

**ADDRESSING FOOD SECURITY AND DEVELOPMENT IN  
GUATEMALA:  
USING LOCAL FEEDS TO PROMOTE AQUACULTURE**

by

**Kirsten E. Roe**

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**STATEMENT OF COMMITTEE APPROVAL**

**Dr. Paul Brown, Chair**

School of Forestry and Natural Resources

**Dr. Kwamena Quagrainie**

School of Agricultural Economics

**Dr. Gerald Shively**

School of Agricultural Economics

**Dr. Hye-ji Kim**

School of Horticulture

**Approved by:**

Dr. Dr. Robert G. Wagner

*Dedicated to God and Honey for helping me through my pain and fatigue.*

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## ACRONYMS

AGR =	Absolute Growth Rate
BB =	Black bean
C1 =	Control 1
C2 =	Control 2
C3 =	Control 3
Ca =	Calcium
CM =	Cricket meal
CP =	Crude Protein
Cu =	Copper
DGC =	Daily Growth Coefficient
DH =	Estimated days to harvest
DO =	Dissolved Oxygen
DW =	Dry Weight
EW =	Earthworm meal
Fe =	Iron
FM =	Fish meal
K =	Potassium
Mg =	Magnesium
Mn =	Manganese
Na =	Sodium
NFE =	Nitrogen-free extract (non-structural carbohydrates)
P =	Phosphorus
Ppm =	parts per million
S =	Sulfur
SGR =	Specific Growth Rate
Zn =	Zinc

## **ABSTRACT**

Food security is an increasingly important global challenge. Population increases, coupled with changing food habits, are placing significant demand on the global food supply. Without significant advances in agricultural techniques and approaches, it will be difficult to feed the global population within several decades. Aquaculture is one underutilized agricultural method which could help alleviate this impending crisis if more farmers were able to implement improved techniques. One of the primary inputs for successful aquaculture is a nutritionally complete feed. However, commercial fish feeds may be prohibitively expensive or unavailable in many locations in the developing world, reducing the ability of farmers to implement economically successful aquaculture ventures. Providing farmers with the ability to produce their own high-nutrition feeds with locally available ingredients would be a key enabler for more widespread successful aquaculture efforts. This dissertation focuses on the development and evaluation of alternative, locally sourced, inexpensive fish feeds to maximize fish production in developing countries.

## **CHAPTER 1. - LITERATURE REVIEW**

### **1.1 Overview**

The human population is increasing rapidly, placing burdens on global resources. Population estimates project a population of about 9.6 billion people by 2050 and between 10 and 12.3 billion people by 2100 (Gerland et al. 2014, Warren 2015, Abel et al. 2016). This will require significantly improved approaches to meet future food security needs because existing techniques are insufficient to meet the future demand (Gerland et al. 2014, Abel et al. 2016). These population estimates are driven by rapid population growth from higher fertility rates, a slowing of fertility decline, a decrease in the relative size of the working-age population compared to the older population, and a decrease in infant mortality (Bongaarts and Casterline 2013, Andreev et al. 2013). In fact, one current projection estimates a population of 3.1 to 5.7 billion people in Africa alone by 2100 at a probability of 95% (Gerland et al. 2014). The primary method of slowing population growth is increasing the use of contraceptives and providing female education (London Summit on Family Planning 2012, Peterson et al. 2013). However, even if local fertility decreases, global fertility rates may still increase. Small populations may maintain high fertility and replace low-fertility populations, leading to continual increases in global population growth rates (Warren 2015).

The recent rate of agricultural yield growth is insufficient to support future projected populations. Currently, the farm yield growth is between 0.6 and 1.1% per annum with staple cereals having a rate of 1.0% or less per annum growth (Lobell et al. 2009, Fischer, Byerlee, and Edmeades 2014). However, these staple crops are showing a decreasing rate of change. In order to prevent increases of more than 30% in real food prices, farm yield must have a linear increase of at least 1.1% per annum growth, meaning there is a current gap between required and actual

growth rates (Fischer, Byerlee, and Edmeades 2014). Closing yield gaps and improving breeding are examples of some methods to help remedy this. Total factor productivity (TFP) is a descriptive measure of efficiency and yield growth. TFP measures the use of resource inputs with respect to the yield where a higher TFP indicates greater efficiency as more output is produced from fewer inputs. For example, reduced land and water requirements of new corn varieties can result in higher yields. With limited resources, increasing TFP for crops is essential for food security. In the past, there was a tripling of crop output with less land expansion, showing a high TFP and crop productivity (Alexandratos and Bruinsma 2012). Over the past 50 years, 86% of output expansion has come from higher yields (77%) or increased cropping intensity (9%).

However, some research has shown that this yield growth increase has been slowing, threatening future food security (Lobell, Cassman, and Field 2009, Fischer, Byerlee, and Edmeades 2014). At least a 150% increase in yield growth in currently cultivated fields would be required to support the projected 2050 population alone (Hertel and Baldos 2016). To meet the future consumption needs of the growing population, an immediate increase in food production capability is required. Additionally, an increase in cultivated land expansion is required to use for additional crops to supplement the more efficient use of existing croplands. This is vital for agricultural growth, but also has implications for other sources of food, such as fish production.

Staple crop research has been an important innovation avenue, but, with current approaches, cereal crop varieties will reach a yield threshold due to constraints with photosynthesis, nutrient use throughout the plant, and physiology. New technologies, such as transgenics, can result in higher productivity, more efficient photosynthesis and nutrient use, drought-tolerance, and pest-tolerance or resistance. This would require less fertilizer, water, and land area, reducing the impact on the environment. However, there are trade-offs related to food production, as well as politics,

negatively affecting new plant breeding technology. In agriculture, the trade-offs include land expansion, irrigation water use, and fertilizer use leading to possible eutrophication of water, decreased water availability, and biodiversity loss (Langley 1987, Power 2010). The trade-off with current technologies is the need to choose between feeding the future population or preserving ecosystems. New, publicly acceptable technologies would lead to fewer compromises between food security and the desire to preserve the environment. Since yield growth rates are slowing for crops, multiple approaches to food production will be needed to meet current and future needs. One of these approaches is using water-based agricultural systems such as aquaculture.

Agriculture improvement and new methods of food production are integrated into development as they help improve food security in impoverished countries. One such developing country is Guatemala, an impoverished nation with high malnutrition rates, government corruption, and climate change-affected agriculture. Guatemala is still within the agricultural economic stage with 70% of the population living in rural areas (WFP 2018). Climate change is further worsening poverty with droughts and flooding that have caused multiple years of crop failures.

## **1.2 Guatemala Status and Agricultural Opportunity**

### **1.2.1 Poverty and Malnutrition**

According to the World Food Program, Guatemala ranks as the second most malnourished country, and the country with the highest child undernutrition, in the western hemisphere (WFP 2018). It also ranks as the sixth most malnourished country and has the fourth highest child undernutrition in the world (WFP 2018). This malnutrition is driven by poverty and discrimination, with the largest malnourished population (70%) living rurally in the mountains. This population consists of indigenous Mayan communities which still suffer from isolation, ethnic discrimination



from the wealthier, more urban Ladino (Spanish-descent) population, exclusion, violence, and social inequality (Steinberg and Taylor 2008, IFAD 2011). Often, girls do not receive a secondary education, but instead get married before turning 18 to reduce the burden on their families, leading to further perpetuation of poverty.

The indigenous population faces high population density over limited land area due to being relegated to a smaller portion of the country compared to the wealthier Ladino population. This results in small farm size per household; 85% have arable land less than 1.4 ha, while 62% have less than 0.7 ha (MAGA 2011). The structural exclusion and isolation reduce access to inputs (i.e., fertilizer, improved seed varieties, pesticides), which coupled with small farm size, limits farm production capability and income.

### **1.2.2 Agriculture**

Agriculture is the primary livelihood for indigenous households, although there is some non-farm income through weaving, construction, and seasonal or permanent migration to other jobs (IFAD 2011). Farm types range from small-scale non-diverse maize to diversified maize, small and large-scale coffee, diverse with other crops, and home gardens. Farm diversity includes food crops (i.e., maize, beans, potatoes), livestock, and cash crops (i.e., coffee, cacao) (Berre et al. 2016). In the Western Highlands of Guatemala, where many indigenous communities live, maize and potatoes are the two primary staple crops, while coffee is the primary cash crop followed by peas, faba beans, and green beans (Lopez-Ridaura 2019). In this region, farms had an average of 2.5 crop types and 26% of land was cultivated as polyculture. Most farms had poultry (77.8%), but only 9.2% had small ruminants (Lopez-Ridaura 2019). Several factors that limit food security include the difficulty in obtaining energy for agriculture (52% of households) and farm

diversification, such as diversified coffee or maize-based farms. Those with specialization in maize and households with resource constraint experienced greater food insecurity (Lopez-Ridaura 2019).

### **1.3 Aquaculture**

Aquaculture is the cultivation of aquatic plants and animals. Aquaculture methods include open-water cages or pens in bodies of water, as well as land-based tanks or ponds. Aquaculture requires some land for tanks or earthen ponds but can also be done in pen or cage systems in natural watersheds. Additionally, aquaculture is a three-dimensional food production system, enabling a high-density food production per unit land surface area compared to field crops. There is an opportunity to enable more widespread adoption of aquaculture, which is a critical component of our ability to meet future food production needs. Additionally, aquaculture products are excellent protein and nutrient sources. Tilapia, for example, contain ~36% protein, vitamins A, C, D, and E, and all the essential amino acids (Easterling 2007, FAO 2009, Rice and Garcia 2011). Therefore, aquaculture represents an opportunity to provide a supply of high-quality nutrition with minimal impact.

Aquaculture represents one of the fastest growing sectors of food production in the world, especially among developing countries, with estimated production increasing from 10.64 million tons in 1987 to 32.2 million tons in 1999, accounting for 26.18% of the world's total fishery production (FAO 2000). By 2016, aquaculture accounts for 47% of the total global fish production with an increase to 53% if non-food uses are excluded (i.e., fish meal and fish oil reduction) (FAO 2018). Aquaculture provides an opportunity to improve food security, nutrition and livelihoods across developing countries (Edwards 2013), where rates of poverty and malnutrition remain high despite growing economies (Marini and Gragnolati 2003).

Otherwise non-arable land can be used for terrestrial aquaculture (Devendra and Thomas, 2002), leaving arable land available to maximize agricultural production. Aquaculture can even be integrated into small-scale rice paddy fields and irrigation systems, offering a sustainable system of food production and livelihood diversification (Bondari et al. 1983, D'Silva and Maughan, 1994 1996, McMurty et al. 1997).

Southeast Asia and Latin America are the largest producers of farmed shrimp in the world (Hall 2004). In Southeast Asia, aquaculture is most developed in Thailand, the Philippines and Indonesia. The total output of Association of Southeast Asian Nations' fisheries was 30.6 million tons, accounting for 18.3% of global production, and there are over 650,000 people employed in fish farms and related industries (FAO 2000). Continued aquaculture development faces barriers in both Latin America and Southeast Asia, including underdeveloped technology and facilities and high dependence on government financial support, especially for shrimp farming as a major source of export earnings (Neiland et al. 2003). The industry growth has been highly uneven in Latin America, with Chile and Brazil accounting for 72% of the region's production (Tacon 2003). Chile produces farmed fish at almost the same quantity as the rest of Latin America combined as of 2016 (1035 thousand tons vs 1667 thousand tons) (FAO 2018)

While other countries in Latin America have seen successful aquaculture development, Guatemala has had very low aquaculture investment and production. However, Guatemala is important in marine shrimp cultivation. Production has increased to the point there is optimism for future increases (FAO, 2013). In Guatemala, fish consumption is 2.0 kg/capita, and less than the consumption of the other main animal proteins. The low protein consumption contributes to the fact that Guatemala is ranked the second largest malnourished country in western hemisphere with malnutrition rates of 15.8% (Marini and Gragnolati 2003). In 2016, food shortage rates

reached 101 kcal/capita/day in Guatemala, further contributing to malnutrition and food insecurity and leading to migration (Beveridge et al. 2019).

### **1.3.1 Economic Feasibility of Aquaculture**

Before discussing the role of aquaculture in development, it is important to address the feasibility of aquaculture itself. Importantly, there is already demand and consumption of fish in daily diets in many regions. Countries that are food and financially insecure use fish as a major protein source. On average, people in Africa and Asia have a greater dependence on fish in diets than those in developed nations. People in these regions obtain essential amino acids, minerals, and vitamins from fish consumption (Easterling 2007, FAO 2009, Rice and Garcia 2011). In 2002, in North and Central America, fish accounted for only 7.1% of the average diet and in Europe 10.3%, but the percentage of diet comprising fish in Africa was 19.4%, in China 21.2%, and in the rest of Asia 23.3% of (FAO 2002). As a relatively extreme example, freshwater fish are an important nutrient source for families in Cambodia, making up 65-75% of the protein in the diet (Guttmann 1999). Natural fisheries have often provided the fish quantity demanded but have reached and exceeded carrying capacities of local fish production rates of 80 million metric tons (mmt) per year (FAO 2010).

This supply issue has resulted in damage to the aquatic ecosystems and reduced catch rates for those who depend on wild-caught fish, decreasing their food security (Manach et al. 2012). To help reduce the demand burden on marine or freshwater fisheries, aquaculture may be a solution, growing on average 8% per year since the 1970s (FAO 2010, Hall et al. 2010). In addition, FAO reported “...*Per caput* food fish supply from aquaculture has increased eightfold, from 0.71 kg in 1970 (0.9 kg from China, 0.6 kg from the rest of the world) to 5.87 kg in 2000 (19.6 kg from China, 2.3 kg from the rest of the world)...” However, since 2013, there has been a decrease in aquaculture

production in several industrialized nations such as the US, Japan, and France (FAO 2014). It is theorized that this reduction may be due to the lower costs of labor and production in developing countries, leading to industrialized countries importing from those areas (FAO 2014). Aquaculture can be used to cultivate other aquatic foods as well, such as seaweed, shellfish, and shrimp. This increases the flexibility of aquaculture to meet the demands and constraints of an area, such as preferred foods, space, water salinity, and location.

### **1.3.2 History of Aquaculture in Development**

Aquaculture has been an important component in developmental activities for many years. Production systems can be simple, conversion of feed to flesh is the best of any animal, and the resulting product is of high quality. Previous studies have shown that cages and pens in irrigation canals can produce between 100 to 250,000 kg/ha/year, depending on intensification level (Costa-Pierce and Effendi 1988, Ishak 1982, 1986). Integrated aquaculture can be used alongside irrigation in crop production (Bondari et al. 1983, D'Silva and Maughan, 1994, 1996, McMurty et al. 1997). In addition, existing systems can produce effective aquaculture yields. For example, in West Java, two hydropower reservoirs utilize cage aquaculture, employing 7000 people, producing 25,000 metric tons of fish, and generating a total of \$24 million in annual gross revenue (Costa-Pierce 1998). This helps avoid the costs incurred when building a pond or tank, prevents the need for determining the water source and flow, and decreases the time required to begin producing fish.

As mentioned previously, unlike field crops, aquaculture uses three-dimensional space, increasing yield while reducing required land size. This is especially important in countries where farm size is limited. The proportion of rural households having less than 0.2 ha has increased from 47.4% in 1988 to 58.9% in 2007 (Hossain and Bayes 2009). Only 1.8% of rural households have landholdings larger than 3 hectares. In Guatemala, this means that maximizing food production on

a small amount of land is vital. One important advantage of aquaculture is the smaller land area required for production, enabling crop production on the remainder of the land, depending on the production possibilities frontier (the optimal level of output given two input levels). Regardless of the method and location, there is demand and a market for aquaculture products as well as a need for it due to overharvested fisheries. Increasing the intensity level of aquaculture with aeration, improved feeds and genetics and disease management could help increase yield.

### **1.3.3 Examples of Aquaculture Success**

Overall, aquaculture production has grown more than seven times faster in developing countries than in developed countries over a thirty-year span. Much of this growth has occurred in mainland China which makes up 91.3% of global aquaculture production by weight (FAO 2000). When mainland China is excluded, global production increased six-fold from 2.23 million metric tons (mmt) to 13.27 mmt from 1970 to 2000 (FAO 2000). Aquaculture also improves food security, nutrition, employment, and income for the rural poor (Edwards 2013). This facilitates the economic and agricultural development of households and the communities, and improves the country's economy by providing jobs, increased fish for consumption, and lower fish cost due to greater supply. Studies of development projects implemented by NGOs and governments have been investigated in Haiti, Malaysia, the Amazon, Timor-Leste, and Bangladesh, among others. These studies show a general trend where household incomes, farm output, and food security increase in parallel with fish production (Hallman, Lewis, & Bugum, 2003; Jahan, Ahmed, & Belton, 2010; Rand & Tarp, 2010; Thompson, Firoz Khan, & Sultana, 2006). Malnourished people in Cambodia showed an increase in vitamin A, riboflavin, and zinc when aquaculture production was implemented (Verbowski et al. 2018). In Zambia, there was an increase in growth in the value chain, fish per capita, and vertical integration with the introduction of aquaculture (Kaminski et al.

2018). Ahmed and Lorica (2002) found that food security improved from aquaculture via income, employment linkages, and consumption (Ahmed and Lorica 2002).

#### **1.3.4 Bangladesh History of Aquaculture**

Bangladesh provides a case study of the potential developmental impact of aquaculture. This country has a history of aquaculture projects from NGOs, foreign funding agencies, and local and foreign governments. A USAID-funded ICLARM project helped implement low input technology, such as using drainage ditches and no feed, and the World Bank helped stock carp fingerlings in local watersheds. Additionally, the FAO implemented training, demonstrations, and seed farms. This was done using a “trickle down” approach where interest-free credit for input resources was offered as an incentive for trainees to subsequently train others in their villages (Kumar 1999). The project was relatively successful in that farmers did indeed train others in their village and fish production increased. However, the benefit in smaller villages was limited as the farmers there could not train as many others to fully utilize the incentives. This history provides opportunities to study effects of aquaculture on overall development.

Shrimp farming along the coast of Bangladesh has been successful. Some of the initial concerns with this effort included instability of markets, society, and livelihood displacement (Bondad-Reantaso and Subasinghe 2008, Paul and Vogl 2011). Lewis (1997) claimed only land-holding, wealthier households would benefit from aquaculture projects due to their hoarding of fish-farming resources. However, in Bangladesh, the Adivasi Fisheries Project was implemented among the landless indigenous Adivasi people. The Adivasi experienced an increase in monthly frequency of fish, meat, and egg consumption, especially for fish. Those families in the project generated increased household income (Pant et al. 2014). Further data from 2000 to 2010 showed

an increase in aquaculture, leading to decreased fish prices due to higher output, more consumption of fish, and it is linked to pro-poor economic growth (Toufique and Belton 2014).

### **1.3.5 Examples from Africa**

African aquaculture expanded from 36,685 tons to 121,905 tons between 1984 to 1995 (FAO 1995). In that same 11-year span, there was an increase in the number of countries using aquaculture and an increase in the number of species cultivated. In many developing countries, there are three economic roles of aquaculture production. The first role is commercial development where profitable species are fed commercial feed. This agribusiness model can help provide employment and can more easily enter the global market (Katz 1995). The second role is rural aquaculture development, which is generally small-scale, small-holder, and dependent on local available resources. Investment is focused on labor, water, and land, and the system is focused on food security. The products are generally eaten by the household or traded in the local economy (Brummett and Williams 2000). One estimate of the potential yield in sub-Saharan Africa in small watersheds was almost one million tons yearly (Coates 1995).

The third role of aquaculture is small-scale commercial systems that are in rural settings - i.e., a combination of the first two approaches. In the 1980s, 90% of foreign investment focused on the small-scale commercial sector (Huisman 1990). From the 1970s to the 1990s, over 300 projects were started, focusing on training, building hatcheries, extension, and promoting proven technology (Brummett and Williams 2000). These projects resulted in few long-lasting benefits due to a lack of addressing local constraints, economic conditions, and cultural values.



### **1.3.6 Challenges to Aquaculture Development**

The disparity in regional aquaculture growth may be due to input differences. For instance, there are many input factors that affect output, including climate, aquaculture intensiveness, market factors, fingerling lines and quality, water source and quality, feed availability, pathogen exposure, and management practices (Kumar and Engle 2016, Bostock et al. 2010, Muir and Young 1998, Nadarajah et al. 2017). Even the simple ability to prevent poaching from humans and predation by birds can lead to significant differences in yield. Since there are stringent quality requirements for import into industrialized countries there is a trade-off for farmers in which they can produce without regulations and sell locally or accept reduced fish growth rates and higher costs to be able to participate in the global market.

Aquaculture production is negatively correlated, though not significantly, to strict environmental regulations. These regulations often require incorporating specific steps in production including preventing fish escape to local watersheds, limiting phosphorus in the effluent water, and handling feed waste and feces (Nadarajah et al. 2017). These regulations increase production costs and labor required while also hindering the local farmers' ability to trade on the global market. However, the regulations are also beneficial in allowing the farmer to meet health requirements for export. In addition, there is non-significant negative correlation between aquaculture growth rate (AGR) and the quality of institutions and governance. These institutions include policies, laws, rules, and regulatory measures, most often demanded by international organizations. This was unexpectedly different from the hypothesis that high-quality institutions would benefit AGR, not hinder it. This hypothesis was based on "resource curse" literature that indicated high-quality institutions use natural resource industries to help improve economic benefits in the country (Mehlum, Moene, and Torvik 2006, Boschini, Pettersson, and Roine 2007).

Even some existing aquaculture literature cites the requirement for good governance policies to help with production (Smith et al. 2010, Hishamunda and Ridler 2004).

While more recent studies provide support for the success of aquaculture, the Lewis (1997) study did highlight several important areas that could affect the efficacy of aquaculture projects. For example, to maintain a consistent, sustainable aquaculture farm, it is necessary for farmers to have a reliable source of fingerlings. Some producers will breed their own fingerlings, maintaining a breeding colony. However, this requires resources such as additional space, water, and feed, as well as training. Those that do not breed fish must purchase fingerlings, requiring a provider in the value chain. If these fingerlings are poorly bred or diseased, this will reduce aquaculture yield. In addition, lack of understanding technical information and disease management, risks from theft or flooding, and trade-offs of water use are limiting factors in local aquaculture success (Lewis 1997).

### **1.3.7 Aquaculture and Trade**

The production of fish to meet subsistence needs is just the first step in aquaculture development. At this point, the farmer is still not reaching the highest utility via consumption by the household. Many farmers may prefer to consume more of some other good or another type of fish, requiring the farmer to enter the market. This allows the farmer to gain an initial benefit and also allows trading the produced good (fish) for something else that improves utility (e.g., money, other foodstuffs or supplies, medical care, etc.) for the household.

However, there is a current theory, sometimes called ‘economic geography’, that considers trade harmful to the undernourished Southern nations. This theory holds that the fish produced in the Southern nations will be mainly exported to wealthier Northern markets and any remaining farmed fish will be consumed by wealthier people in the country, leading to a lack of improvement in meeting the needs of the impoverished and malnourished in those countries (McIntyre et al.

2016, Ponte et al. 2014, Beveridge et al. 2013, Bush 2004, Ahmed and Lorica 2002, Lewis 1997, Hall et al. 2013, Golden et al. 2016). However, this theory does not consider the realities of the domestic vs. traded farmed fish relationship. In the top ten fish-producing countries, many of which are impoverished, around 89% of farmed fish are consumed domestically. Even if global trade of the fish from impoverished countries was harmful economically, these data indicate a significant improvement in available food supplies, countering the idea that most fish are exported (Belton, Bush, and Little 2017).

There are often constraints to agricultural intensification which prevent the immediate jump up the “development ladder” rungs to true intensification of production. These constraints may be lack of land, absence of resources, or lack of training. This leads some groups to suggest that maintaining self-sufficiency is the preferred approach. However, the development ladder enables farmers to understand what they can change and improve, while working around the constraints. This allows for a step-wise climb up the ladder. For instance, if the objective of a development project is to improve yield of maize for farmers, but the farmers are limited in income, the farmers are less likely to absorb the risk of new technology. Instead of suggesting using new tractors or a large amount of fertilizer, it might be better to start with an older machine or something designed for the situation. This will help to begin yield improvement but allow the farmers to be able to improve their future ability to afford the new process or be able to absorb the risk of any potential failure.

An example of this explanation is the aquaculture project implementation in Bangladesh. One analysis suggested that the public sector and NGO methods of implementation for aquaculture intensification in this project did not note resource, social, and economic constraints. Instead, this study suggested that poor farmers would not have an income benefit (Lewis 1997). However, a

closer investigation of the most marginalized, impoverished members of the Bangladesh study showed household income, protein consumption, and food security improvement when part of the aquaculture treatment group (Pant et al. 2013).

The communities investigated consisted of the Adivasi, the previously mentioned ethnic minority that is often marginalized (OPHI 2011). These minorities are mired in a cycle of poverty where 60% lie below the absolute poverty line (IRIN 2011). A fisheries project was implemented among these people that included aquaculture. Those to whom aquaculture and related technologies were introduced saw a significant increase in household incomes as well as an increase in consumption of fish, meat, and eggs, resulting in an overall increase in food security and nutrient intake (Pant et al. 2013). This indicates an increase in food security and development for these communities compared to those without aquaculture production.

In Haiti, discussion of aquaculture as a tool of development led to a workshop for NGOs, businesses, government agencies, and universities. During this workshop, optimal locations for pond aquaculture was discussed. The participants agreed that Haiti would benefit from commercial-scale tilapia aquaculture, helping to improve economic development by providing jobs and improved food security. This would provide a combination of local nutrition and food availability while allowing value-added, marketing, and export opportunities. Regardless of country, identifying optimal locations for aquaculture, such as in the “Haiti recommendation” above, can improve aquaculture development. This depends on availability of water and soil type for pond aquaculture and access to open water for cage or net pen aquaculture. It is also important to note constraints and limitations such as water resources (and current uses), infrastructure, training, suitable sites, and input availability such as feeds or capital (Hargreaves et al. 2012).

To foster intensive aquaculture production, there are several requirements. First, as mentioned above, there is a need for accessible fingerlings. This may be from a private or public hatchery or they may be bred by the farmer. Second, aerated water is necessary to provide adequate oxygen content to maximize fish metabolism and growth rates. Third, there must be a containment system with circulation to allow movement of oxygenated and nutrient-rich water while also providing sufficient space for each fish. Too high of a stocking density can negatively affect the health and growth rate of the fish. Finally, food, or feed, is vital to ensuring fish survival and growth. This dissertation focuses on the feed input in aquaculture.

## **1.4 Tilapia**

### **1.4.1 History of Tilapia Cultivation**

Tilapia species consist of two genera, *Tilapia* and *Oreochromis*, and belong to the Cichlidae family. Cultured species include the Mozambique tilapia *Oreochromis mossambicus*, blue tilapia (*Oreochromis aureus*), and the Nile tilapia *Oreochromis niloticus*. In 2017, Nile tilapia represented 70% of all tilapia production (FAO 2019). Species from Cichlidae are freshwater fish originating from Palestine through Africa and survive in many water sources such as lakes (i.e., deep, shallow, permanent, floodplain), rivers, artificial bodies of water, thermal springs, drainage ditches, rice paddies, and brackish water (Philippart and Ruwet 1982). Tilapia fisheries and culture have contributed to global food security and economic development, especially in tropical developing countries and are found in over 100 countries (Costa-Pierce 2003, de Silva et al. 2004, Canonico et al. 2005, Athauda 2010, Deines et al. 2016).

#### **1.4.2 Tilapia Biology**

The Nile Tilapia, *Oreochromis niloticus*, are resistant to both extremes of dissolved oxygen (DO) levels. They can withstand anoxia or hypoxia events of 0.1-0.5 mg/L and supersaturation of 400% (Magid and Babiker 1975, Tsadik and Kutty 1987, Morgan 1972). If surface air is available, tilapia can tolerate zero DO concentration for short periods of time (Morgan 1972). Even though tilapia survive low DO levels, they thrive at levels of 5.5-6.5 mg/L (Abdel-Tawwab et al. 2015, Tran-Duy et al. 2012).

Intensive aquaculture results in higher ammonia levels due to increased feeding and fish waste. Nile tilapia are tolerant of high levels of ammonia, ranging from 1 to 7.4 mg/L, though optimum levels are less than 0.1 mg/L (Magid and Babiker 1975, Benli and Koksall 2005). In earthen ponds in developing countries, intensive aquaculture promotes excess ammonia due to reduced water circulation, turbid water, feeding to satiation, and high stocking density. This necessitates the use of a species tolerant to periodically elevated ammonia levels, hence the use of Nile tilapia in these systems and in this dissertation.

Aeration is required for culturing fish larger than 100-200g. Feed intake tends to plateau at 3mg/L DO in these larger fish (Tran-Duy et al. 2012). As pond temperature increases, aeration becomes even more important due to reduced DO levels in warmer water (Abdel-Tawwab et al. 2015). This is especially true in tropical developing countries like Guatemala. Optimal temperature for Nile tilapia growth is 27-30°C, but they can survive in water temperature extremes ranging from 10.5°C to 42°C (Fukushu 1968, Denzer 1968, Beamish 1970).

#### **1.4.3 Tilapia Nutrient Requirements**

Since tilapia is the second most farmed fish species in the world, there has been significant research done on their nutrient requirements (FAO 2011). The focus of the dissertation is on Nile

tilapia, the most common tilapia species used in aquaculture (FAO 2011). Fry and spawning females require more dietary crude protein than fingerlings and grow-out fish (30-40% vs 20-30%) (Siddiqui et al. 1998, Sweilum et al. 2005, Abdel-Tawwab et al. 2010). Tilapia require ten essential amino acids: arginine, leucine, valine, isoleucine, threonine, tryptophan, methionine, phenylalanine, lysine, and histidine (Santiago and Lovell 1988). Currently, fish meal is the best source of crude protein and essential amino acids. However, plant and other animal proteins are being investigated as potential replacements. Among plant sources, soybean meal and cottonseed meal have the most complete protein and amino acid content (El-Sayeed 1999, El-Saidy and Saad 2011). One of the issues in alternative protein research is the presence of antinutritional components, deficiencies of one or more amino acids or phosphorus, or poor digestibility or efficiency (El-Sayed et al. 2000, El-Saidy and Gaber 2004, Garcia-Abiado et al. 2004, Yue and Zhou 2008, Zhao et al. 2010). Testing combinations of plant and animal ingredients could help reduce the effects of antinutritional components while providing all the amino acids required (El-Saidy and Gaber 2003, Borgeson et al. 2006, Liti et al. 2006). This approach will be used in Chapter 2 with local ingredients from Guatemala.

Nile tilapia also have a dietary requirement for lipids with a minimum level of 5%-12%, along with polyunsaturated fatty acids such as alpha-linoleic and linolenic acids (Olsen et al. 1990, De Silva et al. 1991). If linoleic acid levels are above the optimal value (0.5%), then fish growth can be hindered (Kanazawa et al. 1980, Takeuchi et al. 1983). Marine fish oil is the primary lipid source in commercial feeds, but its price and environmental effects are increasing (Turchini et al. 2009). Possible alternative sources include soybean oil (Stickney and McGeachin 1983, Gaber 1996, Huang et al. 1998). However, palm oil has become the least expensive and most abundant

plant oil and is suitable for tilapia growth (Ng et al. 2001, 2006, Bahurmiz and Ng 2007, Ng and Gibon 2011).

Tilapia do not have specific carbohydrate requirements, but they are a useful source of energy to allow protein-sparing (acts as the energy source rather than protein) (Shiau and Peng 1993, Wilson 1994). Plant carbohydrate sources include starches and non-starch polysaccharides (NSP), with NSPs being less digestible (Francis et al. 2001, Sinha et al. 2011). Fish are not as efficient as terrestrial animals at utilizing carbohydrates, especially NSPs (Wilson 1994). The gut of Nile tilapia can ferment (due to intestinal bacteria) certain carbohydrates including whole wheat, and wheat starch, but gelatinizing starches prior to feeding can improve fermentation rates, fecal movement efficiency, and fish growth rates (Amirkolaie et al. 2006, Leenhouwers et al. 2008). Other than gelatinizing, pre-treating ingredients, such as chitin and other NSPs, can help increase utilization efficiency of tilapia as well as promote enzyme activity (Ng et al. 2002, Belal 2008, Li et al. 2009, Yigit and Olmez 2011). Pre-treatment includes using enzymes or fungi which both help release nutrients and increase digestibility (Ng et al. 2002, Belal 2008).

Vitamins and minerals are a small, but essential, component of fish nutritional needs. The necessary minerals include calcium (3.5-7 mg/kg), phosphorus (5 g/kg), chromium (204 mg/kg), potassium (2-3 g/kg), magnesium (0.59-0.77 mg/kg), iron (85-160 mg/kg), and zinc (30-79.5 mg/kg). (Robinson et al. 1987, Dabrowska et al. 1989, Eid and Ghonim 1994, do Carmo e Sa et al. 2004, Shiau and Hsieh 2001, Shiau and Su 2003, Shiau and Tseng 2007). Minerals contribute to fish growth, bone development and mineralization, energy production, liver function, and protein utilization. The required vitamins include choline (3000mg/kg), thiamin (3.5 mg/kg), folic acid (0.5-1.0 mg/kg), vitamin C (125mg/100kg), vitamin A (5000 IU/kg), beta-carotene (28.6-44 mg/kg), and vitamin E (20-100 mg/kg) (Soliman et al. 1994, Saleh et al. 1995, Eleraky et al. 1995,



Kasper et al. 2000, Shiau and Huang 2001, Hu et al. 2006, Lim et al. 2011). Vitamin B12 is not required in feed since tilapia can produce their own (Shiau and Lung 1993, Guo et al. 2010).

#### **1.4.4 Tilapia Production in Guatemala**

Currently, aquaculture is not prevalent in Guatemala for smallholders due to expense of feed or overall lack of knowledge about aquaculture methods. Tilapia were introduced to Guatemala over sixty years ago and there are market demands for fish. The Guatemala government has even promoted aquaculture with technical support from UNIPESCA. However, consumption rates are low due to low production with 26,268 metric tons produced in 2018. There has been a steeper increasing trend in production since 2005, indicating that aquaculture is now more prevalent (FAO 2016, World Bank Data [www.data.worldbank.org](http://www.data.worldbank.org)). Most of this growth is from companies like Paraiso Springs, rather than rural households (World Bank Data [www.data.worldbank.org](http://www.data.worldbank.org)). There is still insufficient fish production, and overfishing has reduced wild fish catch over the last decade. Coastal indigenous households have more regular access to fish even though the small fisheries are declining. These communities which consume fish regularly have lower levels of chronic malnutrition, demonstrating the importance of fish consumption for nutrition (Lasso Alcala 2011).

### **1.5 Fish Feed Input and Constraints**

#### **1.5.1 Commercial Fish Feed**

Generally, commercial fish feed is optimized for species and age or size of the individual fish, and high-quality formulations help maximize fish growth rates and yield. For example, first feeding larvae (45-50% protein) and tilapia fingerlings (35-40% protein) require more crude protein than adult tilapia (28-32% protein) (Jauncey 2000, Shiau 2002, El-Sayed 2006, Lim and Webster 2006). The formulated feed will usually consist of 28-32% protein, 10-15% lipids, 40%

carbohydrates, and 8-10% crude fiber (Shiau 1997, Ng and Chong 2004). In addition, the feed contains sufficient essential vitamins and minerals to meet nutritional needs.

Commercial fish feed generally relies on fish meal as a primary ingredient, consisting of wild-caught species such as menhaden, anchovies, and sardines, which are then cooked, pressed, dried, and ground into meal. Fish meal itself is highly nutritious for farm-raised fish, containing high concentrations of crude protein, essential amino acids, and fatty acids while maximizing digestibility and growth rate and minimizing water pollution caused by poor digestion (Miles and Chapman 2005). However, these species are vital for many species of wild fish and ocean mammals and using them in fish feeds can strain fishery stability.

The world's fisheries are at maximum sustainable yield, including the forage fishes used in fish meal. As demand for fish meal increased in response to increased aquaculture production, prices have risen significantly, jeopardizing economic viability of aquaculture. One key issue for local farmers is that commercial fish feed can be cost-prohibitive or hard to access in developing countries. Fish consumption itself is essential for the livelihood of 2.9 billion people globally, providing nutrition and animal protein and helping increase global food security. These fish species are also fished by local fishermen in developing countries, providing a subsistence food and income production. Overfishing for fish meal-sourced fish could further reduce food security for these people (Belton and Thilsted 2014; FAO 2014; Golden et al. 2016).

### **1.5.2 Alternative Fish Feed Ingredients**

There is a need for sustainable and locally available fish feeds that are inexpensive and/or accessible and which provide adequate nutrition to the target animals such that they grow, reproduce and remain healthy. However, there is the risk of competition for ingredients between use for feed or human consumption. There are two potential approaches to creating alternative

feeds that can limit, or replace, fish meal as a required feed ingredient. One option is to enable local fish farmers to create their own optimized feeds using 100% locally available ingredients, enabling them to move up rungs in the development ladder. This could be done by supplementing a portion of the fish meal with other protein components.

Another method would be to completely remove fish meal from the mix and replace it with a combination of the above, or other, ingredients. These may be able to be grown locally or be derived from redirected waste or by-products. Using locally produced ingredients that require little labor or capital could provide a comparative advantage to those in developing countries by lowering labor and input costs for aquaculture compared to those in developed countries. In addition, isolated rural regions could use the locally sourced feeds to farm fish without needing to find a way to access markets. Eventually, these farmers could enter the global marketplace and may be able to afford imported feed. They may also be able to sell locally produced feed or fish as exports both intra- and internationally.

However, there is limited information and research available regarding nutrient content of the alternative ingredients which may be available in developing countries and their correct combination to achieve acceptable fish growth rates vs. commercial fish feed. This dissertation endeavors to better understand the nutritional values and possible usable combinations of these materials. If a viable combination of ingredients can be defined and demonstrated, this could provide a source of food and income for local people. The alternative protein sources and other ingredients could be locally sourced since many options are easy to grow or obtain. The replacement ingredients require little space, water, and nutrient/feed inputs for production and these supplements can be used in other ways, such as human consumption, composting, or fertilizer. Looking further into the future, if they are successful, these ingredients may be able to replace fish

meal and other ingredients used in livestock production such as pig, chicken, or cattle feed, increasing the portion of the population that can move up the development ladder and ultimately increase the economic competitiveness of the country as a whole in the global market.

Another reason to investigate other ingredients for fish feed is to find food sources outside of the human consumption chain. Since it is important to conserve food for human consumption, ideal ingredients would be those that either are waste products with no other use or products found locally in the surrounding environment. Examples of waste products include banana leaves, cacao husks, coffee pulp, edible products not usable on the market, and sweet potato leaves. In addition, locally sourced products such as the leaves, bark, husks, seeds, pulp, or fruit of various plants or trees like *Cecropia* or *Ficus* could be used. Caution must be used if locally sourced ingredients are taken from the surrounding environment to not overharvest or affect the ecosystem. Perhaps in the future, these trees could be cultivated as well to reduce the impact on the environment while maximizing production.

## **1.6 Potential Alternative Ingredients**

### **1.6.1 Insect-Based Ingredients**

Using insect-based ingredients for animal feed is a concept dating from the 1950's and has increased in importance over time. Insects can be used for a variety of food security applications, including human consumption and as a component of animal feed for chickens, fish, and other livestock. Insects are also being studied for use in pet food. Using insects as a basis for feeds can help increase sustainability since raising insects consumes less space, water, and resources compared to other protein sources, increasing suitability for poorer farmers in developing countries. Nutritionally, insects have high levels of protein, polyunsaturated fatty acids, calcium, iron, and

zinc. Environmentally, they emit less greenhouse gases than livestock (methane is only emitted from certain species and only low levels of ammonia are emitted), require less land, and have an efficient feed-to-protein conversion rate (FAO 2013). For instance, house crickets require twelve times less feed than cattle, four times less feed than sheep, and half as much feed as chickens to produce equal quantities of protein (i.e., the cost/g protein is much lower). The feed fed to insects can include waste food products if it is sanitary and has enough nutrients/protein (FAO 2013). Innovation would help increase insect farming sustainability in the future and enable farmers to reach a further rung on the development ladder, just like other livestock production systems.

Over 2000 insect species are consumed globally, whether wild-caught or domesticated (FAO 2013). Some species that have been either traditionally consumed or more recently added to the menu include species in Hymenoptera such as black weaver ants (*Polymachis dives*), which are found in subtropical Asia, such as Bangladesh, Malaysia, and India (Shen, Li, and Ren, 2006), and black soldier fly larvae (*Hermetia illucens*). Other consumed species include those found in Orthoptera, most notably the house cricket (*Acheta domesticus*). This species is an ideal insect to use for farming as both nutritional composition, diet, and rearing mechanisms are known. The life cycle is 3-5 weeks, holometabolous, and can be maintained in a single container.

### **1.6.2 Previously Studied Insects**

Insects can be used as animal feed for a variety of livestock and species, including swine, poultry, and fish. For this project, the first objective is to create a fish feed for use in small-scale aquaculture systems in developing countries. Fish feed normally contains fish meal, and previous research has focused on using other sources of protein in fish feed as a fish meal replacement. In Malaysia, several researchers investigated the optimization of protein content of earthworms for catfish. They found that replacing 25% of fish meal with earthworm powder led to no negative

effects on fish growth. This was equivalent to the replacement percentage for chicken by-products and was greater than the feasible 5.95% soybean waste replacement demonstrated (Zakaria et al. 2012).

Another study investigated the use of 5% earthworm powder and 5% powdered silkworm pupae for chum salmon (*Oncorhynchus keta*). When the powdered earthworm was combined with fishmeal, the effects included greater weight gain, increased feed efficiency, and greater fat content than other treatments or controls. This indicates that adding earthworm powder to fishmeal provided a benefit for the salmon (Akiyama et al. 1984). Several other studies have shown that using up to 50% silkworm pupae (with or without fishmeal) do not cause a significant difference in growth or quality of the fish but could provide more protein and less fat than using fishmeal (Nandeeshha et al. 1990; Nandeeshha et al. 2000). Although silkworm larvae consist of 41% crude protein, while fishmeal consists of 68% crude protein, silkworm protein is more digestible than fish meal, allowing the fish to absorb more protein.

The success of silkworm and earthworm powder as fish feed additives also extends to black soldier fly larvae and crickets. Black soldier fly larvae may be used to replace up to 25% of fishmeal in fish feed without decreasing growth performance or lipid content (St-Hilaire et al 2007). Decreased growth may be related to digestibility or palatability issues with higher fishmeal replacement ratios. To increase palatability, one investigator fed soldier fly larvae two different feeds; fish offal or dairy cow manure. Fish fed larvae that had fed on fish offal appeared to be more palatable, leading to comparable fish growth rate, feed taste, and feed aroma versus fishmeal (Sealey et al. 2011).

It is also possible the chitin of the insect's exoskeleton reduces digestibility due to the absence of chitinase in the fish intestines. Because of this, black soldier fly larvae may only be able to replace a portion of fishmeal in fish feed. There were no significant differences in diets containing 0%, 17%, or 33% insect larvae in feeds, but higher inclusion rates displayed significantly slower fish growth rates (Kroeckel et al., 2012). Termites are also highly proteinaceous with *Macrotermes subhyalinus* comprising 46.3% protein. This termite species is common in Africa and may be suitable to replace up to 50% of fish meal in feed with higher mean weight gains, relative growth rates, specific growth rates, lowest feed conversion and protein efficiency ratios, lowest cost, and highest profit index (Sogbesan and Ugwumba 2008).

### **1.6.3 Cricket Use and Potential**

Field crickets *Gryllus bimaculatus* were tested at 0%, 25%, 50%, 75%, or 100% replacement for fish meal for African catfish *Clarias gariepinus* feed. Fish growth rates and protein use efficiency were comparable up to 50% replacement, at which point, these metrics decreased, resulting in the conclusion that up to 50% fish meal can be replaced with field cricket powder (Taufek et al. 2018). Another species in Orthoptera, the common house cricket (*Acheta domesticus*) is the most common insect species used for human consumption and feed research in the US and has the ability synthesize unsaturated fatty acids (such as C18:2 and C18:3) de novo, which fish require. (Beenackers et al. 1985; Blomquist et al. 1991). However, this species is more difficult to use in developing countries for projects and research since it is not a native species, leading to risks of invasiveness. Additionally, they are not well-adapted or productive for humid tropical conditions. Practically, they chirp much louder, affecting those that work in insect farms and requiring special buildings for noise reduction. Finally, they do not contain as much protein as some other cricket species.

To counteract these difficulties, the use of tropical banded house crickets (*Gryllodes sigillatus*), which are native to many tropical regions may improve production and research. This species has a higher protein content than temperate house crickets. They have high levels of lysine, isoleucine, leucine, and lysine. All amino acid levels were comparable to plant and animal levels, except for a low leucine concentration (Jonas-Levi and Martinez 2017). Based on these considerations, this species was selected for this research.

While temperate house crickets have long wings, tropical house cricket females are wingless, and males' wings are approximately 50% smaller than temperate crickets. This may allow more nutrients to go towards egg and muscle production, resulting in larger crickets. For another species of cricket (*Modicogryllus confirmatus*), it has been found that de-winged longwing females and short-winged females produce more eggs and can produce eggs even without food consumption after maturity (Tanaka 1992). This is due to the use of energy reserves for egg production instead of thoracic muscle formation for flight. If the same concept would work with tropical banded crickets, then the lack of wings in females and shorter wings in males might allow for better nutrient retention and egg production, increasing nutritional content and weight of crickets as feed. In addition, a benefit of fewer wings with tropical banded crickets is the reduced indigestible chitin in the wings.

A fish farmer's willingness to use alternative ingredients is often overlooked. For example, if they do not understand the nutritional value of insects, they might be unwilling to use the insects as feed ingredients. In Uganda, 94.9% of farmers surveyed would willingly use insects for feed, but only 44.8% had ever done so (Ssepuuya et al. 2019). Farmers who knew the nutritional value were more willing to rear or buy insects than those that did not. Additionally, training and



promoting large-scale insect rearing would increase the use of insects as fish feed (Ssepuuya et al. 2019).

#### **1.6.4 Plant- and Algae-Based Ingredients**

Plant-based ingredients have been studied more extensively than insects in feeds, but knowledge is still relatively limited. Soybeans have been the most commonly studied crop. However, globally, there is a wide range of unexplored plant-based resources that are normally regarded and treated as waste. These resources include vegetative growth, fruit or vegetable rinds or non-edible parts, seeds, and seed pods. While these components are generally considered waste and either fed to animals, discarded, or burned, they could be used as feed ingredients for farmed fish or insects.

For example, if a feed was created specifically for fish farmers in Guatemala, the ingredients could be sourced locally. Advantageously, Guatemala has ecosystems and species compositions like other countries in Central America, such as Honduras, ensuring feed design would be applicable across multiple countries. This allows for a low-cost ease of transfer of technology and methods between countries, increasing the reach of the research. Guatemala contains many ecosystems such as dry forests, deserts, rainforests, temperate forests, coastal mangrove forests, coastal bays, and cloud forests. Each ecosystem has its own plant composition and climate, providing great variety in potential ingredients. For example, rain trees are one of the most common species in the dry forests. These nitrogen-fixing trees are in the leguminous family Fabaceae, which produce dry, dark-brown pods. These pods range from 6-36 cm long with a tacky endosperm surrounding the seeds. The endosperm is edible to humans as well as animals. However, it is currently not used as a food or feed source and pods are left for native birds to consume or to decompose, making these resources potential feed ingredients.

### 1.6.5 Ingredient Replacement

Availability, nutritional content, economics, and feed efficiency are factors to consider when choosing alternative feed ingredients. The ingredients must be found locally, either naturally or as cultured resources. For optimal use in feed formulations, these resources must be analyzed to determine nutrient content compared to fish nutritional requirements. Once local ingredients are identified and analyzed, feed can be formulated with these ingredients and evaluated through feeding experiments to determine how palatable, digestible, and utilizable it is to fish. The feeds that promote fish growth can be economically analyzed with cost-benefit ratios and profitability.

In Benin, fish farms currently use imported commercial feeds, but with higher costs (Adeyemi et al. 2020). In this study, three types of feed were used: imported, local feed to complement commercial feeds, and whole local ingredients (i.e., *Moringa* and maggots). Local feeds can help reduce costs but require ingredient identification. An analysis identified both locally available plant and animal protein ingredients. Ingredient sources were ranked according to availability, cost, and nutrient content. Cottonseed meal, soybean meal, cereal bran, maize meal, cassava, and palm kernel cake were the most abundant ingredients. However, there was also trash fish, oyster shell, poultry viscera, snail meal, tapioca, and lafun. The researchers identified the ingredients according to nutritional category. The most promising ingredients for carbohydrates were tapioca, cassava, and lafun and, for proteins, trash fish, soybean, and cottonseed. Oyster shells and snail meat provided ash, or minerals. Other local ingredients fed as-is included *Moringa* leaves, maggots, and cassava leaves.

## **1.7 Development Research Approach**

### **1.7.1 Development Process**

As discussed above, development is a process that requires innovation relevant to the local economy, resources, and culture, which then leads to progress in economics, business, technology, and agriculture. New techniques and/or products enable producers to make step changes in the efficiency of their production and the range of things they can produce, including higher value-added products. This phenomenon is also true in agriculture. For example, there is a current limit to a farmers' ability to specialize due to diminishing marginal production, or the additional benefit from one more product being produced. The only way to move the production frontier outwards (greater production with the same inputs) is to incorporate new technology/innovation. This may include new techniques such as zai holes in arid soils, using irrigation or fertilizer, or using new crop varieties. In addition, another component of development is the transfer of existing technologies to the farmers unaware of such technologies, at a specific step along the development ladder. Transferring a given technology too early could result it in being locally unrepaired or refueled due to lack of finances or knowledge. However, transferring too late, or not at all, results in a potential loss of income, food security, and production. As with most successful development projects, agriculture development efforts must be tailored to the local needs, abilities, and markets. Further, they must be implemented in a manner that provides enough training and support to maximize the ability of the local farmers to sustain the new approaches without continued external support.

### **1.7.2 Development Research vs. Basic Scientific Research**

Scientific research involves analyzing the effects of variable changes in a manner that should allow for differentiation of these effects. However, this is a lengthy process, something that can be counter-productive in development work. In development work, whether agricultural, economic, or other, there is a sense of urgency due to the immediacy of food needs, estimated population growth to 9 to 12 billion people by 2050, and resulting demands on food supplies. This requires larger agricultural output, more water use, greater economic growth, and more land use. Improvement and innovation are necessary now to prepare for the future. This also requires research that is innovative and different from current research methods, which rely on single variable testing. For this fish feed research, while it would be optimal to test each ingredient individually and its effect within the feed on fish growth, it would take years to evaluate every possible alternative fish feed ingredient that could be used in a developing country. Any improvement in a locally producible fish feed would be better than no improvement. This means that even if the analyzed new feeds are less effective than commercial fish feed, yet better than what may be currently used, that would be significant result and may be economically attractive. The feed could subsequently be further improved through systematic optimization of ingredient ratios and types.

Where this method departs from typical scientific methods is the changing of different variables concurrently. This makes it difficult to know exactly what effect each variable has on the dependent variables. While Experiment 1 was based on typical scientific methods, i.e., single variable ingredient changes, Experiment 2 leveraged a more development-focused approach. Linear programming in Excel was used to test models for feed using nutrient values from the different ingredients. These can be put into software to calculate percentage of ingredients used in the feed to provide all the nutrients and protein the fish will require. One can then shift the ratio of

new ingredients after making an initial recipe using known nutritional content. This means one can have different ratios of ingredients without testing only a single variable. This approach proposes a balance between scientific method and development urgency. Even if the feed is improved to, for example, 80% efficiency compared to commercial fish feed, this may be better than having no feed at all in an unmanaged system. Often, either the commercial fish feed is too expensive or inaccessible. Instead, either people just let algae form naturally in the ponds without additional feeding or do no fish farming at all, greatly reducing the potential yield. In this case, an alternative, locally sourced feed would help improve fish growth, output, protein, and profit. This might encourage impoverished people to begin aquaculture for both food and income.

Development projects, by their very nature, generally involve more risk than anticipated by many individuals involved with them. These risks include insufficient or inaccurate knowledge from the experts, project design mistakes or failures, unexpected problems from internal or external sources, failure of techniques or data to transfer to individual projects, failure of long-term sustainability, or cultural disorientation. As development itself is inherently risky, it is logical to expect research in developmental settings and/or for development objectives to involve risks as well. For example, if an experimental fish feed is not 100% as effective as commercial fish feed, slower growth rates or reduced survival may be experienced. However, as long as the less-efficient fish feed is able to increase production compared to the absence of feed (at sufficient levels to reach the break-even line), there would be a benefit to use the fish feed - i.e., the inherent “risks” are justified from a development perspective.

### **1.7.3 Knowledge Gaps**

Individual ingredient sources such as insects, worms, larvae, algae, soybeans, and nuts have been previously researched. These materials or ingredients are generally studied separately in

either direct food form or as a replacement of, or addition to, fish meal in fish feed. For some of the potential “waste” ingredients discussed herein, there is no available nutrient content and they have not been used in any published feeding experiments. Additionally, ingredients from Guatemala have yet to be identified or nutritionally analyzed for potential fish feed sources. Other gaps related to alternative feeds include how to formulate feeds based on nutrient content of ingredients, the impact of these feeds on fish growth, and the economic viability of the feeds. The objective of this dissertation is to address these knowledge gaps and encourage future work for local feed development in Guatemala.

In Chapter 1 of this dissertation, the development ladder, and how it can benefit rural households in Guatemala, will be discussed in more detail, including steps for aquaculture development (i.e., local feeds). In Chapter 2, Experiment 1 evaluates the effects of tropical banded cricket meal substitution of fish meal in fish feeds, using Nile tilapia (*Oreochromis niloticus*) fingerlings. Experiment 2 identifies local ingredients found in Guatemala, provides a nutrient analysis of the ingredients and the feeds formulated from them, and provides results of testing the feeds on Nile tilapia fingerlings. Chapter 3 provides an economic analysis of a small-pond intensive aquaculture system in Guatemala, based on an exemplary tilapia project in that country, to determine at what point are locally produced feeds are economically feasible compared to commercial feeds. Chapter 4 identifies via GIS ideal locations in which to focus aquaculture development in Guatemala based on variables such as watershed distance, land area, climate, and access to the market.

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## **CHAPTER 2. - THE DEVELOPMENT LADDER: AN INTERMEDIATE APPROACH TO DEVELOPMENT**

### **2.1 Objective**

The objective of this dissertation is to provide potential next steps in the development process, or the next rung on the development ladder, through investigating locally sourced fish feed as an intermediate step of intensifying household aquaculture. This dissertation focuses primarily on the agricultural section (and, specifically, aquaculture) of the development ladder for households, and, more particularly, in enabling households to more readily obtain critical nutrients and/or income. For this specific research, the target rungs include achieving market participation and self-sufficiency as shown in the conceptual ladder depicted in Figure 1.1. Subsequent rungs to which households could progress include interactions with neighbors, villages, regions, countries, continents, and the global market. This chapter provides a background on development, how the role of agriculture plays in development, and focuses on the definition of a development ladder, including how it specifically applies to agriculture and how it differs versus other development approaches.

### **2.2 Development**

#### **2.2.1 Basics of Development**

The first definition of development is the historical progression or modernization of a country or society, with a sequence of events or improvement brought on by human activity (Kingsbury et al. 2016). Huntington (1971) defines modernization as a sequence resulting in increased control over social structure and natural environment with the help of improved technology and scientific knowledge. The primary driver of this definition was Rostow who



proposed an economic development process wherein a society initially develops from a traditional society with most of a population involved in agriculture, limited production functions and pre-Newtonian technologies, then to a transitional stage where pre-conditions need to be met (Rostow 1960). These pre-conditions include an increase and creation of social overhead capital (mainly in transportation), improvements in agricultural technology that increases agricultural productivity, and expansion of imports. Once these conditions are met, the society enters the take-off stage where the manufacturing industries have a high growth rate, productive investment reaches 10% of the national income, and improvements in the social and political framework. The fourth step is the drive to maturity, including a more skilled workforce, urbanization, real wage increase, economic security, and industrialization. The last step is the post-maturity phase where the nation grows in power and global influence, the government seeks to achieve a welfare state, and commercial centers of cheaper technologies and goods are developed. At this stage, mass consumption of goods and services occurs, and leisure is more common.

A more pessimistic definition of development views it as exploitation or domination of developing countries by developed countries. In this case, poor countries would be considered underdeveloped, rather than developing, since richer countries have deformed the development process of the underdeveloped countries. This perspective claims global trade and foreign aid keep the countries in a locked-down state of development, meant to be subservient to the richer countries. This definition can also be called the dependency theory, where the rich powerful core countries exploit the poor peripheral countries, forcing the periphery to be relegated to a role as only a supplier of primary products through the imperialistic international economic system, rather than having their own industrial and economic development (Frank 1967). Any foreign aid, investment, or knowledge transfer would be used to increase the core countries' gains from the periphery rather

than help develop the periphery. This perspective is the main driver of opposition to implementing technology and techniques in development projects.

A third position defined development as a method to achieve conditions allowing the “*realization of the potential of human personality*” (Seers 1972). Reaching the potential of a society (or component thereof) requires sufficient food security, which is connected to income from jobs as well as equality. Progress in this development would be a decrease in unemployment, income inequality, and poverty (Seers 1972). This conceptualization led to the International Labour Organization to create a guideline for five basic human needs: basic goods (food, housing), basic services or public goods, role in decision making, basic human rights, and productive employment to earn sufficient income to consume at desired levels (ILO 1976). The United Nations Development Programme (UNDP) shifted the focus from economic to human development, defining the new concept as progress in improving and growing people’s choices. The critical choices, as defined by the UNDP, lead to good education, healthy lives, and acceptable living standards (UNDP 1990: 10)

Amartya Sen defines development as freedom from poor public services and goods, tyranny, poor social status and inequality, and poor living standards and economic position (Sen 1999a). In Sen’s *Development is Freedom*, he writes about “agency aspect” as one of the roles of freedom in development. This phrase refers to a person’s ability to help themselves and improve their position or situation (Sen 1999). This includes influence in politics to achieve better standards of living. Sen’s definition also includes the ability of someone to create change by acting and where successes are evaluated by the person’s own values and goals. The “agency aspect” of individuals in a nation may be disproportionate, with those having more political power owning more “agency” than those with less power (Sen 1999). According to Sen, development or “good

change” helps reduce restrictions that prevent freedom. Political power could be affected by income, proportion of population, or geographical proportion.

Survival ethics theory focuses on development meeting the most basic needs first before addressing ethics and reasoning behind development (i.e., virtue ethics, utilitarianism, colonialism, or guilt) (Verharin et al. 2014). Before debating the reasoning behind development, the priority is to ensure the continued existence of the target group. The principle is “to be good is first to be” (Verharin, *et al.* 2014). The basic needs to be met before determining the correct development approaches include shelter, access to clean water, education, healthcare, temperature control, clean air, and adequate food. It does not ignore a community’s rights and identity; it views these rights as including the aspects of survival (Verharin et al. 2014). A community cannot flourish and evolve its culture and identity without existing or achieving basic survival. In survival ethics, the desirable approach is to create teams of wide-ranging experts, such as water engineers, agronomists, anthropologists, and doctors, which work to meet the needs of communities. Alongside the teams, evaluation and monitoring from the team and universities provide assessments of efficacy (Verharen et al. 2011)

### **2.2.2 Role of Agriculture in Structural Transformation and Development**

Agricultural transition is integrated with structural transformation in the development process of a country. Changes in farm numbers and size should not be feared as it is a natural part of a country’s development. Similar to the first stage of Rostow’s economic theory, countries begin in as an agrarian economy with more than half the population living in rural areas and agriculture representing up to 90% or more of the economy (Tomich, Kilby, and Johnston 1995, Newman, Singhal, and Tarp 2020). Many small farms exist alongside abundant rural labor with each farm sharing a small part of the food production profit. The competition between farmers, coupled with

the small land area each farmer uses, results in some competitors failing (not making a profit) and either children or entire families move to larger cities to find urban work as industry and services surpass farming in impact on GDP (Tomich, Kilby, and Johnston 1995, Norton, Alwang and Masters 2006, Liu et al. 2020, Newman, Singhal, and Tarp 2020). Both day laborers and members of farmer households move to these cities with the incentive of a higher-paying job since, at this point, farms would produce less income than urban jobs (Klliesen and Poole 2000, Liu et al. 2020). This leads to land consolidation and larger farms, allowing remaining farmers to increase their share of the profit and have a higher income that begins to be competitive again with the other economic sectors' incomes, sometimes surpassing them and drawing some labor back to the agricultural sector (Gardner 2000, Newman, Singhal, and Tarp 2020, Liu et al. 2020).

Even with the decrease in agricultural labor and the agricultural share of total exports, agricultural output and value has increased (FAO 2007). At this point, agriculture undergoes a shift where differentiation and specialization occur, ranging from new techniques (i.e., row crops, crop rotations, monocultures, irrigation) to new agribusinesses related to agricultural inputs, food processing, and export (World Bank 2008).

This structural transformation could be the result of various factors. One is related to Engel's law and Bennett's law. Engel's law states that as income increases, demand decreases more for agricultural products than for other goods. Bennett's law states that increases in income lead to variable demand changes so that demand for high value products increases more than for basic staples like cereals, sugar, and eggs. As income increases, demand for variety and diversity in food and goods increases. Wealthier nations spend a smaller share of household incomes on food (from 80% to 20% or less) (De Hoyos and Lessem 2008).

Another explanation is the development of new technology: both farm and non-farm. For on-farm technology, there is a concept called “Cochrane’s treadmill”, proposed by Cochrane in 1958. This concept begins with farmer adoption of new technology, increasing yield, but also farmer debt. Higher yields can lead to overproduction and declining crop prices, causing farmers to lose money and continue to overproduce (Cochrane 1958). The final result would be the departure of the least-efficient farmers to enter non-farm jobs. Even though this concept can lead to excess supply, lower crop prices are important for reducing food cost shares of income while overabundance, combined with international trade, ensures global food security in terms of both food quantity and affordability. The non-farm technology results in drawing rural farm labor to migrate to urban areas, reducing the numbers of laborers and farmers and increasing the income of those that remain (Liu et al. 2020).

The last explanation, with the largest contribution, is the limited amount of arable land available. As mentioned above, the more numerous the farmers, the smaller the average farm size becomes, which then decreases income per acre and farmer. This then pushes the less competitive or efficient farmers out and into non-farm work (Liu et al. 2020).

### **2.2.3 Development Actors**

International and community development covers a complex web of targeted sectors, ranging from health, environment, food security, and political power. As diverse as the actions performed to help improve development, there are just as many development actors, including implementers, policy-makers, donors, and researchers. Government organizations provide large portions of the funding used in development (i.e., USAID), but private donors are becoming ever more influential and essential to development. International NGOs (INGOs), local organizations, and universities also function as important actors in global development efforts.

INGOs are key implementers of development projects, working alongside government organizations and foundations. They also have the freedom to help with power structures in other countries. Governments and politics of developing countries are essential to the process of development, but there can be limitations and constraints that reduce their effectiveness to drive effective and balanced improvement. Due to inefficiencies, corruption, or unequal power distribution, certain populations might be ignored in policy-making or implementation. INGOs can take the role of advocate in developing countries and help provide a voice for the underserved population.

One economic principle relevant to development is referred to as the Pareto equilibrium. This idea was created by Pareto and revolves around efficiency and increasing the size of the economic pie (Lau, Qian, and Roland 2000). Pareto-improving reforms or policies are initiatives that seek to increase efficiency and economic growth. The economic pie should grow and there will be those who experience a benefit (winners) and those who experience a net-zero or negative outcome (losers). For Pareto equilibrium, winners would compensate the losers sufficiently so that the losers have no net losses (remain at the level they were at previously) while the winners are still better off than before (Lau, Qian, and Roland 2000). This allows for overall economic growth without creating negative effects for the losers. This may be a viable way to approach public policy and advocacy.

When an organization or individual advocates for a perspective or side, there will most likely be another side of the story for those who may be negatively affected by the targeted policy change. In this situation, opposing sides could conflict with each other, hindering progress and potentially scaring off potentially desirable government action. Conflict may delay action or even sway action the other direction from the advocate's desired goal. This conflict, sometimes caused

by opposing INGOs, can create losers and winners, if any change can be made at all. Instead of fighting each other, perhaps INGOs should seek a compromise or negotiation. Although there will be losers regardless of which side wins, perhaps the INGOs can ensure losers are compensated in a Pareto-improving reform. This can be economic or outside of economics, like social policies.

#### **2.2.4 Approaches to Knowledge Transfer**

The first approach to economic development focuses on embracing every aspect of the target's indigenous or cultural ways. In many situations, introduction of external resources (i.e. knowledge, technology, or access) is condemned because of past failures and unrealized expectations. The idea that Western imported development projects have failed to reduce poverty and address basic needs has led to a focus on building on local technology and knowledge, using only indigenous knowledge and resources in development (Gooneratne and Mbilinyi 1992, Burkey 1993, Binns 1994, Binns and Nel 1999).

Another approach is merely to help households reach subsistence-level, using only local materials and production, regardless of price or profit changes in resources and goods which can increase costs (Rahul 1997). Some local materials might be more expensive locally compared to global prices, increasing costs or reducing profit. This subsistence state may be dependent on traditional seeds, methods, or technologies. Both approaches focus on activities with low technology (i.e., subsistence farming) and usually discourage or actively oppose the idea of more modern methods or technologies. This does not address the needs of the target group (i.e., survival needs and need for accurate information for decision-making). With the desire to advocate for the indigenous ways of the target group, the development actors may spread incorrect or false information about new technologies and techniques. An example is GMO seeds where INGOs, unknowingly or not, misinform the people about these seeds, claiming they are harmful and pushed

by companies to “enslave” the farmers (gmwatch.com). Instead, the INGOs encourage indigenous methods of seed saving via their false arguments against GMOs, limiting farmers’ abilities to make informed choices based on their needs. This leads to a continuous cycle of poverty and food and economic insecurity.

Another issue with maintaining low technology for agriculture and other development activities is the acting pressure of external technological progress and internal pressure of competition that lead to local or indigenous groups having their own technological development (Lall 1993). Even among smallholder farmers, there is competition for seeds and other inputs, market sales, and even in farming technique. This leads to competitive advantages for a subset of farmers and an “arms-race” of skills acquisition, training, innovation, and variability (Lall 1993). The internal need to feed one’s family triggers farmers to improve and increase crop production. This might mean trying the same method repeatedly, with or without success, or innovating and using a new techniques, tools, or technology. Even without “Western development strategies”, there is pressure to improve, just differing in the scale and speed of that improvement. Providing training, education, and demonstration of new technology (at least new to the target group) helps reduce farmers’ perceived risk of experimenting with new approaches and techniques.

Technology development interacts with economic, social, and agricultural development, helping to support progress (Huntington 1971). Another, more extreme, method is to attempt to drive maximum improvement through a significant leap in technology. This may include providing modern technology such as tractors. However, households, or even communities, in developing countries are often not be able to maintain the technology or even understand or utilize the advantages of such technologies. In other cases, the technology may not even be appropriate for the conditions in the farmers’ fields (e.g., tractors cannot function on the very steep mountainside



maize fields in Guatemala). Other development methods aim for households to enter global market trade immediately without considering the feasibility of implementation or the process. This may lead to unprepared households lacking understanding or motivation to participate in trade-or an understanding of market logistics and government regulations. Local families may not know to produce more specialized goods to trade for more preferred goods, such as value-added goods, commodities, or cash crops (i.e. coffee, cacao), to maximize utility. This could cause farmers, for example, to produce too few of the specialized crops versus a less valuable crop, leading to insufficient income and food availability.

An intermediate method, and the closest to the proposed development ladder approach below, is a technology transfer model that combines understanding the knowledge of the provider and the capacity, needs, and desires of the recipient while minimizing the need for long-term foreign aid (Chege and Wang 2020). The objective of technology transfer is to help improve the capability, skill, human resources, and productivity, reducing the gap in technology between developing and developed countries (World Bank Group 2017). This transfer of technology can help small businesses such as agribusiness (i.e., household farmers, processors, marketers, sellers) improve efficiency and performance, while helping with development efforts in rural regions (Chege and Wang 2020). It increases their competitiveness both in the regional and global market.

To further boost food security, governments can promote agricultural technology transfer through incentives (Chege and Wang 2020). Regardless of approach, the inclusion of the target group's voice and participation is not only essential to long-lasting success of projects due to their ownership of the project, but also helps meet the actual needs and priorities of the target group. The target group needs to be informed of the possibilities and be given a visual demonstration of the possible techniques or knowledge. They may not be able to envision the potential next steps to

meet their needs without these demonstrations and it broadens their choices as well. An example is a demonstration farm where different irrigation methods, seed types, and planting methods are shown so the farmers can see for themselves what the possibilities are for each approach.

### **2.2.5 Factors Affecting Success and Failure**

Development work focusing on technology adoption faces challenges and there are many examples of failures. Development projects fail for a variety of reasons, especially in agriculture, which entails higher risks for farmers (i.e., the risk of failure and lack of food and income for the household). These include issues with the design phase of projects, such as failing to meet the actual need of the targets (rather than a perceived need), the perception the technology is not valuable, the lack of ability to manufacture or use the innovation (e.g., lack of training, no access to fuel for a tractor, etc.), or if the innovation is culturally inappropriate (Gilliam, Nassar, and Mehta 2014). Finally, the innovation may be too complex for set-up, use, or repair.

For the implementation phase, farmers may not have access to capital or credit for adoption or continuation (i.e., not able to refuel or repair) (Gilliam, Nassar, and Mehta 2014). There may also be legal issues related to lack of recognition by the government, or the pricing may be beyond the negligible discretionary budget farmers may have (Gilliam, Nassar, and Mehta 2014). Other factors include poor customer education and demand, quality control issues, and gender dynamics, where women are the main agricultural producers but do not handle the household finances (Gilliam, Nassar, and Mehta 2014). For the final maturity phase, challenges include continued innovation, building supply chains, marketing, competition, standardized operation processes, legal aspects, and management of the innovation (Gilliam, Nassar, and Mehta 2014).

Failure may also occur when development workers do not recognize the complementarity of different innovations (Feder, Just, and Zilberman 1985). For example, the output of high-yielding crop varieties is maximized when fertilizer is used since they have been developed to be especially responsive to the fertilizer (Hiebert 1974). Introducing only one of these technologies prevents realization of the full potential benefit and can result in a lack of adoption since the additional cost of innovation is justified.

Another source of failure stems from the perspective that technology adoption is dichotomous: it is either adopted or it is not (and therefore considered a failure) (Feder, Just, and Zilberman 1985). In reality, there is typically a gradient of adoption where the degree of adoption intensity varies over time. Examples range from slowly increasing fertilizer use, using high-quality seeds on only part of the farm, or using aeration in a tilapia pond only during a portion of the day. This relates to the development ladder concept described later in this chapter. Adoption gradation, or the development ladder, encourages households to use innovations when, and to the degree, their resources can support (i.e., time, labor, income, access to inputs). Additionally, variable prices of inputs and outputs can affect profitability of different innovations (Falcon 1977, Feder, Just, and Zilberman 1985)

Successful development projects are also driven by several factors. Farmers measure performance differently and less precisely than do researchers and may accept small gains for less inputs rather than maximized gains. Usually, they underutilize a new technology or compromise with a less advanced technology that produces sufficient production rather than maximized production (Razanakoto et al. 2018). For example, farmers in Madagascar applied the introduced phosphorus fertilizer at a lower rate than instructed and sometimes only after the organic material

ran out (Razanakoto et al. 2018). This was due to the difficulty farmers had in precisely measuring the field and the fertilizer, as well as their expectation of seeing large improvements with minimal input use.

Several factors influence technology adoption, beginning with the level of comprehension farmers have about the introduced innovation. The greater the understanding, the greater their incentive is to invest in said innovation and proceed to the next step of adoption (Razanakoto et al. 2018). As development projects are temporally constrained, there is tension between helping farmers see the advantage of the innovation and the long innovation process of internalization. This results in both social and opportunity costs (Harnecker et al. 2012). Another success factor is related to allowing the farmers to test the innovation themselves on their own land and the potential resulting profitability, since they are often not persuaded by expert opinions (Razanakoto et al. 2018).

Innovation trials encourage farmers to evaluate the technology and accept the risk temporarily. True adoption after the implementers leave may be limited due to farmers' financial concerns, however. Farmers may be interested in a technology but may not be willing to internalize and invest in it long-term since success is not guaranteed. The incentive to adopt is balanced in their minds by the risks involved (i.e., disease, pests, drought) and farmers often do not prioritize investing (when they have the means) in agriculture (Razanakoto et al. 2018). The target audience, risks, negative impacts, and possible failures or harm should be understood when development work is implemented. Many projects focus on one input or technology without considering other factors affecting food security. Instead, farmers understand the range of factors and are more motivated to adopt when multiple variables are addressed (Razanakoto et al. 2018).

An example of a failed development project is the SODESP project in Senegal (*Société pour le Développement de l'Elevage dans la zone Sylvo-Pastorale*). Ranchers from the United States alongside anthropologists attempted to implement the latest herd-culling techniques among nomadic indigenous cattle herders to promote healthy herds, focusing on herd quality and environmental health (Nolan 2002). However, the project failed due to the experts not understanding the culture. For the cattle herders, quantity was more valued than quality and they focused on maximizing herd size. Larger herds increased prestige, wealth (cattle were equivalent to money), and hedges against disease, drought, and famine (Nolan 2002). This resulted in non-adoption of the approach and project failure.

One dilemma in introducing new technology or techniques is the further advancement of competitiveness and comparative advantage, leading to the technology adopters becoming increasingly efficient. This can lead to the early adopters gaining an income advantage and helping them acquire additional resources, land, and wealth, eventually changing landholder patterns (Feder, Just, and Zilberman 1985). Development project planning must take land and income accumulation changes into account.

The development ladder theory may increase adoption of technology through gradual demonstration and trials. Having step-wise trials and demonstrations would help farmers to see the incremental benefits of additional inputs or technologies, while reducing real and perceived financial risk. This helps with internalization of the innovation among farmers. The theory can also address a target's understanding of different factors affecting food security, rather than focusing on a specific factor (Razanakoto et al. 2018).

## 2.3 Development Ladder

Both extremes of development efforts, inaction and large-scale government-led programs, can fail to drive the intended improvements for large groups of individuals and communities. I propose an intermediate approach which may be called the “development sequence” or “development ladder” (Figure 1.1) that describes a step-wise process of development and may inspire design and implementation of interventions truly relevant to real people in their own unique situations. This ladder may have a nearly infinite number of rungs, each of which represents a state of being for a “household” that includes agriculture, living situation, economics, and social interactions. “Households” in this context refers to one or more people who live and eat together in the same building and usually have one source of income (i.e. most often, a family unit). Each rung includes the household’s income level and economic situation, access to market and transportation, and agricultural capability and resources. For the household’s agricultural situation, many aspects may be taken into account, including type of agriculture (e.g., cereals, row crops, horticulture, orchards, aquaculture), agricultural inputs (i.e., fertilizer use, irrigation, technology), mechanization, crop and seed types, planting methods, local or regional supply and demand, the global market and prices and other agriculturally relevant variables.

## Development Ladder



Figure 2.1 Conceptual Development Ladder

The development ladder's rungs combine to provide a sequence of manageable steps that a household (or, to a larger scale, a community or even country) can take to reach a new level or rung. Depending on what assets or capabilities the household has, the household may be able to climb only one rung at a time or may be able to leapfrog ahead to a much higher rung. This means that development (agriculture-specific) is a process that is dependent on current conditions, but is also flexible in scope and timing, as well as susceptible to external stimuli (i.e., correctly targeted developmental efforts). Assuming a development actor can evaluate the rung a target group occupies, this approach to development increases the likelihood of introducing knowledge or technology that is usable by the target group since it would be more relevant and within their resources to use. It also allows the target group to have autonomy to choose what steps they want to take in their progression up the "ladder", if any. This takes a step back from the idea of

Westernized development strategies, often considered colonialism in nature, being forced onto local people, and provides an opportunity for their own decisions and voice to be heard.

Some development efforts, such as infrastructure development, by their very nature may support movement up the development ladder, regardless of a household's current "rung". Economic aspects of development such as road development interact with social development by increasing access to urban areas, governments, and services. This opens a path to increase power and "voice" due to increased presence in the social sphere through reduced isolation. Isolation can cause governments and authorities to forget or ignore those people, especially if the urban population is unaware of their plight.

Development is not an event that occurs instantaneously, but one that, as the word 'process' implies, requires time, energy, inputs, and commitment. For agriculture-specific development, the long-term objective of the ladder could be merely household food and economic security, or it could be to reach a point of maximum production. Essentially, the ladder has infinite rungs as it is difficult to designate an ideal endpoint for development for a given target group. Further, innovation will continue pushing the production possibilities frontier out, lengthening the ladder and the total potential production output. However, for this paper, the objective of agriculture development is to reach global food security for the estimated population of 9-12 billion by 2050, allowing for sufficient nutritious food at affordable prices. To provide a measurable goal using the development ladder, the long-term goal for development projects is optimal production, where a maximum output occurs with minimum or balanced inputs.

However, this large-scale development process can be divided into sub-ladders or sub-processes focused on varying disciplines or economic activities. The rungs of these ladders are more flexible and interchangeable than those of the main ladder and the order depends on the



specific economic and geographical situation of the household. This creates a dynamic progression in development and can depend on cultural values, resource constraints (e.g. land, inputs, income), and geographical location (e.g. climate, distance to markets, topography).

As an example, consider a hypothetical Guatemalan family whose main livelihood is agriculture. There are a variety of sub-ladders in which they participate, such as education, healthcare, transportation, and, of course, agriculture. Table 2.1 provides a selected portion of the potential rungs for each category.

Table 2.1 Development Ladder Rung Examples

Rung No.	Transportation	Healthcare	Agriculture	Education
Rung 5	Buying a vehicle	Hospital	Diversification/ Selling in market	Higher education
Rung 4	Buying a motorcycle (a common, inexpensive vehicle)	Small local clinic	Irrigation	Apprenticeship
Rung 3	Buying a horse	Traveling medical care	Fertilizer input	Secondary school for boys
Rung 2	Renting a Tuk-Tuk ride	Medical trips to villages	Hectare of maize or beans (no inputs)	Basic primary school
Rung 1	Walking	Pharmacy in the market		

Each of these categories affects the others, either positively or negatively. For example, if a child obtains a complete education, they could get a higher-paying job and send money back to

the family, which increases access to inputs for agriculture, transportation, or healthcare. If the family can produce adequate food and then sell the excess, they might be able to afford more education for the children or better transportation. If they can access better transportation, they could get to better medical clinics or sell their crops in bigger markets to make a larger profit. Of course, better transportation does not matter if the roads wash out in the rainy season and access to healthcare is dependent on quality care being within the family's transportation's range. Some variables are external or exogenous to the family and are the responsibility of government, INGOs, or for-profits who will improve these variables (i.e. road development, hospital construction, medical training).

One important sub-ladder would be an agriculture-specific sub-ladder, focusing on crop production (Figure 2.2). This ladder would be used for households to increase crop production and living standards and would include rungs such as irrigation, fertilizer, better seed varieties, crop diversity, and better planting strategies. For this proposal, an aquaculture-specific sub-ladder was developed, and is described in detail later in this chapter, that could be vital to sufficient protein production for households (Fig. 2.3, Table 2.2). The rungs include aeration, local fish food, formulated fish feed, improved fingerlings, breeding, mono-sex populations, value-added processing, and market participation as a household would progress up the ladder.

## Dynamic Agricultural Development Sub-Ladder

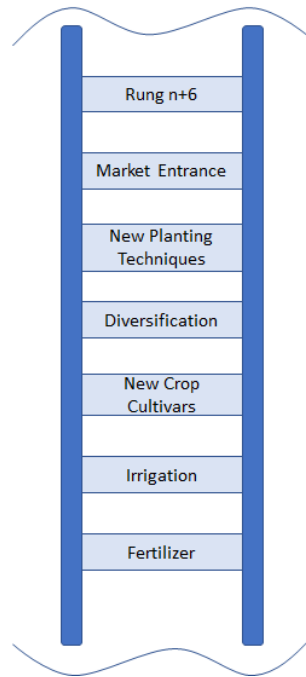


Figure 2.2 Conceptual Dynamic Agricultural Development Sub-Ladder

Households are subsistent if the income from its production are lower relative to its consumption needs, putting it at risk of failure and starvation and limiting consumption to what can be afforded (Pagiola 1995, Sibhatu and Qaim 2017). This is often considered and encouraged as household self-sufficiency. A subsistent household does not meet dietary diversity needs (Sibhatu and Qaim 2017). However, true self-sufficiency may come not from isolation, but from some interaction with markets (Pagiola 1995, FAO 2015). It occurs when the consumer or household is able to trade a portion of goods in order to obtain other goods it needs or wants to reach the optimal utility curve, or what it would prefer to consume rather than what it is limited to from farming (FAO 2015). If production is equal to or greater than consumption (i.e., sell the surplus), then self-sufficiency is met. When households enter the global market, they realize an initial improvement in economic welfare, but also nutritional welfare since purchased foods are

more important for dietary diversity than on-farm subsistence production (Sibhatu and Qaim 2017). This is also the point at which consumption equals production. Each household makes input and purchasing decisions based on what bundle of outcomes (i.e., maximized output or profit) or goods (ex. buying some corn and beans) that helps them reach that greatest utility or most satisfied. There may be multiple decision ratios that could bring the same level of utility. These choices exist in the production possibilities frontier: a range of choices that have equal utility.

As a country develops, not everyone can work as a farmer or produce everything they need on their own land. An agricultural surplus is necessary for the development of a nation to occur in order to provide enough food for non-farm urban workers as well. Continued development of the country also provides farmers access to new prices for buying and selling of food and can participate in trade. One of the benefits of having access to the market is the ability to sell to more consumers, regionally and, sometimes, worldwide. However, broader market participation also brings more competition, which means producers using the market need to have efficient production to lower costs and prices. This is especially true with respect to aquaculture, particularly in developing countries. Fish farmers in these countries generally are not even self-sufficient fish producers as they need to buy other goods, such as other parts of their diets like beans, grains, or meat (net buyers). However, the question is whether these fish farmers can become self-sufficient or even enter the global market both domestically and/or via export. How far-reaching can these farmers become and what benefit is there in participation in these markets?

### **2.3.1 An Agriculture Example of the Development Ladder**

One contributor to food insecurity is post-harvest loss, reducing food availability and profitability for smallholder farmers and their families. In addition, without reliable storage, farmers must sell their crops immediately to prevent damage and lower product quality (Purdue

University 2015). This limitation to sell quickly results in the farmer selling the crops during a high-supply period in the market right after harvest (Baributsa et al. 2019). Large supplies in the market commonly leads to a decrease in selling price. In addition, during the low-supply time between harvests, prices are higher and seasonal food shortages can occur. Farmers with hermetic storage like PICS bags can store their products until later in the season to make a larger profit and have year-round food security without having post-harvest loss from insects or fungi. Another advantage of the PICS bags is the lack of need for chemical insecticide and fungicide treatment of the dry grain (Baributsa et al. 2010, 2014). Independent researchers found competitive profitability of the PICS bags compared to other storage technologies in Tanzania (Jones, Alexander and Lowenberg-DeBoer 2011).

The PICS bag acts as a hermetic storage that uses two inner layers of high-density polyethylene plastic and an outer woven polypropylene bag (Baributsa et al. 2012, 2014). To use the bag, farmers start with the inner bag, pushing out all the air possible, then tying the bag tightly in a knot. This is then done for the other two layers, creating a hermetically sealed bag to prevent oxygen transfer inside. This system may be used for many crop products, including beans (*Phaseolus vulgaris*), maize, cowpeas, groundnut, wheat, and coffee. The hermetic storage helps prevent damage from fungi and insect pests such as Bruchid beetles, weevils, and aflatoxin-producing fungi (Pretari, Hoffman, and Tian 2019). Preventing post-harvest loss improves total yield for farmers, allowing them to consume and sell their products and earn additional income for other needs such as education and health care. This helps reduce food insecurity and may help the farmers realize more of their potential (Seers 1972). The PICS bags project helps cover basic needs and helping the farmers gain more options for their families through additional yield and income (ILO 1976, UNDP 1990).

Initially, researchers at Purdue, including Dr. Larry Murdock, designed the original three-layer bag to store cowpeas and prevent pest damage and loss, specifically for Sub-Saharan Africa (SSA). The bags were tested in the lab, then the field, to confirm efficacy as well as demonstrate ease of use and cultural acceptance of the technology. In the first phase or rung, these tests were run in large-scale randomized control experiments both between and within villages. For the second phase, PICS bags were distributed on a large-scale throughout SSA. In 2007, 33,000 villages had access to PICS bags and, by 2013, over 3.5 million bags have been made and sold.

The third phase was to help establish local factories and distribution businesses for the bags in the target countries. The Purdue/Gates Foundation partnership did not want to just distribute bags for free, especially those made in developed countries. Instead, the project aimed to help create a new industry in each country in which PICS bags were being introduced, improving access to the market and food security for smallholder farmers in Sub-Saharan Africa, including Burkina Faso, Malawi, and Uganda. Several intended outcomes for the developed system included providing jobs, stimulating the economy, maintaining affordable bag prices, and ensuring local production, accessibility, and distribution of the bags. Another objective was to increase hermetic storage use by 20% of on-farm grain (Purdue University 2015). The success of the previous two phases helped guide this third phase to encourage commercialization as discussed above. Objectives included farmer training in 14,000 villages, creating a supply chain for the bags, and integrate extensions and research capacity in those villages and countries (Bill & Melinda Gates Foundation 2010). Communication such as radio, apps, and cell phones have helped increase availability, access, and awareness of the bags.

In this example, Purdue addressed several rungs in the development ladder. The first rung is allowing for food preservation so the family can eat without post-harvest loss or aflatoxin

contamination. The second rung is addressed when farmers use the PICS bags to preserve a surplus of their crops to sell at higher prices when food is scarce. The third rung is the establishment of an “industry” to make the bags, providing jobs for more stable income. This is reached by the establishment of local or regional factories which produce the bags rather than importing them. The fourth rung is the creation of a supply chain with more opportunities for jobs (distributors, delivery, trainers, etc.).

The PICS bag project helps meet the basic need of preventing crop spoilage, while also helping the farmers participate in the broader market and begin to have a voice in politics. As the PICS bags partnership works with the government, it builds a reputation and a connection between the farmers and the government, helping them gain more power (Kingsbury et al. 2016).

## **2.4 Aquaculture Development**

There are many methods of food production, and development approaches usually focus on just one or two of these methods in a project. With so many possible approaches to food security, what role does aquaculture play among cereal crops, horticulture, and domesticated livestock production? Does aquaculture help or harm economic livelihood? What impact does it have on economic development and food security of households and communities?

With the PICS bag example in mind, similar opportunities may exist for alternative protein source development, such as aquaculture. Fish is a nutrient-rich source of protein, fatty acids, vitamins, and minerals, and is an essential food for reducing malnutrition in developing countries (Thilsted et al. 2017, HLPE 2014, Kawarazuka and Bene 2011). Increased fish consumption can help reduce child stunting (Chegere and Stage 2020). Higher consumption levels either require an increase in harvesting wild fisheries or an increase in aquaculture. Globally, most wild fish populations at maximum sustainable harvest or have been overfished. For instance, fear of

overfishing leading to fishery collapse has grown, since approximately 366 global fisheries, or a quarter of the total fisheries, have collapsed since 1950 (Mullon, Freon, and Cury 2005). Aquaculture development has the potential of increasing fish availability, reducing fish prices due to increased supply, and increasing household income and protein consumption.

Aquaculture has the advantage of using non-arable land for terrestrial fish-farming or to use sectioned-off regions of natural bodies of water. This enables the use of agriculturally productive land for other crops while maximizing overall food production and reducing the opportunity cost of land use. The pond effluent can then be used for crop irrigation or aquaponic systems. This may also provide income and/or opportunities for value-chain creation, enabling families to move up the ladder. The next ladder rung would be reaching general self-sustenance. This would be effective if feed is accessible or producible by farmers using locally available resources. Future steps would include access to and incorporation into local, regional, national or global markets, as well as changing production to a combination of goods to allow for consumption to meet utility. However, this dissertation focuses on the next step of self-sustenance and the development and demonstration of a viable locally producible feed.

One perspective on aquaculture as a development tool is that of oppression or colonialism; essentially part of the dependency theory. Several articles have called this oppression “economic geography”, where the undernourished Southern (Global South) nations are taught how to produce farmed fish which are then sold internationally to wealthier Northern countries (McIntyre et al. 2016, Ponte et al. 2014, Beveridge et al. 2013, Bush 2004, Ahmed and Lorica 2002, Lewis 1997, Hall et al. 2013, Golden et al. 2016). They also claim aquaculture development keeps poor indigenous people in a state of landlessness (Golden et al. 2016). In 1997, Lewis argued that



economic gain from non-targeted aquaculture development in Bangladesh leads to wealthier owners claiming the resources and inputs for fish production (Lewis 1997).

However, newer studies support the theory that aquaculture development increases food security and income for households in all economic classes, including the indigenous people (Toufique and Belton 2014, Pant et al. 2014). In Bangladesh, from 2000 to 2010, increases in aquaculture led to reduced fish prices, increased fish consumption, and was linked to economic growth focused on the poor (Toufique and Belton 2014). It also increases frequency of protein consumption (i.e., meat, fish, eggs) while increasing household incomes (Pant et al. 2014). Aquaculture promotes food security through increased income, employment linkages (through the market or input production), and increased consumption (Ahmed and Lorica 2002). Also, tilapia can be produced year-round with multiple harvests, reducing seasonal food supplies and hunger.

Related to the dependency theory, there is the perspective that industrial or commercial-scale, intensive aquaculture development increases poverty through global trade of the farmed fish. Those that hold this viewpoint want to focus on increasing extensive, low-input small ponds to improve food security without considering any inputs (Bondad-Reantaso and Subasinghe 2013, Nayak and Berkes 2011). However, there is an intermediate form of aquaculture development being spurred on by the farmers themselves: a segment of commercial farms, increasingly intensifying with inputs (Belton, Bush, and Little 2017). The fish produced by this segment stays in domestic markets, available for the rural and urban consumers and for the rich and poor, and contributing to food security (Belton, Bush, and Little 2017).

### 2.4.1 Aquaculture Example

Since aquaculture has been shown to be an effective development tool in reducing poverty, food insecurity, and malnutrition, the question is how best to be implement in areas of need. Exemplary rungs on the aquaculture development ladder are shown in Table 2.2.

Table 2.2 Example of Aquaculture Development Rungs

Ladder Rungs	Aquaculture Steps
n+15	Exporting fish or feed
n+14	Processing fish (i.e., smoking, filleting, salting, freezing)
n+13	Selling fish in the market
n+12	Fish breeding
n+11	Effluent Redirection/Reuse
n+10	Increase stocking density (35 fish/ m <sup>2</sup> )
n+9	Commercial feed to satiation
n+8	Community cooperative
n+7	Scale local feed production
n+6	Increase stocking density (20 fish/ m <sup>2</sup> )
n+5	Aeration
n+4	Local feed
n+3	Increase stocking density (10 fish/m <sup>2</sup> )
n+2	Supplementary feeding (i.e., rice bran, maize, commercial feed)
n+1	Fertilization of pond
n	Dig/stock earthen pond (1 fish/m <sup>2</sup> )

## Dynamic Aquacultural Development Sub-Ladder

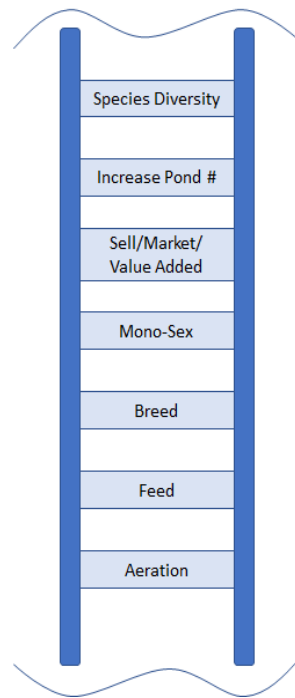


Figure 2.3 Conceptual Aquacultural Development Sub-Ladder

### 2.4.2 Example of Aquaculture Development in Guatemala

Tilapia has been cultivated in Guatemala for over 60 years due to initial efforts by the FAO. No training was provided, however, nor was the program continued, leaving Guatemala with failed ponds and no further implementation or aquaculture growth. The Fisheries and Aquaculture Unit of the Ministry of Agriculture of Guatemala has partnered with UNIPESCA, the Central American organization promoting fish consumption. In addition, the communities where AgInno Institute works have expressed interest in increasing fish consumption through aquaculture production. The theory of change in Figure 2.4 below describes the process and objectives of the aquaculture project. Each level relates to one or more rungs on the aquaculture development ladder (Figure 2.3).

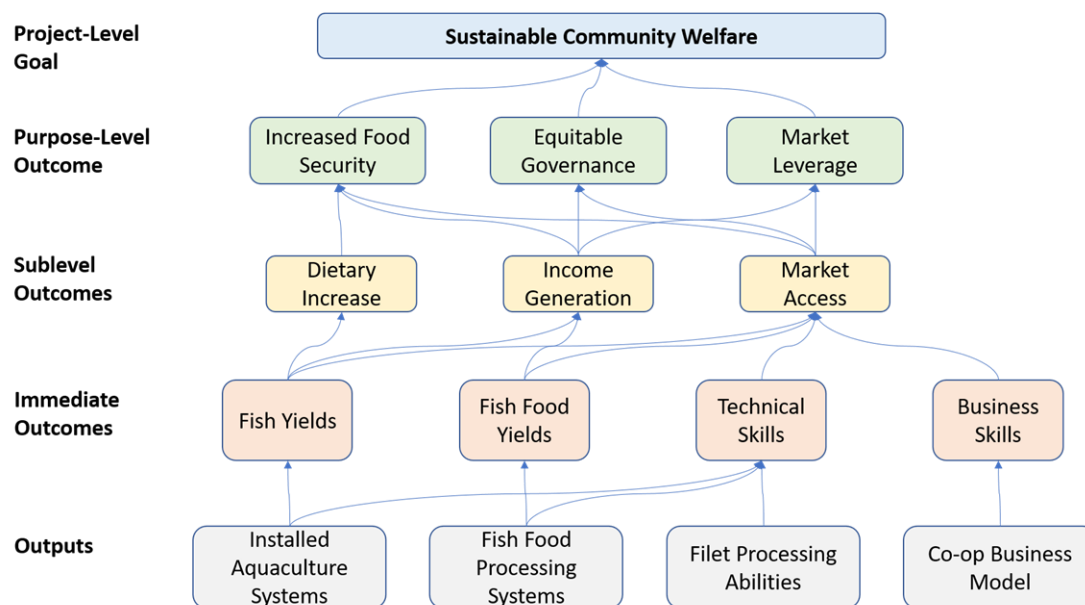


Figure 2.4 Theory of Change

Figure 2.4 above demonstrates the stepwise outputs at the bottom which connect with short-term and long-term outcomes. Each ladder rung helps households and communities improve their welfare (i.e., income, food security, nutrition). In Figure 2.5 below, the different rungs include local feed production, breeding, grow-out, harvesting, processing, and market considerations. While not in order from most basic to more advanced rungs, it provides a clear picture of the final ladder. This would be done in a cooperative approach with the community so that each household has reduced labor and time requirements, ensures viability of the entire system since one household would be unable to do all the steps on their own, and increases scale and efficiency. One or more households could raise the insects/algae for feed production, others would participate in gathering other feed ingredients (i.e., harvesting fruits, connecting with plantations for byproducts or unsellable produce), others would participate in the fish production (i.e., breeding fish, nursery, grow-out, harvesting), and still others would participate in the harvesting/selling stage (i.e., harvesting, preservation, transportation, and selling in the market).

Many households sell their products rather than consuming them, enabling market access and involvement as well as extra income vital to improving nutrition (Smith et al. 2013). Selling surplus fish in the market would increase household income, increasing food security. The surplus fish in the market would increase household income, increasing food security. The additional income could be spent on other protein sources like meat and eggs as well as vegetables, reducing malnutrition and stunting by increasing the protein share of the diet. At first, diets mainly consist of starches, but, as income increases, so does protein purchase and consumption (Smith et al. 2013). Even some consumption of animal-sourced protein can prevent stunting, cognitive development issues, malnutrition, illness morbidity, and birth issues while improving immune responses (Keusch and Farthing 1986, Neumann et al. 2002, Sadler et al. 2013). The scaling-up of local feed production and fish production can also benefit the community by allowing some profit to be used for improving education and health facilities, investing in clean water, and further investment in food production.

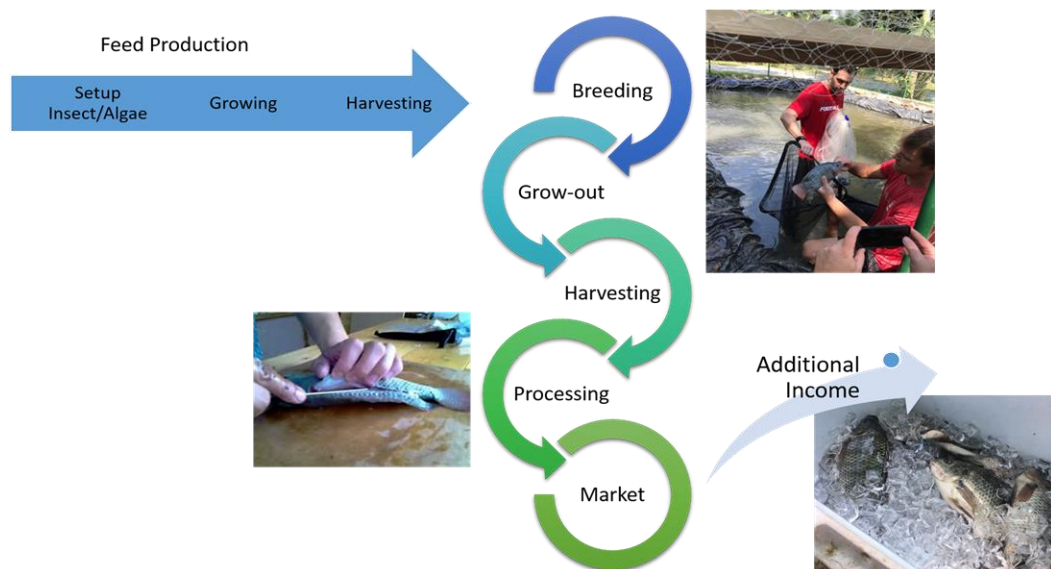


Figure 2.5 Development Ladder Rungs for Aquaculture

### **2.4.3 Intermediate Fish Feed Input as a Rung**

Adding feed as an input is important to intensifying fish farms and reaching the next rung on the ladder. However, commercial feed might be unaffordable or inaccessible, and simply fertilizing a pond to stimulate primary productivity is not effective for increasing production. One solution could be a fish feed that can be locally produced with locally sourced ingredients. The challenge is formulating this type of feed to stimulate profitable fish growth. The objective of Chapter 3 is to analyze several formulations of locally sourced ingredients to define a feed that promotes adequate fish growth, ideally approaching the efficacy of commercial feed. Chapter 4 evaluates the economics of the various feeds in Chapter 2 in the context of an intensive earthen pond system in Guatemala.

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## **CHAPTER 3. - EFFECTS OF LOCALLY SOURCED NONCONVENTIONAL FEED INGREDIENTS ON NILE TILAPIA (OREOCHROMIS NILOTICUS) GROWTH**

### **3.1 Introduction**

Aquaculture can be an important and inexpensive source of protein and micronutrients for humans, but one that is often underutilized and under-appreciated in developing countries such as Guatemala (Bene and Heck 2005). One of the primary constraints associated with aquaculture development as a farm-level activity is the cost of feed, which can account for up to 50-70% of production costs (El-Sayed 2020). This variable cost can prohibit adoption of aquaculture for smallholder farmers. As an example, an earthen aerated pond containing 1200 tilapia fingerlings in Guatemala requires an average of \$32-\$40 per week for feed. In addition to the cost issue, there can also be a logistical barrier. High quality feeds may not be, and often are not, near rural farms. Thus, there is a significant need for feed development within the overall context of fish production in rural developing countries; a similar research line pursued in developing countries since at least the 1980's when cottonseed meal, soybean meal, groundnut cake, rapeseed meal, and copra meal were tested (Jackson et al. 1982, El-Sayed 1987, Hoffman et al. 1997).

The most basic aquaculture system, near the bottom of the aquaculture-specific development ladder, is extensive aquaculture, in which neither supplemental aeration nor feeding is provided. Instead, oxygen is provided to the system through simple diffusion from atmospheric oxygen and phytoplankton, algae, and insects are the source of food. In this scenario, dissolved oxygen (DO) levels may fluctuate widely with lowest values before dawn and higher values in the afternoon (Bhujel 2014). A common occurrence in extensive systems is chronically low DO levels, which can lead to reduced feed intake and digestibility, while increasing energy costs of digestion,

leading to lower growth rates (Wang et al. 2009, Tran-Duy et al. 2012, Duan et al. 2011, Tran et al. 2016). Use of natural food supplies demands lower stocking densities and often results in less than maximal fish growth. Extensive aquaculture generally supports less than one fish/m<sup>2</sup> (10-20 g), while semi-intensive ponds support 1-5 fingerlings/m<sup>2</sup>, and intensive systems can support 5-10 fingerlings/m<sup>2</sup> with values ranging up to 30 (Romana-Eguia et al. 2013). Due to reduced stocking density and reduced growth rates, extensive systems can also only support 0-1 t/ha/yr (Pullin, Rosenthal, and Maclean 1993).

In semi-intensive pond systems, food by-products and chemical or organic fertilizer can be added to stimulate primary productivity of phytoplankton and algae in the water. Tilapia, such as *Oreochromis niloticus*, feed on this nutritious growth as 50-80% of their diet in semi-intensive systems (Schroeder et al. 1990, Abou et al. 2013). Phytoplankton may comprise 12-51% protein, 7.2 to 7.7% lipid, and 8.2 to 27.3% carbohydrate (Kang'ombe et al. 2006, Abou et al. 2013). Semi-intensive systems begin supporting more fish (1-15 t/ha/year) as fertilizer and supplementary feed are added. There are several published studies that have documented increased productivity and growth of fish offered simple feed ingredient inputs compared to a strictly extensive scenario. For example, semi-intensive and intensive systems overlap when nutritionally complete feed and supplemental aeration without recirculation are used, which can support production levels as high as 15-20 t/ha/yr with some systems surpassing this to over 120 t/ha/yr (Pullin, Rosenthal, and Maclean 1993).

In another study illustrating the productivity of semi-intensive ponds, two different feeds were offered to fish (*Oreochromis niloticus*) over a 270-day period. The two treatments produced total average weight gains of 114g and 100.04g, daily mean weight gains of 0.43g/day and 0.38g/day, specific growth rates of 0.92% and 0.89%, and survival of 82.6% and 81.5%,

respectively (Limbu et al. 2016). The lower survival rate and low growth rates observed in this semi-intensive pond approach increase harvest cycle time significantly compared to intensive systems.

Intensive aquaculture includes aeration of the water to increase DO levels, commercial feed, and, oftentimes, all-male (mono-sex) fish to maximize growth and productivity. For small- or medium-scale farms, increasing DO levels above 5mg/L, maintaining water temperature between 27°C and 32°C, using a stocking density of 3-5 fish per square meter, using feed with CP (crude protein) of 25-30%, and using a stocking rate of more than 10 g will help maximize production and fish growth (Mengistu et al. 2020). As of 1993, intensive systems with complete feed, aeration, and recirculation could support between 20 to 100 t/ha/yr in a pond and 100-1000 t/ha/yr in a raceway (Edwards et al. 1988, Pullin, Rosenthal, and Maclean 1993). However, advancements in aquaculture (i.e. technology, feed, aeration systems, breeding) has most likely increased potential yield.

Intensive aquaculture permits scale production of protein, but currently requires commercial feed to maximize fish growth and survival. In terms of the development ladder, extensive fish production is near the bottom rung while intensive aquaculture is closer to the top rung. To bridge this gap, intermediate local or regionally produced feed types can allow for increased fish growth (compared to extensive or semi-intensive production) while reducing feed costs. As described above, not only are commercial feeds expensive to purchase, but are they also difficult for smallholder farmers to replicate since many ingredients are not sold locally. For example, pure vitamin and mineral mixes, choline chloride, and carboxymethylcellulose, which are common additives to commercial feeds, are not available for rural households in countries like Guatemala, but only for established companies. This requires a new approach to identify suitable



replacement protein and micronutrient sources, specifically in a developing country setting, where expedited development and testing is needed to meet food security needs. It also requires new formulation methods, using ingredient nutritional analyses and linear programming to design diets which meet nutritional needs of the target species.

As discussed in the Literature Review, Nile tilapia (*Oreochromis niloticus*) was chosen as the target species in this dissertation as tilapia tolerate wider ranges of water temperature, DO levels, salinity, and turbidity than other farmed species and are a commonly consumed fish in many poorer countries (El-Sayed 2020). The objective of Experiment 1 was to identify a potential source of dietary crude protein, chemically analyze that identified ingredient, and evaluate the effect of dietary inclusion at different levels in diets for tilapia. The tropical banded cricket (*Gryllodes sigillatus*) was identified as a potential source of dietary crude protein. Crickets are known to have relatively high protein content and have been explored by other researchers as potential feed ingredients for terrestrial livestock (Beenackers et al. 1985, Blomquist et al. 1991, Dierenfeld and King 2008, Jonas-Levi and Martinez 2017). The tropical banded cricket is indigenous to many poorer countries such as Guatemala, where it would be available for local farmers to raise as a feedstock. The objective of Experiment 2 was to design a fish feed that can be made and sold locally in developing countries at an affordable price, using only locally sourced ingredients and methods to account for the ingredients to which farmers or local feed producers would feasibly have access.

## **3.2 Methods**

### **3.2.1 Insect Preparation**

Tropical banded crickets, *Gryllodes sigillatus*, were purchased from Josh's Frogs (joshsfrogs.com). After 24 hours of fasting, crickets were frozen at -12°C to euthanize them. Once dead, the crickets were laid out on trays in a single layer and dried in a convection oven at 75°C for eight hours. After cooling, crickets were ground using a countertop blender to form a coarse powder which was later stored in Ziploc gallon freezer bags at -12°C.

### **3.2.2 Fish Preparation**

Tilapia fingerlings were raised in a single five-foot diameter tank at the Aquaculture Research Lab, Purdue University and were maintained at 28°C +/- 2°C with a 12:12 light:dark cycle and fed to satiation (35% Hi Production Fish Feed from Star Milling, Perris, CA). Fingerlings were weighed on the first day of the experiment at around 70 days old. Individual fish were placed in a 3.785l tared bucket containing tank water. A digital scale was used to weigh fish to the nearest 0.01g. For Experiment 1, average weight of fish was 10.10g, with a standard deviation of 2.01g while fish for Experiment 2 had a mean weight of 15.5g and a standard deviation of 5.76g. A transparent plastic box was used for measuring length of fish with a portion of the lid cut to fit widthwise in the box (i.e., as a divider) and enable the fish to be restrained and pressed against the end wall for measurement (A.1 and A.2, respectively). Holes in the lid allowed water to flow through the divider to reduce water pressure as the divider was moved toward the end wall to constrain the fish. For measurements, the box was partially filled with water from the original tank, an individual fingerling placed in the box, and the lid was pulled forward to one end, trapping the fingerling safely against the wall. A clear ruler was then used to measure the standard length of

each fish from nose tip to fin end in millimeters. The Sterilite measurement box had dimensions of 27.3 cm x 11.4 cm x 16.5 cm (Appendix Tables A.1 and A.2).

### **3.2.3 Non-Conventional Ingredient Preparation**

After researching locally available resources in Guatemala, several were selected based on seasonal availability and nutritional concentrations. Tropical banded crickets (*Gryllobates sigillatus*), earthworms (*Lumbriculus variegatus*), and black beans (*Phaseolus vulgaris*) were selected to meet the crude protein requirements of tilapia. Ingredients selected and meeting tilapia carbohydrate requirements included green plantain flour (*Musa paradisiaca*) and sweet potato flour (*Ipomoea batatas*). Watermelon seed flour (*Citrullus lanatus*), whole sapote (*Pouteria sapota*), whole rambutan (*Nephelium lappaceum*), whole longan (*Dimocarpus longan*), starfruit (*Averrhoa carambola*), whole mango (*Mangifera indica*), and mamey papaya (*Carica papaya*) were selected to meet the mineral and vitamin requirements of tilapia. Corn cob powder (*Zea mays*) and carboxycellulose were used as binding agents and fiber in the feed formations. Due to the cost and time of obtaining ingredients directly from Guatemala, the same fruits were instead obtained from Jungle Jim's in Fairfield, Ohio, the sweet potato flour, moringa powder, and green plantain flour was obtained from Anthony's Goods ([anthonysgoods.com](http://anthonysgoods.com)), and the dried earthworms were obtained from California Blackworm Co., CA.

Whole fruits were peeled and flesh was separated from seeds, then the peels, flesh, and skin were cut into thin strips. Seeds and strips were then placed on parchment paper-lined metal trays in a single layer, and the trays were subsequently placed into an industrial convection oven at 65°C for 12 hours. Next, each dried material was separately ground in a kitchen blender and finally pulverized to a fine powder (APWONE 2000g Electric Grain Mills Grinder Powder LCD

Digital). Black beans, crickets, and earthworms were also separately ground in the pulverizer. All ingredients were placed in labeled Ziploc Freezer gallon bags and stored at -12°C.

#### **3.2.4 Ingredient Analysis**

Fish meal was donated by Daybrook Fisheries, Inc., of Empire, LA. All ingredient nutrient analysis was conducted by Midwest Laboratory (Omaha, NE) to provide the basis for the experimental formulations (Tables 3.1, B.1). Final feeds for both experiments were also chemically analyzed by the same commercial laboratory after experiments were completed to compare final nutrient composition.

Table 3.1 Nutrient Concentrations of Tropical Banded Cricket (*Gryllodes sigillatus*) and Fish Meal

Nutrient (% , % CP for amino acids)	<i>Gryllodes sigillatus</i> (dry matter basis)	Fish Meal
Crude protein	69.4	70
Fat	15.7	9.3
Total carbohydrate	N/A	0.2
Crude fiber	7.52	0.82
Ash	4.86	21.9
Alanine	4.55	3.74
Arginine	4.24	3.76
Aspartic acid	5.85	4.98
Cystine	0.53	0.55
Glutamic acid	6.99	7.48
Glycine	3.00	4.67
Histidine	1.57	1.57
Isoleucine	2.74	2.5
Leucine	4.62	4.24
Lysine	3.67	4.63
Methionine	0.94	1.77
Phenylalanine	2.28	2.38
Proline	3.32	3.1
Serine	3.53	2.32
Threonine	1.65	1.73
Tryptophan	0.57	0.58
Tyrosine	3.18	1.81
Valine	3.62	2.84

### 3.2.5 Feed Formulation

For both experiments, linear programming using Solver in Excel was used to formulate diets. Animal feed formulations typically use linear programming in Excel or in commercial software to maximize nutrients and occasionally minimize cost (FAO 1980, Thomson and Nolan 2001, Guevara 2004, van Dooren 2018). For both experiments, cost was set at zero for all ingredients to focus on meeting nutritional requirements while managing ingredient levels. To use linear programming, the nutritional data from Midwest Labs was combined with those from USDA

FoodData Central in Excel to form a sheet with columns indicated unique nutrients (i.e., protein, fat, carbohydrates) and rows indicated ingredients (i.e., wheat flour, soybean meal, fish meal, cricket meal) with both nutrient and ingredient concentrations having constraints. Total weight of ingredients was set at 100g and all ingredients were constrained to specific amounts, except for the main protein and carbohydrate sources. Once a formula was designed, ingredients were maintained at similar rates for all treatments with only slight changes to maintain a total weight of 100g.

For Experiment 1, test diets were formulated to replace 25, 50, 75, or 100% of the fish meal with the tropical banded cricket powder on an isonitrogenous and isoenergetic basis (Treatments 1, 2, 3, and 4, respectively). These formulations shown in Table 3.2 and their respective nutrient compositions in Table 3.3.

Table 3.2 Feed Formulations Used in Experiment 1

Ingredients (%)	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Fish meal	20.0	15.0	10.0	5.0	0.0
Tropical Banded cricket (adult)	0.0	5.0	10.1	15.1	20.2
Soybean meal <sup>a</sup>	32.0	32.0	32.0	32.0	32.0
Wheat flour <sup>b</sup>	36.6	36.6	36.5	36.5	36.4
Soybean oil <sup>b</sup>	5.0	5.0	6.0	7.0	8.0
Choline chloride <sup>c</sup>	0.5	0.5	0.5	0.5	0.5
Vitamin mix	0.4	0.4	0.4	0.4	0.4
Mineral mix	0.1	0.1	0.1	0.1	0.1
Sodium phosphate <sup>c</sup>	1.6	1.6	1.6	1.6	1.6
Carboxymethylcellulose <sup>c</sup>	2.0	2.0	2.0	2.0	2.0
Potassium phosphate <sup>c</sup>	1.6	1.6	1.6	1.6	1.6
Vitamin C <sup>d</sup>	0.2	0.2	0.2	0.2	0.2
Total	100.0	100.0	100.0	100.0	100.0

<sup>a</sup>Cargill, <sup>b</sup>Meijer, <sup>c</sup>Sigma, <sup>d</sup>VitaminPlus

Table 3.3 Nutrient Composition of Feeds (% Dry Weight Basis) of Diets Fed to Tilapia in Experiment 1

Nutrient (% , % protein for amino acids)	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Crude protein	36	35.3	35.4	36.4	33.7
Crude fat	8.59	12.2	12.8	15.1	18.2
Crude fiber	2.8	2.8	3.4	3.7	3.4
Ash	11.1	8.16	7.08	5.86	5.4
Alanine	1.77	1.74	1.77	2	1.85
Aspartic acid	3.18	3.4	3.61	3.38	2.92
Threonine	1.04	1.25	1.14	1.21	0.93
Glutamic acid	6.05	6.13	6.07	6.23	5.4
Serine	1.56	1.71	1.79	1.78	1.55
Proline	1.95	1.94	1.89	2.14	1.78
Cystine	0.44	0.39	0.42	0.43	0.39
Valine	1.54	1.7	1.65	1.92	1.56
Methionine	0.55	0.56	0.54	0.46	0.41
Glycine	1.89	1.92	1.57	1.92	1.41
Isoleucine	1.37	1.56	1.32	1.62	1.25
Leucine	2.5	2.64	2.45	2.81	2.09
Tyrosine	0.97	1.31	1.37	1.53	1.32
Phenylalanine	1.5	1.71	1.54	1.71	1.28
Lysine (total)	2.01	2.14	2.02	2.11	1.65
Histidine	0.72	0.89	0.86	0.9	0.81
Arginine	2.09	2.35	2.23	2.45	1.94
Tryptophan	0.3	0.39	0.43	0.34	0.38
Total digestible nutrients	82.4	89.2	90.6	94	98.3
Digestible energy MJ/kg	15.20	16.40	16.67	17.32	18.15
Metabolizable energy MJ/kg	13.45	14.55	14.83	15.29	16.12
Tryptophan	0.3	0.39	0.43	0.34	0.38
Sulfur	0.4	0.34	0.35	0.31	0.29
Phosphorus	1.41	1.82	1.71	1.44	1.4
Potassium	1.24	1.74	1.73	1.57	1.69
Magnesium	0.26	0.17	0.17	0.15	0.14
Calcium	1.93	1.17	0.88	0.49	0.2
Sodium	0.16	0.84	0.71	0.65	0.63
Iron (ppm)	270	304	155	114	89.4
Manganese (ppm)	83.4	24.4	30.8	23.8	23.6
Copper (ppm)	22.9	9.9	11.3	12.6	14.3



Actual values of certain nutrients were compared to the estimates from the linear programming step. The crude protein concentrations were consistent between estimated formulations and the actual nutrient content (Table 3.4). Fat was higher in the actual feed content than estimated content, especially with Treatments 3 and 4 (almost double the amount). This is most likely due to adding additional oil to bind the mixture for pelletizing. Carbohydrates were consistent between estimated and actual compositions while actual fiber values were lower than the model estimates.

Table 3.4 Comparison of Estimated vs Actual Nutrient Content of Feeds

Nutrient Values	Control		Treatment 1		Treatment 2		Treatment 3		Treatment 4	
	Est.	Actual	Est.	Actual	Est.	Actual	Est.	Actual	Est.	Actual
Protein (%)	34.9	36	34.9	35.3	34.9	35.4	34.9	36.4	34.9	33.7
Fat (%)	7.78	8.59	8.11	12.2	8.44	12.8	8.76	15.1	9.09	18.2
NFE (%)	40.3	41.5	40.3	41.5	40.3	41.3	40.3	38.9	40.3	39.4
Fiber (%)	4.62	2.8	4.96	2.8	5.3	3.4	5.64	3.7	5.97	3.4

For Experiment 2, Treatments 1-4 were formulated using the nutrient analyses of individual ingredients (Appendix Table B.1) and calculating optimal ratios using linear programming in Excel (Table 3.5). The objective was the formulation of the four experimental diets so that they would be isonitrogenous and isocaloric. Another objective was to have the four treatments utilize different protein sources representing a variety of potentially available animal sources in developing countries: fish meal, tropical banded cricket meal, earthworm meal, and a combination of earthworm and black bean powder. While the protein source was varied between diets, micronutrient, carbohydrate, fat, and fiber sources held constant between diets (i.e. ratio of local

ingredients would be equal). Feeds were formulated to be as isoenergetic and isonitrogenous as possible.

Control 1 was a commercial feed, 35% Hi Production Tilapia Food with soybean meal, porcine meat and bone meal, wheat bran, fish meal, vitamins and minerals, and other ingredients (Star Milling). Control 2 was a simulated commercial feed identical to the Control diet in Experiment 1 (Table 3.2). Control 3 comprised 100% algae with a 1:1 ratio of *Chlorella* and *Spirulina*, 5% corn oil, and 30% water mixed together to form a dough-like feed. This feed was not dried in the oven or formulated like the treatments to better simulate the fresh algae fish in extensive systems would consume. The nutrient compositions for the controls and Treatments 1-4 are provided in found in Table 3.6. Figure A.3 shows the final feed product for each group.

Table 3.5 Feed Formulations Used in Experiment 2

Nutrients	Actual Amount (%)			
Upper limits of dietary nutrients (%)	T1	T2	T3	T4
Fish Meal	38.16	0.00	0.00	0.00
Earthworm Meal	0.00	0.00	40.00	20.00
Tropical Banded Cricket (adult)	0.00	40.00	0.00	0.00
Plantain Flour	16.00	16.00	16.00	10.00
Moringa Leaf	16.43	18.36	18.36	16.15
Sweet Potato Flour	14.14	14.14	14.14	10.20
Corn Oil	4.00	4.00	4.00	4.00
Moringa Seed	0.00	1.00	1.00	1.00
Black Bean Powder	0.00	0.00	0.00	32.16
Corn Cob Powder	0.50	0.50	0.50	0.50
Starfruit	0.50	0.50	0.50	0.50
Chlorella	0.00	0.00	0.00	0.00
Sapota	1.00	1.00	1.00	1.00
Longan	0.50	0.50	0.50	0.50
Rambutan	0.50	0.50	0.50	0.50
Watermelon Seeds	0.50	0.50	0.50	0.50
Mango	0.50	0.50	0.50	0.50
Mamey Papaya	0.50	0.50	0.50	0.50
Carboxymethylcellulose	2.00	2.00	2.00	2.00

Table 3.6 Nutrient Composition of Feeds (% Dry Weight Basis) of Diets Fed to Tilapia in Experiment 2.

Nutrient (%, % protein for amino acids)	Control 1	Control 2	Control 3	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Protein	37	36	61.7	34.9	35.3	32	27.7
Crude fat	5.01	8.59	3.32	11.3	14.8	9.2	7.92
Crude fiber	7.4	2.8	32.6	3.7	4.8	6.6	9.4
Moisture	9.98	5.34	43.9	8.05	5.1	8.72	6.21
Ash	7.98	11.1	7.7	12.8	5.53	12.6	7.87
Aspartic acid	3.26	3.18	4.83	2.97	3.12	3.24	2.95
Threonine	1.1	1.04	1.82	0.89	0.86	0.99	0.96
Serine	1.67	1.56	2.41	1.32	1.71	1.52	1.42
Glutamic acid	6.15	6.05	6.92	4.14	3.64	4.38	3.86
Proline	2.27	1.95	2.24	1.66	1.62	1.25	1.09
Glycine	2.23	1.89	2.78	2.48	1.61	1.63	1.42
Alanine	2.02	1.77	4.62	1.91	2.41	1.98	1.56
Cystine	0.46	0.44	0.62	2.45	0.3	0.4	0.3
Valine	1.68	1.54	3.37	1.57	1.78	1.69	1.47
Methionine	0.71	0.55	1.16	6.48	0.41	0.39	0.27
Isoleucine	1.22	1.37	2.9	1.26	1.22	1.53	1.29
Leucine	2.95	2.5	4.97	2.39	2.21	2.67	2.37
Tyrosine	1	0.97	2.12	0.84	1.45	1.02	0.9
Phenylalanine	1.57	1.5	2.53	1.26	1.2	1.41	1.34
Lysine	2.03	2.01	2.71	2.16	1.53	1.99	1.7
Histidine	0.88	0.72	0.93	0.7	0.77	0.72	0.65
Arginine	2.22	2.09	3.69	1.91	1.94	2.12	1.9
Tryptophan	0.42	0.3	0.52	0.24	0.28	0.35	0.29
Metabolizable energy MJ/kg	12.99	13.45	10.87	13.72	15.38	13.36	14.00
S	0.4	0.38	0.92	0.59	0.5	0.49	0.46
P	1.41	2.2	1.24	1.84	0.58	0.39	0.45
K	1.24	1.91	0.65	1.49	1.52	1.31	1.4
Mg	0.26	0.17	0.49	0.23	0.22	0.35	0.28
Cal	1.93	1.64	0.38	3.29	0.63	1.16	0.72
Na	0.16	0.72	1.74	0.62	0.34	0.28	0.3
Fe (ppm)	270	201	411	299	83.7	3620	750
Mn (ppm)	83.4	25.4	60.2	33.3	37.2	104	41.1
Cu (ppm)	22.9	8.6	1.9	7.4	23.7	15.7	11.2
Zn (ppm)	120	44	11.3	53.9	80.4	67.6	46.3
NFE	48.31	44.67	40.92	42.3	42.65	44.9	50.39

Most of the diets in Experiment 2 had comparable measured protein levels, ranging from 27.7% for Treatment 4 to 37% for Control 1. However, the algae in Control 3 had a dry weight (DW) crude protein content of 61.7%, but the “as fed” crude protein levels were much closer to those in the other treatments. Crude fat content was more variable across all diets, and, other than Control 3, all treatments had higher lipid levels than Control 1. Fiber content was also highly variable among the experimental feeds with Control 2 (2.8%), Treatment 1 (3.7%), Treatment 2 (4.8%), and Treatment 3 (6.6%) having lower levels than Control 1 (7.4%), while Control 3 (32.6%) and Treatment 4 (9.4%) had higher fiber levels than Control 1.

Serine, proline, lysine, and aspartic acid levels were comparable between the diets. Glutamic acid was lower in the negative control and the treatments compared to Controls 1 and 2. Total digestible nutrient content was lowest for Control 3 (39.6%) and highest for Treatment 2 (89%). Metabolizable energy was comparable among diets, except for Control 3, which had a lower level. Control 1 had a significantly higher zinc content than the other diets. Phosphorus content was comparable between the three controls and Treatment 1 (mean of 1.67%), but was lower for Treatment 2, 3, and 4 (0.58%, 0.39%, and 0.28%). Potassium content was comparable among diets except for a lower level for Control 3. Treatment 1 had higher levels of calcium than Controls 1 and 2, while Control 3 and Treatments 2 and 4 had lower levels than those controls. Iron content was very high for Treatments 3 and 4 (3620ppm and 750ppm, respectively), low in Treatment 2 (83.7ppm), and high in Control 3 (411ppm), compared to the other diets (between 201 and 299ppm).

Estimates for various nutrient compositions for Control 2 and Treatments 1-4 (i.e., the formulated feeds) from the linear programming step and the actual measurements are shown in Table 3.7. Actual measured protein levels differed from the estimated protein levels by as little as

0.1% for Treatment 1, and as much as 5.6% for Treatment 2, but appeared close to the expected values overall. Actual fat content values were higher than the estimates for all the formulated diets, possibly due to the addition of vegetable oil as a binder and extrusion aid. Metabolizable energy levels were all well within 20% of the estimated values. Phosphorous content diverged more significantly among the experimental diets compared to the predicted levels.

Table 3.7 Actual (Meas.) vs Estimated (Est.) Proximate Composition of Diets Evaluated in Experiment 2

Nutrient Values	Control 2		Treatment 1		Treatment 2		Treatment 3		Treatment 4	
	Est.	Meas.	Est.	Meas.	Est.	Meas.	Est.	Meas.	Est.	Meas.
Protein (%)	34.9	36	35	34.9	29.7	35.3	30.9	32.0	24.7	27.7
Fat (%)	7.78	8.59	8.9	11.3	9.99	14.8	7.73	9.2	6.9	7.92
NFE (%)	40.3	44.7	35	42.3	34.5	42.7	35	44.9	48	50.4
Fiber (%)	4.62	2.8	5	3.7	7.4	4.8	4.77	6.6	5.4	9.4
Metabolizable energy (MJ/kg)	18.1	15.20	17.7	13.72	16.8	15.38	16.3	13.36	16.8	14.00
Phosphorus (%)	0.6	2.2	0.38	1.84	0.1	0.58	0.39	0.39	0.35	0.45

### 3.2.6 Feed Preparation

The dry ingredients used in larger quantities (e.g., at least about 5% of the formula weight) were combined and mixed thoroughly by hand based on feed formulations (Tables 3 and 5). The remaining dry ingredients were also combined and mixed thoroughly by hand prior to adding to the mixture of the “major” dry ingredients and mixing homogeneously. Vegetable oil and water were added and kneaded manually once the dry ingredients were thoroughly mixed, then the

mixture, having a homogenous dough-like consistency, was extruded through a meat grinder with round orifices of 2 mm diameter. Post-extrusion, the resulting long strands were laid out in a single layer on a tray with parchment paper and dried in an industrial convection oven at 65°C overnight for 12 hours. Afterwards, the dried feed was chopped and ground to 1.5-2 mm length pellets in a kitchen blender and sieved in a 0.5mm sieve to remove excess powder. Finished pellets were placed in Ziploc gallon freezer bags and stored at -12°C.

### **3.2.7 Experimental Design**

#### **3.2.8 Experiment 1-**

Twenty 50-gallon plastic tanks were arranged in two rows with recirculating water maintained at 29°C +/- 2°C. Each tank was aerated with a single aeration stone (3.5 cm square cross-section, 7.6 cm in length) to maintain a DO level of 7.5mg/L +/- 0.5 mg/L. Fish were removed from the larger tank in groups of 20 and were measured in randomized groups of five, five, and seven fingerlings to ensure similar average fingerling weights per tank. All dietary treatments were randomly assigned among the three tanks with 17 fish per tank. Fish in each tank were fed at 3% of total initial fish weight/tank with a daily increase of 0.5g/tank of feed to reflect daily fish growth. Initial and final weights were averaged per tank and used in the metrics below. Population means were used since independent initial and final weights of individual fish were unknown.

For Experiment 1, each of the treatment groups had four replicates in order to spread potential environmental and system variability more evenly among the treatments and reduce the probability of losing one treatment entirely (if a tank “failed”). The data for the replicates for each treatment were combined, creating populations of 67-68 fish per treatment for analysis. Average

changes in length and weight were measured by subtracting the average initial weight from the average final weight. Each variable was analyzed to test relationships with treatment type by running an ANOVA with a Tukey test and a linear model.

### **3.2.9 Experiment 2-**

Twenty-one 50-gallon plastic tanks were arranged in two rows with circulating water and aeration stones (the same tanks, aeration stones, and water parameters as used in Experiment 1). Fish were measured in randomized groups of five, five, and seven fingerlings to ensure similar average weights per tank. All dietary treatments were randomly assigned to three tanks with 17 fish per tank, creating populations of 51 fish per treatment. Fish in each tank were fed daily 3% of total initial fish weight/tank with an increase of 0.5 g/tank per day to reflect daily fish growth. Initial and final weights were averaged per tank and used in the metrics below. Averages were used since independent initial and final weights of fish are unknown. Average changes in fish length and weight were measured by subtracting the average initial weight from the average final weight. Each variable was analyzed to test relationships with treatment type by running an ANOVA with a Tukey test and a linear model.

### **3.2.10 Data Analysis and Statistics**

To provide broader perspective on the relative efficacy of the various feeds, several approaches from the literature were used to calculate growth rate, including absolute growth rate and specific growth rate. Absolute growth rate has units of grams per day and assumes a linear growth rate pattern in fish. It can be used for analyses within the duration of the experiment. However, the usual assumption is that tilapia grow exponentially when smaller (10-30g) and follow a power growth curve once larger (Iwama and Tautz 1981, Kauffman 1981, Terpstra 2015).



SGR is the percent weight increase per unit time (in units of days for these experiments) (Houde and Schekter 1981; Crane, Ogle, and Shoup 2019). This growth metric permits extrapolation past experiment duration due to the use of an exponential function. This accounts for the assumption of exponential growth over short time periods for smaller fish (Crane, Ogle, and Shoup 2019).

A basic function for length-to-weight relationships is the length:weight ratio where length is divided by weight. A more advanced and useful function is the Fulton's K coefficient, also called Condition Factor (CF) which assumes allometric growth of  $1:1^3$  for weight to length, forming an exponential growth curve. It acts as the coefficient  $b$  in the allometric growth equation (AGE), but with an approximate estimate of 3,  $b$  can be held constant. The original logarithmic regression form is  $W=aL^b$ . Variable  $b$  is the intercept, or regression coefficient, and  $a$  is the slope (Froese 1990). To find projected days to harvestable size (DH), the daily growth coefficient (DGC) was determined using the power growth curve. This then was used to estimate DH.

For graphs below, the letters reflect statistical significance at  $p\text{-value} < 0.05$ . Average length and weight gain were measured by subtracting the average initial weight from the average final weight. Each variable was analyzed to test relationships with treatment type by running an ANOVA with a Tukey test. All analyses for both experiments were performed in R, Version 3.5.1 (R Core Team 2018).

### 3.2.11 Equations

$$(1) \text{ Absolute Growth Rate (AGR)} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{time}} \text{ with time} = 56 \text{ days}$$

$$(2) \text{ Specific Growth Rate (SGR)} = \frac{\log(\text{Final Weight}) - \log(\text{Initial Weight})}{\text{time} * 100} \text{ or}$$

$$100 * \left[ \left( \frac{\text{Final Weight}}{\text{Initial Weight}} \right)^{\frac{1}{\text{time}}} \right] - 1$$

$$(3) \text{ General allometric growth equation (AGE)} = W = aL^b$$

where  $W$  is weight (g),  $a$  is the growth coefficient,  $L$  is length (cm), and  $b$  is an estimate based on the population.

(4) Fulton's coefficient (K):  $\frac{Weight}{Length(cm)^3} * 100$  with both initial and final weight and length

included. The cubic exponent comes from the estimate that allows for isometric growth in fish.

(5) Daily Growth Coefficient (DGC)=  $100 * \frac{\frac{(Final\ Weight)^{1/3}}{(Initial\ Weight)^{1/3}}}{t}$  where  $t$  = total days of

experiment which was 56 days. DGC is the slope of the power growth curve multiplied by 100.

(6) Number of days to 500g (harvestable size) (DH) =  $100 * \frac{Desired\ Weight^{1/3} - Initial\ Weight^{1/3}}{DGC}$

(7) Feed Conversion Ratio = Weight of Feed/Weight of Product Produced (Inputs/Outputs)

### 3.3 Results and Discussion

#### 3.3.1 Experiment 1

#### 3.3.2 Comparison of Initial and Final Weights and Lengths

As confirmation that all treatments began the experiment with similarly sized fingerlings, initial weights or lengths of the fish in each experimental treatment were compared. The average fingerling lengths and weights for each of the five treatment populations at  $t=0$  were not significantly different (Figure 3.1).

Statistically significant differences were observed among the treatments in both weight and length at  $t=8$  weeks (56 days). Final average fish weights for Treatments 4 and 5 (75% and 100% replacement of fish meal with cricket powder, respectively) were significantly lower than the fish in the other treatments. In terms of length, there appeared to be a more monotonic decrease in fish

length with increasing replacement of fish meal with cricket powder (Figure 3.2, Tables 3.8 and 3.9).

As the rate of cricket meal substitution increased, the average length gain decreased, reflecting a negative correlation between cricket meal concentration and lengthwise fish growth ( $F(4,14)=20.13$ ,  $p<0.001$ ,  $R^2 = 0.8519$ ). In the ANOVA analysis, there were significant differences in average length change between treatments ( $F(4,14)=20.13$ ,  $p<0.001$ ). The Tukey test also showed reduced length growth as cricket meal concentration increased, confirming the linear regression test. No significant differences were observed in increases in length between the Control and Treatments 1 and 2, Treatments 1 and 2, and Treatments 2 and 3 ( $P= 0.556$ ,  $0.070$ ,  $0.644$ , and  $0.605$ , respectively) (Table 3.9). The fish in Treatments 3 and 4 exhibited significantly smaller length increases compared to the Control ( $p=0.004$  and  $<0.001$ , respectively). Fish in Treatment 4 were smaller in length at 56 days than those in Treatments 1, 2, and 3 ( $p<0.001$ ,  $<0.001$ , and  $0.005$ ). While the fish in Treatment 3 grew significantly more slowly than the fish on the Control diet, those fed the Treatment 4 diet grew at only half the rate as those in the Control 3 treatment. (Tables 3.8 and 3.9).

Increase in length represents skeletal growth, so smaller length changes might be caused by insufficient micronutrients or amino acids in cricket meal important for skeletal growth. For example, insufficient levels of Ca, which is essential for bone mineralization, and P can lead to lower bone growth (Robinson et al. 1987, Shiau and Tseng 2007). Even with enough Ca and P, if there are insufficient levels of vitamin D, those minerals may be less usable in bone mineralization and growth will be limited (Shiau and Hwang 1993). Feeding to satiation might help reduce this effect by increasing the overall concentration of these nutrients. However, another possibility is

the chitin in the cricket meal may be decreasing the digestibility of the measured protein in the cricket powder containing feeds or could be acting as an antinutrient, reducing nutrient absorption.

There was a negative correlation between cricket meal substitution and average weight gain ( $F(4,14)=26.93$ ,  $p<0.001$ ,  $R^2 = 0.8519$ ). The Tukey test of the ANOVA analysis showed no significant difference in the increase in fish weight between the Control and Treatment 1, Treatments 1 and 2, Treatments 1 and 3, and Treatments 2 and 3 ( $P = 0.126, 0.907, 0.102$ , and  $0.383$ , respectively) (Table 3.9). Weight gain for fish in Treatments 2-4 was significantly lower compared to the Control ( $p=0.027$ ,  $p<0.001$ ,  $p<0.001$ , respectively). The fish in Treatment 4 were significantly smaller than all other treatments ( $p<0.001$ ,  $<0.001$ , and  $p=0.001$ , respectively), exhibiting weight gains only half that of the fish in the next lowest group, Treatment 3. Lower weight gains indicate poorer muscle formation as cricket meal concentration increases above 10% of the diet, possibly due to difficulty in digesting the chitin protein or due to insufficient calories and nutrients by feeding only at 3% body weight, rather than to satiation. In any event, higher inclusion rates of cricket meal had notable negative effects on growth. The fish in Treatment 4 experienced weight and length gains less than 50% of those in Treatment 3 (Tables 3.8 and 3.9).

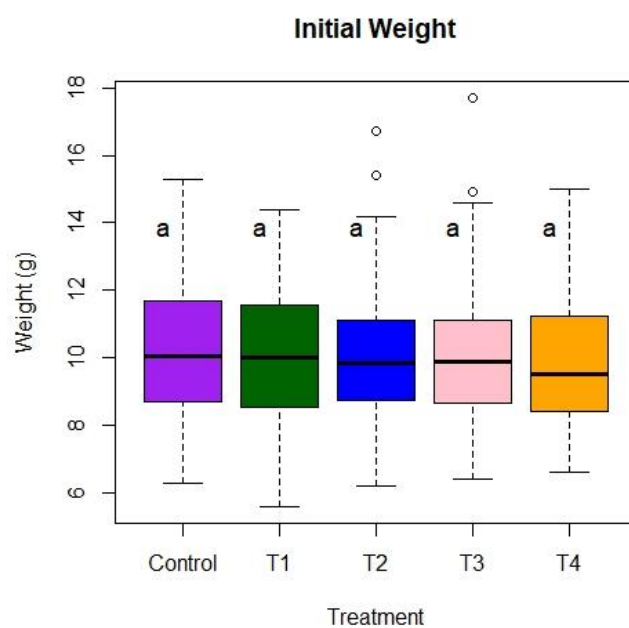


Figure 3.1 Initial Weight of Juvenile Tilapia in Experiment 1

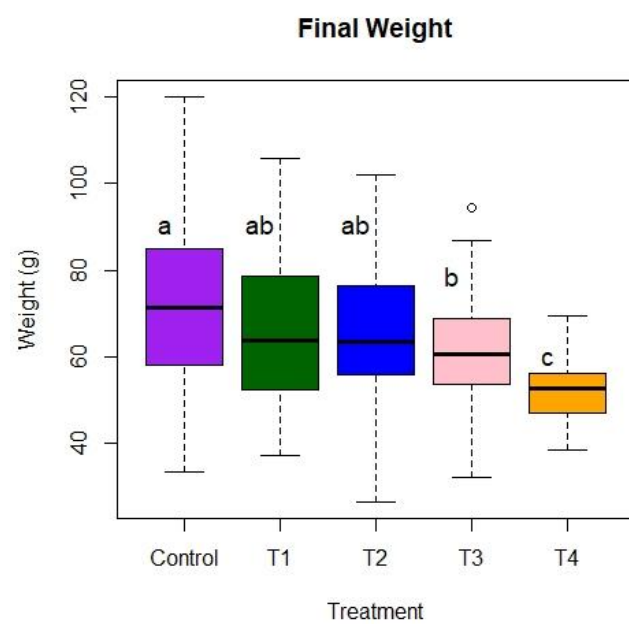


Figure 3.2 Final Weight of Juvenile Tilapia in Experiment 1

Table 3.8 Mean (Standard Deviation) Initial and Final Weight (g) and Length (mm) for Experiment 1

	Initial Weight	Initial Length	Final Weight	Final Length
Control	10.3 (1.99)	80.5 (4.88)	70.8 (19.4)	151 (13.8)
Treatment 1	10.2 (2.05)	80.2 (5.27)	66.3 (17.2)	148 (13.4)
Treatment 2	10.1 (2.01)	80.9 (5.01)	64.9 (14.7)	146 (11.9)
Treatment 3	10.2 (2.08)	81.1 (5.30)	61.7 (12.5)	144 (10.5)
Treatment 4	9.84 (1.91)	80.2 (5.04)	52.1 (7.23)	135 (7.5)

Table 3.9 Mean Weight (g), Length (mm) Gain, and Length-to-Weight Growth Ratio (LWGR) for Experiment 1

		Length Gain		Weight Gain		LWGR	
Groups		Mean Difference	p-value	Mean Difference	p-value	Mean Difference	p-value
Control	Treatment 1	-2.700	0.556	-4.429	0.126	0.044	0.146
	Treatment 2	-5.132	0.702	-5.901	0.027*	0.032	0.389
	Treatment 3	-7.684	0.004**	-9.076	<0.001***	0.057	0.041*
	Treatment 4	-15.70	<0.001***	-18.35	<0.001***	0.130	<0.001***
Treatment 1	Treatment 2	-2.434	0.644	-1.472	0.907	-0.012	0.962
	Treatment 3	-4.985	0.081	-4.647	0.102	0.013	0.947
	Treatment 4	-13.20	<0.001***	-13.92	<0.001***	0.086	0.003**
Treatment 2	Treatment 3	-2.551	0.605	-3.175	0.383	0.024	0.647
	Treatment 4	-10.77	<0.001***	-12.44	<0.001***	0.098	0.001**
Treatment 3	Treatment 4	-8.216	<0.001***	-9.269	0.001**	0.074	0.012**

\*p&lt;0.05, \*\*p&lt;0.01, \*\*\*p&lt;0.001

### 3.3.3 Length-Weight Relationship

The length-to-weight-growth ratio (LWGR), or the change in length divided by the change in weight as used in this analysis, increased as degree of cricket meal substitution increased. ANOVA analysis indicated LWGR differed significantly between treatments so that treatments with higher cricket meal concentrations had significantly larger LWGRs ( $F(4)=12.52$ ,  $P<0.001$ ). The Tukey test indicated no significant differences in LWGR between the Control and Treatments 1 and 2 ( $P = 0.146$  and  $0.389$ , respectively). Treatment 4 had a significantly larger LWGR than the Control and other treatments ( $P < 0.001$ ,  $0.003$ ,  $0.001$ , and  $0.012$ , respectively). In the linear model, there was a positive correlation between cricket meal concentration and LWGR ( $F(4,14)=12.52$ ,  $P<0.001$ ,  $R^2=0.7816$ ). As cricket meal concentration increased, weight gain compared to length gain decreased. With increasing cricket meal substitution for fish meal, both length and weight decreased compared to the Control, but weight decreased at a faster rate than length. Lower weight gain is related to reduced muscle gain, possibly resulting in smaller filets. This might be due to insufficient energy, protein, or micronutrients in the cricket meal or due to feeding at only a 3% of body weight rate rather than satiation.

The Condition Factor (K) was not significantly different between the experimental groups ( $F(4,639)=1.229$ ,  $p=0.298$ ). No significant differences were observed between the treatments and Control. As cricket meal concentration increased, the K coefficient increased slightly, but not significantly. Even though the basic LWGR was significantly different between the Control and Treatments 3 and 4, no significant differences in K coefficient were observed among the diet groups. Since the calculation of K involves cubing the length and multiplying the ratio by 100,000, inter-treatment differences were minimized. This indicates that, overall, cricket meal concentration did not significantly affect the K coefficient, i.e., the relationship between length and weight.



### 3.3.4 Projected Time to Harvest

No significant differences in the Daily Growth Coefficient (DGC) were observed between the Control and Treatments 1 and 2, Treatments 1 and 2, Treatments 1 and 3, or Treatments 2 and 3 ( $p = 0.549, 0.204, 0.943, 0.156, \text{ and } 0.454$ , respectively) (Table 3.10). The DGC was significantly larger for the Control fish compared to those in Treatments 4 and 5 ( $p = 0.009$  and  $<0.001$ , respectively). There were no significant differences in DGC between Treatments 1 and 3 and Treatment 2 and 3 ( $p = 0.156$  and  $0.454$ , respectively). Treatment 4 exhibited a significantly smaller DGC than Treatments 1-3 ( $p < 0.001, 0.001, \text{ and } 0.019$ , respectively). The DGC ranged in individual fish from the highest value of 3.46 in the control group to 3.04 in Treatment 4 (Figure 3.3, Table 3.11). As a comparable reference datapoint, tilapia raised by TilAqua and Zemach have respective DGCs of 3.70 and 2.88, and all treatments in Experiment 1 fall within this range (Terpstra 2015).

The projected number of days to harvest (DH) were not significantly different between the Control and Treatments 1 and 2, Treatments 1 and 2, Treatments 1 and 3, and Treatments 2 and 3 ( $P = 0.351, 0.098, 0.921, 0.105, \text{ and } 0.371$ , respectively) (Table 3.11, Figure 3.4). Treatments 1-3 would require fewer days to harvest than Treatment 4 ( $p < 0.001, <0.001, \text{ and } 0.002$ , respectively) (Table 3.11). The Control DH was 169 days, while the treatments increase in mean DH values as cricket meal concentration increases, from 173 DH for Treatment 1 to 186 days for Treatment 4 (Figure 3.4, Table 3.11). Once again, Treatment 4 had twice the mean difference from the Control compared to Treatment 3, with Treatment 4 having an average DH of 186 and Treatment 3 with an average DH of 177. Fish fed the Treatment 4 diet would take 10% more time to reach harvestable weight – 17 more days vs the 169 of the Control. While the results also indicate DH for Treatment 3 may be significantly different than the Control, it may be biologically sufficient as a replacement for commercial feed with only five additional days to harvestable size. For both

the DGC and DH, there may be estimation errors due to the model design. However, the results allow useful comparisons between groups and provide an indication of potential grow-out times.

As cricket meal concentration increased, there was an increase in the estimated number of days until tilapia reached minimum harvestable size of 500g. In a commercial aquaculture setting, the harvestable size is larger (1000-2000g), lengthening the time to harvest for fish fed higher amounts of cricket meal and resulting in economic losses due to increased feed costs and reduced harvest cycles. Commercial systems could handle feed with up to 50% cricket meal substitution without significant differences in harvest time and fish growth. In development settings, fish farmers may be able to absorb the costs of increased harvest time and decreased fish growth if those costs do not outweigh commercial feed costs. Since in many developing countries the preferred harvest size is only 500 g, this could also facilitate the use of higher cricket meal utilization in the feeds.

Table 3.10 Mean Daily Growth Coefficients (DGC) and Days to Harvest for Tilapia Fed Graded Concentrations of Cricket Meal

Group	DGC	DH
Control	3.40 (0.038)*	169 (1.68)
Treatment 1	3.35 (0.067)	173 (2.49)
Treatment 2	3.32 (0.045)	175 (2.40)
Treatment 3	3.26 (0.039)	177 (2.24)
Treatment 4	3.11 (0.066)	186 (3.12)

\*Mean (SD)

Table 3.11 Statistical Comparisons of Mean Daily Growth Coefficients (DGC) and Days to Harvest for Tilapia Fed Graded Concentrations of Cricket Meal

		DGC		Days to Harvest	
Groups		Mean Difference	p-value	Mean Difference	p-value
Control	Treatment 1	-0.056	0.549	3.224	0.351
	Treatment 2	-0.083	0.204	4.594	0.098
	Treatment 3	-0.146	0.009*	7.749	0.003**
	Treatment 4	-0.288	<0.001***	16.68	<0.001***
Treatment 1	Treatment 2	-0.027	0.943	1.370	0.921
	Treatment 3	-0.089	0.156	4.525	0.105
	Treatment 4	-0.232	<0.001***	13.46	<0.001***
Treatment 2	Treatment 3	-0.062	0.454	3.155	0.371
	Treatment 4	-0.205	0.001**	12.09	<0.001***
Treatment 3	Treatment 4	-0.143	0.019*	8.933	0.002**

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001

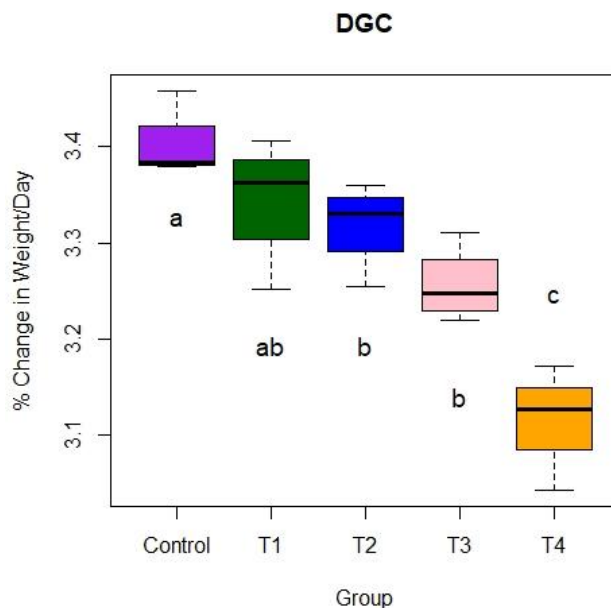


Figure 3.3 Daily Growth Coefficients (DGC) for Tilapia Fed Graded Concentrations of Cricket Meal

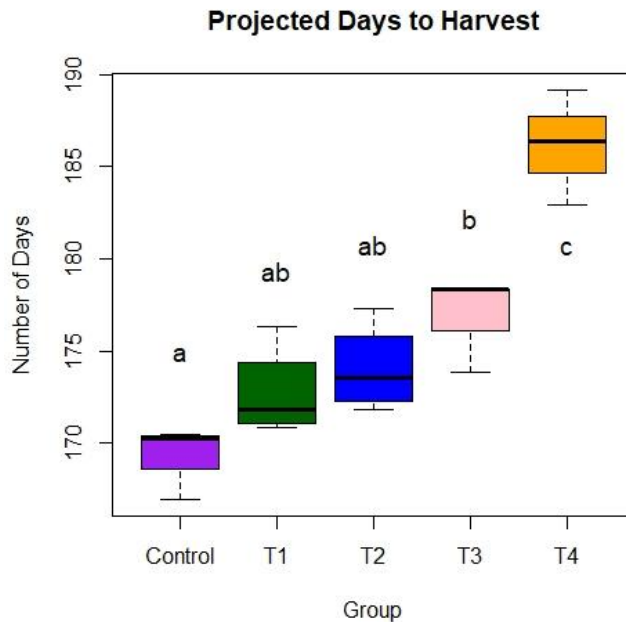


Figure 3.4 Mean Projected Days to Harvest for Tilapia Fed Graded Concentrations of Cricket Meal

### 3.3.5 Growth Rates

Absolute growth rate, AGR, was analyzed by performing an ANOVA between AGR and treatment. There was a significant difference between groups for AGR ( $F(4,16)=26.93$ ,  $p<0.001$ ). No significant differences in AGR were observed between Control 1 and Treatment 1, Treatments 1 and 2, Treatments 1 and 3, and Treatments 2 and 3 ( $P = 0.126$ ,  $0.907$ ,  $0.102$ , and  $0.383$ , respectively) (Figure 3.5, Tables 3.12, 3.13). The AGR for Treatments 2-4 were significantly than Control 1 (Figure 2.5). The AGR for Treatment 4 was significantly lower than for the other diets in this experiment.

Specific growth rate, SGR, was significantly different among the diet groups ( $F(4,16)=15.63$ ,  $p<0.001$ ) (Figure 3.6, Table 3.12). No significant differences in SGR were observed between Control 1 and Treatment 1, Control 1 and Treatment 2, Treatments 1 and 2, Treatments 1 and 3, and Treatments 2 and 3 ( $P = 0.570$ ,  $0.223$ ,  $0.948$ ,  $0.164$ , and  $0.460$ , respectively) (Figure 6).

AGR values for Treatments 3 and 4 were significantly smaller compared to Control 1 ( $p = 0.011$  and  $p < 0.001$ , respectively). AGR for Treatment 4 was significantly smaller compared to the control and the other treatments ( $p < 0.001$ , except Treatment 3 ( $p = 0.016$ )) (Figure 3.6).

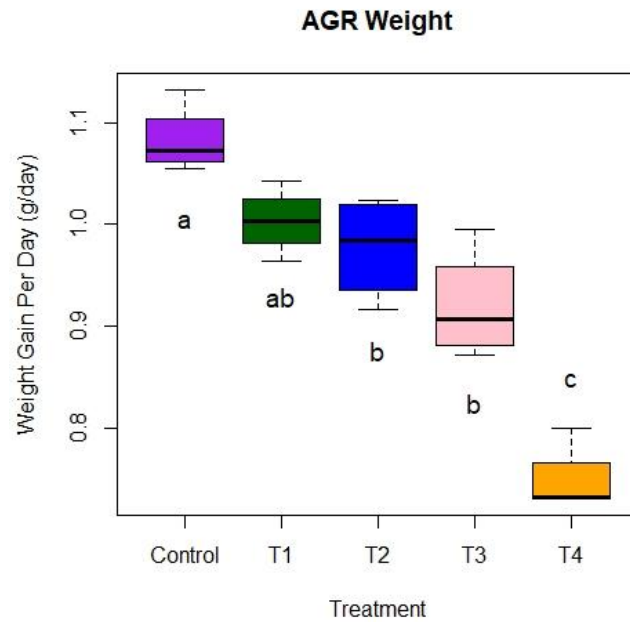


Figure 3.5 Mean Absolute Growth Rate Weight for Tilapia Fed Graded Concentrations of Cricket Meal

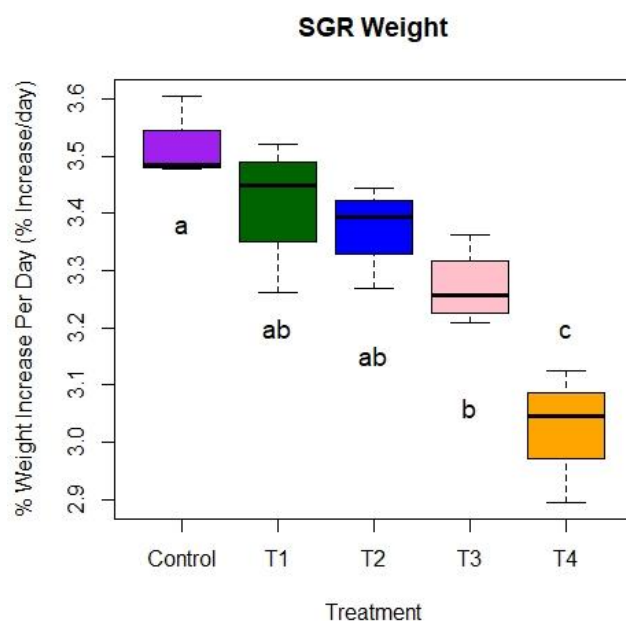


Figure 3.6 Mean Specific Growth Rate of Tilapia Fed Graded Concentrations of Cricket Meal

Table 3.12 Statistical Comparisons of AGR and SGR for Tilapia Fed

		AGR		SGR	
Groups		Mean Difference	p-value	Mean Difference	p-value
Control	Treatment 1	-0.079	0.126	-0.093	0.570
	Treatment 2	-0.105	0.027*	-0.137	0.223
	Treatment 3	-0.162	<0.001***	-0.242	0.011*
	Treatment 4	-0.328	<0.001***	-0.491	<0.001***
Treatment 1	Treatment 2	-0.026	0.907	-0.044*	0.948
	Treatment 3	-0.083	0.102	-0.149	0.164
	Treatment 4	-0.249	<0.001***	-0.398	<0.001***
Treatment 2	Treatment 3	-0.057	0.383	-0.105	0.460
	Treatment 4	-0.222	<0.001***	-0.353	<0.001***
Treatment 3	Treatment 4	-0.166	0.001**	-0.248	0.016*

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001

The results indicate that substituting 25% and 50% cricket meal for fish meal (i.e., Treatments 1 and 2) does not significantly affect the specific growth rate compared to 100% fish meal as the protein source (i.e., the control in this experiment. While small reductions in growth rates were observed which may affect harvest timing, replacing up to 50% of fish meal with cricket powder would reduce the costs associated with making feed in developing countries without significantly affecting production yield. Future experiments to feed fish to satiation with the treatments may provide a more complete picture since differences between groups may be partially mitigated with increased protein and micronutrient concentration through increased feed intake.

The SGR analysis showed no significant difference between the fish in Treatment 2 compared to the Control, unlike the AGR analysis which did show a significant difference between the two treatments. AGR may be a standard method of growth measurement used for aquaculture but has a systematic error in underestimating intermediate data points between two time points. This is due to the assumption of a linear relationship between growth and time (g/day) and especially underestimates weight relationships (Hopkins 1992, Lugert 2015). There is typically an inflection point in the weight vs time relationship where the growth rate changes as fish develop and age (Lugert 2015). The data before the inflection point is overestimated by the AGR analysis, while those after it are underestimated. Therefore, AGR is not recommended for long-term experiments and for projecting future growth. Instead, it is useful for shorter portions of growth curves, shorter experiments, and correspondent studies (Lugert 2015). SGR is more flexible and is useful for comparing fish groups in nutrition experiments and for juvenile fish in their exponential phase of growth. SGR, using an exponential function, is still imprecise, potentially leading to underestimation of intermediate data and overestimation of future growth (Lugert 2015).

Comparing a logistic function, a Gompertz function, a von Bertalanffy growth function, and the Schnute function would greatly reduce over- and underestimation. However, with only two time points (before and after the experiment), these functions would still result in some type of estimation error (Lugert 2015). For this reason, and the fact this was a nutritional study, the SGR was used for comparisons between treatments.

### 3.3.6 Feed Conversion Ratio

The feed conversion ratio (FCR) was significantly different between groups ( $F(4,14) = 31.43$ ,  $p < 0.001$ ). Treatments 1 and 2 were not significantly different from the control for diet FCRs in fish ( $p = 0.2843$  and  $0.0773$ , respectively). Treatments 3 and 4 had significantly larger FCRs compared to the control ( $p = 0.003$  and  $< 0.001$ , respectively).

Table 3.13 Mean (Standard Deviation) of FCRs

Group	Means (SD)
Control	1.17 (0.036)
Treatment 1	1.26 (0.040)
Treatment 2	1.30 (0.068)
Treatment 3	1.38 (0.078)
Treatment 4	1.68 (0.085)



Table 3.14 Statistical Comparison of FCRs Between Groups

FCR			
Groups	Group Comparison	Mean Difference	p-value
Control	Treatment 1	0.092	0.284
	Treatment 2	0.128	0.077
	Treatment 3	0.208	0.003**
	Treatment 4	0.509	< 0.001***
Treatment 1	Treatment 2	0.036	0.926
	Treatment 3	0.116	0.122
	Treatment 4	0.417	<0.001***
Treatment 2	Treatment 3	0.081	0.406
	Treatment 4	0.381	<0.001***
Treatment 3	Treatment 4	0.301	<0.001***

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001

### **3.3.7 Experiment 2**

### **3.3.8 Comparison of Feed Nutrient Compositions**

All measured nutrients (Midwest Laboratories) for the feeds developed for Experiment 2 were provided above in Table 3.6. Fat increased as cricket meal concentration increased, resulting in 8.59% fat content for the Control diet and 18.2% for the Treatment 4 diet. This is likely related to the high fat content of the cricket meal (15.9% fat content) compared to the 9.3% fish meal fat concentration. It could also be the result of the feed preparation since increasing cricket meal levels resulted in a drier mix (hydrophobic) and required a few extra grams of vegetable oil and water to hold the material together for pelletizing. Total digestible nutrients, digestible energy, and tyrosine also increased with increasing cricket meal levels (Midwest Laboratories). Methionine levels decreased with increasing cricket meal content.

Phosphorus and potassium concentrations for Treatments 1 and 2 were both higher than the Control. Phosphorus content was lowest in Treatments 3 and 4, while potassium remained higher in Treatments 3 and 4 compared to the Control. Calcium content decreased with increasing cricket meal content, resulting in the lowest value in Treatment 4 (0.2% vs the Control value of 1.93%). Protein content was comparable between all groups, ranging from 33.7% to 36.4%. Differences between Treatments 3 and 4 compared to the Control could be due to their lower calcium and phosphorus concentrations which are necessary for fish growth, energy use, and bone growth, resulting in lower growth rates (Robinson et al. 1987).

### **3.3.9 Comparison of Initial and Final Weights and Lengths**

Initial weights of fingerlings were not significantly different between treatments or replicates, eliminating initial weight as a variable ( $F(6,14)=0.417$ ,  $p=0.856$ ) as shown in Figure

3.7 and Table 3.14. Final fish weights were significantly different between treatments ( $F(6,14)=55.32$ ,  $p<0.001$ ), as shown in Figure 2.8. Initial lengths also were not significantly different between treatments ( $F(6,14)=0.372$ ,  $p=0.885$ ), while final lengths were significantly different between treatments ( $F(6,14)=87.71$ ,  $p<0.001$ ). The final lengths of fish fed Control diet 1 were significantly greater than those for the Control 2 diet. However, the final weights of the Control 1 and 2 diets were not significantly different ( $p=0.021$  and  $0.262$ , respectively). (Table 3.15) The weight of the fish in Control 1 were significantly different from those in Treatment 1, but the length of the fish was not ( $p=0.015$  and  $0.051$ , respectively). Neither the weight nor length of the fish in Control 2 were significantly different compared to those in Treatment 1 ( $p=0.646$  and  $0.999$ , respectively). Final weights and lengths provide an incomplete analysis of fish growth since they do not incorporate initial weight/length ratios.

Weight gain between the groups was significantly different, reflecting the final weight differences ( $F(6,14)=60.46$ ,  $p<0.001$ ) (Figure 3.9). The final weights of the fish fed the Controls 1 and 2 diets were not significantly different compared to each other ( $p=0.158$ ), while the fish in all treatments exhibited significantly smaller weight gains than Control 1 ( $p<0.001$ ,  $0.008$ ,  $<0.001$ , and  $<0.001$ , respectively).

Table 3.15 Mean (SD) Initial and Final Weight (g) and Length (mm) Values for Experiment 2

	Initial Weight	Initial Length	Final Weight	Final Length
Control 1	15.6 (4.45)	96.6 (9.43)	109.0 (30.6)	177 (18.4)
Control 2	16.5 (5.98)	97.3 (10.8)	95.9 (31.6)	163 (16.3)
Control 3	15.1 (5.84)	94.7 (12.4)	60.4 (19.7)	148 (15.8)
Treatment 1	16.0 (6.18)	96.1 (11.8)	85.0 (25.6)	165 (17.5)
Treatment 2	15.2 (6.48)	94.1 (12.0)	30.8 (12.3)	114 (13.6)
Treatment 3	15.2 (6.27)	95.2 (12.5)	41.8 (18.7)	129 (17.6)
Treatment 4	15.0 (5.14)	94.7 (10.4)	31.4 (11.9)	116 (13.0)

Table 3.16 Mean Weight and Length Gain for Experiment 2

Groups	Group Comparison	Length Gain (mm)		Weight Gain (g)	
		Mean Difference	p-value	Mean Difference	p-value
Control 1	Control 2	-15.52	0.003**	-15.72	0.168
	Control 3	-28.04	<0.001***	-49.76	<0.001***
	Treatment 1	-12.50	0.016*	-25.54	0.008**
	Treatment 2	-61.45	<0.001***	-79.55	<0.001***
	Treatment 3	-46.84	<0.001***	-67.82	<0.001***
	Treatment 4	-59.69	<0.001***	-78.71	<0.001***
Control 2	Control 3	-12.52	0.016	-34.05	<0.001***
	Treatment 1	3.020	0.951	-9.82	0.633
	Treatment 2	-45.93	<0.001***	-63.83	<0.001***
	Treatment 3	-31.32	<0.001***	-52.11	<0.001***
	Treatment 4	-44.17	<0.001***	-62.99	<0.001***
Control 3	Treatment 1	15.54	0.003**	24.22	0.013 *
	Treatment 2	-33.41	<0.001***	-29.79	0.002**
	Treatment 3	-18.80	<0.001***	-18.06	0.086
	Treatment 4	-31.65	<0.001***	-28.94	0.003**
Treatment 1	Treatment 2	-48.95	<0.001***	-54.01	<0.001***
	Treatment 3	-34.34	<0.001***	-42.28	<0.001***
	Treatment 4	-47.19	<0.001***	-53.17	<0.001***
Treatment 2	Treatment 3	14.61	0.005**	11.73	0.446
	Treatment 4	1.76	0.997	0.84	1.000
Treatment 3	Treatment 4	-12.85	0.013*	-10.88	0.527

\*p&lt;0.05, \*\*p&lt;0.01, \*\*\*p&lt;0.001

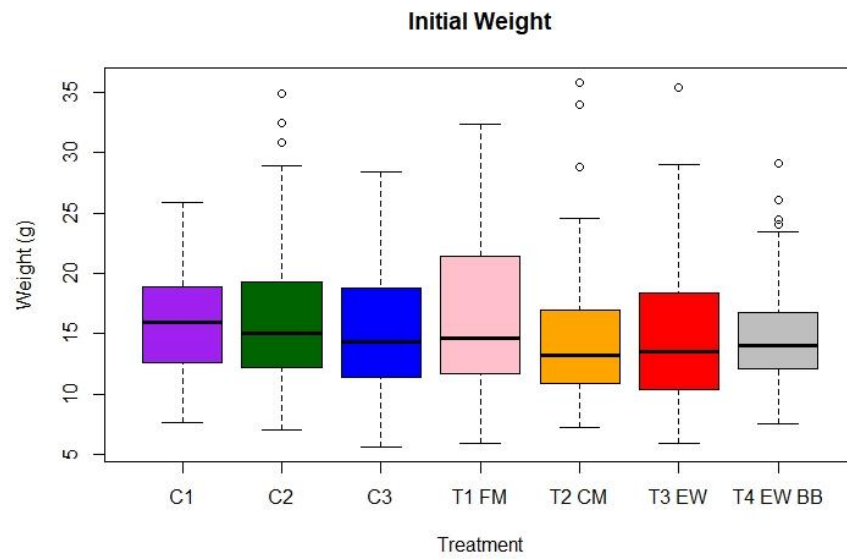


Figure 3.7 Initial Weight of Juvenile Tilapia in Experiment 2

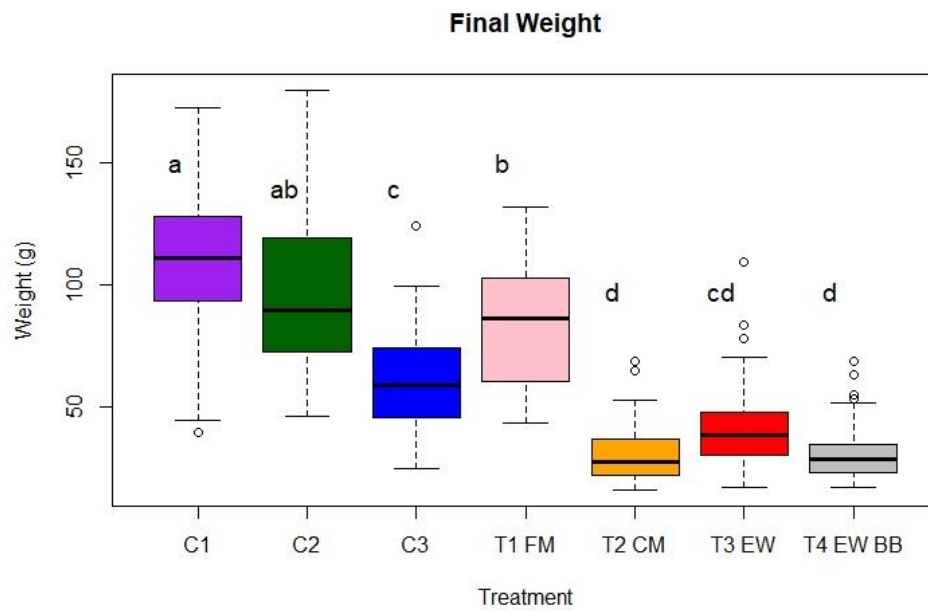


Figure 3.8 Final Weight of Juvenile Tilapia in Experiment 2

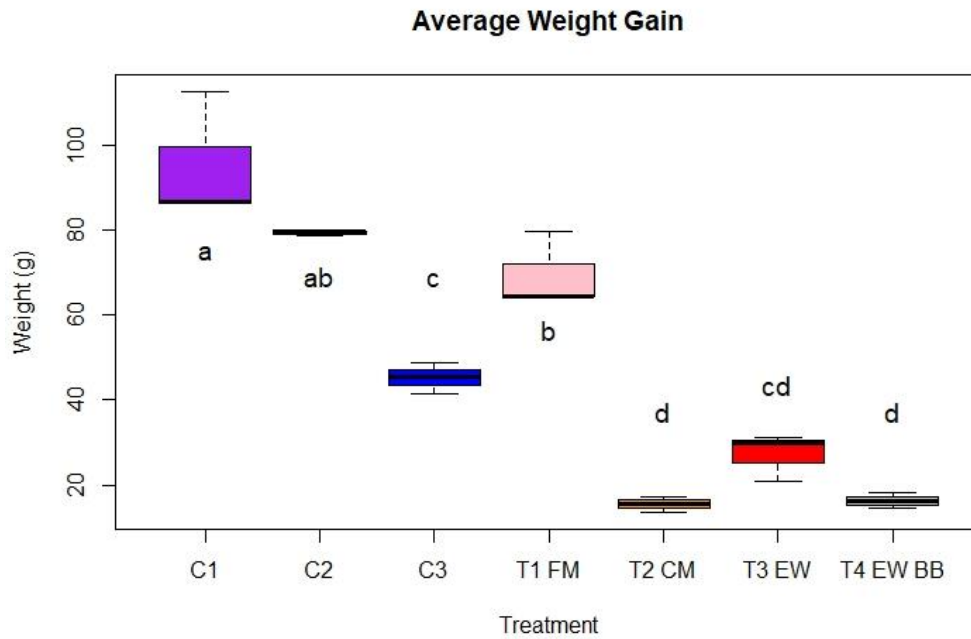


Figure 3.9 Average Weight Gain of Juvenile Tilapia in Experiment 2

### 3.3.10 Length-Weight Relationship

Differences among the treatments for the basic length-to-weight ratio (LWR) were significant ( $F(6,14)=52.28$ ,  $p<0.001$ ). There were no significant differences in LWR between Controls 1 and 2, and Control 1 and Treatment 1 ( $p=0.999$  and  $0.575$ , respectively). Control 1 had the smallest LWR values with Treatments 2-4 having more than twice the LWR of Control 1.

Relative to Fulton's Constant, or condition factor (K), the K coefficients were significantly different among the groups ( $F(6,14) = 21.24$ ,  $p < 0.001$ ). All groups had K values less than 3, making them poorly balanced in growth between length and weight. A value of 3 is considered isometric, or optimal allometric, growth so that the fish does not grow unbalanced between the two dimensions. Interestingly, the results indicate that Control 2 (mean = 2.2) and Treatment 2 (mean = 2.05) had the closest K values to the optimal allometric value. Control 1 and Treatment 1

were both below an average of 2.0. However, as explained in the Experiment 1 section, feeding rate can impact K values.

### **3.3.11 Projected Time to Harvest**

The DGC values were significantly different among the groups ( $F(6,14) = 68.07, p < 0.001$ ) with significantly larger values for Controls 1 and 2 than for the treatments and Control 3. The DGC for Control 1 was significantly greater than that of Treatment 1 at a significance level of 0.05 ( $p = 0.029$ ) (Figure 3.10, Table 3.16). However, the mean difference was -0.298 compared to Control 2 with a mean difference of -0.215 (Table 3.17).

DH was significantly smaller for the controls compared to the four treatments ( $F(6,14) = 112.8, p < 0.001$ ). DH values ranged from 150 DH for Control 1 to 242 DH for Treatment 2 (Figure 3.11, Table 3.16). There were no significant differences in DH between the Control 1 and either Control 2 or Treatment 1 diets ( $p = 0.475$  and  $0.078$ , respectively) (Figure 3.11, Table 3.17). There were significant differences between the DH values for Control 1 and Treatments 2-4 with differences in DH of 83, 59, and 81 days, respectively ( $p < 0.001$  for all three treatments). The DH for Treatment 3 was significantly shorter compared to Treatments 2 and 4 (20-24 days earlier estimated harvest). However, the DH for Control 3 was significantly longer than that of Control 1, with a mean difference of 34 days ( $p < 0.001$ ).

Interestingly, while DGC values for the fish in Treatment 1 were significantly lower than for those in Control 1, there was no significant difference in DH between the two groups. This indicates that small statistical differences in DGC may not translate to biological significance, i.e., harvest dates may be similar despite having small differences in DGC. However, in general, and with much larger absolute differences in DGC values, high DGC indicates faster growth, which leads to shorter grow-out periods and earlier harvest (e.g., the large DGC differences between the

Control 1 diet and Treatments 2-4 result in large differences in projected harvest dates). This reduces feed costs due to feeding fewer days and increases the annual number of grow-out cycles and harvests for fish farms.

The DH was 169 days for the Control in Experiment 1 and 168 days for Control 2 in Experiment 2 (i.e., virtually identical values). Since these two feeds had identical feed formulations, the results indicate a high degree of inter-experiment consistency. Also, in Experiment 1, DH for Treatments 1-3 ranged from 173-177 days, while Treatment 1 in Experiment 2 had almost identical DH (173 days). This indicates that the effect on fish growth of replacing 25-75% of the fish meal with cricket meal is similar to that of replacing the commercial micronutrients and carbohydrates with local ingredients in the simulated commercial feed formula. For all graphs for Experiment 2, notation is the following: C1 (Control 1-Commercial Feed), C2 (Control 2-Homemade Control), C3 (Negative Control-Algae), T1-FM (Treatment 1-Local Ingredients with Fish Meal), T2-CM (Treatment 2-Local Ingredients with Cricket Meal), T3-EW (Treatment 3-Local Ingredients with Earthworm Meal), and T4-EW BB (Treatment 4-Local Ingredients with Earthworm and Black Bean Meal).

Table 3.17 Comparison of DGC and DH Values for the Diets in Experiment 2

	DGC	DH
Control 1	3.43 (0.182)*	159 (7.41)
Control 2	3.21 (0.028)	168 (0.87)
Control 3	2.84 (0.083)	193 (4.33)
Treatment 1	3.13 (0.147)	173 (5.49)
Treatment 2	2.26 (0.0141)	242 (4.34)
Treatment 3	2.51 (0.049)	218 (8.57)
Treatment 4	2.28 (0.054)	240 (4.85)

\*Mean (SD)



Table 3.18 Tukey Post-Hoc Results DGC and DH

Groups	Group Comparisons	DGC		DH	
		Mean Difference	p-value	Mean Difference	p-value
Control 1	Control 2	-0.215	0.174	8.996	0.475
	Control 3	-0.592	<0.001***	33.91	<0.001***
	Treatment 1	-0.298	0.029*	14.48	0.078
	Treatment 2	-1.169	<0.001***	83.00	<0.001***
	Treatment 3	-0.917	<0.001***	58.80	<0.001***
	Treatment 4	-1.144	<0.001***	80.70	<0.001***
Control 2	Control 3	-0.376	0.005**	24.92	0.001**
	Treatment 1	-0.083	0.938	5.486	0.884
	Treatment 2	-0.954	<0.001***	74.00	<0.001***
	Treatment 3	-0.701	<0.001***	49.81	<0.001***
	Treatment 4	-0.929	<0.001***	71.70	<0.001***
Control 3	Treatment 1	0.294	0.033*	-19.43	0.011
	Treatment 2	-0.578	<0.001***	49.08	<0.001***
	Treatment 3	-0.325	0.016*	24.89	0.001**
	Treatment 4	-0.552	0.001**	46.78	<0.001***
Treatment 1	Treatment 2	-0.871	<0.001***	68.52	<0.001***
	Treatment 3	-0.619	<0.001***	44.32	<0.001***
	Treatment 4	-0.846	<0.001***	66.22	<0.001***
Treatment 2	Treatment 3	0.253	0.080	-24.20	0.002**
	Treatment 4	0.025	0.999	-2.300	0.998
Treatment 3	Treatment 4	-0.227	0.137	21.89	0.004**

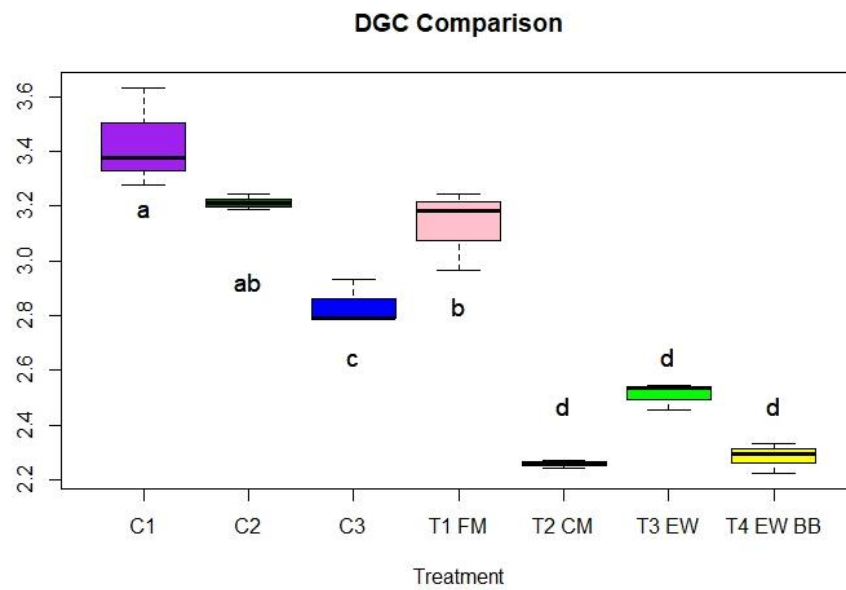


Figure 3.10 DGC Values for the Diets in Experiment 2

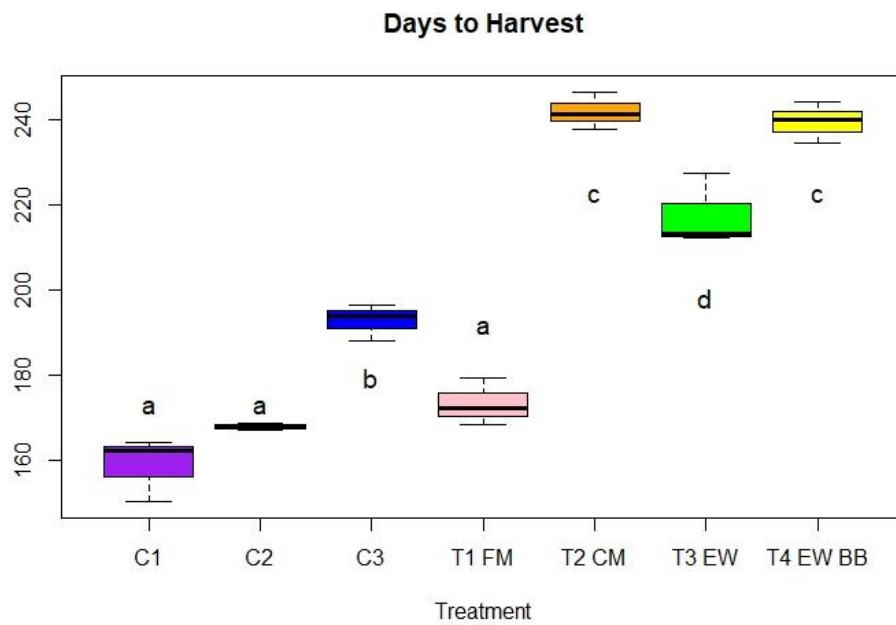


Figure 3.11 DH for the Diets in Experiment 2

### 3.3.12 Growth Rates

AGR was analyzed by performing an ANOVA between AGR and treatment. Significant differences were observed between the various treatments and controls ( $F(6,14) = 60.46$ ,  $p < 0.001$ ) (Figure 3.11). No significant differences were observed between Controls 1 and 2, Control 2 and Treatment 1, Control 3 and Treatment 3, Treatments 2 and 3, Treatments 2 and 4, and Treatments 3 and 4 ( $P = 0.168, 0.633, 0.086, 0.446, 0.999$ , and  $0.527$ , respectively). AGR for Control 1 was significantly greater than for Treatment 1 ( $p = 0.009$ ) (Figure 3.12, Table 3.18). However, there was only a mean difference of  $-0.456$  compared to the mean difference with Control 2 of  $-0.281$ . AGR may be statistically different, but not biologically significant. The AGR for Treatment 1 was significantly higher than the negative Control 3 and Treatments 2, 3, and 4, making it a feasible feed option and showing the efficacy of local ingredients combined with a highly digestible protein (i.e., fish meal, in this case) (all at  $p < 0.001$ ).

SGR was analyzed by performing an ANOVA between SGR and treatment. A TukeyHSD test allowed for a Tukey multiple comparison of means between treatments at a 95% confidence level. Once again, there were significant differences between the SGR values for the controls and treatments ( $F(6,14) = 85.51$ ,  $p < 0.001$ ) (Figure 3.12). No significant differences in SGR were observed between Controls 1 and 2, Control 2 and Treatment 1, and Treatments 2 and 4 ( $p = 0.228, 0.934$ , and  $0.999$ , respectively) (Table 3.18).

The commercial control (Control 1) and simulated commercial feed control (Control 2) are comparable, indicating that the simulated commercial feed in the first experiment was a viable control comparison for the treatments (Table 3.4). Growth rates for Control 3, the negative control, were significantly smaller than Controls 1 and 2, confirming that simulated algae in fertilized ponds would result in slower fish growth compared to commercial feeds, even under optimistic conditions. Both SGR and AGR values for Control 3 were significantly lower compared to

Treatment 1. AGR for Control 3 was significantly greater than that of Treatment 3 This might be due to digestibility issues with Treatments 2-4 or Control 3 providing excess nutrition compared to a fertilized pond situation.

Fish were fed the Control 3 diet at the same rate as the other feeds in Experiment 2 and the feed was provided in aggregated form, improving ease of access and consumption. In an extensive pond setting, however, algae availability would be significantly lower than with the Control 3 diet under the current experimental conditions (i.e., the algae for Control 3 were concentrated and fed at a level higher vs. what fish would experience in a fertilized pond, and the water had a higher DO level than a non-aerated pond). Consequently, in an extensive pond setting, stocked tilapia would have less available food compared to the 3% of body weight feeding rate in this experiment. Nutrition of the algae in such a pond would be greatly diminished compared to the concentrated algae powder used in Control 3, further decreasing fish growth. However, this data indicates that cultured algae ingredients may be locally produced by farmers and become a viable sustainable fish feed ingredient.

Treatment 1 resulted in significantly faster growing fish with respect to both AGR and SGR compared to fish fed the diets in Treatments 2-4 ( $p = 0.013$  and  $0.025$ ) (Table 3.18, Figures 3.12, 3.13). Additionally, AGR and SGR for Treatment 1 were not significantly different compared to Control 2 ( $p = 0.228$ , respectively), suggesting that the local ingredients (non-fish meal ingredients) in Treatment 1 are comparable to conventional supplemental feed ingredients used in Control 2. The only differing ingredient between Treatment 1 and Treatments 2-4 was the selected protein source (i.e., cricket, earthworm, and black beans).

This indicates that the chosen protein source can significantly affect the fish growth efficiency of a local-ingredient feed. Previous research has shown reduced digestibility of

earthworms and house crickets (*Acheta domesticus*) due to chitinous exoskeletons or other antinutrients, but for this experiment, the most basic version of the protein sources, similar to what would be available for use by farmers in Guatemala, formed the basis for the Treatments 2, 3, and 4. Additionally, black beans contain antinutrients like lectins that have the potential to inhibit nutrient absorption or digestion. Treatment 1, while not significantly different than Control 2 in fish growth rate, was significantly different compared to Control 1, possibly due to antinutrients present in the local ingredients. For example, moringa leaves consist of 2.75% phenolics, 6.38% saponins, 2.25% phytic acid, and 0.53% tannins (Richter et al. 2002).

Table 3.19 Tukey Post-Hoc Results AGR and SGR

Groups	Group Comparison	AGR		SGR	
		Mean Difference	p-value	Mean Difference	p-value
Control 1	Control 2	-0.281	0.168	-0.355	0.228
	Control 3	-0.889	<0.001***	-1.043	<0.001***
	Treatment 1	-0.456	0.008**	-0.503	0.039*
	Treatment 2	-1.421	<0.001***	-2.284	<0.001***
	Treatment 3	-1.211	<0.001***	-1.708	<0.001***
	Treatment 4	-1.405	<0.001***	-2.225	<0.001***
Control 2	Control 3	-0.608	<0.001***	-0.688	0.004**
	Treatment 1	-0.175	0.633	-0.148	0.934
	Treatment 2	-1.140	<0.001***	-1.930	<0.001***
	Treatment 3	-0.930	<0.001***	-1.353	<0.001***
	Treatment 4	-1.125	<0.001***	-1.870	<0.001***
Control 3	Treatment 1	0.433	0.013*	0.540	0.024*
	Treatment 2	-0.532	0.002**	-1.241	<0.001***
	Treatment 3	-0.322	0.086	-0.666	0.005**
	Treatment 4	-0.517	0.002	-1.182	<0.001***
Treatment 1	Treatment 2	-0.964	<0.001***	-1.782	<0.001***
	Treatment 3	-0.755	<0.001***	-1.206	<0.001***
	Treatment 4	-0.950	<0.001***	-1.722	<0.001***
Treatment 2	Treatment 3	0.209	0.446	0.576	0.015*
	Treatment 4	0.015	0.999	0.060	0.999
Treatment 3	Treatment 4	-0.194	0.527	-0.517	0.033*

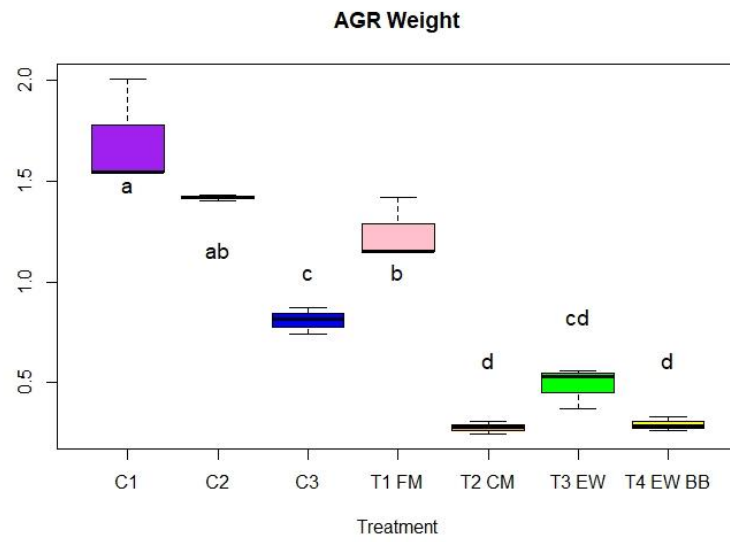


Figure 3.12 AGR Values for Juvenile Tilapia in Experiment 2

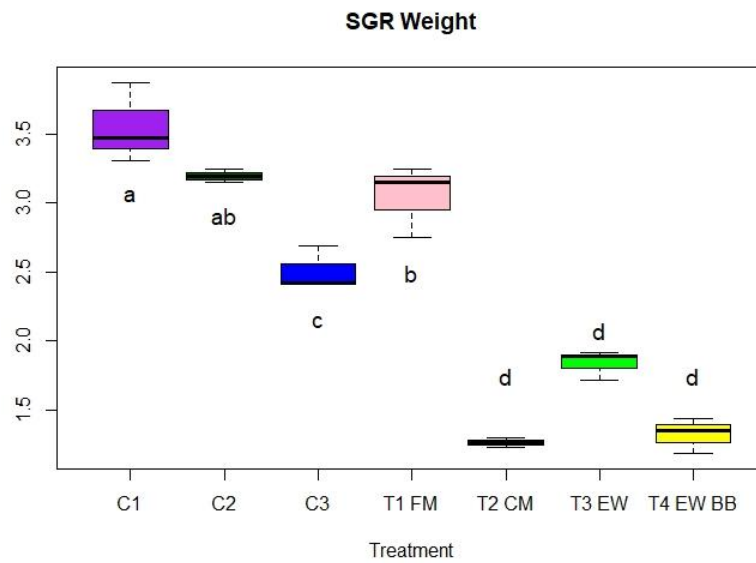


Figure 3.13 SGR Values for Juvenile Tilapia in Experiment 2

### 3.3.13 Feed Conversion Ratio

The feed conversion ratio (FCR) is the ratio of inputs to outputs and is related to how efficient an animal is at utilizing the energy and protein of feeds consumed (Table 3.19). The greater the efficiency (i.e., the smaller the FCR), the more the feed is utilized and not wasted and the more cost-effective and sustainable a cultured animal. On average, fish have a smaller food conversion ratio (1.6) and greater feed efficiency (62.5%) compared to other animal-based protein sources such as beef (9.0 and 11.1%), hog (4.9 and 20.4%), and chicken (2.4 and 41.7%) (Ensminger and Olentine 1978, Parker 1987).

The FCR (feed conversion ratio) is smaller, in part, due to the reduced feeding amount since FCR is related to feeding rate (de Verdal et al. 2018). In these experiments, daily feeding rate was 3% of body weight, over 2 feeding periods a day, compared to the preferred rate of 1.5-5% over 3-4 feeds per day (Ng and Romano 2013). Feed cost is the largest cost of a fish farm with crude protein being the costliest part of feed (El-Sayed 1999, Craig 2009). There is also a balance between the optimal feeding rate minimizing the feed conversion rate and the feeding rate that would maximize fish growth, with an inflection point in crude protein levels such that up to that point growth rate increases and after which growth rate decreases (Lovell 1989, Kim et al. 2002).

FCR values for the experimental diets in Experiment 2 are provided in Table 3.19. FCR values for fish in Controls 1-3 and Treatment 1 were smaller than the FCR values for fish (1.6) reported by Ensminger and Olentine (1978) as well as Parker (1987). Treatments 2-4 had higher values ranging from 2.68 to 4.59. There were significant differences between the groups ( $F(6,14) = 58.04, p < 0.001$ ). Treatment 1 was not significantly different than the controls while fish in the other treatments were, indicating Treatment 1 had comparable feed utilization and efficiency to



the controls and to the reported value above. Treatments 2 and 4 had the largest mean differences from Control 1 (Table 3.20).

Table 3.20 FCR Values for Fish Fed the Diets in Experiment 2

Group	FCR
Control 1	0.76 (0.11)
Control 2	0.89 (0.01)
Control 2	1.57 (0.126)
Treatment 1	1.03 (0.12)
Treatment 2	4.59 (0.53)
Treatment 3	2.68 (0.63)
Treatment 4	4.36 (0.50)

Table 3.21 FCR Mean Differences and P-values among Groups

FCR			
Groups	Group Comparison	Mean Difference	p-value
Control 1	Control 2	0.14	0.999
	Control 3	0.81	0.178
	Treatment 1	0.27	0.968
	Treatment 2	3.84	<0.001***
	Treatment 3	1.93	<0.001***
	Treatment 4	3.60	<0.001***
Control 2	Control 3	0.68	0.343
	Treatment 1	0.14	0.999
	Treatment 2	3.70	<0.001***
	Treatment 3	1.79	<0.001***
	Treatment 4	3.46	<0.001***
Control 3	Treatment 1	-0.54	0.583
	Treatment 2	3.02	<0.001***
	Treatment 3	1.11	0.032
	Treatment 4	2.79	<0.001***
Treatment 1	Treatment 2	3.56	<0.001***
	Treatment 3	1.66	0.001**
	Treatment 4	3.33	<0.001***
Treatment 2	Treatment 3	-1.91	<0.001***
	Treatment 4	-0.24	0.984
Treatment 3	Treatment 4	1.67	0.001**

### **3.3.14 Effects of Experimental Diet Nutritional Differences**

Referring to the nutrient content of the diets in Experiment 2 (Table 3.6), it does not appear that metabolizable energy or total digestible nutrients were factors in the observed differences in growth between groups since the former was comparable between all diets and the latter was highest in Treatment 2 which performed similarly to Treatment 4. Treatment 1 performed the best of the treatments, possibly due to its high calcium level and comparable potassium and phosphorus levels (vs Controls 1 and 2). Both Control 2 and all treatments had higher levels of fat content due to small extra amounts of oil added during preparation. This could explain reduced growth and could result in a fatty fish (de Silva et al. 1991, Chou and Shiau 1996). Treatments 2, 3, and 4 all had lower values of phosphorus and calcium which is important for fish growth. Phosphorus is important for ATP and bone production, affecting body and bone growth and energy production (which affects growth as well) (Robinson et al. 1987). Calcium is vital for bone mineralization and growth (Robinson et al. 1987, Shiau and Tseng 2007). This could explain the significantly smaller fish growth metrics of these treatments. Additionally, the chitin of the cricket meal and earthworms could reduce protein digestibility and absorption.

## **3.4 Conclusion**

A previous study had similar objectives to these experiments: identify potential locally available unconventional protein sources in a developing country setting (Haiti) to improve tilapia production and increase affordability of aquaculture (St. Martin Francois 2012). In that study, brewer's yeast, breadfruit, and blood meal as ingredients in an experimental diet, resulting in negative growth rates and weight loss of up to 8% body weight during the experimental time period. In contrast, the experimental diets in this dissertation resulted in significant fish weight gains. One potential factor in the success of the feeds in Experiments 1 and 2 relative to the referenced

experiment may be the use of linear programming involving the individual ingredient nutrient compositions. Unlike the diets created in the current research, the experimental feeds in the paper by St. Martin Francois were not optimized for tilapia nutrient requirements.

The fish growth data for the experimental diets evaluated in Experiment 1 indicate cricket meal may be substituted for up to 50% of the fish meal in a tilapia diet (i.e., 10% of the total formulation) without significant differences in tilapia growth rates, estimated days to harvest, or the fish length-weight relationship compared to the Control. Replacing 75% of the fish meal in the Control diet with cricket meal (i.e., Treatment 3) did significantly reduce fish growth. However, the estimated DH is not significantly different compared to Treatments 1 and 2. When 100% of the fish meal in the Control diet was replaced with cricket meal (i.e., 20% of the total feed) in Treatment 4, the fish growth rate was halved compared to the treatment with next lowest growth rate (Treatment 3).

However, even Treatment 4 could be an economically viable option for local farmers since the time required to reach a 500g harvestable weight was estimated to be only 17 days longer than the Control. Future research could include the evaluation of a “dose-response curve”, testing intermediate amounts of cricket meal replacement between 50% to 100% for a more precise analysis. Fish could also be fed to satiation to maximize fish growth for each diet and/or the grow-out period could be extended to measure the actual time required for tilapia to reach a 500g harvestable weight. This would facilitate both a more accurate economic comparison of the various diets, as well as inform improvements to the growth extrapolation model for future shorter-duration experiments.

In Experiment 2, the Control 3 represented a “best possible case” aerated extensive pond system (dependent on primary productivity, rather than feeding). Fish fed the Control 3 diet were

significantly smaller at the end of the experiment, with a much lower growth rate, than fish fed the Control 1, Control 2, and Treatment 1 diets, all of which included fish meal as the primary protein source. However, fish fed the Control 3 diet grew significantly faster, with a shorter estimated grow-out period (DH), compared to fish fed the Treatment 2, 3, or 4 diets, which utilized the alternative protein ingredients. As mentioned above, the Control 2 and Treatment 1 diets yielded similar fish growth rates. These two diets used the same primary protein source (fish meal), differing in the other supplementary ingredients. These data would suggest that the diet ingredient with the greatest impact on fish growth rate was the primary protein source. The other “local” ingredients appear to have been successfully selected to provide at least a reasonable approximation of the “commercial” ingredients, including the vitamin and mineral premixes.

A primary objective of Experiment 2 was to formulate a locally sourced feed, using available and inexpensive ingredients to replace standard ingredients and vitamin/mineral mixes used in commercial feeds. Fruits and other ingredients with high concentrations of specific vitamins, micronutrients, and amino acids were identified then the diets were optimized to reduce cost while maximizing protein and nutrient levels. The local ingredient-mix was proven to be effective as an ingredient mix for intensive aquaculture use when formulated with fish meal as the primary protein ingredient.

Several of the fruit choices for the local feed experiment are seasonal, available primarily during certain portions of the year. Future research could investigate other ingredient choices such as jackfruit, jackbean (ice cream-tree fruit or *Inga edulis*), avocados, palm hearts, and other mango varieties. Another ingredient readily available in Guatemala that could be included in fish feed is crude palm oil, which contains sufficient vitamin E and could replace fish oil in feed (Ng and Romano 2013).

The DH of the diets with alternative primary protein sources and locally available supplemental ingredients (i.e., Treatments 2-4) ranged from 218 to 242 days, while the DH for the fish meal containing diets (Controls 1 and 2, Treatment 1) ranged from 159-173 days. Depending on the relative costs of the diets, and the daily operating costs of the pond, a 45 to 83 day longer grow-out period may, or may not, be economically attractive to local farmers in developing countries. This is explored in the next chapter in this dissertation.

Other researchers have suggested that tilapia feed costs could be reduced by leveraging the rhythmicity of protein digestibility of tilapia. De Silva suggests that periodically a lower protein (i.e., lower cost) diet may be substituted for a higher protein (i.e., higher cost) diet. One potential diet rotation would involve feeding tilapia a low protein diet (e.g., 18% protein) for two days, then switch to a high protein diet (e.g., 30% protein) for three days in an ongoing cycle. Another potential approach would be to feed the fish the low and high protein diets at alternating times of the day (e.g., the high-protein diet in the morning and the low-protein diet in the afternoon) (De Silva and Perera 1984). Rotational feeding approaches like this have shown the potential to reduce production costs by 20% without a significant reduction in fish growth rates (De Silva 1985).

Overall these experimental results demonstrate the viability of locally sourced feeds utilizing alternate sources of protein and other supplemental nutrients available in developing countries to produce fish growth significantly greater than extensive pond systems.

### **3.5 Works Cited**

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## **CHAPTER 4. ECONOMIC ANALYSIS OF LOCALLY SOURCED FISH FEEDS IN GUATEMALA**

### **4.1 Introduction**

Chapter 2 of this dissertation outlined various approaches to development as described in the literature, the important potential role of aquaculture in developing countries, and introduced the concept of the Development Ladder, including the aquaculture-specific sub-ladder. Since active feeding of cultured fish represents an important, higher, rung on the aquaculture ladder, and a key barrier to feeding by local farmers is the cost and availability of feed, the need for a locally sourced, nutritionally complete feed was established. Chapter 3 described the formulation of several locally sourced feeds and their effects on fish growth. Several of these feeds yielded tilapia growth rates and projected harvest times like those provided by a commercial feed. This chapter assesses the economic viability of the feeds described in Chapter 3 based on recent tilapia grow-out experimentation in Guatemala by a small NGO.

### **4.2 Background**

Several NGOs have explored aquaculture systems at various scales in developing countries. AgInno Institute, the sponsor of the research in this dissertation, is one of these. The overall objective of AgInno Institute (AgInno) is to apply state-of-the-art agricultural research to the problem of global food security, using innovative solutions and the latest scientific knowledge to develop sustainable techniques for individuals and communities in developing countries. One focus for AgInno is the development of realistic and sustainable aquaculture systems viable for local farmers or small communities in rural locations in Guatemala that struggle with food and income security. Over the past several years, AgInno has gained practical

experience in intensive small-pond tilapia aquaculture in a coastal region of Guatemala. Since feed cost has been one of the largest production costs in this pond system, AgInno has had interest in identifying alternate feeds that could be produced by the local farmers using ingredients available, or producible, in that region.

The project-level objective of this dissertation aligns with AgInno Institute's objective: to help provide sustainable community welfare, specifically in Guatemala. The long-term objective is to address different struggles in the community, such as food insecurity, malnutrition, and low-income levels. The defined strategy to reach this objective is to leverage aquaculture as a sustainable source of protein and income for rural Guatemalan communities. The overall objective of sustainable community welfare is dependent on household food security and market access. Aquaculture can help provide additional protein and micronutrients for families and potential discretionary income through selling surplus fish. This can improve the household's standard of living while also helping the community. Related important potential outcomes include increased fish yields, fish feed production, and the development or improvement of individuals' technical and business skills. The outputs of the development work include installed aquaculture systems, fish food processing systems, filet processing skills, and a co-op business model.

As described above, while the dissertation research focuses on the fish feed, a holistic approach is required in using aquaculture in development. It is critical to consider market needs, infrastructure availability, mobility, and expertise levels in the creation of a viable aquaculture business. For example, if fish feed can be made locally, but there is no market demand for fish or an available aquaculture system, then the effort would be unproductive. Instead, according to the Theory of Change, local fish feed production would be one step of a broader action plan to further household and community welfare.

There are several possible outcomes related to the utility of locally sourced fish feeds in countries like Guatemala, depending on the efficacy and economics of the experimental feeds. First, if locally sourced and produced fish feed is comparable to commercial fish feed in fish growth rate and yield, the feed could be sold on the market throughout the country or exported (as “fair trade” or “local” product made using sustainable ingredients). In this scenario, increased market access could lead to increased food security, equitable governance for indigenous people, and market leverage. Currently, there is high demand for sustainable fish feed ingredients, and current sustainable ingredients (i.e. soybeans) are edible for humans. This results in competition for soybean supply between animal and human food. An alternative would be a comparable completely locally sourced feed from a country like Guatemala, which may be one of the optimal locations to initiate this feed production method.

Labor is inexpensive in Guatemala while capital is costly, making the time-consuming physical process of feed production less costly than in a country with higher labor costs. In addition, Guatemala has several climates and ecosystems, allowing for feed to be created from each region’s plant and agribusiness resources. For example, the feeds from the dry forest region could include parts of rain trees (*Samanea saman*), *Cecropia* trees, cacti of the family Cactaceae, and other vegetation. Guatemala has a wide variety of agribusinesses and plantations, where food waste could be redirected to fish feed. For households that own arable land, they can grow some of their own ingredients to ensure a stable supply. The comparable local feed could then be made by rural households and used in personal aquaculture, sold to other fish farmers within the country, or even exported to earn additional income. This would take time, effort, connections, and government collaboration, but is feasible as well.

In a second scenario, if local feed is less effective than commercial feed, i.e., yields slower growing fish, it still may be viable as a national or regionally sold product. If the profit from fish production using a lower performing locally produced feed is greater than the costs of fish production, including the opportunity costs of producing something else with the same time, space, and resources, then efficacy differences may be insignificant and the feed may have a positive market value.

Finally, in a third scenario, if the feed is not as effective as commercial feed and there are no market opportunities for the feed itself, the benefits from farming the fish using the feed may allow smallholder farmers to raise enough income to move their families up the development ladder. While a fish farmer may need to use more local feed to satisfy fish yield, the decreased feed cost may still enable a profit for the farmer. The yield might not be as great as with optimized commercial fish feed, but the farmer may still be accessing the next small step on the developmental ladder and will still benefit with increased food security per the Theory of Change (Figure 1.5). In time, increased income could allow the farmer either to improve the nutritional value of their self-produced feed (i.e., with improved ingredient opportunities) or choose to purchase commercial feeds. It is critical, therefore, to apply appropriate economic analysis techniques, such as benefit-cost analysis, to the proposed small-holder farmer aquaculture systems in Guatemala.

#### **4.2.1 Benefit-Cost Analysis**

Benefit-cost analysis (BCA) is a technique that combines monetary assignment and option comparison. Typically, a new policy, project, or technology will be assigned monetary value to allow incremental benefits and costs to be calculated. This analysis often helps in decision-making for public policies which have social costs and benefits to society, helping

improve allocative efficiency and social value (Boardman et al. 2018). In this case, the net social benefit would equal benefits minus costs. Opportunity costs and externalities can be included in BCA, if they can be adequately valued. If benefits outweigh the costs, then the policy or project would be advantageous to the society/target group even if it is conferring private costs (Barbier, Markandya, and Pearce 1990).

As an example, development projects can be analyzed with BCA to judge whether the project should be completed. This prevents waste of time and money in beginning a project that would be insufficiently beneficial. For example, if a new solar-powered tilapia pond aeration system had a higher net benefit than a current system, then it would be best to install the new system, regardless of opportunity costs (which must be accounted for in the calculation). For public-sector investments, BCA has been used for development projects, using shadow-pricing (Kirkpatrick and Weiss 1996). BCA helps address externalities and other market failures but needs to address world prices as a measure of international trade, scarcity of domestic savings, and assign value to distributional project effects (Kirkpatrick and Weiss 1996). BCA for economic aspects of projects often include financing, impact on public finance and foreign exchange, economic position of the nation, cash flows, and expenditures (Tang and Phataralaoha 1987).

For instance, if there was a large development project aimed to establish smallholder fish farmers in Guatemala, a BCA would help determine whether it would be viable or worthwhile. If farmers would have greater costs than benefits from switching from corn to fish farming, it might be better to remain corn farmers. Opportunity costs for the new project may include profit from corn farming and other non-farm income sources. Factors quantified would include profit, costs

of inputs (i.e., aeration, feed, labor, maintenance, fingerlings), and externalities (i.e. pollution of watersheds from nutrient-rich pond effluent and overfishing for fish meal in feed).

One of the disadvantages to BCA, using externalities as costs, is the potential prioritization of social net benefit over private costs. Impoverished people in developing countries do not have the flexibility to absorb private costs to help improve overall social benefits. In addition, for development projects, making decisions based on social net benefits can leave the impoverished people in an unescapable situation. In these cases, one must be careful to understand the tension between a private cost and social benefit. For example, if a hydroelectricity dam would benefit farmers by providing irrigation but affect native biodiversity, i.e., would have a net social cost, an ethical approach would need to address the farmers' needs directly by providing another solution for them with a lower social cost.

Another obstacle to using BCA is the requirement to assign monetary value to all variables. This may work for variables such as material inputs or technology, but, when addressing a situation where value cannot be easily assigned, this prevents the full use of BCA. One example might be malaria-preventing bed nets where the number of lives saved would not be monetizable. Additionally, there may be a preference to consider the financial costs for the new program or policy, rather than include other costs such as externalities, opportunity costs, labor, or time. As another example, if a government is trying to decide whether a wind farm should be permitted, and they only addressed the cost of production and not externalities (such as danger to birds and bats or the effects on land use and rent). Valuation may also be difficult due to imprecision and subjective differences, leading to over- or under-estimation, depending on who is assigning the valuation. Every person could monetarily value something differently since it is often a subjective process.



An alternative to BCA is a cost-effectiveness analysis (CEA) can be used when monetary value cannot be placed on the factors, such as number of lives saved from a new technology (Boardman et al. 2018). CEA is used when the factors are impossible to estimate monetarily or is considered unethical to do so. The measures of the new program or technology would need measured shadow prices, resulting in some monetization when concerned with efficiency. Due to excluded variables, shadow prices, and opportunity costs, the alternative solution may not be allocatively efficient. In development projects, monetizing the number of people saved from malaria-preventing mosquito nets may be unsettling for society. Instead of using BCA in this case, CEA might be a preferable method of analysis. CEA would allow comparison of different techniques and technologies that prevent malaria from mosquito bites compared to no treatment at all. In addition, CEA prevents the need to subjectively and inaccurately assign value as it creates a cost-effectiveness ratio with a monetary cost and units of effectiveness (Boardman et al. 2018). The higher the ratio, the higher the net benefit for the alternative.

However, a weakness for CEA lies in the sensitivity analysis, where the ratios do not have a known, or always normal, distribution and the mean ratio may not equal the ratios of the means of costs and efficacy (Boardman et al. 2018). CEA typically analyzes one factor for effectiveness compared to all costs, while BCA can include multiple factors. For optimal results and analysis, both approaches would be ideal. For argument's sake, choosing one method would depend on the factors measured or evaluated. If the factors included were non-monetizable, these would require a CEA. If the factors were monetizable, then BCA would suffice. For BCA, the factors would need to include variables such as cost of labor, feed, aeration, and fingerlings, all of which are already monetized. Externalities of water pollution from nutrient-rich effluent

would be included to address social costs. Social benefits could include overall fish and protein supply. However, if the analysis was intended to compare numbers of lives lost or affected by malnutrition (i.e. death rates, stunting, morbidity), then CEA would be preferable as it does not require monetization of the variables.

In this project, the primary focus is on monetizable factors, so BCA was used to analyze the viability of local fish feed for aquaculture development in Guatemala and determine if replacing all the fish feed ingredients with locally accessible sources is viable for entering or expanding aquaculture. It can be used to determine if farmers would benefit from switching to fish farming by using local feeds. If not, then the new feed is net costly and not viable as a solution to reduce costs and increase profits for farmers. It will be difficult to include the externality of water quality effects but can be estimated to allow for a preliminary analysis of social net benefits.

#### **4.2.2 Guatemalan Tilapia Pond Research Background**

The previously referenced tilapia research project experience in Guatemala provided the basis for the valuation of a number of the inputs required for the BCA of the experimental feeds in Chapter 2, including capital investment cost for the pond system, commercial feed cost, electricity cost, fish market value, and harvestable fish size. The Guatemala experiment is described below.

#### **4.2.3 Study Site**

The study by AgInno Institute was conducted at Mision El Faro in Punta de Palma, Izabal, Guatemala, 15 minutes by boat from Puertos Barrios, over a period of two years (October 2016 to

July 2018). The ambient temperatures in this area average 27.2°C with seasonal rain (270 cm annually) and humidity levels of 83% or higher.

#### 4.2.4 Pond Set-up

The stream-fed earthen pond (flow-controllable inlet) measured 5x7m with a depth ranging from 30 cm to about 1.5m, lined with a black plastic tarp which was scrubbed periodically to remove algal and plant growth (shown in Figure 4.1). Overflow was directed through a screen-covered pipe to the creek downstream of the inlet. Forced aeration was accomplished using two different systems.



Figure 4.1 Tilapia Pond at Mision El Faro

Initially, a Big Max pump ([fishpondaerators.com](http://fishpondaerators.com)) was used to force air through two separate aerator rings with small holes. The rings were weighted with rocks at the pond bottom while air was blown through the small holes to form oxygen-containing bubbles. The smaller and more plentiful the bubbles, the more oxygen dissolves in the water. The aerators maintained the

dissolved oxygen levels at 5.4mg/L +/- 1mg/L (depending on rain amount and electricity availability). Once, the pump failed and resulted in an extreme anoxia event of 0.8mg/L with fish at the water surface gasping for air. The Big Max pump was replaced with a new pump (Hiblow HP-80 Septic Air pumps from Septic Solutions), resulting in increased pump efficacy and efficiency with 50% less electricity utilization compared to the BigMax pump. The aerator rings were replaced with 30.48 cm diameter aeration disks. The aeration disks and HiBlow pump are shown in Figures 3.2 and 3.3. This is vital for countries like Guatemala where electricity prices can be four times the cost in the United States. While events such as pump failures are not desirable, this is a reality local farmers could encounter and need to overcome.

#### **4.2.5 Tilapia Pond Aeration Equipment**



Figure 4.2 Matala 12" Round Rubber Fine Bubble Disk Diffuser



Figure *Error! No text of specified style in document.*3 HiBlow Air Pump

#### 4.2.6 Fish Selection and Preparation

For the first replicate, 1200 tilapia (*Oreochromis niloticus*) fingerlings (2.5cm) were donated by Paraiso Springs (fish hatchery and grow-out farm), in Peten, Guatemala. The fingerlings were transported from the hatchery to the pond site in large 50-gallon plastic bags filled with water from the hatchery pond. After the fingerlings adjusted to the pond water, fish predation by birds was observed, leading to the placement of a fishing line net over the pond for protection. Fish were fed a commercial feed to satiation on a twice-daily basis.

#### 4.2.7 Fish Measurements

Fish measurements from the pond project was used to determine productivity of the pond system with inputs. Fish were caught with seine nets and the weight (g) and length (mm) were measured as well as the fish count per harvest.

For the economic analysis, fish measurement variables (DH and growth rate) were used from Experiment 2 in Chapter 3. The control was the commercial control feed tested from Star Milling and the treatments were the locally sourced feeds (Treatments 1-4) with varying protein

sources (40% fish meal, 40% cricket meal, 40% earthworms, and 20% earthworms/20% black beans).

#### **4.2.8 Ingredient Selection for the Experimental Feeds in Chapter 3**

While ingredient selection for these feeds was described in Chapter 3, it is important for the economic analysis to penetrate the details further to provide the basis for the BCR. For example, it is important to consider ingredient availability, fresh ingredient moisture content (which affect the quantities required to achieve the target dry basis for the feeds), and differences in nutrition values in the different parts of a fruit. Several advantages of the selected ingredient sources include the local availability in rural Guatemala and the usability of food by-products such as rinds, seeds, skin, and damaged flesh, which reduces ingredient costs and agricultural waste while producing a consumable or marketable good.

#### **4.2.9 Calculations**

The fresh:dry weight ratio is the conversion value of dry matter (DM) from fresh material to dried material and is calculated by dividing the dried material %DM by the fresh material %DM. The fresh and dry nutrient values and the fresh:dry weight ratios for the ingredients in the Experiment 2 feeds in Chapter 3 are provided in Table 4.1.

Table 4.1 Calculation of the Dry Matter (DM) Conversion of Ingredients

	Fresh DM % (USDA FoodData, unless noted otherwise)	Dried DM% (Midwest Laboratories)	Fresh:Dry Weight Ratio (calculated)
Moringa leaves	25	93.9	3.76
Moringa seeds	86.4	95	1.1
Sweet potato flour	23.1	94.4	4.1
Black bean	89	99	1.1
Sapota	74	86.12	1.16
Mamey papaya	11.4	87.04	7.63
Rambutan	18	87.8	4.9
Longan	17.2	91.1	4.1
Corn cob	60.1	90.2	1.5
Earthworms	14.5 <sup>a</sup>	89.36	6.2
Crickets	16.1	80.7	5
Mango	16.5	83.4	5.05
Green plantain	38.9	87.2	2.24
Starfruit	8.6	82.3	9.6
Watermelon seeds	91	94.9	1.04
Fish meal	18.5	92.7	5

It is important to note that it is possible to use all the parts of a given fruit when making a fish feed, and not only the commonly eaten portions. I.e., portions of the fruit that are commonly considered “waste”, also can provide nutrition in a fish diet. For example, there are only slight differences between the shells or rinds and the flesh of rambutan and longan, as shown in Table 3.2. The nutritional similarities indicate the utility of fruit by-products (i.e., rinds, shells, seeds) as feed ingredients and the possibility of obtaining “free” ingredients for feeds.

Table 4.2 Comparison of Nutrient Composition of Exemplary Portions of Two Fruits

Sample ID	Longan Flesh and Seed	Longan Shell	Rambutan flesh seeds	Rambutan Rind
Moisture AR %	10.11	7.84	12.94	11.64
Dry matter AR %	89.89	92.16	87.06	88.36
Protein (crude) DW %	8.57	8.43	7.18	5.98
Fat (crude) DW %	1.36	0.52	6.25	5.09
Fiber (crude) DW %	6.34	32.3	8.58	8.61
Ash DW %	3.01	4.75	2.79	2.49
Sulfur (total) DW %	0.09	0.09	0.09	0.09
Phosphorus (total) DW %	0.22	0.16	0.14	0.11
Potassium (total) DW %	1.29	1.12	0.88	0.76
Magnesium (total) DW %	0.08	0.11	0.12	0.1
Calcium (total) DW %	0.1	1	0.34	0.29
Iron (total) DW ppm	21	15.5	28.8	23
Manganese (total) DW ppm	7.2	8	37.8	13.7
Copper (total) DW ppm	10	7	11.2	11.8
Zinc (total) DW ppm	14.2	10.3	19	13.7

#### 4.2.10 Ingredient Availability and Opportunity Cost

Ingredient availability significantly impacts the economic viability of locally sourced feeds. An assessment by the author of the ingredients selected for the experimental feeds in Experiment 3 and is provided in Table 4.3. Most of the opportunity costs are related to labor costs (i.e., time, not working elsewhere) and whether the product was in competition with human consumption. For example, moringa seeds can be used for flocculation and purification of water for human consumption.



Table 4.3 Availability and Opportunity Cost of Nonconventional Ingredients in Guatemala

Ingredient	Availability	Opportunity Cost Basis
Moringa leaf	Environment < 400 m altitude in Guatemala, cut back annually to 1-2 meters to regrow pods and leaves	Harvesting time
Moringa seed	Same as above	Using seeds for water purification
Sweet Potato Flour	Uncommon	Not consumed directly
Black Bean	Common, usually for human consumption	Not consumed directly
Sapote	Common, sold on roadside	Collection labor
Mamey Papaya	Common	Collection labor
Rambutan	Common, sold on roadside	Collection labor
Longan	Available in small regions	Collection labor
Corn Cob	Common	Other animal feed
Earthworms	Culturable	Labor costs of culturing
Crickets	Culturable	Labor costs of culturing
Mango	Common	Collection labor
Green plantain	Common	Collection labor
Corn oil	Common	Not consumed directly
Starfruit	Uncommon	Collection labor
Watermelon seeds	Common, all seeded watermelons	Collection labor
Fish Meal	Less common	Collection labor

For several ingredients, the by-products (i.e., seeds, rind, skin) can also be used for feed. These ingredients include mango, mamey papaya, corn cob, rambutan, sapote, and longan (see Table 4.2 above). Other ingredients are available as surplus, defective, or spoiled. For example, plantain plantations will often have bruised or damaged plantains that are not marketable for human consumption. Instead of disposing of this produce as waste, the bruised plantains can be used for fish feed. Additionally, most fruit orchards, whether commercial or small-scale, have excess output, leading to the products falling to the ground and rotting. Instead, these products, such as mangoes, rambutan, starfruit, and starfruit, can be used in feed, rather than attracting pathogens and insect pests that could harm the fruit trees. In these examples, ingredients may be obtained at minimal or no cost.

Fish meal is obtainable from markets or households from fish by-products or waste. Depending on location, it might be obtained from regional fish meal factories, but at a higher cost. Fish farmers can also combine fish meal and one or more other protein sources (i.e., crickets, beans, earthworms, or algae) as described in Chapter 3 to decrease the quantity of fish meal required for feed production. One disadvantage of culturing crickets, earthworms, and algae is the required training, resources, and the start-up of the colony. However, if several people in a region are culturing the same species, exchanging portions of the colony amongst households would increase genetic diversity and reduce inbreeding. Once colonies are established, these species can be quite productive and raised at minimal cost since they are typically fed waste products. One advantage of algae culture is the ability to reuse the effluent from the fishponds and use the fish waste and nutrients to fertilize algal growth. This also would reduce excessive nitrogen, organic matter, and phosphorus that enters the natural watershed, decreasing or preventing eutrophication of the water systems.

As mentioned in the Discussion portion of Chapter 3, other seasonal sources could be tested or used as replacements for other fruit ingredients. Jackfruit produces large rinds, pulp, and seeds which can be used as ingredients. Heart of palms and bamboo, bananas, seeds and fruit of *Inga edulis* (Jackbean fruit), cacao and coffee pulp or cake, brewer's grain, macadamia nuts, palm kernel husk cake, rain tree fruits, chayote rinds, dragonfruit rinds or juice, lychee, breadfruit, tamarinds, sapodilla, cecropia, Chaya, or different species of algae (i.e., *Spirulina*, *Chlorella*, *Ulva*, *Sargassum*, and *Caulerpa*) are also available in Guatemala as putative ingredients. Other commonly cultured insects include mealworms and black soldier fly larvae.

#### 4.2.11 Basis of Price Levels and Cost Analysis

The premise of varying ingredient price levels originated from Tudor et al. (1996) and St. Martin Francois (2012). Additionally, since rural market prices are unknown and there are different situations (if ingredients are found locally or not), a range of estimated price levels are used to provide a more thorough analysis. The approach using the costs of a full system of pond creation, aeration, and feeding is found in several literature resources (Shang 1990, Tudor et al. 1996, Mazid et al. 1997). One of the objectives of AgInno is to design a model aquaculture system that can be globally implemented. This system would be an affordable, intensive system with pond creation, aeration, and feeding for maximized yield. The analysis allows for AgInno to understand what development step for feed would be most affordable for rural households. This is the reason the economic analysis includes fixed costs for pond creation and electricity.

#### 4.2.12 Costs of Aeration Equipment for the Mision El Faro Experimental Tilapia Pond

The purchase prices (in US \$) for the most efficient pumps, hoses, and air diffusers used in the experimental tilapia pond system in Guatemala are shown in Table 4.4 and are used as the basis for the analysis in this chapter.

Table 4.4 Aeration Equipment Costs

	Price (\$)	Quantity	Total (\$)
Hosing	25.90	1	25.90
Septic Solutions pump	325	1	325
Matala Air Diffuser 12"	51	2	102

A ten-year investment cost assessment for a pond system like the one used at Mision El Faro, but also including equipment to locally produce feeds, was completed and shown in Table 4.5. Fixed costs included costs of digging the pond (to pay laborers), nets, aeration equipment, and feed production equipment for the feeds to be made on the farm. The annual operating costs for the ponds using commercial feed will be \$30 less, since feed would not have to be made on the farm. All costs and estimates were calculated for a 10-year period - fixed costs were annualized based on their expected usable lifespans to calculate annual operating costs.

#### **4.2.13 Cost Calculations**

The time required to manually dig a 5m X 7m X 1m pond by four laborers is estimated to be four days. The daily average wage for a rural Guatemalan laborer is \$1.49. Fixed and variable costs (i.e., commercial feed, electricity, pond preparation, fingerlings, equipment), fish yield, and feeding rates were taken from the AgInno experiment and used in the benefit-cost analysis. Experimental feed costs were estimated at four price levels by using identical feeding rates as commercial feed in the pond system.

Cost of pond= #laborers\*#days\*average daily wage= 4 workers\* 4 days \* \$6.49/day = \$103.84

Table 4.5 Investment and Annual Costs over 10 Years

Fixed Costs	Initial Investment (\$)	Estimated Life (yrs)	Quantity over 10 years	10-year Total (\$)	Annualized Investment Cost (\$)
Ponds	103.84	10	1	103.84	10.38
Nets	150	10	1	150	15
Aerator Membrane Discs (2)	102	10	2 sets	204	20.40
Air tubing	25.90	10	1	25.90	2.59
Pump + spare baffles (for repairs)	405	10	1	405	40.5
Meat Grinder/Extruder	50	5	1	100	10.00
Pulverizer	100	5	1	200	20.0
Drying Oven	200	10	1	200	20.00
Total Cost					138.87

Variable costs include electricity for the aeration system, fingerlings acquired from a hatchery, and feed. Annual electricity and fingerling costs are identical between feed groups, but these costs per harvest are dependent on feed type, DH, and number of harvests annually. These are shown in Table 4.6.

Table 4.6 Variable Costs

Variable Cost	Price (\$)	Cost	Total (\$)
Electricity	\$0.29/kWhr	\$3.32/day, \$1211.80/yr	Depends on grow-out period
Fingerlings	\$0.20/fingerling	1200	\$240/batch

Farmers are assumed to have three options for disposition of the fish produced: directly selling in a market, selling them whole-sale to a middle-man, and consuming on-farm. AgInno has observed that all these options are utilized in Guatemala. While one farmer may not utilize all three, the combination in this analysis reflects the range of possible options. Obviously, personal sales in a market generate the highest revenue. On-farm fish consumption can be considered an opportunity cost compared to selling the fish in the market but can also be considered a revenue source since the household is not using income to purchase fish or other dietary protein sources. Gross revenue per harvest is held constant among feed groups in this analysis. However, net annual revenue is dependent on feed type due to the differences in fish growth rate, DH, and number of possible annual harvests. Potential gross revenue for each of the three possible uses of the fish are shown in Table 4.7. In all calculations in this dissertation, prices are shown in \$ USD (i.e., prices in Guatemalan Quetzales are converted using an exchange rate of Q7.7 per USD). Selling price of fish is provided in terms of price per pound since that is a common practice in Guatemala and since a 1 lb (~454 g) fish represents a commonly marketed size as it fits the Guatemalan dinner plate.

Table 4.7 Calculated Potential Gross Revenue for Various Uses of Farmed Fish at an Average Weight of 454g

Fish utilization	Selling Price or opportunity cost (\$/lb)	Quantity (fish)	Gross revenue per 1000 fish (\$)	Gross revenue per 1200 fish (\$)
Selling Price in market	1.69	400	676	811.20
Selling price for whole-sale	1.23	400	492	590.40
Consumed on Farm	1.69	200	338	405.60
Total Value of Fish			1506	1807.20

#### 4.2.14 Guatemala Project Results

The two tilapia batches raised in Guatemala during the above-referenced AgInno research provided the basis for determining the total productivity, or yield, of an aerated intensive system in earthen ponds in that environment. The two grow-out cycles in said research in Mision El Faro yielded 709 and 837 harvested tilapia, respectively. Based on a target marketable weight of 500 g, the pond productivity was 109 mt/ha (in the 0.0035 ha pond).

The first harvest cycle resulted in a net operating loss due to the use of the much less energy efficient BigMax pump, leading to increased electricity costs during the long grow-out period to achieve an average fish weight of almost 800g. After the first cycle, further market research indicated demand for a smaller fish (~500g), which shortened the length of the second grow-out cycle and reduced feed costs. The second grow-out cycle resulted in a profit of \$334 (over \$0.40/fish), which represents about 17% of an average annual wage in Guatemala. The second

cycle was more profitable due the use of the more efficient HiBlow pump and the shorter grow-out period. Intensive aquaculture in rural areas in Guatemala is feasible, but can be refined to reduce costs (e.g., more efficient aeration equipment, local formulated feed, solar power). On average, the earthen pond was able to support up to 560 kg of tilapia with no apparent overcrowding or ammonia saturation.

#### **4.2.15 Feed Costs**

The net cost of tilapia production using the Experiment 2 feeds (Chapter 3) was analyzed under various cost assumptions for the various ingredients, termed “Low”, “High”, and “Intermediate” in this analysis. “Low” ingredient costs were based on the assumption that the ingredients are readily available and can be harvested, collected, or cultured locally by the farmer at no cost (Tables 4.8 and 4.9). The “High” ingredient cost scenario entails US-based ingredient costs for all ingredients. Cricket prices were obtained from purchasing tropical banded crickets for the cricket meal research (Joshfrogs.com) while the other ingredients’ prices were obtained from Jungle Jims, an international market store in Fairfield, OH. This represents a scenario in which either US ingredients are imported to Guatemala or the opportunity cost of farmers not exporting the product to the US is high (Table 4.8).

“Intermediate costs” reflect the ingredient prices in the tourist city Antigua, Guatemala (and are therefore at the high end of the local ingredient price range) (Table 4.8). The mixed feed factor (MX) ingredient cost assumption represents a local feed made for which either moringa harvested for free (since moringa leaves are one of most expensive components) or it is replaced with another cheaper or free ingredient. This would help the local feeds approach the cost per kilogram of commercial feed (~\$3.30). The total feed amount used in the analysis was the daily



average feed weight used in the Guatemala project. Based on this, costs for each feed type were estimated, keeping daily feeding rate consistent.

Table 4.8 Price Levels

Ingredient Cost Level	Basis
Low	Free (harvested or cultured locally)
Middle	Antigua market prices (higher than average for Guatemala)
High	US-based prices – Jungle Jim’s
Mixed	Moringa price removed (assumed to be harvestable)-identical feed price to commercial feed Representative of village market prices

The Intermediate feed cost for treatments 1, 3, and 4 from Experiment 2 were similar, with respective costs of \$15.47, \$14.08, and \$13.75 per kilogram. The highest costs for these treatments were the protein sources: earthworms and fish meal, primarily due to the high fresh:dry conversion ratio (i.e., both animals have high moisture content), quantity of fresh ingredients needed, and unit cost. Treatment 2 had a much lower intermediate cost of \$7.33/kg based on the low protein cost of crickets. Moringa leaves were the second most expensive ingredient (Intermediate cost) for Treatments 1, 3, and 4 (\$3.98, \$4.44, and \$3.91/kg, respectively) and the most expensive ingredient for the Intermediate cost Treatment 2 at \$4.44/kg. Crickets are less expensive than other protein sources since there are no alternative uses for them currently in Guatemala, while black beans and moringa leaves are commonly consumed by people and earthworms are often used for vermicompost.

Tables 4.9 to 4.12 list for each ingredient the Low, High, and Intermediate ingredient costs and the ratio of the grams of fresh ingredient required to obtain one gram of the dried ingredient (based on water content of the fresh ingredient). These tables also include the percentage of the feed comprising each individual ingredient (i.e., the formula from Experiment 2) and the weights in both dry and fresh basis of each ingredient required to achieve that percentage in one kilogram of finished feed (i.e., the required dry weight is multiplied by the fresh:dry ratio). Finally, the tables include the Low, High, and Intermediate costs of the required fresh ingredients. A total cost for each feed is calculated based on these values for each ingredient price category.

Table 4.9 Treatment 1 Ingredient Costs

Ingredient	Ingredient Pricing (\$/kg)			Ratio fresh:dry weights	% of feed (g/100g)	Quantity (g) dry ingredient 1 kg feed	Quantity (g) of fresh ingredient 1 kg feed	Cost of fresh ingredients (\$/kg finished feed)		
	Low	High	Intermed.					Low	High	Intermed.
Moringa leaf	0	50	6.49	3.73	16.43	164.3	612.8	0	30.64	3.98
Moringa seed	0	30	5.19	1.1	1	10.0	11.0	0	0.33	0.07
Sweet Potato Flour	0	1	0.91	4.1	14.14	141.4	579.7	0	0.58	0.53
Black Bean	0	2	1.69	1.1	0	0	0	0	0	0
Sapote	0	1	2.34	1.16	1	10.0	11.6	0	0.2	0.02
Mamey Papaya	0	1	0.13	7.63	0.5	5.0	38.2	0	0.04	0.04
Rambutan	0	1	1.82	4.9	0.5	5.0	24.5	0	0.02	0.04
Longan	0	1	4.16	4.1	0.5	5.0	20.5	0	0.02	0.09
Corn Cob	0	1	0.13	1.5	0.5	45.0	7.5	0	0.007	0.001
Earthworms	0	20	6.49	6.2	0	0	0	0	0	0
Crickets	0	100	0.65		0	0	0	0	0	0
Mango	0	1	.091	5.05	0.5	5.0	25.3	0	0.02	0.02
Green plantain	0	1	1.69	2.24	16	160.0	358.4	0	0.36	0.64
Corn oil	0	2	0.13	1	4	40.0	40.0	0	0.09	0.005
Starfruit	0	20	1.69	9.6	0.5	5.0	4.8	0	0.97	0.09
Watermelon seeds	0	30	12.99	1.04	0.5	5.0	5.2	0	0.16	0.07
Fish Meal	0	2	5.19	5	38.16	381.6	1908	0	3.82	9.90
Total Cost								0	37.26	15.47

Table 4.10 Treatment 2 Ingredient Costs

Ingredient	Ingredient Pricing (\$/kg)			Ratio fresh:dry weights	% of feed (g/100 g)	Quantity (g) dry ingredient 1 kg feed	Quantity (g) of fresh ingredient 1 kg feed	Cost of fresh ingredients (\$/kg finished feed)		
	Low	High	Intermed.					Low	High	Intermed.
Moringa leaf	0	50	6.49	3.73	18.36	183.6	684.8	0	34.24	4.44
Moringa seed	0	30	5.19	1.1	1	10.0	11.0	0	0.33	0.07
Sweet Potato Flour	0	1	0.91	4.1	14.14	141.4	579.7	0	0.58	0.53
Black Bean	0	2	1.69	1.1	0	0	0	0	0	0
Sapote	0	1	2.34	1.16	1	10.0	11.6	0	0.02	0.02
Mamey Papaya	0	1	0.13	7.63	0.5	5.0	38.2	0	0.04	0.05
Rambutan	0	1	1.82	4.9	0.5	5.0	20.5	0	0.02	0.04
Longan	0	1	4.16	4.1	0.5	5.0	20.5	0	0.02	0.09
Corn Cob	0	1	0.13	1.5	0.5	5.0	7.5	0	0.007	0.001
Earthworms	0	20	6.49	6.2	0	0	0	0	0	0
Crickets	0	100	0.65	5	40	400.0	2000	0	200.00	1.30
Mango	0	1	.091	5.05	0.5	5.0	25.3	0	0.02	0.02
Green plantain	0	1	1.69	2.24	16	160.0	358.4	0	0.36	0.61
Corn oil	0	2	0.13	1	4	40.0	40.0	0	0.09	0.005
Starfruit	0	20	1.69	9.6	0.5	5.0	48.0	0	0.96	0.08
Watermelon seeds	0	30	12.99	1.04	0.5	5.0	5.2	0	0.16	0.07
Fish Meal	0	2	5.19	5	0	0	0	0	0	0
Total Cost								0	236.85	7.33

Table 4.11 Treatment 3 Ingredient Costs

Ingredient	Ingredient Pricing (\$/kg)			Ratio fresh:dry weights	% of feed (g/100 g)	Quantity (g) dry ingredient 1 kg feed	Quantity (g) of fresh ingredient 1 kg feed	Cost of fresh ingredients (\$/kg finished feed)		
	Low	High	Intermed.					Low	High	Intermed.
Moringa leaf	0	50	6.49	3.73	18.36	183.6	684.8	0	34.24	4.44
Moringa seed	0	30	5.19	1.1	1	10	11.0	0	0.33	0.07
Sweet Potato (Flour)	0	1	0.91	4.1	14.14	141.4	579.7	0	0.58	0.53
Black Bean	0	2	1.69	1.1	0	0	0	0	0	0
Sapote	0	1	2.34	1.16	1	10.0	11.6	0	0.02	0.02
Mamey Papaya	0	1	0.13	7.63	0.5	5.0	38.2	0	0.04	0.04
Rambutan	0	1	1.82	4.9	0.5	5.0	24.5	0	0.02	0.04
Longan	0	1	4.16	4.1	0.5	5.0	20.5	0	0.02	0.09
Corn Cob	0	1	0.13	1.5	0.5	5.0	7.5	0	0.007	0.001
Earthworms	0	20	6.49	6.2	40	400.0	2480.0	0	49.60	8.05
Crickets	0	100	0.65		0	0	0	0	0	0
Mango	0	1	.091	5.05	0.5	5.0	25.3	0	0.02	0.02
Green plantain	0	1	1.69	2.24	16	160.0	358.4	0	0.36	0.61
Corn oil	0	2	0.13	1	4	40.0	40.0	0	0.09	0.005
Starfruit	0	20	1.69	9.6	0.5	5.0	48.0	0	0.96	0.09
Watermelon seeds	0	30	12.99	1.04	0.5	5.0	5.2	0	0.15	0.07
Fish Meal	0	2	5.19	5	0	0	0	0	0	0
Total Cost								0	86.45	14.08

Table 4.12 Treatment 4 Ingredient Costs

Ingredient	Ingredient Pricing (\$/kg)			Ratio fresh:dry weights	% of feed (g/100g)	Quantity (g) dry ingredient 1 kg feed	Quantity (g) of fresh ingredient 1 kg feed	Cost of fresh ingredients (\$/kg finished feed)		
	Low	High	Intermed.					Low	High	Intermed.
Moringa leaf	0	50	6.49	3.73	16.15	161.5	602.4	0	30.12	3.91
Moringa seed	0	30	5.19	1.1	1	10.0	11.0	0	0.33	0.07
Sweet Potato (Flour)	0	1	0.91	4.1	10.2	102.0	418.2	0	0.42	0.38
Black Bean	0	2	1.69	1.1	32.16	321.6	353.8	0	0.71	0.60
Sapote	0	1	2.34	1.16	1	10.0	11.6	0	0.02	0.02
Mamey Papaya	0	1	0.13	7.63	0.5	5.0	38.2	0	0.04	0.04
Rambutan	0	1	1.82	4.9	0.5	5.0	24.5	0	0.02	0.04
Longan	0	1	4.16	4.1	0.5	5.0	20.5	0	0.02	0.09
Corn Cob	0	1	0.13	1.5	0.5	5.0	7.5	0	0.007	0.0009
Earthworm s	0	20	6.49	6.2	20	200.0	1240.0	0	24.80	8.05
Crickets	0	100	0.65		0	0	0	0	0	0
Mango	0	1	.091	5.05	0.5	5.0	25.3	0	0.02	0.02
Green plantain	0	1	1.69	2.24	10	100.0	224.0	0	0.22	0.38
Corn oil	0	2	0.13	1	4	40.0	40.0	0	0.09	0.004
Starfruit	0	20	1.69	9.6	0.5	5.0	48.0	0	0.96	0.08
Watermelo n seeds	0	30	12.99	1.04	0.5	5.0	5.2	0	0.16	0.07
Fish Meal	0	2	5.19	5	0	0	0	0	0	0
Total Cost/lb								0	57.94	13.75

Commercial feed is sold in larger towns and cities for 300-400Q per 100lb bag, depending on protein content (higher protein feeds are more expensive), resulting in a mean price of \$3.30/kg for feed. Up until 2015, all fish feed was imported, but there have recently been two fish feed mills established in Guatemala (ARECA and Cargill). If local feed fails to compete with commercial feed in an earthen pond setting, local feed could be used as a lower rung on the development ladder with commercial feed representing a higher rung. Since feed mills are low localized in Guatemala, fish farmers are not forced to purchase the even more expensive imported feed. If local feed production or specific local ingredients are successful, future opportunity partnership could be created between the feed mills and local feed producers to implement the locally sourced recipe or some of the constituent ingredients into a full-scale feed production line. Feed producers may also be motivated to market some of these lower cost feeds to reduce the barrier-to-entry for local farmers to begin aquaculture production, with the intent to have them “trade up” to existing commercial feeds when they become more experienced and have met their families’ basic needs.

BCA can include opportunity costs for family labor to simulate the head worker working on the fish farm instead of at a job providing a daily income. In some larger Guatemalan villages, the household head would earn on average ~50Q (or \$6.49) a day as a laborer. Of course, this may not be true for all communities or households due to job variability access. Family labor is the opportunity cost of one family member spending labor and time on fish farming rather than a daily job where he/she would make 50Q a day or about \$6.49 a day. While there can be an opportunity cost with family labor in fish farming, this may be avoided if the primary wage earner continues working, while the other partner or youth run the fish farm. In Cambodia, combining horticulture, poultry farming, and aquaculture has helped empower women as well as increase food security and income (Dragojlovic et al. 2020).

The 10-year variable and fixed costs for the hypothetical 5m X 7m pond (replicating the conditions of the AgInno research) are shown in Tables 4.12 and 4.13. If the Guatemala government or an INGO would provide the equipment and covers the fixed costs, the annual profit would increase by \$138.87 which is equivalent to almost 7% of the average annual Guatemalan income of ~\$2000. The additional profit would benefit the household by covering the children's' school supplies, allowing purchase of other livestock, or implementation of a home garden, all furthering income and food security.

For the following tables, the following labels are used for simplicity: Commercial Feed = C, Treatment 1 = T1, Treatment 2 = T2, Treatment 3 = T3, Treatment 4 = T4, Low Cost = L, Intermediate Cost = M, High Cost = H, Mixed Cost = MX. Treatments T1-T4 are the four experimental treatments from Experiment 2, Chapter 3, and their associated calculated DH values are used in the economic analysis. While electricity is a variable cost on a per harvest basis, annually it is treated as a fixed cost if the ponds are cycled continuously. Local farmers would be motivated to minimize the time between grow-out cycles used to empty, clean, and refill the pond.



Table 4.13 Variable Costs for Grow-out of Tilapia Using Treatment 1-4 Diets

Diet Code	DH (# days to 500g)	# of Harvests/Year	Electricity (\$)/harvest	Annual Electricity Cost (\$)	Fingerling Cost (\$)/Harvest	Annual Fingerling Cost (\$)	Total Cost (\$)/Harvest	Total Annual Cost (\$)
C	159	2.3	527.88	1211.80	240.00	552.00	767.88	1763.80
T1L	173	2.11	574.36	1211.80	240.00	506.40	814.36	1718.20
T1M	173	2.11	574.36	1211.80	240.00	506.40	814.36	1718.20
T1H	173	2.11	574.36	1211.80	240.00	506.40	814.36	1718.20
T1MX	173	2.11	574.36	1211.80	240.00	506.40	814.36	1718.20
T2L	242	1.51	803.44	1211.80	240.00	362.40	1043.44	1574.20
T2M	242	1.51	803.44	1211.80	240.00	362.40	1043.44	1574.20
T2H	242	1.51	803.44	1211.80	240.00	362.40	1043.44	1574.20
T2MX	242	1.51	803.44	1211.80	240.00	362.40	1043.44	1574.20
T3L	218	1.67	723.76	1211.80	240.00	400.80	963.76	1612.60
T3M	218	1.67	723.76	1211.80	240.00	400.80	963.76	1612.60
T3H	218	1.67	723.76	1211.80	240.00	400.80	963.76	1612.60
T3MX	218	1.67	723.76	1211.80	240.00	400.80	963.76	1612.60
T4L	240	1.52	796.80	1211.80	240.00	364.80	1036.80	1576.60
T4M	240	1.52	796.80	1211.80	240.00	364.80	1036.80	1576.60
T4H	240	1.52	796.80	1211.80	240.00	364.80	1036.80	1576.60
T4MX	240	1.52	796.80	1211.80	240.00	364.80	1036.80	1576.60

Table 4.14 Total Feed Costs for Grow-out of Tilapia Using Treatment 1-4 Diets

Diet Code	Feed Ingredient Cost (\$)/kg	Average feeding weight (kg/day)	DH (# days to 500g)	Total Feed Cost (\$)/Harvest	# of Harvests/ Year	Annual Feed Cost (\$)
C	3.3	0.61	159.00	322.17	2.30	740.98
T1 L	0	0.61	173.00	0.00	2.11	0.00
T1 M	15.47	0.61	173.00	1643.25	2.11	3467.27
T1 H	37.26	0.61	173.00	3957.83	2.11	8351.02
T1 MX	3.3	0.61	173.00	350.53	2.11	739.62
T2 L	0	0.61	242.00	0.00	1.51	0.00
T2 M	7.33	0.61	242.00	1089.15	1.51	1644.62
T2 H	236.85	0.61	242.00	35193.07	1.51	53141.53
T2 MX	3.3	0.61	242.00	490.34	1.51	740.41
T3 L	0	0.61	218.00	0.00	1.67	0.00
T3 M	14.08	0.61	218.00	1884.64	1.67	3147.34
T3 H	86.45	0.61	218.00	11571.51	1.67	19324.41
T3 MX	3.3	0.61	218.00	441.71	1.67	737.66
T4 L	0	0.61	240.00	0.00	1.52	0.00
T4 M	13.75	0.61	240.00	2026.20	1.52	3079.82
T4 H	57.94	0.61	240.00	8538.04	1.52	12977.82
T4 MX	3.3	0.61	240.00	486.29	1.52	739.16

The annual costs of the control/commercial feed were comparable to the mixed cost of each treatment, ranging from ~\$738 to \$740, even with the differences in DH between the formulations. However, the cost per harvest for these treatments was higher with the MX pricing, due to smaller growth rates and increased DH, increasing length of grow-out to harvest and reducing the number of harvests a year. Obviously, if the feeds were produced by the farmer at zero out-of-pocket cost, these would be more cost-effective than the commercial feed, but Treatments 2-4 (Experiment 2, Chapter 3) would only deliver only one complete harvest a or, on average, 15 harvests over 10 years, rather than the approximately 20 harvests/10 years for the control and Treatment 1. The profit per harvest and total annual profit are shown in Tables 4.14 and 4.15, respectively.

Table 4.15 Total Profit Per Harvest Using Treatment 1-4 Diets

Diet Code	Fixed Cost (\$)/harvest	Variable Costs – Electricity/Fingerling (\$)	Variable Costs – Feed (\$)	Total Costs (\$)	Revenue (\$)	Profit (\$)
C	60.38	767.88	322.17	1,150.42	1,807.20	656.78
T1L	65.82	814.36	0.00	880.18	1,807.20	927.02
T1M	65.82	814.36	1,643.25	2,523.43	1,807.20	-716.23
T1H	65.82	814.36	3,957.83	4,838.01	1,807.20	-3,030.81
T1MX	65.82	814.36	350.53	1,230.71	1,807.20	576.49
T2L	91.97	1,043.44	0.00	1,135.41	1,807.20	671.79
T2M	91.97	1,043.44	1,089.15	2,224.56	1,807.20	-417.36
T2H	91.97	1,043.44	35,193.07	36,328.47	1,807.20	- 34,521.27
T2MX	91.97	1,043.44	490.34	1,625.75	1,807.20	181.45
T3L	83.16	963.76	0.00	1,046.92	1,807.20	760.28
T3M	83.16	963.76	1,884.64	2,931.55	1,807.20	-1,124.35
T3H	83.16	963.76	11,571.51	12,618.42	1,807.20	- 10,811.22
T3MX	83.16	963.76	441.71	1,488.63	1,807.20	318.57
T4L	91.36	1,036.80	0.00	1,128.16	1,807.20	679.04
T4M	91.36	1,036.80	2,026.20	3,154.36	1,807.20	-1,347.16
T4H	91.36	1,036.80	8,538.04	9,666.20	1,807.20	-7,859.00
T4MX	91.36	1,036.80	486.29	1,614.45	1,807.20	192.75

With respect to profit per harvest, the “high cost” assumptions for feed prices proved impractical, with projected deficits for each feed (up to \$34,521). If there was an opportunity cost to selling the local ingredients rather than using in the feed (high-cost ingredient option), it would be more profitable for households to sell the ingredients and perhaps purchase commercial fish feed.

For the “low cost” approach, all treatment feeds result in higher profit per harvest than the system with the commercial feed. Treatment 1 feed’s system resulted in the highest profit (\$927/harvest) of the treatment feeds due to its lower fixed and variable costs resulting from the reduced grow-out period. Even though the control system has smaller fixed and variable (electricity) costs, Treatment 1 feed was produced at no cost, leading to an overall smaller total cost. The use of the other “Low” cost feeds resulted in profits ranging from \$671 to \$760, depending on how many days each feed required to complete a harvest cycle.

The “Intermediate” costs (M) were based on ingredient prices from a market in a tourist city in Guatemala, which fails to perfectly reflect rural prices (i.e., is a very conservative set of assumptions). It is a reasonable comparison to make, but results may be underestimating profit if ingredient prices are lower for rural households. No feed using Intermediate ingredient price assumptions were profitable. This indicates if certain ingredients are too expensive, like moringa and protein sources, they should be replaced, if possible.

Although Treatment 1 in Experiment 2 resulted in fish with a lower DH and faster growth rates compared to the other treatments and a statistically comparable DH to the fish fed the positive controls, the economic analysis shows that Treatment 2 would be cheaper for the Intermediate cost than Treatment 1 (\$2225 vs \$2523, respectively). However, if each feed ingredient was available to local farmers at zero cost, Treatment 1 would be a better choice than the other treatments as the system would complete two full harvest cycles annually, rather than just one.

The “mixed cost” (MX) reflected the replacement of an expensive ingredient to reduce feed production costs. The feed cost is assumed to be comparable to the commercial feed cost (~\$3.30/kg). Only Treatment 1 would provide a profit for each harvest with the MX price assumptions.

Table 4.16 Annual Total Profit Using Treatment 1-4 Diets

Diet Code	Annual Fixed Costs (\$)	Variable Costs (\$)– Electricity/Fingerling	Variable Costs (\$) - Feed	Total Costs (\$)	Annual Revenue (\$)	Profit (\$)
C	138.87	1763.80	740.98	2643.65	4156.56	1512.91
T1L	138.87	1718.20	0.00	1857.07	3813.19	1956.12
T1M	138.87	1718.20	3467.27	5324.34	3813.19	-1511.14
T1H	138.87	1718.20	8351.02	10208.09	3813.19	-6394.90
T1MX	138.87	1718.20	739.62	2596.69	3813.19	1216.50
T2L	138.87	1574.20	0.00	1713.07	2728.87	1015.80
T2M	138.87	1574.20	1644.62	3357.69	2728.87	-628.81
T2H	138.87	1574.20	53141.53	54854.60	2728.87	-52125.73
T2MX	138.87	1574.20	740.41	2453.48	2728.87	275.39
T3L	138.87	1612.60	0.00	1751.47	3018.02	1266.55
T3M	138.87	1612.60	3147.34	4898.81	3018.02	-1880.79
T3H	138.87	1612.60	19324.41	21075.88	3018.02	-18057.86
T3MX	138.87	1612.60	737.66	2489.13	3018.02	528.90
T4L	138.87	1576.60	0.00	1715.47	2746.94	1031.47
T4M	138.87	1576.60	3079.82	4795.29	2746.94	-2048.35
T4H	138.87	1576.60	12977.82	14693.29	2746.94	-11946.34
T4MX	138.87	1576.60	739.16	2454.63	2746.94	292.32

For annual profit (averaged over a 10-year period), the “High” ingredient cost assumptions were for treatments once again impractical, with deficits for each feed (up to \$52,000). If there was an opportunity cost to selling the local ingredients rather than using in the feed (high-cost ingredient option), it would be more profitable to sell the ingredients and perhaps purchase commercial fish feed instead.

For the “Low” ingredient cost assumptions, only Treatment 1 resulted in higher profit than the control, though the other treatments feeds still were profitable. Treatment 1 resulted in lower total costs (at zero feed ingredient costs) than the control and both these feeds resulted in, on average, two harvests/year, and leading to higher profit for the system using Treatment 1. The other treatments resulted in smaller profits than the control this time due to resulting in only about 1 to 1.5 harvests a year. They might have been less expensive than the control feed but provided

less annual revenue due to fewer fish harvests. Using the “Intermediate” cost assumptions, all treatments resulted in an annual deficit.

In summary, Treatment 1 with the “Low” ingredient price assumptions would be the most profitable tilapia diet option among those evaluated in this research in both per harvest and annual profit analyses. The other “Low” cost treatments were still profitable, even if somewhat less so than the commercial feed. The treatments under the “Intermediate” and “High” cost ingredient assumptions were unprofitable.

Based on this analysis, if commercial feed was too expensive or inaccessible and local ingredients were inexpensive, locally sourced feeds could still produce a profit comparable to that expected using a commercial feed. The profitability of pond systems using locally produced feeds is affected by feed prices (a positive effect) and longer harvest times (a negative effect). Since Treatment 1 had similar harvest rates to the commercial feed, so lower-cost ingredients resulted in additional profitability. The other treatments were hindered by the slower growth rates and fewer harvests per year. More optimal balances between harvest cycle time (DH) and feed ingredient prices, compared to Treatments 1-4, may be possible. For example, supplementation of the Treatment 1-4 formulas with algae grown by the local farmer would likely, based on the Control 3 diets fish growth results, decrease DH, while maintaining the lowest possible feed prices.

Beyond feed prices, farmers have other options to increase the profitability of their aquaculture pond systems. For example, since electricity in Guatemala is expensive, and a large portion of fish production costs, the use of more efficient aerator designs or solar power may enable some of the feeds in this analysis to become profitable. Another AgInno funded project (outside the scope of the current research) is directed toward scaling down the size of commercial paddle-

wheel aerators (shown to require 50-60% less electricity than forced air systems) to be appropriate for small ponds, such as the ones described in this dissertation.

One disadvantage of Treatment 1 is the use of fish meal. Fish meal is more challenging to obtain than beans or earthworms, but not impossible. However, fish meal can be obtained from local restaurants, the community, or saved at the household level. In the future, it would be interesting to test other animal-based protein sources such as poultry or pig viscera since these livestock are more common in rural areas.

Table 4.17 Benefit-Cost Ratios

Diet Code	Benefit:Cost
C	1.57
T1L	2.05
T1M	0.72
T1H	0.37
T1MX	1.47
T2L	1.59
T2M	0.81
T2H	0.05
T2MX	1.11
T3L	1.73
T3M	0.62
T3H	0.14
T3MX	1.21
T4L	1.60
T4M	0.57
T4H	0.19
T4MX	1.12

Treatment 1 at Low cost had the highest benefit-cost ratio (BCR) at 2.05, more than twice the revenue compared to costs (Table 4.16). Even the Mixed cost level for Treatment 1 resulted in a comparable BCR to the other Treatments at their Low cost. This indicates Treatment 1 was the most profitable of the local feeds. The other treatments still had BCRs above 1.0 for the Low and Mixed cost levels. If the cost levels are Intermediate or High, however, the fish farm would have a deficit. It is important for the more costly ingredients to either be found or grown locally (rather than purchased) or substituted with cheaper ingredients. Even if the substitutions were less effective for fish growth, it can still result in a profit for the farmers.

#### 4.2.16 Approaches to Improve Aquaculture Affordability and Profit

Intensive aquaculture is capital-dependent, and, due to the initial fixed costs, ongoing variable costs like feed and electricity, and the risk of unreliable markets or prices, it is challenging



for impoverished rural households to implement fish farms without additional guidance or assistance. Government or INGOs can help reduce risks for farmers and rural households while incentivizing adding fish production to their farms by helping fund or subsidize pond production and equipment purchases (i.e., nets, aeration systems, grinders) while subsidizing inputs such as fish feed can help increase production and economic gain (Dragojlovic et al. 2020). For example, if the Guatemala government or an INGO provides the equipment and covers the fixed costs, the annual profit increases by \$138.87 which is almost 7% of the average Guatemalan income of ~\$2000. The additional profit would benefit the household by covering the children's school supplies, allowing purchase of other livestock, or implementation of a home garden, all furthering income and food security.

Governments can also provide indirect financial services via providing an unconstrained credit supply without collateral. Many farmers do not have adequate valuable assets to use as collateral, leading to lack of credit access and capital to establish or improve a fish farm. This leads to dependency on donations or some form of savings for farm establishment (Mitra 2019). In Ghana and Vietnam, the lack of credit was a primary constraint since loans can help increase profits due to higher input use (Ly and Nguyen 2014, Antwi et al. 2017). In Kenya, Quagrainie et al. (2010) determined that factors such as increases in fish sales and pond size helped increase the probability of getting credit, but higher labor costs decreased the probability. Without credit access, there is a credit constraint, leading to little or no input use which further reduces yields and profit (Mitra 2019). Lower yields result in no surplus income to use as investment or savings, further preventing collateral (Mitra 2019).

The development ladder approach helps those without credit access to make progress toward improvement or implementation of farms (both agriculture and aquaculture). As discussed

in Chapter 2, if a Guatemalan household does not have credit or savings to purchase commercial feed immediately or a larger pond, they can begin with the basic rungs of a small pond with fertilizer or locally produced feed. The local feed is a rung between pond fertilizer and commercial feed, helping reduce gaps between rungs without burdening the household financially.

### **4.3 Conclusion**

Commercial fish feeds are optimized to meet nutritional requirements for fish to maximize growth, yield, and profit. In developing countries, rural or impoverished households might be unable to afford commercial fish feed at first. Currently, the alternative is to use extensive pond systems with little to no supplemental feeding with agricultural by-products, resulting in slow growth rates, long grow-out periods, and little income. However, alternative complex feeds, made using inexpensive or free locally available ingredients, could offset costs while increasing fish growth and output. Chapter 3 demonstrated the efficacy of a formulated feed using local ingredients while this chapter demonstrated the profitability of a “Low” cost Treatment 1 feed compared to the commercial feed. All treatments at “Low” and “Mixed” cost levels resulted in profits, indicating the feasibility of using local feeds in aquaculture. Even though Treatment 1 (Experiment 2) resulted in the highest profit and the best fish growth of the treatments, the other treatments were still profitable and are viable alternative feeds to use in aquaculture development. Depending on the situation, it might be easier to raise or find the protein sources for the other treatments than for the fish meal in Treatment 1. In those cases, it might be wise to accept the smaller profit to reduce costs and time obtaining ingredients.

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## CHAPTER 5. CONCLUSION

Chapter 1 consisted of a literature review, covering development, aquaculture, tilapia nutrition, and alternative fish feed ingredients. Chapter 2 continued discussing development with associated definitions, approaches, and success factors. Conventional development approaches range from the promotion of traditional knowledge and methods (and resisting the introduction of new technology) to the deployment of high-tech innovations that are unusable either due to cultural, price, or maintenance constraints. An intermediate approach, the development ladder theory, was also presented as an approach to all aspects of development, including education, agriculture, economics, and potable water. Step-wise implementation of new technology or methods can help improve long-term adoption and ensure each rung of the ladder is affordable and usable by households, while improving their standard of living.

Chapter 3 described the effects of tropical banded cricket meal replacement of fish meal, and completely locally sourced feeds, on tilapia growth. In Experiment 1, cricket meal replacement up to 50% had no significant effect on fish growth metrics. In Experiment 2, fish fed Treatment 1 had growth rates comparable to the fish fed the homemade Control 2 diet in all metrics to the fish fed the commercial feed Control 1 for the projected days to harvest and FCR, as well as for SGR at the significance level of 0.01. This indicates that local ingredients are suitable for alternative feeds. Fish in the other treatment groups had significantly smaller growth metrics than those in Treatment 1 and the controls, indicating that the protein source is important for determining the efficacy of the feed. A combination of a higher quality protein source and local ingredients can produce a fish feed that is suitable for aquaculture in developing country settings.

In Chapter 4, different assumptions for fish feed ingredient prices (i.e., low, intermediate, high, and mixed) for the various Experiment 2 diets resulted in varying degrees of projected

profitability. Both intermediate and mixed costs resulted in large deficits greater than \$700. Even though Treatment 1 (Experiment 2) resulted in the highest profit and the best fish growth of the treatments, the other treatments were still profitable and are viable alternative feeds for use in aquaculture development. Depending on the situation, it might be easier to raise or find the protein sources for the Treatment 2-4 diets than for the fish meal in Treatment 1. In those cases, it might be wise to accept the smaller profit to reduce costs and time obtaining ingredients.

## APPENDIX A

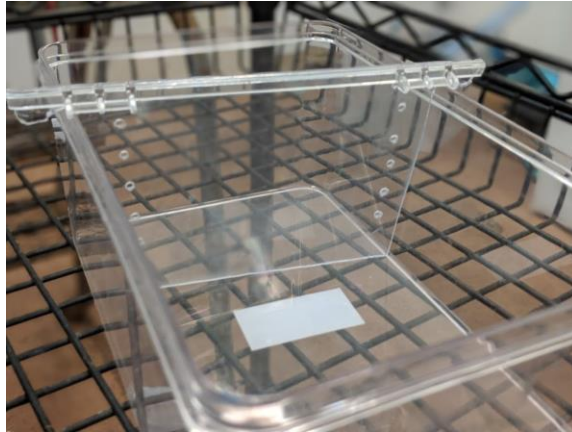


Figure A. 1 Measurement Box



Figure A. 2 Divider



Figure A.3

From Right to Left: A=Control 1 (Commercial feed), B=Control 2( Handmade Commercial feed), C=Control 3 (Negative Control=Algae), D=Treatment 1 (40% fish meal), E=Treatment 2 (40% cricket meal), F=Treatment 3 (40% earthworm meal), G=Treatment 4 (20% earthworm/20% black bean)



# APPENDIX B

Table B. 1 Nutrient Composition of Ingredients (% DW) of Diets Fed to Tilapia in Experiment 2

Nutrients (in %, unless noted)	Earthworm Meal	Black bean powder	Plantain Flour	Coconut Flour	Sweet Potato Flour	Corn Oil	Avocado Oil	Corn cob	Starfruit	Chlorella	Sapote	Longan	Rambutan	Watermelon Seeds	Mango	Mamey Papaya
Crude protein	60.1	21.1	4.32	28.57	6.15	0	0	7.98	6.41	47.82	4.69	7.74	5.77	42	6.67	7.48
Crude fat	4.96	0.96	0.31	14.2	0.9	100	100	8.28	1.99	13.82	2.57	0.85	4.97	12	0	1.37
NFE	10.12	66.09	79.52	14.29	78.87	0	0	58.04	65.13	8.08	63.6	61.15	67.12	8	68.1	66.1
Crude fiber	0.68	3.94	0.71	42.86	3.96	0	0	13.8	5.31	0	12	18	8	0	9	7
Potassium	0.74	1.34	1.05		1.89			0.63	1.52		1.23	1.095	0.765			2.31
Iron (mg/100g)	120	6.04	3.31		3.45			2.46	3.58		1.36	1.66	2.405			2.94
Calcium	0.57	0.15	0.02		0.2			0.02	0.05		0.12	0.505	0.295			0.19
Phosphorus	0.77	0.39	0.12		0.18			0.23	0.12		0.09	0.175				0.18