Grains Project uses a multi-disciplinary approach to develop of plant cultivars with improved nutritional value, develop ingredient processing technologies, quantify the nutritional value of ingredients, define the nutritional requirements, and breed trout for improved performance when fed plant based diets.

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