ENHANCING THE NUTRITIONAL VALUE OF TILAPIA FOR HUMAN HEALTH

Sustainable Feed Technology and Nutrient Input Systems/Experiment/13SFT02PU

Collaborating Institutions and Lead Investigators

Kwame Nkrumah University of Science and Technology (Ghana)	Nelson W. Agbo Regina Edziyie
University of Arkansas at Pine Bluff (USA)	Rebecca Lochman

Objectives

1. Assess the availability and distribution of potential fish feed ingredients containing n-3 fatty acids for use in Nile tilapia diets in Ghana.
2. Determine the proximate composition and fatty acid composition of potential feed ingredients, and recommend specific inclusion rates for diets to be tested in feeding trials with Nile tilapia in Ghana.
3. Analyze the proximate and fatty acid composition of experimental diets and fish from feeding trials in Ghana and determine cost-of-gain of the different diets.
4. Determine the amount of tilapia that would need to be consumed by humans to obtain the target amount of n-3 fatty acids (500-1500 mg/day) for health benefits.

The null hypothesis for the feeding trial is that there will be no difference in n-3 fatty acid content among fish fed diets with different ingredients.

Significance

Data on food intake in Ghana and other developing countries suggests that many people do not get enough essential fatty acids. Both n-3 and n-6 fatty acids are essential nutrients for people, but there is a striking imbalance in the intake of n-3 and n-6 fatty acids in the US and many developing countries. Intake of n-6 fats far exceeds that of n-3 fats due to the widespread use of plant oils and grains that contain more n-6 than n-3 fatty acids (Trushenski and Lochmann 2009). Thus, traditional diets can lead to marginal to severe deficiencies of n-3 fatty acids and a variety of associated health problems such as cardiovascular disease, arthritis, atherosclerosis, diabetes, and cancer (Horrocks and Yeo 1999; Arterburn et al. 2006; Simopoulos 2008). In infants, the n-3 fatty acids are crucial for normal brain development, behavior and cognitive ability.

In Ghana, n-3 deficiency is more common in infants and pregnant or lactating women than in adult males (Siekmann and Huffman 2011). Deficiency of n-3 fatty acids during infancy can be especially destructive, as fatty acids such as DHA are crucial for normal brain development and cognitive function (Innis 2007).

Fish are the primary practical source of n-3 fatty acids in most countries (Tocher 2003), and fish supplies approximately 60% of the protein for the population of Ghana. However, farm-raised fish that are fed diets high in n-6 fatty acids will accumulate high levels of n-6 fatty acids in their tissues. Farmed tilapia have received criticism for being too high in n-6 and too low in n-3 fatty acids (Weaver et al. 2008). This is a function of their diets, which typically contain large amounts of plant ingredients high in n-6 fatty acids. Although these ingredients are considered environmentally sustainable to use in fish diets, they lack the healthy n-3 LC-PUFA that are found in marine fish products. There is a tuna processing plant in Tema, Ghana that provides some fishmeal for tilapia diets. However, catches of tuna are declining and it would be preferable to identify viable plant sources of n-3 fatty acids for long-term growth and sustainability of the aquaculture industry in Ghana. Fortunately, tilapia can elongate and desaturate 18:3n-3 found in plant oils to form n-3 LC-PUFAs such as 20:5n-3 and 22:6n-3. Therefore, inclusion of preformed LC-PUFAs is not necessary for the general performance or health of the fish (NRC 2011).

It is well known that fish acquire the fatty acid signatures of their diets (they "are what they eat")(Turchini et al. 2011). The degree of enrichment and the time needed to achieve a target level of enrichment will depend on the amount of n-3 fatty acids in the diet, the feeding rate, and length of time the fish are fed the diets. The diets will also need to support optimal growth, feed conversion and survival of the tilapia to minimize cost-of-gain and support production profitability. In this project, we will identify and characterize locally available, cost-effective ingredients that will add n-3 fatty acids to the diets of tilapia, and create an n-3 enhanced product that is a preferred fish to Ghanians but has greater potential to enhance their health.

The potential health impact can be assessed by comparing the concentration of n-3 fatty acids in the fish to the daily recommended intake of n-3 fatty acids for good health in humans from medical and health organizations such as the American Heart Association and the World Health Organization. Currently, the recommended range is from 500-1500 mg/day depending on factors such as age, gender, reproductive status, and prior history of coronary problems. The number of servings of fish with a given concentration of n-3 fatty acids that would need to be consumed to improve health status can then be calculated. This metric is easy for the layperson to understand and can be included in health education materials such as pamphlets and bulletins.

The suitability of feed ingredients for commercial production of both fish and feed are based not only on nutrient content, but on economics and availability, as well as palatability to the fish (Hardy and Barrows 2002). In Ghana, a large number of oilseed and cereal by-products are available for screening as potential sources of n-3 fatty acids for fish feeds (Nelson and Wallace, 1998; Hecht, 2007). A few plants found in Ghana such as *Leucaena leucocephala* and *Moringa oleifera* contain more than 30% 18:3n-3 in their lipids. There is some information on the feeding value of *Leucaena* and *Moringa* in Nile tilapia (Adeparusi and Agbede 2005; Madalla 2008), but more information is needed to optimize the inclusion levels of these leaf meals in diets to obtain both profitable production and improved product quality (i.e., enhanced content of n-3 fatty acids). A recent study at the University of Arkansas at Pine Bluff (Kasiga 2012) showed that leaf meals made from these plants could be substituted for up to 30% of the protein in soybean meal in diets of Nile tilapia without reducing fish performance. The leaf-meal diets also significantly increased the concentration of total n-3 fatty acids and n-3 LC-PUFAS in the fish. These preliminary results should be expanded to include other variables. For example, the basal diet included some fish oil because the primary focus of the study was on use of the leaf meals as protein sources. It would be preferable to test the leaf meals in diets without any fishmeal or oil in line with the global trend toward fishmeal and oil reduction in aquafeeds. Also, Kasiga (2012) did not grow the fish to market size, so diets containing these leaf meals need to be tested in fish for a full growing season, and the fatty acid composition and taste of the market-size fish should also be assessed. In addition to the leaf meals, a survey should be conducted to identify any other potential feed ingredients available in Ghana that would enhance the n-3 fatty acid content of the fish and meet the other criteria discussed previously.

Aside from whole plant ingredients, it is possible to add isolated lipid sources to the diets also, such as flaxseed oil. This oil contains more than 50% 18:3n-3, and does not inhibit tilapia growth (Karapanagiotidis et al. 2007). There is a commercial producer of flaxseed oil in Cameroon, which currently sells the oil for $350/MT. This might be close enough for Ghana to consider using it in tilapia diets. It is also possible to maximize the retention of the desirable n-3 LC-PUFAs in fish by supplying most of their dietary fat as saturated fat such as coconut oil or palm kernel oil (Trushenski et al. 2009). These oils are widely available in tropical regions and could be used in combination with an n-3 lipid like flaxseed oil to optimize the n-3 content of the fish while keeping diet cost as low as possible. This strategy has not been tested in tilapia using diets without fishmeal and oil, so further verification of the strategy using diets without marine products is needed.

In summary, the ability to produce tilapia enriched with n-3 acids as a functional food could be a key factor in mitigating widespread health problems associated with essential fatty acid deficiencies in Ghana and other developing countries. The challenge is to identify n-3 sources that will support fish performance and enhance the quality of farmed tilapia for human health while maintaining production profitability.

Quantified Anticipated Benefits

The study will produce information useful for practical diet development for Nile tilapia in Ghana that will result in production of a fish with a healthier lipid profile (enriched in n-3 fatty acids) compared to conventionally grown fish. The number of servings of fish needed to provide the recommended daily intake of n-3 fatty acids for health benefits will be calculated for each diet. The study will also emphasize the importance of using environmentally sustainable and cost-effective ingredients to accomplish the nutrient enhancement of tilapia.

Production of healthier tilapia is part of a larger strategy to improve the nutritional status of people in developing countries such as Ghana. The information will be summarized in lay publications suitable for distribution at workshops or posting on websites. The information will also be prepared for publication in peer-reviewed journals.

Quantifiable

The number of suitable ingredients identified and characterized in the survey as having good potential to enhance n-3 fatty acids in tilapia (target = 5 minimum); number of lay publications produced (at least one); number of journal articles produced (at least one).

Deliverables

At least one fact sheet or article for posting on website (for the layperson or farmer); at least one journal article (in Journal of Applied Aquaculture or other suitable peer-reviewed journal).

Research Design/Activity Plan

Location

This study will be conducted in Ashanti and Brong Ahafo regions of Ghana and the feed trial in the FRNR facilities in the KNUST. UAPB will assist in the survey to identify new feed ingredients, and will analyze the proximate composition and fatty acid composition of the ingredients. Following analysis, UAPB will advise HC personnel on inclusion levels of the most promising ingredients to include in feeding trials. After diet formulation at KNUST, UAPB will also analyze the proximate and fatty acid composition of the finished diets. Following each feeding trial, UAPB will analyze samples of fish for proximate and fatty acid composition.

Methods

Feeding trials (KNUST): One feeding trial will be conducted in year 2, and another in year 3. Both trials will be conducted in hapas placed in earthen ponds at the KNUST aquaculture facility in Kumasi (Ashanti region). Based on the composition of potential feed ingredients from UAPB, the 2-3 most promising ones will be selected for the first feeding trial (Year 2). Experimental diets will be formulated by substituting the appropriate amounts of the new ingredients for traditional ingredients with the goal of increasing the total n-3 content of the diets. Diets will be formulated to meet or exceed the known nutrient requirements of Nile tilapia (NRC 2011) and will be as similar as possible in total protein and energy content. The control diet will be a locally produced tilapia feed (Raanan Feeds). Proximate and fatty acid analysis of five fish will be conducted before the experiment begins (baseline data), and at the end of each experiment to test for differences in composition due to diet. Ten-gram fish will be stocked at 30/hapa (1m X 1m X 1m), and each treatment will have four replicates. Fish will be fed 4-6% body weight daily (based on fish size). Water quality will be monitored throughout the feeding trials. Dissolved oxygen, temperature and pH (5-7 days a week), nitrite, total ammonia, and chlorophyll a will be determined weekly. At two-week intervals during each trial 15 fish from each hapa will be randomly selected and weighed to track growth. Trial 1 will last 8-12 weeks. At the end of the trial all fish will be counted and weighed. Weight gain, survival and feed conversion will be analyzing using 1-way ANOVA and differences in means will be considered significant at $P<0.05$. Specific differences in means will be identified using Fisher's LSD test. Based on the results of the first feeding trial, new diets will be formulated to refine the results in the second feeding trial (year 3), which will last 6 months or until fish achieve market size. A very basic consumer taste test will be run at the end of the second trial to assess acceptability on the market. However, in Ghana fish size is more important than taste.

Proximate and fatty acid analysis (UAPB): Dry matter and ash will be determined by drying the sample at 135^{o}C for 2 h, and burning the sample at 600^{o}C for 3 h, respectively (AOAC 1995). Crude fiber (of feed ingredients and diets only) will be determined according to Ankom filter bag technique (AOCS 2005; Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, New York). Crude protein will be analyzed with the Macro-Kjeldahl method of total nitrogen analysis and the protein content in samples determined by multiplying the nitrogen values by 6.25. Total lipid will be determined using chloroform/methanol (Folch et al. 1957). The nitrogen free extract (NFE), a measure of soluble carbohydrates, will be calculated by subtracting the percentage protein, lipid, ash, fiber and moisture from 100. Lipid extracts from the diet ingredients, diets and fish will be used for fatty acid analysis. Ten mL of the lipid extracts will be evaporated under nitrogen and then trans-esterified with 14% boron trifluoride. The resulting fatty acid methyl esters (FAMEs) will be analyzed (Morrison and Smith 1964) using a flame ionization gas chromatograph with helium as the carrier gas. The FAMEs will be separated on a fused silica capillary column (15 m x 0.25 mm internal diameter). The injection volume will be 1µl, with an injector and detector temperature of 250^{o}C and 315^{o}C, respectively. The column temperature will be held initially at 100^{o}C for 10 min, increased to 160^{o}C at a rate of 15^{o}C/min and held for 4 min, then increased to 250^{o}C at a rate of 2.5^{o}C/min. The FAMEs will be identified and quantified by comparing the retention time and peak area to those of serially diluted mixtures of reference standards. After the feeding trials and all analyses, the cost-of-gain will be calculated by determining the cost of the amount of feed (kg) required to produce 1 kg of tilapia. The costs of the diets and feed conversion data will be used to generate cost-of-gain. Based on the total n-3 composition of the fish, the amount of fish that would have to be consumed to provide the recommended daily intake of n-3 fatty acids for human health will also be quantified.

Schedule

Start date: The study will begin July 1, 2013, and end September 30, 2015.

Anticipated timetable

July 1 - December 31, 2013 - conduct survey, obtain feed ingredients, and analyze their proximate and fatty acid composition (KNUST - obtain and send feed ingredients; UAPB - lab analysis of ingredients.)

January 1 - December 31, 2014 - Formulate diets (UAPB and KNUST) and conduct initial feeding trials with tilapia at KNUST. Send feed and fish samples to UAPB for analysis. Formulate diets for second feeding trial (UAPB and KNUST).

January 1 - September 30, 2015 - Conduct second feeding trial (KNUST). Send feed and fish samples to UAPB for analysis. Analyze results statistically and prepare them for presentation and publication.