Use of Metabolically Modified Canola Oil as a Replacement for Fish Oil in Practical Diets

of Pacific White Shrimp *Litopeneaus vannamei*

By

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ABSTRACT

The rapid growth of the aquaculture sector can be stifled by the inadequate supply of fish oil, leading the sector to search for alternative oil sources. The efficacy of metabolically modified canola oil (MCO) has not been extensively tested in shrimp. Hence, the objective of this research was to quantify the effect of MCO on shrimp growth performance at various menhaden fish oil (FO) and menhaden fishmeal (FM) replacement levels. FO was replaced by MCO, while FM was replaced by poultry meal (PM). Ten diets were formulated to contain 8% lipid and 36% protein with varying levels of fish oil and tested in both clear and green water tank systems. In the first experiment, all ten diets were evaluated under clear water conditions using indoor glass aquaria. Results indicated shrimp reared on treatments with above 90% FO replacement had significantly lower ($p<0.05$) growth and feed utilization likely due to a nutritional deficiency of long chain highly unsaturated fatty acid (LC-HUFA), docosahexaenoic acid (DHA). In the second experiment, five experimental diets were utilized in a green water growth trial, with FO replacement levels from 75% to 95%. At conclusion, no significant differences were observed between treatments that indicated an effect of treatment on shrimp growth and survival, suggesting that all replacement levels were successful. Shrimp tissues from both experiments were analyzed for fatty acid (FA) profiles.

Lipid results indicated that the diets containing 90% FO replacement or higher in the clear water trial were very close to the nutritional LC-HUFA requirement but were deficient in DHA. Significant differences in shrimp growth were observed in the clear water growth trial, but not in the green water growth trial. This difference could possibly be attributed to the consumption of algae from the system, supplementing the contribution from the diets and meeting nutritional requirement for limiting fatty acids like DHA. Whole shrimp were kept from the green water

growth trial to be used for human sensory analysis. Cooked shrimp samples were evaluated on appearance, juciness, texture, flavor, and overall acceptability. Shrimp fed FM-MCO received consistent complaints of less desirable texture, but there were no significant differences between sensory parameters. Diets FM-FO, FM-MCO, PM-FO, and PM-MCO were used for a shrimp palatability trial to determine if growth differences could be attributed to poor palatability of MCO or PM. No significant differences were observed in amount of feed consumed by the shrimp, indicating that all diets were equally palatable.

Results of this research confirm that albeit MCO has sufficient DHA as a pure oil source, the dilution effect of native oils results in the 100% replacement being deficient in DHA. MCO is not yet suitable to fully replace fish oil in shrimp diets, but is able to be replaced at levels up to 90% without large sacrifices in growth and survival. Because results from these experiments are conflicting, more research with this product is warranted. Further experiments in green water conditions should evaluate the lipid profile of shrimp that are not fed any feed in order to determine how much of the EFA nutritional requirement is fulfilled by the natural foods present in green water systems.

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Chapter 1

Introduction

As the world population continues to increase and capture fisheries have reduced landings, the aquaculture industry is expanding to meet the increasing demand for high-quality seafood products. Aquaculture production has surpassed capture fisheries, and is now the main producer of seafood for human consumption (Garlock et al. 2019). It also plays a crucial role in increasing food security in developing countries by providing jobs and improving nutrition (Bénéa et al. 2016). Intensification of the aquaculture industry aims to increase overall production while optimizing land and water usage (Avnimelech and Kochba 2009).

Fish oil has been an important feed ingredient in complete aquaculture feeds because of its high concentration of omega-3 (n-3) long chain highly unsaturated fatty acids (LC-HUFA) such as eicosapentaenoic acid (EPA, 20:5n- 3) and docosahexaenoic acid (DHA, 22:6n-3) (Asdari, Aliyu-Paiko et al. 2011). Aside from its use in aquaculture, fish oil is a popular human supplement, as it is known to benefit human health and decrease risk of certain diseases like cardiovascular and brain disease (Kris-Etherton et al. 2002). These findings have encouraged people to consume more fish products, which stimulates the aquaculture industry (Nasopoulou and Zabetakis 2012). Fish oil is also an increasingly popular pet food additive, further increasing demand for fish oil in the market.

The Pacific white shrimp (*Litopaneaus vannamei)* is an important species to world aquaculture production, accounting for around 80% of global farmed shrimp (Panini, Freitas et al. 2017). While the dominant shrimp culture species used to be the black tiger shrimp (*Penaeus monodon*), the industry has shifted to producing *L. vannamei* due to their ability to produce specific pathogen free post-larvae, more efficient growth rates, low salinity tolerance, and

tolerance to high stocking density (Cuzon et al. 2004, Bondad-Reantaso et al. 2012, Liu et al. 2017). The shrimp sector of the aquaculture industry is growing even faster than aquaculture as a whole, with an average annual growth rate of 14.5% since 1950 (Garlock et al. 2019). Though aquaculture production has increased, the industry continues to rely heavily on capture fisheries for feed ingredients like fish oil, which is problematic because 80% of wild fish stocks are fully exploited (Swartz, Sumaila et al. 2010). Furthermore, aquaculture utilizes over 75% of the fish oil currently in the market, meaning that it is not a sustainable feed ingredient for the future as there is not enough fish oil to sustain the expansion of aquaculture (Nasopoulou and Zabetakis 2012). Currently, a wide range of production intensities are practiced when culturing shrimp. Intensive shrimp farming relies on commercially produced complete feeds to supply animals with a nutritionally complete diet to allow for optimal growth and feed conversion. Aside from the advantage of being more productive, intensive shrimp farming is also more environmentally friendly, with options like biofloc technology removing ammonia and thus preventing ammonia pollution in the environment (El-Sayed 2021).

As aquaculture expands and intensifies to meet the increasing worldwide demand, the demand for feed ingredients like fish oil has increased as well, causing a subsequent increase in prices (Shepherd and Bachis, 2014). Increasing prices and the lack of increasing availability of these feed ingredients has led to a need for an acceptable replacement that meets the nutritional needs of the animals. Fish oil production is projected to decrease (Tacon and Metian 2008), while oils like canola have been increasing steadily and are projected to continue to increase (Rosillo-Calle et al. 2009). Camelina oil has been successfully used as a fish oil replacement in diets for salmonid production, but was unsuccessful in Atlantic cod because of salmonid's ability to desaturate and elongate long chain fatty acids from dietary sources (Hixson et al. 2014).

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Pacific white shrimp are also not capable of synthesizing DHA and EPA from fatty acids provided in the diet, meaning that the diet must satisfy their requirements to result in adequate growth performance (Perez-Velazquez and Lawrence 2004). In Gonzalez-Felix et al. (2003a) the nutritional requirement for DHA and EPA for juvenile *L. vannamei* was at least 0.5% of the lipid profile.

The limiting factor in using most terrestrial plant oils as complete fish oil replacements in shrimp diets is the lack of EFAs. This has encouraged the use of advanced breeding technologies to increase naturally occurring levels of HUFA in these plants (Turchini, Torstensen et al. 2009). Canola is one of the plants that has been genetically manipulated to naturally produce omega-3 long-chain polyunsaturated fatty acids EPA and DHA which are essential to shrimp development, growth, and survival (Rainuzzo et al. 1997). The canola plant was modified by engineering the plant to have a microalgal polyketide synthase-like polyunsaturated fatty acid (PUFA) synthase system, comprising three multidomain polypeptides and an accessory enzyme that synthesizes DHA and EPA de novo from malonyl-CoA without substantially altering plastidial fatty acid production (Walsh et al. 2016). This breeding technology is promising, because canola oil is a more cost efficient, sustainable, and available ingredient than fish oil for long-term use in aquatic animal diets. While genetically modified canola oil has been successfully used in salmon rearing, it should be tested on other species such as Pacific white shrimp in order to determine the efficacy of the oil as a replacement in practical diets. Previous experiments with this oil determined that there may be nutritional or palatability concerns when applied to shrimp production (Gia Vo et al. 2021b). Thus, these experiments were designed with the following objectives:

1. Determine efficacy of modified canola oil as a fish oil replacement and estimate replacement levels at which nutritional deficiencies are observed, resulting in depressed growth or survival of shrimp.

2. Evaluate the effects of canola oil on feed consumption in juvenile shrimp.

3. Observe growth performance of shrimp under green water tank conditions.

4. Determine the consumer sensory acceptability of shrimp fed with varying levels of modified canola oil.

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Chapter II

Use of Modified Canola Oil as a Replacement for Fish Oil in Practical Diets of Whiteleg Shrimp

Litopeneaus vannamei Reared in Clear-Water Conditions

Abstract

   With advanced breeding technologies, the canola plant has been modified to naturally produce more of the essential fatty acids (EFAs) needed for shrimp, namely DHA (Docosahexaenoic acid, C22:6n-3) and EPA (Eicosapentaenoic acid, C20:5n-3). This experiment was designed to evaluate the efficacy of genetically modified canola oil in shrimp diets. Treatments were designed with the goal of observing a depression in shrimp growth and FCR, indicating the level at which EFA requirements are not met. Ten diets were formulated, one series of fishmeal-based diets, and one series of poultry meal-based diets. Various levels of modified canola oil (MCO) were then used to replacing fish oil (FO) on an isolipidic basis. Juvenile shrimp (average weight 0.22g) were cultured in an indoor recirculating aquaculture system for 7 weeks. A significant increase ($p<0.05$) in FCR and depression in final weight of the shrimp was seen after 90% replacement of FO with MCO in the poultry meal-based diets. This suggests that diets above 90% replacement were nutritionally deficient in EFAs. Diets FM15-FO; FM15-100MCO; PM-100FO & PM-100MCO were also used to evaluate diet palatability. No significant differences ($p>0.05$) were observed in feed consumption, indicating that all diet ingredients were equally palatable to the shrimp and that depressions in growth are not due to consumption issues. Based on these results, MCO can be used as a primary replacement for FO. However, DHA nutritional requirements appear to be considerably higher than previously reported values. Growth and feed conversions were depressed when DHA levels fell below 2.47% in test diets.

1. Introduction

Aquaculture has been the fastest growing sector for human food production for several decades, growing at an average rate of 8% annually since 1970 (Garlock et al. 2019). As technology and management practices have advanced, the industry has shifted from largely extensive farming to intensive and large-scale culture operations. One of the most expensive feed ingredients is fishmeal, and its derivative, fish oil. The market for fishmeal is not able to expand, as it is produced by capture fisheries which are at near capacity, so alternative feed ingredients must be found.

Fishmeal has been successfully replaced in diets for many fish species, but aquatic feeds continue to rely on fish oil as a lipid source because of the high concentration of omega-3 long chain highly unsaturated fatty acids (LC-HUFA) like docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n- 3) (Asdari et al. 2011). For many species, terrestrial oil sources do not meet the nutritional requirement of highly unsaturated essential fatty acids (EFA) for shrimp like DHA and EPA. Canola oil is an attractive fish oil replacement, as it has increased in global production and is projected to continue increasing in the future (Rosillo-Calle et al. 2009). Traditional canola oil does not contain DHA in high enough quantities to meet nutritional requirements, so the canola oil plant has been metabolically manipulated to produce higher concentrations of LC-HUFA (Walsh et al. 2016). Oil from the modified plant is a promising candidate for fish oil replacement in aquatic diets, and has been successfully used as a replacement in juvenile salmon research (Ruyter et al. 2019). However, salmon are able to desaturate and elongate fatty acids, whereas shrimp are not (Perez-Velazquez and Lawrence 2004, Turchini and Francis 2009). The purpose of this study was to evaluate the growth performance of Pacific white shrimp cultured using diets with various replacement levels of fish

oil with modified canola oil, and to observe palatability differences between dietary protein and lipid sources.

2. Materials and Methods

2.1 System Setup

The growth trial was carried out at the E.W. Shell Fisheries Station (Auburn, AL USA). The trial was conducted in an indoor recirculating aquaculture system consisting of 40 glass aquaria (50 x 50 x 50 cm) filled with 100 L of water with constant aeration, salinity of 8 ppt and temperature maintained near 28 °C. Juvenile shrimp were hand sorted to uniform size (0.22g) and randomly stocked into aquaria at 15 shrimp per tank. Shrimp were fed one of ten experimental diets, with each treatment being replicated four times.

2.2 Diet Preparation

Diet formulations are presented in Table 1. A total of ten experimental diets were made for growth trials, including three fishmeal-based diets and seven poultry meal-based diets. All diets were formulated to contained 36% protein and 8% lipid. Soybean meal and corn protein concentrate were the primary plant-based protein sources, with fishmeal and poultry meal being the primary animal-based protein sources. Menhaden fish oil was the primary lipid source, and was replaced at varying levels with the modified canola oil (Table 1). Pre-ground dry ingredients and oil were weighed and mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 min. Boiling water (30-40% by weight) was then blended into the mixture to attain a consistency appropriate for pelleting. Finally, all diets were pressure-pelleted using a meat grinder with a 3-mm die, dried in a forced air oven (50 °C) to a moisture content of less than 10% and stored at -30°C.

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(0.22g initial weight) and grown for 7 weeks. Ingredient sources are shown below the table. Values are reported as percentage of the diet. All diets were formulated to contain 36% protein and 8% lipid on an "as is" basis.

Diet	FM15-FO	FM15-100MCO	FM6-100MCO	FM3-100MCO	PM-FO	PM-75MCO	PM-85MCO	PM-90MCO	PM-95MCO	PM-100MCO
Menhaden Fishmeal[1]	15.00	15.00	6.00	3.00	0.00	0.00	0.00	0.00	0.00	0.00
Poultry Meal[2]	0.00	0.00	0.00	3.00	6.00	6.00	6.00	6.00	6.00	6.00
Soybean Meal[3]	49.25	49.25	50.10	50.10	50.10	50.10	50.10	50.10	50.10	50.10
Corn Protein Concentrate[4]	0.00	0.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Menhaden Fish Oil[5]	5.09	0.00	0.00	0.00	5.51	1.38	0.83	0.55	0.28	0.00
Lecithin (soy)[6]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Latitude Oil[7]	0.00	5.09	5.77	5.63	0.00	4.13	4.68	4.96	5.23	5.51
Cholesterol[8]	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Corn Starch[8]	0.33	0.33	1.31	1.55	2.67	2.67	2.67	2.67	2.67	2.67
Whole Wheat[9]	25.61	25.61	25.10	25.00	24.00	24.00	24.00	24.00	24.00	24.00
Mineral Premix[10]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin Premix[11]	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Choline Chloride[12]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay-C 35%[13]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-dibasic[14]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

FM-Fishmeal
FO-Fish Oil
PM- Poultry Meal
MCO- Modified canola oil
1.Special Select™, Omega Protein Inc., Hammond, Louisiana, USA. 2.Chicken by product meal (Darling ingredient) 3. De-hulled solvent-extracted soybean meal, Bunge Limited, Decatur, AL, USA 4. CPC - Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.5. Omega Protein, Inc., Houston, TX, USA 6. The Solae Company, St. Louis, MO, USA. 7. Cargill Crop 2019 Latitude, FC012320APFO. 8. MP Biomedicals Inc., Santa Ana, CA, USA. 9. Bob's Red Mill Natural Foods, Milwaukie, OR, USA. 10. Mineral premix (g/100 g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.550; Ferrous sulfate, 2.000; Magnesium sulfate anhydrous, 13.862; Manganese sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate heptahydrate, 13.193; Alpha- cellulose, 69.664. 11.

25.00; Vitamin A acetate (500,000 IU/g), 0.32; Vitamin D3 (1,000,000 IU/g), 80.00; Menadione, 0.50; Alpha-cellulose, 856.81. 12. Amresco Inc., Solon, Ohio, USA. 13. Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), DSM Nutritional Products., Parsippany, NJ, USA. 14. Alfa Aesar, Ward Hill, MA, USA

2.3 Water Quality and Management

The shrimp rearing system included a circulation pump, submersible heater, bead filter and fluidized bed biological filter for maintaining water quality. Dissolved oxygen, water temperature, pH and salinity were measured twice daily using a YSI multi-parameter instrument (YSI, Yellow Springs, OH, USA). Total ammonia nitrogen and nitrite were analyzed twice per week using YSI 9500 photometer (YSI, Yellow Springs, OH, USA).

2.4 Feed and Treatments

Commercial feed (Ziegler 50% protein, 15% fat) was fed to post-larval shrimp in a nursery system from arrival until system stocking. For the duration of the experiment, shrimp were fed one of ten experimental diets outlined in Table 1. Feeds were formulated using menhaden fishmeal, poultry meal, menhaden fish oil, and genetically modified canola oil (Latitude Oil FC012320APFO) as primary protein and lipid sources, respectively.

Feed inputs were based on a preprogramed standard feeding protocol that assumes shrimp double their weight until reaching 0.8g, then gain 0.8g per week for the remainder of the trial and have an expected FCR of 1.8.

2.5 Termination

Shrimp in each tank were captured, counted and group weighed to calculate survival, shrimp biomass, mean weight, FCR and weight gain. After weighing and counting the shrimp, shrimp were frozen to be used for fatty acid profile analysis.

 2.6 Fatty Acid Analysis

Fatty acids were extracted from feeds and body samples at Auburn University and sent to Cargill to analyze the fatty acid profile. Whole shrimp samples were stored at -80C, and thawed directly before analyzing. Three shrimp from each tank were pooled into a representative sample

and homogenized. From each sample of shrimp body and diets, two random sub-samples were taken with a weight of 2g each from shrimp whole body, and an approximate weight of 0.6g per sub-sample of feed. These sub- samples were extracted using the methods of (Folch et al. 1957). In short, weighed tissue or feed was homogenized using a Polytron homogenizer in 20 mL of chloroform/methanol (2:1) for 1.5 minutes. The homogenate was filtered through sintered glass filter covered with a glass microfiber filter paper into a screw cap test tube. The residue was re-extracted with 14 mL of chloroform/methanol (2:1) with a Polytron homogenizer for 1.5 minutes and again filtered through the sintered glass filter into the screw cap test tube. Then, the screw cap test tube filled with the filtrate was brought to 40 mL volume with chloroform/methanol (2:1). To this, 8 mL distilled water was added and flushed with nitrogen, then the test tube was capped and inverted to mix. This was stored in a refrigerator (dark) overnight to allow phases to separate. The upper phase was then removed with a pipette and the lower phase washed with fresh upper phase (chloroform: methanol: water 3:48:47) three times by gently allowing it to flow down the side of the test tube. A minimum amount of methanol was added to make one phase. Then, 0.5 g sodium sulfate was added, and the solution decanted to a dried pre-weighed test tube. The chloroform was evaporated using a heated water bath and stream of nitrogen gas, the tubes were then dried and weighed. The percent (%) lipid was then calculated (on a dry weight basis). After the extractions, oils from sub-samples were transferred to 2 mL vials, dried by the nitrogen evaporator, and flushed with nitrogen gas. The samples were stored at -80°C in an ultra-freezer and sent to Cargill's oil division laboratory, Colorado, USA for fatty acid composition analysis. The fatty acid compositions of the samples were analyzed by gas chromatography (GC) method. Total lipid content was expressed as percent of wet tissue or dry diet. First, the extracted oil samples from shrimp or diets were suspended by 500 uL of Isooctane

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with 100 ppm Butylated Hydroxytoluene (BHT). Second, 100 uL of the suspended sample was added to a 15mL polypropylene conical tube, along with 1mL of Isooctane and 100 uL of 1N potassium hydroxide in methanol. Then, samples were vortexed at 3000 rpm for 30 seconds and centrifuged at 3000rpm for 5 mins. Five-hundred microliter of supernatant (isooctane layer) was removed from conical and added to GC vial and crimp capped. Then, all samples were analyzed using an Agilent 7890B with a Flame Ionization Detector. Retention time confirmation was induced by using Nu-Check GLC566 FAME Standard. BHT peak was removed from chromatograms of samples prior to analysis. Individual fatty acid methyl esters (FAMEs) were calculated as percent of total peak area.

2.7 Palatability of diets

The system was prepared by hand sorting shrimp to uniform size (mean weight 5.38g) and stocking 15 shrimp per tank into a series of 40 glass aquaria (40 total, n=10). Shrimp were fed one of four experimental diets, FM-FO, FM-MCO, PM-FO, PM-MCO (Table 1). Shrimp were acclimated to the experimental diets for two days prior to collecting data. To determine diet palatability, each tank was given two grams of experimental feed weighed on a dry weight basis at 8am, and leftover feed was collected after allowing shrimp 30 minutes to consume the feed. Feed was siphoned from the bottom of the tank onto a coffee filter, which was then dried in an oven at 100C overnight and weighed on a 4-decimal scale. Feed and empty coffee filters were weighed in order to calculate the amount of dry feed leftover after the consumption period. Each treatment was replicated ten times daily, for two days, leading to twenty observations per treatment.

2.8 Statistical Analysis

Data was subjected to a two-way analysis of variance (ANOVA) using SAS (V9.4, SAS Institute, Cary, NC, USA) to determine the differences in percent survival, mean initial weight, final biomass, biomass gain, final mean weight, weight gain, percent gain, weekly gain, feed offered, feed conversion ratio, and feed conversion ratio based on shrimp biomass. Student-Newman-Keuls multiple range test was also performed on the data to determine differences in the means between treatments.

3. Results

Water quality parameters were acceptable for growth and typical for this type of system (Table 2). Growth performance and survival results are presented in Table 3. There were no significant differences in survival, ranging from 83.3-95% (P=0.641). Growth results indicated that at the end of the culture period, the basal diet had significantly higher shrimp biomass (67.03g/tank) (P<0.0001) than the full replacement diet (36.85 g/tank). Shrimp individual gain was observed to decrease as fish oil inclusion decreased, with slight increases seen when fishmeal was included in diets 9 and 10. Furthermore, there were no significant differences seen in shrimp final weight between the FM15-FO diet and PM-FO. The basal diet also had the most desirable FCR (1.78), as compared to the highest, seen in shrimp fed diet PM100MCO (3.26).

Palatability results are presented in Table 4. Results indicate that there were no differences in shrimp consumption (p=0.5414) between the four diets that were observed (FM15FO; FM15-100MCO; PM100FO & PM100MCO).

Fatty acid profile results from shrimp whole body tissue are presented in Table 5. Percentage of DHA ranged from 2.04-10.83 (p=0.8173), with FM15FO having the highest concentration, and PM100MCO having the lowest. The relationship between DHA % of the oil in experimental

diets and shrimp final weight is presented in figure 1. DPA ranged from 1.55-2.23 (p=0.8832), with the full fish oil diets (FM100FO, PM100FO) having the lowest amounts, and diet FM3PM3100MCO having the highest levels. EPA ranged from 9.97-13.19 (p=0.4027), with PM75MCO having the lowest, and FM15FO having the highest. There were no significant differences in these oil concentrations between treatments. ARA levels ranged from 1.88-3.34 (p=0.6642), with PM100FO having the lowest, and PM100MCO having the highest. The relationship between EPA and shrimp final weight is modeled in Figure 5, with the lowest concentration of EPA in experimental feeds being 6.43% in PM-100MCO. Furthermore, EPA concentrations in FM15-100MCO and FM6-100MCO were very similar, having 7.66% and 7.39% respectively, but had significantly different growth responses with final weights being 4.08g and 3.23g, respectively.

Fatty acid analysis of experimental diets is presented in Table 6. Generally, n-3 fatty acids decreased, and n-6 fatty acids increased as supplementation of MCO increased. As was expected, diet PM100MCO had the lowest n-3 fatty acid concentration and highest n-6 concentration. Particularly, DHA was highest in FM100FO (10.22) and lowest in PM100MCO (0.44). Concentrations of n3, n6, and other fatty acids are modeled in Figure 6. The n3/n6 ratio in whole shrimp samples also not found to be significant, ranging from 0.31-1.98 (p=0.7285). The highest n3/n6 ratio was seen in the basal FM100FO, with a ratio of 1.98, and the lowest was seen in PM100MCO with a ratio of 0.31. The n3/n6 ratio decreased as fish oil inclusion decreased, with slight increases being seen when fishmeal was included.

Table 2. Water quality parameters throughout the culture period of post-larval shrimp (0.22g initial weight) stocked at 15 shrimp per tank and grown for 49 days in a clear-water RAS system.

Water parameters	Growth Trial
Dissolved Oxygen (mg/L)	6.81 ± 0.52
Temperature (°C)	28.16 ± 1.83
Salinity (ppt)	8.22 ± 1.60
pH	8.24 ± 0.28
Total Ammonia nitrogen (mg/L)	0.18 ± 0.22
Nitrite nitrogen (mg/L)	0.53 ± 0.14

shrimp per tank and grown for 7 weeks in a clear-water RAS system. Letters denote statistical differences between pairwise comparisons.

Diet	Initial weight (g)	Survival (%)	Final Biomass (g)	Final Weight (g)	Weight Gain (g)	FCR
FM15-100FO	0.23^a	93.33^a	67.03^a	4.78^a	4.55^a	1.78^a
FM15-100MCO	0.21^a	86.67^a	52.68^{bc}	4.08^b	3.87^b	2.26^{ab}
FM6-100MCO	0.22^a	88.33^a	42.53^{cd}	3.23^{cd}	3.01^{cd}	2.79^{bc}
FM3PM3-100MCO	0.24^a	86.67^a	40.8^{cd}	3.13^{cd}	2.89^{cd}	2.97^{bc}
PM-FO	0.21^a	91.67^a	64.20^{ab}	4.67^a	4.47^a	1.85^a
PM-75MCO	0.21^a	88.33^a	53.68^{bc}	4.04^b	3.83^b	2.25^{ab}
PM-85MCO	0.22^a	90.00^a	48.18^d	3.85^{cd}	3.64^{cd}	2.53^c
PM-90MCO	0.23^a	95.00^a	53.3^{bc}	3.71^{bc}	3.49^{bc}	2.29^{ab}
PM-95MCO	0.23^a	83.33^a	40.85^{cd}	3.26^{cd}	3.03^{cd}	2.94^{bc}
PM-100MCO	0.22^a	83.33^a	36.85^d	2.95^d	2.74^d	3.26^c
PSE	0.02	9.58	6.22	0.30	0.30	0.37
p-value	0.7201	0.6408	<0.0001	<0.0001	<0.0001	<0.0001

Table 4. Palatability results of post-larval shrimp (5.38g weight) stocked at 15 shrimp per tank and given 30 minutes to consume feed that was offered.

Diet	Amount Fed (g)	Average Remaining %
FM15-FO	2.0	35.59[a]
FM-100MCO	2.0	40.97[a]
PM-FO	2.0	36.70[a]
PM-100MCO	2.0	37.52[a]
PSE	0.0	12.22
p-value	n/a	0.5414

diets containing various levels of modified canola oil and poultry meal as replacements for fish oil and fishmeal, respectively. Results are presented as mean ± *SE*.

Fatty Acids	FM15-FO	FM15-100MCO	FM6-100MCO	FM3PM3-100MCO	PM-FO	PM-75MCO	PM-85MCO	PM-90MCO	PM-95MCO	PM-100MCO	p-value
C14:0	10.83 ± 0.80	4.95 ± 0.84	4.06 ± 0.50	2.70 ± 0.34	9.81 ± 0.16	4.03 ± 0.09	3.60 ± 0.75	3.60 ± 0.55	2.92 ± 0.81	2.04 ± 0.12	<0.0001
C14:1n-5	13.19 ± 1.10	11.19 ± 0.29	11.30 ± 0.16	11.77 ± 0.51	11.57 ± 0.38	9.97 ± 0.92	11.48 ± 0.63	11.02 ± 0.24	11.31 ± 0.55	11.23 ± 0.36	0.4027
C15:0	0.26 ± 0.03	0.13 ± 0.04	0.12 ± 0.02	0.09 ± 0.03	0.24 ± 0.00	0.11 ± 0.04	0.10 ± 0.01	0.11 ± 0.06	0.10 ± 0.04	0.08 ± 0.04	0.8475
C16:0	17.62 ± 1.60	13.42 ± 1.43	12.52 ± 0.71	12.25 ± 1.16	18.10 ± 0.26	13.39 ± 1.34	12.79 ± 0.29	13.68 ± 1.63	12.85 ± 1.58	12.21 ± 1.58	0.8130
C16:1n-7	2.99 ± 0.32	1.03 ± 0.70	0.84 ± 0.28	0.35 ± 0.52	2.96 ± 0.04	0.98 ± 0.61	0.69 ± 0.18	0.85 ± 0.80	0.52 ± 0.68	0.42 ± 0.68	0.8973
C17:0	0.89 ± 0.13	0.51 ± 0.07	0.49 ± 0.02	0.42 ± 0.09	0.73 ± 0.02	0.43 ± 0.02	0.46 ± 0.14	0.44 ± 0.02	0.43 ± 0.13	0.41 ± 0.11	0.7575
C18:0	8.01 ± 0.86	6.72 ± 0.25	6.50 ± 0.26	7.21 ± 0.29	7.03 ± 0.33	6.07 ± 0.64	7.31 ± 0.41	6.95 ± 0.19	6.97 ± 0.34	6.99 ± 0.21	0.1793
C18:1n-9	12.11 ± 2.51	20.62 ± 2.02	21.51 ± 0.92	22.44 ± 2.27	14.46 ± 0.34	22.70 ± 3.05	22.02 ± 0.45	22.06 ± 3.08	22.80 ± 2.72	23.61 ± 2.37	0.8103
C18:1n-7	3.71 ± 0.24	2.91 ± 0.16	2.91 ± 0.05	2.72 ± 0.18	3.42 ± 0.03	2.77 ± 0.30	2.83 ± 0.04	2.79 ± 0.29	2.79 ± 0.23	2.69 ± 0.21	0.9062
C18:2n-9	0.09 ± 0.29	0.35 ± 0.07	0.33 ± 0.04	0.34 ± 0.06	0.06 ± 0.01	0.33 ± 0.08	0.30 ± 0.02	0.29 ± 0.06	0.32 ± 0.07	0.35 ± 0.08	0.4100
C18:2n-6	14.89 ± 3.37	21.55 ± 1.39	22.47 ± 0.74	22.24 ± 1.65	16.56 ± 0.47	23.43 ± 2.36	21.73 ± 0.70	22.03 ± 0.2.23	22.58 ± 2.07	23.01 ± 1.67	0.6170
C18:3n-6	0.16 ± 0.32	0.29 ± 0.04	0.27 ± 0.03	0.25 ± 0.04	0.11 ± 0.05	0.29 ± 0.06	0.20 ± 0.05	0.20 ± 0.03	0.20 ± 0.04	0.25 ± 0.04	0.3015
C18:3n-3	1.06 ± 0.47	1.24 ± 0.04	1.19 ± 0.05	1.05 ± 0.04	1.12 ± 0.10	1.28 ± 0.08	1.08 ± 0.02	1.11 ± 0.07	1.10 ± 0.03	1.13 ± 0.04	0.1155
C18:4n-3	0.34 ± 0.03	0.10 ± 0.08	0.08 ± 0.03	0.00 ± 0.06	0.30 ± 0.02	0.09 ± 0.07	0.03 ± 0.03	0.07 ± 0.11	0.02 ± 0.08	0.01 ± 0.08	0.8362
C20:0	0.22 ± 0.05	0.30 ± 0.03	0.29 ± 0.02	0.32 ± 0.02	0.20 ± 0.02	0.28 ± 0.02	0.29 ± 0.00	0.29 ± 0.01	0.29 ± 0.03	0.30 ± 0.03	0.6414
C20:1n-9	0.79 ± 0.12	1.03 ± 0.06	1.09 ± 0.04	1.10 ± 0.08	0.85 ± 0.01	1.01 ± 0.09	1.11 ± 0.02	1.03 ± 0.06	1.02 ± 0.08	1.07 ± 0.06	0.8397
C20:2n-6	1.69 ± 0.64	2.80 ± 0.41	3.07 ± 0.18	3.34 ± 0.30	1.82 ± 0.06	2.52 ± 0.39	3.12 ± 0.25	2.85 ± 0.40	3.02 ± 0.36	3.11 ± 0.38	0.7446
C20:3n-6	0.56 ± 0.27	1.90 ± 0.39	1.97 ± 0.19	2.12 ± 0.31	0.51 ± 0.04	1.90 ± 0.44	1.85 ± 0.07	1.81 ± 0.44	2.04 ± 0.44	2.13 ± 0.44	0.8426
C20:4n-6 ARA	2.04 ± 0.25	2.77 ± 0.37	2.78 ± 0.16	3.27 ± 0.29	1.88 ± 0.19	2.46 ± 0.32	3.15 ± 0.26	2.96 ± 0.40	3.17 ± 0.30	3.30 ± 0.38	0.6642
C20:4n-3	0.80 ± 0.05	0.78 ± 0.08	0.74 ± 0.01	0.78 ± 0.05	0.88 ± 0.02	0.77 ± 0.05	0.69 ± 0.04	0.68 ± 0.03	0.73 ± 0.06	0.70 ± 0.04	0.0191
C20:5n-3 EPA	13.19 ± 1.10	11.19 ± 0.29	11.30 ± 0.16	11.77 ± 0.51	11.57 ± 0.38	9.97 ± 0.92	11.48 ± 0.63	11.02 ± 0.24	11.31 ± 0.55	11.23 ± 0.36	0.4027
C22:0	0.19 ± 0.02	0.18 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.17 ± 0.00	0.17 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.15 ± 0.02	0.16 ± 0.01	0.3495
C22:1n-9	0.07 ± 0.00	0.08 ± 0.05	0.07 ± 0.04	0.18 ± 0.01	0.07 ± 0.05	0.05 ± 0.04	0.13 ± 0.02	0.12 ± 0.03	0.12 ± 0.02	0.11 ± 0.06	0.9287
C23:0	0.04 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.00 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.00 ± 0.02	0.01 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.3882
C22:4n-6	0.25 ± 0.03	0.24 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.29 ± 0.00	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.02	0.25 ± 0.01	0.27 ± 0.01	0.9313
C22_5 (n-3) DPA	1.55 ± 0.00	1.83 ± 0.17	1.96 ± 0.00	2.23 ± 0.14	1.55 ± 0.07	1.71 ± 0.20	1.95 ± 0.13	1.95 ± 0.13	2.05 ± 0.13	2.17 ± 0.18	0.883

C22_6 (n-3) DHA	10.83 ± 1.45	4.95 ± 1.70	4.06 ± 0.84	2.70 ± 1.72	9.81 ± 0.22	4.03 ± 2.20	3.60 ± 0.48	3.60 ± 2.21	2.92 ± 2.07	2.04 ± 1.96	0.817 3
C24_1 (n-9)	0.17 ± 0.0	0.16 ± 0.02	0.14 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.12 ± 0.02	0.15 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.605 5

FM = Fish Meal

PM = Poultry Meal

FO = Fish Oil

MCO = Modified Canola Oil

shrimp (initial weight 0.22g) offered feeds using modified canola oil (MCO) to replace

menhaden fish oil (FO) and grown for 7-weeks in a clear water RAS system.
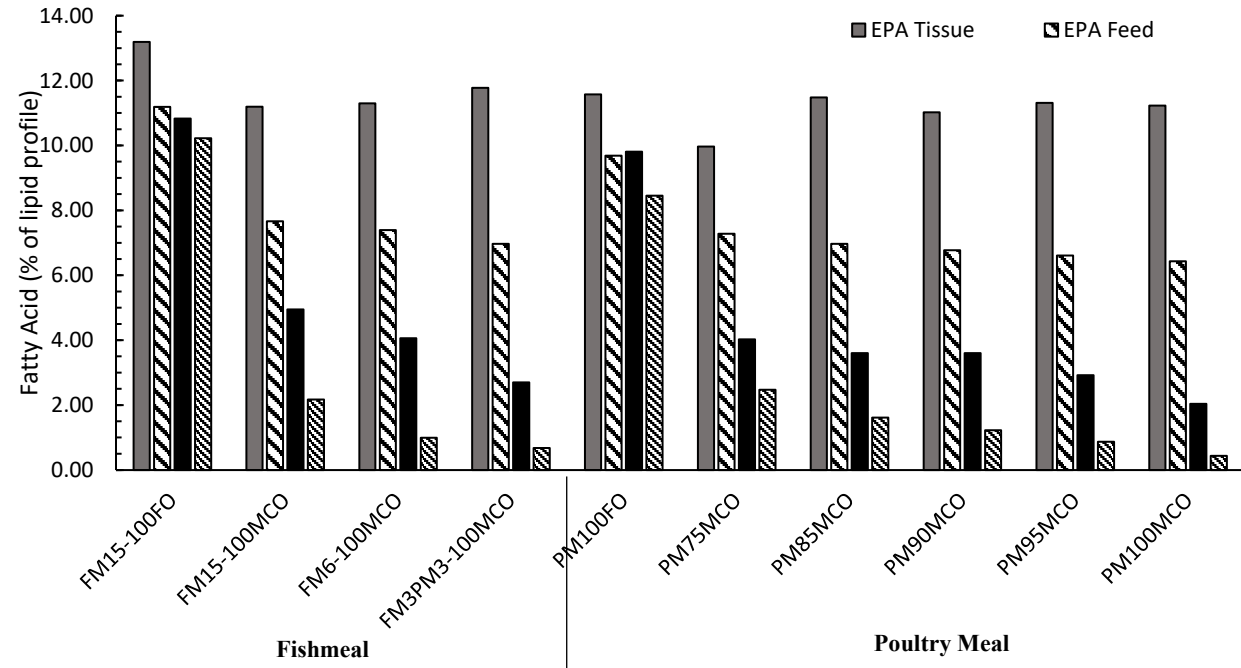
(initial weight 0.22g) cultured for 7 weeks in a clear water RAS system. with various

replacement levels of FO and FM with MCO and PM, respectively. Results are presented as

mean ± *SE*.

	FM-15FO	FM15-100MCO	FM6-100MCO	FM3P M3-100MCO	PM-FO	PM-75MCO	PM-85MCO	PM-90MCO	PM-95MCO	PM-100MCO
C14_0	10.22	2.17	0.99	0.68	8.45	2.47	1.61	1.22	0.87	0.44
C14_1 (n-5)	11.19	7.66	7.39	6.97	9.68	7.28	6.97	6.77	6.61	6.43
C15_0	0.51	0.10	0.05	0.04	0.42	0.12	0.08	0.07	0.05	0.03
C16_0	16.65	8.61	7.29	7.83	16.94	10.77	9.87	9.60	9.15	8.53
C16_1 (n-7)	8.68	1.24	0.54	0.61	8.13	2.57	1.80	1.46	1.12	0.73
C17_0	0.69	0.15	0.10	0.07	0.58	0.18	0.13	0.11	0.09	0.06
C18_0	3.20	3.17	2.98	3.08	3.44	3.37	3.32	3.35	3.31	3.23
C18_1 (n-9)	7.05	24.54	27.13	28.27	10.96	24.48	26.26	27.07	27.74	28.86
C18_1 (n-7)	2.60	2.15	2.18	2.18	2.47	2.24	2.19	2.18	2.17	2.15
C18_2 (n-9)	0.03	1.37	1.52	1.46	0.03	1.06	1.20	1.27	1.33	1.39
C18_2 (n-6)	13.41	31.64	33.68	33.90	15.94	29.30	31.23	32.06	32.82	33.85
C18_3 (n-6)	0.24	1.47	1.61	1.55	0.24	1.18	1.31	1.37	1.42	1.47
C18_3 (n-3)	2.61	3.34	3.27	3.16	2.53	2.97	3.04	3.06	3.09	3.11
C18_4 (n-3)	2.48	0.46	0.26	0.19	2.13	0.63	0.43	0.33	0.25	0.14
C20_0	0.19	0.49	0.51	0.48	0.19	0.41	0.43	0.44	0.45	0.47
C20_1 (n-9)	0.59	0.57	0.59	0.55	0.56	0.55	0.56	0.55	0.55	0.54
C20_2 (n-6)	0.18	0.09	0.08	0.08	0.17	0.10	0.09	0.09	0.09	0.08
C20_3 (n-6)	0.16	2.49	2.75	2.64	0.20	1.94	2.20	2.26	2.41	2.55
C20_4 (n-6) ARA	0.83	1.48	1.54	1.51	0.83	1.32	1.40	1.40	1.46	1.49
C20_3 (n-3)	0.18	0.05	0.04	0.04	0.15	0.07	0.05	0.04	0.04	0.03
C20_4 (n-3)	1.13	1.00	0.99	0.92	1.01	0.91	0.89	0.86	0.88	0.86
C20_5 (n-3) EPA	11.19	7.66	7.39	6.97	9.68	7.28	6.97	6.77	6.61	6.43
C22_0	0.20	0.26	0.25	0.24	0.19	0.22	0.22	0.22	0.23	0.23
C22_1 (n-9)	0.10	0.07	0.08	0.07	0.09	0.10	0.09	0.08	0.09	0.09
C22_2 (n-6)	0.04	0.05	0.05	0.03	0.03	0.04	0.04	0.03	0.03	0.05
C23_0	0.10	0.11	0.09	0.08	0.10	0.09	0.09	0.08	0.08	0.08
C22_4 (n-6)	0.15	0.28	0.30	0.28	0.19	0.26	0.28	0.29	0.30	0.29
C22_3 (n-6)	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
C22_5 (n-3)	1.89	1.63	1.63	1.50	1.65	1.45	1.45	1.43	1.43	1.41

C22_6 (n-3) DHA	10.22	2.17	0.99	0.68	8.45	2.47	1.61	1.22	0.87	0.44
C24_1 (n-9)	0.28	0.10	0.09	0.07	0.21	0.11	0.09	0.08	0.07	0.07

FM = Fish Meal
PM = Poultry Meal
FO = Fish Oil
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4. Discussion

Feed is a major expense of shrimp production costs (Davis et al. 2008). Fishmeal accounts for 15-25% of many feed formulations representing about 40% of total feed costs (Borski et al. 2011). This means that one way to make shrimp farming more cost efficient is to find acceptable fishmeal replacements for shrimp diets, including fish oil replacement options, being that fish oil is derived from fishmeal. While fishmeal has been successfully replaced in shrimp diets (Cheng et al. 2002), replacing fish oil has been more problematic due to limited choices. This is because shrimp have a dietary requirement for LC-PUFA, and are not able to synthesize these fatty acids from dietary sources (Perez-Velazquez and Lawrence 2004). Presently, fish oil is the primary source of LC-PUFA in aquaculture diets, making it both a limiting as well as expensive ingredient.

In previous experiments with MCO obtained from research varieties of modified canola, growth was significantly depressed when fish oil was completely removed from shrimp diets and substituted with either standard canola oil or modified canola oil (MCO) (Gia Vo et al. 2021b). They identified a probable deficiency of DHA but did not identify the maximum level of inclusions of MCO. Presently commercial lines of modified canola have been produced and oil is becoming available on the market. Hence there is a need to evaluate these oils and to identify maximum levels of replacement in feed formulations. For the current work, fish oil was substituted with modified canola oil at various levels in order to determine how much of the oil is able to be substituted in shrimp diets without sacrificing animal growth.

The first four diets included a diet with 15% fishmeal and 5.1% fish oil (FM-FO) followed by three diets with the fish oil replaced by MCO but containing 15, 6 and 3% fishmeal (FM15-100MCO, FM6-100MCO; FM3-100MCO). Thus, allowing for the evaluation of the effects of

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fishmeal, and the oil it contains, in combination with MCO. The second series of diets were PM based, and had various levels of FO replacement with MCO (75-100%) with a goal to determine the acceptable replacement ratio with no other source (except FO and MCO) of DHA. These diets were intentionally formulated to observe nutritional deficiencies, so we would be able to predict the replacement value of MCO level more closely.

There are many factors that could be responsible for poor growth and feed conversion seen in the shrimp growth trial. These include the dietary protein source, lipid source, attractability, palatability, and vitamin deficiencies. When final weights of shrimp fed fishmeal-based diets were compared, a significant decrease in final weights of the shrimp is observed (Figure 2). This suggests that removal of fishmeal and the subsequent shift in nutrients could be responsible for poor growth performance of shrimp. A significant decrease was also seen in shrimp final weights when poultry meal-based diets are compared corresponding to decreases in the concentration of fish oil (Figure 3). However, there were no significant differences in the final weight of shrimp offered diets utilizing FM or PM as the protein source with fish oil as the lipid supplement. This indicated that growth differences are not due to the basal protein source and are instead related to the level and type of lipids. This is supported by other work that used non marine protein sources in combination with fish oil (Amaya et al. 2007) as well as alternative sources of HUFA such as algae meals (Kristy et al. 2019).

Figure 2. Relationship between fishmeal inclusion in experimental diets and final shrimp weights (mean weight 0.22g) cultured for 7 weeks in a clear water recirculating aquaculture system.
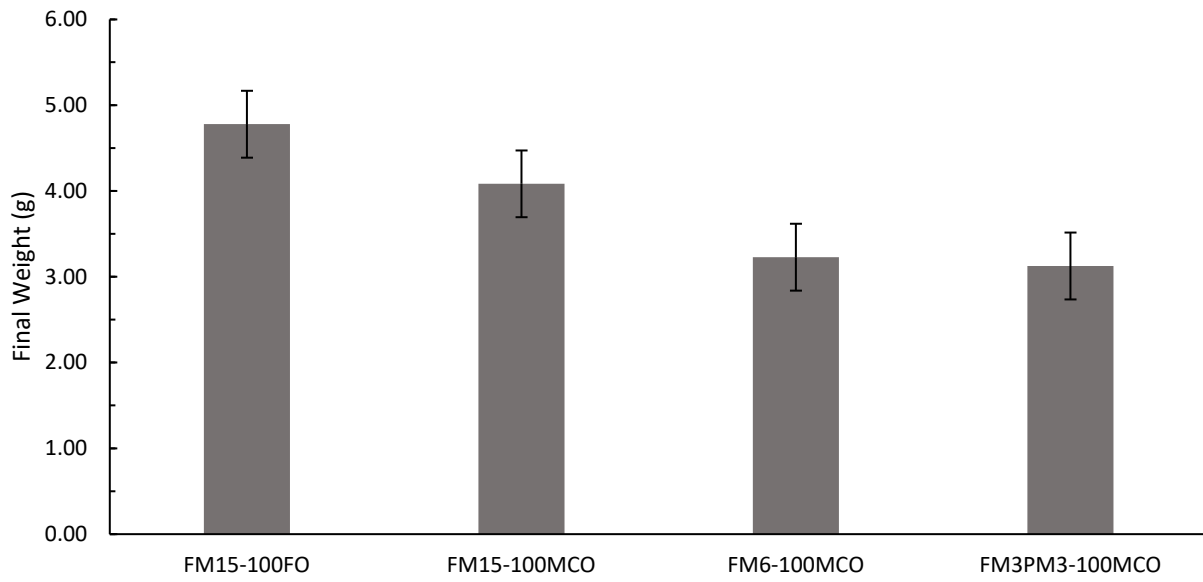
Figure 3. Relationship between poultry meal inclusion in experimental diets and shrimp final weights (mean weight 0.22g) cultured for 7 weeks in a clear water recirculating aquaculture system. Letters denote significant differences in shrimp final weight.
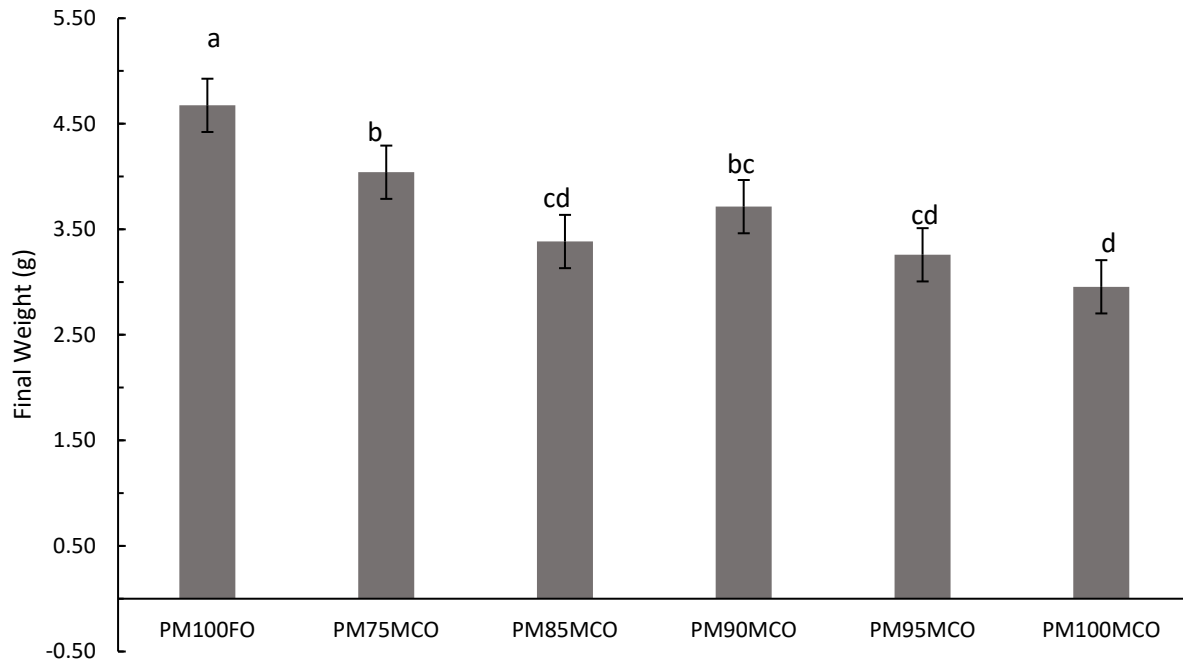
Cholesterol is required nutrient for shrimp (Gong et al. 2000) and is naturally found in fish oil, so as fish oil was removed from the diet, cholesterol levels would also decrease. Hence, cholesterol was supplemented at 0.12% in order to eliminate cholesterol as a growth limiting factor. FO is also a good source of vitamins which were supplemental equally across diet formulations, eliminating vitamin deficiencies as a factor that could be limiting shrimp growth performance.

Another possibility is that marine oils are considered attractants and could influence feed consumption. Results from the palatability trial indicate that there are no issues in attractability as there were no significant differences in consumption between dietary treatments (Table 4). Palatability results contrast with Cruz-Suárez et al. (2007), which found significant differences in consumption when shrimp were offered diets with various levels of replacement of FM with a PM blend. The shifts in palatability of the feeds were not apparent and also do not seem to be responsible for the differences in shrimp growth response.

Higher feed conversion ratios and lower final weights seen in shrimp that were fed diets with low or no inclusion of FO, specifically diet PM-95MCO and PM-100MCO, indicate that those diets did not meet the dietary requirement of LC-HUFA for shrimp growth. This is similar to previous research with this oil, finding that full replacement led to nutritional deficiencies, while partial replacement (up to 75%) was successful (Gia Vo et al. 2021b).

In previous research with plant oils such as soybean oil and rapeseed oil, substitution levels have been limited as the pacific white shrimp have dietary requirements for DHA and EPA. EPA and DHA are recommended to be at least 0.5% of the lipid (Gonzalez-Felix et al. 2003a). In that experiment, diets had a lower total lipid content (5%) than formulations used for this research (8%). Basal protein sources were also processed to remove trace lipids, meaning that all of the

lipids in the diets were supplemental. Whereas in the present work we utilized practical ingredients which have background levels of lipids.

Some previous research has suggested that ARA is also of concern in shrimp diets (Araújo et al. 2020). However, ARA levels increased in diets as MCO inclusion increased, which indicates that ARA is not limiting. The relationship between ARA concentration in experimental feeds and shrimp growth response is modeled in Figure 4. Thus, ARA does not appear to limit growth in this experiment, which agrees with other studies, suggesting that when present in shrimp diets, DHA is limiting rather than ARA (Glencross 2009). EPA is considered to be a growth limiting EFA for shrimp, but being that inclusion levels in this experiment were well over the nutritional requirement (0.5% of lipid profile) it was not considered to limit shrimp performance. DPA is not considered an essential fatty acid for shrimp growth, but is typically evaluated alongside the essential fatty acids being that it is known to be a precursor in DHA metabolism and play a role in immune function (Dyall 2015). The relationship between DPA concentration in experimental feeds and shrimp final weights is graphically presented in figure 6. Though there does seem to be some relationship between DPA concentration and shrimp weight, DPA is not recognized as an EFA for shrimp growth, and was eliminated as a growth limiting factor. In diets that contained very similar levels of DPA, the growth response was significantly different, meaning that another fatty acid must be responsible. In diets FM15-100MCO and FM6-100MCO, concentrations of DPA were the same at 1.63%, but final weights were different at 4.08g and 3.23g, respectively. DHA is most commonly recognized as the EFA of the most concern when lipid sources are exchanged (Feng et al. 2020). We found a strong relationship between DHA inclusion levels and shrimp growth response, with shrimp fed the highest levels at 10.22% and 8.45%, also having

the highest final weights at 4.78g and 4.67g, respectively. This relationship between DHA concentration in the feed and shrimp final weight is presented graphically in figure 7.

Fatty acid analysis of the experimental diets used for this trial (Table 6) show that PM100MCO was below the recommended DHA level, with a value of 0.44 and would be expected to be marginally deficient, yet diets with higher DHA levels also had poor growth. Figure 8 demonstrates the logarithmic relationship between feed DHA levels and shrimp final weight, showing that there is a relationship between DHA in the feed and shrimp final weights. This analysis was also performed on DPA, ARA, and EPA, but a strong correlation was not observed. This indicates that DHA is the limiting EFA, as we expected. Diets PM90MCO and PM95MCO had DHA values of 0.99 and 0.87 respectively, and demonstrated significantly depressed growth values, meaning that the nutritional requirement may be higher than previously predicted. Based on figure 8, the nutritional requirement may be closer to 2% as that is where the slope appears to change, and higher levels lead to diminishing returns in terms of growth. Attempts were made to statistically model the DHA requirement, but this experiment did not yield a wide enough range to make these models successful. More research with a wider range of DHA levels is necessary in order to effectively model the DHA requirement. Samocha et al. (2010) found that shrimp were able to be fed diets with marine oils completely replaced, as long as fermented products were added to the diet to provide a source of LC-HUFA, specifically ARA and DHA. Similarly, Soller et al. (2017) reported that shrimp could be fed diets with complete replacement of marine ingredients, with use of genetically modified products or fermented products as an EFA source. In general, fatty acid levels in feeds correlated to the level that was found in whole body tissue (Figure 1), with diet PM100MCO having the lowest dietary and tissue levels of DHA.

Figure 4. The relationship between final body weight (g) and ARA (Arachidonic acid, C20:4n-6) level (% of lipid profile) in test diets using modified canola oil (MCO) to replace menhaden fish oil (FO) in feeds offered to juvenile shrimp (mean weight 0.22g) cultured for 7 weeks in a clear water recirculating aquaculture system. Letters denote significant differences between final weights.
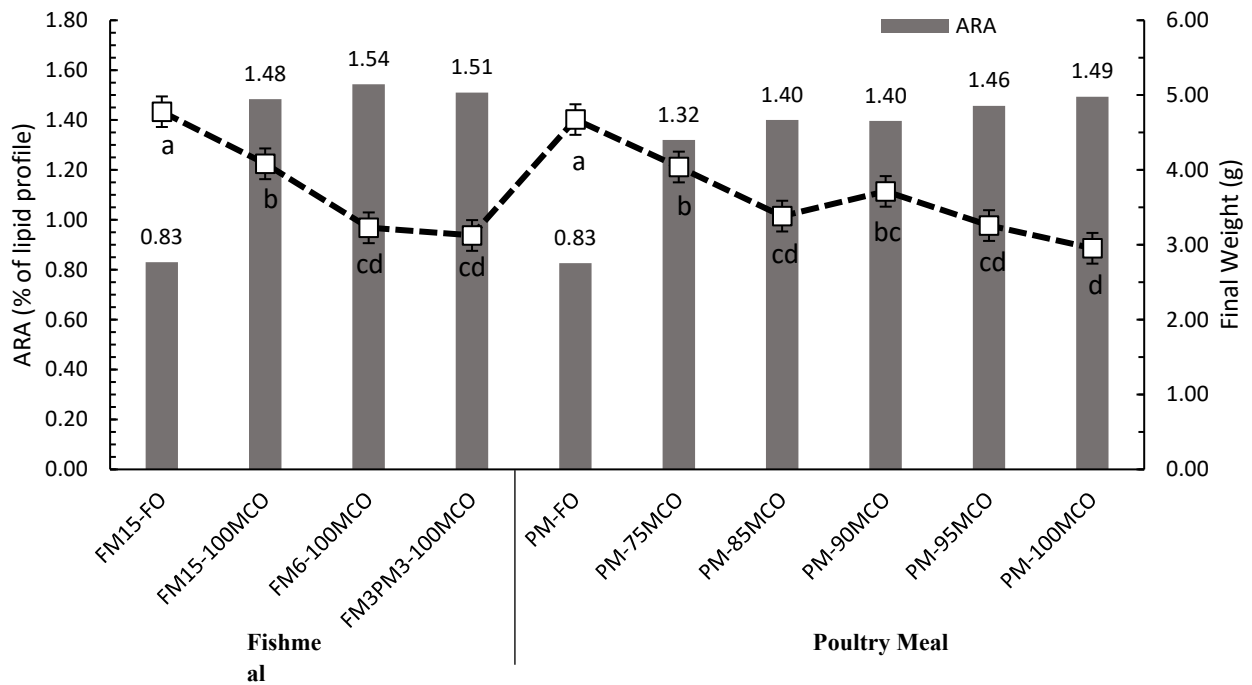
Figure 5. The relationship between final body weight (g) and EPA (Eicosapentaenoic acid, C20:5n-3) level (%) in test diets using modified canola oil (MCO) to replace menhaden fish oil (FO) in feeds offered to juvenile shrimp (mean weight 0.22g) cultured for 7 weeks in a clear water recirculating aquaculture system. Letters denote significant differences between final weights.
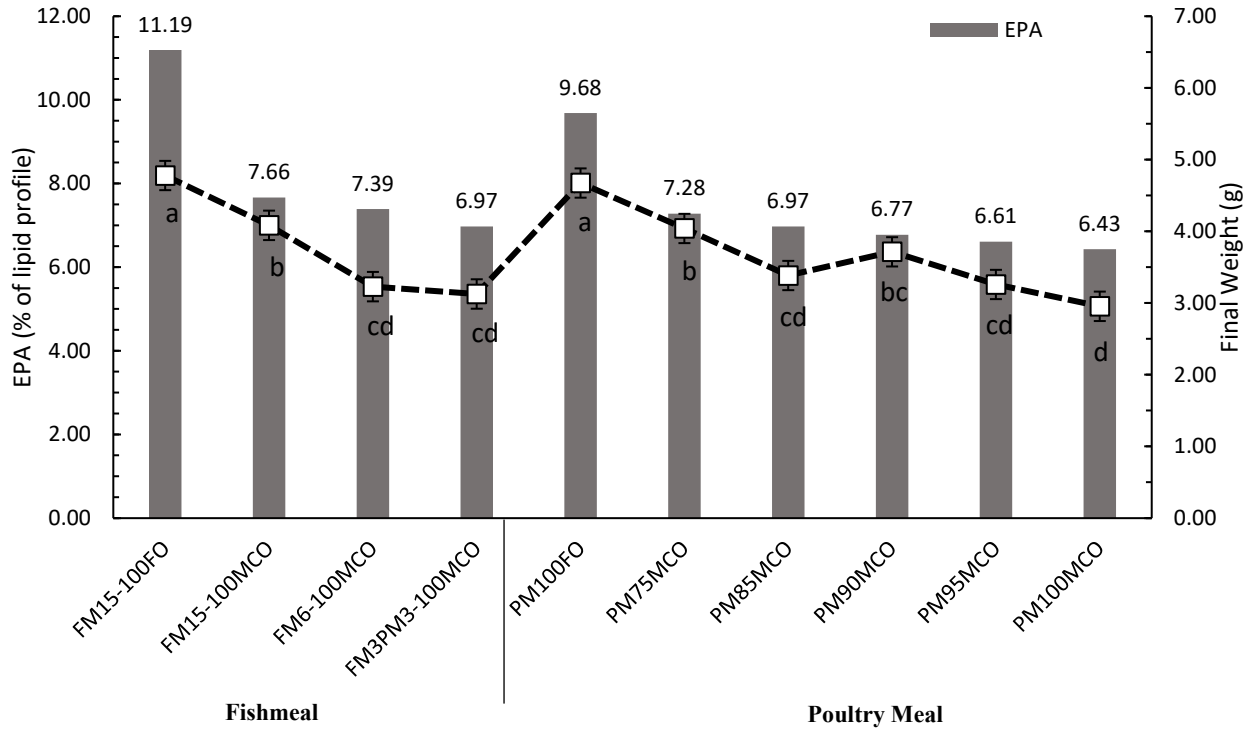
Figure 6. The relationship between final body weight (g) and DPA (Docosapentaenoic acid, C22: 5n-3) level (%) in test diets using modified canola oil (MCO) to replace menhaden fish oil (FO) in feeds offered to juvenile shrimp (mean weight 0.22g) cultured for 7 weeks in a clear water recirculating aquaculture system. Letters denote significant differences between final weights.
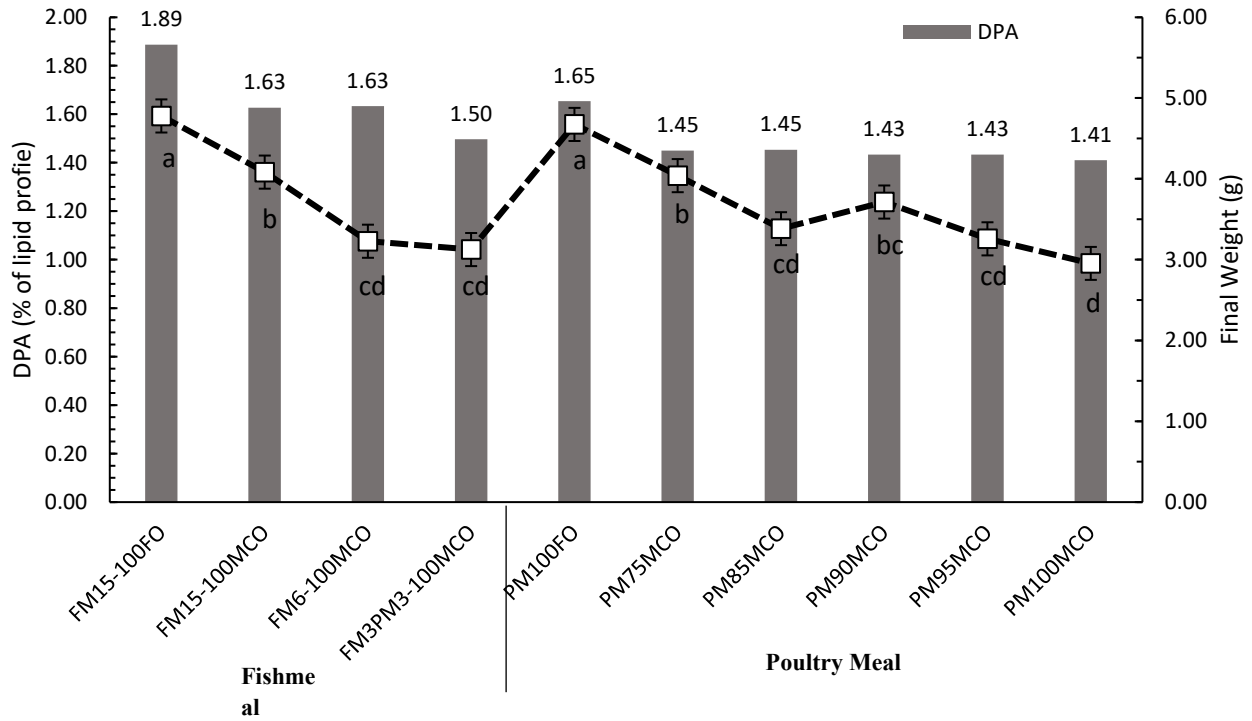
Figure 7. The relationship between final body weight (g) and DHA (Docosahexaenoic acid, C22:6n-3) level (%) in test diets using modified canola oil (MCO) to replace menhaden fish oil (FO) in feeds offered to juvenile shrimp (mean weight 0.22g) cultured for 7 weeks in a clear water recirculating aquaculture system. Letters denote significant differences between final weights.
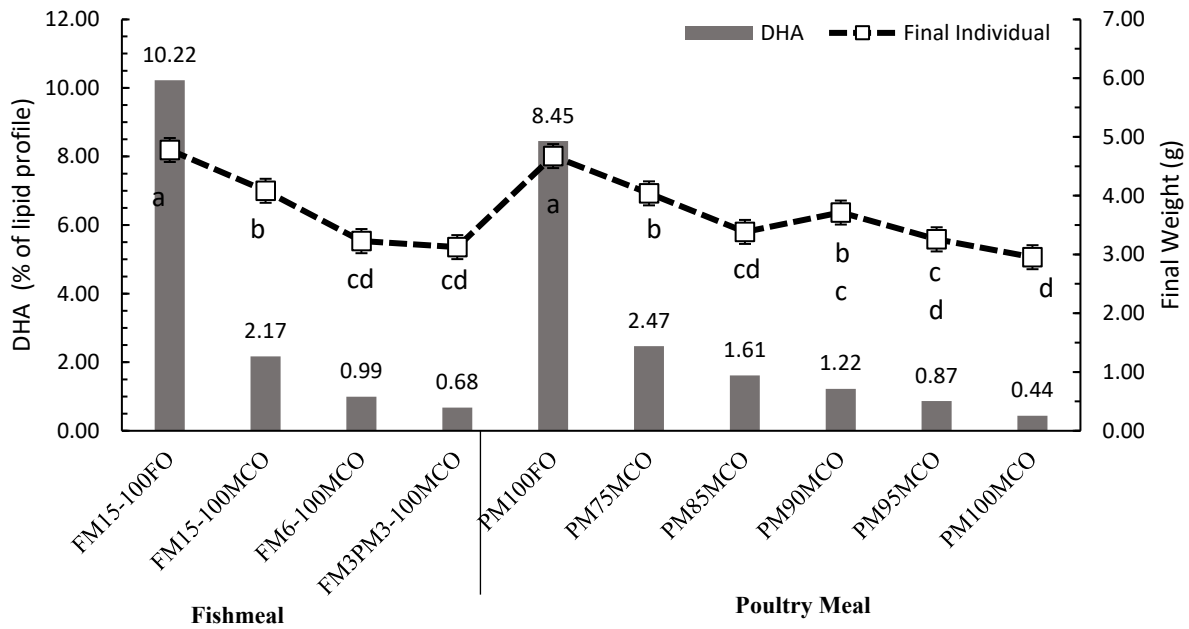
Figure 8. Relationship between DHA (% of lipid profile) in experimental feeds and final weights of shrimp (mean weight 0.22g) offered feeds with various levels of fish oil replacement with modified canola oil and cultured for 7 weeks in a clear water recirculating aquaculture system.

$y = 3.3531x^{0.1542}$
$R^2 = 0.8273$
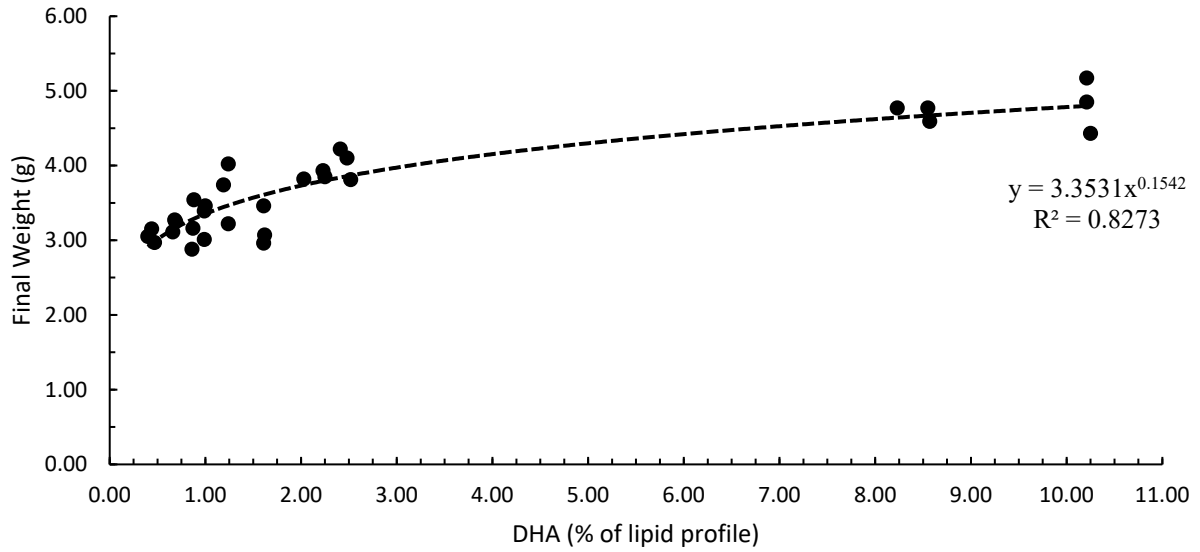
The n3/n6 ratio decrease that was observed in test diets as MCO supplementation increased was also observed in shrimp tissue. This is similar to that of Gonzalez-Felix et al. (2003a), which found that increases in LC-HUFA in diets led to a corresponding increase in LC-HUFA in shrimp tissues. Though this relationship between feed and tissue levels of fatty acids has been well established, Browdy et al. (2007) concluded that the ratio of n3/n6 fatty acids did not affect shrimp production efficiency. Tissue levels of LC-HUFA like DHA were higher than the levels of the same fatty acids in experimental diets, which is in agreement with the results from Araujo et al. (2019). Lim et al. (1997) suggests that shrimp fed diets higher in n3 fatty acids resulted in better growth. The lowest n3/n6 ratio was seen in the PM100MCO diet, as compared to the basal diet with a ratio of 1.97. Gonzalez-Felix et al. (2009) states that the n3/n6 ratio plays a role in shrimp growth, but it is unclear how it affects growth and survival, with supplementation of ARA and DHA being more effective at promoting shrimp growth. Because of this, it is unlikely that n3/n6 ratio is responsible for poor growth and performance with high MCO inclusion.

In Gonzalez-Felix et al. (2003b), the dietary requirement for EPA and DHA was determined to be 0.5% of the lipid content of the diet, as previously mentioned. However, that experiment did not test the requirement using practical protein sources which contribute to the lipid profile, instead extracting oils from protein sources so all oil in the feeds were supplemental. In our experiment, basal protein sources contributed about 50% of the lipid profile, which had a dilution effect on the fatty acid profile of the MCO which contained 0.61% DHA. This suggests that under experimental conditions, the nutritional requirement may be 0.5% of the lipid profile, but under practical conditions the requirement must be higher to account for dilution of the oil profile by native oil sources in protein ingredients. Our results suggest that the nutritional

requirement for DHA in practical conditions is closer to 2% of the lipid profile. After 2% inclusion, shrimp had significantly higher final weights, but growth returns were diminishing.

5. Conclusion

Results from this growth trial indicate that MCO is able to be used at a replacement level of up to 90%. The basal diet and PM100FO performed the best in terms of growth, which was expected because those diets contained full levels of fish oil. From 75%-90% replacement of FO with MCO, shrimp growth was significantly lower than the two aforementioned diets, but was still somewhat acceptable. After 90% replacement, however, animal growth was significantly depressed and feed conversion increased significantly, indicating that growth was stunted due to a nutritional EFA deficiency, likely DHA. The feed palatability experiment did not indicate any significant differences in shrimp consumption, or attractability between diet ingredients, meaning that the decrease in growth and feed conversion must be due to nutritional factors. Our results indicate that the nutritional requirement for DHA is higher than previously determined (0.5%) and is instead closer to 2% because of dilution of the oil profile from native oil sources in practical feed formulations. Additional research with this product is warranted to confirm results and determine the DHA requirement under more practical conditions. More research should also be done to explore other options for fishmeal and fish oil free diets, such as including fermented products as an EFA source when FO is removed.

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Chapter III

Use of Genetically Modified Canola as a Replacement for Fish Oil in Practical Diets for

Litopeneaus vannamei Reared in Green Water Conditions

Abstract

With current advancements in technology allowing for genetic modification of crops, canola has been modified to contain higher levels of n3 long chain fatty acids like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The purpose of this study was to determine the efficacy of using genetically modified canola oil as a DHA replacement for Menhaden fish oil in practical diets for Pacific white shrimp *L. vannamei.* This trial was conducted using 24, 750L tanks stocked at 40 shrimp per tank, and grown for 63 days. Shrimp were fed one of five experimental diets with varying levels of replacement of fish oil as the treatments. Two of the diets were fishmeal-based, while the other three diets utilized poultry meal as the protein source. Shrimp were harvested and frozen after nine weeks to be used for protein retention, lipid extraction and analysis, and taste and texture analysis by an untrained panel to mimic consumer responses. No significant differences were seen between treatments in final individual weight (9.21-10.14) (p=0.1323), final biomass (362.28-387.28) (p=0.3886), feed conversion ratio (1.18-1.23) (p=0.3988), or survival (95.50-98.75) (p=0.4364). Human sensory analysis did not yield significant differences between measured taste parameters, but multiple panelists expressed negative responses to shrimp texture from diet FM-100MCO. Lipid extraction and analysis results showed significant differences in DHA, EPA, ARA, and DPA concentrations from whole shrimp samples.

1. Introduction

The Pacific white shrimp (*Litopaneaus vannamei*) is an increasingly important species to world aquaculture production, accounting for around 80% of globally farmed shrimp (Panini et al. 2017). Though aquaculture production has increased, the industry continues to rely heavily on capture fisheries for feed ingredients like fish oil, which is problematic because 80% of wild fish stocks are fully exploited (Swartz et al. 2010). Additionally, fish oil supply is projected to decrease in the future (Tacon and Metian 2008). Shrimp is the most common frozen seafood product sold in the US, and demand for seafood is expected to continue increase (Dey, Surathkal et al. 2017). Because capture fisheries are fully exploited and demand is continuing to increase for seafood products, the demand must be met by increasing aquaculture production. Intensive shrimp farming relies on commercially produced complete feeds to supply animals with a nutritionally complete diet to allow for optimal growth and feed conversion. Fish oil has been an important feed ingredient in complete aquaculture feeds because of its high concentrated of omega-3 highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (EPA, 20:5n- 3) and docosahexaenoic acid (DHA, 22:6n-3) (Asdari, Aliyu-Paiko et al. 2011). As the aquaculture industry expands and intensifies, the demand for these feed ingredients has increased which has caused a subsequent increase in prices (Shepherd and Bachis 2014). Increasing prices and competition from other sectors like human and pet food make it necessary to find an acceptable replacement that satisfies the nutritional needs of the animals (De Silva and Turchini 2008, Shepherd and Bachis 2014).

Many terrestrial plant oilseeds lack HUFA, specifically DHA, which is essential for growth and survival of aquatic species (Higgs et al. 2006). This problem has encouraged the use of advanced breeding technologies to increase naturally occurring levels of HUFA in plants

(Turchini, Torstensen et al. 2009). Canola is one of the plants that has been genetically manipulated to naturally produce the fatty acids essential to shrimp growth because of its continual increase in global production, and availability for future use (Rosillo-Calle et al. 2009). This study is a continuation of previous research to determine the efficacy of this oil as a replacement for fish oil in practical diets for *L. vannamei*.

2. Materials and Methods

2.1 System Setup

Post-larval shrimp were obtained from American Penaeid (St. James City, Florida, USA) and offered commercial feed (Ziegler 50% protein, 15% fat) in a nursery system for twenty-six days prior to stocking. The growth trial was conducted in a green water outdoor recirculation system at Claude Peteet Mariculture Center (Gulf Shores, AL USA). The research system consisted of a central reservoir (~1,000 L), a 1/3 horsepower circulation pump, 24 circular polyethylene tanks (750 L, 0.85 m height x 1.22 m upper diameter, 1.04 m lower diameter, lower surface area 0.84m^2) and supplemental aeration via air stones. A second sump pump was used to move unfiltered water from a shrimp production pond (thus transferring natural productivity) to the central reservoir at a rate of ~8 L min. This pump was running for approximately 4 hours per day. Juvenile shrimp were hand-sorted to uniform size (0.1g) and 40 shrimp were stocked into each tank. Shrimp in this system were fed one of five experimental diets, with treatments one through four being replicated five times, and the fifth treatment being replicated four times.

2.2 Diet Preparation

A total of five experimental diets were made for this growth trial, including two fishmeal-based diets (FM-FO and FM-MCO) and three poultry meal-based diets (PM-FO, PM-75MCO, PM-95MCO). All diets were formulated to contain 36% protein and 8% lipid. Soybean meal and

corn protein concentrate were the primary plant-based protein sources, with fishmeal and poultry meal being the primary animal-based protein sources. Menhaden fish oil was the primary lipid source and was replaced at varying levels with the modified canola oil (Table 1). Pre-ground dry ingredients and oil were weighed and mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 min. The lipid sources and then boiling water (30-40% by weight) were then blended into the mixture to attain a consistency appropriate for pelleting. Finally, all diets were pressure-pelleted using a meat grinder with a 3-mm die, dried in a forced air oven (50°C) to a moisture content of less than 10% and stored at -4°C.

2.3 Water Quality and Management

During the growth trial, dissolved oxygen, water temperature, pH and salinity were measured twice daily using a YSI multi-parameter instrument (YSI, Yellow Springs, OH, USA). Total ammonia nitrogen was analyzed once per week using a Thermo Orion ISE probe, while nitrite and nitrate were analyzed once per week using a WaterLink SpinTouchFF meter (LaMotte Company Chesterton, MD, USA).

2.4 Feed and Treatments

Commercial feed (Ziegler 50% protein, 15% fat) was fed for twenty-six days, from arrival until system stocking. When experimental treatments were assigned, feed inputs were based on a preprogramed standard feeding protocol for which we assume shrimp doubled their weight until reaching 1.3g. After reaching 1.3g shrimp were assumed to gain 1.3g per week for the remainder of the trial and have an assumed FCR of 1.2.

Table 1. Formulation of experimental diets used for green water growth trial of post larval shrimp stocked at 40 shrimp per tank (53 shrimp/m^2) (0.10g initial weight) and grown for 10 weeks. Values are reported as percentage of the diet. All diets were formulated to contain 36% protein and 8% lipid on an "as is" basis.

Ingredient (g/100g as is)	FM-FO	FM-MCO	PM-FO	PM-75MCO	PM-95MCO
Menhaden Fishmeal[1]	15.00	15.00	0.00	0.00	0.00
Poultry Meal[2]	0.00	0.00	6.00	6.00	6.00
Soybean Meal[3]	49.25	49.25	50.10	50.10	50.10
Corn Protein Concentrate[4]	0.00	0.00	7.00	7.00	7.00
Menhaden Fish Oil[5]	5.09	0.00	5.51	1.38	0.28
Lecithin (soy)[6]	1.00	1.00	1.00	1.00	1.00
Latitude Oil[7]	0.00	5.09	0.00	4.13	5.23
Cholesterol[8]	0.12	0.12	0.12	0.12	0.12
Corn Starch[8]	0.33	0.33	2.67	2.67	2.67
Whole Wheat[9]	25.61	25.61	24.00	24.00	24.00
Mineral Premix[10]	0.50	0.50	0.50	0.50	0.50
Vitamin Premix[11]	1.80	1.80	1.80	1.80	1.80
Choline Chloride[12]	0.20	0.20	0.20	0.20	0.20
Stay-C 35%[13]	0.10	0.10	0.10	0.10	0.10
CaP-dibasic[14]	1.00	1.00	1.00	1.00	1.00

FM-Fishmeal
FO-Fish Oil
PM-Poultry Meal
MCO-Modified Canola Oil

1. Special Select™, Omega Protein Inc., Hammond, Louisiana, USA. 2.Chicken by product meal (Darling ingredient) 3. De-hulled solvent-extracted soybean meal, Bunge Limited, Decatur, AL, USA 4. CPC - Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.5. Omega Protein, Inc., Houston, TX, USA 6. The Solae Company, St. Louis, MO, USA. 7. Cargill Crop 2019 Latitude, FC012320APFO. 8. MP Biomedicals Inc., Santa Ana, CA, USA. 9. Bob's Red Mill Natural Foods, Milwaukie, OR, USA. 10. Mineral premix (g/100 g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.550; Ferrous sulfate, 2.000; Magnesium sulfate anhydrous, 13.862; Manganese sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate heptahydrate, 13.193; Alpha- cellulose, 69.664. 11. Vitamin premix (g kg-1 premix): Thiamin. HCl, 4.95; Riboflavin, 3.83; Pyridoxine. HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Bio n, 0.50; folic acid, 4.00; Cyanocobalamin, 0.05; Inositol, 25.00; Vitamin A acetate (500,000 IU/g), 0.32; Vitamin D3 (1,000,000 IU/g), 80.00; Menadione, 0.50; Alpha-cellulose, 856.81. 12. Amresco Inc., Solon, Ohio, USA. 13. Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), DSM Nutritional Products., Parsippany, NJ, USA. 14. Alfa Aesar, Ward Hill, MA, USA

2.5 Termination

Shrimp in each tank were captured, counted and group weighed to calculate survival, biomass, mean weight, FCR and weight gain. After weighing and counting the shrimp, shrimp were frozen to be used for lipid profile analysis and human sensory analysis. Ten shrimp per tank were frozen for Cargill sensory analysis, twenty were selected to be used for sensory analysis at Auburn University, and the remaining shrimp were frozen to be used for lipid analysis. Shrimp selected for taste analysis were frozen in plastic bags filled with water and kept in cardboard boxes at -80°C to preserve the flesh. Shrimp selected for lipid extraction were frozen inside plastic bags.

2.6 Fatty Acid Analysis

Fatty acids were extracted from feeds and body samples at Auburn University and sent to Cargill to analyze the fatty acid profile. Shrimp samples were stored at -80°C in plastic bags, removed one hour prior to analysis, and placed in a warm water bath to thaw. Three shrimp from each tank were pooled into a representative sample and homogenized. Shrimp hepatopancreas and tail meat samples were also analyzed. From each sample of shrimp body and diets, two random sub-samples were taken with a weight of 2g each from shrimp whole body and tail meat, and an approximate weight of 0.6g per sub-sample of feed or hepatopancreas. These sub-samples were extracted using the methods of (Folch et al. 1957). In short, weighed tissue or feed was homogenized using a Polytron homogenizer in 20 mL of chloroform/methanol (2:1) for 1.5 minutes. The homogenate was filtered through sintered glass filter covered with a glass microfibre filter paper into a screw cap test tube. The residue was re-extracted with 14 mL of chloroform/methanol (2:1) with a Polytron homogenizer for 1.5 minutes and again filtered through the sintered glass filter into the screw cap test tube. Then, the screw cap test tube filled

with the filtrate was brought to 40 mL volume with chloroform/methanol (2:1). To this, 8 mL distilled water was added and flushed with nitrogen, then the test tube was capped and inverted to mix. This was stored in a refrigerator (dark) overnight to allow phases to separate. The upper phase was then removed with a pipette and the lower phase washed with fresh upper phase (chloroform: methanol: water 3:48:47) three times by gently allowing it to flow down the side of the test tube. A minimum amount of methanol was added to make one phase. Then, 0.5 g sodium sulfate was added, and the solution decanted to a dried pre-weighed test tube. The chloroform was evaporated using a heated water bath and stream of nitrogen gas, the tubes were then dried and weighed. The percent (%) lipid was then calculated (on a dry weight basis). After the extractions, oils from sub-samples were transferred to 2 mL vials, dried by the nitrogen evaporator, and flushed with nitrogen gas. The samples were stored at -80°C in an ultra-freezer and sent to Cargill's oil division laboratory, Colorado, USA for fatty acid composition analysis. The fatty acid compositions of the samples were analyzed by gas chromatography (GC) method. Total lipid content was expressed as percent of wet tissue or dry diet. First, the extracted oil samples from shrimp or diets were suspended by 500 uL of Isooctane with 100 ppm Butylated Hydroxytoluene (BHT). Second, 100 uL of the suspended sample was added to a 15mL polypropylene conical tube, along with 1mL of Isooctane and 100 uL of 1N potassium hydroxide in methanol. Then, samples were vortexed at 3000 rpm for 30 seconds and centrifuged at 3000rpm for 5 mins. Five-hundred microliter of supernatant (isooctane layer) was removed from conical and added to GC vial and crimp capped. Then, all samples were analyzed using an Agilent 7890B with a Flame Ionization Detector. Retention time confirmation was induced by using Nu-Check GLC566 FAME Standard. BHT peak was removed from chromatograms of

samples prior to analysis. Individual fatty acid methyl esters (FAMEs) were calculated as % of total peak area.

2.7 Sensory Analysis

Sensory analysis was conducted in two sessions within the same day. Panelists were not trained in order to mimic the response of a typical consumer. Shrimp samples were assigned a random three-digit code each session in order to prevent panelists from identifying treatment.

Shrimp were frozen into water and stored in a cardboard box at -80°C in order to preserve flesh quality. Twenty-four hours in advance of the sensory analysis evaluation, samples were thawed in a refrigerator. Prior to cooking, random subsamples were taken from each tank in order to form a representative sample reflecting treatment to cook for panelists. Shrimp were de-headed and cooked with the shell on. Shrimp samples were boiled for 1 minute and 15 seconds and internal temperature was measured to ensure thorough cooking. After being removed from the boiling water, shrimp were placed in an ice bath and then peeled and refrigerated until being served. Each panelist received one shrimp from each treatment, and each treatment received 33 responses, 17 in the AM session, and 16 in the PM session. Panelists evaluated both taste and texture characteristics. Cooked shrimp were evaluated for appearance, flavor, texture, and juiciness.

2.8 Statistical Analysis

All growth data was analyzed using SAS (V9.3. SAS Institute, Cary, NC, USA). Data was subjected to one-way analysis of variance (ANOVA) to determine significant differences ($P<0.05$) among treatments, followed by Tukey's multiple comparison test to determine differences between treatment means. These tests were also conducted on sensory analysis

results to determine if there are differences between treatment means. An independent T-test was performed to compare diets in terms of shrimp growth performance.

3. Results

Water quality parameters were within the acceptable range for growth and survival throughout the growth trial, with values presented in Table 2. Growth performance results are presented in Table 3. No significant differences between treatments ($P<0.05$) were seen between survival (95.5-98.7) (p=0.4364), final biomass (362.28-387.28) (p=0.3886), feed conversion ratio (1.17-1.23) (p=0.3988), or final individual weight (9.21-10.14) (p=0.1323). Fatty acid profile results from experimental feeds are presented in Table 4. Fatty acid profiles from shrimp whole body are presented in Table 5. Whole body EFA values were higher than the values in the feeds, which is to be expected. Whole body EFA values followed the expected trends, with diets high in certain fatty acids leading to tissues high in the same fatty acids. For example, in experimental diets, DHA ranged from 0.72 in PM-95MCO to 9.44 in FM-FO, while whole body values ranged from 3.62 in PM-95MCO to 8.92 in PM-FO. This demonstrates the trend that the diets low in DHA led to whole body tissues low in DHA, while diets high in DHA led to shrimp tissues high in DHA. Similarly, EPA feed values ranged from 6.31 in PM-95MCO to 10.91 in FM-FO, while whole body tissue values ranged from 13.78 in FM-FO to 12.35 in FM-MCO. DPA feed values ranged from 1.37 in PM-95MCO to 1.82 in FM-FO, and tissue values ranged from 1.14 in FM-MCO to 2.06 in PM-95MCO. ARA values ranged from 0.89 in FM-FO to 1.48 in FM-MCO, and tissue values ranged from 1.45 in FM-FO to 4.66 in PM-MCO. DHA, DPA, and ARA values were all significantly different between treatments (p<0.0001). EPA values were not significantly different between treatments, with a p-value of 0.0819.

Table 2. Water quality parameters throughout the culture period of post-larval shrimp (0.10g initial weight) stocked at 40 shrimp per tank and grown for 10 weeks in a green-water system.

Water parameters	Growth Trial
Dissolved Oxygen (mg/L)	6.94 ± 0.36
Temperature (°C)	28.17 ± 0.54
Salinity (ppt)	5.99 ± 0.24
pH	7.49 ± 0.59
Ammonia (mg/L)	0.26 ± 0.17
Nitrite (mg/L)	0.03 ± 0.01

Table 3. Growth performance results of juvenile shrimp stocked at 40 shrimp per tank (53 shrimp/m^2) and grown for 10 weeks in a green-water system. Data was subjected to a two-way ANOVA and a Tukey's test to determine significantly different means. Letters denote statistical differences (p<0.05) between variables.

Treatment	Individual Initial	Initial Biomass	Survival %	Final Biomass	Final Weight (g)	FCR
FM-FO	0.10[a]	4.06[a]	95.5[a]	387.28[a]	10.14[a]	1.17[a]
FM-MCO	0.10[a]	4.05[a]	98.0[a]	362.28[a]	9.21[a]	1.23[a]
PM-FO	0.10[a]	3.92[a]	95.5[a]	368.92[a]	9.67[a]	1.22[a]
PM-75MCO	0.10[a]	3.96[a]	97.0[a]	371.28[a]	9.52[a]	1.22[a]
PM-95MCO	0.10[a]	4.06[a]	98.7[a]	383.15[a]	9.70[a]	1.18[a]
p-value	0.89	0.90	0.44	0.39	0.13	0.40
PSE	0.01	0.29	3.15	21.76	0.53	0.07

Table 4. Fatty acid profile (% of lipid profile) of experimental diets offered to juvenile shrimp during a 10-week culture period with various replacement levels of fish oil and fishmeal with modified canola and poultry meal, respectively. Lipids were extracted from diets in triplicates. Data was subjected to a two-way ANOVA and a Tukey's test to determine significantly different means. Results are presented as mean ± *SE*.

Fatty Acid	FM-FO	FM-MCO	PM-FO	PM-75MCO	PM-95MCO
C14:0	9.44	2.04	7.38	2.28	0.72
C14:1n-5	10.91	7.78	9.21	7.15	6.31
C15:0	0.50	0.10	0.42	0.13	0.02
C16:0	16.96	8.69	17.10	10.72	8.87
C16:1n-7	9.15	1.25	8.50	2.68	1.08
C17:0	0.66	0.14	0.51	0.18	0.08
C18:0	3.22	3.26	3.43	3.46	3.36
C18:1n-9	7.55	24.87	11.43	24.16	28.53
C18:1n-7	2.67	2.34	2.48	2.25	2.21
C18:2n-9	0.06	1.47	0.08	1.08	1.36
C18:2n-6	13.42	31.21	17.03	29.24	32.99
C18:3n-6	0.26	1.54	0.27	1.24	1.43
C18:3n-3	2.43	3.12	2.42	2.89	2.98
C18:4n-3	2.37	0.47	1.95	0.62	0.23
C20:0	0.23	0.50	0.23	0.42	0.48
C20:1n-9	0.56	0.58	0.52	0.56	0.56
C20:2n-6	0.16	0.09	0.15	0.10	0.09
C20:3n-6	0.21	2.51	0.24	1.98	2.42
C20:4n-6 ARA	0.89	1.48	0.90	1.36	1.42
C20:3n-3	0.16	0.06	0.13	0.06	0.03
C20:4n-3	1.08	0.99	0.93	0.88	0.85
C20:5n-3 EPA	10.91	7.78	9.21	7.15	6.31
C22:0	0.19	0.25	0.17	0.22	0.23
C22:1n-9	0.10	0.08	0.08	0.09	0.08
22:2n-6	0.03	0.04	0.00	0.01	0.03
23:0	0.09	0.09	0.08	0.08	0.08
22:4n-6	0.16	0.28	0.18	0.27	0.28
22:3n-6	0.05	0.01	0.00	0.00	0.00
22:5n-3 DPA	1.82	1.62	1.53	1.46	1.37
24:0	0.12	0.13	0.09	0.11	0.11

22:6n-3 DHA	9.44	2.04	7.38	2.28	0.72
24:1n-9	0.20	0.07	0.17	0.10	0.08

FM = Fishmeal
PM = Poultry meal
FO = Fish Oil
MCO = Modified Canola Oil

Table 5. Fatty acid profile (% of lipid profile) of lipids extracted from whole shrimp (mean initial weight 0.1g) grown in green water for 10 weeks and fed diets with various replacement levels of fish oil and fishmeal with modified canola and poultry meal, respectively. Data was subjected to a two-way ANOVA and a Tukey's test to determine significantly different means. The results are presented Mean ± *SE*.

Fatty Acid	FM-FO	FM-MCO	PM-FO	PM-75MCO	PM-95MCO	p-value
C14_0	4.28 ± 0.08	0.30 ± 0.03	1.14 ± 0.16	0.32 ± 0.06	0.13 ± 0.03	<0.0001
C15_0	0.72 ± 0.03	0.26 ± 0.06	0.36 ± 0.05	0.21 ± 0.04	0.18 ± 0.04	<0.0001
C16_0	26.33 ± 0.20	15.80 ± 0.22	20.19 ± 0.19	16.68 ± 0.33	15.29 ± 0.27	<0.0001
C16_1 (n-7)	6.03 ± 0.24	0.78 ± 0.09	2.71 ± 0.16	0.94 ± 0.61	0.53 ± 0.34	<0.0001
C17_0	1.22 ± 0.06	0.87 ± 0.05	1.15 ± 0.02	0.76 ± 0.03	0.71 ± 0.04	<0.0001
C18_0	3.66 ± 0.02	8.80 ± 0.01	10.24 ± 0.02	9.12 ± 0.02	9.19 ± 0.03	0.0015
C18_1 (n-9)	8.13 ± 0.15	17.49 ± 0.34	12.02 ± 0.16	17.59 ± 0.66	18.72 ± 0.30	<0.0001
C18_1 (n-7)	3.10 ± 0.02	3.22 ± 0.01	3.33 ± 0.02	3.05 ± 0.02	3.03 ± 0.02	<0.0001
C18_2 (n-9)	0.04 ± 0.03	0.43 ± 0.03	0.09 ± 0.03	0.31 ± 0.04	0.35 ± 0.05	<0.0001
C18_2 (n-6)	13.47 ± 0.02	18.33 ± 0.3	13.94 ± 0.2	18.13 ± 0.7	18.50 ± 0.3	<0.0001
C18_3 (n-6)	0.14 ± 0.01	0.26 ± 0.01	0.17 ± 0.02	0.20 ± 0.01	0.22 ± 0.01	0.0050
C18_3 (n-3)	1.48 ± 0.03	1.06 ± 0.02	0.96 ± 0.03	0.97 ± 0.04	0.95 ± 0.01	0.1627
C18_4 (n-3)	0.82 ± 0.04	0.01 ± 0.06	0.23 ± 0.06	0.02 ± 0.07	0.00 ± 0.10	<0.0001
C20_0	0.32 ± 0.02	0.40 ± 0.01	0.34 ± 0.04	0.35 ± 0.06	0.36 ± 0.04	<0.0001
C20_1 (n-9)	0.72 ± 0.08	0.72 ±0.09	0.61 ± 0.14	0.69 ± 0.27	0.74 ± 0.13	0.0016
C20_2 (n-6)	1.01 ± 0.01	2.21 ± 0.1	1.35 ± 0.1	2.17 ± 0.1	2.39 ± 0.1	<0.0001
C20_3 (n-6)	0.24 ± 0.01	1.27 ± 0.01	0.23 ± 0.02	1.09 ± 0.02	1.36 ± 0.02	<0.0001
C20_4 (n-6) ARA	1.45 ± 0.26	4.06 ± 0.13	2.97 ± 0.49	4.13 ± 0.50	4.66 ± 0.30	<0.0001
C20_3 (n-3)	0.28 ± 0.02	0.25 ± 0.02	0.26 ± 0.05	0.24 ± 0.03	0.24 ± 0.04	<0.0001
C20_4 (n-3)	0.54 ± 0.03	0.40 ± 0.03	0.33 ± 0.03	0.37 ± 0.04	0.44 ± 0.02	0.0015
C20_5 (n-3) EPA	13.78 ± 0.00	12.35 ± 0.02	12.60 ± 0.00	12.78 ± 0.00	13.30 ± 0.00	0.0819
C22_0	0.28 ± 0.00	0.23 ± 0.00	0.32 ± 0.00	0.20 ± 0.00	0.22 ± 0.00	0.1120
C22_1 (n-9)	0.19 ± 0.03	0.03 ± 0.02	0.04 ± 0.02	0.08 ± 0.03	0.06 ± 0.02	0.3833
C22_4 (n-6)	0.12 ± 0.00	0.16 ± 0.00	0.07 ± 0.00	0.15 ± 0.00	0.24 ± 0.00	0.0002
C22_5 (n-3) DPA	1.50 ± 0.04	1.62 ± 0.03	1.14 ± 0.04	1.59 ± 0.09	2.06 ± 0.03	<0.0001
C24_0	0.11 ± 0.01	0.12 ± 0.00	0.13 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.0112
C22_6 (n-3) DHA	7.84 ± 0.29	5.48 ± 0.18	8.92 ± 0.51	5.30 ± 0.40	3.62 ± 0.21	<0.0001
C24_1 (n-9)	0.18 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.8212

Tail meat fatty acid analysis results are presented in Table 6, and hepatopancreas results are presented in Table 7. Whole body, tail meat, and hepatopancreas DPA, DHA, ARA, and EPA results are modeled in Figures 1-3. Results show that EFA concentrations were highest in tail meat tissues compared with whole body and hepatopancreas values. For example, in FM-FO, DHA was 7.84% of the oil in the hepatopancreas and whole body, but 11.42% in the tail meat. DPA, however, was the only EFA that tended to have lower or equal values in the tail meat as opposed to the hepatopancreas and whole body. In FM-FO, DPA was 0.96% of the oil in the tail meat but was 1.50% of the oil in the hepatopancreas and whole body. In order to visualize the relationship between EFAs ARA, EPA, DPA, and DHA, shrimp final weight is compared to each respective lipid level in the diets in figures 4-7.

Results from the human taste panel are presented in Table 8. No sensory differences were indicated between treatments. However, consistent complaints of less than desirable "mushy" texture experienced when eating shrimp fed FM-MCO were received in the comment section available on the sensory scoring sheet.

Table 6. Fatty acid profile (% of lipid profile) of lipids extracted from tail meat of shrimp (mean initial weight 0.1g) grown in green water for 10 weeks and fed diets with various replacement levels of fish oil and fishmeal with modified canola and poultry meal, respectively. Data was subjected to a two-way ANOVA and a Tukey's test to determine significantly different means. The results are presented Mean $\pm$ *SE*.

Fatty Acid	FM-FO	FM-MCO	PM-FO	PM-75MCO	PM-95MCO	p-value
C14_0	0.88 ± 0.09	0.18 ± 0.05	0.71 ± 0.02	0.12 ± 0.05	0.05 ± 0.03	<0.0001
C15_0	0.24 ± 0.07	0.13 ± 0.05	0.32 ± 0.02	0.11 ± 0.03	0.13 ± 0.05	0.0273
C16_0	20.28 ± 0.48	17.32 ± 0.57	20.95 ± 0.15	17.69 ± 0.14	16.33 ± 0.36	<0.0001
C16_1 (n-7)	2.76 ± 0.10	0.70 ± 0.06	2.24 ± 0.02	0.73 ± 0.02	0.41 ± 0.03	<0.0001
C17_0	1.28 ± 0.04	1.00 ± 0.05	1.21 ± 0.03	0.79 ± 0.04	0.74 ± 0.05	<0.0001
C18_0	10.87 ± 0.17	10.21 ± 0.24	10.89 ± 0.11	10.16 ± 0.18	10.39 ± 0.10	0.0114
C18_1 (n-9)	10.30 ± 0.38	16.90 ± 0.37	11.82 ± 0.16	16.32 ± 0.35	17.08 ± 0.33	<0.0001
C18_1 (n-7)	3.91 ± 0.07	3.46 ± 0.08	3.52 ± 0.06	3.32 ± 0.03	3.15 ± 0.08	<0.0001
C18_2 (n-9)	0.06 ± 0.02	0.26 ± 0.07	0.00 ± 0.00	0.18 ± 0.05	0.26 ± 0.03	0.0006
C18_2 (n-6)	13.05 ± 0.35	16.64 ± 0.49	14.01 ± 0.25	16.19 ± 0.15	16.01 ± 0.16	<0.0001
C18_3 (n-6)	0.10 ± 0.04	0.07 ± 0.05	0.09 ± 0.02	0.04 ± 0.03	0.11 ± 0.04	0.6969
C18_3 (n-3)	1.01 ± 0.06	0.90 ± 0.05	0.91 ± 0.03	0.82 ± 0.03	0.79 ± 0.04	0.0242
C18_4 (n-3)	0.10 ± 0.04	0.00 ± 0.00	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.0080
C20_0	0.13 ± 0.05	0.24 ± 0.06	0.17 ± 0.03	0.19 ± 0.05	0.30 ± 0.01	0.1845
C20_1 (n-9)	0.37 ± 0.09	0.46 ± 0.12	0.42 ± 0.02	0.42 ± 0.11	0.62 ± 0.00	0.3826
C20_2 (n-6)	1.20 ± 0.04	2.08 ± 0.06	1.27 ± 0.04	1.93 ± 0.04	2.49 ± 0.05	<0.0001
C20_3 (n-6)	0.12 ± 0.05	1.14 ± 0.04	0.19 ± 0.04	1.08 ± 0.13	1.18 ± 0.03	<0.0001
C20_4 (n-6) ARA	2.74 ± 0.10	4.10 ± 0.09	2.95 ± 0.09	4.19 ± 0.06	4.83 ± 0.05	<0.0001
C20_3 (n-3)	0.21 ± 0.06	0.16 ± 0.05	0.22 ± 0.02	0.47 ± 0.23	0.24 ± 0.03	0.3626
C20_4 (n-3)	0.19 ± 0.06	0.31 ± 0.09	0.31 ± 0.03	0.40 ± 0.06	0.45 ± 0.05	0.0682
C20_5 (n-3) EPA	15.22 ± 0.22	14.54 ± 0.48	15.17 ± 0.15	15.89 ± 0.21	16.08 ± 0.29	0.0123
C22_0	0.06 ± 0.03	0.01 ± 0.01	0.07 ± 0.04	0.01 ± 0.01	0.08 ± 0.03	0.1750
C22_1 (n-9)	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.04 ± 0.02	0.4048
C22_4 (n-6)	0.00 ± 0.00	0.03 ± 0.02	0.04 ± 0.03	0.04 ± 0.02	0.12 ± 0.05	0.0946
C22_5 (n-3) DPA	0.96 ± 0.00	1.52 ± 0.02	0.93 ± 0.03	1.50 ± 0.02	2.17 ± 0.05	<0.0001
C24_0	0.09 ± 0.03	0.10 ± 0.05	0.08 ± 0.03	0.08 ± 0.04	0.08 ± 0.05	0.9377
C22_6 (n-3) DHA	11.42 ± 0.38	6.34 ± 0.28	9.73 ± 0.14	5.97 ± 0.23	4.28 ± 0.33	<0.0001
C24_1 (n-9)	0.17 ± 0.04	0.15 ± 0.03	0.13 ± 0.03	0.11 ± 0.03	0.14 ± 0.02	0.7188

Table 7. Fatty acid profile (% of lipid profile) of lipids extracted from hepatopancreas of shrimp (mean initial weight 0.10g) grown in green water for 10 weeks and fed diets with various replacement levels of fish oil and fishmeal with modified canola and poultry meal, respectively. Data was subjected to a two-way ANOVA and a Tukey's test to determine significantly different means. The results are presented Mean $\pm$ *SE*.

Fatty Acid	FM-FO	FM-MCO	PM-FO	PM-75MCO	PM-95MCO	p-value
C15_0	0.72 ± 0.02	0.34 ± 0.02	0.67 ± 0.08	0.32 ± 0.01	0.25 ± 0.02	<0.0001
C16_0	26.33 ± 0.25	16.09 ± 0.19	26.20 ± 1.88	18.83 ± 0.46	16.42 ± 0.41	<0.0001
C16_1 (n-7)	6.03 ± 0.19	1.25 ± 0.05	5.20 ± 0.77	1.88 ± 0.04	0.97 ± 0.07	<0.0001
C17_0	1.22 ± 0.13	0.60 ± 0.03	0.99 ± 0.16	0.54 ± 0.05	0.40 ± 0.05	<0.0001
C18_0	3.66 ± 0.10	2.71 ± 0.11	3.54 ± 0.21	2.60 ± 0.06	2.41 ± 0.05	<0.0001
C18_1 (n-9)	8.13 ± 0.31	19.95 ± 0.47	10.99 ± 2.02	20.74 ± 0.45	21.58 ± 1.73	<0.0001
C18_1 (n-7)	3.10 ± 0.10	2.28 ± 0.04	2.66 ± 1.59	2.22 ± 0.05	4.10 ± 2.03	0.4429
C18_2 (n-9)	0.04 ± 0.01	0.60 ± 0.03	0.04 ± 0.10	0.44 ± 0.02	0.57 ± 0.02	<0.0001
C18_2 (n-6)	13.47 ± 0.09	28.04 ± 0.23	15.41 ± 2.56	26.42 ± 0.19	29.73 ± 0.44	<0.0001
C18_3 (n-6)	0.14 ± 0.01	0.66 ± 0.03	0.13 ± 0.10	0.51 ± 0.02	0.63 ± 0.02	<0.0001
C18_3 (n-3)	1.48 ± 0.09	1.85 ± 0.03	1.35 ± 0.17	1.64 ± 0.08	1.63 ± 0.17	0.0155
C18_4 (n-3)	0.82 ± 0.02	0.15 ± 0.01	0.71 ± 0.12	0.20 ± 0.01	0.08 ± 0.00	<0.0001
C20_0	0.32 ± 0.02	0.34 ± 0.01	0.33 ± 0.02	0.30 ± 0.01	0.27 ± 0.02	0.0832
C20_1 (n-9)	0.72 ± 0.02	0.71 ± 0.03	0.70 ± 0.05	0.76 ± 0.03	0.79 ± 0.04	0.8584
C20_2 (n-6)	1.01 ± 0.12	1.43 ± 0.31	0.69 ± 0.31	1.77 ± 0.11	2.05 ± 0.10	0.0023
C20_3 (n-6)	0.24 ± 0.04	1.57 ± 0.05	0.26 ± 0.29	1.42 ± 0.07	1.69 ± 0.13	<0.0001
C20_4 (n-6) ARA	1.45 ± 0.10	2.17 ± 0.14	1.45 ± 0.08	1.82 ± 0.11	1.86 ± 0.11	0.0004
C20_3 (n-3)	0.28 ± 0.03	0.21 ± 0.02	0.27 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	0.0331
C20_4 (n-3)	0.54 ± 0.03	0.51 ± 0.01	0.48 ± 0.05	0.50 ± 0.03	0.52 ± 0.06	0.7410
C20_5 (n-3) EPA	7.60 ± 0.03	7.54 ± 0.45	6.88 ± 0.20	6.52 ± 0.09	6.03 ± 0.09	0.0023
C22_0	0.28 ± 0.05	0.31 ± 0.07	0.27 ± 0.05	0.15 ± 0.01	0.13 ± 0.02	0.0966
C22_1 (n-9)	0.19 ± 0.05	0.16 ± 0.05	0.12 ± 0.05	0.13 ± 0.04	0.16 ± 0.09	0.8584
C23_0	0.05 ± 0.03	0.00 ± 0.02	0.05 ± 0.03	0.02 ± 0.01	0.05 ± 0.01	0.1069
C22_4 (n-6)	0.12 ± 0.00	0.26 ± 0.00	0.18 ± 0.00	0.27 ± 0.00	0.32 ± 0.00	<0.0001
C22_5 (n-3) DPA	1.50 ± 0.03	1.58 ± 0.05	1.40 ± 0.01	1.41 ± 0.02	1.47 ± 0.02	0.0018
C24_0	0.11 ± 0.01	0.08 ± 0.00	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.00	0.0066
C22_6 (n-3) DHA	7.84 ± 0.33	3.09 ± 0.13	6.75 ± 0.80	2.84 ± 0.05	1.47 ± 0.09	<0.0001
C24_1 (n-9)	0.18 ± 0.02	0.08 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	<0.0001

Figure 1. Fatty acid levels (% of lipid profile) extracted from the tail meat of shrimp (mean initial weight 0.1g) grown in green water for 10 weeks and fed diets with various replacement levels of fish oil and fishmeal with modified canola and poultry meal, respectively.
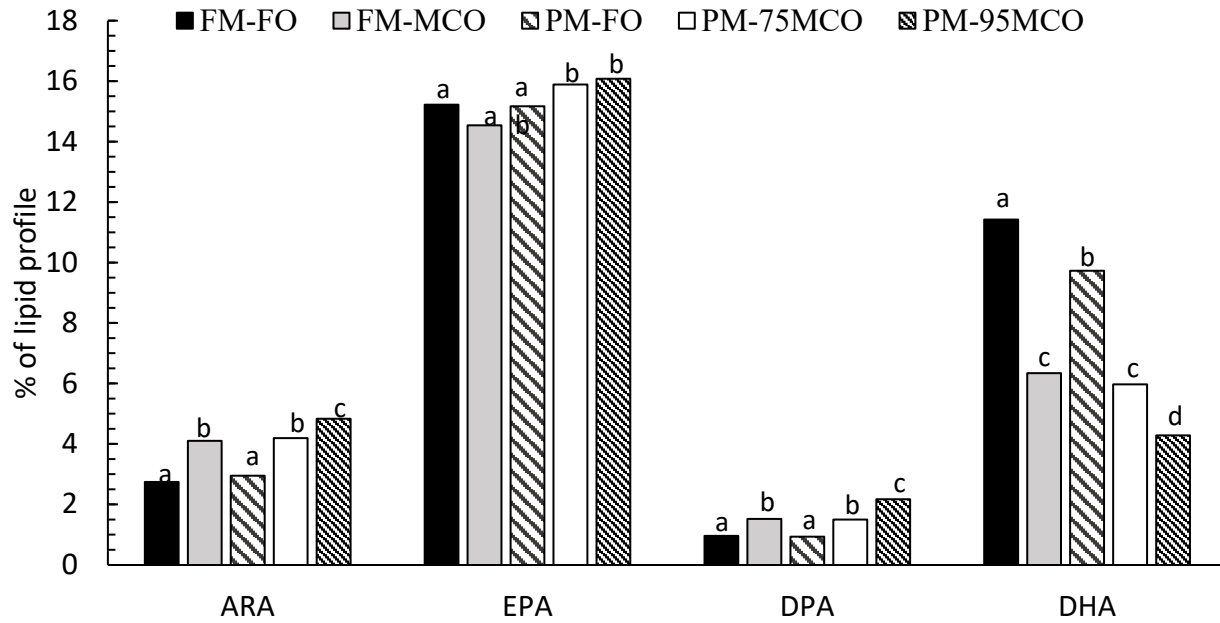
Figure 2. Fatty acid levels (% of lipid profile) extracted from the hepatopancreas of shrimp (mean initial weight 0.1g) grown in green water for 10 weeks and fed diets with various replacement levels of fish oil and fishmeal with modified canola and poultry meal, respectively.
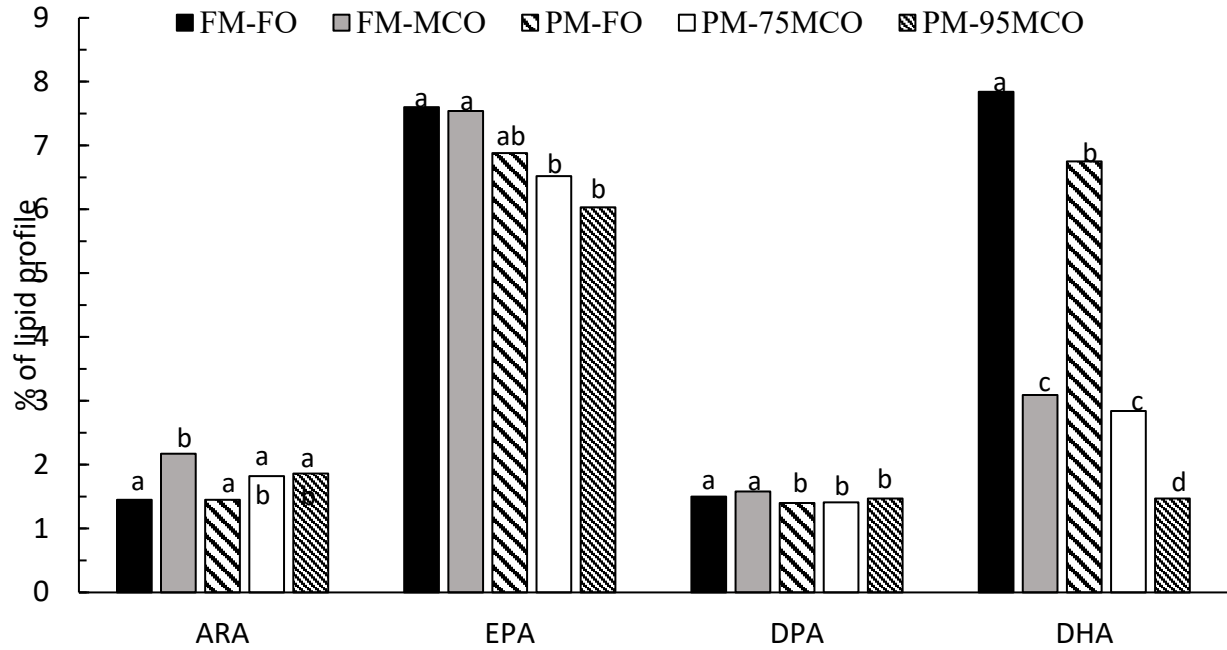
Figure 3. Fatty acid levels (% of lipid profile) extracted from the whole body of shrimp (mean initial weight 0.10g) grown in green water for 10 weeks and fed diets with various replacement levels of fish oil and fishmeal with modified canola and poultry meal, respectively.
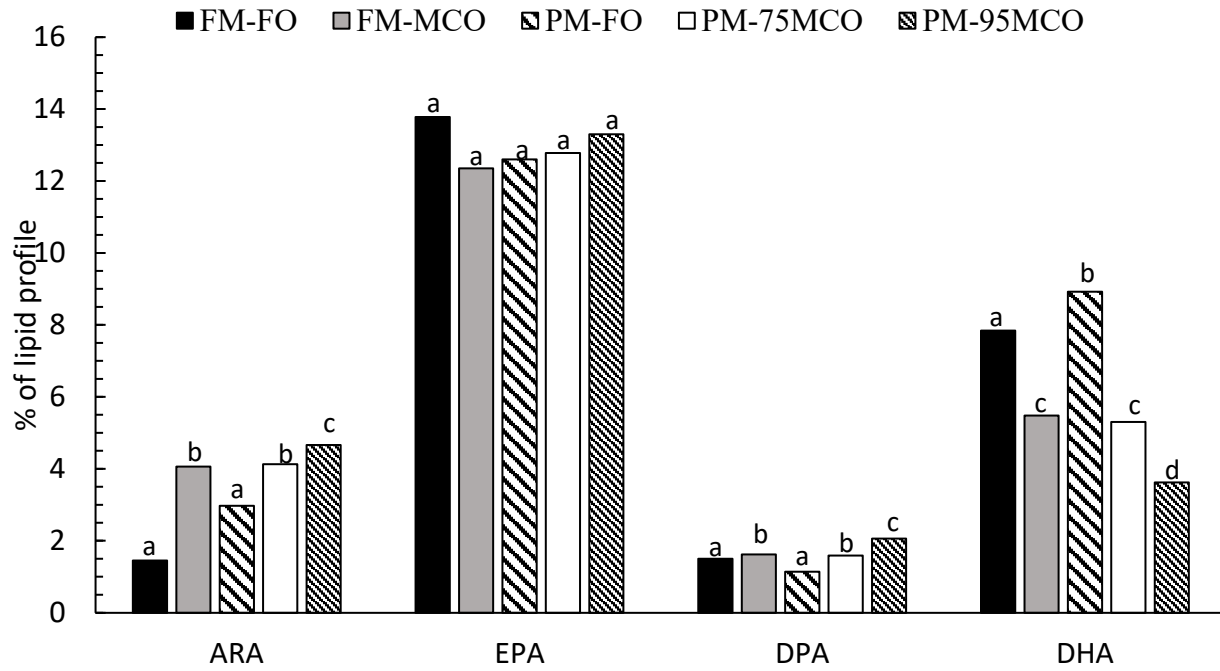
Figure 4. The relationship between final body weight (g) and ARA (Arachidonic acid, C20:4n-6) level (% of lipid profile) in test diets using modified canola oil (MCO) to replace menhaden fish oil (FO) in feeds offered to juvenile shrimp (mean weight 0.10g) cultured for 10 weeks in a green water recirculating aquaculture system. Data was subjected to a two-way ANOVA and a Tukey's test to determine significantly different means.
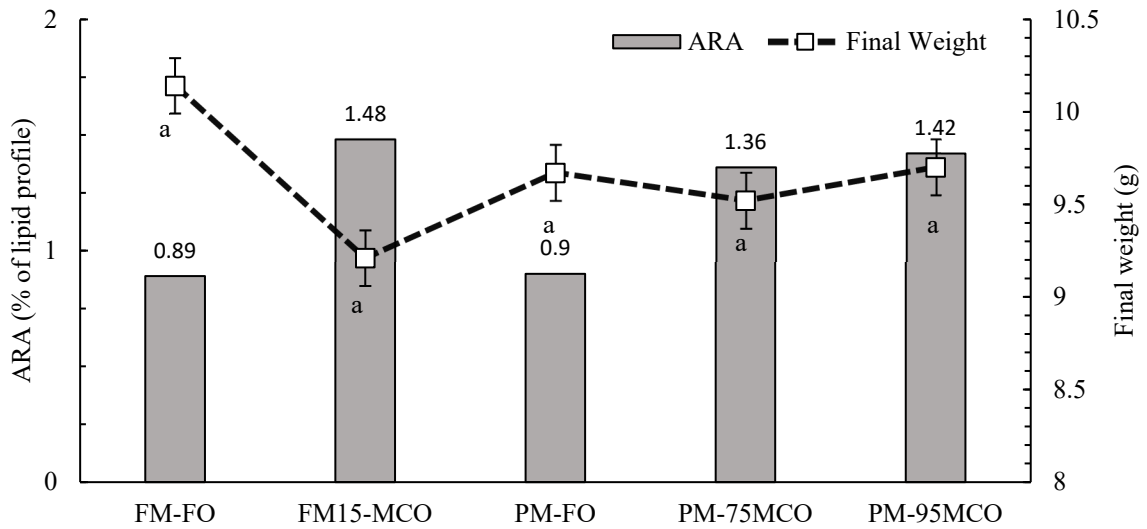
Figure 5. The relationship between final body weight (g) and EPA (Eicosapentaenoic acid, C20:5n-3) level (%) in test diets using modified canola oil (MCO) to replace menhaden fish oil (FO) in feeds offered to juvenile shrimp (mean weight 0.10g) cultured for 10 weeks in a green water recirculating aquaculture system.
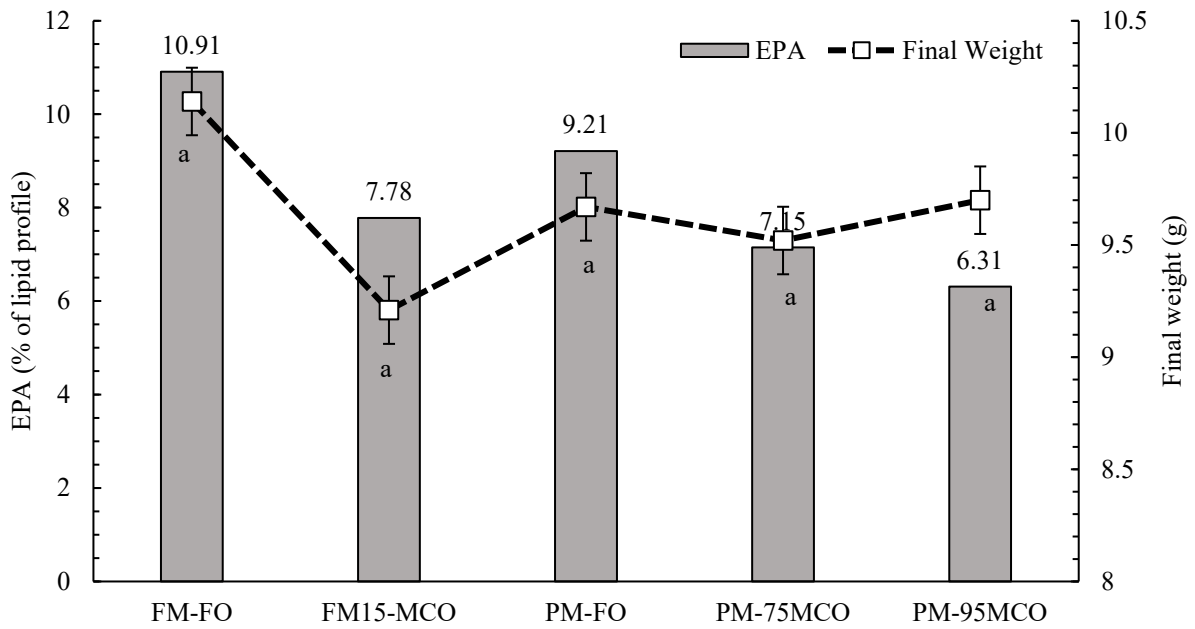
Figure 6. The relationship between final body weight (g) and DPA (Docosapentaenoic acid, C22:5n-3) level (%) in test diets using modified canola oil (MCO) to replace menhaden fish oil (FO) in feeds offered to juvenile shrimp (mean weight 0.10g) cultured for 10 weeks in a green water recirculating aquaculture system.
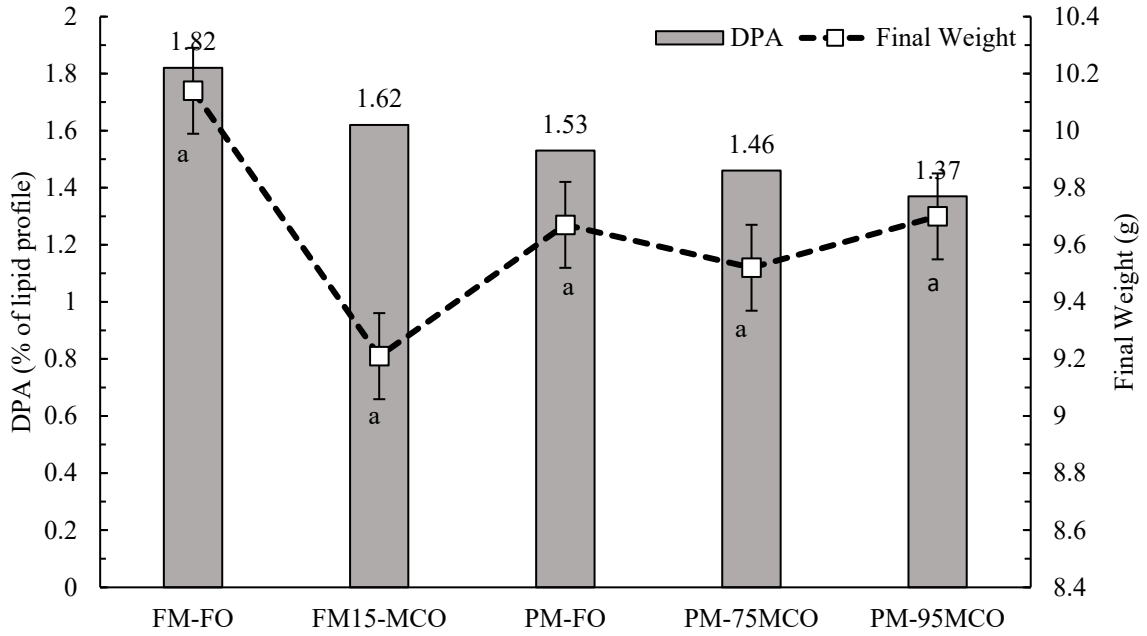
Figure 7. The relationship between final body weight (g) and DHA (Docosahexaenoic acid, C22:6n-3) level (%) in test diets using modified canola oil (MCO) to replace menhaden fish oil (FO) in feeds offered to juvenile shrimp (mean weight 0.10g) cultured for 10 weeks in a green water recirculating aquaculture system.
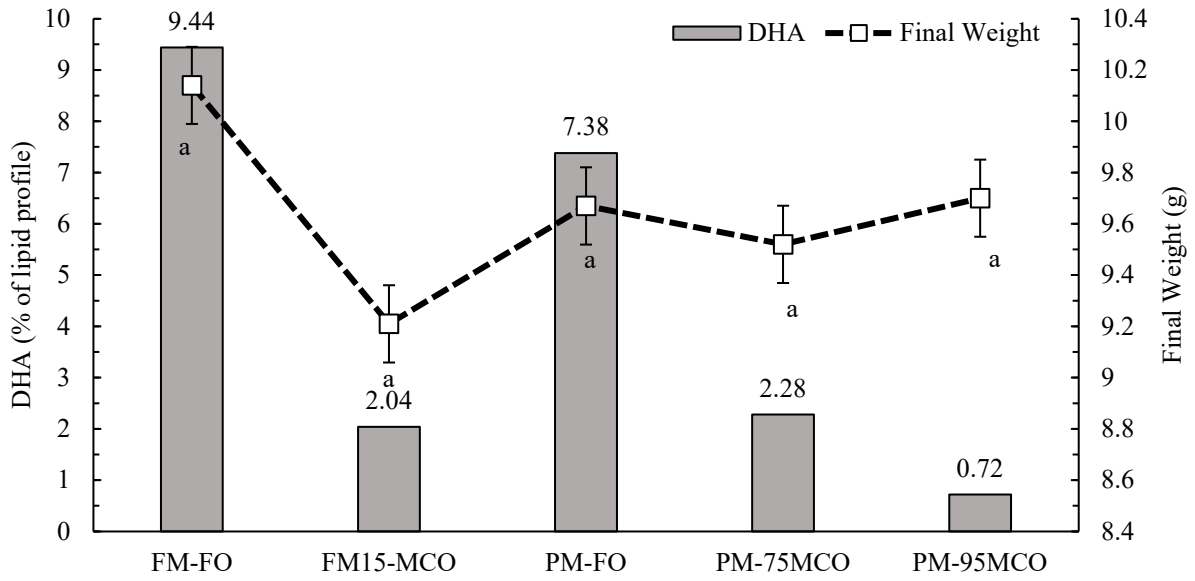
Table 8. Sensory analysis results from untrained panelists who evaluated shrimp (initial weight 0.10g) that were stocked at a density of 40 shrimp per tank and grown for 10 weeks in green water, fed diets with varying levels of fishmeal and fish oil. Data was subjected to a Kruskal Wallis test to determine statistical differences between parameters. No statistically significant differences were observed.

Treatment	FM-FO	FM-MCO	PM-FO	PM-75MCO	PM-95MCO	**p-value**
Appearance	6.27	5.94	6.25	6.24	6.49	0.78
Texture	6.19	6.44	6.56	6.49	6.21	0.52
Juiciness	6.10	6.50	6.42	6.32	5.96	0.40
Flavor	5.97	5.60	5.85	5.94	6.10	0.95
Overall acceptability	5.87	5.45	5.88	5.91	6.07	0.84

4. Discussion

As aquaculture continues to grow and intensify, the need for sustainable and cost-efficient feeds and feed ingredients grows simultaneously (Tacon 2011). This is because intensified aquaculture systems require nutritionally complete feeds that satisfy all dietary requirements for growing aquaculture species (NRC 2011). Pacific white shrimp are one of the most commonly farmed aquaculture species in the world, meaning that a focus on their dietary requirements has increased as production of them has intensified (Tacon 2019). One of the most important and most expensive feed ingredients is lipids. Fish oil has been the most widely used oil in aquatic diets due to its high concentration of LC-HUFA that are essential for fish growth (Kok et al. 2020). As capture fisheries are the leading source for fish oil, and the majority of capture fisheries are fully or over-exploited; hence, the supply of fish oil from wild sources cannot increase (Ye et al. 2017). This means that affordable, sustainable, and nutritionally adequate replacements for fish oil must be found in order for aquaculture production to flourish. Many terrestrial plant oils have been tested as fish oil replacements in the past, but they are lacking in LC-HUFA that are required by some aquatic animals (Turchini et al. 2009). While oil from terrestrial oil seeds have been successful as partial fish oil replacements, there has yet to be a terrestrial oil source that is able to fully replace fish oil without inducing an EFA nutritional deficiency and causing poor growth performance. This is partially due to the fact that essential fatty acid requirements have not been fully understood in many aquatic species, and because terrestrial sources lack the naturally high levels of LC-HUFA that are found in marine sources.

In the current work, five experimental diets were formulated to observe the efficacy of MCO as a FO replacement in shrimp aquaculture feeds. The first set of diets were FM based, with full inclusion of FO and MCO, respectively. FM was removed from the fishmeal basal diet and

replaced with PM to observe complete replacement of FO, being that FM contains FO. Hence, the next series of diets was PM based, with one diet having full inclusion of FO, 75% replacement of FO with MCO, and 95% replacement of FO with MCO. Shrimp were stocked at a density of 40 shrimp per tank (initial weight 0.10g) and grown for 10 weeks for which an average shrimp biomass gain of 9583% observed without significant differences (p>0.05) in growth or survival irrespective of the treatments. The current work indicates that this oil is effective at a level up to 95% which was 20% higher than previously reported by Gia Vo et al. (2021a) for shrimp (*Litopenaeus vannamei*) in in clear water systems This suggests that the effective replacement level is even higher than previously expected. The findings also reinforce previous reports that fishmeal/fish oil can be replaced by non-marine protein (Amaya et al., 2007) and lipid sources (Soller et al., 2019).

Previous research with plant-based fish oil replacements indicates that there is a dietary requirement for DHA, EPA, and ARA for pacific white shrimp (Lim et al. 1997). EPA and DHA are recommended to be at least 0.5% of the lipid profile to fulfill the nutritional needs for maximum growth (Gonzalez-Felix et al. 2003a). Although, the aforementioned work was conducted in clear water systems, Izquierdo et al. (2006) concluded that even in outdoor green water systems that DHA was required in the diet and EFA deficiencies could be induced. This experiment also found nutritional deficiencies in clear water systems, while a corresponding trial in green water resulted in no significant differences in growth, final weight, or survival. This suggests that natural productivity present in green water systems may allow diets to be formulated with lower levels of DHA and still be successful, with the natural productivity in the system allowing the animals to meet the nutritional requirement and achieve acceptable growth.

Fatty acid analysis was performed on the hepatopancreas, tail meat, and whole-body shrimp samples, and are presented in tables 9-11. In general, fatty acid levels in all three tissues followed the trends of the fatty acids offered in experimental diets. This is supported by An et al. (2020) which found that fatty acid profiles of whole shrimp reflected the fatty acid profile of the experimental diet, and Gonzalez-Felix et al. (2003b) which found the same result for hepatopancreas and muscle tissue. While there were no significant differences in shrimp final weights, the strongest relationship between EFA and final weight was DHA. This was to be expected, with (Araujo et al. 2019) and (Kontara et al. 1995) finding that DHA seems to be the most important EFA in relation to shrimp growth performance.

Other experiments have suggested that natural productivity found in outdoor 'green water' systems can serve as an additional feed source for shrimp, but because the tissue EFA levels followed the trends of EFAs offered in feeds, this demonstrates the fact that supplemental feeds are the major source of EFAs (Browdy et al. 2007, Tacon et al. 2002). However, PM95MCO was expected to be deficient based on diet formulations, but did not result in significant growth differences as compared to the other dietary treatments. This suggests that shrimp are able to consume natural productivity in order to supplement their EFA intake in their diet, at least marginally, to meet their nutritional requirements for growth. This is supported by Moreno-Arias et al. (2018) which found that shrimp reared in biofloc systems were able to supplement their EFA intake with fatty acids available in the biofloc which were lacking in feeds. So, though the majority of EFAs are obtained from supplemental feeds in green water systems, it is possible that shrimp fed diets that are marginally lacking in certain EFAs may be able to consume natural productivity to reach nutritional requirements which results in adequate growth. Though natural productivity should not be considered a major source of EFAs, more research is warranted in this

area to determine the contribution of natural productivity to shrimp EFA levels in tissue. With more research to quantify the contribution of natural productivity to EFA profile, practical diets may be able to be formulated with higher levels of alternative oils than are successful in clear water, without inducing nutritional deficiencies that stunt shrimp growth.

Because shrimp are destined for human consumption, it is essential that alternative oil sources meet the nutritional needs of the animal for growth, while also avoiding negative effects on shrimp flesh quality and consumer acceptability. It is typical for consumers to report a "milder" taste from shrimp cultured at lower salinities, but previous work at low salinities done by Silva et al. (2013) found that trained panelists were unable to distinguish between shrimp fed diets with alternative oil sources. This suggests that external factors like salinity may influence shrimp taste and texture more than the oil source in the diet. Brookmire et al. (2013) found that boiling shrimp had a negative effect on shrimp firmness. In the current work, consistent complaints of "mushy" texture of shrimp fed diet FM-MCO suggests that the dietary treatment has an effect on consumer acceptability even though there was no effect on growth and feed conversion. This could be a result of freezing, thawing, or cooking method, but because all complaints were received from the same treatment, this suggests that the difference was due to dietary treatment instead of shrimp preparation methods. This result is similar to that of Turchini et al. (2009), which found that a significantly softer texture was reported for fillets of Atlantic salmon fed canola compared with fish fed fish oil or soybean oil. If this is the case, alternative oil replacements that have this effect are not acceptable, even if they do not compromise growth performance or survival. More research with consumer acceptability and effects of MCO on shrimp texture are warranted to rule out other factors that could influence texture characteristics.

5. Conclusion

Results from the growth trial indicate that under practical conditions, MCO is able to successfully replace FO at a level of at least 95% without compromising growth, feed conversion, or survival. Results from the sensory analysis panel indicate that dietary treatment may have affected consumer acceptability, notably with FM-100MCO receiving consistent complaints of less than acceptable texture. Further research is warranted to observe response to full fish oil replacement with modified canola oil under practical condition, as well as more research observing the effect of fish oil replacement effects on taste and texture to consumers.

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CHAPTER IV

Summary and Conclusion

Lipids are extremely important to animal rearing, playing a role in both cellular structures and as an energy source to support growth and development. In aquaculture and culture of the Pacific white shrimp, fish oil is the leading lipid source in complete feeds for commercial use. Fish oil (FO) provides all of the essential fatty acids (EFA) needed for shrimp growth, specifically long chain highly unsaturated fatty acids (LC-HUFA) like EPA and DHA. These are important for Pacific white shrimp specifically because of their limited capacity to desaturate and chain elongate fats that are provided in the diet. Because FO is in high demand for aquaculture, pet food, and human industries and supply is not able to increase, finding an acceptable alternative oil source for aquatic diets is necessary.

One solution to this problem is to use advanced plant breeding technologies to create terrestrial plants that produce higher quantities of LC-HUFA that can then be extracted and used as replacement oil sources in fish diets. Canola has been modified this way, so that the oil harvested from the plant contains adequate levels of EFAs to be used as partial or complete fish oil alternatives. This metabolically modified plant is a new product, and must be evaluated in vivo to assess its efficacy as a fish oil replacement in shrimp diets.

In the current work, the first experiment was designed to evaluate nutritional value of the canola oil (MCO) as opposed to the basal diet containing FO, as well as to determine palatability of the oil. This experiment was conducted in indoor glass aquaria, with 15 shrimp (0.22g initial weight) stocked per aquaria. Two series of diets were formulated, one fishmeal (FM) based, and one poultry meal (PM) based. All diets were formulated to contain 8% lipid and 36% protein Four FM based diets were formulated, a basal diet with full levels of FM (15%) and FO,

followed by a diet with full FM levels and complete replacement of FO by MCO. The next two diets were formulated to have lower levels of FM (6% and 3%) with full replacement of FO by MCO. All PM based diet formulations contained full levels of PM, with various replacement levels of FO. The first diet was formulated with full levels of PM, and full levels of FO. The remaining five diets contained 75%, 85%, 90%, 95%, and 100% replacement of FO by MCO. There was a significant depression in shrimp growth at replacement levels above 90%. Palatability testing was conducted on FM-FO, PM-FO, FM-MCO, and PM-MCO. The results from the palatability trial indicated no significant differences in palatability between the diets that were evaluated, which suggests that there are no issues with palatability of the test ingredients. A relationship between DHA level in the diet and shrimp final weight was observed, with shrimp fed diets having below 2% DHA as a percentage of the lipid profile resulting in significantly smaller shrimp by final weight. These results indicate that growth differences should be attributed to a nutritional deficiency rather than exterior factors like palatability.

The second experiment was designed in order to evaluate five of the ten test diets from the first experiment, but under practical conditions. While the first experiment was conducted in indoor glass aquaria, this experiment was conducted in an outdoor green water recirculation aquaculture system. These conditions are more comparable to practical industry conditions with natural productivity present in the culture system. The diets used for this experiment were FM-FO, FM-MCO, PM-FO, PM-75MCO, and PM-95MCO. Results from this trial indicated no significant differences in growth or survival, meaning that the replacement here was successful. This suggests that this oil can be successfully replaced under practical conditions at least up to 95%.

Knowing that algae contain EPA and DHA (Williams and Burdge 2006), one explanation for the lack of significant differences seen in the green water system that were seen in the clear water system is that the shrimp consumed algae and subsequently fulfilled the nutritional need for LC HUFA, allowing for acceptable growth rates in all feeding treatments. This suggests that the modified canola oil was very close to meeting the nutritional requirements for growth, so when shrimp supplemented their diets with algae, the nutritional requirements were met. This was not able to happen in clear water conditions with no primary productivity present, so nutritional deficiencies caused stunted growth. Further experimentation in outdoor systems is necessary to determine the acceptable replacement level and to confirm that there are no differences in growth between basal and experimental diets. Future experiments in green water conditions should include a treatment that is not offered any feed so that the lipid profile can be analyzed. This would help to determine the availability of EFAs in algae that is present in the culture system. This canola oil product is a promising tool for shrimp aquaculture because of its potential to alleviate pressure on fish oil in the market, through partial or full replacement options.

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