

Development of Omega-3-Fatty Acid Enriched Finishing Feed and Value
Added Tilapia Product

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ACADEMIC ABSTRACT

Despite being a low fat fish and consequently a low omega-3 fish, tilapia have widespread consumer acceptability due to its mild taste, cheap price and low mercury content. However farmed tilapia can be detrimental to human health due to high omega-6:3 ratios and low omega-3 content specifically eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid. The objective of this study was to create an omega-3 enriching feed that would increase omega-3 content in tilapia and subsequently decrease the omega-6:3 ratio. An 8 week feeding trial was conducted. Tilapia were cultured in a recirculating aquaculture system on one of eight diets (control, commercial, 1, 3, 5% fish oil or 1.75, 5.26, 8.77% ALL-G-Rich (algae). Water quality, selected fish biometrics and growth performance were recorded. Fillet and rib meat tissues were collected at weeks 4 and 8, and liver and mesenteric fat tissues were collected at week 8. Fatty acids were extracted, methylated and identified using gas chromatography–mass spectrometry. Docosahexaenoic acid increased in concentration in all tissues as percent fish oil and ALL-G-Rich increased in the diets with 8.77% ALL-G-Rich resulting in significantly ($P<0.0001$) greater concentrations in the fillet and mesenteric fat compared to all other diets after 8 weeks. The 8.77% ALL-G-Rich diet resulted in significantly ($P=0.003$) greater cumulative accumulation of EPA, DPA and DHA on a mg/4oz fillet basis after 4 weeks compared to control. The results of this study suggest that an ALL-G-Rich finishing feed could be produced that would result in a value added farmed tilapia fillet.

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GENERAL AUDIENCE ABSTRACT

Tilapia are an economically important species of fish with global production exceeding 2.3 million metric tons annually. However, tilapia are a low fat and subsequently an omega-3 deficient fish. Omega-3 fatty acids including EPA, DPA and DHA have numerous human health benefits including decreased cholesterol, decreased inflammation, and are important for fetal neural development. For these benefits the FDA recommends two 4oz portions of fatty fish (salmon, trout etc) per week. The aim of this study was to develop a tilapia feed that would increase the fillet omega-3 content. It was found that one of the experimental ingredients, an algae meal, was effective in enhancing omega-3 content in tilapia fillets. It performed similarly fish oil, when fed over the same length of time. This means that feed manufacturers could potentially develop an omega-3 enriching diet, based on algae, with an effort to develop a more healthy tilapia product for the consumer.

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Introduction and Justification

Aquaculture is the process of farming aquatic organisms such as fish, shellfish, and aquatic plants. Since the 1980s, aquaculture has been expanding rapidly and now accounts for more than half of all seafood produced for commercial consumption with the remainder being produced from wild fisheries (Granata and others 2012). Compared to traditional mammalian or poultry agriculture, aquaculture is significantly more efficient because fish are more effective at converting food into consumable protein. Tilapia for example have a feed conversion ratio of 1.2, meaning it takes roughly 1.2 pounds of feed to increase fish weight of the animal by 1 pound (NOAA 2011). Meanwhile, food conversion ratios of poultry, pigs, and cows are 1.9, 5.9, and 8.7 respectively (NOAA 2011). Therefore fish are extremely efficient protein converters. US aquaculture production is far behind other nations, with the US only producing between 5-7% of their demand for seafood. The remainder of the demand is met with imports (FAO 2011). In fact the US imports approximately 15 billion pounds of seafood annually valued at \$18.9 billion USD in 2014. Replacing a fraction of this with domestically produced seafood would be economically favorable for the US. Currently approximately $\frac{3}{4}$ of US aquaculture consists of catfish, trout, and tilapia, all of which are freshwater fish. Of these three species, tilapia stands out as an excellent candidate for value added experimentation due to its high marketability and low cost of production. Despite being one of the top produced species domestically, tilapia imports in the US have increased from \$450 mil in 2009 to more than \$750 mil in 2013 (USDA 2014). The majority of frozen tilapia found in the US are imported from Asia, while the majority of fresh tilapia are imported from Central and South America. This illustrates one of the many reasons tilapia are an ideal

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candidate for value added experimentation, their market applicability and popularity.

Tilapia are an optimal fish to experiment on due to their market applicability including a relatively cheap price, mild flavor, and low mercury levels. Producing tilapia in the US would be advantageous in the following ways: Increased fish quality, food safety, and production traceability. The producers would be closer to large domestic markets meaning the fish sold in these markets would be fresher. Also domestically producing more of our food supply would increase the food security of the US.

In order to reduce imports and become commercially competitive in the growing aquaculture industry, the US must improve the economic viability of domestic production. One way to do this is to develop “value added” products. By adding inherent value, from either a health, sensory, or environmentally favorable standpoint, US producers would be able to justify higher prices as compared to foreign competitors. One of the promising fields of value added products are enhanced healthy fats (omega-3). Tilapia typically has very low levels of long chain omega 3 fatty acids (LCO3), making them a great candidate for LCO3 enrichment. While it is difficult to ascertain a true accurate value of LCO3 composition in market tilapia, since LCO3 composition varies with diet, production climate and genetic variation, it is estimated to be around 0.32% weight of total fish weight (<200mg/3oz fillet serving) (Hearn and others 1987).

Another field that needs to be explored is finding uses for traditionally discarded portions of fish. For example, tilapia will typically yield fillets that are 35% of their total weight. The other 65% is discarded despite most of it being edible. Further research is warranted to convert discarded portions of fish into useful products. Perhaps even these by-products could be value added with LCO3.

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It has been well established that the fat composition of meat is heavily influenced by the diet of said animal. Therefore in this experiment the lipid source of the feed will be the experimental variable. Alternative lipid sources (from industry partners); microalgae (Alltech, Nicholasville, Kentucky, US), macroalgae (Ocean Harvest, Galaway, Ireland), fish oil (Omega Protein, Houston, Texas, US) were provided and were evaluated as test ingredients. The fish oil diets were supplemented with 1, 3 and 5% by dry weight of each diet, and the algae diets were supplemented with 1.75, 5.26, and 8.77% ALL-G-Rich by dry weight while being isonitrogenous and isocaloric to the fish oil diets. The macroalgae diets were composed at a similar ratio with 3% of the macroalgae diets being fish oil by weight. These 9 experimental diets were compared to each other in terms of LCO3 fillet and tissue deposition as well as to an isonitrogenous and isocaloric control diet supplemented with corn oil, a typical commercial diet, and an additional macroalgae-fish oil diet of 3% macroalgae and 1% fish oil. A secondary objective of this study was to not compromise fish health or growth.

The primary objective of this study was to determine which of the experimental diets results in the greatest increase in LCO3 acids in the fillet, rib meat, mesenteric fat, and liver tissues of tilapia

- The null hypothesis will be that there is no difference between any of the experimental diets in terms of LCO3 deposition in any of the tissues samples
- The alternative hypothesis is that there will be some difference between any of the experimental diets in terms of LCO3 deposition in any of the tissues samples

Chapter 1–Literature Review

Global Tilapia Aquaculture

Tilapia are one of the most widely cultured fish and as of 2010 with a global production estimated to be over 2.3 million metric tons annually across 74 countries (Bostock and others 2010). This is an increase of ~8.6% from 2009. China is the major producer and consumer. In 2003 China and Taiwan cumulatively produced almost 1 million metric tons. Put into perspective global tilapia production in 2002 was 1.5 million metric tons meaning that cumulatively China and Taiwan accounted for ~52% of total tilapia production. Tilapia when sold in groceries is typically sold as either frozen or fresh fillets. In America, frozen tends to be more popular, possibly due to its convenience for consumers, ease of buying in bulk and low price. Between 2010-2014 US tilapia frozen fillet imports grew from 611 million USD to 828 million USD with a sharp decrease to 707 million USD in 2015 (USDA 2016). Fresh fillet imports also increased from 2010-2014 from 168 million USD to 196 million USD with a slight decline in 2015 to 191 million USD. This could possibly be due to the many popular press articles published in late 2014 early 2015 claiming that tilapia is worse for your health than bacon (HealthPost 2014; Axe 2015; Eating 2015) due to its high omega-6:3 ratio.

Frozen fillets in the US are largely imported from China, Indonesia, and Taiwan which combined make up a total of >70% total frozen tilapia imports. Frozen fillets are typically vacuum packaged individually as a means of primary packaging and then secondary packaged in a large polyethylene bag. Fillets are also sometimes packaged with carbon monoxide which helps retain color during storage and distribution.

Fresh fillets typically are imported from Honduras, Colombia, Costa Rica, and Mexico which combined are proportional to >50% total fresh tilapia imports. Most fresh fillets available in America are “refreshed” which means that they are frozen from harvest through transportation and then thawed out “fresh” for display in a marketplace. This technique is used since non-frozen seafood demands a higher price, however it severely limits the shelf life of tilapia fillets.

Domestic production of tilapia accounts for less than 20% of domestic supply (Timmons and Northeastern Regional Aquaculture 2002). That is not surprising when the cost of production is considered. As of 2003, the cost of production in China and Honduras was \$0.70/kg and \$0.90/kg respectively, whereas the cost of production in the US was \$2.00/kg (Fitzsimmons 2003). This is because production in the US is mostly intensive and in many cases RAS.

Tilapia have widespread acceptability in the United States because of its mild, sweet flavor and tender, flaky texture. Historically tilapia has always had great palatability, with some historical records claiming that tilapia was the fish multiplied by Jesus of Nazareth to feed to masses (The Editors of Seafood 2010).

Recirculating Aquaculture Systems vs. Pond Systems

There are many commercial methods for rearing tilapia including pond systems (both semi intensive and intensive, cage systems and recirculating systems. In pond systems the ponds are intentionally build from aquaculture and usually have a production rate of 3 metric tons/hectare annually (Lucas and Southgate 2012a). Ponds are often fertilized in order to maintain phytoplankton growth as tilapia are partially planktivorous,

and feed is supplemented but not the main food source. Cage systems allow for the culture of tilapia in a naturally occurring body of water such as a lake, river or ocean. While cage culture of tilapia is common in Brazil, Colombia, Thailand and China most tilapia is produced in semi intensive pond systems. Recirculating aquaculture systems (RAS) permit the most intensive culturing for tilapia, where fish are raised in tanks, where water quality is continuously monitored and environment and feed are controlled. Indoor RAS is growing in developed countries due to the increased control of environmental conditions and enhanced biosecurity.

Recirculating aquaculture systems (RAS) as stated previously is one of many methods of rearing fish for industrial production. In RAS, fish are raised in tanks, in an environmentally controlled space, typically indoors. Water circulates through the system with only a small percentage of the water being changed daily ($0.1\text{--}1\text{ m}^3/\text{kg feed}$) (Martins and others 2010). Water quality is monitored and controlled in order to promote optimal fish health. Solid wastes are removed via some sort of mechanical filter, and biological wastes are passed through a biological filter which oxidize ammonia into nitrate. Gas exchanged is also implemented in order to aerate the system with oxygen and strip carbon dioxide from the water. RAS has many advantages including higher stocking density potential, a controllable environment, and uses approximately 10% of the water and land used in traditional pond aquaculture (Timmons and Northeastern Regional Aquaculture 2002). One of the determining factors of fish flavor and texture are water quality during production. Poor water quality, which is more common in pond systems than in RAS results in muddy and grassy off flavors similar to those of wild

catfish (The Editors of Seafood 2010). Therefore, another benefit of RAS over pond is a more reliable and acceptable product quality.

Recirculating aquaculture has a significantly lower environmental impact compared to pond aquaculture. This is largely due to the higher stocking density and low water exchange rate. Phillips and others (1991) have established that tilapia raised in a RAS can be raised at a production density of 1,340,000 kg/ha/y and have a water requirement of 100 L/kg. This is a staggering improvement compared to tilapia produced in a pond system where the production intensity is only 17,400 kg/ha/y with a water requirement of 21,000 L/kg. RAS production has a higher cost of startup and overall production compared to pond systems and typically are only highly profitable when they are working at their maximum production capacity.

While recirculating aquaculture is more environmentally conscious and can produce higher volumes of fish faster and with a more consistent quality, it has not been widely adopted in many countries. Currently only 6% of China's over 800,000 metric tons of tilapia produced annually is cultured in RAS, the rest is various pond aquaculture (Basu 2015). The majority of Central American production, including Honduras, is also various pond aquaculture. The studies performed herein were conducted exclusively in RAS.

RAS Engineering

Recirculating aquaculture systems are designed in order to have segmented units perform unit processes. These processes include, aeration, disinfection, solids removal,

and biofiltration. Each unit process is important to maintain water quality throughout the RAS.

Aeration is important to maintain adequate dissolved oxygen and limit carbon dioxide accumulation. This is because dissolved oxygen is typically what limits carrying capacity in properly designed RAS systems (Timmons and Northeastern Regional Aquaculture 2002). Standard sources of air include blowers and compressors. Blowers provide high volumes of air at low pressure while compressors provide low volumes of air at high pressures. Depth of the RAS system is considered when determining what type of air source to use as the aeration needs to have enough air pressure to overcome the surrounding water pressure. Diffusers are typically used in low density, high exchange rate systems due to their cheap cost and maintenance.

Bacterial and viral issues are serious problems for intensive RAS systems, partially due to the use of municipal water which can harbor fish pathogenic microorganisms. For this reason, disinfection of recirculating water is crucial to RAS, and one of two methods are conventionally utilized; ozonation and UV irradiation. The experiments in the following studies used the later. Ozonation utilizes ozone, an extremely reactive oxidant to act as a microbiological control measure. Ozone is generated on site typically with electric corona discharge of oxygen/air. It is then fed into the system through normal water gas exchange. UV irradiation damages bacteria by damaging DNA specifically causing alterations to the nucleic acids. UV irradiation is commonly used in RAS systems although they must be carefully engineered. Turbidity can be an issue with UV irradiation due to low transmittance of UV light through multiple mediums (Summerfelt 2003). Because of this the lowest expected transmission

rate must be used to determine the UV intensity applied, which will ensure an adequate lethality to microorganisms.

Solids removal, or mechanical filtration, is necessary to reducing total suspended solids (TSS). TSS is both organic and inorganic, with the organic portion contributing to oxygen depletion and TAN, and both the organic and inorganic portions contributing to sludge formation. There are many methods of mechanical filtration for RAS including granular (sand) filtration, gravity separation and flotation. The method used in the experiments described in this thesis are bead filtration. In bead filters, water enters through the bottom of the filter through a mesh retaining screen, it then passes through thousands of 3-5mm polyethylene beads which compress near the outlet at the top of the filter. Particles are captured as the water moves through the floating bead bed. (Malone and Beecher 2000). These captured solids are then backwashed out of the system by reversing the flow out through the bottom of the filter and into a drain.

Biofiltration is necessary for closed RAS systems as it helps control toxic ammonia by using nitrifying bacteria to oxidize ammonia into nitrite (i.e *Nitrosomonas* spp.) and nitrite into nitrate (i.e *Nitrobacter* spp.). Biofiltration units are comprised of small (7-10mm) polyethylene or polypropylene media. These media are designed to provide the greatest surface area to volume ratio, allowing for optimal bacterial adhesion. These bacteria are usually limited by one of a few factors, dissolved oxygen, and surface area of biological media. The biological filter used in the following studies is a moving-bed biofilm reactor. It uses tubular media which are mixed in a tank by heavy aeration. Since aeration is used to keep the bed of media in constant motion, solids do not accumulate and dissolved oxygen is not a limiting factor (Rusten and others 1998).

Water Quality in RAS

Water quality of a recirculating aquaculture system can determine if said system is successful or not. Water quality is a general term and is used to describe a variety of chemical parameters associated with culture water. Some of the most important parameters monitored are dissolved oxygen (DO), nitrogen metabolites including: ammonia/ammonium or total ammonia nitrate (TAN), nitrite, nitrate, as well as pH, water temperature and alkalinity (Colt 2006). All of these parameters are often interconnected. In general, the minimum water quality required to maintain fish health are: DO (5ppm), pH (6.7-8.6), ammonia or the unionized fraction of TAN(0.02ppm or less), alkalinity (at least 20ppm) (Pillay and Kutty 2005). Tilapia are a relatively hardy species and as such have a minimum water quality of DO (>3 ppm), pH (6.5-8.5), ammonia (<1 ppm), alkalinity (>20ppm) and temperature (26-32C) (Lucas and Southgate 2012b).

Fish use oxygen in order to undergo respiration and promote growth. Dissolved oxygen concentrations that are suboptimal will result in stunted growth and unhealthy fish. However, bioavailability of dissolved oxygen is dependent on more than just total concentration, but also the solubility of that oxygen in the system. The solubility of oxygen in water, like all gases, is dependent on the partial pressure of those gasses above the water and the temperature of that water. This phenomenon uses principals described in Henry's Law. In general gas solubility decreases as temperature increases and at a constant temperature the solubility of a gas is directly proportional to the partial pressure of that gas at equilibrium with the liquid. If saturation of oxygen is too low, it can reduce the bioavailability of oxygen despite the concentration being above 5ppm (Summerfelt and others 2000). Carbon dioxide concentration is also important to consider. Low

concentrations of carbon dioxide can stress fish and interfere with respiration by forming calcareous kidney deposits (Lucas and Southgate 2012a). Conversely, high concentrations of dissolved carbon dioxide will reduce carbon dioxide excretion potential at the fish gill interface. Carbon dioxide then becomes elevated in blood plasma causing blood acidosis and reduction of hemoglobin's affinity to oxygen. This reduces oxygen uptake from the water to the gills (Ishimatsu and others 2004).

Nitrogen metabolites that originate from fish wastes have a negative effect on fish health. These metabolites include ammonia/ammonium (TAN), nitrite and nitrate, each with decreasing toxicity respectively. Ammonia increases oxygen utilization by tissues and can impair gill function. Ammonia originates from fish as excrement, and uneaten feed decomposing. Nitrite and nitrate are produced by nitrifying bacteria, which in a RAS system are concentrated in a biological filter. Nitrite when absorbed into blood plasma oxidizes ferrous iron in hemoglobin into ferric iron, forming methemoglobin. Methemoglobin can not bind with oxygen, thus diminishing overall oxygen carrying capacity of the circulatory system. It can often take a few weeks for the heterotrophic and nitrifying bacteria to become acclimated to their environment. Since *Nitrosomonas sp.* the microorganism that oxidizes ammonia to nitrite develops faster than *Nitrobacter sp.* and *Nitrobacter sp.* is inhibited by ammonia, the *Nitrosomonas sp.* needs to be well established before the full ammonia to nitrate pathway can be utilized effectively. The bacteria oxidize ammonia into nitrite, and then oxidize nitrite into nitrate (Summerfelt and Sharrer 2004). In this way nitrite is the intermediate during the nitrification process. Nitrate can not be oxidized any further. In general, warm water fish like tilapia are more tolerant to high ammonia concentrations compared to cold water species.

pH is a measure of acidic and basic conditions of a system. Prolonged exposure to an extreme acidic (<6) or basic (>8) pH will typically result in high mortality and stunted growth. This is due to how pH affects other water quality parameters such as ammonia/ammonium ratio, and dissolved carbon dioxide (Wurts 2003). Ammonia has a greater mole fraction in an unionized form at high pH and a greater mole fraction in an ionized form at low pH. The unionized form is more toxic to fish (Lucas and Southgate 2012a). pH is also affected by concentrations of dissolved carbon dioxide, since when dissolved, carbon dioxide can protonate and become carbonic acid, a weak acid which lowers pH. pH can be affected by a number of parameters and can have a drastic effect on overall water toxicity.

Temperature can have a large impact on fish health both directly and indirectly. Fish are poikilotherms, meaning their internal temperature varies according to ambient temperature (Guschina and Harwood 2006). This is one of the reasons fish are such efficient food to mass converters, because they do not expend energy regulating body temperature. Every fish species has an optimal growth temperature which is indicative of the environment in which that species developed, i.e. tilapia are warm water fish because they originate in northern Africa. Attempting to rear fish in a temperature outside the optimum range for that species will result in mortality, stunted growth and other health deficits (Timmons and Northeastern Regional Aquaculture 2002). As already stated solubility of dissolved oxygen is directly related to temperature, with solubility decreasing as temperature increases. The mole fraction of ammonia to ammonium increases as temperature decreases, resulting in ammonia toxicity being greater as temperature decreases. Nitrifying bacteria can also be inhibited at low temperatures,

meaning that the oxidation of TAN into nitrite and nitrate can be stunted in recirculating aquaculture systems at low temperatures.

Alkalinity is a measure of total buffer in the system in question and how resistant that system is to large pH shifts (Wurts 2002). The greater the alkalinity, the more resistant that system is to fluctuations in pH. Alkalinity is often thought of as concentrations of carbonate and bicarbonate. Carbon dioxide, carbonate and bicarbonate are all varying species of inorganic carbon, with carbon dioxide being dominate at low pH, bicarbonate being dominate at neutral pH and carbonate being dominate at high pH. When carbon dioxide dissolves into water it evolves into carbonic acid which then reacts with calcium carbonate to form calcium bicarbonate. If the system shifts to be more acidic, calcium carbonate will dissolve, and store protons in bicarbonate ions. If something is added which makes the system more basic, the reverse happens and bicarbonate ions dissociate into carbonate ions and water molecules (McLarney 1998). This equilibrium of weak acids and bases results in a buffered system, meaning that it is resistive to pH change. Alkalinity in aquaculture is often regulated by adding calcium carbonate or sodium bicarbonate to the system, thus fortifying alkalinity when it is depleted.

Aquaculture wastewater can be damaging to the environment if not handled properly. Compared to municipal wastewater, aquaculture wastewater is low in solids and ammonia but high in nitrogen and phosphorous. In aquaculture wastewater total phosphorous is typically 1.7% solid mass where in municipal wastewater it is 0.7% (Timmons and Northeastern Regional Aquaculture 2002). Nitrogen and phosphorous can cause algae blooms when introduced to an environment. Algae blooms are responsible

for eutrophication, a process where water bodies become more eutrophic and plentiful of plant life (Cloern 2001). The following decomposition of this plant life results in oxygen depletion of the surrounding environment. In order to avoid such environmental issues nitrogen is removed by wastewater treatment plants. In some cases, the nutrient rich waste water is used to hydroponically grow crops, a practice known as aquaponics.

Tilapia Nutrition

Total global aquafeed production is expected to be valued at 165 billion USD by 2022 (2015). This is not surprising considering that aquafeed accounts for between 30-60% of total production costs in aquaculture (El-Sayed 2006). One of the reasons aquafeed is so expensive is fluctuating ingredient prices, with some ingredient prices i.e. fish oil and fish meal trending more expensive over the past decade (Turchini and others 2011). For this reason, many efforts have been made to develop more sustainable and economically viable macronutrient alternatives.

Tilapia are omnivorous, and in the wild subsist on a diet consisting mostly of plankton, aquatic plants and filamentous algae (Ng and Romano 2013). They are efficient utilizers of both aquatic and terrestrial macronutrients, with their protein typically coming from; plant sources like soybean meal or wheat, as well as animal sources including feather meal and meat and bone meal. Dietary protein requirement for tilapia is affected by a variety of factors including, age, sex, water quality, salinity, temperature and dissolved oxygen being the major factors. Some studies have shown that the optimal dietary protein level for fry stage tilapia is between 50-55% BW where as the optimal dietary protein level for fingerling and larger tilapia is somewhere between 25-40%

depending on the size and age of the fish (Hooley and others 2014; Siddiqui and others 1988). Growth tends to be positively correlated to protein levels in the feed (Abdel-Tawwab 2012). This is likely due to the fact that tilapia are efficient protein utilizers, more so than they are for lipids or carbohydrates. However, since protein sources are typically more expensive than carbohydrate, it is often more cost beneficial to use a low protein diet and have a longer production cycle than a high protein diet with a shorter production cycle. Excess protein diets can have the added problem of increasing FCRs and stunted growth in tilapia as well as increasing unionized ammonia concentration in the water (Robert R Stickney 1979; Abdel-Tawwab and others 2010).

Like many organisms, tilapia dietary lipid requirements vary with growth, with the highest amount of dietary lipid required being 10% when they are fry or fingerling. This dietary lipid requirement lowers to between 6-8% from 2g to harvest size (Alceste 2000). Excess dietary lipid levels can be detrimental to fish health though, with upwards of 12% BW feeds resulting in stunted growth (Jauncey and Ross 1982). High dietary lipid and carbohydrate levels have a sparring effect on dietary protein utilization (Lim and others 2011; Shiau and Peng 1993). This means that an increase in dietary lipid and carbohydrate levels in a diet allows for the dietary protein level to be proportionally decreased (Li and others 1991). Similar to other fish, tilapia can not synthesize 18:2 n-6, or 18:3 n-3 and must acquire these fatty acids through their diet (National Research Council and National Research Council . Committee on Animal 1993). Omega-3 and omega-6 fatty acids should be included in the diet as some research suggests that both series of fatty acids are essential and in total should be included at a minimum of 1% of the diet (Chou and Shiau 1999). Despite being required at such a low level, they clearly

have a serious effect on overall health, tilapia fed diets deficient in omega-6 and omega-3 fatty acids presented with anorexia, poor growth, and swollen, pale and fatty livers (Webster and Lim 2006). Omega-3 and omega-6 fatty acids are essential because tilapia are unable to efficiently elongate and desaturate shorter polyunsaturated fatty acids such as linoleic and linolenic acids. Marine species are able to elongate and desaturate linoleic (18:2 n-6) and linolenic (18:3 n-3) acids into longer chain PUFAs more efficiently than tilapia. Despite the presence of enzymes necessary for this transformation tilapia are not efficient bioconverters. This bioconversion is rather inefficient and is inhibited by the presence of PUFAs (Olsen and others 1990). This is likely caused by a feedback mechanism where in a high concentration of PUFAs deactivates the transcription of desaturase and elongase enzymes necessary for PUFA bioconversion. Due to environmental and evolutionary factors, omega-3 and omega-6 PUFAs are produced in high concentrations in marine algae, another reason they are not typically found in high concentrations in freshwater organisms such as tilapia (Ackman 1989; Parrish 2013).

Tilapia do not have a specific dietary requirement for carbohydrates, and in fact have been shown to grow well when on carbohydrate free feed (Wilson 1994). However, carbohydrates are always added to diets since they are the most economical source of energy. They also function well as a feed binder helping pellets and extruded feed keep their shape. Tilapia can metabolize carbohydrates relatively well, with complex carbohydrates in general being more digestible than simple carbohydrates. Many commonly used commercial sources of complex carbohydrates; corn, wheat, soybean meal have varying levels of digestibility with decreasing digestibility respectively (Popma 1982). High fiber feed ingredients have been found to decrease diet and protein

digestibility with > 10% dietary fiber resulting in depressed growth (Dioundick and Stom 1990). As such the maximum crude fiber content of many grow out feeds is ~7% (El-Sayed 2006).

Tilapia have diverse and complex dietary requirements and the goal of a producer is to develop the lowest cost feed that results in the quickest growth, and greatest fillet yield. Being deficient in protein will likely result in smaller fillets i.e. worse fillet yield. However, since protein is usually the most expensive macronutrient to incorporate into a feed, having too high of protein levels in a feed will usually result in a feed that is not economically viable. That is why some research studies have focused on creating omega-3 enhanced tilapia, because a value added product would help offset the cost of a high protein feed.

Omega-3 Feed Trials in Fish

Other researchers have understood the importance of increasing omega-3 fatty acids in seafood and their potential health benefits (Kris-Etherton and others 2002). Typically lipid sources for finfish have been derived from production fisheries in the form of fish oil. Many studies have found that partial replacement of fish oil with canola, soybean, and linseed oils did not negatively affect feed conversion ratios, weight gain or overall health (Trushenski and others 2006). In general research has shown that fillet fatty acid composition follows dietary fatty acid source (Raso and Anderson 2003). In this way, diets where the lipid source came from a terrestrial plant such as soybean oil produced a lower omega-3 composition in the fillet (Trushenski and others 2006). One study in particular demonstrated that tilapia with dietary lipid replacement with

soybean oil, tea seed oil, and pork lard had significantly lower omega-3 fillet content as compared to fish oil (Han and others 2013). One lipid replacement that has been proven to be efficient in increasing tilapia omega-3 fillet content is replacement with flaxseed oil (de Souza and others 2007; Tonial and others 2012). This is because flaxseed oil is primarily linolenic acid which is the shortest omega-3 fatty acid and can be elongated and desaturated into polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA 20:5 n-3), docosapentaenoic acid (DPA 22:5 n-3), and docosahexaenoic acid (DHA 22:6 n-3). However, when compared to fish fed fish oil, other vegetable oil sources do not result in higher omega-3 fillet content. One study compared fish oil, linseed oil and palm olein oil as dietary lipid replacement for tilapia (*O. niloticus*). It was found that after 20 weeks none of the vegetable based diets resulted in higher omega-3 fillet content (EPA and DHA) when compared to the fish oil (Karapanagiotidis and others 2007). This is likely because tilapia have a limited capacity to elongate and desaturate shorter chain omega-3 fatty acids into longer chain omega-3 fatty acids. Since no terrestrial lipid sources have proven to be effective at increasing long chain omega-3 fatty acids i.e. EPA and DHA it would be interesting to look at alternative marine lipid sources which is what this research project aims to do.

Human Health Benefits of Omega-3 Fatty Acids

For decades' omega-3 and other long chain polyunsaturated fatty acids (PUFAs) have been recognized for their health benefits. Of the many PUFAs, EPA, DPA, and DHA have been extensively researched for their potential health effects. By the early

1980s there was evidence that heart disease risk decreased when consumption of EPA and DHA acids increased (Cahu and others 2004). For this reason the American Heart Association (AHA) recommends two 4 oz servings of fish (preferably fatty fish i.e. salmon) per week (Kris-Etherton and others 2002). This serving size should provide the consumer with at least 250mg/day of EPA and DHA which is what the AHA claims are enough to provide cardiovascular protective effects. However a recent meta-analysis determined that the cardiovascular beneficial effects from omega-3 fatty acids plateau after 200mg/day (Trikalinos Ta 2012). Since there is such a variation in what dose of omega-3 fatty acids provides the optimal benefit, there is no yet defined dietary reference intake (Flock and others 2013). The reason salmon are recommended is because they have a favorable ratio of omega-3 to omega-6 fatty acids, and they are widely accessible throughout the US (Harris 2008).

Docosahexaenoic acid (DHA) has been associated with numerous health benefits. Studies have demonstrated a link between DHA content and fetal neural development as well as fetal retina development (Jørgensen and others 2001). More than EPA, chronic DHA supplementation has been associated with lowered blood pressure (Morris and others 1993). Long chain polyunsaturated fatty acids like DHA have a positive effect on a number of other diseases including: arthritis, hypertension, diabetes, thrombosis and some cancers (Horrocks and Yeo 1999). However some of these benefits are only present as dietary levels far above average nutritional levels(>2g/day)

Docosapentaenoic acid (DPA) while less studied than EPA and DHA has been associated with numerous health benefits. When applied to platelets *in vitro* DPA was found to significantly decrease platelet aggregation (Phang and others 2009). Platelet

aggregation was more inhibited by DPA than both EPA and DHA (Akiba and others 2000). Other *in vitro* studies concluded that DPA when applied to liver cells decreased synthesis of triglycerols and cholesterol (Nagao and others 2014). It is for these reasons that DPA is believed to be linked to decreased risk of cardiovascular disease (Hino and others 2004).

EPA has also been associated with decreased risk of thrombosis since EPA can lead to the production of anti-thrombotic prostaglandins (Dyerberg and Bang 1979; Dyerberg and others 1978). Prostaglandins (PG) are one of the classes of molecules generated by the oxidative pathway of arachidonic acid, a member of the omega-3 and omega-6 biochemical pathway. Other classes of molecules generated by this pathway include leukotrienes and (LT) and lipoxins (LP). All of these molecules act as signal molecules and specifically as a primary messenger in intercellular communication (Harizi and others 2008). The signaling can be summarized as such, a g-protein membrane receptor is activated by attachment of a substrate, and this triggers the release of arachidonic acid from the phospholipid bilayer. This AA molecule is then converted into a PG, LT, or LP molecule via a transformative enzyme reaction, mediated by cyclooxygenases, lipoxygenases, or cytochrome p450. These signal molecules then trigger the release of secondary messengers usually cAMP or Ca^{2+} causing a cascade effect transducing the signal throughout the cell. This signaling often plays a role in chronic issues including inflammation, cancer, allergies, and autoimmune diseases (Harizi and others 2008).

Certain plant sources of omega-3 fatty acids such as flax seed oil are beneficial to human health (Rajaram 2014). The justification for this is that flax seed oil contains high

levels of alpha linolenic acid (ALA, 18:3 n-3). Linolenic acid is the shortest chain omega 3 fatty acid. The benefits of high ALA foods is often exaggerated since the rate of conversion of linolenic acid into EPA and DHA in humans is very low, approximately 5% and <1% respectively (Finnegan and others 2003).

Fish Oil

Fish oil is a byproduct of the reduction process used to create fish meal. The most prominent species used in the reduction industry for the production of fish meal and fish oil are; Peruvian anchovy, mackerel, sand eel, capelin, and menhaden, all of which are considered fatty fish by having over 8% fat BW (Turchini and others 2011). The majority of fish oil is produced through a process called wet pressing (FAO 1986). The main steps of this process are; cooking, and centrifugation. Cooking coagulates proteins and frees bound water and lipids. This water and oil mixture is then centrifuged to separate the two liquids. Fish oil often has synthetic antioxidants added in order to prevent rancidity and oxidation (Lundebye and others 2010). These include ethoxyquin and butylated hydroxytoluene, which have been found to be potentially carcinogenic and tumor promoting (Botterweck and others 2000; Iverson 1999). Global fish oil production has fluctuated greatly over the past few decades, with a boom in the late 1980s of 1.6 million metric tons. However, since 2005 global fish oil production has steadily been declining and is currently valued at less than one million metric tons. Despite global fish oil production declining, the use of this commodity has been increasing. As the culture aquaculture industry expands, producers that raise species that require fish oil for optimal growth require greater volume of the commodity in order to meet production deadlines and costs. This static supply and increasing demand has caused the price of fish oil and

fish meal to fluctuate dramatically. Between 2007-2008, fish oil price almost doubled from \$894 USD/metric ton to 1,700 USD/metric ton with a current value of approximately \$3,800 USD/ metric ton (FAO 2009; DBL 2016).

Chemically, fish oil is mostly triacylglycerols (TAG) with some oils being ~90%TAG. Phospholipids (PL) are a whole order of magnitude less abundant in bulk fish oils compared to TAG (Haraldsson and Hjaltason 2001). In general fish oil a low omega-6 composition (18:2 n-6 <3%, 20:4 n-6 <1%) and high in beneficial long chain polyunsaturated fatty acids (20:5 n-3 4-22%, 22:5 n-3 0.6-2%, 22:6 n-3 2-14%) (Ackman 1976; Gunstone and others 2007; Hertrampf and Piedad-Pascual 2012). Overall marine based lipid sources have a higher concentration of long chain omega-3 fatty acids compared to terrestrial oils.

Micro- and Macro-Algae

Several lipid sources have been proposed as replacement lipid sources in fish feed. Some of these include microalgae, macroalgae (kelp) (Turchini and others 2009; Turchini and others 2011). Both of these would be excellent alternatives to fish oil since they can be produced intensively in aquaculture farms. Microalgae are grown in intensive bioreactors where they can be optimized for biofuel, or marine oil production. Macroalgae can be grown intensively in along Atlantic coastlines and can provide a beneficial habitat for the marine ecosystem. The microalgae that would make the most sense as a fish oil replacement would be *Schizochytrium sp.* a marine algae very high in 22:6 n-3 fatty acid (Manikan and others 2014). While not much research has been done on *Schizochytrium sp.* being used as a lipid replacement in aquaculture feeds, one study conducted on salmon indicate that this would indeed be a viable alternative (Miller and

others 2007). Salmon were feed *Schizochytrium sp.* oil and fish oil as two different experimental groups. Those fed the *Schizochytrium sp.* diet showed significantly higher omega-3 muscle composition as compared to fish oil. This is particularly impressive when noted that salmon have a much higher PUFA requirement as compared to tilapia.

The other alternative proposed would be in inclusion of macroalgae (kelp). Ocean Harvest Technology, a macroalgae producer out of Ireland already has found success with macroalgae added to salmon diets. While the macroalgae does not act as a lipid replacement, as it is so low in fat, it does appear to act as a modulator of lipid storage in salmon (Wilke and others 2015). Some of the success they have observed is that they can increase omega-3 content of salmon fillets by 30%. While their specific mixture of macroalgae is proprietary it is likely they use a variety of species that are prevalent in coastal temperate Atlantic waters including: *Laminaria sp.*, *Fucus sp.*, *Ascophyllum nodosum*, *Chondrus crispus*, *Porphyra sp.*, *Ulva sp.*, *Sargassum sp.*, *Gracilaria sp.* and *Palmaria palmate*. Also while the mechanism of action as to how these macroalgae influence fatty acid metabolism is unknown there are a number of bioactive compounds that could be responsible. These include polysaccharides, dietary fiber, alginates, vitamins and minerals and antioxidants (Holdt and Kraan 2011).

Aside from possibly being a more economically viable alternative to fish oil, both of these options would provide a more ecologically beneficial alternative. With a rising demand for fish oil both for supplements and aquaculture feeds the chance of overfishing to meet demand increases. These alternatives are relatively easy to cultivate and harvest and can be done in a controlled environment where certain environmental stresses can be applied in order to increase production of PUFAs (Ren and others 2014). In this way

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production of omega-3 fatty acids can be done ethically and without devastating effects to natural marine ecosystems.

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Chapter 2–Production of Omega-3 Enriched Tilapia Through the Use of A Commercially Available *Schizochytrium Sp.* Additive

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Abstract

The objective of this work was to compare fish oil with an algae product for enhancing omega-3 fatty acids in tilapia. Tilapia were raised in a recirculating aquaculture system for 8 weeks and were fed one of eight experimental diets (control, commercial, 1,3,5% fish oil or 1.75,5.26,8.77% ALL-G-Rich. Water quality, selected fish biometrics and growth performance were recorded. Fillet and rib meat tissues were collected at weeks 4 and 8, and liver and mesenteric fat tissues were collected at week 8. Fatty acids were extracted, methylated and identified with gas chromatography–mass spectrometry. There were no significant differences in fish growth or biometrics. The 8.77% ALL-G-Rich diet resulted in significantly ($P=0.003$) greater accumulation of beneficial omega-3 per 4oz fillet after 4 weeks compared to the control. Strong linear relationships ($R^2>0.98$) were observed between % ALL-G-Rich in the diet and beneficial omega-3 fatty acid deposition in the fillet at weeks 4 and 8.

Introduction

The health benefits of omega-3 fatty acids to humans include prevention of cardiovascular disease, improvement of visual acuity, and fortification of mental health (Dyerberg, Eskesen, Andersen, Astrup, Buemann, Christensen, et al., 2004; Heude, Ducimetière, Berr, & Study, 2003; Jørgensen, Hernell, Hughes, & Michaelsen, 2001). For this reason the American Heart Association (AHA) recommends two 4 oz servings of fish (preferably fatty fish i.e. salmon) per week (Kris-Etherton, Harris, Appel, & Committee, 2002). Omega-3 fatty acids include, among others, alpha linolenic acid (ALA 18:3 n-3), eicosapentaenoic acid (EPA 20:5 n-3), docosapentaenoic acid (DPA 22:5 n-3) and docosahexaenoic acid (DHA 22:6 n-3). However, not all omega-3 fatty acids are equally beneficial to humans (Sinclair, Attar-Bashi, & Li, 2002). Due to the low efficiency of converting ALA into longer chain omega-3 fatty acids (<10%), ALA is of relatively little benefit to humans (Burdge, 2006). The longer chain omega-3 fatty acids, EPA, DPA, and DHA are significantly more beneficial to human health and development.

Tilapia is the second most cultivated freshwater fish worldwide, typically yielding between 30-40% fillet yield leaving 60-70% processing waste (El-Sayed, 2006; Silva, Ribeiro, Silva, Cahú, & Bezerra, 2014). Tilapia is a relatively low-fat fish and is reportedly high in omega-6 and low in omega-3 (Young, 2009) fatty acids. High dietary n-6:n-3 ratios lead to human health deficits including inflammation, asthma and reduced kidney function (Wang & DuBois, 2008). Some recent popular press have even claimed that tilapia is worse for you than bacon (Essex, 2014). Enrichment of omega-3 fatty acids in tilapia would provide beneficial omega-3 fatty acids and reduce the omega-6:3 ratio which would also provide human health benefits.

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The marine algae, *Schizochytrium sp.* has been found to be a possible feed ingredient for enriching beneficial omega-3 fatty acids in channel catfish, Atlantic salmon and seabream (Ganuza, Benítez-Santana, Atalah, Vega-Orellana, Ganga, & Izquierdo, 2008; Li, Robinson, Tucker, Manning, & Khoo, 2009; Miller, Nichols, & Carter, 2007). Attempts to enrich beneficial omega-3 fatty acids in tilapia using plant oil alternatives have been relatively unsuccessful. Diets supplemented with flaxseed have been found to increase ALA but not significantly increase beneficial omega-3's (de Souza, Matsushita, de Oliveira, Franco, & Visentainer, 2007; Visentainer, de Souza, Makoto, Hayashi, & Franco, 2005). Fish oil has been found to enrich beneficial omega-3's, however the use of fish oil for enrichment of tilapia is likely not economically viable (Teoh, Turchini, & Ng, 2011). Economic viability requires a cost benefit analysis. However, the majority of studies aiming to enhance omega-3 fatty acids in tilapia have focused solely on the fillet. Even though the cost for algae meal is currently high, these costs will undoubtedly be reduced in the future as we scale up and develop new technologies for algae production, harvest, and processing. The cost of an omega-3 enriched feed could also be reduced by utilizing the rest of the tilapia frame, 60-70% after filleting, in value-added products. The aim of this study was to evaluate if diets supplemented with *Schizochytrium sp.* can provide a similar enrichment of beneficial omega-3 fatty acids and reduction of omega-6:3 ratio compared to fish oil supplementation and to examine byproduct tissues of tilapia including rib meat, liver and mesenteric fat.

Methods and Materials

Fish and culture system

Juvenile tilapia (*Oreochromis niloticus*, ~11 grams each) were shipped from Spring Genetics (Akvaforsk Genetics Center, Miami, Florida) to Virginia Tech's aquaculture facilities (Blacksburg, Virginia). Fish were acclimated and conditioned for 4 weeks until they reached ~150 grams for experiment initiation. Fish were cultured in an indoor recirculating aquaculture systems (RAS) equipped with 16, 1-meter-diameter polyethylene tanks (~250 liters each), bubble-bead filters for mechanical filtration, fluidized-bed bioreactors for biological treatment, UV disinfection units, heat exchangers, and distributed diffuse aeration.

Water quality in the RAS was rigorously monitored throughout the nutritional study. All water quality parameters were analyzed using methods adapted from APHA (2012) and HACH (2015). Dissolved oxygen and temperature were monitored daily. Alkalinity, total ammonia nitrogen (TAN), nitrite, nitrate and pH values were analyzed three times a week.

Diets

All experimental diets were formulated on an isonitrogenous and isocaloric basis except for the commercial feed (Production 35, Rangen Inc., Buhl, ID, USA) which was utilized as received. The pelleted experimental feed formulations are presented in Table 1. The independent variable for this experiment was the lipid composition of the eight diets, and the dependent variables were: survival rate, growth, biometrics, performance indices, feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), and nutritionally relevant fatty acid profiles.

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All diets were analyzed to confirm their proximate nutritional values and essential amino acids using a commercial lab (Midwest Labs, Omaha, NE, USA). Feeding rates were determined for all treatment groups on a percent of body weight per day basis. Monitoring the amount of feed consumed allowed FCR, SGR and PER to be determined. Tilapia were group-weighed on a per tank basis weekly to enable appropriate feed adjustments. Feeding rates were 4, 3.75, 3.25, 3.25, 2.375, 2, and 1.85 percent body weight per day (% BW/d) for weeks 1, 2, 3, 4, 5, 6, 7 and 8, respectively.

Biometrics

Fillet yield, hepatosomatic index (HSI), viscerasomatic index (VSI), and mesenteric fat index (MFI) were determined by dividing the fillet/muscle tissue, liver, total viscera mass, and mesenteric/visceral fat by the whole weight of the fish, respectively (Ighwela, Alhadi, Abol-Munafi, 2014).

Tissue Sampling

Fatty acid profiles were determined for fish fillets, rib meat, liver, and mesenteric fat at the end of the 8-week trial. Additional profiles were determined at week 4 for the fillets and rib meat only. Rib meat for the purposes of this study is comprised of the pin bones and belly meat ventral to the fillet. Tissue samples were collected on a per tank/diet basis with two samples for each tissue originating from each tank. Each of these two samples were a pooled sample containing tissues from two fish of the same treatment. Samples were vacuum packed with 10 ml of methanol in order to deactivate enzymes and then quick-frozen in a bath of isopropanol and dry ice. These samples were then stored at -80 °C until analysis. Fatty acid extraction was

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performed according to Bligh and Dyer (1959) and methyl esters were formed according to Ackman (1998). The AOCS (1998) method Ce-1b-89 was used with a Shimadzu QP-5050 GCMS (Kyoto, Japan) to determine the fatty acid profiles of each sample.

Data analysis

Statistical analysis was performed using JMP Pro 11 for Apple (Cary, NC, USA). One-way ANOVA was utilized to determine dietary effects on dependent variables (fish performance, biometrics, tissue fatty acid composition). When appropriate, Tukey's post-hoc test was applied to determine where the significant ($P < 0.05$) differences occurred amongst the means.

Results

Water quality averages were: temperature 29.4 °C, pH 7.79, dissolved oxygen 5.45 mg/L, alkalinity 199 mg/L, total ammonia-N 0.35 mg/L, nitrite-N 0.06 mg/L, and nitrate-N 11.8 mg/L in the RAS over the experimental period. These conditions are considered optimal for tilapia culture (El-Sayed, 2006; Rakocy & Brunson, 1989). Fish performance and biometric results are presented in Table 3. No significant differences between survival, FCR, PER, SGR, or any biometrics were observed for fish fed experimental diets. Weight gain and SGR slightly increased as percent fish oil increased in the diet. Conversely, weight gain and SGR marginally decreased as percent ALL-G-Rich increased in the diet.

Fillet and rib meat nutritional fatty acid data from fish fed experimental diets for 4 weeks are displayed in Table 4a. After 4 weeks of being fed experimental diets, fish fed the control diet had significantly ($P=0.036$) greater quantities of EPA, expressed as mg EPA per 4-oz serving of fillet, as compared to those fed 3% fish oil. EPA also was found to decrease in the

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fillet as percent ALL-G-Rich in the diets increased. Total omega-3 was found to have significantly ($P=0.020$) higher deposition in fillets of fish fed the 8.77% ALL-G-Rich diet compared to 3% fish oil diet. Omega-3 deposition also was found to increase as percent ALL-G-Rich increased in the diet. This trend was also observed for beneficial omega-3 fatty acids which also increased in the fillet as percent ALL-G-Rich increased in the diet. Since DHA, DPA and EPA are of the greatest benefit to humans, those fatty acids have been combined and defined as “beneficial omega-3 fatty acids”. Beneficial omega-3 fatty acids also were found to be significantly ($P=0.003$) higher in fillets of fish fed the 8.77% ALL-G-Rich diet as compared to those fed the 3% fish oil, 1% fish oil or commercial diet. Omega-6 composition in fillets was found to decrease as percent ALL-G-Rich increased in the diet, with 1.75% ALL-G-Rich and control diets resulting in significantly ($P<0.0001$) greater omega-6 per 4-oz fillet as compared to 3% and 5% fish oil, and 8.77% ALL-G-Rich. The ratio of omega-6:3 was found to decrease in fillets on as percent fish oil and percent ALL-G-Rich increased in the diets, with 5% fish oil and 8.77% ALL-G-Rich diets resulting in significantly ($P<0.0001$) lower ratios of omega-6:3 in fillets compared to 1.75% ALL-G-Rich, 1% and 3% fish oil and control diets. DHA deposition in fillet increased as percent ALL-G-Rich increased in the diet, with 8.77% ALL-G-Rich diet resulting in significantly ($P<0.0001$) greater DHA compared to 1.75% ALL-G-Rich, 1% and 3% fish oil, commercial and control diets. DPA also was found to be significantly ($P<0.0001$) greater in fillets of fish fed a 5% fish oil diet for 4 weeks than any other diet.

A significantly ($P=0.009$) greater deposition of DPA was also found in rib meat of fish fed the 5% fish oil diet for 4 weeks compared to those fed a 1.75%, 5.26%, and 8.77% ALL-G-Rich diet, commercial, and control diets. DPA was found to increase in deposition in rib meat as percent fish oil in the diet increased. In rib meat, the omega-6:3 ratio was found to decrease as

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percent ALL-G-Rich increased in the diet. Omega-6:3 was significantly ($P=0.001$) greater in rib meat of fish fed the control diet for 4 weeks as compared to those fed the commercial, 1 and 5% fish oil, as well as 5.26 and 8.77% ALL-G-Rich diets. ALA and EPA also were found to decrease in rib meat as percent fish oil increased in the diet.

The fillet and rib meat fatty acid data of tilapia fed experimental diets for 8 weeks are presented in Table 4b. After 8 weeks of being fed treatment diets, DPA and DHA had increasing deposition in fillets as percent fish oil and percent ALL-G-Rich increased in the diet. DPA was significantly ($P<0.0001$) greater in fillets of fish fed a 5% fish oil diet than all other diets. DHA was significantly ($P<0.0001$) greater in fillets of fish fed a 8.77% ALL-G-Rich diet compared to those fed 1.75% ALL-G-Rich, 1% and 3% fish oil, commercial and control diets. Omega-6:3 ratio decreased as percent fish oil and percent ALL-G-Rich increased in the diet, with 5% fish oil and 8.77% ALL-G-Rich resulting in significantly ($P<0.0001$) lower omega-6:3 ratio compared to 5.26% ALL-G-Rich, 3% fish oil commercial and control diets. EPA decreased deposition in fillets at 8 weeks as percent ALL-G-Rich increased in the diet. Omega-3 increased as percent ALL-G-Rich increased in the diet. Omega-6 decreased deposition in the fillet as percent fish oil and percent ALL-G-Rich increased in the diet. Beneficial omega-3 fatty acids increased as percent fish oil and percent ALL-G-Rich increased in the diet.

After 8 weeks of being fed treatment diets, omega-6:3 deposition decreased in rib meat as percent fish oil and percent ALL-G-Rich increased in the diet, with 8.77% ALL-G-Rich having a significantly ($P=0.001$) lower omega-6:3 ratio compared to 1.75% ALL-G-Rich, 1% fish oil and control diets (Table 4b). Meanwhile, total omega-3, DPA, DHA and beneficial omega-3 fatty acids increased deposition in rib meat as percent fish oil and percent ALL-G-Rich increased in the diet. EPA decreased in deposition as percent ALL-G-Rich increased in the diet.

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Tilapia liver and mesenteric lipid fatty acid data are delineated in Table 4c. While there were no significant ($P<0.05$) findings for tilapia liver fatty acid deposition after 8 weeks, several trends were present. ALA, EPA, DPA, DHA and total omega-3 all increased as percent fish oil increased in the diet. Omega-6:3 decreased as percent fish oil was increased in the diet.

Deposition in mesenteric fat followed expected trends with ALA, and EPA decreasing as percent ALL-G-Rich increased in the diet. Also omega-3 had increased deposition as percent ALL-G-Rich increased in the diet. DPA and DHA had increased deposition in mesenteric fat as percent ALL-G-Rich increased in the diet. DPA had significantly ($P<0.0001$) greater deposition in the mesenteric fat of fish fed the 5% fish oil diet compared to all other diets. DHA had significantly ($P=0.001$) greater deposition in fish fed 8.77% ALL-G-Rich diet compared to 1.75% ALL-G-Rich, 1% and 3% fish oil, commercial and control diets. Omega-6:3 decreased as percent ALL-G-Rich increased in the diet with 8.77% ALL-G-Rich having significantly ($P<0.0001$) lower omega-6:3 compared to 1.75% ALL-G-Rich and control diets.

Discussion

Fish demonstrated excellent growth and performance throughout the 8-week feeding trial. Survival ranged from 96%-100%, indicating that fish health was not compromised. Specific growth rate was greater between the initiation of the study and week 4 than between weeks 4 and 8. The mean growth rate of fish fed the commercial feed in this study was 38 g/week, this was greater than tilapia fed commercial diets in other studies, in similar RAS systems for similar lengths of time. Similar feeding trials had mean growth rates of 25 g/week and 14 g/week, respectively (Arredondo-Figueroa, Núñez-García, Ponce-Palafox, & Irene de Los Ángeles, 2015; Luo, Gao, Wang, Liu, Sun, Li, et al., 2014). While it is understood that increased feeding frequency can possibly increase feed conversion efficiency (FCE) (Dong, Yang, Yao, Chen, Bu,

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Li, et al., 2015) it was also suggested that feeding too frequently, more than once every 4-5 hours could decrease FCE in tilapia (Riche & Garling, 2003). This is due to tilapia reaching their gastrointestinal capacity causing some feed to rapidly pass through the stomach, however the tilapia in this trial were fed continuously for 18 hours daily with excellent feed conversion ratios (Table 3). Tilapia in this trial also demonstrated above average fillet yields of ~45% (Table 3), where average fillet yields for tilapia typically range from 33%-40% (Borderías & Sánchez-Alonso, 2011; El-Sayed, 2006).

Numerous efforts have been made to try to increase the omega-3 content of tilapia fillets. Addition of flaxseed oil, which is rich in alpha linolenic acid (18:3 n-3) has been found to moderately increase concentration of ALA in tilapia fillets, but this approach does little to increase beneficial omega-3 fatty acids (de Souza, Matsushita, de Oliveira, Franco, & Visentainer, 2007; Visentainer, de Souza, Makoto, Hayashi, & Franco, 2005). This is likely due to that fact that tilapia are limited in their ability to elongate and desaturate (18:3 n-3 and 18:3 n-6) into longer chain polyunsaturated fatty acids (20:4 n-6, 20:5 n-3, 22:5 n-3, 22:6 n-3) (Teoh, Turchini, & Ng, 2011). This limited ability to synthesize long- chain polyunsaturated fatty acids in tilapia is also present in humans and as a result, tilapia rich in 18:3 n-3 are of little nutritional benefit to humans (Graham C. Burdge & Calder, 2005). Other vegetable oil replacements, including palm oil and sunflower oil, have resulted in similar beneficial omega-3 deficits (Bahurmiz & Ng, 2007; Justi, Hayashi, Visentainer, de Souza, & Matsushita, 2003).

The study of *Schizochytrium sp.* as an alternative to fish oil for boosting beneficial omega-3 has not been studied extensively. It has been deemed feasible from a fish health perspective that *Schizochytrium sp.* meal could function as a replacement for fish meal and fish oil (Sarker, Gamble, Kelson, & Kapuscinski, 2016). Watters, Rosner, Franke, Dominy, Klinger-

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Bowen, and Tamaru (2013) determined that *Schizochytrium* sp. and fish oil can boost omega-3 fatty acids in tilapia; however, their study took 6 months to reach ~200 mg/4oz fillet of beneficial omega-3 fatty acids. In contrast, fish in this study reached the same omega-3 levels in only 8 weeks (Table 4b). Watters' study also concluded that commercial feed resulted in the fastest growth compared to any experimental fish oil or *Schizochytrium* sp. based feeds. This was likely because the commercial feed used was designed for trout, not tilapia which may have a higher digestible fatty acid requirement. The method of incorporating *Schizochytrium* sp. into the diet in that study was with an oil blended top coating; whereas, in this study the *Schizochytrium* sp. meal was blended into the diet before pelleting. The commercial diet used in this study resulted in significantly lower ($P=0.022$) average weight gain per week compared to 1.75% ALL-G-Rich. While there were no significant differences in SGR, FCR or fillet yield, all performance and biometrics were above average for tilapia (El-Sayed, 2006).

In the wild, tilapia fatty acid composition fluctuates with location and season (Kwetegyeka, Mpango, & Grahl-Nielsen, 2008; Rasoarahona, Barnathan, Bianchini, & Gaydou, 2005) However in controlled RAS systems, other factors affect fatty acid metabolism including feeding frequency, starvation, and water temperature (De Silva, Gunasekera, & Austin, 1997; Ma, Qiang, He, Gabriel, & Xu, 2015). All of these conditions factor into how tilapia utilize dietary fatty acids and proteins as energy sources. The colder the water temperature, the more efficient tilapia are at converting saturated fatty acids into monounsaturated and polyunsaturated fatty acids (Ma, Qiang, He, Gabriel, & Xu, 2015). This is possibly due to the need to keep cell membranes fluid at lower temperatures, and polyunsaturated fatty acids provide greater membrane fluidity (Muriana & Ruiz-Gutierrez, 1992). Since tilapia were kept ~29 °C throughout this study, it is likely that this moderate temperature did not inhibit the desaturation and

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elongation of saturated fatty acids to mono- and polyunsaturated fatty acids. Starvation resulted in utilization of fatty acids in the liver as an energy source as opposed to muscle fatty acids (De Silva, Gunasekera, & Austin, 1997). Because fish in this study were not starved prior to sampling, livers were observed to be high in fat (Table 4c).

Compared to fish fed a commercial diet for 4 weeks, fish fed 1%, 3% or 5% fish oil or 1.75%, 5.26%, 8.77% ALL-G-Rich for 4 weeks resulted in greater beneficial omega-3 fillet composition (Table 4a). Beneficial omega-3 fillet composition was significantly ($P=0.003$) greater in fish fed 8.77% ALL-G-Rich compared to those fed a commercial diet for 4 weeks (Table 4a). Fish fed a commercial diet for 8 weeks, and fish fed 1%, 3% or 5% fish oil or 5.26% or 8.77% ALL-G-Rich for 8 weeks resulted in greater beneficial omega-3 fillet composition (Table 4b). At 4 and 8 weeks fish fed a 5% fish oil diet or 8.77% ALL-G-Rich diet had lower omega-6:3 ratios compared to those fed the control diet.

Other tissues that would normally be considered byproducts including rib meat, mesenteric fat and liver could also be developed into value-added products. Rib meat could be formulated into sausages, surimi or salt-biscuits as all of these products have been created with positive sensory characteristics (Ibrahim, 2009; Kim & SpringerLink, 2014; Oliveira Filho, Maria Netto, Ramos, Trindade, & Viegas, 2010). Liver and mesenteric fat tissues could also be incorporated into a high omega-3 pet food (Folador, Karr-Lilienthal, Parsons, Bauer, Utterback, Schasteen, et al., 2006). At 8 weeks, the liver tissue of tilapia fed experimental fish oil and ALL-G-Rich diets had a similar composition of beneficial omega-3 fatty acids to fillets of tilapia fed the same diets at the same time, between~100-200 mg (Tables 4b,4c). Szabo, Mezes, Hancz, Molnar, Varga, Romvari, et al. (2011) found when tilapia were fed various vegetable oils, long-chain polyunsaturated fatty acids would accumulate in the liver as opposed to the fillet. This

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study demonstrates that it is possible for tilapia to store beneficial omega-3 fatty acids in both liver and fillet tissues. Also at 8 weeks the mesenteric fat was saturated with beneficial omega-3 fatty acids with 1362 mg per serving in fish fed 5% fish oil and 1504 mg per serving in fish fed 8.77% ALL-G-Rich (Table 4c).

Beneficial omega-3 composition is observed to increase linearly with percent ALL-G-Rich at 4 weeks with a line equation of $y = 8.8525x + 63.657$ and an $R^2=0.9946$, calculated from Table 4a. The same trend is observed at 8 weeks with a line equation of $y = 14.973x + 78.091$ and an $R^2=0.9885$, calculated from Table 4b. This indicates that percent ALL-G-Rich in the diet and beneficial omega-3 content in the fillet are strongly positively correlated, regardless of duration on ALL-G-Rich feed. This was also indicative that tilapia fed increasing percent ALL-G-Rich diet do not readily utilize the beneficial omega-3 fatty acids themselves, but instead store them. This is possibly due to the high protein content of the feed, between 35.5-37.3%. As tilapia grow, their relative protein requirement decreases and the required digestible energy can be replaced with carbohydrate (Ng & Romano, 2013). It is recommended that commercial tilapia feeds for fry are typically between 30 and 40% protein and for fingerlings to harvest size between 25-30% (Orachunwong, Thammasart, & Lohawatanakul, 2001). This is true for both outdoor pond cultivation and RAS cultivation; however, the quality and purity of protein used in RAS systems is generally higher than in pond production because in pond production fish can supplement feed with environmental protein sources. Because these diets were so high in energy from protein sources relative to the fishes' nutritional requirement, the majority of high energy polyunsaturated fatty acids including beneficial omega-3's were able to be stored in tissues instead of being utilized as an energy source.

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Fish fed either the 8.77% ALL-G-Rich, or the 5% fish oil diets resulted in >200mg DHA per 4oz serving. This is more than commercially available channel catfish, Atlantic and Pacific cod (137mg, 154mg, and 173mg respectively) (USDA, 2005). This demonstrates that farmed tilapia fed these diets show a nutritional improvement over other low fat white fish. Future research would include the economic feasibility of a high percent ALL-G-Rich diet compared to the added value to consumers of omega-3 enriched tilapia fillets. This would solidify the use of practical alternatives to fish oil as a method of modifying omega-3 content of tilapia fillets. New advancements in the production of *Schizochytrium sp.* could lead to the rapid, sustainable, and economical cultivation of DHA-rich microalgae (Ling, Guo, Liu, Zhang, Wang, Lu, et al., 2015; Xu, Weathers, Xiong, & Liu, 2009). Also observing if the linear trend of beneficial omega-3 fillet content continues with increasing percent of ALL-G-Rich beyond 8.77% should be pursued. If the trend continues, it may be possible to develop a finishing feed with very high ALL-G-Rich (possibly 10-15% of diet) that deposits the desired quantity of beneficial omega-3 into the fillet quicker and therefore more cost-effectively.

Conclusion

Overall the experimental diets presented in this study show promise as a feasible method of enriching beneficial omega-3 content in tilapia fillets. The continuous feeding along with moderate temperatures, high protein and high-omega-3 diets resulted in rapid fish growth and beneficial omega-3 enriched fillets. This study also suggests that tilapia fed these diets could produce value-added byproducts, by using omega-3 enriched rib meat, liver and mesenteric fat tissues in other processed foods. However, further study is needed in order to determine the economic reality of switching to an ALL-G-Rich based diet on a semi-intensive or intensive scale.

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Appendix A

Table 1: Composition of experimental feeds on a g/100 g (%) as-is basis.

Table 2a: Experimental and commercial diet nutritional proximates and minerals on a dry-matter basis.

Table 2b: Essential amino acid (%) profiles of the experimental and commercial feed on a dry-matter basis.

Table 2c: Composition of fatty acids (%) in experimental and commercial feeds as-is.
SFA:Saturated fatty acids, MUFA:Monounsaturated fatty acids, PUFA:Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA.

Table 3: Fish growth and biometrics.

Table 4a: Nutritional fatty acid profiles of tilapia fillet and rib meat after 4 weeks of dietary treatments. All values are presented on a per 4oz serving basis. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Table 4b: Nutritional fatty acid profiles of tilapia fillet and rib meat after 8 weeks of dietary treatments. All values are presented on a per 4oz serving basis. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Table 4c: Nutritional fatty acid profiles of tilapia liver and mesenteric fat after 8 weeks of dietary treatments. All values are presented on a per 4oz serving basis. Means followed by different letters in a row are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Table 1: Composition of experimental feeds on a g/100 g (%) as-is basis.

Ingredients	Control	Treatment diets [g/100g as fed]					
		Fish Oil			Alltech (All-G-Rich)		
		1%	3%	5%	1.75%	5.26%	8.77%
Soybean (46.5%) ¹	37.0	37.0	37.0	37.0	37.0	37.0	37.0
Wheat ²	37.7	37.7	37.7	37.7	37.5	37.2	37
Meat and Bone Meal ³	13.8	13.8	13.8	13.8	13.4	12.1	10.9
Menhaden fish meal ⁴	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Corn Oil ⁵	6.3	5.3	3.3	1.3	5.1	3.2	1.1
Vitamin Premix ⁶	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Mineral Premix ⁶	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Fish oil ⁴	0	1	3	5	0	0	0
All-G-Rich ⁷	0.0	0.0	0.0	0.0	1.75	5.26	8.77

¹ ADM Alliance Nutrition

² Southern States Cooperative

³ Smithfield - Farmland, Smithfield, VA, US

⁴ Omega Protein, Houston, Texas, US

⁵ Kroger, Cincinnati, Ohio, US

⁶ Purina, St. Louis, Missouri, US

⁷ Alltech, Nicholasville, Kentucky, US

Table 2a: Experimental and commercial diet nutritional proximates and minerals on a dry-matter basis.

Parameter	Control	Commercial	Treatment Diets					
			Fish Oil			ALL-G-Rich		
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.75% ALL-G-Rich	5.26% ALL-G-Rich	8.77% ALL-G-Rich
Caloric Content (cal/g)								
Total Calories ¹	4940	4860	4830	4810	4930	4900	4880	4850
Proximate and mineral levels								
Crude protein	37.0	37.1	36.5	35.5	35.7	37.3	35.5	36.5
Carbohydrate ¹	33.7	41.2	36.6	37.7	37.3	36.4	37.9	35.7
Total ash	9.18	7.49	9.19	9.12	8.96	8.80	8.55	8.38
Crude fat	10.2	4.31	9.17	9.55	9.56	10.2	10.3	10.6
Crude fiber	4.00	8.70	4.50	4.00	3.80	5.10	3.90	5.80
Calcium	2.08	1.19	2.14	2.11	2.10	2.10	2.01	1.91
Phosphorus	1.41	1.31	1.45	1.42	1.40	1.40	1.33	1.27
Potassium	1.31	1.62	1.25	1.28	1.29	1.30	1.26	1.29
Magnesium	0.24	0.34	0.23	0.24	0.24	0.24	0.27	0.26
Sodium	0.16	0.09	0.15	0.15	0.16	0.14	0.16	0.15
Trace element levels (ppm)								
Iron	226	270	215	224	223	222	224	208
Copper	24	15	21	22	21	21	21	21
Zinc	305	190	285	317	272	274	299	271
Manganese	101	388	71	88	122	101	83	92

¹ Calculated value (Merrill and Watt, 1973):

carbohydrate = total - (ash + crude protein + moisture + total fat)

Table 2b: Essential amino acid (%) profiles of the experimental and commercial feed on a dry- matter basis.

Essential Amino Acid	Treatment Diets							
	Control	Commercial	Fish Oil			ALL-G-Rich		
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.75% ALL-G-Rich	5.26% ALL-G-Rich	8.77% ALL-G-Rich
Arginine ¹	2.57	2.50	2.48	2.46	2.47	2.35	2.47	2.43
Histidine ²	0.84	0.83	0.81	0.77	0.83	0.84	0.81	0.78
Isoleucine ³	1.45	1.35	1.22	1.32	1.44	1.25	1.40	1.24
Leucine ⁴	2.38	2.40	2.30	2.27	2.41	2.38	2.30	2.25
Lysine ⁵	1.99	1.86	1.90	1.89	2.01	2.00	1.89	1.92
Methionine ⁶	0.49	0.47	0.46	0.48	0.48	0.42	0.41	0.44
Phenylalanine ⁷	1.61	1.57	1.55	1.56	1.62	1.58	1.49	1.54
Threonine ⁸	1.36	1.32	1.35	1.29	1.33	1.39	1.25	1.36
Tryptophan ⁹	0.35	0.35	0.25	0.23	0.31	0.30	0.27	0.26
Valine ¹⁰	1.64	1.94	1.68	1.61	1.81	1.65	1.77	1.42

¹ (204)Method: AOAC 994.12 (Alt. III) total amino acids-hydrolysis Units: %Basis

² (209)Method: AOAC 994.12 (Alt. III) total amino acids-hydrolysis Units: %Basis

³ (210)Method: AOAC 994.12 (Alt. III) total amino acids-hydrolysis Units: %Basis

⁴ (211)Method: AOAC 994.12 (Alt. III) total amino acids-hydrolysis Units: %Basis

⁵ (total) (195)Method: AOAC 994.12 (Alt. III) total amino acids-hydrolysis Units: %

⁶ (212)Method: AOAC 994.12 (Alt. I) - cystine & methionine Units: %Basis

⁷ (213)Method: AOAC 994.12 (Alt. III) total amino acids-hydrolysis Units: %Basis

⁸ (217)Method: AOAC 994.12 (Alt. III) total amino acids-hydrolysis Units: %Basis

⁹ (218)Method: AOAC 988.15 - tryptophan Units: %Basis

¹⁰ (220)Method: AOAC 994.12 (Alt. III) total amino acids-hydrolysis Units: %Basis

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Table 2c: Composition of fatty acids (%) in experimental and commercial feeds as-is. SFA:Saturated fatty acids, MUFA:Monounsaturated fatty acids, PUFA:Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA.

Fatty Acid	Treatment Diets							
	Control	Commercial	Fish Oil			ALL-G-Rich		
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.75% ALL-G-Rich	5.26% ALL-G-Rich	8.77% ALL-G-Rich
Total SFA	23.22	26.31	26.00	29.34	35.22	28.08	39.95	46.78
12:0	0.00	0.00	0.04	0.08	0.10	0.00	0.08	0.13
14:0	0.78	3.02	2.26	4.28	6.54	1.45	2.99	4.45
15:0 ANTEISO	0.00	0.19	0.20	0.39	0.56	0.28	0.81	1.24
15:0 ISO	0.00	0.00	0.06	0.14	0.21	0.00	0.00	0.00
16:0	17.07	18.33	16.30	17.16	20.76	21.75	30.60	36.18
17:0	0.10	0.17	0.25	0.44	0.49	0.00	0.34	0.43
18:0	4.88	4.43	6.36	6.43	6.28	4.30	4.77	4.08
20:0	0.39	0.17	0.53	0.42	0.28	0.30	0.36	0.27
Total MUFA	31.66	42.46	32.37	32.12	32.13	28.92	20.48	14.85
14:1 n-5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11
16:1 n-11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16:1 n-9	0.00	0.21	0.19	0.13	0.19	0.00	0.00	0.00
16:1 n-7	1.05	5.24	3.12	5.90	8.88	0.98	0.93	0.96
18:1 n-9	28.77	24.56	26.04	22.40	19.04	26.41	18.26	12.72
18:1 n-7	1.44	4.00	2.35	2.91	3.23	1.21	1.03	0.90
20:1 n-11	0.00	5.68	0.00	0.00	0.00	0.00	0.00	0.00
20:1 n-9	0.40	2.77	0.67	0.78	0.79	0.32	0.26	0.16
Total PUFA	45.12	31.23	41.62	38.55	32.65	43.01	39.58	38.37
18:2 n-6	43.53	25.26	36.47	29.41	21.10	38.91	26.73	16.82
18:3 n-6	0.00	0.00	0.05	0.13	0.15	0.00	0.00	0.00
18:3 n-3	1.59	3.02	2.09	2.12	1.93	1.07	1.06	0.88
20:2 n-6	0.00	0.00	0.18	0.22	0.20	0.00	0.00	0.00
20:3 n-3	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00
20:5 n-3	0.00	0.23	0.40	0.70	0.92	0.00	0.00	0.00
22:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:5 n-6	0.00	0.00	0.00	0.00	0.00	0.00	2.23	4.00
22:5 n-3	0.00	0.00	0.42	1.08	1.43	0.00	0.00	0.00
22:6 n-3	0.00	2.72	2.01	4.78	6.92	3.03	9.56	16.67
% Omega 3	1.59	5.97	4.92	8.79	11.20	4.10	10.62	17.55
% Omega 6	43.53	25.26	36.70	29.76	21.45	38.91	28.96	20.82
Omega 6:3	27.38	4.23	7.46	3.39	1.92	9.49	2.73	1.19
% Beneficial Omega-3	0.00	2.95	2.83	6.56	9.27	3.03	9.56	16.67

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Table 3: Fish growth and biometrics.

	Treatment Diets								P	Root Mean Square Error
	Control	Commercial	Fish Oil			ALL-G-Rich				
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.75% ALI G-Rich	5.26% ALI G-Rich	8.77% ALI G-Rich		
<u>Tilapia Performance</u>										
Survival (%)	100	96	98	100	100	100	98	100	0.202	1.5
Initial weight (g)	161	157	156	156	158	162	155	157	0.134	2.4
4 week weight (g)	331	345	333	329	311	333	329	311	0.855	21.4
8 week Weight (g)	521	464	504	513	540	562	521	484	0.436	41.0
Average Weight Gair (g/Week)	45	38	43	46	41	50	46	41	0.521	5.3
SGR (%/Day)	2.09	1.98	2.09	2.13	2.19	2.22	2.16	2.01	0.974	0.69
FCR	1.46	1.50	1.31	1.31	1.24	1.26	1.30	1.37	0.398	0.36
<u>Biometrics at 8 weeks</u>										
Fillet Yield	45.3	45.8	43.9	43.8	44.3	43.9	44.6	43.6	0.147	2.48
Hepatosomatic Index	1.6	1.83	1.52	1.61	1.59	1.61	1.65	1.71	0.522	0.28
Viscerasomatic Index	3.04	2.45	2.83	2.97	2.61	2.64	3.06	2.9	0.749	0.80
Mesenteric Fat Index	0.87	0.98	0.98	1.03	1.05	1.17	0.85	0.87	0.647	0.36

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Table 4a: Nutritional fatty acid profiles of tilapia fillet and rib meat after 4 weeks of dietary treatments. All values are presented on a per 4oz serving basis. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

			Treatment Diets						<i>P</i>	Root Mean Square Error
			Fish Oil			ALL-G-Rich				
	Control	Commercial	1% Fish Oil	3% Fish Oil	5% Fish Oil	1.75% ALL-G-Rich	5.26% ALL-G-Rich	8.77% ALL-G-Rich		
Week 4										
Fillet										
g Fat	2.10	1.23	1.84	1.55	1.70	2.41	1.48	1.87	0.183	0.59
mg ALA	14	12	13	6	13	14	10	11	0.209	4.56
mg EPA	32 _A	13 _{A,B}	24 _{A,B}	12 _B	17 _{A,B}	24 _{A,B}	17 _{A,B}	17 _{A,B}	0.036	8.44
mg DPA	8 _B	11 _B	14 _B	9 _B	37 _A	7 _B	7 _B	9 _B	<0.0001	6.93
mg DHA	29 _C	27 _C	34 _{B,C}	33 _{B,C}	72 _{A,B,C}	50 _{B,C}	84 _{A,B}	117 _A	<0.0001	22.3
mg Omega-3	115 _{A,B}	76 _{A,B}	94 _{A,B}	69 _B	154 _{A,B}	120 _{A,B}	132 _{A,B}	166 _A	0.020	40.8
mg Omega-6	349 _A	139 _B	225 _{A,B}	103 _B	174 _B	340 _A	230 _{A,B}	192 _B	<0.0001	61.4
mg Omega-6:3	3.07 _A	1.83 _{B,C}	2.40 _{A,B}	1.97 _B	1.11 _C	2.89 _A	1.75 _{B,C}	1.18 _C	<0.0001	0.32
mg Beneficial Omega-3	70 _{A,B}	51 _B	66 _B	54 _B	126 _{A,B}	81 _{A,B}	108 _{A,B}	143 _A	0.003	32.4
Rib Meat										
g Fat	4.32	1.27	3.73	4.99	4.34	3.55	4.03	2.53	0.060	1.56
mg ALA	49	11	85	68	57	36	57	23	0.326	43.0
mg EPA	60	16	78	56	47	32	45	15	0.203	34.9
mg DPA	1 _B	15 _B	60 _{A,B}	68 _{A,B}	140 _A	2 _B	17 _B	0 _B	0.009	51.5
mg DHA	18	41	135	126	224	85	304	179	0.077	129
mg Omega-3	190	89	437	384	507	190	480	227	0.296	276
mg Omega-6	1182	185	1175	1222	702	781	1109	408	0.169	614
mg Omega-6:3	5.87 _A	2.62 _B	3.03 _B	3.12 _{A,B}	1.45 _B	3.42 _{A,B}	2.59 _B	1.51 _B	0.001	1.12
mg Beneficial Omega-3	80	71	272	250	411	119	366	194	0.166	197

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Table 4b: Nutritional fatty acid profiles of tilapia fillet and rib meat after 8 weeks of dietary treatments. All values are presented on a per 4oz serving basis. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

			Treatment Diets						<i>P</i>	Root Mean Square Error
	Control	Commercial	Fish Oil			ALL-G-Rich				
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.75% ALL-G-Rich	5.26% ALL-G-Rich	8.77% ALL-G-Rich		
Week 8:										
Fillet										
g Fat	2.21	2.43	2.71	2.54	3.07	2.83	2.52	2.63	0.734	0.66
mg ALA	21	23	20	15	19	12	12	12	0.373	8.25
mg EPA	57	30	45	25	28	29	19	18	0.174	20.7
mg DPA	11 _C	26 _{B,C}	16 _{B,C}	35 _B	65 _A	8 _C	9 _C	12 _C	<0.0001	8.19
mg DHA	44 _D	60 _{C,D}	70 _{B,C,D}	80 _{B,C,D}	130 _{A,B}	70 _{B,C,D}	122 _{A,B,C}	183 _A	<0.0001	27.5
mg Omega-3	180	163	179	176	262	148	179	237	0.343	71.1
mg Omega-6	613	260	478	285	256	344	295	260	0.373	243
mg Omega-6:3	3.28 _A	1.60 _{B,C}	2.65 _{A,B}	1.63 _{B,C}	0.97 _C	2.31 _{A,B}	1.64 _{B,C}	1.10 _C	<0.0001	0.47
mg Beneficial Omega-3	112	116	131	140	222	108	150	213	0.014	49.6
Rib Meat										
g Fat	5.22	4.44	4.70	4.25	5.74	5.07	5.44	4.12	0.903	1.89
mg ALA	23	28	11	26	23	22	24	24	0.946	18.1
mg EPA	36	21	15	23	17	19	19	16	0.660	15.5
mg DPA	6	21	8	38	68	4	12	17	0.064	28.8
mg DHA	33	41	21	64	69	48	163	191	0.031	76.3
mg Omega-3	142	130	72	178	194	151	241	262	0.545	132
mg Omega-6	656	325	274	443	300	559	591	325	0.587	328
mg Omega-6:3	3.92 _A	2.36 _{A,B,C}	3.69 _A	2.47 _{A,B,C}	1.62 _{B,C}	3.49 _{A,B}	2.44 _{A,B,C}	1.09 _C	0.001	0.86
mg Beneficial Omega-3	74	82	45	125	154	70	194	223	0.208	104

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Table 4c: Nutritional fatty acid profiles of tilapia liver and mesenteric fat after 8 weeks of dietary treatments. All values are presented on a per 4oz serving basis. Means followed by different letters in a row are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

	Treatment Diets									Root Mean Square Error
	Control	Commercial	Fish Oil			ALL-G-Rich			P	
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.75% ALL-G-Rich	5.26% ALL-G-Rich	8.77% ALL-G-Rich		
Week 8:										
Liver										
g Fat	3.67	7.63	3.10	5.17	5.39	3.78	4.20	3.31	0.363	2.79
mg ALA	7	21	1	6	9	6	4	6	0.081	8.24
mg EPA	45	61	28	31	34	45	23	25	0.483	26.3
mg DPA	3	20	1	12	32	1	1	7	0.028	13.6
mg DHA	46	128	49	93	143	99	96	136	0.586	81.6
mg Omega-3	123	255	88	150	228	167	132	183	0.670	132
mg Omega-6	270	374	105	173	160	246	157	161	0.524	182
mg Omega-6:3	1.94	1.36	1.18	1.06	0.56	1.59	1.12	1.55	0.120	0.61
mg Beneficial Omega-3	94	208	78	135	210	145	120	168	0.673	116
Mesenteric Fat										
g Fat	49.1	28.1	36.4	46.6	30.8	38.6	31.8	31.7	0.862	21.8
mg ALA	281	321	154	84	219	195	159	158	0.345	139
mg EPA	232	107	120	48	111	118	83	68	0.130	81.8
mg DPA	77 _B	222 _B	147 _B	126 _B	565 _A	99 _B	105 _B	127 _B	<0.0001	118
mg DHA	148 _B	257 _B	218 _B	174 _B	687 _{A,B}	364 _B	802 _{A,B}	1308 _A	0.001	357
mg Omega-3	1134	1096	862	491	1724	1001	1309	1760	0.263	727
mg Omega-6	6930	3124	2293	1523	2594	4461	3294	2483	0.148	2558
mg Omega-6:3	6.37 _A	4.04 _{A,B,C}	2.42 _{B,C}	2.95 _{B,C}	1.50 _C	4.62 _{A,B}	2.49 _{B,C}	1.37 _C	<0.0001	1.23
mg Beneficial Omega-3	456	586	485	348	1362	581	991	1504	0.016	494

Chapter 3—Algae and Fish Oil Increase Tissue Specific Lipid Deposition of Docosahexaenoic Acid in Tilapia (*Oreochromis niloticus*)

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Chapter 3—Algae and Fish Oil Increase Tissue Specific Lipid Deposition of Docosahexaenoic Acid in Tilapia (*Oreochromis niloticus*)

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Abstract

An 8 week feeding trial was conducted to determine the effect algae or fish oil as dietary lipid sources have on tissue specific lipid deposition in tilapia. Seven isocaloric and isonitrogenous diets were supplemented with corn oil (control), corn oil and: 1%, 3%, or 5% fish oil by dry weight, or corn oil and: 1.75%, 5.26%, or 8.77% algae by dry weight. An additional commercial diet was also evaluated. After 8 weeks, tissue samples of fillet, rib meat, liver and mesenteric fat were analyzed for fatty acid composition. No arachidonic acid (20:4n-6) was found in any tissues of fish fed any diet. Docosahexaenoic acid (22:6n-3) increased in concentration in all tissues as percent fish oil and ALL-G-Rich increased in the diets with 8.77% ALL-G-Rich resulting in significantly ($P < 0.0001$) greater concentrations in the fillet and mesenteric fat compared to all other diets.

Introduction

Tilapia are an economically important species for worldwide aquaculture production. As of 2010, tilapia aquaculture production was 2.3 million metric tons across 74 countries [1]. Over the past few decades, selective breeding efforts have produced genetically improved farmed tilapia (GIFT) [2]. While typical traits selected for include, growth rate, hardiness, and domesticated behavior, any changes in genetics could influence fatty acid metabolism [3]. Some GIFT strains have been documented as having different fatty acid metabolism characteristics compared to their wild counterpart [4].

Tilapia, like all fish, are poikilotherms, meaning their internal body temperature varies considerably [5]. Poikilotherms typically store lipids in a variety of tissues including light and dark muscle, liver, and mesenteric fat [6]. Lipids aid in regulating body temperature, cellular membrane fluidity, and energy for growth and reproduction [7]. Some fatty acids such as long chain omega-3 (20:5n-3, 22:5n-3, 22:6n-3) are utilized by poikilotherms to increase membrane fluidity at low temperatures and provide the insulation required to maintain an electrical potential difference in neural myelin [8]. Tilapia however, being a low fat fish cultured in warm waters (22-29 °C) typically contain low levels of 20:5n-3, 22:5n-3, 22:6n-3. This makes them a less valued fish from the consumer standpoint for omega-3 health benefits.

Omega-3 fatty acids, including 20:5n-3 (eicosapentaenoic acid, EPA), 22:5n-3 (docosapentaenoic acid, DPA) and 22:6n-3 (docosahexaenoic acid, DHA) have been associated with a wide variety of human health benefits. All three have been associated with decreased risk of cardiovascular disease [9, 10]. High levels of EPA have been associated with decreased risk of

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thrombosis, due to cyclooxygenases in the prostaglandin production pathway preferentially reacting with EPA as opposed to arachidonic acid (20:4n-6). When cyclooxygenases utilize AA as a substrate, prostaglandins are produced leading to increased inflammation and an increased risk of inflammatory chronic diseases [11], whereas utilization of omega-3 fatty acids leads to production of less pro-inflammatory prostaglandins [12]. While tilapia are typically low in omega-3 fatty acids they are conversely high in omega-6 fatty acids like AA [13]. This makes them a less nutritionally beneficial species compared to high fat omega-3 rich fish like salmon or trout.

Changes in dietary lipid sources often have a significant impact on tissue composition in tilapia. Studies have shown that increases in dietary linolenic acid (18:3n-3) decreased the deposition of longer chain polyunsaturated fatty acids in tilapia fillets [14]. Other studies demonstrate that supplementation with algae or fish oil, two lipid sources rich in long chain omega-3 fatty acids, results in increased fillet concentrations of EPA, DPA, and DHA [15, 16]. However, no studies have explored how these different lipid sources impact fat deposition in typical lipid deposition sites in tilapia.

The aims of this study were to: (1) evaluate the effect of algae and fish oil on the deposition of fatty acids in known fatty deposits in tilapia, (2) to study how dietary fatty acid compositions affect tissue fatty acid profiles from a mass balance perspective, and (3) Determine which dietary composition would provide the best nutritional fillet product for consumers.

Methods and Materials

Fish and culture system

Juvenile tilapia (*Oreochromis niloticus*, ~11 g each) were obtained from Spring Genetics (Akvaforsk Genetics Center, Miami, Florida). Fish were acclimated and conditioned for 4 weeks until they grew to ~150 grams for experiment initiation. Fish were reared in an indoor recirculating aquaculture system (RAS) equipped with 16, 1-meter-diameter polyethylene tanks (each ~250 liters), bubble-bead filters for mechanical filtration, fluidized-bed bioreactors, UV disinfection units, heat exchangers, and distributed diffuse aeration via air stones. Stocking density of the systems started at ~16 kg/m³, and approached ~44 kg/m³ after 8 weeks.

Water quality in the RAS was monitored during the 8 week study. All water quality factors were evaluated using methods modified from APHA [17] and HACH [18]. Dissolved oxygen and temperature were examined daily. Alkalinity, total ammonia nitrogen (TAN), nitrite, nitrate and pH were evaluated three times a week. This animal study was approved by Virginia Tech IACUC, protocol # 14-211.

Diets

All treatment diets were prepared to be isonitrogenous and isocaloric except for the commercial feed (Production 35, Rangen Inc., Buhl, ID, USA) which was used as received. Algae was obtained from Alltech (All-G-Rich, Nicholasville, Kentucky, USA), and fish oil from Omega Protein (Houston, Texas, USA). The fish oil was extracted from menhaden, and the algae

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was *Schizochytrium spp.* Experimental feeds were then pelleted into sinking ~3 mm pellets. The pelleted treatment diet formulations are offered in Table 1. The independent variable for this experiment was the lipid composition of the eight diets, and the dependent variables were: survival rate, growth, biometrics, performance indices, feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), and nutritionally relevant fatty acid profiles.

All diets, including the commercial diet, were analyzed to verify proximate nutritional composition and essential amino acids using a commercial lab (Midwest Labs, Omaha, NE, USA). All diets also had their fatty acid profiles determined via gas chromatography-mass spectrometry. Tilapia were group-weighted on a per tank basis weekly to enable appropriate feed adjustments. Feeding rates were determined for all treatment groups on a percent of body weight per day basis. Feeding rates were 4, 3.75, 3.25, 3.25, 2.375, 2, and 1.85 percent body weight per day (% BW/d) for weeks 1, 2, 3, 4, 5, 6, 7 and 8, respectively. This adjustment was done to maximize feed conversion efficiency. Fish were fed once an hour for 18 hours a day. Measuring feed consumption allowed FCR, SGR and PER to be calculated.

Biometrics

Fillet yield, hepatosomatic index (HSI), viscerasomatic index (VSI), and mesenteric fat index (MFI) were calculated by dividing the fillet/muscle tissue, liver, total viscera mass, and mesenteric/visceral fat by the whole weight of the fish, respectively [19] All filleting and tissue differentiation was done by one individual in order to avoid differences in technique.

Tissue Sampling

Fatty acid profiles were determined for fish fillets, rib meat, liver, and mesenteric fat at the end of the 8-week trial. Additionally, profiles were measured at week 4 for the fillets and rib meat only. Rib meat for the purposes of this study is comprised of the pin bones and belly meat ventral to the fillet. Tissue samples were collected on a per tank/diet basis with two replicate samples collected for each tissue. Each replicate was a pooled sample, consisting of tissue from two fish of the same treatment. Tissues were vacuum packed with 10 ml of methanol in an effort to neutralize enzymes and then quick-frozen in an immersion of isopropanol and dry ice. Tissues were then stored at -80°C pending evaluation. Fatty acid extraction was done according to Bligh EG and Dyer WJ [20] and methyl esters were prepared using the AOCS official method Ce-1b-89 [21]. A Shimadzu QP-2020 GCMS (Kyoto, Japan) was used with a 60m $\times$ 0.25mm i.d., 0.25 μm film Phenomenex Zebron Carbowax column operated with He carrier gas at 30cm/second linear velocity.

Data analysis

Statistical evaluation was conducted using JMP Pro 11 for Apple (Cary, NC, USA). The fatty acid profiles of each of the four pooled samples per tissue per diet were averaged. One-way ANOVA was utilized to determine dietary effects on dependent variables (fish performance,

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biometrics, tissue fatty acid composition). When applicable, Tukey's post-hoc test was used to determine where significant ($P < 0.05$) differences occurred between the means.

Results

Fish demonstrated excellent growth throughout the trial with no significant differences in survival, specific growth rate (SGR), food conversion ratio (FCR), or average weight gain per week (Table 2). Water quality was optimal for tilapia growth, mean values ($\pm$ standard error) over the duration of the trial were the following: temperature 29.4 ± 0.06 °C, pH 7.79 ± 0.02 , dissolved oxygen 5.45 ± 0.04 mg/L, alkalinity 199 ± 5.9 mg/L, total ammonia-N 0.35 ± 0.02 mg/L, nitrite-N 0.06 ± 0.005 mg/L, and nitrate-N 11.8 ± 0.6 mg/L. Formulated diets were confirmed to be isonitrogenous and isocaloric. The lipid composition of the diets did vary.

Dietary Lipid Composition

The lipid composition of the diets can be referenced in Table 3. In total: percent saturated fatty acids, 14:0, 16:0, 17:0, and percent omega-3 increased as inclusion of fish oil and ALL-G-Rich were increased in the diets. Percent polyunsaturated fatty acids, 18:2n-6, 20:1n-9, percent omega-6, and omega-6:3 ratio decreased as levels of fish oil and ALL-G-Rich increased in the diets. Percent 18:1n-7 and 20:1n-9 increased as fish oil increased in the diet and decreased as ALL-G-Rich increased in the diet. A few fatty acids, iso 15:0, 18:3n-6, 20:2n-6, and 22:5n-3 were not present in control, commercial or any level of ALL-G-Rich diets with iso 15:0, 18:3n-6 and 22:5n-3 increasing as fish oil increased in the diet. Level of monounsaturated fatty acids and 18:3n-3 decreased as ALL-G-Rich increased in the diets. Proportion of 16:1n-7 increased and percent 20:0 decreased as fish oil increased in the diets. Some fatty acids were not found in any

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of the diets including; 16:1n-11 and 22:4n-3. 14:1n-5 was only present in the 8.77% ALL-G-Rich diet. The fatty acids 16:1n-9, and 20:5n-3 were not found in control or ALL-G-Rich diets with percent 20:5n-3 increasing as fish oil increased in the diet. Some fatty acids including anteiso 15:0 and 22:6n-3 were not present in the control diet and increased in percentage as level of fish oil and ALL-G-Rich increased in the diets. Docosapentaenoic acid, 22:5n-6, was not present in control, commercial or fish oil diets but proportion of 22:5n-6 increased as inclusion rate of ALL-G-Rich increased in the diets. One fatty acid, 12:0 was not present in the control or commercial diets but increased in percentage as inclusion rates of fish oil and ALL-G-Rich increased in the diets. One monounsaturated fatty acid, 20:1n-11 was only present in the commercial diet and 20:3n-3 was only present in the 3% fish oil diet.

Fillet Lipid Composition

The lipid composition of the fillets of tilapia fed experimental diets for 8 weeks can be observed in Table 4. Saturated fatty acids, 12:0, 14:0, anteiso 15:0, 17:0, 16:1n-7, 22:5n-3, omega-3 fraction and beneficial omega-3 (EPA, DPA, DHA) increased as fish oil and ALL-G-Rich increased in the diets. Total polyunsaturated fatty acids, 18:1n-9, 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-3, 20:5n-3, 22:4n-3, omega-6 and omega-6:3 ratio decreased as inclusion rate of fish oil and ALL-G-Rich increased in the diets. Percent 22:5n-6 decreased as fish oil increased in the diets and increased as ALL-G-Rich increased in the diets. Fish fed the control diet had significantly greater polyunsaturated fatty acids compared to those fed the commercial, 3% fish oil and 5% fish oil ($P=0.003$). Fish fed the commercial diet had significantly greater ($P<0.0001$) 18:3n-3 in their fillets after 8 weeks compared to all the other diets. The control diet resulted in significantly higher ($P<0.0001$) proportions of 18:3n-6 compared to all other diets except for

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commercial, 20:2n-6 compared to all other diets except 1.75% ALL-G-Rich, and 20:3n-3 compared to 3% fish oil, 5% fish oil, 5.26% ALL-G-Rich and 8.77% ALL-G-Rich. Fish fed the control diet also had significantly ($P=0.0002$) higher proportions of 22:4n-3 compared to those fed the 3% fish oil, 5% fish oil, 5.26% ALL-G-Rich and 8.77% ALL-G-Rich diets. Accretion of 22:5n-3 was significantly ($P<0.0001$) greater in fillets of fish fed the 5% fish oil diet, compared to all other diets. The 8.77% ALL-G-Rich diet resulted in significantly greater ($P<0.0001$) concentrations of 22:5n-6 and 22:6n-3 compared to all other diets. Proportion of omega-6 was significantly ($P<0.0001$) greater the fillet of fish fed the control diet compared to those fed any other diet.

Rib Meat Lipid Composition

The lipid composition of the rib meat is presented in Table 5. Several trends, albeit not reaching significance, were observed. Percent 20:1n-11, 20:3n-3 and 20:5n-3 decreased as proportion of fish oil in the diet increased. Proportion of polyunsaturated fatty acids decreased as ALL-G-Rich increased in the diets. Percent 18:2n-6, 20:2n-6, 22:4n-3, omega-6 and omega-6:3 ratio decreases as fish oil and ALL-G-Rich increased in the diets. Fraction of 14:0, 15:0, 22:5n-3, 22:6n-3, total omega-3 and beneficial omega-3 increased as fish oil and ALL-G-Rich increased in the diets. Fraction of 22:5n-6 decreased as fish oil increased in the diets and increased as ALL-G-Rich increased in the diets. The rib meat of fish fed the 5% fish oil diet had significantly ($P<0.0001$) greater proportions of 22:5n-3 compared to all other diets. Proportion of omega-6 was significantly ($P=0.004$) lower in fish fed the 8.77% diet compared to 1% fish oil and control diets. The omega-6:3 ratio of fish fed the 8.77% ALL-G-Rich diet was significantly ($P=0.001$) lower compared to those fed the control, 1% fish oil, 5% fish oil and 1.75% ALL-G-Rich diets.

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Liver Lipid Composition

The lipid composition of tilapia livers is presented in Table 6. Numerous trends were observed. Percent 18:2n-6, 18:3n-6, 20:2n-6 and total omega-6 decreased as inclusion rates of fish oil and ALL-G-Rich increased in the diets. Composition of 20:3n-3, 22:4n-3 and omega-6:3 ratio decreased as fish oil increased in the diets. Total polyunsaturated fatty acids, 18:1n-9 and 20:5n-3 decreased in percentage as ALL-G-Rich increased in the diets. Percent iso 15:0, 17:0, and 22:5n-3 increased as % fish oil increased in the diets. Fraction of 22:5n-6 decreased in as inclusion rate of fish oil increased and increased as inclusion rate of ALL-G-Rich increased in the diets. Some significant differences in fatty acid composition were observed. The amount of 18:2n-6 was significantly ($P=0.017$) greater in fish fed the control diet compared to those fed the 5% fish oil diet. Percent omega-6 was significantly ($P=0.001$) greater in fish fed the control diet compared to those fed the commercial, 5% fish oil and 8.77% ALL-G-Rich diets. Deposition of 18:3n-6 was significantly ($P=0.009$) higher in control livers compared to 5% fish oil, and 8.77% ALL-G-Rich. Deposition of 20:2n-6 was significantly ($P<0.0001$) greater in fish fed the control diet compared to those fed the commercial, 3% fish oil, 5% fish oil, 5.26% ALL-G-Rich, and 8.77% ALL-G-Rich diets. Proportion of 22:4n-3 was significantly ($P=0.001$) greater in control fish livers compared to all other diets except 1% fish oil. Accretion of 22:5n-3 was significantly ($P<0.0001$) greater in fish fed 5% fish oil compared to all other diets. Percent 22:5n-6 was significantly ($P<0.0001$) greater in fish fed the control diet compared to those fed the commercial, 3% fish oil and 5% fish oil.

Mesenteric Fat Lipid Composition

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The lipid composition of mesenteric fat is presented in Table 7. Some not significant trends were observed. Percent 12:0, 14:0, 15:0, 17:0, 14:1n-5, 16:1n-9, and omega-3 increased as inclusion rate of ALL-G-Rich increased in the diets. Proportion of 18:1n-9, 18:2n-6, 20:5n-3, 22:4n-3, omega-6 and omega-6:3 ratio decreased as inclusion rate of ALL-G-Rich increased in the diets. Fraction of 16:0, 18:3n-6, 20:2n-6 and 20:3n-3 decreased as fish oil and ALL-G-Rich increased in the diets. Percent 22:5n-3, 22:6n-3 and beneficial omega-3s increased as fish oil and ALL-G-Rich increased in the diets. Proportion of 22:5n-6 decreased as fish oil increased in the diets and increased as ALL-G-Rich increased in the diets. Some significant differences were also observed. Accretion of 18:3 n3 was significantly ($P=0.003$) greater in the mesenteric fat of tilapia fed the commercial diet compared to all other diets except 5% fish oil. Many polyunsaturated fatty acids were in greater concentration in fish fed the 1% fish oil diet. Accumulation of 18:3n-6 was significantly ($P<0.001$) greater in 1% fish oil fed fish compared to those fed the commercial, 3% fish oil, 5% fish oil, and all the ALL-G-Rich diets. Deposition of 20:2n-6 was significantly ($P<0.0001$) greater in fish fed the 1% fish oil diet, compared to those fed commercial, 3% fish oil, 5% fish oil, 5.26% ALL-G-Rich, and 8.77% ALL-G-Rich diets. Deposition of 20:3n-3 and 20:5n-3 were significantly ($P=0.001$, $P=0.002$ respectively) greater in fish fed the 1% fish oil compared to all other diets except the control. Proportionally 22:4n-3 was significantly ($P=0.005$) greater in the mesenteric fat of fish fed 1% fish oil diet compared to all other diets except the control and 1.75% ALL-G-Rich diets. Accretion of 22:5n-3 was significantly ($P<0.001$) greater in fish fed 5% fish oil compared to all other diets. Percent 22:5n-6 and 22:6n-3 were significantly ($P<0.001$) greater in fish fed 8.77% ALL-G-Rich compared to all other diets. Deposition of omega-3 was significantly ($P<0.001$) greater in fish fed 8.77%

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ALL-G-Rich diet compared to all other diets except 5% fish oil. Deposition of omega-6 was significantly ($P=0.024$) lower in fish fed the 1% fish oil diet compared to those fed the control diet. Omega-6:3 ratio was significantly ($P<0.001$) lower in fish fed 8.77% 8.77% ALL-G-Rich and 5% fish oil compared to those fed the control or 1.75%, 8.77% ALL-G-Rich diets. Beneficial omega-3s was significantly ($P<0.001$) greater in 8.77% ALL-G-Rich compared to all other diets.

Discussion

Fish store lipids in a variety of tissues including fillet (dark muscle), rib meat (light muscle), liver and mesenteric fat. Each of these tissues provides a different function for lipid storage and processing. Mesenteric fat typically provides long-term storage of lipids, liver performs lipid processing, and light and dark muscle functions as more short-term storage for localized energy requirements [7]. Tilapia tend to have more fat stored in liver compared to muscle, on a %wt basis [22]. The results of this study agree with this, every diet resulted in a greater % lipid in liver compared to fillet. Since all of the fish in this study came from the same broodstock and were exposed to the same environmental conditions, any differences in tissue lipid deposition *de novo* synthesis or metabolism was likely due to differences in the diet.

Effects of Fish Oil Diets on Fatty Acid Deposition in Tilapia

The fish oil diets were the only diets to contain 22:5n-3 (Table 3). As such, it was unsurprising that 5% fish oil had significantly higher concentrations of 22:5n-3 in all tissues

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observed (Tables 4-7). The order of average tissue concentration of 22:5n-3 from highest to lowest was fillet (4.47%), mesenteric fat (3.94%), rib meat (3.78%) and liver (2.09%). This trend of preferential deposition in the fillet was consistent across most diets, with mesenteric fat being the preferred deposition location in 1% fish oil and 8.77% ALL-G-Rich. This indicates that rather than process or store this lipid for enzymatic breakdown, tilapia prefer to store this fatty acid in muscle for quick energy, or in mesenteric fat for long-term storage. The results seem to suggest that low exogenous concentrations of 22:5n-3 (1% fish oil) trigger storage in mesenteric fat, whereas higher concentrations (5% fish oil) trigger storage in fillet muscle. Deposition of 22:5n-3 in mesenteric fat increased as percent ALL-G-Rich increased in the diet, possibly indicating some bioactive compounds in the algae are causing this long-term storage.

The fish oil diets were also the only diets to contain 20:2n-6. Menhaden contain over 20 unique unsaturated fatty acids with 20:2n-6 occurring at <1% whole body lipid [23]. Interestingly, as percent fish oil increased in the diets as percent 20:2n-6 decreased in all tissues (Tables 4-7). This trend also occurs in fish fed the ALL-G-Rich diets. This could mean that the biochemistry of tilapia metabolism is much more extensive than previously thought. The fatty acid 20:2n-6 is the result of elongation of 18:2n-6, which was present in all of the diets.

Effects of ALL-G-Rich Diets on Fatty Acid Deposition in Tilapia

ALL-G-Rich diets were the only diets to contain 22:5n-6 (Table 3). Omega-6 docosapentaenoic acid was found in highest concentration in the fillets of fish fed 8.77% ALL-G-Rich diets (3.72%) and second highest in the livers of fish fed the control diet (3.25) (Tables 4,6). This finding indicates that tilapia are capable of *de novo* synthesis of 22:5n-6 from 18:2n-6. Since tissue concentrations of 22:5n-6 were greater in control fish compared to fish oil fed fish,

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and tissue concentrations of 22:5n-6 in fish oil fed fish decreased as percent fish oil in the diet increased it would appear that this *de novo* synthesis was inhibited by the presence of omega-3 polyunsaturated fatty acids. Omega-6 docosapentaenoic acid (22:5n-6) is the product of elongation and desaturation of 20:4n-6 (arachidonic acid) [24]. Arachidonic acid is typically found in high relatively high concentrations in both wild and farmed tilapia [25]. In this study it was specifically searched for using authentic for retention time and mass spectra. Despite this, arachidonic acid was not detected in any tissues across all diets. The explanation the data presents was that any arachidonic acid generated from elongation and desaturation of 18:2n-6 (which was present in all diets) was further elongated and desaturated into 22:5n-6. Some studies have suggested that genetic differences can account for within species differences in tilapia metabolism [4]. It was entirely possible that the generically improved farmed tilapia (GIFT) strain used in this study possess more active elongase and desaturase enzymes which convert any arachidonic acid formed into 22:5n-6. This conclusion would need to be confirmed by enzyme assay in a future study.

Effects of a Commercial Diet on Fatty Acid Deposition in Tilapia

The commercial diet was the only diet that contained 20:1n-11, an unusual fatty acid believed to only be synthesized by zooplankton (Table 3) [26]. However, all diets and all tissues resulted in some deposition of 20:1n-11, with the commercial diet resulting in significantly higher concentrations compared to all other diets in fillet and liver tissues (Tables 4-7). This suggests that tilapia have limited ability to *de novo* synthesize 20:1n-11. This particular fatty acid has not been found to be synthesized by tilapia previously [27]. The monounsaturated fatty acid, 20:1n-11 is thought to provide little biological functionality and is typically metabolized by fish

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into longer chain polyunsaturated fatty acids[27]. It could also mean that some 20:1n-11 was accumulated before the trial began while the fish were being acclimated to the RAS system being fed a commercial diet and was not catabolized. There is no documented definitive role 20:1n-11 plays in the function and growth of zooplankton.

Effects of the Control Diet on Fatty Acid Deposition in Tilapia:

The control diet contained no long chain polyunsaturated fatty acids but rather only contained shorter chain 18:2n-6 and 18:3n-3 (Table 2). However, in all tissues, fish fed the control diet maintained relatively high levels of all polyunsaturated fatty acids, with some fatty acids in some tissues being significantly higher than in fish fed diets high in polyunsaturated fatty acids (Tables 4-7). This appears to confirm that long chain polyunsaturated fatty acids inhibit the *de novo* synthesis of long chain polyunsaturated fatty acids in tilapia [27]. This is due to a feedback mechanism, high concentrations of polyunsaturated fatty acids inhibit elongase and desaturase enzymes necessary for conversion of short chain PUFA to long chain PUFA. Conversely a deficit in long chain PUFA promotes the transcription and translation of elongases and desaturases [28].

Comparison of Wild, Farmed, and Experimental Tilapia

Tilapia in this study were found to have higher concentrations of polyunsaturated fatty acids compared to wild caught tilapia [29]. However the omega-6:3 ratio of fish in this study were higher than wild caught tilapia [30]. Intensively cultured tilapia typically have lower

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muscle lipid proportions of 18:3n-3, 20:4n-6, 20:5n-3, and 22:6 n-3 compared to wild caught tilapia, although this depends on the diet [31]. On average wild tilapia have lipid proportions of 3%, 5.5%, 2% and 12% for 18:3n-3, 20:4n-6, 20:5n-3 and 20:6n-3 respectively [31]. Intensively culture tilapia from other studies have typical lipid proportions of 0.8%, 1.5%, 0.7%, 8.3% for 18:3n-3, 20:4n-6, 20:5n-3 and 20:6n-3 respectively [31]. Virtually no 20:4n-6 was present in any tissues of fish from this study. Fish fed an 8.77% ALL-G-Rich demonstrated a fillet 22:6n-3 composition of 11.5%, a similar proportion to wild tilapia. Fish fed the control diet (Table 4) had greater fillet proportions of 20:5n-3 (2.69%) compared to both wild and intensively cultured fish from other studies. This was exceptional since the control diet contained no 20:5n-3 (Table 3) meaning all 20:5n-3 occurred due to *de novo* synthesis or from pre-study fatty acids stores.

In general, the lowest omega-6:3 ratio was present in the liver and the highest in mesenteric fat. This suggests that more omega-6 fatty acids were partitioned for long-term storage compared to omega-3. This could also indicate a preference to utilize omega-3 fatty acids as short-term energy sources compared to omega-6 fatty acids. Overall the fish in this study demonstrated the ability to elongate and desaturate shorter chain polyunsaturated fatty acids into longer chain polyunsaturated fatty acids. This study also furthers the understanding of lipid metabolism in tilapia by demonstrating the potential ability to *de novo* synthesize fatty acids including 20:2n-6 and 20:1n-11. Tilapia in this study in general had a greater composition of 22:5n-6 and 22:6n-3 compared to other n-6 and n-3 polyunsaturated fatty acids respectively. This possibly was indicative of more active elongase and desaturase enzymes in this particular strain of GIFT tilapia. In salmon, fish oil based diets resulted in depressed elongase and desaturase activity in liver tissues compared to vegetable oil based diets [32]. Temperature is also known to have an effect on fatty acid metabolism in tilapia, with lower temperatures ~22 °C producing

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greater proportions of polyunsaturated fatty acids compared to higher temperatures ~28 °C [33].

This indicates that the large proportion of polyunsaturated fatty acids found in this study could be even further enhanced by culturing at lower temperatures. Further research would examine the relationship between specific diet fatty acid deposition and temperature. Also tissue specific gene expression and elongase/desaturase activity should be quantified. Understanding how tilapia deposit specific fatty acids in various tissues could influence how future value added diets are formulated in order to maximize beneficial omega-3 fatty acid deposition in fillets. In addition, only total lipids were analyzed, it would be interesting to separately analyze phospholipids and triacylglycerols. Looking at these lipids separately may provide more clues to how tilapia partition omega-6 and omega-3 fatty acids in tissues.

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Conflict of Interest

There were no perceived or actual conflicts of interest in this study.

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Appendix B

Table 1: Composition of experimental feeds on a g/100g (%) as-is basis.

Table 2: Fish growth and performance measurements.

Table 3: Composition of fatty acids (%) in experimental and commercial feeds as-is. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA.

Table 4: Fillet fatty acid profile (% sum fatty acid) of fish fed experimental diets for eight weeks. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Table 5: Rib meat fatty acid profile (% sum fatty acid) of fish fed experimental diets for eight weeks. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Table 6: Liver fatty acid profile (% sum fatty acid) of fish fed experimental diets for eight weeks. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Table 7: Mesenteric fat fatty acid profile (% sum fatty acid) of fish fed experimental diets for eight weeks. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

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Table 1: Composition of experimental feeds on a g/100g (%) dry matter basis.

Ingredients	Treatment diets [g/100g as fed]						
	Control	Fish Oil			Alltech (All-G-Rich)		
		1%	3%	5%	1.75%	5.26%	8.77%
Soybean (46.5%) ¹	37.0	37.0	37.0	37.0	37.0	37.0	37.0
Wheat ²	37.7	37.7	37.7	37.7	37.5	37.2	37
Meat and Bone Meal ³	13.8	13.8	13.8	13.8	13.4	12.1	10.9
Menhaden fish meal ⁴	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Corn Oil ⁵	6.3	5.3	3.3	1.3	5.1	3.2	1.1
Vitamin Premix ⁶	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Mineral Premix ⁶	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Fish oil ⁴	0	1	3	5	0	0	0
All-G-Rich ⁷	0.0	0.0	0.0	0.0	1.75	5.26	8.77

¹ ADM Alliance Nutrition

² Southern States Cooperative

³ Smithfield - Farmland, Smithfield, VA, US

⁴ Omega Protein, Houston, Texas, US

⁵ Kroger, Cincinnati, Ohio, US

⁶ Purina, St. Louis, Missouri, US

⁷ Alltech, Nicholasville, Kentucky, US

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Table 2: Mean values of fish growth and performance measurements.

			Treatment Diets						<i>P</i>	Root Mean Square Error
			Fish Oil			ALL-G-Rich				
	Control	Commercial	1% Fish Oil	3% Fish Oil	5% Fish Oil	1.75% ALI G-Rich	5.26% ALI G-Rich	8.77% ALI G-Rich		
Tilapia Performance										
Survival (%)	100	96	98	100	100	100	98	100	0.202	1.5
Initial weight (g)	161	157	156	156	158	162	155	157	0.134	2.4
4 week weight (g)	331	345	333	329	311	333	329	311	0.855	21.4
8 week Weight (g)	521	464	504	513	540	562	521	484	0.436	41.0
Average Weight Gain (g/Week)	45	38	43	46	41	50	46	41	0.521	5.3
SGR (%/Day)	2.09	1.98	2.09	2.13	2.19	2.22	2.16	2.01	0.974	0.69
FCR	1.46	1.50	1.31	1.31	1.24	1.26	1.30	1.37	0.398	0.36

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Table 3: Composition of fatty acids (mean %) in experimental and commercial feeds as-is. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA.

Fatty Acid	Control Commercial		Treatment Diets					
			Fish Oil			ALL-G-Rich		
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.75% ALL-G-Rich	5.26% ALL-G-Rich	8.77% ALL-G-Rich
Total SFA	23.22	26.31	26.00	29.34	35.22	28.08	39.95	46.78
12:0	0.00	0.00	0.04	0.08	0.10	0.00	0.08	0.13
14:0	0.78	3.02	2.26	4.28	6.54	1.45	2.99	4.45
15:0 ANTEISO	0.00	0.19	0.20	0.39	0.56	0.28	0.81	1.24
15:0 ISO	0.00	0.00	0.06	0.14	0.21	0.00	0.00	0.00
16:0	17.07	18.33	16.30	17.16	20.76	21.75	30.60	36.18
17:0	0.10	0.17	0.25	0.44	0.49	0.00	0.34	0.43
18:0	4.88	4.43	6.36	6.43	6.28	4.30	4.77	4.08
20:0	0.39	0.17	0.53	0.42	0.28	0.30	0.36	0.27
Total MUFA	31.66	42.46	32.37	32.12	32.13	28.92	20.48	14.85
14:1n-5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11
16:1n-11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16:1n-9	0.00	0.21	0.19	0.13	0.19	0.00	0.00	0.00
16:1n-7	1.05	5.24	3.12	5.90	8.88	0.98	0.93	0.96
18:1n-9	28.77	24.56	26.04	22.40	19.04	26.41	18.26	12.72
18:1n-7	1.44	4.00	2.35	2.91	3.23	1.21	1.03	0.90
20:1n-11	0.00	5.68	0.00	0.00	0.00	0.00	0.00	0.00
20:1n-9	0.40	2.77	0.67	0.78	0.79	0.32	0.26	0.16
Total PUFA	45.12	31.23	41.62	38.55	32.65	43.01	39.58	38.37
18:2n-6	43.53	25.26	36.47	29.41	21.10	38.91	26.73	16.82
18:3n-6	0.00	0.00	0.05	0.13	0.15	0.00	0.00	0.00
18:3n-3	1.59	3.02	2.09	2.12	1.93	1.07	1.06	0.88
20:2n-6	0.00	0.00	0.18	0.22	0.20	0.00	0.00	0.00
20:3n-3	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00
20:5n-3	0.00	0.23	0.40	0.70	0.92	0.00	0.00	0.00
22:4n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:5n-6	0.00	0.00	0.00	0.00	0.00	0.00	2.23	4.00
22:5n-3	0.00	0.00	0.42	1.08	1.43	0.00	0.00	0.00
22:6n-3	0.00	2.72	2.01	4.78	6.92	3.03	9.56	16.67
% Omega 3	1.59	5.97	4.92	8.79	11.20	4.10	10.62	17.55
% Omega 6	43.53	25.26	36.70	29.76	21.45	38.91	28.96	20.82
Omega 6:3	27.38	4.23	7.46	3.39	1.92	9.49	2.73	1.19
% Beneficial Omega-3	0.00	2.95	2.83	6.56	9.27	3.03	9.56	16.67

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Table 4: Fillet fatty acid profile (mean % sum fatty acid) of fish fed experimental diets for eight weeks. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Fatty Acid	Treatment Diets								P	Root Mean Square Error
	Control	Commercial	Fish Oil			ALL-G-Rich				
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.75% ALL-G-Rich	5.26% ALL-G-Rich	8.77% ALL-G-Rich		
Total SFA	21.67 _C	28.81 _{A,B}	27.61 _{B,C}	29.83 _{A,B}	30.33 _{A,B}	28.41 _{A,B}	31.22 _{A,B}	34.16 _A	0.0002	2.74
12:0	0.03 _C	0.04 _{B,C}	0.03 _C	0.04 _{B,C}	0.06 _{A,B}	0.04 _{B,C}	0.06 _{A,B}	0.07 _A	<0.001	0.01
14:0	1.93 _D	2.66 _{B,C}	2.53 _{B,C,D}	3.10 _B	4.01 _A	2.12 _{C,D}	2.78 _{B,C}	3.02 _B	<0.001	0.28
15:0 ANTEISO	0.13 _F	0.22 _{D,E,F}	0.18 _{E,F}	0.31 _{C,D}	0.40 _{B,C}	0.28 _{D,E}	0.48 _{A,B}	0.58 _A	<0.001	0.05
15:0 ISO	0.01 _C	0.04 _{B,C}	0.05 _{B,C}	0.08 _{A,B}	0.13 _A	0.04 _{B,C}	0.06 _{B,C}	0.01 _C	<0.001	0.02
16:0	13.45 _C	18.28 _B	17.78 _{B,C}	18.52 _{A,B}	18.04 _B	18.57 _{A,B}	20.74 _{A,B}	22.87 _A	<0.001	1.95
17:0	0.13 _C	0.25 _{A,B,C}	0.21 _{B,C}	0.32 _{A,B}	0.39 _A	0.25 _{A,B,C}	0.30 _{A,B}	0.32 _{A,B}	0.0005	0.07
18:0	5.80	7.15	6.63	7.22	7.10	6.89	6.59	7.09	0.473	0.95
20:0	0.20	0.18	0.21	0.23	0.21	0.23	0.24	0.21	0.962	0.07
Total MUFA	35.55 _A	35.87 _A	32.98 _{A,B,C}	33.81 _{A,B}	34.09 _{A,B}	31.81 _{B,C,D}	29.80 _{C,D}	28.67 _D	<0.001	1.52
14:1n-5	0.00 _C	0.00 _C	0.00 _C	0.00 _C	0.00 _C	0.00 _C	0.05 _B	0.08 _A	<0.001	0.00
16:1n-11	0.03	0.06	0.09	0.04	0.02	0.05	0.00	0.02	0.368	0.05
16:1n-9	0.63	0.67	0.63	0.66	0.69	0.60	0.58	0.56	0.107	0.07
16:1n-7	2.91 _D	4.30 _{B,C}	3.89 _{C,D}	5.02 _B	6.59 _A	3.02 _D	3.56 _{C,D}	3.60 _{C,D}	<0.001	0.47
18:1n-9	27.45 _A	23.62 _B	23.83 _B	23.32 _B	21.32 _B	23.80 _B	21.64 _B	20.55 _B	<0.001	1.50
18:1n-7	2.93 _D	3.85 _{A,B}	3.12 _{C,D}	3.43 _{B,C}	4.04 _A	2.78 _D	2.72 _D	2.66 _D	<0.001	0.20
20:1n-11	0.13 _B	1.59 _A	0.16 _B	0.12 _B	0.18 _B	0.16 _B	0.09 _B	0.15 _B	<0.001	0.09
20:1n-9	1.48 _{A,B}	1.78 _A	1.28 _{B,C}	1.23 _{B,C}	1.25 _{B,C}	1.41 _{A,B,C}	1.16 _{B,C}	1.06 _C	0.0002	0.17
Total PUFA	42.79 _A	35.32 _B	39.41 _{A,B}	36.37 _B	35.58 _B	39.79 _{A,B}	38.98 _{A,B}	37.18 _{A,B}	0.003	2.44
18:2n-6	27.52 _A	18.91 _{C,D,E}	24.07 _{A,B}	20.17 _{B,C}	15.46 _{D,E}	23.16 _{A,B,C}	19.88 _{B,C,D}	15.19 _E	<0.001	1.99
18:3n-6	1.38 _A	0.88 _{B,C}	1.21 _{A,B}	0.75 _{C,D}	0.73 _{C,D}	0.90 _{B,C}	0.57 _{C,D}	0.42 _D	<0.001	0.15
18:3n-3	1.15 _{B,C,D}	1.92 _A	1.19 _{B,C}	1.19 _{B,C}	1.31 _B	0.98 _{D,E}	1.01 _{C,D,E}	0.95 _E	<0.001	0.08
20:2n-6	1.79 _A	1.00 _{C,D,E}	1.32 _{B,C}	0.93 _{C,D,E}	0.70 _E	1.64 _{A,B}	1.11 _{C,D}	0.76 _{D,E}	<0.001	0.17
20:3n-3	1.29 _A	1.17 _{A,B}	1.09 _{A,B,C}	0.90 _{B,C}	0.82 _{C,D}	1.18 _{A,B}	0.81 _{C,D}	0.60 _D	<0.001	0.12
20:5n-3	3.07 _A	2.54 _{A,B}	2.82 _{A,B}	2.01 _{B,C,D}	1.92 _{B,C,D}	2.44 _{A,B,C}	1.60 _{C,D}	1.39 _D	<0.001	0.39
22:4n-3	1.36 _A	0.84 _{A,B,C}	0.89 _{A,B,C}	0.69 _{B,C}	0.59 _{B,C}	1.02 _{A,B}	0.56 _{B,C}	0.38 _C	0.0002	0.24
22:5n-6	2.08 _B	0.89 _C	1.19 _C	0.65 _C	0.64 _C	2.05 _B	2.65 _B	3.72 _A	<0.001	0.35
22:5n-3	0.65 _C	2.17 _B	1.20 _C	2.74 _B	4.47 _A	0.69 _C	0.76 _C	0.89 _C	<0.001	0.32
22:6n-3	2.49 _E	5.00 _{D,E}	4.45 _{D,E}	6.35 _{C,D}	8.96 _{B,C}	5.74 _D	10.04 _B	12.90 _A	<0.001	1.20
% Omega 3	10.02 _D	13.64 _{B,C,D}	11.63 _{C,D}	13.87 _{A,B,C,D}	18.07 _A	12.05 _{C,D}	14.77 _{A,B,C}	17.09 _{A,B}	<0.001	1.80
% Omega 6	32.78 _A	21.67 _{C,D}	27.78 _B	22.49 _C	17.52 _D	27.74 _B	24.21 _{B,C}	20.09 _{C,D}	<0.001	1.91
Omega 6:3	3.28 _A	1.60 _{B,C,D}	2.65 _{A,B}	1.63 _{B,C,D}	0.97 _D	2.31 _{A,B,C}	1.64 _{B,C,D}	1.21 _{C,D}	<0.001	0.48
% Beneficial Omega-3	6.21 _D	9.72 _{B,C,D}	8.48 _{C,D}	11.09 _{B,C}	15.35 _A	8.86 _{B,C,D}	12.39 _{A,B}	15.18 _A	<0.001	1.59

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Table 5: Rib meat fatty acid profile (mean % sum fatty acid) of fish fed experimental diets for eight weeks. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Fatty Acid	Treatment Diets								P	Root Mean Square Error
	Control	Commercial	Fish Oil			ALL-G-Rich				
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.75% ALL-G-Rich	5.26% ALL-G-Rich	8.77% ALL-G-Rich		
Total SFA	32.86	47.66	43.59	35.51	48.57	39.62	35.03	37.07	0.427	11.68
12:0	0.04 _{A,B}	0.03 _B	0.04 _{A,B}	0.06 _{A,B}	0.06 _A	0.04 _{A,B}	0.06 _{A,B}	0.06 _A	0.007	0.01
14:0	2.60	5.26	3.41	4.18	5.65	2.94	3.22	3.51	0.030	1.32
15:0 ANTEISO	0.31	0.50	0.34	0.40	0.61	0.45	0.53	0.55	0.325	0.19
15:0 ISO	0.03 _C	0.00 _C	0.06 _{A,B,C}	0.11 _{A,B}	0.13 _A	0.04 _{B,C}	0.04 _{B,C}	0.03 _C	0.0001	0.03
16:0	21.55	30.32	27.17	22.03	28.69	24.65	23.83	24.58	0.549	6.71
17:0	0.27	0.53	0.45	0.41	0.66	0.45	0.31	0.33	0.248	0.22
18:0	7.75	10.79	11.56	7.96	12.29	10.48	6.79	7.74	0.433	4.10
20:0	0.30	0.24	0.56	0.36	0.49	0.56	0.26	0.26	0.252	0.23
Total MUFA	28.24	23.95	20.62	28.56	23.74	25.97	31.81	34.70	0.812	12.76
14:1n-5	0.02	0.02	0.00	0.01	0.00	0.02	0.05	0.06	0.042	0.03
16:1n-11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
16:1n-9	0.59	0.47	0.65	0.79	0.63	0.66	0.56	0.62	0.515	0.19
16:1n-7	3.61 _C	4.77 _{A,B,C}	3.78 _{B,C}	5.82 _{A,B}	6.91 _A	3.40 _C	3.67 _{B,C}	4.46 _{B,C}	0.0001	0.94
18:1n-9	19.96	14.05	12.09	17.97	11.58	17.97	23.88	24.26	0.674	11.76
18:1n-7	2.67	2.55	2.78	2.27	3.48	2.52	2.44	3.04	0.745	0.99
20:1n-11	0.12	1.05	0.51	0.21	0.09	0.44	0.10	0.78	0.379	0.67
20:1n-9	1.28	1.05	0.82	1.49	1.05	0.97	1.12	1.47	0.691	0.58
Total PUFA	38.90	28.39	35.79	35.94	27.69	34.42	33.16	28.24	0.178	6.65
18:2n-6	23.72 _A	18.32 _{A,B}	25.15 _A	23.08 _A	15.49 _{A,B}	22.64 _A	20.27 _{A,B}	11.47 _B	0.004	4.59
18:3n-6	1.12	0.61	1.25	1.08	0.66	1.01	0.54	0.65	0.189	0.44
18:3n-3	1.08	1.50	1.16	1.51	0.93	1.10	0.98	1.52	0.640	0.57
20:2n-6	1.27	0.55	1.29	1.11	0.54	1.40	0.91	0.76	0.022	0.39
20:3n-3	0.96	0.65	0.95	0.92	0.61	0.99	0.63	0.65	0.293	0.30
20:5n-3	1.72	1.59	1.87	1.35	1.20	1.56	0.82	0.91	0.086	0.52
22:4n-3	0.81	0.41	0.85	0.67	0.32	0.81	0.36	0.29	0.053	0.31
22:5n-6	2.05	0.29	0.48	0.39	0.18	1.31	1.76	2.10	0.011	0.89
22:5n-3	0.52 _C	1.08 _{B,C}	0.61 _C	2.16 _B	3.78 _A	0.43 _C	0.56 _C	1.06 _{B,C}	<0.001	0.51
22:6n-3	5.67	3.40	2.18	3.68	3.99	3.18	6.34	8.85	0.273	3.73
% Omega 3	10.75	8.63	7.63	10.29	10.82	8.06	9.68	13.27	0.488	3.74
% Omega 6	28.15 _A	19.77 _{A,B}	28.17 _A	25.64 _{A,B}	16.87 _{A,B}	26.36 _{A,B}	23.48 _{A,B}	14.97 _B	0.004	5.04
Omega 6:3	3.92 _A	2.36 _{A,B,C}	3.69 _A	2.47 _{A,B,C}	1.62 _{B,C}	3.39 _{A,B}	2.44 _{A,B,C}	1.09 _C	0.001	0.85
% Beneficial Omega-3	7.91	6.07	4.67	7.19	8.96	5.16	7.71	10.81	0.392	3.85

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Table 6: Liver fatty acid profile (mean % sum fatty acid) of fish fed experimental diets for eight weeks. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Fatty Acid			Treatment Diets							P	Root Mean Square Error
			Fish Oil			ALL-G-Rich					
	Control	Commercial	1% Fish Oil	3% Fish Oil	5% Fish Oil	1.75% ALL-G-Rich	5.26% ALL-G-Rich	8.77% ALL-G-Rich			
Total SFA	36.44	37.21	40.99	48.90	43.13	39.24	45.43	40.55	0.749	10.82	
12:0	0.02	0.04	0.00	0.01	0.01	0.02	0.02	0.03	0.287	0.02	
14:0	2.78 _B	4.22 _A	2.62 _B	3.62 _{A,B}	3.34 _{A,B}	3.31 _{A,B}	3.26 _{A,B}	3.44 _{A,B}	0.024	0.58	
15:0 ANTEISO	0.18 _C	0.13 _C	0.12 _C	0.31 _{A,B,C}	0.31 _{A,B,C}	0.24 _{B,C}	0.38 _{A,B}	0.47 _A	<0.001	0.86	
15:0 ISO	0.08	0.03	0.05	0.06	0.07	0.05	0.01	0.03	0.604	0.05	
16:0	20.38	21.69	21.60	26.58	22.04	22.76	24.42	23.29	0.752	5.01	
17:0	0.29	0.19	0.32	0.47	0.53	0.29	0.49	0.47	0.274	0.21	
18:0	12.53	10.84	16.07	17.48	16.70	12.47	16.58	12.64	0.614	5.78	
20:0	0.18	0.09	0.22	0.38	0.14	0.10	0.27	0.19	0.558	0.21	
Total MUFA	30.69	39.78	24.65	23.13	27.32	32.50	26.53	33.17	0.469	10.99	
14:1n-5	0.00 _B	0.00 _B	0.00 _B	0.00 _B	0.00 _B	0.00 _B	0.01 _B	0.05 _A	0.001	0.02	
16:1n-11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
16:1n-9	0.92	0.99	0.92	0.83	0.89	1.08	1.08	1.13	0.641	0.25	
16:1n-7	3.28	5.11	2.77	4.52	4.78	3.35	3.47	4.25	0.095	1.18	
18:1n-9	22.68	26.53	17.47	13.45	17.22	24.09	18.30	17.64	0.678	10.40	
18:1n-7	2.60	3.86	2.47	3.23	3.49	2.58	2.59	8.68	0.441	4.11	
20:1n-11	0.08 _B	1.72 _A	0.00 _B	0.38 _B	0.00 _B	0.00 _B	0.00 _B	0.00 _B	<0.001	0.35	
20:1n-9	1.13	1.58	1.03	0.72	0.95	1.41	1.08	1.44	0.141	0.43	
Total PUFA	32.88	23.00	34.36	27.98	29.56	28.27	28.03	26.28	0.272	6.16	
18:2n-6	15.34 _A	10.90 _{A,B}	14.35 _{A,B}	12.69 _{A,B}	8.98 _B	13.92 _{A,B}	11.05 _{A,B}	9.42 _{A,B}	0.017	2.66	
18:3n-6	0.81 _A	0.59 _{A,B}	0.61 _{A,B}	0.51 _{A,B}	0.29 _B	0.49 _{A,B}	0.43 _{A,B}	0.27 _B	0.009	0.19	
18:3n-3	0.47	0.68	0.26	0.47	0.45	0.38	0.33	0.39	0.426	0.24	
20:2n-6	1.33 _A	0.71 _{C,D}	1.27 _{A,B}	0.70 _D	0.51 _D	1.21 _{A,B,C}	0.79 _{B,C,D}	0.71 _{C,D}	<0.001	0.22	
20:3n-3	0.90	0.83	0.96	0.73	0.61	0.61	0.67	0.54	0.066	0.20	
20:5n-3	4.60	2.60	4.90	2.79	3.23	3.05	2.50	2.37	0.017	1.09	
22:4n-3	0.92 _A	0.06 _B	0.53 _{A,B}	0.21 _B	0.00 _B	0.28 _B	0.09 _B	0.10 _B	0.001	0.26	
22:5n-6	3.25 _A	0.30 _C	2.33 _{A,B}	0.58 _{B,C}	0.32 _C	1.57 _{A,B,C}	2.16 _{A,B}	3.20 _A	<0.001	0.77	
22:5n-3	0.26 _B	0.79 _B	0.22 _B	0.89 _B	2.09 _A	0.09 _B	0.05 _B	0.40 _B	<0.001	0.44	
22:6n-3	5.01	5.55	8.95	8.42	13.09	6.69	9.98	8.88	0.062	3.45	
% Omega 3	12.15	10.51	15.81	13.51	19.47	11.08	13.61	12.68	0.173	4.51	
% Omega 6	20.73 _A	12.49 _{B,C}	18.55 _{A,B}	14.48 _{A,B,C}	10.09 _C	17.19 _{A,B,C}	14.43 _{A,B,C}	13.61 _{B,C}	0.001	3.04	
Omega 6:3	1.94	1.36	1.18	1.06	0.56	1.59	1.12	1.55	0.120	0.61	
% Beneficial Omega-3	9.86	8.94	14.06	12.10	18.41	9.82	12.53	11.65	0.097	4.28	

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Table 7: Mesenteric fat fatty acid profile (mean % sum fatty acid) of fish fed experimental diets for eight weeks. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Fatty Acid	Treatment Diets								P	Root Mean Square Error
	Control	Commercial	Fish Oil			ALL-G-Rich				
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.75% ALL-G-Rich	5.26% ALL-G-Rich	8.77% ALL-G-Rich		
Total SFA	29.03	35.94	40.19	35.33	32.95	32.89	34.02	33.65	0.648	7.42
12:0	0.04 _B	0.05 _B	0.07 _{A,B}	0.04 _B	0.07 _{A,B}	0.05 _{A,B}	0.07 _{A,B}	0.09 _A	0.006	0.02
14:0	2.57 _B	3.65 _{A,B}	4.22 _{A,B}	4.10 _{A,B}	4.95 _A	3.36 _{A,B}	3.44 _{A,B}	3.96 _{A,B}	0.008	0.73
15:0 ANTEISO	0.20 _B	0.26 _B	0.42 _B	0.38 _B	0.47 _B	0.37 _B	0.51 _B	0.91 _A	0.0001	0.16
15:0 ISO	0.05 _B	0.05 _B	0.11 _{A,B}	0.07 _B	0.15 _A	0.07 _B	0.04 _B	0.06 _B	0.002	0.04
16:0	19.33	23.73	25.02	22.18	19.64	21.75	22.40	22.09	0.722	4.75
17:0	0.22	0.18	0.46	0.36	0.40	0.27	0.31	0.49	0.053	0.14
18:0	6.35	7.85	9.36	7.94	7.01	6.73	6.98	5.69	0.402	2.17
20:0	0.28	0.18	0.54	0.26	0.25	0.30	0.28	0.36	0.184	0.17
Total MUFA	36.25	33.40	34.65	35.44	36.82	34.46	33.45	31.47	0.947	6.30
14:1n-5	0.00 _B	0.00 _B	0.04 _B	0.00 _B	0.00 _B	0.01 _B	0.06 _{A,B}	0.10 _A	<0.0001	0.03
16:1n-11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
16:1n-9	0.62	0.71	0.99	0.61	0.73	0.63	0.64	0.76	0.071	0.17
16:1n-7	3.76 _C	5.89 _{A,B,C}	6.30 _{A,B}	5.56 _{A,B,C}	7.73 _A	4.21 _{B,C}	4.21 _{B,C}	4.70 _{B,C}	<0.001	0.96
18:1n-9	27.74	19.92	21.08	24.90	23.13	25.53	24.54	21.60	0.787	7.03
18:1n-7	2.58 _B	3.74 _{A,B}	4.06 _A	3.10 _{A,B}	3.81 _{A,B}	2.66 _{A,B}	2.60 _{A,B}	2.82 _{A,B}	0.007	0.62
20:1n-11	0.13	1.29	0.89	0.05	0.17	0.12	0.15	0.13	0.069	0.62
20:1n-9	1.43	1.86	1.30	1.22	1.27	1.32	1.25	1.35	0.363	0.38
Total PUFA	34.72	30.66	25.17	29.24	30.23	32.65	32.54	34.89	0.085	4.42
18:2n-6	26.71 _A	22.29 _{A,B}	12.48 _B	20.17 _{A,B}	16.81 _{A,B}	24.05 _{A,B}	20.27 _{A,B}	16.02 _{A,B}	0.035	5.67
18:3n-6	1.38 _{A,B}	0.88 _{B,C}	1.71 _A	0.79 _{B,C}	0.66 _C	0.88 _{B,C}	0.65 _C	0.51 _C	<0.001	0.29
18:3n-3	1.21 _B	2.27 _A	1.21 _B	1.21 _B	1.53 _{A,B}	1.15 _B	1.14 _B	1.34 _B	0.003	0.36
20:2n-6	1.40 _{A,B}	0.84 _{B,C}	1.75 _A	0.72 _C	0.62 _C	1.18 _{A,B,C}	0.93 _{B,C}	0.82 _C	<0.001	0.28
20:3n-3	0.96 _{A,B}	0.81 _B	1.35 _A	0.65 _B	0.60 _B	0.77 _B	0.72 _B	0.59 _B	0.001	0.22
20:5n-3	0.98 _{A,B}	0.78 _B	1.27 _A	0.73 _B	0.78 _B	0.70 _B	0.60 _B	0.59 _B	0.002	0.20
22:4n-3	0.69 _{A,B}	0.35 _B	0.99 _A	0.36 _B	0.40 _B	0.55 _{A,B}	0.43 _B	0.33 _B	0.005	0.23
22:5n-6	0.45 _C	0.05 _C	0.57 _C	0.09 _C	0.05 _C	0.65 _C	1.37 _B	2.72 _A	<0.001	0.26
22:5n-3	0.31 _D	1.11 _{B,C,D}	1.56 _{B,C}	1.91 _B	3.94 _A	0.55 _D	0.78 _{C,D}	1.09 _{B,C,D}	<0.001	0.40
22:6n-3	0.63 _D	1.29 _{C,D}	2.28 _C	2.62 _C	4.85 _B	2.17 _{C,D}	5.69 _B	10.87 _A	<0.001	0.66
% Omega 3	4.78 _D	6.60 _{C,D}	8.66 _{B,C}	7.48 _{C,D}	12.10 _{A,B}	5.90 _{C,D}	9.33 _{B,C}	14.82 _A	<0.001	1.51
% Omega 6	29.94 _A	24.06 _{A,B}	16.51 _B	21.75 _{A,B}	18.13 _{A,B}	26.75 _{A,B}	23.21 _{A,B}	20.07 _{A,B}	0.024	5.20
Omega 6:3	6.37 _A	4.04 _{A,B,C}	2.42 _{B,C}	2.95 _{B,C}	1.50 _C	4.62 _{A,B}	2.49 _{B,C}	1.37 _C	<0.001	1.23
% Beneficial Omega-3	1.92 _E	3.18 _{D,E}	5.11 _{C,D}	5.27 _{C,D}	9.56 _B	3.42 _{D,E}	7.06 _{B,C}	12.55 _A	<0.001	1.14

Conclusions & Summary

The aim of this research was to determine if algae could provide a similar or increased beneficial omega-3 concentration in tilapia compared to fish oil. After 8 weeks of being fed experimental diets, there was no significant differences in total lipid fillet content (2.21-3.07g/4oz serving). Fish fed 8.77% algae, were found to have significantly ($P<0.001$) greater proportions of DHA, in fillets, compared to all other diets. This change in lipid composition was also observed on a mg fatty acid/4oz serving basis. The 8.77% algae diet resulted in greater beneficial omega-3 (EPA, DPA, DHA) fatty acids on a mg/4oz serving basis, compared to all other diets except 5% fish oil, albeit not significant. Compared to what is currently available in the marketplace, the tilapia in this study showed increased omega-3 fillet concentrations and decreased omega-6:3 ratios. A comparison between other tissues examined in this study (liver, mesenteric fat, rib meat) and analogous tissues in commercial tilapia is not possible because that data is not available.

On a mg/4oz fillet basis, the accretion of beneficial omega-3 fatty acids (EPA, DPA, and DHA) approximately doubled from weeks 4 to 8 for most diets. While a linear correlation can not be made with only the two time points available, the data suggests that the deposition of omega-3 fatty acids is correlated to time. However this correlation would need more time points to be verified. Based on the data collected, the ALL-G-Rich additive has potential to be the basis of an omega-3 enriching finishing diet. There is a strong correlation between %ALL-G-Rich in the diet and mg omega-3 deposited into the fillet. By using this correlation and the suggested relationship between time on diet and increase of fillet deposition, a diet containing double 8.77% ALL-G-Rich (17.54%) could potentially result in the same omega-3 content in half the

Conclusions & Summary

time tested in this experiment. Although this would need to be studied further in a future experiment.

Future Work

While the work in this thesis was comprehensive there are a number of potential future research projects that would add to the overall understanding of this value added product potential. Firstly, how these various diets affect sensory attributes including: texture analysis, taste and appearance should be examined. Many consumers who prefer tilapia, do so because of its mild flavor and soft texture. Anecdotal sensory evaluation of the fish cultured in this study indicate that there is a noticeable difference in taste between fish fed different diets.

Approximately 3 kg of fillet meat was collected from fish fed the 5% fish oil, 8.77% ALL-G-Rich, 8.65% kelp, and control diets at the end of the 8 week trial. Plans are already underway for a sensory trial.

In order to determine what other factors affect fatty acid deposition in tilapia. Fish would need to be raised from fry (0.5-10g) through market size on various diets of the same composition. Different diets that could be utilized by the fish as it grows would be necessary, and tissue samples from different life stages could give insight as to how omega-3 fatty acids are partitioned and applied in tilapia. Gender could also play a role in how tilapia deposit omega-3 fatty acids. Commercially males are preferred for production because they grow larger, faster, on the same diet compared to females. This size dimorphism is caused by females utilizing energy to prepare for reproduction. By conducting a similar study to this, but with a mixed sex population of fish and examining the gonadal tissues for omega-3 deposition, a better understanding of how tilapia utilize omega-3 fatty acids for reproduction. Since genetics are also an important factor on fatty acid metabolism, a better understanding of how the species strain we used differs from other farmed tilapia would be interesting. To do this, fry or juvenile tilapia

Future Work

could be collected from various tilapia farms both nationally and internationally. These fish would then be raised in the same recirculating environment, and a similar tissue extraction and analysis would be performed. This would provide insight into how genetics amongst tilapia fed the same experimental diets affect partitioning of fatty acids into various tissues.

Another potential future study would be examining blood and gut tissue samples. During sampling, blood and gut sections were excised and preserved for future examination. For blood, serum fatty acid and cholesterol concentrations could be examined in relation to diet fatty acid composition. The gut samples would be examined under microscopy and could provide a mechanism for any differences in growth observed during the feeding trial. Previous work done by this laboratory has examined how microvilli length is affected by dietary supplementation of probiotics. It is possible that some bioactive compounds in the kelp, specifically polysaccharides, influence bioavailability of nutrients in the feed to the fish. This theory has already been investigated by Ocean Harvest, the company who provided the kelp additive, and it would be interesting to examine that mechanism in tilapia. Lastly a market study to determine consumer acceptability of a value added tilapia product would be useful. This would help producers determine if their market demographic would actively purchase the value added product and what they would be willing to pay.

Appendix C

Appendix C.1: Composition of experimental feeds on a g/100 g (%) on a dry-matter basis.

Appendix C.2: Experimental and commercial diet nutritional proximates and minerals on a dry-matter basis.

Appendix C.3: Composition of fatty acids (%) in experimental and commercial feeds as-is. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA.

Appendix C.4: Fish growth and biometrics.

Appendix C.5: Nutritional fatty acid profiles of tilapia fillet and rib meat after 4 weeks of dietary treatments. All values are presented on a per 4oz serving basis. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Appendix C.6: Nutritional fatty acid profiles of tilapia fillet and rib meat after 8 weeks of dietary treatments. All values are presented on a per 4oz serving basis. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Appendix C.7: Nutritional fatty acid profiles of tilapia liver and mesenteric fat after 8 weeks of dietary treatments. All values are presented on a per 4oz serving basis. Means followed by different letters in a row are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Appendix C.8: Fillet fatty acid profile (% sum fatty acid) of fish fed experimental diets for eight weeks. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Appendix C.9: Rib meat fatty acid profile (% sum fatty acid) of fish fed experimental diets for eight weeks. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Appendix C.10: Liver fatty acid profile (% sum fatty acid) of fish fed experimental diets for eight weeks. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA:

Appendix

Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Appendix C.11: Mesenteric fat fatty acid profile (% sum fatty acid) of fish fed experimental diets for eight weeks. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Appendix C.12: Example of arachidonic acid on GC chromatograph and mass spectra (Top) and of tissue sample and mass spectra (Bottom) at the same retention time.

Appendix

Appendix C.1: Composition of experimental feeds on a g/100 g (%) on a dry matter basis.

Ingredients	Treatment diets [g/100g as fed]							
	Control	Fish Oil			Ocean Harvest (Kelp)			
		1%	3%	5%	1.70%	5.19%	8.65%	5.19% Kelp 1% Fish Oil
Soybean (46.5%) ¹	37.0	37.0	37.0	37.0	37.0	37.0	37.0	37.0
Wheat ²	37.7	37.7	37.7	37.7	36.1	32.61	29.45	32.61
Meat and Bone Meal ³	13.8	13.8	13.8	13.8	13.7	13.7	13.4	13.8
Menhaden fish meal ⁴	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Corn Oil ⁵	6.3	5.3	3.3	1.3	3.3	3.3	3.3	5.2
Vitamin Premix ⁶	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Mineral Premix ⁶	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Fish oil ⁴	0	1	3	5	3	3	3	1
Kelp ⁷	0.0	0.0	0.0	0.0	1.70	5.19	8.65	5.19

¹ ADM Alliance Nutrition

² Southern States Cooperative

³ Smithfield - Farmland, Smithfield, VA, US

⁴ Omega Protein, Houston, Texas, US

⁵ Kroger, Cincinnati, Ohio, US

⁶ Purina, St. Louis, Missouri, US

⁷ Alltech, Nicholasville, Kentucky, US

Appendix

			Treatment Diets						
Parameter	Control	Commercial	Fish Oil			Ocean Harvest (Kelp)			
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.70%	5.19%	8.65%	5.19% Kelp 1% Fish Oil
Caloric Content (cal/g)									
Total Calories	4940	4860	4830	4810	4930	4710	4640	4770	4870
Proximate and mineral levels									
Crude protein	37.0	37.1	36.5	35.5	35.7	36.1	37.1	35.3	36.3
Carbohydrate ¹	33.7	41.2	36.6	37.7	37.3	38.4	34.6	33.3	36.47
Total ash	9.18	7.49	9.19	9.12	8.96	9.26	10.70	12.90	11.00
Crude fat	10.2	4.31	9.17	9.55	9.56	8.96	9.82	9.65	8.40
Crude fiber	4.00	8.70	4.50	4.00	3.80	4.00	4.30	5.30	5.3
Calcium	2.08	1.19	2.14	2.11	2.10	2.02	2.04	1.83	2.36
Phosphorus	1.41	1.31	1.45	1.42	1.40	1.34	1.30	1.08	1.5
Potassium	1.31	1.62	1.25	1.28	1.29	1.41	1.62	1.50	1.79
Magnesium	0.24	0.34	0.23	0.24	0.24	0.26	0.30	0.28	0.34
Sodium	0.16	0.09	0.15	0.15	0.16	0.22	0.32	0.36	0.37
Trace element levels (ppm)									
Iron	226	270	215	224	223	319	516	473	537
Copper	24	15	21	22	21	23	22	18	26
Zinc	305	190	285	317	272	318	304	251	288
Manganese	101	388	71	88	122	111	178	192	216

¹ Calculated value (Merrill and Watt, 1973):

carbohydrate = total - (ash + crude protein + moisture + total fat)

Appendix C.2: Experimental and commercial diet nutritional proximates and minerals on a dry-matter basis.

Appendix

Appendix C.3: Composition of fatty acids (%) in experimental and commercial feeds as-is. SFA:Saturated fatty acids, MUFA:Monounsaturated fatty acids, PUFA:Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA.

Fatty Acid	Treatment Diets								
	Control	Commercial	Fish Oil			Ocean Harvest			
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.70% Kelp	5.19% Kelp	8.65% Kelp	5.19% Kelp 1% Fish Oil
Total SFA	23.22	26.31	26.00	29.34	35.22	30.83	30.96	30.63	25.00
12:0	0.00	0.00	0.04	0.08	0.10	0.06	0.08	0.06	0.00
14:0	0.78	3.02	2.26	4.28	6.54	4.17	4.65	4.18	1.87
15:0 ANTEISO	0.00	0.19	0.20	0.39	0.56	0.35	0.42	0.33	0.14
15:0 ISO	0.00	0.00	0.06	0.14	0.21	0.12	0.15	0.11	0.00
16:0	17.07	18.33	16.30	17.16	20.76	19.71	17.88	19.59	17.01
17:0	0.10	0.17	0.25	0.44	0.49	0.30	0.46	0.29	0.18
18:0	4.88	4.43	6.36	6.43	6.28	5.78	6.86	5.75	5.39
20:0	0.39	0.17	0.53	0.42	0.28	0.34	0.46	0.32	0.41
Total MUFA	31.66	42.46	32.37	32.12	32.13	31.92	28.48	32.33	32.68
14:1 n-5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16:1 n-11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16:1 n-9	0.00	0.21	0.19	0.13	0.19	0.27	0.13	0.27	0.16
16:1 n-7	1.05	5.24	3.12	5.90	8.88	5.38	0.25	5.50	2.61
18:1 n-9	28.77	24.56	26.04	22.40	19.04	23.31	23.96	23.52	27.48
18:1 n-7	1.44	4.00	2.35	2.91	3.23	2.38	3.25	2.42	1.90
20:1 n-11	0.00	5.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20:1 n-9	0.40	2.77	0.67	0.78	0.79	0.58	0.89	0.62	0.53
Total PUFA	45.12	31.23	41.62	38.55	32.65	37.24	40.55	37.06	42.31
18:2 n-6	43.53	25.26	36.47	29.41	21.10	30.75	30.38	30.43	38.81
18:3 n-6	0.00	0.00	0.05	0.13	0.15	0.00	0.16	0.00	0.00
18:3 n-3	1.59	3.02	2.09	2.12	1.93	1.70	2.25	1.68	1.67
20:2 n-6	0.00	0.00	0.18	0.22	0.20	0.00	0.22	0.00	0.00
20:3 n-3	0.00	0.00	0.00	0.11	0.00	0.00	0.12	0.00	0.00
20:5 n-3	0.00	0.23	0.40	0.70	0.92	0.52	0.80	0.57	0.29
22:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:5 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00
22:5 n-3	0.00	0.00	0.42	1.08	1.43	0.52	1.26	0.76	0.00
22:6 n-3	0.00	2.72	2.01	4.78	6.92	3.75	5.17	3.62	1.54
% Omega 3	1.59	5.97	4.92	8.79	11.20	6.49	9.60	6.63	3.50
% Omega 6	43.53	25.26	36.70	29.76	21.45	30.75	30.95	30.43	38.81
Omega 6:3	27.38	4.23	7.46	3.39	1.92	4.74	3.22	4.59	11.09
% Beneficial Omega-3	0.00	2.95	2.83	6.56	9.27	4.79	7.23	4.95	1.83

Appendix C.4: Fish growth and biometrics.

	Treatment Diets											
			Fish Oil			Ocean Harvest					P	Root Mean Square Error
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.70% Kelp	5.19% Kelp	8.65% Kelp	5.19% Kelp 1% Fish Oil			
Tilapia Performance												
Survival (%)	100	96	98	100	100	100	96	100	98	0.044	1.3	
Initial weight (g)	161	157	156	156	158	156	159	160	161	0.285	2.4	
4 week weight (g)	331	345	333	329	311	341	334	337	331	0.779	19.5	
8 week Weight (g)	521 _{A,B}	464 _B	504 _{A,B}	513 _{A,B}	540 _{A,B}	573 _A	567 _A	528 _{A,B}	538 _{A,B}	0.038	24.8	
Average Weight Gair (g/Week)	45 _{A,B}	38 _B	43 _{A,B}	46 _{A,B}	41 _{A,B}	52 _A	51 _{A,B}	46 _{A,B}	47 _{A,B}	0.048	3.2	
SGR (%/Day)	2.09	1.98	2.09	2.13	2.19	2.28	2.23	2.18	2.15	0.966	0.65	
FCR	1.46	1.50	1.31	1.31	1.24	1.25	1.25	1.32	1.27	0.259	0.34	
Biometrics at 8 weeks												
Fillet Yield	45.3	45.8	43.9	43.8	44.3	44.4	43.2	44.4	44.1	0.282	2.80	
Hepatosomatic Index	1.6	1.83	1.52	1.61	1.59	1.59	1.57	1.62	1.53	0.593	0.28	
Viscerasomatic Index	3.04	2.45	2.83	2.97	2.61	2.94	2.92	3.76	2.64	0.131	0.83	
Mesenteric Fat Index	0.87	0.98	0.98	1.03	1.05	0.91	0.90	0.91	0.80	0.834	0.32	

Appendix

Appendix C.5: Nutritional fatty acid profiles of tilapia fillet and rib meat after 4 weeks of dietary kelp treatments. All values are presented on a per 4oz serving basis. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

	Treatment Diets									<i>P</i>	Root Mean Square Error
			Fish Oil			Ocean Harvest					
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.70% Kelp	5.19% Kelp	8.65% Kelp	5.19% Kelp 1% Fish Oil		
Control	Commercial										
Week 4											
Fillet											
g Fat	2.10	1.23	1.84	1.55	1.70	1.73	1.82	1.51	1.47	0.715	0.59
mg ALA	14	12	13	6	13	14	14	11	11	0.426	5
mg EPA	32	13	24	12	17	22	22	19	17	0.105	9
mg DPA	8 _C	11 _{B,C}	14 _{B,C}	9 _C	37 _A	26 _{A,B,C}	29 _{A,B}	22 _{A,B,C}	11 _{B,C}	<0.001	8
mg DHA	29 _{A,B}	27 _B	34 _{A,B}	33 _{A,B}	72 _A	56 _{A,B}	64 _{A,B}	56 _{A,B}	30 _{A,B}	0.009	19
mg Omega-3	115	76	94	69	154	136	149	124	85	0.057	43
mg Omega-6	349 _A	139 _B	225 _{A,B}	103 _B	174 _{A,B}	236 _{A,B}	246 _{A,B}	195 _{A,B}	221 _{A,B}	0.012	79
mg Omega-6:3	3.07 _A	1.83 _{B,C}	2.40 _{A,B}	1.97 _{A,B,C}	1.11 _C	1.76 _{B,C}	1.69 _{B,C}	1.58 _{B,C}	1.93 _{A,B,C}	0.002	0.52
mg Beneficial Omega-3	70	51	66	54	126	104	115	97	59	0.013	32
Rib Meat											
g Fat	4.32	1.27	3.73	4.99	4.34	4.61	4.9	4.02	4.71	0.085	1.59
mg ALA	49	11	85	68	57	97	47	55	37	0.215	42
mg EPA	60	16	78	56	47	70	42	43	29	0.263	33
mg DPA	1 _B	15 _{A,B}	60 _{A,B}	68 _{A,B}	140 _A	143 _A	51 _{A,B}	78 _{A,B}	16 _{A,B}	0.012	57
mg DHA	18 _B	41 _{A,B}	135 _{A,B}	126 _{A,B}	224 _{A,B}	227 _A	131 _{A,B}	139 _{A,B}	23 _{A,B}	0.010	87
mg Omega-3	190	89	437	384	507	624	297	353	139	0.071	242
mg Omega-6	1182	185	1175	1222	702	1387	835	925	740	0.153	559
mg Omega-6:3	5.87 _A	2.62 _B	3.03 _{A,B}	3.12 _{A,B}	1.45 _B	2.34 _B	2.85 _B	2.69 _B	3.75 _{A,B}	0.005	1.24
mg Beneficial Omega-3	80	71	272	250	411	439	224	260	69	0.026	168

Appendix

Appendix C.6: Nutritional fatty acid profiles of tilapia fillet and rib meat after 8 weeks of dietary kelp treatments. All values are presented on a per 4oz serving basis. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

			Treatment Diets								<i>P</i>	Root Mean Square Error
			Fish Oil			Ocean Harvest						
	Control	Commercial	1% Fish Oil	3% Fish Oil	5% Fish Oil	1.70% Kelp	5.19% Kelp	8.65% Kelp	5.19% Kelp 1% Fish Oil			
Week 8:												
Fillet												
g Fat	2.21	2.43	2.71	2.54	3.07	3.20	2.40	3.17	2.42	0.410	0.63	
mg ALA	21	23	20	15	19	20	14	19	9	0.343	8	
mg EPA	57	30	45	25	28	31	24	28	24	0.339	20	
mg DPA	11 _D	26 _{B,C,D}	16 _{C,D}	35 _{B,C}	65 _A	46 _{A,B}	36 _{B,C}	47 _{A,B}	13 _{C,D}	<0.001	10	
mg DHA	44 _{B,C}	60 _{B,C,D}	70 _{B,C}	80 _{A,B,C}	130 _A	100 _{A,B}	81 _{A,B,C}	97 _{A,B}	40 _C	0.0003	24	
mg Omega-3	180	163	179	176	262	224	174	218	104	0.173	71	
mg Omega-6	613	260	478	285	256	362	262	363	219	0.331	234	
mg Omega-6:3	3.28 _A	1.60 _{B,C}	2.65 _{A,B}	1.63 _{B,C}	0.97 _C	1.63 _{B,C}	1.51 _{B,C}	1.66 _{B,C}	1.58 _{B,C}	0.0002	0.57	
mg Beneficial Omega-3	112 _{A,B}	116 _{AB}	131 _{A,B}	140 _{A,B}	222 _A	177 _{A,B}	141 _{A,B}	172 _{A,B}	77 _B	0.016	49	
Rib Meat												
g Fat	5.22	4.44	4.70	4.25	5.74	6.27	3.85	5.80	6.67	0.742	2.14	
mg ALA	23	28	11	26	23	27	19	23	11	0.917	20	
mg EPA	36	21	15	23	17	25	18	22	20	0.884	18	
mg DPA	6	21	8	38	68	54	40	48	10	0.185	36	
mg DHA	33	41	21	64	69	92	68	78	30	0.685	58	
mg Omega-3	142	130	72	178	194	226	164	195	89	0.833	141	
mg Omega-6	656	325	274	443	300	503	361	454	295	0.852	357	
mg Omega-6:3	3.92 _A	2.36 _{A,B}	3.69 _{A,B}	2.47 _{A,B}	1.62 _B	2.21 _{A,B}	1.83 _{A,B}	2.33 _{A,B}	2.30 _{A,B}	0.031	0.97	
mg Beneficial Omega-3	74	82	45	125	154	171	125	148	60	0.664	106	

Appendix

Appendix C.7: Nutritional fatty acid profiles of tilapia liver and mesenteric fat after 8 weeks of dietary kelp treatments. All values are presented on a per 4oz serving basis. Means followed by different letters in a row are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

			Treatment Diets								<i>P</i>	Root Mean Square Error
			Fish Oil			Ocean Harvest						
	Control	Commercial	1% Fish Oil	3% Fish Oil	5% Fish Oil	1.70% Kelp	5.19% Kelp	8.65% Kelp	5.19% Kelp 1% Fish Oil			
Week 8:												
Liver												
g Fat	3.67	7.63	3.10	5.17	5.39	2.68	4.71	2.62	3.41	0.246	2.46	
mg ALA	7 _{A,B}	21 _A	1 _B	6 _{A,B}	9 _{A,B}	4 _{A,B}	6 _{A,B}	4 _{A,B}	2 _B	0.043	8	
mg EPA	45	61	28	31	34	41	27	18	24	0.432	26	
mg DPA	3	20	1	12	32	15	15	9	1	0.083	14	
mg DHA	46	128	49	93	143	126	82	54	46	0.282	68	
mg Omega-3	123	255	88	150	228	199	142	93	76	0.387	122	
mg Omega-6	270	374	105	173	160	129	191	108	102	0.379	171	
mg Omega-6:3	1.94 _A	1.36 _{A,B}	1.18 _{A,B}	1.06 _{A,B}	0.56 _B	0.63 _B	1.19 _{A,B}	0.81 _B	1.04 _{A,B}	0.006	0.44	
mg Beneficial Omega-3	94	208	78	135	210	181	124	81	70	0.381	106	
Mesenteric Fat												
g Fat	49.15	28.13	36.41	46.58	30.84	45.78	43.21	38.55	24.41	0.309	49.53	
mg ALA	281	321	154	84	219	265	121	257	64	0.173	147	
mg EPA	232	107	120	48	111	142	67	155	38	0.100	87	
mg DPA	77 _B	222 _{A,B}	147 _{A,B}	126 _{A,B}	565 _A	427 _{A,B}	179 _{A,B}	413 _{A,B}	198 _{A,B}	0.022	199	
mg DHA	148 _B	257 _{A,B}	218 _{A,B}	174 _{A,B}	687 _A	574 _{A,B}	269 _{A,B}	513 _{A,B}	68 _B	0.004	218	
mg Omega-3	1134	1096	862	491	1724	1623	735	1592	428	0.118	726	
mg Omega-6	6930	3124	2293	1523	2594	4051	2061	3508	1297	0.172	2709	
mg Omega-6:3	6.37	4.04	2.42	2.95	1.50	2.71	3.25	2.08	4.28	0.160	2.27	
mg Beneficial Omega-3	456	586	485	348	1362	1143	515	1081	304	0.020	462	

Appendix

Appendix C.8: Fillet fatty acid profile (% sum fatty acid) of fish fed experimental kelp diets for eight weeks. SFA:Saturated fatty acids, MUFA:Monounsaturated fatty acids, PUFA:Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Fatty Acid	Treatment Diets										Root Mean Square Error
	Control	Commercial	Fish Oil			Ocean Harvest				P	
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.70% Kelp	5.19% Kelp	8.65% Kelp	5.19% Kelp 1% Fish Oil		
Total SFA	21.67 _B	28.81 _A	27.61 _A	29.83 _A	30.33 _A	29.59 _A	29.11 _A	28.78 _A	27.34 _{A,B}	0.002	2.50
12:0	0.03 _C	0.04 _{A,B,C}	0.03 _{B,C}	0.04 _{A,B,C}	0.06 _A	0.05 _{A,B}	0.04 _{A,B,C}	0.05 _{A,B}	0.03 _{A,B,C}	0.002	0.01
14:0	1.93 _F	2.66 _{C,D,E}	2.53 _{D,E,F}	3.10 _{B,C,D}	4.01 _A	3.56 _{A,B}	3.16 _{B,C,D}	3.26 _{B,C}	2.30 _{E,F}	<0.001	0.27
15:0 ANTEISO	0.13 _C	0.22 _{B,C}	0.18 _C	0.31 _{A,B}	0.40 _A	0.33 _{A,B}	0.32 _{A,B}	0.30 _{A,B}	0.23 _{B,C}	<0.001	0.05
15:0 ISO	0.01 _D	0.04 _{B,C,D}	0.05 _{B,C,D}	0.08 _{A,B}	0.13 _A	0.10 _{A,B}	0.07 _{B,C}	0.08 _{A,B,C}	0.02 _{C,D}	<0.001	0.02
16:0	13.45 _B	18.28 _A	17.78 _A	18.52 _A	18.04 _A	18.16 _A	17.91 _A	18.02 _A	17.17 _{A,B}	0.015	1.80
17:0	0.13 _C	0.25 _{A,B,C}	0.21 _{B,C}	0.32 _{A,B}	0.39 _A	0.32 _{A,B}	0.33 _{A,B}	0.29 _{A,B}	0.27 _{A,B,C}	0.0002	0.06
18:0	5.80	7.15	6.63	7.22	7.10	6.84	7.04	6.58	7.06	0.484	0.91
20:0	0.20	0.18	0.21	0.23	0.21	0.24	0.24	0.22	0.27	0.825	0.07
Total MUFA	35.55	35.87	32.98	33.81	34.09	34.39	32.99	34.71	32.53	0.103	1.68
14:1 n-5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
16:1 n-11	0.03	0.06	0.09	0.04	0.02	0.02	0.05	0.00	0.05	0.473	0.05
16:1 n-9	0.63	0.67	0.63	0.66	0.69	0.65	0.65	0.62	0.61	0.762	0.07
16:1 n-7	2.91 _D	4.30 _{B,C}	3.89 _{C,D}	5.02 _B	6.59 _A	5.39 _B	5.04 _B	5.18 _B	3.47 _{C,D}	<0.001	0.47
18:1 n-9	27.45 _A	23.62 _B	23.83 _B	23.32 _B	21.32 _B	23.31 _B	22.59 _B	24.10 _{A,B}	23.89 _B	0.0006	1.44
18:1 n-7	2.93 _E	3.85 _{A,B}	3.12 _{C,D,E}	3.43 _{B,C,D}	4.04 _A	3.58 _{B,C}	3.41 _{B,C,D}	3.43 _{B,C,D}	3.06 _{D,E}	<0.001	0.19
20:1 n-11	0.13 _B	1.59 _A	0.16 _B	0.12 _B	0.18 _B	0.15 _B	0.10 _B	0.09 _B	0.14 _B	<0.001	0.09
20:1 n-9	1.48 _{A,B}	1.78 _A	1.28 _B	1.23 _B	1.25 _B	1.30 _B	1.15 _B	1.30 _B	1.32 _B	0.002	0.18
Total PUFA	42.79 _A	35.32 _B	39.41 _{A,B}	36.37 _B	35.58 _B	36.03 _B	37.91 _{A,B}	36.51 _B	40.13 _{A,B}	0.002	2.39
18:2 n-6	27.52 _A	18.91 _{C,D}	24.07 _{A,B}	20.17 _{B,C}	15.46 _D	19.81 _{B,C,D}	20.34 _{B,C}	20.32 _{B,C}	23.74 _{A,B}	<0.001	1.88
18:3 n-6	1.38 _A	0.88 _{B,C}	1.21 _{A,B}	0.75 _C	0.73 _C	0.91 _{B,C}	0.85 _{B,C}	0.89 _{B,C}	1.12 _{A,B}	<0.001	0.15
18:3 n-3	1.15 _{B,C}	1.92 _A	1.19 _{B,C}	1.19 _{B,C}	1.31 _B	1.23 _{B,C}	1.21 _{B,C}	1.19 _{B,C}	1.11 _C	<0.001	0.08
20:2 n-6	1.79 _A	1.00 _{C,D}	1.32 _{B,C}	0.93 _D	0.70 _D	0.87 _D	0.89 _D	0.90 _D	1.41 _B	<0.001	0.16
20:3 n-3	1.29 _A	1.17 _{A,B}	1.09 _{A,B,C}	0.90 _{B,C}	0.82 _C	0.94 _{B,C}	0.93 _{B,C}	0.94 _{B,C}	1.19 _{A,B}	<0.001	0.12
20:5 n-3	3.07 _A	2.54 _{A,B,C}	2.82 _{A,B}	2.01 _{B,C}	1.92 _{B,C}	1.90 _{B,C}	2.14 _{A,B,C}	1.77 _C	2.87 _{A,B}	0.0004	0.42
22:4 n-3	1.36 _A	0.84 _{A,B}	0.89 _{A,B}	0.69 _B	0.59 _B	0.73 _B	0.73 _B	0.75 _B	1.11 _{A,B}	0.001	0.22
22:5 n-6	2.08 _A	0.89 _B	1.19 _B	0.65 _B	0.64 _B	0.65 _B	0.69 _B	0.58 _B	1.28 _B	<0.001	0.32
22:5 n-3	0.65 _F	2.17 _{C,D}	1.20 _{E,F}	2.74 _{B,C}	4.47 _A	2.85 _{B,C}	3.12 _B	2.99 _B	1.55 _{D,E}	<0.001	0.32
22:6 n-3	2.49 _D	5.00 _C	4.45 _C	6.35 _{B,C}	8.96 _A	6.16 _{B,C}	7.04 _{A,B}	6.18 _{B,C}	4.74 _C	<0.001	0.81
% Omega 3	10.02 _C	13.64 _{B,C}	11.63 _{B,C}	13.87 _{B,C}	18.07 _A	13.79 _{B,C}	15.15 _{A,B}	13.83 _{B,C}	12.58 _{B,C}	<0.001	1.68
% Omega 6	32.78 _A	21.67 _{C,D}	27.78 _B	22.49 _C	17.52 _D	22.23 _C	22.76 _C	22.68 _C	27.55 _B	<0.001	1.79
Omega 6:3	3.28 _A	1.60 _{B,C,D}	2.65 _{A,B}	1.63 _{B,C,D}	0.97 _D	1.63 _{B,C,D}	1.51 _{C,D}	1.66 _{B,C,D}	2.20 _{A,B,C}	<0.001	0.46
% Beneficial Omega-3	6.21 _D	9.72 _{B,C,D}	8.48 _{C,D}	11.09 _{B,C}	15.35 _A	10.90 _{B,C}	12.28 _{A,B}	10.95 _{B,C}	9.17 _{B,C,D}	<0.001	1.45

Appendix

Appendix C.9: Rib meat fatty acid profile (% sum fatty acid) of fish fed experimental kelp diets for eight weeks. SFA:Saturated fatty acids, MUFA:Monounsaturated fatty acids, PUFA:Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test

Fatty Acid	Treatment Diets										Root Mean Square Error
	Control	Commercial	Fish Oil			Ocean Harvest				P	
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.70% Kelp	5.19% Kelp	8.65% Kelp	5.19% Kelp 1% Fish Oil		
Total SFA	32.86	47.66	43.59	35.51	48.57	35.75	34.55	40.11	34.99	0.487	12.18
12:0	0.04	0.03	0.04	0.06	0.06	0.05	0.03	0.05	0.03	0.032	0.02
14:0	2.60	5.26	3.41	4.18	5.65	4.13	3.26	4.26	3.05	0.053	1.34
15:0 ANTEISO	0.31	0.50	0.34	0.40	0.61	0.38	0.32	0.43	0.32	0.283	0.17
15:0 ISO	0.03 _{B,C}	0.00 _C	0.06 _{A,B,C}	0.11 _{A,B}	0.13 _A	0.09 _{A,B,C}	0.04 _{B,C}	0.07 _{A,B,C}	0.05 _{A,B,C}	0.001	0.04
16:0	21.55	30.32	27.17	22.03	28.69	22.22	22.07	24.76	22.30	0.535	7.07
17:0	0.27	0.53	0.45	0.41	0.66	0.38	0.27	0.46	0.38	0.336	0.22
18:0	7.75	10.79	11.56	7.96	12.29	8.19	8.42	9.68	8.60	0.706	4.10
20:0	0.30	0.24	0.56	0.36	0.49	0.32	0.14	0.40	0.26	0.262	0.22
Total MUFA	28.24	23.95	20.62	28.56	23.74	33.55	27.17	31.00	27.42	0.919	12.68
14:1 n-5	0.02	0.02	0.00	0.01	0.00	0.00	0.02	0.00	0.00	0.729	0.02
16:1 n-11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
16:1 n-9	0.59	0.47	0.65	0.79	0.63	0.57	0.52	0.54	0.61	0.406	0.18
16:1 n-7	3.61 _B	4.77 _{A,B}	3.78 _B	5.82 _{A,B}	6.91 _A	5.28 _{A,B}	4.73 _{A,B}	4.78 _{A,B}	4.13 _B	0.002	1.00
18:1 n-9	19.96	14.05	12.09	17.97	11.58	23.51	18.05	21.97	18.58	0.843	11.79
18:1 n-7	2.67	2.55	2.78	2.27	3.48	3.03	2.94	2.69	2.96	0.747	0.86
20:1 n-11	0.12 _B	1.05 _A	0.51 _{A,B}	0.21 _{A,B}	0.09 _B	0.05 _B	0.05 _B	0.03 _B	0.05 _B	0.012	0.38
20:1 n-9	1.28	1.05	0.82	1.49	1.05	1.12	0.88	0.99	1.09	0.563	0.44
Total PUFA	38.90	28.39	35.79	35.94	27.69	30.71	38.28	28.90	37.59	0.120	6.84
18:2 n-6	23.72 _{A,B}	18.32 _{A,B}	25.15 _A	23.08 _{A,B}	15.49 _B	19.39 _{A,B}	21.50 _{A,B}	18.63 _{A,B}	24.04 _{A,B}	0.021	3.87
18:3 n-6	1.12	0.61	1.25	1.08	0.66	0.79	0.76	0.76	1.08	0.217	0.38
18:3 n-3	1.08	1.50	1.16	1.51	0.93	1.12	1.12	1.04	1.19	0.444	0.39
20:2 n-6	1.27	0.55	1.29	1.11	0.54	0.70	0.72	0.65	1.04	0.019	0.36
20:3 n-3	0.96	0.65	0.95	0.92	0.61	0.71	0.83	0.67	0.98	0.368	0.81
20:5 n-3	1.72	1.59	1.87	1.35	1.20	1.10	2.28	1.03	2.22	0.334	0.85
22:4 n-3	0.81	0.41	0.85	0.67	0.32	0.46	0.26	0.44	0.76	0.056	0.29
22:5 n-6	2.05 _A	0.29 _{A,B}	0.48 _{A,B}	0.39 _{A,B}	0.18 _B	0.27 _{A,B}	0.37 _{A,B}	0.13 _B	0.77 _{A,B}	0.044	0.77
22:5 n-3	0.52 _D	1.08 _{C,D}	0.61 _D	2.16 _{B,C}	3.78 _A	2.23 _{B,C}	2.85 _{A,B}	2.17 _{B,C}	1.56 _{B,C,D}	<0.001	0.59
22:6 n-3	5.67	3.40	2.18	3.68	3.99	3.95	7.61	3.38	3.97	0.711	3.82
% Omega 3	10.75	8.63	7.63	10.29	10.82	9.57	14.94	8.73	10.67	0.595	4.63
% Omega 6	28.15 _A	19.77 _{A,B}	28.17 _A	25.64 _{A,B}	16.87 _B	21.14 _{A,B}	23.34 _{A,B}	20.16 _{A,B}	26.93 _{A,B}	0.006	4.36
Omega 6:3	3.92 _A	2.36 _{A,B,C}	3.69 _{A,B}	2.47 _{A,B,C}	1.62 _C	2.21 _{A,B,C}	1.83 _{B,C}	2.33 _{A,B,C}	2.77 _{A,B,C}	0.008	0.84
% Beneficial Omega-3	7.91	6.07	4.67	7.19	8.96	7.28	12.74	6.59	7.74	0.489	4.80

Appendix

Appendix C.10: Liver fatty acid profile (% sum fatty acid) of fish fed experimental kelp diets for eight weeks. SFA:Saturated fatty acids, MUFA:Monounsaturated fatty acids, PUFA:Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

Fatty Acid	Treatment Diets									P	Root Mean Square Error
	Control	Commercial	Fish Oil			Ocean Harvest					
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.70% Kelp	5.19% Kelp	8.65% Kelp	5.19% Kelp 1% Fish Oil		
Total SFA	36.44	37.21	40.99	48.90	43.13	34.65	47.34	53.55	32.27	0.184	11.51
12:0	0.02	0.04	0.00	0.01	0.01	0.00	0.02	0.01	0.00	0.100	0.02
14:0	2.78 _{B,C}	4.22 _A	2.62 _{B,C}	3.62 _{A,B}	3.34 _{A,B,C}	2.79 _{B,C}	3.72 _{A,B}	3.47 _{A,B,C}	2.18 _C	0.001	0.57
15:0 ANTEISO	0.18 _{A,B}	0.13 _B	0.12 _{B,C}	0.31 _{A,B}	0.31 _{A,B}	0.23 _{A,B}	0.31 _{A,B}	0.40 _A	0.14 _B	0.005	0.10
15:0 ISO	0.08	0.03	0.05	0.06	0.07	0.08	0.02	0.02	0.00	0.366	0.06
16:0	20.38	21.69	21.60	26.58	22.04	18.64	25.05	27.33	18.04	0.133	5.00
17:0	0.29	0.19	0.32	0.47	0.53	0.35	0.57	0.72	0.28	0.115	0.25
18:0	12.53	10.84	16.07	17.48	16.70	12.58	17.38	21.23	11.63	0.328	6.25
20:0	0.18	0.09	0.22	0.38	0.14	0.00	0.29	0.37	0.00	0.078	0.20
Total MUFA	30.69	39.78	24.65	23.13	27.32	29.10	25.52	18.62	30.74	0.543	12.67
14:1 n-5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
16:1 n-11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
16:1 n-9	0.92	0.99	0.92	0.83	0.89	0.78	0.94	0.76	1.02	0.779	0.23
16:1 n-7	3.28	5.11	2.77	4.52	4.78	3.82	4.62	3.33	2.72	0.047	1.18
18:1 n-9	22.68	26.53	17.47	13.45	17.22	20.64	14.86	11.21	23.78	0.539	10.84
18:1 n-7	2.60	3.86	2.47	3.23	3.49	3.03	3.74	2.56	2.36	0.046	0.74
20:1 n-11	0.08 _B	1.72 _A	0.00 _B	0.38 _B	0.00 _B	0.00 _B	0.22 _B	0.00 _B	0.00 _B	<0.001	0.36
20:1 n-9	1.13	1.58	1.03	0.72	0.95	0.85	1.15	0.76	0.86	0.368	0.49
Total PUFA	32.88 _{A,B}	23.00 _B	34.36 _A	27.98 _{A,B}	29.56 _{A,B}	36.25 _A	27.15 _{A,B}	27.84 _{A,B}	37.00 _A	0.003	4.68
18:2 n-6	15.34 _{A,B}	10.90 _{B,C}	14.35 _{A,B,C}	12.69 _{A,B,C}	8.98 _C	12.16 _{B,C}	12.71 _{A,B,C}	10.51 _{B,C}	17.76 _A	0.0004	2.29
18:3 n-6	0.81 _A	0.59 _{A,B}	0.61 _{A,B}	0.51 _{A,B}	0.29 _B	0.37 _B	0.64 _{A,B}	0.45 _{A,B}	0.68 _{A,B}	0.011	0.18
18:3 n-3	0.47	0.68	0.26	0.47	0.45	0.40	0.38	0.36	0.46	0.432	0.22
20:2 n-6	1.33 _A	0.71 _C	1.27 _{A,B}	0.70 _C	0.51 _C	0.81 _{B,C}	0.77 _C	0.66 _C	1.29 _A	<0.001	0.19
20:3 n-3	0.90 _{A,B}	0.83 _{A,B}	0.96 _A	0.73 _{A,B}	0.61 _B	0.83 _{A,B}	0.74 _{A,B}	0.68 _{A,B}	0.84 _{A,B}	0.024	0.13
20:5 n-3	4.60 _A	2.60 _{A,B}	4.90 _A	2.79 _{A,B}	3.23 _{A,B}	4.53 _A	1.89 _B	3.13 _{A,B}	4.68 _A	0.002	1.08
22:4 n-3	0.92 _A	0.06 _B	0.53 _{A,B}	0.21 _{A,B}	0.00 _B	0.31 _{A,B}	0.34 _{A,B}	0.10 _{A,B}	0.12 _{A,B}	0.023	0.35
22:5 n-6	3.25 _A	0.30 _C	2.33 _{A,B}	0.58 _C	0.32 _C	0.62 _C	0.29 _C	0.49 _C	1.58 _{B,C}	<0.001	0.60
22:5 n-3	0.26 _C	0.79 _{B,C}	0.22 _C	0.89 _{B,C}	2.09 _A	1.62 _{A,B}	1.22 _{A,B,C}	1.26 _{A,B,C}	0.32 _C	<0.001	0.44
22:6 n-3	5.01 _B	5.55 _B	8.95 _{A,B}	8.42 _{A,B}	13.09 _A	14.62 _A	8.20 _{A,B}	10.22 _{A,B}	9.28 _{A,B}	0.0009	2.84
% Omega 3	12.15 _B	10.51 _B	15.81 _{A,B}	13.51 _{A,B}	19.47 _{A,B}	22.30 _A	12.75 _B	15.75 _{A,B}	15.70 _{A,B}	0.004	3.80
% Omega 6	20.73 _A	12.49 _C	18.55 _{A,B}	14.48 _{B,C}	10.09 _C	13.95 _{B,C}	14.40 _{B,C}	12.10 _C	21.30 _A	<0.001	2.35
Omega 6:3	1.94 _A	1.36 _{A,B}	1.18 _{A,B}	1.06 _{A,B}	0.56 _B	0.63 _B	1.19 _{A,B}	0.81 _B	1.38 _{A,B}	0.0005	0.38
% Beneficial Omega-3	9.86 _{B,C}	8.94 _C	14.06 _{A,B,C}	12.10 _{A,B,C}	18.41 _{A,B}	20.77 _A	11.30 _{B,C}	14.61 _{A,B,C}	14.28 _{A,B,C}	0.003	3.83

Appendix

Appendix C.11: Mesenteric fat fatty acid profile (% sum fatty acid) of fish fed experimental kelp diets for eight weeks. SFA:Saturated fatty acids, MUFA:Monounsaturated fatty acids, PUFA:Polyunsaturated fatty acids. Beneficial omega-3 includes EPA, DPA and DHA. Means followed by different letters are significantly ($P < 0.05$) different by one-way ANOVA with post-hoc analysis by Tukey's test.

	Treatment Diets										Root Mean Square Error
Fatty Acid	Control	Commercial	Fish Oil			Ocean Harvest				P	
			1% Fish Oil	3% Fish Oil	5% Fish Oil	1.70% Kelp	5.19% Kelp	8.65% Kelp	5.19% Kelp 1% Fish Oil		
Total SFA	29.03	35.94	40.19	35.33	32.95	31.34	37.60	32.22	31.15	0.604	7.92
12:0	0.04	0.05	0.07	0.04	0.07	0.06	0.04	0.07	0.02	0.041	0.02
14:0	2.57 _B	3.65 _{A,B}	4.22 _{A,B}	4.10 _{A,B}	4.95 _A	3.87 _{A,B}	4.16 _{A,B}	4.31 _{A,B}	2.67 _B	0.005	0.81
15:0 ANTEISO	0.20	0.26	0.42	0.38	0.47	0.40	0.38	0.46	0.16	0.018	0.13
15:0 ISO	0.05 _{A,B}	0.05 _{A,B}	0.11 _{A,B}	0.07 _{A,B}	0.15 _A	0.12 _{A,B}	0.07 _{A,B}	0.11 _{A,B}	0.03 _B	0.019	0.05
16:0	19.33	23.73	25.02	22.18	19.64	19.47	23.70	20.44	20.88	0.746	5.38
17:0	0.22	0.18	0.46	0.36	0.40	0.36	0.38	0.44	0.18	0.070	0.15
18:0	6.35	7.85	9.36	7.94	7.01	6.84	8.45	6.03	6.95	0.460	2.14
20:0	0.28	0.18	0.54	0.26	0.25	0.24	0.43	0.38	0.25	0.277	0.20
Total MUFA	36.25	33.40	34.65	35.44	36.82	36.12	29.65	39.10	22.14	0.327	9.19
14:1 n-5	0.00	0.00	0.04	0.00	0.00	0.01	0.00	0.01	0.00	0.244	0.02
16:1 n-11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
16:1 n-9	0.62	0.71	0.99	0.61	0.73	0.70	0.61	0.82	0.55	0.075	0.19
16:1 n-7	3.76 _C	5.89 _{A,B,C}	6.30 _{A,B,I}	5.56 _{A,B,C}	7.73 _A	6.04 _{A,B,C}	5.91 _{A,B,I}	6.69 _{A,B}	4.38 _{B,C}	0.001	1.10
18:1 n-9	27.74	19.92	21.08	24.90	23.13	24.66	19.43	26.16	14.19	0.561	8.98
18:1 n-7	2.58 _{A,B}	3.74 _{A,B}	4.06 _A	3.10 _{A,B}	3.81 _{A,B}	3.34 _{A,B}	2.37 _{A,B}	3.72 _{A,B}	1.76 _B	0.024	0.94
20:1 n-11	0.13	1.29	0.89	0.05	0.17	0.08	0.42	0.11	0.59	0.119	0.64
20:1 n-9	1.43	1.86	1.30	1.22	1.27	1.31	0.92	1.60	0.67	0.122	0.52
Total PUFA	34.72 _{A,B}	30.66 _{A,B}	25.17 _B	29.24 _{A,B}	30.23 _{A,B}	32.54 _{A,B}	32.75 _{A,B}	28.69 _B	46.72 _A	0.031	7.55
18:2 n-6	26.71	22.29	12.48	20.17	16.81	21.57	23.29	16.36	23.02	0.441	8.60
18:3 n-6	1.38 _{A,B}	0.88 _{B,C}	1.71 _A	0.79 _{B,C}	0.66 _C	0.98 _{B,C}	0.83 _{B,C}	1.15 _{A,B,I}	0.91 _{B,C}	0.001	0.30
18:3 n-3	1.21 _B	2.27 _A	1.21 _B	1.21 _B	1.53 _{A,B}	1.49 _{A,B}	1.34 _{A,B}	1.64 _{A,B}	1.30 _{A,B}	0.024	0.41
20:2 n-6	1.40 _{A,B}	0.84 _{B,C}	1.75 _A	0.72 _{B,C}	0.62 _C	0.83 _{B,C}	0.83 _{B,C}	1.02 _{A,B,I}	1.05 _{A,B,I}	0.001	0.31
20:3 n-3	0.96 _{A,B}	0.81 _{A,B}	1.35 _A	0.65 _B	0.60 _B	0.75 _B	0.70 _B	0.94 _{A,B}	0.75 _B	0.004	0.23
20:5 n-3	0.98 _{A,B}	0.78 _{A,B}	1.27 _A	0.73 _B	0.78 _{A,B}	0.80 _{A,B}	0.78 _{A,B}	0.98 _{A,B}	0.77 _{A,B}	0.037	0.22
22:4 n-3	0.69 _{A,B}	0.35 _B	0.99 _A	0.36 _B	0.40 _{A,B}	0.39 _{A,B}	0.34 _B	0.68 _{A,B}	0.29 _B	0.011	0.26
22:5 n-6	0.45	0.05	0.57	0.09	0.05	0.13	0.00	0.21	5.23	0.439	3.33
22:5 n-3	0.31	1.11	1.56	1.91	3.94	2.39	1.88	2.34	12.54	0.552	7.87
22:6 n-3	0.63 _E	1.29 _{C,D,E}	2.28 _{B,C,I}	2.62 _{B,C,D}	4.85 _A	3.21 _{A,B}	2.78 _{B,C}	3.36 _{A,B}	0.89 _{D,E}	<0.001	0.76
% Omega 3	4.78	6.60	8.66	7.48	12.10	9.04	7.81	9.95	16.53	0.596	7.60
% Omega 6	29.94	24.06	16.51	21.75	18.13	23.50	24.94	18.74	30.19	0.070	6.72
Omega 6:3	6.37	4.04	2.42	2.95	1.50	2.71	3.25	2.08	5.44	0.044	2.07
% Beneficial Omega-3	1.92	3.18	5.11	5.27	9.56	6.41	5.44	6.68	14.20	0.555	7.79

Appendix

Appendix C.12: Example of arachidonic acid on GC chromatograph and mass spectra (Top) and of tissue sample and mass spectra (Bottom) at the same retention time.



Macroalgae Results and Discussion

For methodology on fish culture, lipid extraction or analysis, see chapters 2 or 3. The purpose of this appendix is to summarize the results of, and briefly discuss the macroalgae (kelp) diets. See chapters 2 or 3 for results and discussion of fish oil diets.

The composition of experimental kelp feeds is presented in Appendix A. The kelp diets were isocaloric and isonitrogenous to the fish oil diets and control diet (Appendix B). The 1.7%, 5.19%, and 8.65% kelp diets each contained 3% fish oil by dry weight. Strangely, only the 5.19% kelp diet had a lipid composition similar to the 3% fish oil diet (Appendix C). All of the kelp diets that contained 3% fish oil should have had a similar fatty acid profile but the 1.7% and 8.65% kelp diets had similarly depressed concentrations of a number of fatty acids including: 20:5 n-3 and 22:6 n-3. Since the diets increased in %kelp, but did not increase in proportion of any fatty acid, no trends were observed.

Tilapia growth and performance metrics can be seen in Appendix D. Fish cultured on kelp diets demonstrated excellent survival (96-100%). There were also some significant differences observed at 8 week weight gain and average weight gain per week. Fish fed the 1.7% or 5.19% kelp diets were significantly ($P=0.038$) heavier after 8 weeks, compared to those fed the commercial diet, despite starting out at approximately the same weight. Similarly, the 1.7% kelp diet resulted in significantly ($P=0.048$) greater average weight gain per week compared to the commercial diet. According to Dr. Stefan Kraan, the co-founder of Ocean Harvest, this is likely due to the polysaccharides in the kelp increasing the bioavailability of the proteins in the feed. Although these findings have yet to be published. There were no other significant differences in performance and biometrics, with fish demonstrating excellent FCR, SGR, and fillet yield.

Macroalgae Results and Discussion

For all fatty acid comparisons, the 1.7%, 5.19% and 8.65% kelp diets will be compared to the 3% fish oil diet, since all the aforementioned kelp diets also contained 3% fish oil. Nutritional fatty acid profiles for fish fed the kelp diets are presented in Appendices E-G. After four weeks of being fed experimental diets, fish fed the 5.19% kelp diet, had significantly ($P < 0.001$) greater mg DPA/4oz fillet serving compared to those fed the 3% fish oil diet. No other significant differences were observed between the kelp diets and the 3% fish oil diet on a mg fatty acid/ 4 oz serving basis.

The tissue specific fatty acid profiles for fish fed experimental diets for eight weeks can be found in Appendices H-K. There were no significant differences in fatty acid proportion between the 1.7%, 5.19% and 8.65% kelp diets and the 3% fish oil diet in the fillet at 8 weeks. This trend was also observed in rib meat, liver and mesenteric fat tissues after 8 weeks. This would seem to indicate that addition of kelp does not modulate fatty acid deposition in tilapia.