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Using Zebrafish (Danio rerio) to Evaluate the Use of Food Waste Based Feed in Sustainable Small-Scale Aquaculture

Caitlin May

USING ZEBRAFISH (*DANIO RERIO*) TO EVALUATE THE USE OF FOOD WASTE BASED FEED IN SUSTAINABLE SMALL-SCALE AQUACULTURE

by

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(Under the Direction of Vinoth Sittaramane)

ABSTRACT

Food waste is rich in nutrients and a valuable resource, but currently its primary method of disposal in the United States (US) is unsustainable. In 2018 the US Environmental Protection Agency (EPA) estimated that the commercial, institutional, and residential sectors of the US economy wasted 63 million metric tons of food, most of which was sent to landfills. Instead, food waste could be recycled and used to supplement or replace commercial feed in aquaculture to sustainably stimulate production of fish. Zebrafish (*Danio rerio*) are a well characterized animal model closely related to other fish species that are important in aquaculture. In this study, food waste was collected, processed, and dehydrated into Converted Fish Flakes (CFF) with a protein ratio appropriate for zebrafish. It was hypothesized that the nutrition provided by a CFF supplemented diet is suitable for zebrafish growth without significant adverse effects on health. Zebrafish were fed diets with increasing ratios of CFF to a commercial flake feed (0%, 25%, 50%, and 100%) for 32 days, and monitored for survival and growth, which was measured with weight and length gain. The viscerosomatic index (VSI) was calculated to determine whether weight was gained in muscle (more desirable) or viscera (less desirable) and reproductive ability was evaluated. Finally, histological analysis was performed to assess the health of the intestine and liver. Zebrafish fed CFF diets had high survival rates and growth that was comparable to the commercial feed. CFF diets did not significantly alter zebrafish fecundity or VSI. Histology revealed an increased amount of goblet cells in the intestine and fat deposits in the liver associated with a 50% CFF diet. These results indicate that food waste could be recycled into a sustainable alternative feed ingredient for small-scale freshwater aquaculture.

INDEX WORDS: Sustainability, Aquaculture, Food waste, Diet, Zebrafish, Recycling

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CHAPTER 1
INTRODUCTION

Food Waste

Production and Disposal

In 2010, food waste in the United States (US) was estimated to be nearly 219 pounds of food per person per year, corresponding to 133 billion pounds of food worth $161 billion (USDA 2021). This food is valuable, nutritious, and should be viewed as a resource instead of as waste to be managed (EPA 2021). The United Nations' Food and Agriculture Organization estimated in 2011 that nearly one-third of the global food supply is lost or wasted annually (FAOa 2011). Food waste is a complex global issue and is a result of the modernization of food systems, individual and cultural attitudes and behaviors, and economic factors (Thyberg and Tonjes 2016). Food loss and/or waste occurs at every level of the food production and supply chain, due to spoilage or issues in transporting or processing food (USDA 2021). However, industrialized countries waste more food per person than less industrialized countries (Figure 1) (FAOa 2011). Food waste in industrialized countries is usually the result of consumer behavior and a lack of coordination between different stages of the food supply chain (FAOa 2011). Food waste in less industrialized countries is usually connected to lack of infrastructure and limitations in harvesting, storing, transporting, and marketing, which results in most food loss occurring at the early and middle stages of the supply chain (FAOa 2011).

In industrialized nations like the US, households and food services at institutions (such as schools, colleges and universities, military installations, office buildings, correctional facilities, and medical facilities) are a significant source of food waste (Wilkie et al. 2015). It was estimated in 2018 that the US commercial, institutional, and residential sectors produced 63 million metric tons of wasted food, of which nearly 56% was landfilled (Figure 1). Food from schools and other institutions is ideal for recycling because the food waste is concentrated at a single point, while residential food waste is spread over hundreds of households, making efficient collection difficult (Wilkie et al. 2015). Universities in industrialized nations have recently increased their campus food sustainability efforts (Grech et al. 2020). Campus dining facilities may contribute significantly to regional food waste, with an estimated 17,491,813 college students enrolled in the US in fall 2020 (Burton et al. 2016, National Student Clearinghouse Research Center 2020). A 2017-2018 study conducted at Georgia Southern University estimated that between 2,100 to 2,400 pounds (over one metric ton) of food is wasted at dining halls per day, with the average student wasting between 0.6 and 0.54 pounds of food per day (Table 1). A 2015 food waste audit at three Florida elementary, middle, and high schools revealed that food waste was the largest component (between 47% to 57%) of cafeteria waste streams, with the average student wasting about 0.12 pounds per day (Wilkie et al. 2015). The same study estimated that over 75% of the cafeteria food waste could be recycled (Wilkie et al. 2015).

When food is wasted the limited resources (land, water, energy, labor) used to produce, harvest, and transport the food are also wasted, and fees are usually incurred to collect and dispose of it (FAOa 2011). Incineration is not an efficient disposal option because of food waste's high moisture content, its variable composition, and its low calorific value (Mo et al. 2018, Pham et al. 2015). Furthermore, incineration of solid organic waste like food can produce polychlorinated dibenzo-p-dioxins (PCDDs) and other toxic gases (Sharma et al. 2019). Nonhazardous waste including food waste from households, restaurants, and institutions is often sent to municipal solid waste landfills which are regulated by the EPA (EPA 2021).

Depositing food waste in landfills is unsustainable for several reasons. First, the food waste takes up limited space needed for other municipal solid wastes. In the US, fees to dispose of waste in landfills have been increasing as the landfills approach capacity (EPA 2020). Food that has been isolated in landfills is unavailable for the natural cycling that would return valuable nutrients to agricultural soils (EPA 2021). Farmers often use synthetic fertilizers to make up for poor soil quality. Additionally, decaying food in landfills results in the production of methane, a greenhouse gas 25 times more potent than carbon dioxide (FAOa 2011). It is estimated that the global carbon footprint of food waste is equivalent to 3.3 metric tons of carbon dioxide annually (FAOa 2011). Because of its high moisture content, food waste is reported to contribute to the leachate and gas generation of landfills (Jordan et al. 2020). According to the EPA's Food Recovery Hierarchy, the most preferred method to reduce food waste and loss is to reduce the volume of surplus food at the source, followed by donating surplus food to hungry people, then using surplus food to feed animals. Less desirable are industrial uses (such as converting into biodiesel) and composting of food waste. Least desirable of all methods is to incinerate or dispose of food in a landfill (EPA 2021).

Historically, composting has been a popular way to repurpose food waste. Composting is a form of controlled decomposition where organic wastes are broken down into a nutrient rich soil additive, although a bulking agent is usually needed to absorb moisture and add physical structure when composting food (UGA 2017). Properly composting and utilizing food waste can improve soil structure and health, reducing the need for fertilizers and pesticides and improving soil water retention (EPA 2021). Vermicomposting, utilizing earthworms and microbial activity to help break down organic matter, can be used to produce vermicompost which similarly increases soil health and crop yields (Sharma et al. 2019).

Anaerobic digestion involves bacterial fermentation of organic waste by redox pathways, producing various acids, gases, and finally methane, which can be used as fuel (Sharma et al. 2019).

Food waste from agriculture and food processing by-products is rich in carbon and nitrogen, and has the potential to be converted into biofuel (Ng et al. 2020). Fermentation using carbohydrate rich food waste could also be used to produce bioethanol while anaerobic digestion could be used to produce biogas, both of which can be used as fuel to reduce reliance on petroleum products (Ng et al. 2020). Other potential uses for food waste include extraction of useful compounds for use in the pharmaceutical and cosmetic industry (Osorio et al. 2021). Food waste can also be used as a food source for microbes used in the process of microbial fermentation to produce useful bioactive compounds, such as enzymes, antioxidants, and pigments (Ng et al. 2020). Of the more than 63 million tons of food wasted by the US commercial, residential, and institutional sectors in 2018, just 18% was recycled through biochemical processing, anaerobic digestion, composting, or used as animal feed (EPA 2018).

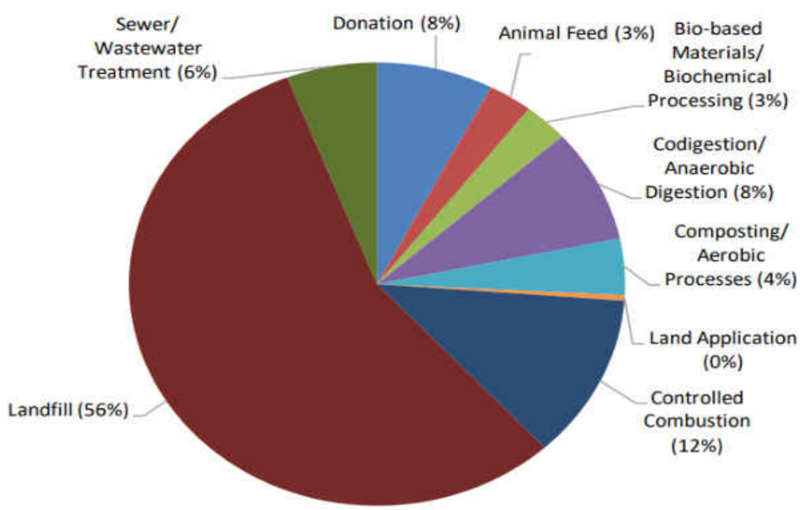

Figure 1. Percentage Distribution of Wasted Food Management, Excluding the Industrial Sector (2018). From the EPA's 2020 Wasted Food Report: Estimates of generation and management of wasted food in the United States in 2018.

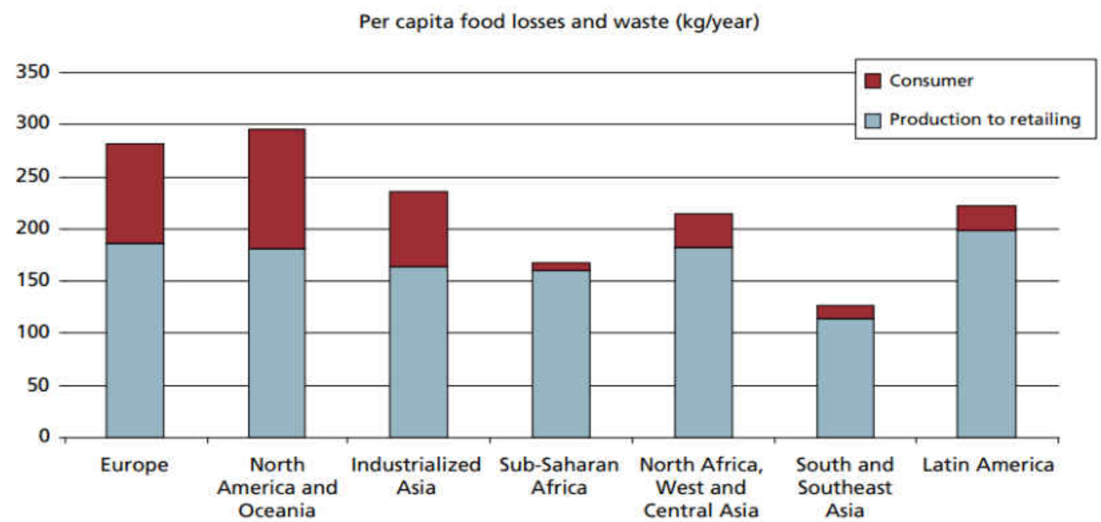

Figure 2. Per capita food losses and waste, at consumption and pre-consumptions stages, in different regions. From the FAO's 2011 report: Global food losses and food waste – Extent, causes and prevention.

Description	Fall 2017	Spring 2018
Food waste per student per day (lbs)	0.60	0.54
Food waste for all students per day (lbs)	2,400	2160
Food waste for all students per month (lbs)	72,000	64,800
Food waste for all students per semester for Dining Commons (lbs)*	288,000	259,200
Food waste for all students per semester for Lakeside and Dining Commons**	504,000	453,600

Average number of students that visit Dining Commons in a day is 4,000

*****Average number of students that visit Dining Commons and Lakeside combined is 7,000***

Table 1. Estimated food waste at Georgia Southern University for the 2017-2018 academic year. From E Afriyie-Gyawu, 2019 [Personal communication].

Use in Aquaculture

Some food by-products are used as aquaculture feed ingredients, for example meals or oils recovered from processing seeds, livestock, and fish products (Naylor 2020). Most crustacean, marine, and other intensively cultured fishes require high quality feed, which usually contain fish meal or fish oil (a fish processing by-product) resulting in the aquaculture industry consuming 71% of globally available fish meal and 90% of globally available fish oil (Bostock et al. 2010). Fish meal is a popular ingredient in formulated fish feeds because of its high protein content and digestibility (Jiang et al. 2019). However, global capture fisheries are nearing maximum sustainable yields, with an estimated 60% of fish stocks classified as maximally sustainably fished, and more than 34% of fish stocks were fished at unsustainable levels (FAOb, 2020). This indicates that fishmeal will become an expensive and unsustainable feed ingredient in the future. While carnivorous fish species require higher levels of protein and are typically fed diets containing fish meal when cultured, for herbivorous or omnivorous species fish meal is unnecessary and is only used because it is currently economically viable (Bostock et al. 2010). In China, where aquaculture has historic roots, the aquaculture industry has previously utilized waste as feed (including animal waste, food waste, agricultural byproducts, and grass clippings) (Mo et al. 2018). Recently, more intensive culture methods that require commercial, formulated pellet feeds are used (Mo et al. 2018).

Demand, and therefore prices, for crops (especially soy) as aquaculture feed ingredients is expected to increase as aquaculture production expands to meet the rising demand for seafood (Naylor 2020). It is estimated that purchasing feed represents more than half of the production cost of aquaculture and that feed cost is a major limiting factor in the growth of aquaculture in developing countries (Nasser et al. 2018). Utilizing nontraditional feeds such as food waste to supplement or replace commercial feed should decrease the cost and increase the sustainability and stability of aquaculture (Nasser et al. 2018). Unlike the production of capture fisheries which are based on the natural productivity of local ecosystems, aquaculture is dependent on external feed inputs (Tacon and Metian 2015). More than 70% of global aquaculture production is dependent on such external feed inputs (Tacon and Metian 2015). There has been advancement in substitution of the nutrients provided by fish meal with different sources of protein (such as bioethanol by-product and seed or legume meals) (Bostock et al. 2010). Several plant and animal-based alternatives to traditional feed ingredients are available and used in aquaculture feeds, and with appropriate economic and regulatory incentives, transitioning to alternative feeds could accelerate (Naylor et al. 2009). The global aquaculture industry is diverse, ranging from small ponds producing a few kilograms of fish to international companies producing $1 billion worth of fish per year (Bostock et al. 2010). It is estimated that globally, more than 20 million people worked (full time, part time, or occasionally) in aquaculture, and most were employed as small-scale fishers and aquaculture workers (FAOb 2020). Small-scale aquaculture is one of the few economic activities available in rural, coastal locations and is especially important to produce subsistence and cash crops in countries like Bangladesh, India and Vietnam (Bostock et al. 2010). Additionally, small-scale aquaculture is used in hatch-and-release programs to rebuild vulnerable wild stocks and conserve aquatic habitats using species like salmon, abalone and oysters (NOAA 2020). Small-scale aquaculture is also used in the ornamental fish trade, which uses a majority of the same feed ingredients as commercial foodfish aquaculture, specifically fishmeal (Sicuro 2018). Freshwater species make up the majority of the ornamental fish trade and are usually produced in small-scale aquaculture facilities (Livengood and Chapman 2020). These facilities primarily utilize small, outdoor ponds dug into the ground, but can also utilize indoor recirculating systems (Watson and Shireman 2002, Martinez 2019).

The US is the largest importer of ornamental fish, and it is estimated that the global value of ornamental fish and invertebrate imports corresponds to $278 million (Livengood and Chapman 2020). Additionally, the US state Florida is a major producer of tropical ornamental fish (95% of the country's domestic ornamental fish production), with an estimated value of $175 million annually (Watson and Shireman 2002, Martinez 2019). Although the Florida ornamental fish industry had existed since the 1930s, recently it has expanded, with more than 700 varieties and 400 species of ornamental fish that are sold globally (Watson and Shireman 2002, Martinez 2019). Minor production of ornamental fish also occurs in the western US (Watson and Shireman 2002). Ornamental fish aquaculture is also practiced in Thailand, Singapore, Indonesia, and Malaysia (Watson and Shireman, 2002). Regardless of location, ornamental fish culture is dependent on fish breeding and the production of eggs, larvae, and/or fry (Martinez 2019). It has been reported that the fecundity of green swordtail increased when fed a diet with more than 30% protein (Velasco-Santamaría and Corredor-Santamaría 2011). Farmed ornamental fish are often raised on the same commercially available, conventional aquaculture feeds used in foodfish aquaculture (Sicuro 2018). However, feed marketed toward ornamental fish is often priced much higher than conventional feeds due to packaging and commercialization (Sicuro 2018).

Food waste and food by-products have been successfully used to replace or supplement fish feed in several studies. There is also interest in replacing the fish meal component of ornamental fish feeds with alternative and less costly protein sources, such as soybean or insect meals (Sicuro, 2018). A study involving grass carp reported desirable protein utilization, weight gain and FCR, as well as enhanced immunity from bacterial infection with a food waste-based diet using plant protein supplemented with yeast and enzymes (Mo et al. 2020). A study indicated that up to 35% corn germ meal (a by-product of milling corn) can be included in a diet for channel catfish without effect on survival, weight gain, feed conversion, or nutritional value (Li et al. 2013). Another study with channel catfish found that the fish meal component of a 32% protein diet could be totally replaced with pork meat and bone meal (a pork processing by-product) without effect on survival, weight gain, or feed conversion (Li et al. 2020). Nile tilapia were fed diets with recycled food waste with no significant differences in growth performance (Bake et al. 2013); similarly in a separate study no significant differences in weight gain or specific growth rate were seen in tilapia fed food waste (Al-Ruqaie 2007). Low trophic level fish including grass carp, grey mullet, bighead carp, and tilapia were fed a 70% food waste feed with acceptable levels of nutrients for growth (Mo et al. 2014).

The growth of fish fed a food waste-based feed may currently be comparable to those fed a commercial feed. However, increased growth and other positive effects should encourage the use of food waste-based feed, diverting food from landfills and increasing the sustainability of aquaculture. Using supplements such as yeast and enzymes in food waste could improve fish growth and weight gain (Mo et al. 2020). Other supplements that could increase the growth of fish fed food waste-based diets include vitamin and mineral mixes, prebiotics which promote the growth of beneficial microorganisms in the gut, and medicinal herbs (Wong et al. 2016). Another process to increase the quality of food waste-based fish feed is fermentation, where fungal microorganisms are grown on organic substrates with minimal water (Lateef et al. 2008). Fermentation was found to increase the protein content, decrease the fiber content, and improve antioxidant activity of agricultural wastes (such as cocoa, cassava, and palm by-products) (Lateef et al. 2008).

Zebrafish

Zebrafish have become a common model organism in academic research because of their high spawning rate, short generation time, and small size (Ulloa et. al. 2011). Zebrafish are also used as a model in aquaculture research. As freshwater teleost fish, information from zebrafish can be generalized to some popularly cultured fish such as tilapia, carp, and catfish (Ulloa et. al. 2011). Their small size and rapid growth rate make zebrafish preferable for aquaculture research compared to other fish, which may take months to reach adulthood and require more food and space.

Wild zebrafish (native to south Asia) are reported through field observation and gut analysis to consume zooplankton and phytoplankton, insects (especially larvae but also eggs), arachnids, algae and plant material, spores, and material such as fish scales, detritus, sand, and mud (Watts et al. 2012). Notably, the diversity of this diet indicates that zebrafish feed at the surface, throughout the water column, and at the benthos of their habitat (Watts et al. 2012). This omnivorous feeding strategy allows zebrafish to utilize nutrients from a variety of sources, a trait common in the cyprinid family, which includes zebrafish, carp, and minnows (Watts et al. 2012).

Despite their popularity in research, the daily dietary requirements of zebrafish at any life stage (larvae, juvenile, or adult) are not yet known (Watts et al. 2012). In an effort to address this, a study using zebrafish juveniles found that weight gain, protein retention, and feed efficiency increased with protein content up to 40% (Fernandes et al. 2016). It also found that maximum weight gain and protein retention corresponded with protein contents of 37.6% and 44.8% respectively (Fernandes et al. 2016). Therefore, the target protein level for the CFF produced for this study was set at 38%, so that a diet formulated with 100% CFF would meet the minimum protein requirement for optimal weight gain in zebrafish.

Nutrient requirements for fish depend on a variety of factors including oxygen consumption, temperature, and fish maturity (Watts et al. 2012). Energy use and metabolic demand are sensitive to temperature, which also influences the feeding activity and reproduction of zebrafish (Watts et al. 2012). A deficit or excess of digestible energy can reduce growth rates and lead to deposition of body fat (National Research Council 1993). In fish diets, too little protein leads to suboptimal growth and weight, while too much protein leads to excessive ammonia excretion and poor water quality (Fernandes et al. 2016). Protein requirements for zebrafish have been found to be related to growth rates, both of which decrease as the organism ages (Fernandes et al. 2016). Zebrafish have also been observed to have sex-specific growth rates (and therefore, protein requirements), with females having higher growth rates than males at the same age (Fernandes et al. 2016). Most freshwater fish require 30-53% protein, and up to 35% carbohydrate in their diets (Watts et al. 2012). Proteins and lipids are both utilized as energy sources by fish, but the use of carbohydrate varies between species (National Research Council 1993). Omnivorous fish species, similar to zebrafish, were reported to digest more than 70% of the gross energy provided by carbohydrates in feed, while rainbow trout, a carnivore, digested less than 50% (National Research Council 1993). Generally, formulated fish diets are not supplemented with trace minerals (such as magnesium, calcium, and phosphorus) because these compounds are incorporated into the water, where fish can absorb them via the gill membranes and intestinal epithelium (Velasco-Santamaría and Corredor-Santamaría 2011).

For fish, ten amino acids have been reported to be essential, as they cannot be synthesized in the body and must be ingested. For juvenile common carp, a cyprinid like zebrafish, reported amino acid requirements are: 1.6% Arginine, 0.8% Histidine, 0.9% Isoleucine, 1.3% Leucine, 2.2% Lysine, 1.2%

Methionine, 2.5% Phenylalanine, 0.3% Threonine, 0.3% Tryptophan, and 1.4% Valine (National Research Council 1993). For essential fatty acids, common carp (a relative of zebrafish) also require 1% linoleic acid and 1% linolenic acid (National Research Council 1993). Some fish species grow optimally with up to 20% lipid in the diet, but excessive lipid levels may result in fat deposition (National Research Council 1993).

Rationale and Purpose of the Study

Due to its abundance and difficulty of disposal, there is an urgent need to manage food waste (Wong et al. 2016). While reducing food waste at the source is most desirable, using excess food to feed people or animals is preferred to isolating it in a landfill (EPA 2021). However, there are issues with storage and transportation as well as health and safety concerns when donating food waste for human consumption. The increasing demand for fish products and the rising costs of fish feed ingredients makes recycling food waste into aquaculture feed an attractive and sustainable option (Nasser 2018, Naylor 2020).

Fish are an excellent candidate for food waste-based feed. Ruminants like cows, sheep, and goats are vulnerable to bovine spongiform encephalopathy (BSE or mad cow disease) spread by producing feed from infected animal blood, bone, and tissue. Fish are not known to be vulnerable to this disease (Wong et al. 2016) and as such should be able to safely consume feed made from ruminant meat. Furthermore, fish rely on external temperatures and therefore are more efficient at converting feed into body weight compared to warm-blooded farmed animals like mammals and birds (Robinson and Li 2015). Pre-consumer food waste is thought to be the most effective target for recycling because it is usually separated from the rest of an institution's waste stream during food preparation, decreasing the food waste's contamination with undesirable materials such as plastics, metals, glass, and chemicals (UGA 2017). Post-consumer food waste is more difficult to separate, usually because the consumer has contaminated the food with undesirable materials and because the consumer must decide to separate the uneaten food from the rest of the waste stream (UGA 2017). Schools and universities represent ideal opportunities to recycle food waste as cafeterias and dining halls are controlled environments with regular, daily consumers, often the same consumers for consecutive years (Wilkie et al. 2015). Also, educational campaigns can be incorporated into the existing curricula to promote sustainability and environmental conservation, raising awareness of food waste (Wilkie et al. 2015).

Using pre-consumer food waste is preferable to post-consumer food waste (food that is served but not eaten) for aquaculture feed because food waste that has not been in contact with a consumer's hands and mouth is more hygienic. It also allows for more control over the macronutrient levels (protein, carbohydrate, fat) in the collected food waste and resulting feed. Bromelain, an enzyme found in pineapple, was shown to enhance the feed conversion ratio and immune response of grass carp and grey mullet when added to a food waste-based feed (Wong et al. 2016), therefore pineapple was selected as an ingredient in the CFF used in this study. Purple cauliflower, another ingredient selected for the CFF used here, contains anthocyanins, compounds known to reduce oxidative stress in tissues by neutralizing free radicals and thought to have other health benefits as well (Singh et al. 2020). After collection, freezing the collected food waste is an important step. In addition to preserving the fresh food, freezing improves the bioavailability of nutrients as ice crystals expand and puncture the cell walls of the fruits and vegetables (Jin and Chen 2018). This also helps to produce a smoother and more uniform texture in the CFF. After being frozen, food waste was thawed and then cooked thoroughly with a hot water bath. Treating food waste with heat is crucial when recycling food into animal feed because heat inactivates anti-nutritional factors such as tannins or alkaloids that may be present in peels, leaves, and root tubers (García et al. 2005). Cooking also increases the digestibility of carbohydrates by fish (National Research Council 1993). Further, heating food reduces undesirable microbial populations that may cause illness (García et al. 2005).

As protein is essential for somatic growth, protein levels are important considerations when formulating a diet. However, excessive protein intake can lead to increased ammonia excretion, which is toxic to fish and can decrease water quality (Fernandes et al. 2016). Protein requirements for zebrafish have been found to be related to growth rates, both of which decrease as the organism ages (Fernandes et al. 2016). Zebrafish have also been observed to have sex-specific growth rates (and therefore, protein requirements), with females having higher growth rates than males at the same age (Fernandes et al. 2016). Several substitutes for traditional protein sources in aquaculture feed have been studied. For instance, it was found that soybean by-product and food waste from restaurants and hotels were acceptable protein replacements for fishmeal in diets for tilapia without reducing growth (Bake et al. 2009). In a study using recycled food waste with protein sources including beef, pork, chicken, salmon, and grouper, the cultured fish exhibited similar or better growth compared to a commercial diet (Cheng et al. 2015). These results indicate that the protein component of fish feed can be replaced with a food waste-based protein source without adversely affecting fish growth. Therefore, beef was chosen for the CFF in this study due to its abundance at the Dining Commons where the food waste for this study was sourced. Beef is reported to contain both linoleic acid and linolenic acid, essential fatty acids for common carp, and it is a complete protein, containing all essential amino acids for fish (Wood et al 2008, Wu et al 2016, National Research Council 1993).

Efficiently utilizing food waste as aquaculture feed is difficult because the current waste collection system usually mixes organic and inorganic waste and transports waste from the source point to a waste processing plant (García et al. 2005). Another challenge to widespread incorporation of food waste-based feed in aquaculture is the need to separate and sort the food waste at the source to formulate feeds with acceptable nutrient profiles. A food waste audit at an elementary school found significant variability in the types of post-consumer food wasted during the week, with vegetable waste ranging from 26.1% to 80% of overall food waste depending on the day, showing a strong effect from consumer preference and behavior (Wilkie et al. 2015). In another food waste audit, unsorted food waste was found to contain only about 20% protein, which is inadequate for even low trophic level fish, which have lower protein requirements than carnivorous fish such as salmon (Mo et al. 2018). Because of these challenges, food waste-based feeds are most appropriate for less intensive, small-scale aquaculture. Small-scale aquaculture excludes fish production from large commercial fisheries, but includes fish production for recreational and ecological purposes, as well as pet and aquarium trade, and small-scale aquaculture in less industrialized nations. Utilizing food waste, which can be obtained for free while being diverted from landfills, should sustainably increase production in small scale aquaculture. Food waste-based feeds should also increase the stability of small-scale aquaculture, by increasing profits and reducing farmers' reliance on commercial feeds (Nasser et al. 2018).

Objectives, Hypotheses, and Predictions

The main objectives of this study were to (1) determine the feasibility of recycling university pre-consumer food waste into Converted Fish Flakes (CFF), and (2) to determine if CFF is appropriate for supplementing or replacing commercial feed in small-scale aquaculture using zebrafish as a model. With the cooperation of the university dining staff, it was hypothesized that (1) food waste could be collected and recycled into CFF with a protein level appropriate for juvenile zebrafish. Since the CFF used in this study was formulated with previously reported optimal protein levels for zebrafish (Fernandes et al. 2016), it was further hypothesized that (2) CFF diets are suitable for zebrafish somatic growth and function.

Correspondingly, it was predicted that (1) CFF can replace 100% of commercial feed without a significant effect on zebrafish survival rates and that (2) CFF with adequate protein can replace up to 50% of commercial feed without reducing weight and length gain in zebrafish. Further, it was predicted that (3) zebrafish fecundity would not be decreased with diets containing up to 100% CFF, and that (4) VSI of zebrafish fed CFF diets would not be significantly different from those fed a commercial diet. Finally, it was predicted that (5) CFF diets would not cause morphological changes in the intestine or liver.

CHAPTER 2
METHODS

Production of Converted Fish Flakes (CFF)

Fruit, vegetable, and meat (including fat) trimmings were collected on a weekly basis from the Georgia Southern University Dining Commons by coordinating with the Dining Commons employees to obtain the desired foods according to the kitchen's preparation schedule over several weeks. Certain foods were excluded, such as red onion peels and bell pepper stems and seeds (Figure 3A), to avoid pungent smells or tastes which might affect the palatability of the CFF. To create nutritionally consistent CFF, three foods were manually separated from the rest of the kitchen's food waste for processing (Figure 3B): pineapple cores, cauliflower (including purple cauliflower) leaves and stems, and beef flank steak. Because the steak trimmings contained fat as well as meat, it was assumed that the CFF would also have some amount of fat after processing, and fat was not specifically collected for the production of CFF.

After collection from the Dining Commons, food was weighed and frozen until adequate portions of each food type had been collected. After the food had been collected, the food was thawed and cooked using a hot water bath at 70 °C for 3-4 hours. The cooked food was then combined by weight to a proportion of 38:62 of meat:fruit/vegetable to obtain a mixture of food with a protein level of 38%. As fruits and vegetables contain a very high proportion of water, the final carbohydrate content of the CFF was expected to substantially decrease. After combining, the food mixture was processed with a food processor into a smooth paste (Figure 3C).

After processing into a uniform paste, the food mixture was very thinly spread onto nonstick silicone sheets. Finally, the processed food mixture was dried with a dehydrator at 66°C for 24 hours to produce thin sheets which were broken into small flakes (Figure 3D). One pound of food waste produced approximately half a pound of flakes. Portions of CFF were mixed with the control feed to produce treatment diets containing by weight 25% CFF and 50% CFF. The control feed selected was Egg Yolk Flakes (Pentair, Minneapolis, MN, US). Samples of the control and treatment diets were sent to RL Food Testing Laboratory (Newbury Park, CA, US) for a guaranteed analysis of the macronutrients contained in a daily serving (2 grams) (Table 2). Each treatment diet exceeded the target protein level of 38%, therefore the study proceeded using the ratios 0% CFF (Control), 25% CFF, 50% CFF, and 100% CFF.

Figure 3. Collection and production of food waste-based feed for a small-scale aquaculture study using zebrafish. (A) Some parts of food, such as peels, rinds, and stems, are separated during food preparation as pre-consumer food waste and are collected in designated bins to reduce contamination with undesirable materials such as paper, plastic, and chemicals. (B) To produce palatable and consistent feed, only a few food items were selected for processing. (C) After collection food was frozen, then cooked, and portioned to contain 38% protein by weight. The cooked portions were then processed with a food processor into a smooth paste, which was dried in a dehydrator (D-E) to produce Converted Fish Flakes (CFF), which were used to feed zebrafish (*Danio rerio*) (F).

	Treatment			
	Control	25% CFF	50% CFF	100% CFF
Nutrient				
Minimum Crude Fat (%)	9.0	18.6	26.8	39.8
Minimum Crude Protein (%)	50.5	48.4	46.2	39.7
Maximum Fiber (%)	1.8	3.1	3.1	2.8
Moisture (%)	6.3	3.0	3.0	5.1
Ash (%)	14.1	11.3	8.2	2.8

Table 2. Nutrient analysis of control and treatment diets from RL Food Testing Laboratory for a daily serving (2 grams).

Animal Husbandry

In this study, juvenile wildtype (AB, non-transgenic) zebrafish were randomly added to 2.8 liter tanks in a recirculating water system (Aquaneering, San Diego, CA, US), as seen in Figure 5. Fish were fed *ad libitum*, approximately 1 gram of flakes every morning and evening for a total of 2 grams per tank per day. The first trial evaluated the growth and reproductive performance of zebrafish fed a Control (0% CFF), 25% CFF, 50% CFF, or 100% CFF diet for 32 days. In the second trial, zebrafish were fed a Control, 25% CFF, or 50% CFF diet for 32 days and growth was again measured. Additionally, the viscerosomatic index (VSI) was calculated and a histological analysis of the liver and intestine was performed to determine if there were morphological changes associated with stress or excessive lipid deposition.

Suitable water quality parameters for zebrafish include temperature between 26 to 28.5 °C, pH between 6.8 to 7.5, nitrite levels less than 0.1 ppm, nitrate levels less than 50 ppm, and ammonia levels less than 0.02 ppm (Avdesh et al. 2012). For Phase 1, experimental groups (25% CFF, 50% CFF, and 100% CFF) were isolated from the Control group, which was a part of a larger tank system which housed other zebrafish stocks (Aquaneering, San Diego, CA, US) to evaluate the effect of CFF diets on water quality. The 100% CFF diet was omitted from Phase 2 of this study to evaluate the effects of CFF diet supplementation, rather than replacement, and the control and experimental tanks were integrated into the same tank system. Water quality parameters for this study can be seen in Table 3.

Trial	Temperature (C)	pH	High pH	Nitrites (ppm)	Nitrates (ppm)	Ammonia (ppm)
1	25.1 ± 0.35	7.6 ± 0.15	7.5 ± 0.22	0.3 ± 0.46	10.0 ± 21.71	4.7 ± 3.08
2	26.1 ± 0.69	7.4 ± 0.21	7.4 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.2 ± 0.17

Table 3. Average experimental water quality parameters, tested weekly with API Freshwater Master Test Kit (Chalfont, PA, US), ± one standard deviation.

Data Collection

Trial 1

An overview of the experiments can be seen in Figure 4. In this study growth was evaluated using the difference between total length (snout to tail tip) and body weight at the beginning and end of the study. Groups were monitored for individual mortality over the course of the trials to determine the survival rate.

Length was measured by first transferring zebrafish (by group) into a tank dosed with 0.01% tricaine, then catching individual sedated fish with a small mesh net, gently blotting away excess water, and transferring the fish to a weighing paper. While on the paper, the fish was quickly aligned with a ruler and total length was noted. Weight was determined immediately after measuring length by placing each fish, on the weighing paper, on a tared scale to obtain individual body weight in grams. After obtaining starting lengths and weights, zebrafish were released into their usual tank to recover from sedation. After all fish had recovered, tanks were reconnected to the recirculating tank system.

In aquaculture, an important measure of fish health and fishery production is the ability to produce viable offspring. To evaluate the reproductive ability of zebrafish fed CFF, at least two males and two females from each group were paired and allowed to breed. The resulting embryos were collected, counted, and observed for 5 days at standard conditions for survival. This experiment was performed after final weight and length were measured due to the high metabolic cost of reproduction, which could affect the weight gain of the fish.

Trial 2

In the second trial, survival rates, weight gains, and length gains were measured again using the same methods as in Trial 1. Additionally, at the end of 32 days of feeding, three male and three female zebrafish from each treatment group were euthanized using standard procedures and fixed in 37% formaldehyde. Fixed zebrafish were sent to a histology laboratory (College of Veterinary Medicine, University of Georgia, GA, US) for paraffin embedding, sectioning, staining with Hemotoxylin & Eosin and Sudan IV dye in slides. Stained histology slides were received and observed for the presentation of normal and abnormal histology of the liver and intestine. Liver sections were checked for excess lipid accumulation, and intestines were checked for signs of inflammation. Sections from approximately the same location of the liver and intestine for each fish were selected and quantification of the histological analysis was performed by manually counting the number of fat-filled vacuoles in the upper right quadrant of the liver sections and by manually counting the number of goblet cells in the intestine sections.

At the conclusion of the study, a sample of five male fish per treatment were dissected and weighed again after the viscera was removed to obtain the viscerosomatic index, or VSI. The VSI is a useful measure of the distribution of body weight, either in muscle or in viscera. Male fish were chosen because female zebrafish have large ovaries, which could potentially bias the results toward a smaller VSI.

Calculations and Statistical Analysis

Data was input, visualized, and calculations were performed with Excel 2015 (Microsoft, Redmond, WA, US). The survival rate was calculated as ((final # / start #) * 100). Embryo survival rates were calculated as ((# surviving at day 5 / # total) * 100). Individual starting and final weights and lengths were averaged by replicate, and percent change in weight and length was calculated as ((average final – average start) / average start * 100). Fecundity was measured by manually counting the total number of embryos produced by each breeding pair. Individual VSI were calculated as ((viscera weight / final body weight) * 100).

Statistical analysis was performed using JMP (SAS, Cary, NC, US). Fecundity, embryo survival rates, and liver vacuole counts met the assumptions of normality with the Shapiro-Wilk test and equal variance with the Levene test. Percent change in weight for both trials, percent change in length for Trial 1, survival rates, and intestine goblet counts were log transformed to meet the assumptions of normality and equal variance. These data were analyzed with a one-way ANOVA to test for differences by treatment (Control, 25% CFF, 50% CFF, and 100% CFF). Percent change in length for Trial 2 and VSI did not meet the assumption of equal variance for ANOVA after log transformation, so a nonparametric Kruskal-Wallis test was used for differences by treatment for these data. Statistical outputs can be seen in Table 4.

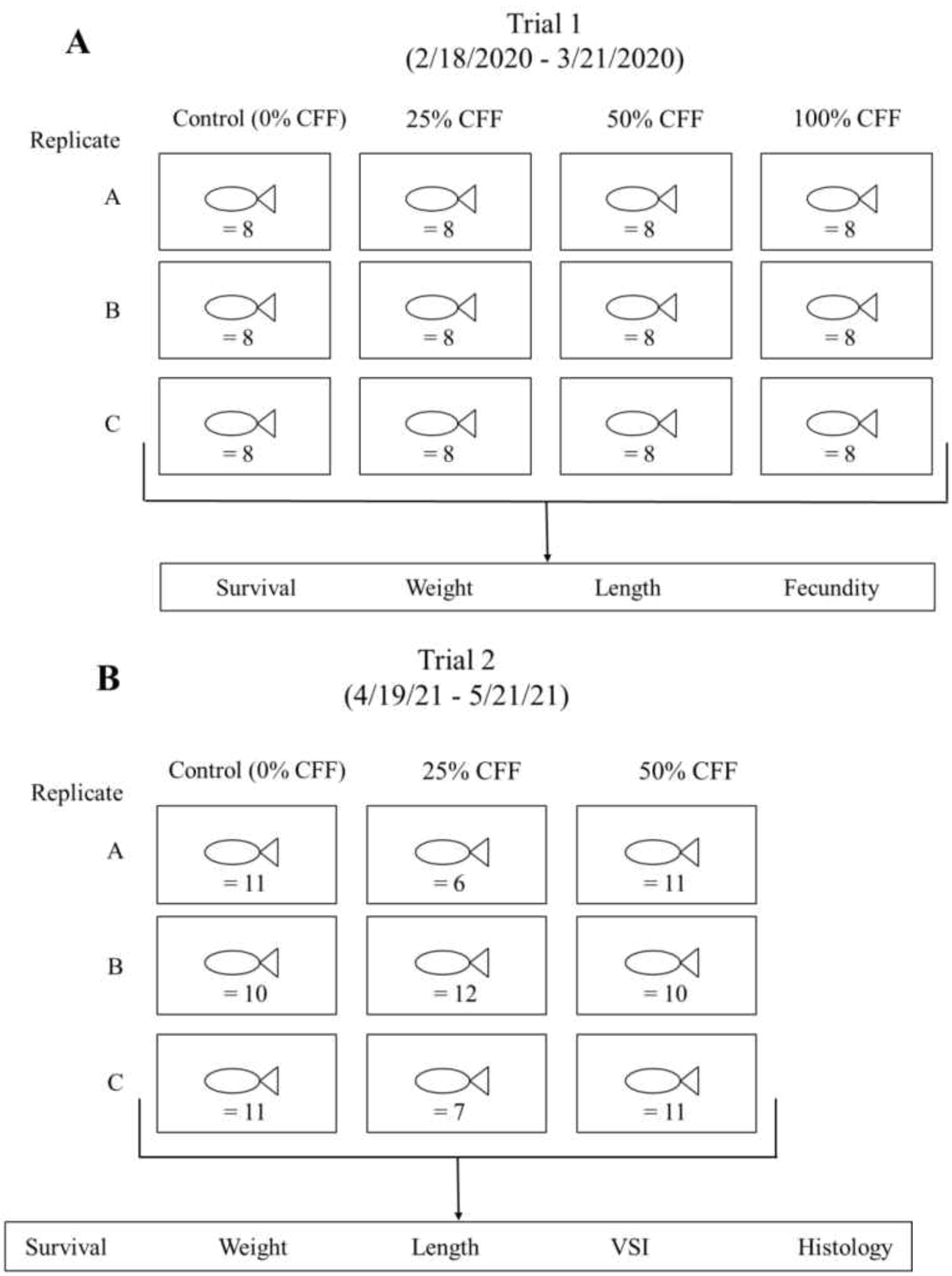

Figure 4. Experimental overview of this study: The first trial (A) evaluated zebrafish survival, growth, and fecundity when fed increasing proportions of food waste-based feed (25% CFF, 50% CFF, and 100% CFF for 32 days. The second trial (B) used the same Control, 25% CFF, and 50% CFF feeds and in addition to measuring survival and growth, evaluated the viscerosomatic index (VSI) as well as morphology of the intestine and liver with a histological analysis.

Trial		df (Effect, Total)	F	*p*>F
1	Survival	3, 11	0.47	0.71
	Weight	3, 11	5.61	0.0228*
	Length	3, 11	4.87	0.0326*
	Fecundity	3, 11	0.47	0.71
	Embryo Survival	3, 11	0.55	0.66
2	Survival	3, 11	0.55	0.66
	Weight	2, 8	1.35	0.33
	Goblet Cells	2, 17	35.16	<0.0001*
	Vacuoles	2, 17	14.72	0.0003*

Trial		df	ChiSquare	*p*>ChiSq
2	Length	2	2.22	0.33
	VSI	2	0.38	0.83

Table 4. Statistical outputs for one-way ANOVA and Kruskal-Wallis tests for differences between groups of zebrafish fed increasing proportions of food waste-based Converted Fish Flakes (CFF) for 32 days. Asterisks represent statistical significance.

CHAPTER 3

RESULTS

High Survival Rates in Zebrafish Fed CFF Diets

CFF diets did not affect the average survival rates in either trial (Figure 5). Survival rates were not significantly different between control and treatment groups in Trial 1 (p=0.71) (Table 4). No mortality was observed in any replicate in the Control, 50% CFF, and 100% CFF groups in Trial 1. Likewise, the 25% CFF group exhibited a high survival rate, and this treatment group had slightly more variation in survival rates between replicates (Figure 5A).

Similarly, survival rates were not significantly different between diet treatments in Trial 2 (p=0.66) (Table 4). As in Trial 1, the Control group had a 100% survival rate in every replicate (Figure 5B). In Trial 2, the 25% CFF and 50% CFF groups had similarly high survival rates, with the 25% CFF group again exhibiting a slightly higher variation in survival rates between replicates (Figure 5B).

CFF Diets and Changes in Zebrafish Growth

In Trial 1, average percent change in weight was highest in the 25% CFF group, and this group also exhibited more variation in percent change in weight (Figure 6A.) The lowest average percent change in weight was in the 50% CFF group (Figure 6A). A significant difference (p=0.0228, Table 4) was found between groups in percent change in weight in this trial. The 25% CFF and 100% CFF group shared a significance level, and the 100% CFF, 50% CFF, and Control groups shared a different significance level (Figure 6A). The 25% CFF group seemed to be the most different from the 50% CFF and Control group, as these groups did not share a significance level.

However, in Trial 2, the highest average percent change in weight was seen in the Control group, while the lowest percent change in weight was again seen in the 50% CFF group (Figure 6B). The Control group exhibited the lowest level of variation in percent change in weight, while the 50% CFF group exhibited the highest level of variation (Figure 6B). The 25% CFF group had a similar average percent change in weight compared to the first trial, and the Control and 50% CFF groups had much higher average percent changes in weight compared to the first trial (Figure 6A, B). However, in this trial, no significant difference (p=0.33, Table 4) was found between groups in percent change in weight for Trial 2.

In length for Trial 1, similar to the change in weight, the highest average percent change was also seen in the 25% CFF group (Figure 6C). The lowest average percent change was found in the 100% CFF group (Figure 6C). A significant difference (*p*=0.326, Table 4) was found between groups in percent change in length. The 25% CFF, 50% CFF, and Control groups shared a significance level, and the 50% CFF, 100% CFF, and Control groups shared a different significance level (Figure 6C). The 100% CFF and Control groups also had higher levels of variation than the other treatment groups. Interestingly, the 25% CFF and 100% CFF groups seem the most different in percent change in length, as these groups did not share a significance level.

There was no significant difference (*p*=0.33, Table 4) found between groups in percent change in length for Trial 2. In this trial, the Control group had the highest average percent change in length, and this group also had an extremely high level of variation compared to the other groups (Figure 6D). The lowest average percent change in length was in the 50% CFF group (Figure 6D). The 25% CFF and 50% CFF groups had similar average percent changes in length, and these groups also has similar levels of variation, which were much smaller than the level seen in the Control group (Figure 6D). The 25% CFF and 50% CFF groups also had higher average percent changes in length compared to the first trial (Figure 6B, D).

Zebrafish Fecundity is Not Altered with CFF Diets

Paired zebrafish from the 25% CFF group produced the highest average amount of embryos, and this group also had the lowest level of variation (Figure 7A). The Control, 50% CFF, and 100% CFF groups produced similar amounts of embryos, and had similar levels of variation (Figure 7A). However, no significant difference (*p*=0.71, Table 4) was found between treatment groups in total number of embryos.

The 25% CFF group also had the highest average embryo survival rate (Figure 7B). The 25% CFF, 50% CFF, and Control groups had similar levels of variation, while the 100% CFF group had a lower level of variation (Figure 7B). Again, no significant difference was found between groups for embryo survival rates (*p*=0.66, Table 4).

CFF Diets Do Not Affect Zebrafish VSI

Similar to what was observed in percent change in length (Figure 6B, D), the Control group had the highest average VSI, but also exhibited a high level of variation (Figure 8). However, no significant difference (*p*=0.83, Table 4) was found between groups in VSI. The 25% CFF and 50% CFF groups had a similar average VSI, and these groups also had similar levels of variation (Figure 8).

50% CFF Diet Associated with Increased Goblet Cell and Lipid Accumulation

A histological analysis of zebrafish intestine revealed differences between the control and treatment diets. The intestines of the Control and 25% CFF groups (Figure 9A, D and Figure 9 B, E) were similar, with many column-shaped enterocytes and few goblet cells observed in the villi, comparable to the reported normal zebrafish intestinal anatomy (Menke et al 2011). In the 50% CFF group more goblet cells (Figure 9C, black arrows) were observed in the villi, compared to the Control and 25% CFF groups as well as to the reported normal anatomy (Menke et al 2011). These changes seem to be more evident in females (Figure 9F) than in males (Figure 9C). Additionally, in female intestines from the 50% CFF group, pink-stained protein granules were observed (Figure 9F, black arrows) in the villi.

The livers of the Control group in both males and females show a majority of normal hepatocytes, few fatty deposits, and clear spaces were observed between the cells (Figure 10A, D), which are comparable to the reported normal liver morphology for zebrafish (Menke et al 2011). Similarly, in the 25% CFF group a majority of normal hepatocytes were observed, with slightly more fatty deposits (Figure 10B, E, black arrows). In the 50% CFF group, many more fatty deposits (vacuoles) were observed (Figure 10C, F, black arrows) compared to the Control and 25% CFF groups as well as to the reported morphology (Menke et al 2011).

Quantification of the goblet cells in the intestine sections revealed a significant difference between the approximate number of intestinal goblet cells in the 50% CFF group compared to the Control and 25% CFF group (Figure 11A). While the Control and 25% CFF groups had about the same average number of goblet cells and shared a level of significance, the 50% CFF group had a significantly ($p<0.0001$, Table 4) higher average number of goblet cells (Figure 11A). The 50% CFF group also had a higher variation in number of goblet cells (Figure 11A). Females in the 50% CFF group had a much higher average number of goblet cells (more than double) compared to males from the same group, confirming initial observations (Figure 11A and Figure 9C, F).

Similarly, quantification of the approximate number of fat-filled vacuoles in liver sections revealed a significant difference between the 50% CFF group compared to the Control and 25% CFF groups (Figure 11B). The Control and 25% CFF groups had about the same average number of fat-filled vacuoles and shared a level of significance. The 50% CFF group had a significantly ($p=0.0003$, Table 4) higher average number of fat-filled vacuoles in the hepatocytes, confirming initial observations (Figure 11B and Figure 10). Interestingly, the liver sections of males from the 50% CFF group were observed to have a slightly higher average number of fatty liver deposits than females, while in the Control and 25%

CFF groups females had slightly higher average numbers of fatty deposits (Figure 11B). Males in both the 25% CFF and the 50% CFF groups also had higher variation in number of fat-filled vacuoles (Figure 11B).

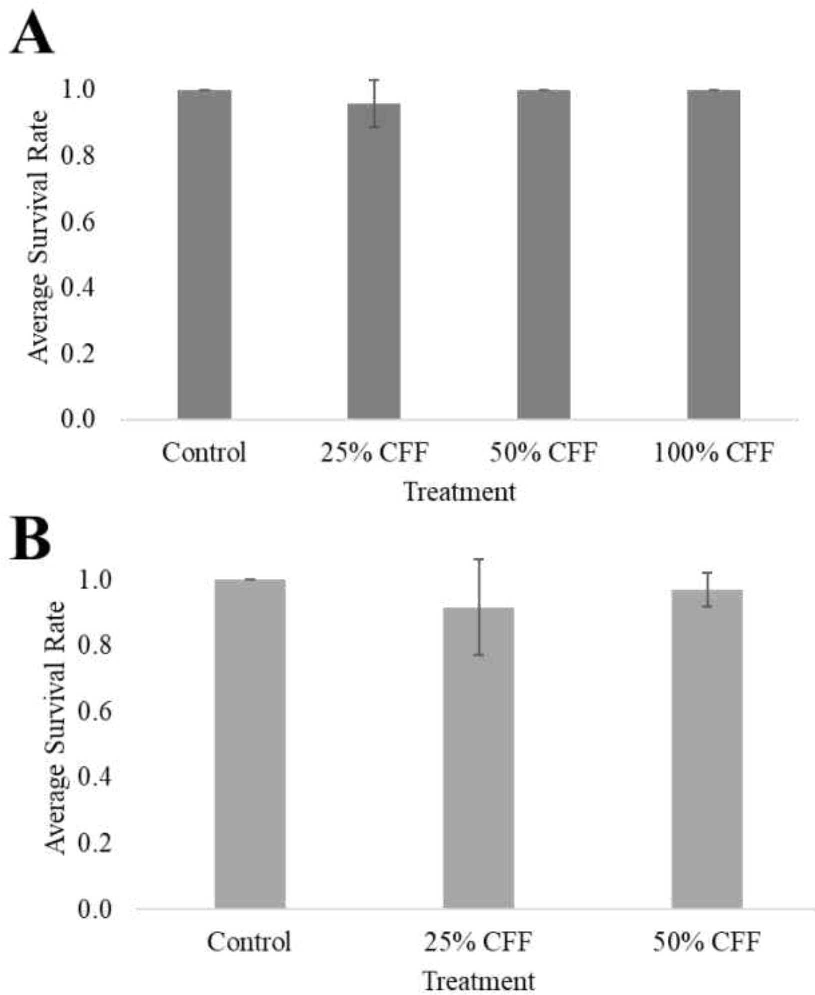

Figure 5. Average survival rates in Trial 1 (A) and Trial 2 (B) for zebrafish fed diets containing increasing proportions of Converted Fish Flakes (CFF) for 32 days. n=3. Error bars represent ± one standard deviation. No significant difference was found between groups in either trial using a one-way ANOVA.

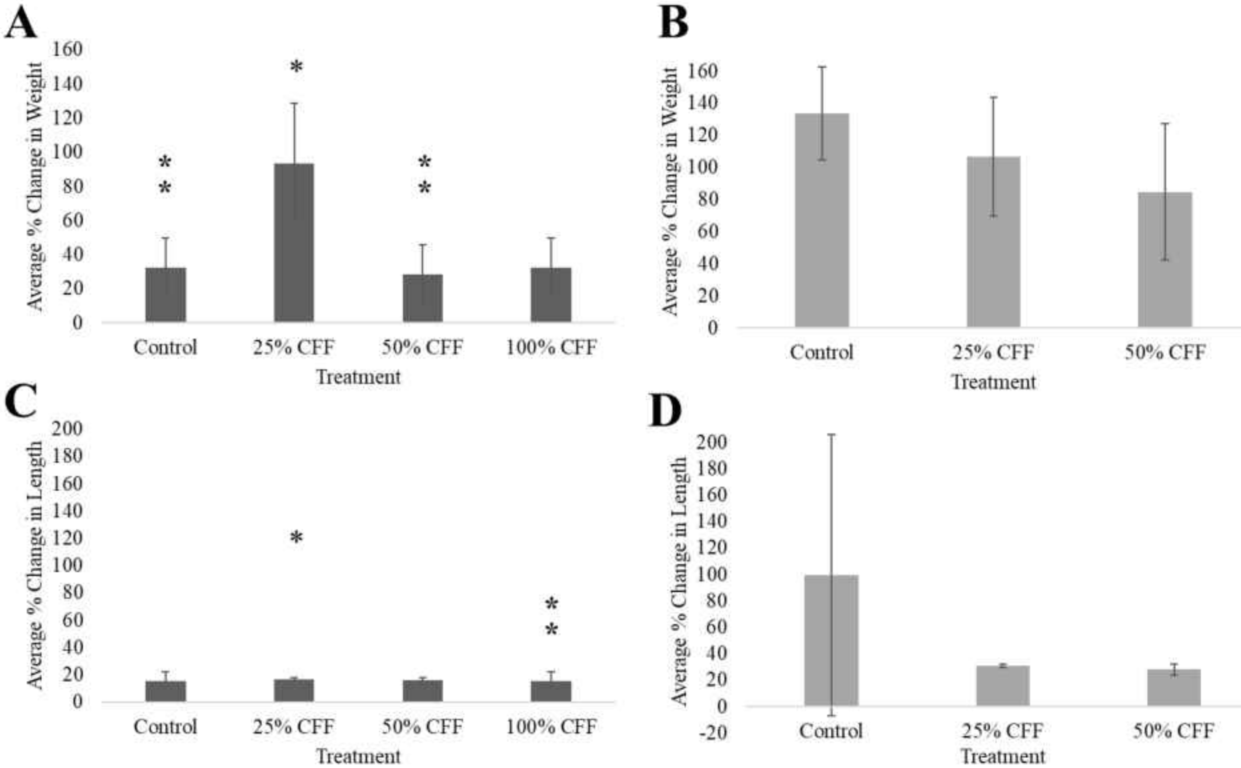

Figure 6. Growth in zebrafish fed diets with increasing proportions of Converted Fish Flakes (CFF) for 32 days. Average percent change in weight in Trial 1 (A) and Trial 2 (B). Average percent change in length in Trial 1 (C) and Trial 2 (D). Error bars represent ± one standard deviation, n=3. Groups with the same asterisk (* or **) share the same level of statistical significance, groups without an asterisk share both levels.

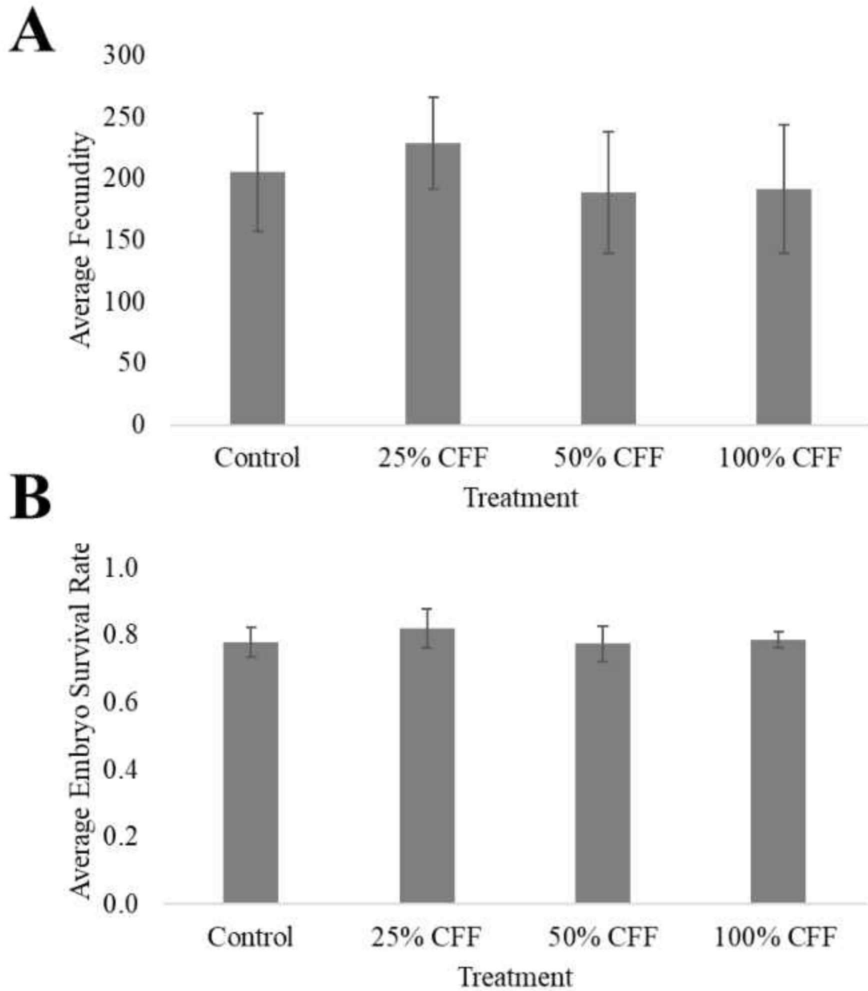

Figure 7. Average fecundity and embryo survival rates in zebrafish fed diets containing increasing proportions of Converted Fish Flakes (CFF) for 32 days. n=3. Error bars represent ± one standard deviation. No significant difference was found between groups using a one-way ANOVA.

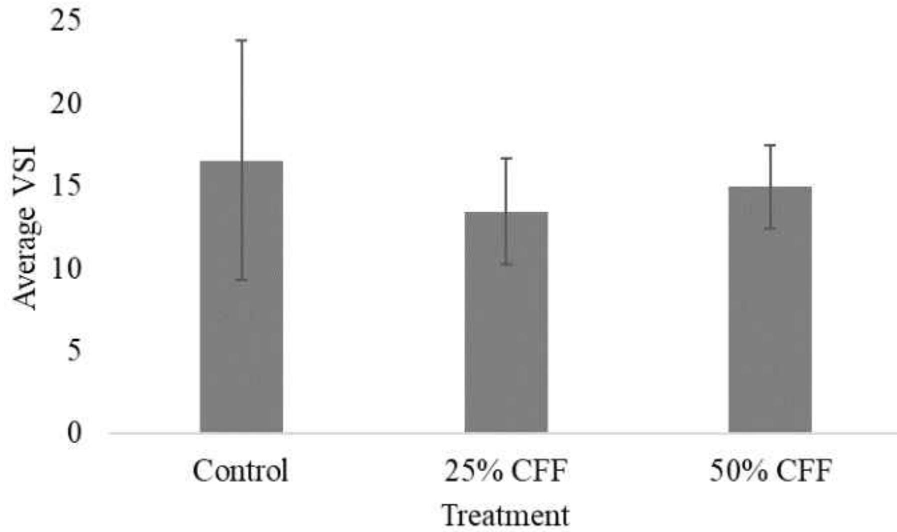

Figure 8. Average VSI in zebrafish fed diets containing increasing proportions of Converted Fish Flakes (CFF) for 32 days. n=3. Error bars represent ± one standard deviation. No significant difference was found between groups using a Kruskal-Wallis test.

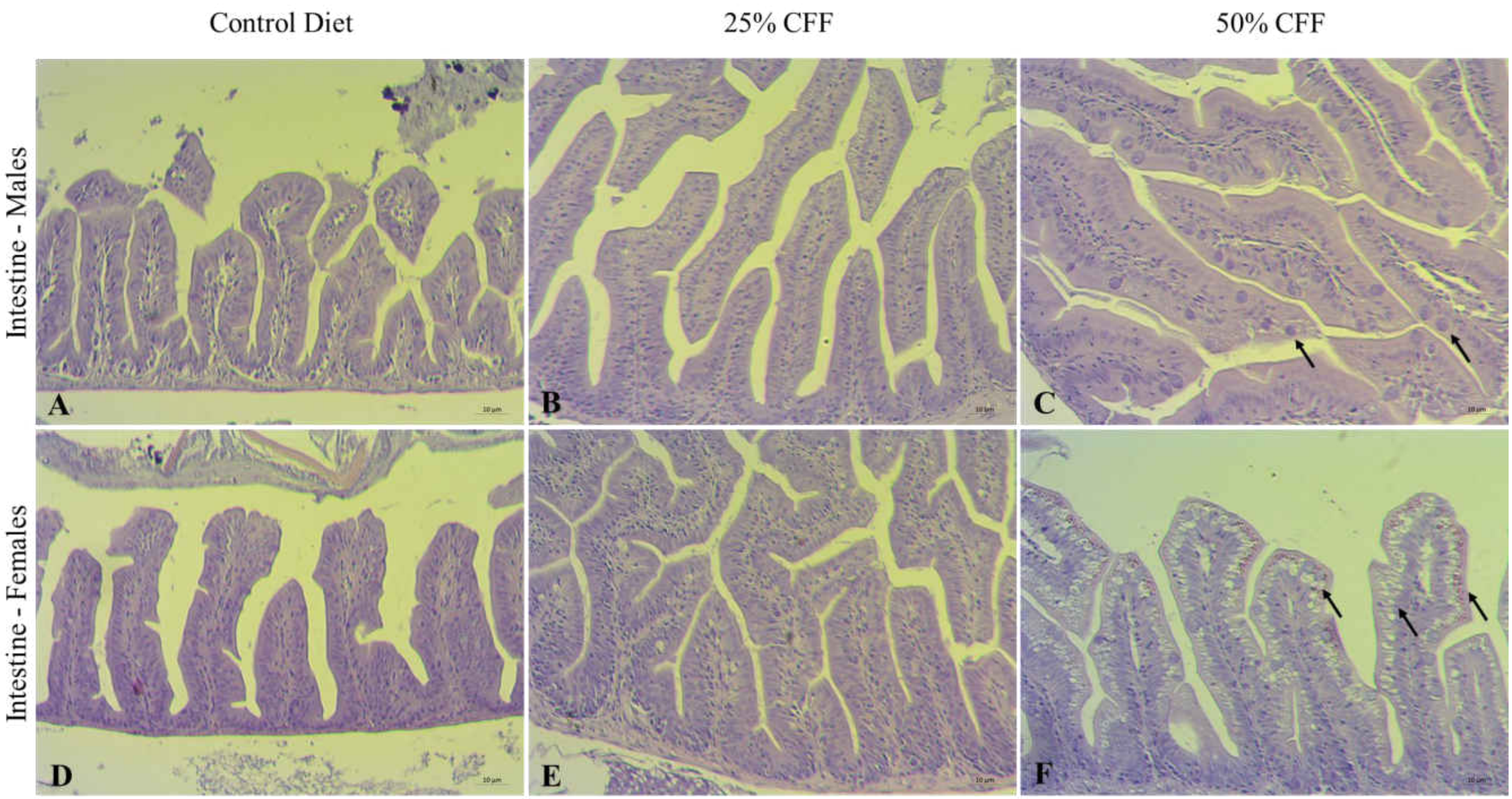

Figure 9. Representative images from sample of zebrafish fed increasing proportions of food waste-based Converted Fish Flakes (CFF) for 32 days sent for histological analysis of intestine (paraffin embedding, 5 µm sections, stained with Hemotoxylin & Eosin and Sudan IV) of intestines of male (A-C) and female (D-F).

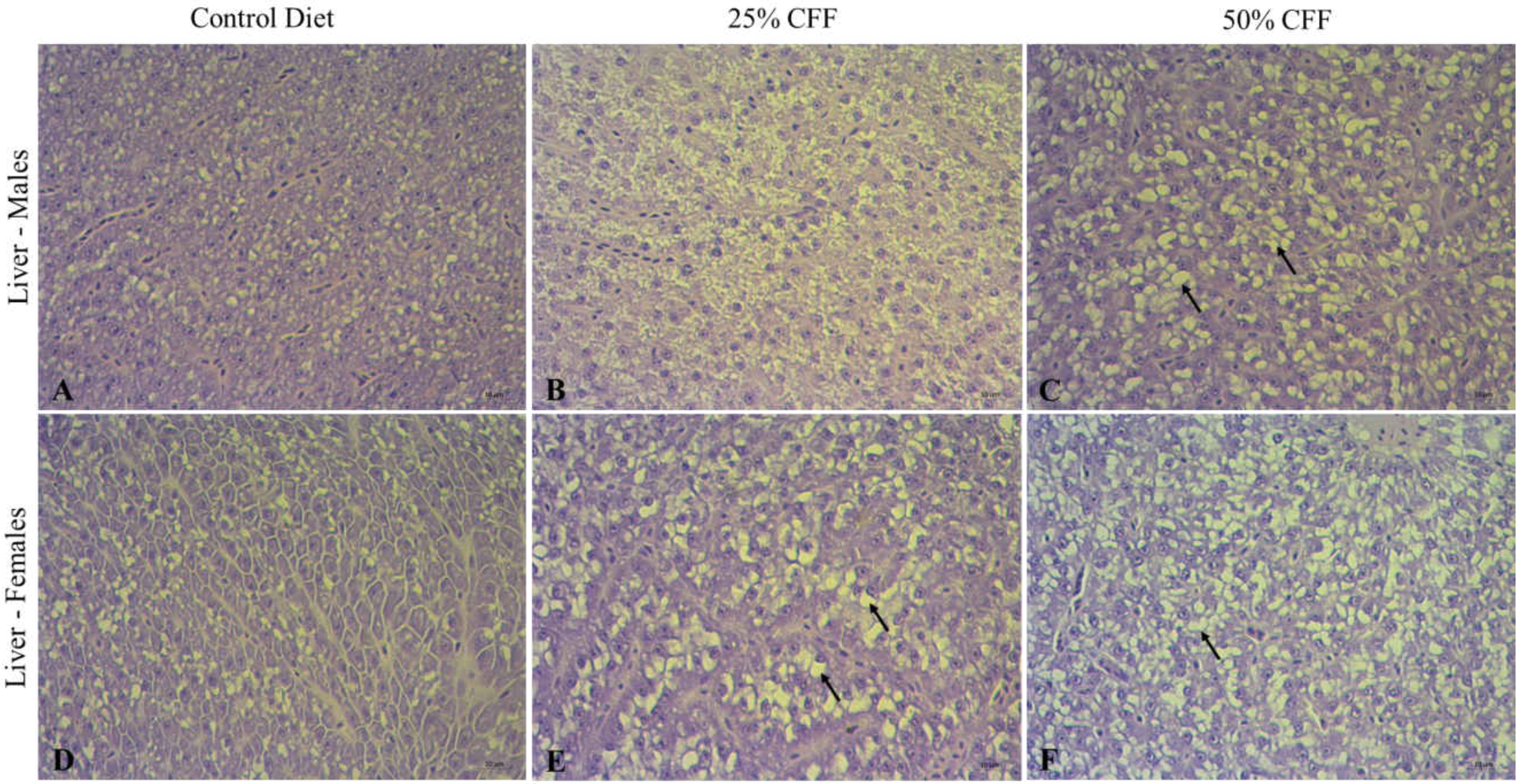

Figure 10. Representative images from sample of zebrafish fed increasing proportions of food waste-based Converted Fish Flakes (CFF) for 32 days sent for histological analysis of liver (paraffin embedding, 5 µm sections, stained with Hemotoxylin & Eosin and Sudan IV) of livers of male (A-C) and female (D-F).

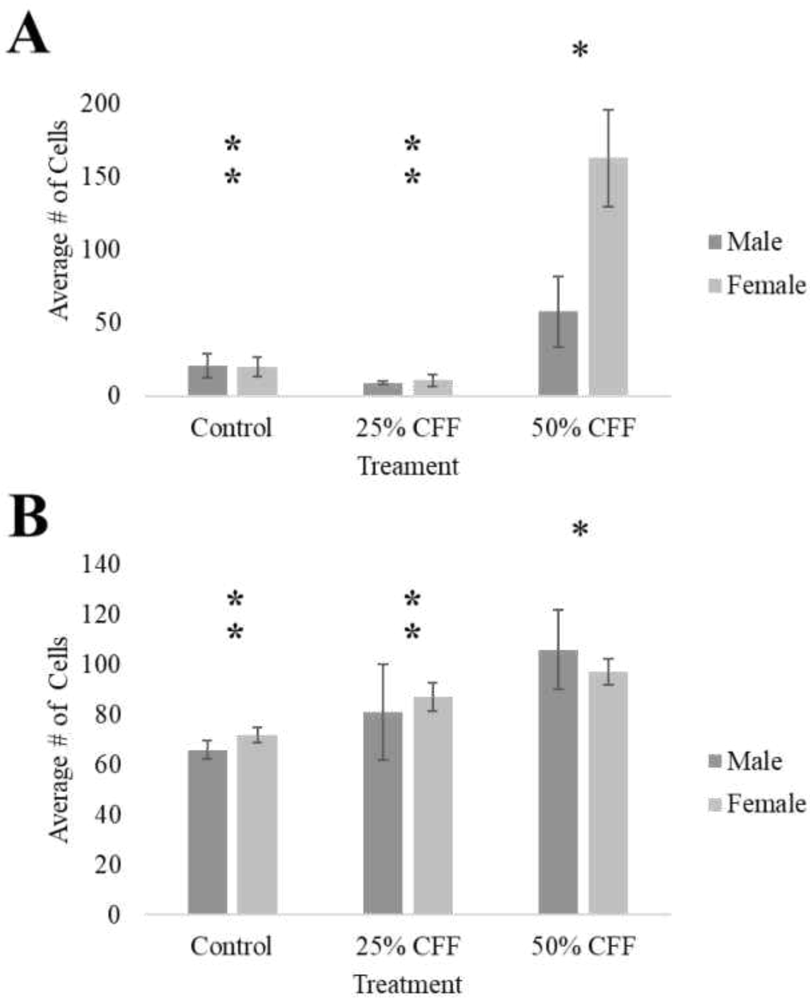

Figure 11. From manual counts of selected tissue sections of zebrafish fed increasing proportions of food waste-based Converted Fish Flakes (CFF) for 32 days. Average number of goblet cells in intestine sections (A) and average number of hepatocytes with vacuolar lipid accumulation in liver sections (B). Error bars represent ± one standard deviation, n=3. Groups with the same asterisk (* or **) share the same level of statistical significance.

CHAPTER 4
DISCUSSION

Food Waste Could Be Recycled into Aquaculture Feed

Overall, the high survival rates and production of viable offspring, as well as the growth in weight and length, of fish fed CFF diets indicate that food waste could be used to supplement commercial feed in freshwater aquaculture. The process used to produce CFF in this study was streamlined and straight forward and can be easily scaled up or down depending on the amount of CFF needed. This makes it an ideal process for use in aquaculture, where fish feed is sometimes produced on-farm (Tacon and Metian 2015). As the demand for fish products increases, demand and prices for crops as fish feed will also increase (Naylor 2020), making food waste an attractive alternative to traditional feed ingredients.

Additionally, these results demonstrate the feasibility of sustaining a small colony of zebrafish with high survival and reproductive rates at a university using campus-generated food waste, supporting the hypotheses of this study. Here, zebrafish fed a 100% CFF diet had high survival rates (Figure 5A), weight and length gains comparable to a commercial feed (Figure 6A, B), and had high fecundity and embryo survival rates (Figure 7). Zebrafish are widely used as a model in research due to their small size and rapid growth and reproduction (Ulloa et. al. 2011), and recently zebrafish are being used in K-12 schools so that students can use them in hands-on experiments (Wilk et al. 2018). Already two district-wide projects (BioEYES and InSciEd Out), and additional smaller projects, have brought zebrafish to high school students (Wilk et al. 2018). Using a food waste-based feed could decrease the cost and increase the sustainability of zebrafish colonies used for teaching students in these projects. Further study is needed to determine if food waste is an appropriate feed or feed supplement for zebrafish larvae or as a long-term feed for zebrafish adults.

In the first experiment of this study, excessive buildup of uneaten CFF, likely from the 100% CFF feed, was observed in the recirculating system, which is a possible explanation for the increased levels of ammonia, nitrates, and nitrites observed (Table 3). Suitable water quality parameters for zebrafish include temperature between 26 to 28.5 °C, pH between 6.8 to 7.5, nitrite levels less than 0.1 ppm, nitrate levels less than 50 ppm, and ammonia levels less than 0.02 ppm (Avdesh 2012). This may indicate that the 100% CFF diet used in this study contained more nutrients than the fish could consume and utilize. Due to this, in the second experiment the 100% CFF treatment was omitted, resulting in greatly improved water quality (Table 3). One strategy to improve water quality when culturing fish is to use a polyculture, or polytrophic, system where multiple species that occupy different trophic levels are fed in the same system. For instance, a common setup involves grass carp which consume aquatic plants and feed pellets, bighead carp which consume plankton as filter feeders, and mud carp which are detritivores (Cheng et al. 2015). Similarly, culturing channel catfish with paddlefish and freshwater mussels was shown to offset the nitrogen output of the fish which would otherwise decrease water quality (Wurts, 2010). This allows for the culture system to efficiently utilize all the nutrients provided by the feed without compromising water quality. These results may indicate that polytrophic aquaculture could be an efficient way to manage food waste while mitigating potential water quality issues.

Food Waste Does Not Affect Zebrafish Survival and Growth

As predicted, no significant differences were found between the survival rates of zebrafish fed a diet supplemented or replaced with recycled food waste and those fed a commercial feed in this study (Figure 5). Similarly, a diet containing 35% corn germ meal, a corn by-product, with 28% protein had no effect on the survival of channel catfish (Li 2013). Another study with channel catfish showed that the fish meal and protein content of an aquaculture feed could be replaced with pork by-product without effect on survival (Li et al. 2020). The high survival rates observed in these results indicate that food waste-based feed with adequate protein, regardless of the source, could be used to supplement or replace traditional feeds in aquaculture with freshwater, herbivorous or omnivorous fish without adverse effects on fish mortality.

Food waste-based feeds may not be ideal for foodfish at large-scale commercial fisheries, but it could be used in the production of fish for the ornamental trade. Production of these fish is usually at small facilities (Livengood and Chapman 2007), where a sub-maximum weight gain may be acceptable if the feed results in high survival and satisfactory growth in size and weight. In this study, zebrafish fed a diet containing CFF had an average percent weight gain that was similar to (in the case of 50% CFF) or higher (in the case of 25% CFF) than the weight gain seen with the commercial diet alone (Figure 6A, C), supporting initial predictions. It was also found that CFF diets resulted in an average percent length gain similar to the commercial diet (Figure 6B, D). This is consistent with other studies, which found that grass carp and Nile tilapia fed a food waste-based diet had favorable growth and weight gain (Mo et al. 2020, Bake et al. 2013, Al-Ruqaie 2007). These results may indicate that food waste could be used as a feed supplement in small-scale aquaculture without major adverse effects on growth in size and weight.

Zebrafish Fed Food Waste Do Not Show Adverse Health Effects

The results seen in the histological analysis can be expected from a high-fat diet, as in the CFF diets (Table 3). Dietary fats are absorbed in the intestine and transported to the liver, and finally to extra-hepatic tissues for storage (Turola et al. 2015). Consumption of diets high in fats or sugar can result in hepatic steatosis in zebrafish (Chen et al. 2018). Excessive dietary fats can also result in lowered digestion and nutrient absorption (Nasser et al. 2018). The fat content in the CFF diet (about 27% in the 50% CFF diet, Table 2) may have resulted in the increase of fatty liver deposits observed in the 50% CFF group (Figure 10). A study found that zebrafish fed a 24% fat diet showed fat accumulation in the liver (Dai 2015). In a study using recycled food waste to feed grass carp, the high fat content in diets containing meat waste may have caused an increase in body fat in the fish and reduced growth (Choi et al. 2016). A different study involving restaurant food waste recycled into feed for tilapia also found that the high-fat food waste-based diet resulted in reduced growth (Nasser et al. 2018). Similarly, histological analysis of zebrafish in this study indicated that increasing proportions of CFF resulted in increased fat deposits in the liver (Figure 10, Figure 11B). These changes were not anticipated during initial predictions. However, it is unclear if these changes negatively impacted the 50% CFF group, as this group also exhibited high survival rates as well as VSI and weight gain comparable to the Control group (Figures 5, 6, 7).

Dietary lipid has been reported to influence reproductive performance in some fish. For instance, increasing fat levels from 12% to 18% in rabbitfish diets was associated with increased fecundity, hatching rates, and fry survival (Izquierdo et al. 2001). Conversely, excessive levels of certain fatty acids in gilthead seabream diets reduced the amount of eggs produced (Izquierdo et al. 2001). However, in this study, no significant differences in zebrafish fecundity and embryo survival rates were found between control and treatment diets (Figure 7), which varied in lipid levels (Table 2). The comparable fecundity between control and treatment diets indicates that the CFF diets were not deficient in vitamins E or C, which also affect fish fecundity (Izquierdo et al. 2001).

In the case of the intestine, the increase in goblet cells seen with the 50% CFF diet seemed to be more pronounced in females than in males (Figure 11C, F). This could be due to inherent differences in zebrafish livers between the sexes due to the differing metabolic needs of male and female reproduction (Zheng et al. 2013). A longer-term study is needed to determine if these changes are persistent or harmful. These results may indicate that excess fats should be removed when processing food waste into aquaculture feed, or that enzymes should be added to pre-digest the food waste in order to improve lipid utilization by the fish (Choi 2016). This may increase the cost and effort of recycling food waste into aquaculture feed, but the abundance of food waste, which in this study was obtained at no cost, makes it an alternative to conventional aquafeed ingredients, which will likely increase in price in the future (FAOb, 2020, Naylor 2020). Therefore, food waste-based feed could be useful in increasing the profitability of small-scale aquaculture, as feed can represent up to half of production costs (Craig et al.2017).

Zebrafish fed diets supplemented with recycled food waste in this study had similar reproductive abilities compared to those fed a commercial diet, as predicted (Figure 7). The high fecundity of fish fed CFF indicates that recycled food waste could be used to supplement aquaculture feed without adversely affecting fish reproduction and embryo survival. Likewise, no significant differences were observed in the VSI of zebrafish fed a commercial or a food waste-based diet, also supporting the initial prediction (Figure 8). No publications were found reporting a standard VSI for zebrafish, however tilapia are closely related to zebrafish and were found to have a VSI ranging from 9.91 (± 0.28) to 11.04 (± 0.51) when fed control and experimental diets containing niacin (Liu et al. 2020), which seems comparable to the values found in this study which range from 13.46 (±3.24) to 16.56 (±7.25).

Food Waste Could Increase the Sustainability of Small-Scale Aquaculture

Food waste is an abundant and nutritious resource, with the US producing an estimated annual 133 billion pounds of food, about 219 pounds per person (USDA 2021). Utilizing food waste, which can be obtained without cost while being diverted from landfills, as a feed or feed supplement should increase the sustainability and stability of aquaculture (Nasser 2018). However, there are some challenges to using food waste in aquaculture: food waste is usually mixed with inorganic wastes such as plastic or glass and transported away from the source, and once obtained food waste can be variable in the amount and type of nutrients. Although food waste might not be suitable for large-scale, intensive aquaculture at commercial fisheries, the results from this study may indicate that food waste could be utilized as a feed supplement in small-scale aquaculture without significant adverse effects to sustainably increase fish production.

Ornamental fish farming, which is usually in small-scale facilities (Livengood and Chapman 2007), can be expected to increase in the future as consumer preferences shift with regard to impact on natural ecosystems: 76% of interviewed aquarium owners preferred farmed fish over captured fish, and 30% were willing to pay a premium price for farmed fish (Sicuro, 2018). In the ornamental aquaculture industry, there is interest in reducing the use of fish meal, due to its heavy impact on marine resources (Sicuro, 2018). Because ornamental fish are often valued for their pigmentation, important ingredients in ornamental fish feeds are carotenoids, which cannot be synthesized by fish and must be provided in the diet (Velasco-Santamaría and Corredor-Santamaría 2011). Carotenoids are pigments synthesized by plants, algae, plankton, and crustaceans (Velasco-Santamaría and Corredor-Santamaría 2011). In addition to coloration, which may affect fish sexual behavior, carotenoids provide a variety of health benefits such as antioxidant activity as well as enhanced immune response and growth (Velasco-Santamaría and Corredor-Santamaría 2011). However, the cost of synthetic pigments for feed ingredients have directed interest toward natural compounds found in yeast, bacteria, algae and plant extracts (Velasco-Santamaría and Corredor-Santamaría 2011). Foods rich in carotenoids include red, orange, and yellow vegetables such as carrots and squash (Micronutrient Information Center 2021), and carotenoid-containing foods could be manually separated from food waste streams for use in fish feeds. Homogenizing and cooking foods increase the bioavailability of carotenoids due to the disruption of the plant matrix (Micronutrient Information Center 2021). Therefore, food waste processed as demonstrated in this study and others could be a useful and sustainable feed ingredient in small-scale aquaculture of ornamental fish.

Future Directions

If the aquaculture industry is to grow, the supply of nutrient and feed inputs will have to grow at a similar rate, while the capture fishery production that supplies fish meal and oil remains static (Tacon & Metian 2015). Capture fisheries have reached their maximum potential production and will likely not be able to meet the increasing demand for fish products (Subasinghe et al. 2009). Overharvesting of wild fish populations has already resulted in dramatic losses in abundances that endanger local seafood industries (CNBC, 2018). Several plant and animal-based feed alternatives are available for use in aquaculture feeds, and with appropriate economic and regulatory incentives, transitioning to alternative feedstuffs could accelerate (Naylor et al., 2009). In the future, aquaculture will likely require expansion into new environments and an increase in efficiency and intensiveness to ensure cost effective and sustainable production (Bostock et al., 2010).

Food waste is a ubiquitous, accessible global resource; however, there are a few challenges to the widespread incorporation of food waste into aquaculture feed. Because food waste may vary in chemical composition depending on where and when it is collected, predicting the composition of processed food waste is difficult (Ng et al. 2020). There is a need for more information on different processes to efficiently convert food waste into aquaculture feed (Mo et al. 2018). To standardize the nutrition derived from food waste, a promising new method involves the use of food waste as a feed and substrate to raise black soldier fly larvae which are then used as fish feed. However, insects lack the polyunsaturated fatty acids which are essential for fish growth and they contain chitin, which is not well digested by all fish species and may decrease feed intake and therefore growth (Zarantoniello et al. 2020). Still, in a study using black soldier fly larvae reared on coffee by-product substituted for 25% and 50% of fish meal in zebrafish feed, no significant adverse effects were found in stress, inflammation, or immune response, although there was a slight reduction in overall growth (Zarantoniello et al. 2018). Another study, involving Siberian sturgeon, found an improved weight gain and growth rate using black soldier fly larvae meal (Rawski et al 2020).

There is already interest in the use of food processing by-products to replace traditional aquafeed ingredients (Bostock et al. 2010, Naylor et al. 2009). Fruit and vegetable wastes from institutions and restaurants are usually rich in carbohydrates and could be used as feed ingredients for herbivorous or omnivorous fish (Garcia et al. 2005). Food waste from institutions is also ideal for efficient collection and recycling (Wilkie et al. 2015). There are several promising methods, such as the addition of enzymes, yeast, vitamin/mineral fortification, or the use of fermentation, to increase the growth of fish fed a food waste-based diet (Wong et al. 2016, Lateef et al. 2008). For fish that require higher levels of protein, livestock processing waste is higher in protein and could be used as fish feed (Garcia et al. 2005, Li et al. 2020). Or, as demonstrated by this study, manually separating food waste at the source allows for control over the protein level in the resulting feed. In conclusion, with certain processing methods food waste could be recycled into nutritious fish feed, instead of wasted into landfills, and used as a supplement in small-scale aquaculture of freshwater herbivorous or omnivorous fish without major adverse effects on survival, growth, or reproduction.

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