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Research Article

Use of Genetically Modified Canola Oil as a Replacement for Fish Oil in Practical Diets for Whiteleg Shrimp *Litopeneaus vannamei* Reared in Green Water Conditions

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With current advancements in technology allowing for genetic modification of crops, canola has been modified to contain n3 long-chain polyunsaturated fatty acids such as 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 and EPA replacement for Menhaden fish oil (FO) in practical diets for Pacific white shrimp *Litopeneaus vannamei*. This trial was conducted using twenty-four 750 L tanks stocked at 40 shrimp per tank (0.1 ± 0.007 g initial weight) and grown for 63 days. Shrimp were fed one of five experimental diets (36% protein, 8% lipid) with supplemental FO replacement varying from 75% to 100% twice daily (7 a.m. and 7 p.m.). Two of the diets contained 15% fishmeal (FM) as the basal protein source, while the other three diets were FM free, allowing for complete removal of DHA sources in the basal formulation. While basal FO was removed, none of the experimental diets were completely devoid of FO. Shrimp were harvested and frozen after 9 weeks to be used for lipid extraction and taste and texture analysis by an untrained panel to mimic consumer responses. Results were subjected to a two-way analysis of variance, with significant differences observed in final mean weight (8.47–10.59 g) ($p = 0.0275$), individual weight gain (8.37–10.48 g) ($p = 0.0279$), and weekly gain (0.84–1.05 g) ($p = 0.0378$). Human sensory analysis did not yield significant differences between measured taste parameters ($p > 0.05$). Lipid extraction and analysis results showed that fatty acid concentrations from whole shrimp samples correlate with diet lipid profiles except for EPA. There is no significant difference ($p > 0.05$) in EPA concentrations in whole shrimp samples regardless of diet. These results suggest that Latitude™ oil can be successfully used as a partial replacement for FO in commercial shrimp diets.

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 [1]. Though aquaculture production will continue to increase, the industry continues to rely heavily on capture fisheries for feed ingredients such as fish oil (FO), which is problematic because 80% of wild fish stocks are fully exploited [2]. In addition, FO supply is projected to decrease in the future [3].

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. FO has been an important feed ingredient in aquaculture feeds because of its high concentration of omega-3 long-chain highly unsaturated fatty acids (LC-PUFA) such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) [4]. As the aquaculture industry expands and intensifies, the demand for these feed ingredients has increased while supply was maintained over a few decades which has caused a subsequent increase in prices [5]. Increasing prices and competition from other sectors like human and pet food make it necessary to find acceptable replacements for marine oils that satisfy the nutritional needs of the animals [5, 6].

Terrestrial plant oilseeds lack LC-PUFA, specifically DHA, which is essential for the growth and survival of aquatic species [7]. This problem has encouraged the use of genetic modification to create transgenic plants able to naturally produce LC-PUFA in plants [8]. The canola plant has been genetically manipulated to produce LC-PUFA essential to shrimp growth. As global canola production expands, so will the availability for future use [9] in an aquaculture setting. This study builds upon previous research to determine the efficacy and applicability of genetically modified (GM) canola oil as a replacement for FO in practical diets for *L. vannamei*. Diets used for this trial utilized fishmeal (FM) and poultry meal (PM) as dietary protein sources, and FO and genetically modified canola oil (MCO) (Latitude™, Cargill) as dietary lipid sources. PM was used in place of FM in some diets to remove background sources of FO naturally present in FM. As FM naturally contains approximately 8% FO, FM must be removed in order to create diets that are truly free of FO. Shrimp were reared in green water conditions to most closely mimic commercial production systems such as ponds.

Because commercially produced shrimp are bound for human consumption, it's important to consider the n-3 fatty acid enrichment for health benefits to the end consumer. In addition, as oil replacement can sometimes affect the sensory characteristics of fish tissue [10], sensory analysis was conducted after trial completion.

2. Materials and Methods

2.1. System Setup. Postlarval shrimp were obtained from American Penaeid (St. James City, FL, USA) and offered commercial feed (Ziegler 50% protein, 15% fat) in a green water nursery system for 26 days before 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 × 1.22 m upper diameter, 1.04 m lower diameter, and lower surface area 0.84 m^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 hr/day. Juvenile shrimp were hand-sorted to uniform size (0.1 ± 0.007 g) and 40 shrimp were group weighed and randomly stocked into each tank. Shrimp in this system were fed one of five experimental diets, with treatments FM-FO, FM-MCO, PM-FO, and PM-75MCO were fed to five tanks each (five replicates per treatment), and PM-95MCO was fed to four tanks (four replicates).

2.2. Diet Preparation. A total of five experimental diets were made for this growth trial, including two FM-based diets (FM-FO and FM-MCO) and three PM-based diets (PM-FO, PM-75MCO, and PM-95MCO). All diets were formulated to contain 36% protein and 8% lipid which is adequate for the shrimp species and life stage. Soybean meal and corn protein concentrate were the primary plant-based protein sources, with FM and PM being the primary animal-based protein sources. Menhaden FO was the primary lipid source and was replaced at varying levels with GM canola oil (Table 1). PM was used to replace FM in order to remove background sources of FO and allow us to formulate diets that were truly FO free. The PM-FO diet was used as a second control diet, as both the protein source and lipid source were manipulated in this trial. As the diets were formulated to be isolipidic, they did not contain the same level of oil but instead the same percentage of oils (8%). FM and PM contain different levels of oil, so the total quantity of supplemental oil levels differed between diets. Preground 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 multiparameter 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. For the duration of the experiment, shrimp were fed one of five experimental diets shown in Table 2. Feed inputs were based on a preprogrammed standard feeding protocol for which we assume shrimp doubled their weight weekly until reaching 1.3 g. After reaching 1.3 g shrimp were assumed to gain 1.3 g per week for the remainder of the trial and have an assumed feed conversion ratio (FCR) of 1.2. These assumptions are based off of Davis et al. [11] The calculations used for FCR and survival are listed below:

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed offered (g)}}{\text{Biomass gain (g)}}, \quad (1)$$

$$\text{Survival (\%)} = \left(\frac{\text{Final number of individuals}}{\text{Initial number of individuals}}\right) \times 100. \quad (2)$$

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, the shrimp were frozen to be used for lipid profile analysis and human 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

Table 1: Fatty acid profile (% of total fatty acids) of test oils and experimental diets offered to juvenile shrimp during a 10-weeks culture period with various replacement levels of fish oil (FO) and fishmeal (FM) with Latitude^{TM} oil (MCO) and poultry meal (PM), respectively.

	Oil source		Diets				
Fatty acid	FO	MCO	FM-FO	FM-MCO	PM-FO	PM-75MCO	PM-95MCO
C14:0	8.3	0.06	9.44	2.04	7.38	2.28	0.72
C14:1n-5	0.08	0.00	10.91	7.78	9.21	7.15	6.31
C15:0	0.73	0.04	0.50	0.10	0.42	0.13	0.02
C16:0	16.55	4.16	16.96	8.69	17.10	10.72	8.87
C16:1n-7	11.13	0.14	9.15	1.25	8.50	2.68	1.08
C17:0	0.58	0.05	0.66	0.14	0.51	0.18	0.08
C18:0	3.11	3.08	3.22	3.26	3.43	3.46	3.36
C18:1n-9	7.42	30.98	7.55	24.87	11.43	24.16	28.53
C18:1n-7	3.06	2.57	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	1.48	28.34	13.42	31.21	17.03	29.24	32.99
C18:3n-6	0.38	2.10	0.26	1.54	0.27	1.24	1.43
C18:3n-3	1.34	2.77	2.43	3.12	2.42	2.89	2.98
C18:4n-3	0	–	2.37	0.47	1.95	0.62	0.23
C20:0	0.23	0.67	0.23	0.50	0.23	0.42	0.48
C20:1n-9	0.81	0.71	0.56	0.58	0.52	0.56	0.56
C20:2n-6	2.96	0.11	0.16	0.09	0.15	0.10	0.09
C20:3n-6	0	3.69	0.21	2.51	0.24	1.98	2.42
C20:4n-6 ARA	1.09	2.02	0.89	1.48	0.90	1.36	1.42
C20:3n-3	0	0.06	0.16	0.06	0.13	0.06	0.03
C20:4n-3	0	0.06	1.08	0.99	0.93	0.88	0.85
C20:5n-3 EPA	13.9	9.27	10.91	7.78	9.21	7.15	6.31
C22:0	0.17	0.26	0.19	0.25	0.17	0.22	0.23
C22:1n-9	0.13	0.00	0.10	0.08	0.08	0.09	0.08
22:2n-6	1.4	0.08	0.03	0.04	0.00	0.01	0.03
23:0	–	0.00	0.09	0.09	0.08	0.08	0.08
22:4n-6	0	0.42	0.16	0.28	0.18	0.27	0.28
22:3n-6	0	0.12	0.05	0.01	0.00	0.00	0.00
22:5n-3 DPA	2.34	2.19	1.82	1.62	1.53	1.46	1.37
24:0	0.11	0.12	0.12	0.13	0.09	0.11	0.11
22:6n-3 DHA	10.48	0.61	9.44	2.04	7.38	2.28	0.72
24:1n-9	0.45	0.12	0.20	0.07	0.17	0.10	0.08

Note: FM = Menhaden fishmeal; PM = poultry meal; FO = Menhaden fish oil; MCO = GM canola oil (Latitude^{TM}, Cargill).

at −80°C to preserve the flesh. Shrimp selected for lipid extraction were frozen without addition of water.

2.6. Fatty Acid Analysis. Lipids 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 1 hr before analysis, and placed in a warm water bath to thaw. Three shrimp from each tank were pooled into a representative sample and homogenized for whole-body analysis. Whole body samples consisted of shrimp as harvested; nothing was removed from the shrimp before homogenization. Shrimp hepatopancreas and tail meat samples were also analyzed. From each sample of shrimp body and diets, two random subsamples were taken with a weight of 2 g each from shrimp whole body and tail meat, and an approximate weight of 0.6 g per subsample of feed or hepatopancreas. These subsamples were extracted using the methods of [12]. In short, weighed tissue or feed was homogenized using a polytron homogenizer in 20 mL of chloroform/methanol (2 : 1) for 1.5 min. 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 min 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 was washed with fresh upper phase (chloroform : methanol : water 3 : 48 : 47) three times by gently allowing it to flow down the side of

Table 2: Formulation of experimental diets used for green water growth trial of postlarval shrimp stocked at 40 shrimp per tank (53 shrimp/m^2) (0.10 g initial weight) and grown for 10 weeks.

Ingredient (g/100 g as is)	FM-FO	FM-MCO	PM-FO	PM-75MCO	PM-95MCO
Menhaden fishmeal[a]	15.00	15.00	0.00	0.00	0.00
Poultry meal[b]	0.00	0.00	6.00	6.00	6.00
Soybean meal[c]	49.25	49.25	50.10	50.10	50.10
Corn protein concentrate[d]	0.00	0.00	7.00	7.00	7.00
Menhaden fish oil[e]	5.09	0.00	5.51	1.38	0.28
Lecithin (soy)[f]	1.00	1.00	1.00	1.00	1.00
Latitude^{TM} oil[g]	0.00	5.09	0.00	4.13	5.23
Cholesterol[h]	0.12	0.12	0.12	0.12	0.12
Corn starch[h]	0.33	0.33	2.67	2.67	2.67
Whole wheat[i]	25.61	25.61	24.00	24.00	24.00
Mineral premix[j]	0.50	0.50	0.50	0.50	0.50
Vitamin premix[k]	1.80	1.80	1.80	1.80	1.80
Choline chloride[l]	0.20	0.20	0.20	0.20	0.20
Stay-C 35%[m]	0.10	0.10	0.10	0.10	0.10
CaP-dibasic[n]	1.00	1.00	1.00	1.00	1.00

Note: [a]Special Select™, Omega Protein Inc., Hammond, LA, USA. [b]Chicken by-product meal (Darling ingredient). [c]Dehulled solvent-extracted soybean meal, Bunge Limited, Decatur, AL, USA. [d]CPC–Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.[e]Omega Protein, Inc., Houston, TX, USA. [f]The Solae Company, St. Louis, MO, USA. [g]Cargill Crop 2019 Latitude, FC012320APFO. [h]MP Biomedicals Inc., Santa Ana, CA, USA. [i]Bob's Red Mill Natural Foods, Milwaukie, OR, USA. [j]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. [k]Vitamin premix (g/kg 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. [l]Amresco Inc., Solon, OH, USA. [m]Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), DSM Nutritional Products, Parsippany, NJ, USA. [n]Alfa Aesar, Ward Hill, MA, USA. Values are reported as a percentage of the diet. All diets were formulated to contain 36% protein and 8% lipid on an "as is" basis. Diets were subjected to proximate composition analysis to confirm the formulation.

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 was decanted into a dried preweighed test tube. The chloroform was evaporated using a heated water bath and stream of nitrogen gas, the tubes were then dried and weighed. The percentage (%) lipid was then calculated (on a dry weight basis). After the extractions, oils from subsamples 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 μL of isooctane with 100 ppm butylated hydroxytoluene (BHT). Second, 100 μL of the suspended sample was added to a 15 mL polypropylene conical tube, along with 1 mL of isooctane and 100 μL of 1N potassium hydroxide in methanol. Then, samples were vortexed at 3,000 rpm for 30 s and centrifuged at 3,000 rpm for 5 min. Five hundred microliter of supernatant (isooctane layer) was removed from the conical and added to a 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 the Nu-Check GLC566 FAME Standard. BHT peak was removed from chromatograms of samples before analysis. Individual fatty acid methyl esters (FAMEs) were calculated as % of total peak area.

Table 3: Water quality parameters throughout the culture period of postlarval shrimp (0.10 g 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

2.7. Sensory Analysis. Sensory analysis was conducted in two sessions on the same day at 9 a.m. and 12 p.m. 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.

Twenty-four hours in advance of the sensory analysis evaluation, samples were thawed in a refrigerator. Before cooking, random subsamples were taken from each tank in order to form a representative sample reflecting the treatment to cook for panelists. The shrimp were deheaded and cooked with the shell on. Shrimp samples were boiled for 1 min and 15 s and internal temperature was measured to ensure thorough cooking. After being removed from the boiling water, the shrimp were placed in an ice bath and then peeled and refrigerated until being served. Each panelist received one shrimp from each

Table 4: 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.

Treatment	Initial weight (g)	Initial biomass (g)	Survival (%)	Final biomass (g)	Final mean weight (g)	Individual gain (g)	Weekly gain (g)	FCR
FM-FO	0.10	4.06	95.5	387.28	10.14	10.04	1.00	1.17
FM-MCO	0.10	4.05	98.0	362.28	9.21	9.11	0.91	1.23
PM-FO	0.10	3.92	95.5	368.92	9.67	9.57	0.96	1.22
PM-75MCO	0.10	3.96	97.0	371.28	9.52	9.42	0.94	1.22
PM-95MCO	0.10	4.06	98.7	383.15	9.70	9.60	0.96	1.18
p-Value	0.89	0.90	0.06	0.32	0.03	0.03	0.04	0.30
PSE	0.01	0.29	3.15	21.76	0.53	0.33	0.03	0.07

Note: Data was subjected to a two-way ANOVA and a Tukey's test to determine significantly different means.

Table 5: Fatty acid profile (% of total fatty acids) of lipids extracted from whole shrimp (mean initial weight 0.1 g) grown in green water for 10 weeks and fed diets with various replacement levels of fish oil (FO) and fishmeal (FM) with Latitude^{TM} oil (MCO) and poultry meal (PM), respectively.

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

Note: Data was subjected to a two-way ANOVA and a Tukey's test to determine significantly different means. The results are presented as mean ± SE.

treatment, and each treatment received 33 responses, 17 in the a.m. session, and 16 in the p.m. session. Cooked shrimps were evaluated for appearance, flavor, texture, and juiciness. Each variable evaluated by panelists was rated on a scale of 1–8, with 1 being "dislike extremely" and 8 being "like extremely."

2.8. Statistical Analysis. All growth data was analyzed using SAS (V9.3. SAS Institute, Cary, NC, USA). Data was treated for homogeneity and normality and then subjected to two-way analysis of variance (ANOVA) to determine significant differences ($p < 0.05$) among treatments. Sensory analysis

Table 6: Fatty acid profile (% of total fatty acids) of lipids extracted from tail meat of shrimp (mean initial weight 0.1 g) grown in green water for 10 weeks and fed diets with various replacement levels of fish oil and fishmeal with Latitude™ oil and poultry meal, respectively.

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

Note: Data was subjected to a two-way ANOVA and a Tukey's test to determine significantly different means. The results are presented as mean ± SE.

data was subjected to a Kruskal–Wallis test to determine statistical differences between parameters.

3. Results

Water quality parameters were within the acceptable range for growth and survival throughout the growth trial, with values presented in Table 3. Growth performance results are presented in Table 4. Significant differences were observed in final mean weight (9.21–10.14 g) ($p = 0.0275$), individual weight gain (9.11–10.04 g) ($p = 0.0279$), and weekly gain (0.91–1.00 g) ($p = 0.0378$). Fatty acid profiles from shrimp whole body are presented in Table 5. Whole body essential fatty acid (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 to provide the unit of fatty acids. This demonstrates the trend that diets low in DHA led to whole-body tissues low in DHA, while diets high in DHA led to shrimp tissues high in DHA. EPA feed values ranged from 6.31% in PM-95MCO to 10.91% in FM-FO, while whole body tissue average values ranged from 13.78% in FM-FO to 12.35% in FM-MCO and were not statistically significant. 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.

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.

Table 7: Fatty acid profile (% of total fatty acids) of lipids extracted from hepatopancreas of shrimp (mean initial weight 0.10 g) grown in green water for 10 weeks and fed diets with various replacement levels of fish oil and fishmeal with Latitude^{TM} oil and poultry meal, respectively.

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

Note: Data was subjected to a two-way ANOVA and a Tukey's test to determine significantly different means. The results are presented as mean ± SE.

Results from the human taste panel are presented in Table 8. No sensory differences were indicated between treatments.

4. Discussion

While oil from terrestrial oil seeds has been successful as a partial FO replacement, there has yet to be a terrestrial oil source that is able to fully replace FO without inducing an EFA nutritional deficiency and causing poor growth performance.

In the current work, five experimental diets were formulated to observe the efficacy of MCO as a FO replacement in shrimp aquaculture feeds. Significant differences were observed in final mean weight, individual weight gain, and weekly gain, indicating that GMO canola is an acceptable partial replacement for FO but more research is needed with this oil. This is supported by Gia Vo et al. [13], which found that in clear water systems, this GM canola oil was an acceptable partial replacement (up to 75% of the supplemental FO). Our results are also supported by Amaya et al. [14] found that FM can be completely replaced by nonmarine protein sources, and Soller et al. [15] concluded that alternative lipid sources are able to be used to partially replace FO in nonmarine-based diets. These studies show successful replacement of FM by alternative protein sources, and FO replacement by alternative oil sources, such as palm oil.

The significant difference in final mean weight confirms our expectation that oil sources play a larger role in growth than protein sources. The shrimp fed PM diets with various levels of FO replacement outperformed shrimp fed FM diets with full FO replacement. Previous research with plant-based FO replacements indicates that there is a dietary requirement for DHA, EPA, and ARA for Pacific white shrimp [16]. EPA and DHA are recommended to be at least 0.5% of the dry weight of the diet to fulfill the nutritional needs for maximum growth [17]. Although the aforementioned work was conducted in clear water systems, Izquierdo et al. [18] concluded that even in outdoor green water systems, DHA was required in the diet and EFA deficiencies could be induced. Izquierdo et al. [18] 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

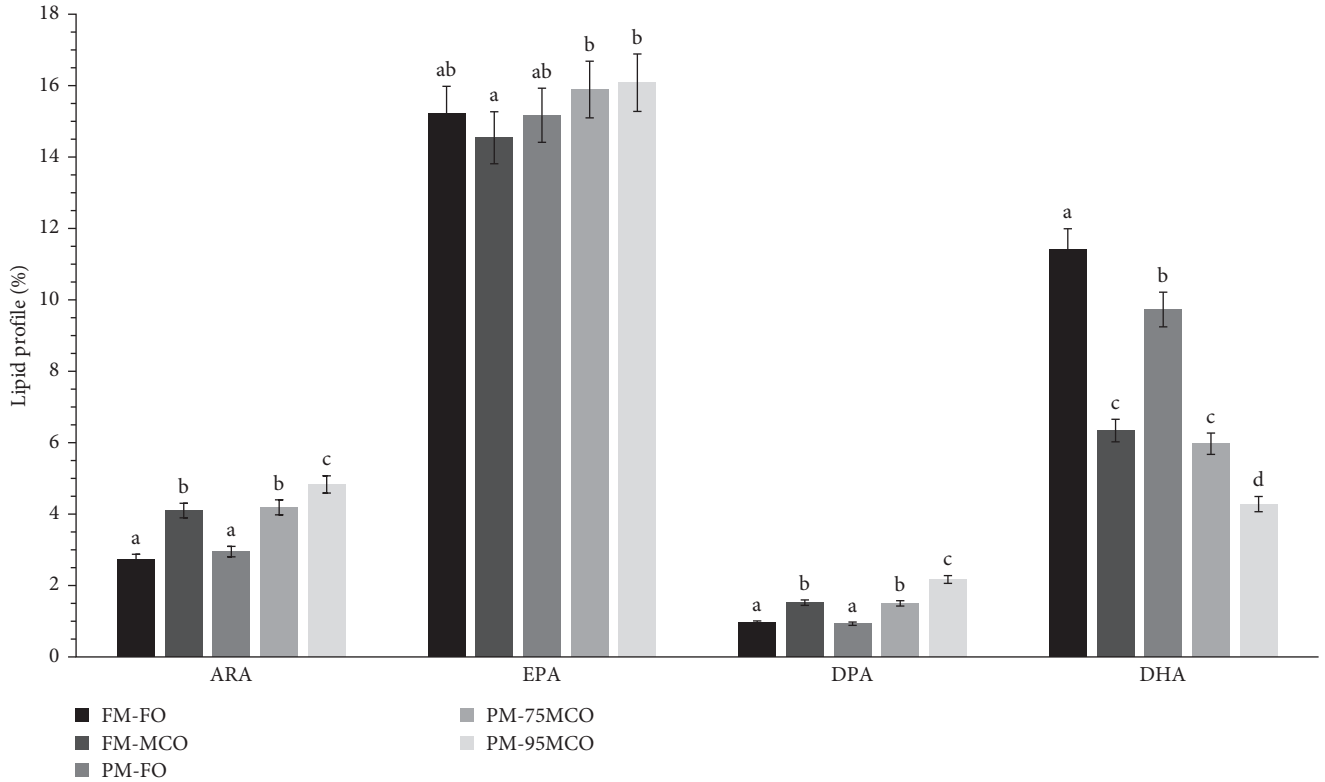

FIGURE 1: Fatty acid levels (% of total fatty acids) extracted from the tail meat of shrimp (mean initial weight 0.1 g) grown in green water for 10 weeks and fed diets with various replacement levels of fish oil (FO) and fishmeal (FM) with Latitude^{TM} oil (MCO) and poultry meal (PM), respectively. (a, b, c, d, and ab) Letters denote statistical differences between fatty acid levels by treatment.

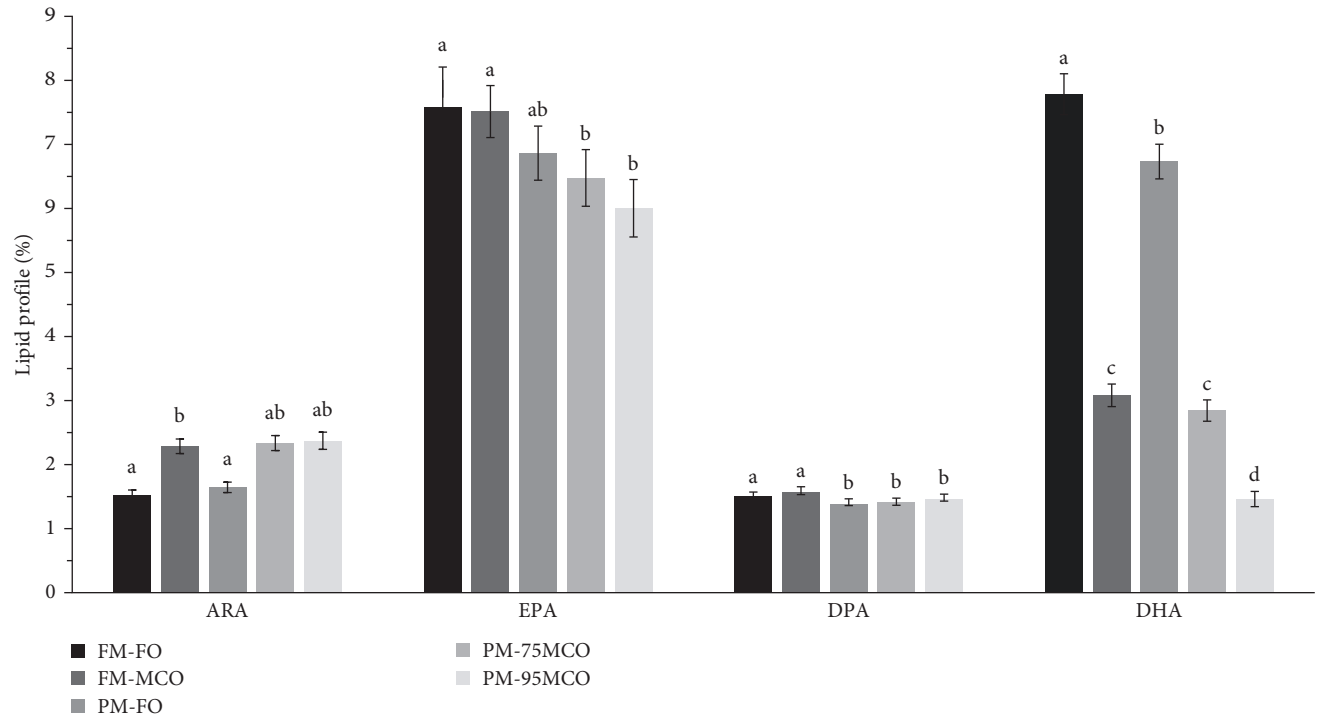

FIGURE 2: Fatty acid levels (% of total fatty acids) extracted from the hepatopancreas of shrimp (mean initial weight 0.1 g) grown in green water for 10 weeks and fed diets with various replacement levels of fish oil (FO) and fishmeal (FM) with Latitude^{TM} oil (MCO) and poultry meal (PM), respectively. (a, b, c, d, and ab) Letters denote statistical differences between fatty acid levels by treatment.

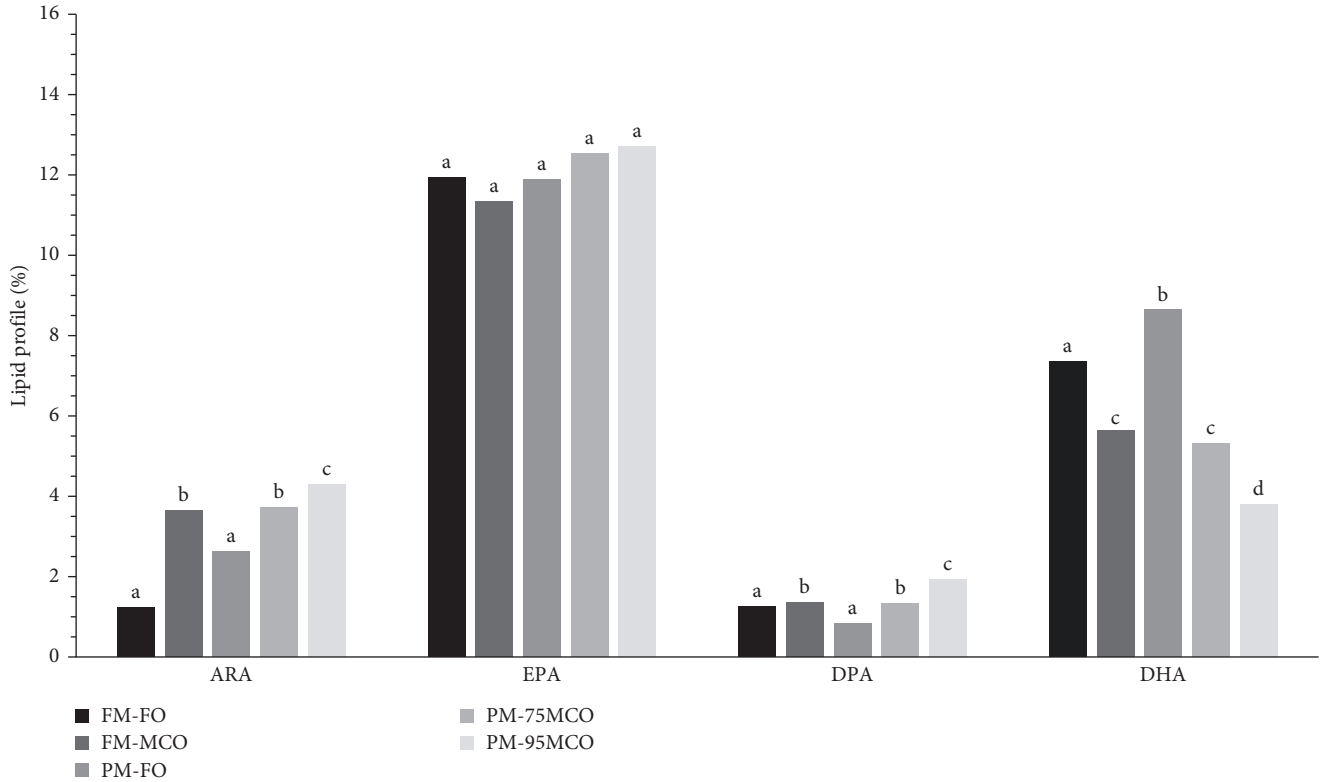

Figure 3: Fatty acid levels (% of total fatty acids) extracted from the whole body of shrimp (mean initial weight 0.10 g) grown in green water for 10 weeks and fed diets with various replacement levels of fish oil (FO) and fishmeal (FM) with Latitude^{TM} oil (MCO) and poultry meal (PM), respectively. (a, b, c, and d) Letters denote statistical differences between fatty acid levels by treatment.

Table 8: Sensory analysis results from untrained panelists who evaluated shrimp (initial weight 0.10 g) 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.

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

Note: Data was subjected to a Kruskal–Wallis test to determine statistical differences between parameters. No statistically significant differences were found.

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 [18].

In general, fatty acid levels in all three shrimp tissues followed the trends of the fatty acids offered in experimental diets, with the exception of DPA. This was to be expected, as shrimp fed higher lipid levels will deposit more of that lipid into their tissues. This is supported by An et al. [19] which found that fatty acid profiles of whole shrimp reflected the fatty acid profile of the experimental diet, and Gonzalez-Felix et al. [17] which found the same result for hepatopancreas and muscle tissue. In order to visualize the relationship between EFAs ARA, EPA, DPA, and DHA, the shrimp final weight is presented with each respective lipid level in the diets in Figure 4(a)–4(d).

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 indicates that artificial feeds are the major source of EFAs [20, 21]. So, though the majority of EFAs are obtained from artificial 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

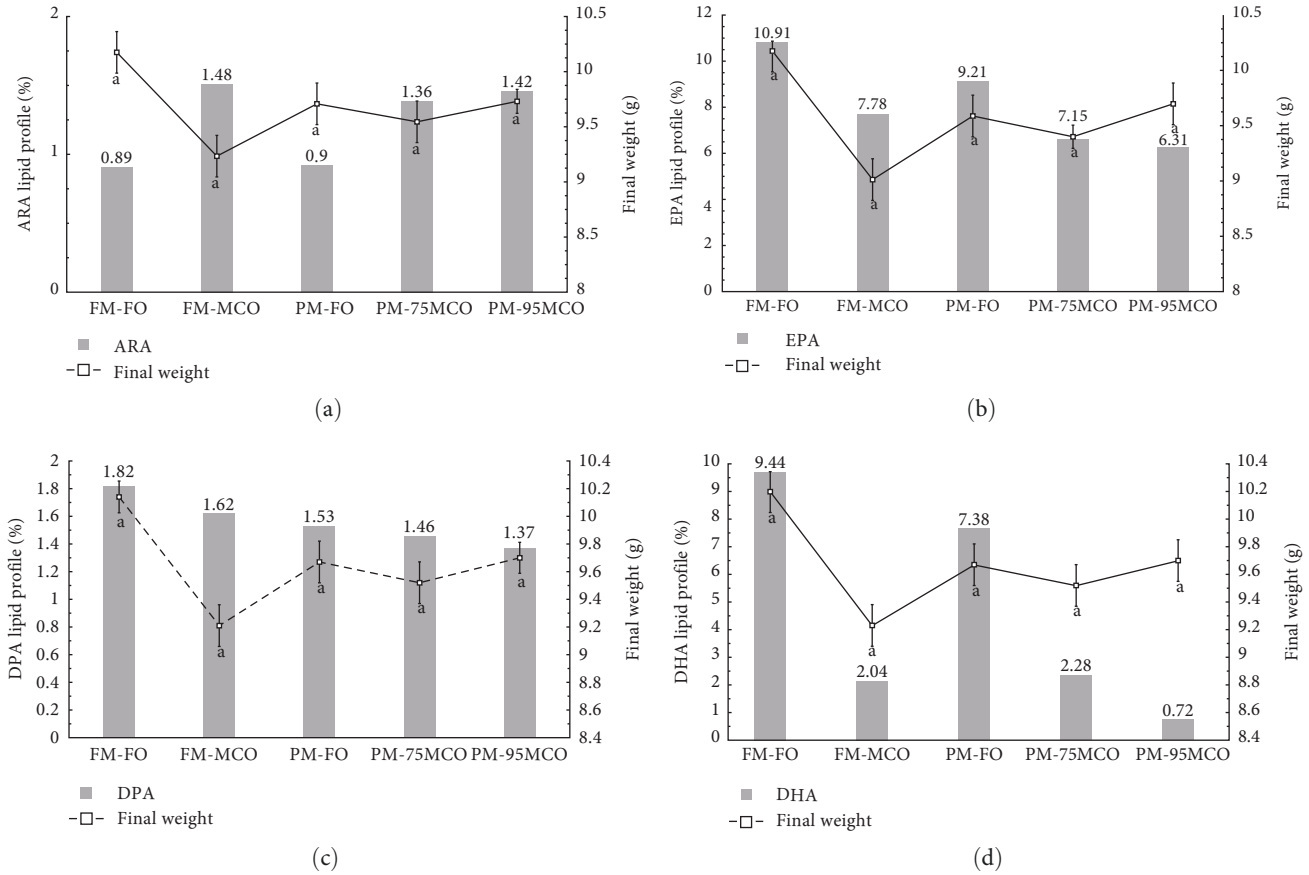

FIGURE 4: (a) Shrimp final body weight (g) and ARA (arachidonic acid, C20:4n-6) level (% of total fatty acids) in test diets using Latitude^{TM} oil to replace Menhaden fish oil (FO) in feeds offered to juvenile shrimp (mean weight 0.10 g) cultured for 10 weeks in a green water recirculating aquaculture system. Data was subjected to a two-way ANOVA and a Tukey's test to determine significantly different means. (b) Shrimp final body weight (g) and EPA (eicosapentaenoic acid, C20:5n-3) level (%) in test diets using Latitude^{TM} oil to replace Menhaden fish oil (FO) in feeds offered to juvenile shrimp (mean weight 0.10 g) cultured for 10 weeks in a green water recirculating aquaculture system. (c) Shrimp final body weight (g) and DPA (docosapentaenoic acid, C22:5n-3) level (%) in test diets using Latitude^{TM} oil to replace Menhaden fish oil (FO) in feeds offered to juvenile shrimp (mean weight 0.10 g) cultured for 10 weeks in a green water recirculating aquaculture system. (d) Shrimp final body weight (g) and DHA (docosahexaenoic acid, C22:6 n-3) level (%) in test diets using Latitude^{TM} oil to replace Menhaden fish oil (FO) in feeds offered to juvenile shrimp (mean weight 0.10 g) cultured for 10 weeks in a green water recirculating aquaculture system. Letter a denotes statistical differences between shrimp final weight by treatment.

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 Soller Dias da Silva [22] 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. [23] found that boiling shrimp had a negative effect on shrimp firmness. In the current work, there were no significant differences in scores across all treatments. Oil sources are the most likely ingredient to have an effect on tissue quality and consumer acceptability, with Turchini et al. [8], finding that a "significantly 'softer' texture has been reported for fillets of Atlantic salmon fed C/RO compared with fish fed FO or SBO" (C/RO = Canola/rapeseed oil, FO = Fish oil, and SBO = Soybean oil). In our case, we did not observe significant difference in texture or any other sensory parameters between tested diets and overall, all averaged scores were in the "liking" zone of the hedonic scale. Results suggest that GM canola oil does not have a negative impact on cooked shrimp sensory performance. More research with consumer acceptability and effects of MCO and other blends of oil sources on shrimp texture are warranted.

5. Conclusion

Results from the growth trial indicate that under practical conditions, MCO can 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 with Latitude oil does not have a negative

impact on cooked shrimp sensory performance. Further research is warranted to observe the response to full FO replacement with GM canola under practical conditions, as well as more research observing the effect of FO replacement effects on taste and texture to consumers. Finally, more research should be conducted to explore both the true DHA requirements for white shrimp and the contribution of natural foods present in green water systems to shrimp EFA consumption.

Data Availability

Data are not freely available due to commercial confidentiality.

Disclosure

This research was previously published as an MS thesis but has been revised and improved for this manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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