THE EFFICIENCY OF MODIFIED CANOLA OIL AS A REPLACEMENT OF MENHADEN FISH OIL IN PRACTICAL DIET FOR PACIFIC WHITE SHRIMP, *Litopenaeus vannamei*

By

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ABSTRACT

Lipids are an essential component of the diet serving as source of energy and components of cell structures. Marine species require a certain level of specific fatty acids in their diet as they have limited ability to synthesize and/or transform precursors into essential fatty acids (EFA). As fish oil is rich in EFA it has served as the primary lipid source in aquaculture diets. Due to fish oils advantages of omega-3 (n-3) and omega-6 (n-6) long-chain highly unsaturated fatty acids (LC-HUFAs), EPA (Eicosapentaenoic acid, C20:5n-3), DHA (Docosahexaenoic acid, C22:6n-3) and ARA (Arachidonic acid, C20:4n-6), aquaculture currently uses 90% of this resource. In order to expand the aquaculture industry, fish oil supply should be replaced by a source of lipids which are suitable to meet the shrimp requirements for fatty acids, and more available in terms of production. Canola oil is not only cheaper than fish oil, but also more available and sustainable in terms of production for a long-term period. As with other terrestrial oil sources, the limited availability of LC-HUFA in canola oil means it cannot meet the EFA requirements of the shrimp. However, with the development of technology to genetically modify lipid metabolism of canola, the fatty acid profile is modified to produce higher level of n3 fatty acids, in particular DHA and EPA, and can possibly serve as a true alternative to fish oil. The development of modified canola oil is a relatively new technology which must be validated and is thus the subject of this work.

The objective of the first study was to estimate the efficacy of genetically modified canola oil as a replacement for fish oil in Pacific white shrimp feeds. In this experiment, a series of fishmeal free diets were formulated to contain 36% protein and 8% lipid. The lipid from menhaden fish oil (MFO, 5.45 g/100g diet) was replaced by the modified canola oil (MCO-1)

at 25%, 50%, 75%, and 100% in the first series of diets. A diet of MCO-2 replaced 100% MFO was also formulated. In the second series, MFO was replaced by 50%, 75%, and 100% using standard canola oil (SCO). At the conclusion of a 6-week growth trial, there was a significant depression in final weights of shrimp maintained on diets containing 100% MCO-1, MCO-2, or SCO as compared to shrimp reared on the basal diet with MFO. The reduced performance at high levels of replacement is likely caused by low levels of DHA not meeting the threshold of the requirement for this EFA. Another possible explanation was due to rancidity of the oil which could be an issue as peroxide value of this diet was considerably higher than that of the menhaden fish oil-based diet. Hence, palatability or toxicity of the oxidized products could have contributed to the poor response at 100% inclusion.

The second study was similar to the first but with a new source of the modified canola oil, which was stabilized to reduce oxidation. As before, MFO was replaced with graded level of MCO-3 up to 100% replacement. The first five of the eight diets were designed with fishmeal, soybean meal and corn protein concentrate as the primary protein sources while the other three were fishmeal free based. In theory, fishmeal does not only contain protein, but also provide lipid and fatty acids which contribute to EFA levels of the diets. In all diets, menhaden fish oil served as the primary lipid source and was sequentially replaced with a modified canola oil at 25%, 50%, 75%, and 100%. Then, the three fishmeal-free based diets were evaluated using 75% MCO-3 as well as 75% MCO-3 in combination with hydrolyzed salmon (amino salmon, AS) by product meal which served as an attractant to replace MFO. Results of the 6-week growth trial indicate that 100% of MFO could be successfully replaced by the MCO-3 without causing deficiencies in shrimp performance, survival, and FCR. Whether or not palatability of the diet was affected by the MCO-3 in the diet was not clearly detected; however, mean final weights of the shrimp offered the diet containing AS did not show a significant improvement.

Finding an alternative source of lipid to replace fish oil in commercial shrimp feeds is required if the industry is to continue to expand. According to the overall results of this study, the modified canola oil has a higher level of n3 fatty acids and an increased level of EFA which are required by many species. Results of this work confirm that the MCO can be used as the primary lipid source in production diets for shrimp. As there were slight reductions in performance, further research into the efficacy of the MCO is warranted. It is also recommended that work should be conducted in more practical conditions to demonstrate the efficacy under less controlled conditions.

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INTRODUCTION

Lipid requirements differ due to species, culture conditions, and stage of life cycle. Lipid is one of the more important nutrients required for growth and development. It is a major source of metabolic provision of energy, up to 20% wet weight, which is used for maintaining bodily functions, growth, and reproduction (Tocher, 2003). Lipid is also a source of fatty acids that play important roles in cell structures of tissues. Fatty acids such as LC-HUFAs (long chain highly unsaturated fatty acids) and PUFAs (polyunsaturated fatty acids) play specific roles in physiological processes. While HUFAs provide necessary fatty acids for the development of structures and functions of the cell membranes (Tocher, 2003), PUFAs take part in improving the immune system through their antibody actions (Das, 2006). Lipids can be found in variable sources from animals to plants. Canola oil, flaxseed oil, linseed oil and most of the vegetable oils are rich in α-linolenic acid (LNA, C18:3n3) and *cis*-linoleic acid (LOA, C18:2n-6; (Das, 2006) while fish oils have larger quantities of LC-HUFA specific to DHA (Docosahexaenoic acid, C22:6n-3) , EPA (Eicosapentaenoic acid, C20:5n-3), and ARA (Arachidonic acid, C20:4n-6; Silva *et al.*, 2011; Tocher, 2003). Many studies have been conducted with the purpose of replacing fish oil with different alternative lipid resources mainly from plant oils (González-Félix *et al.*, 2010) in diets for marine species, particularly shrimp. The results vary depending on the types of species. In Pacific white shrimp, the results also showed that marine oils could be replaced without showing deficiencies in growth and survival as long as the essential fatty acid (EFA) requirements were reached (González-Félix *et al.*, 2010; Soller *et al.*, 2017). According to Soller et a*l.* (2017) fish oil could be completely

replaced by genetically modifying lipid sources of plant oils or organisms with low potential of negative impacts on final product qualities.

In terms of production, lipids serve as an energy source and EFA source for development, growth, and survival of marine species (Rainuzzo *et al.*, 1997). There are many sources of lipid that have been used in formulated feeds including oils extracted from marine species, terrestrial animals and plants. Fish oil has been used as the most popular lipid source in aquaculture because of its fatty acid profile. It does not just provide energy, it is also known as a rich resource for omega-3 (n-3) LC-HUFAs, which are considered essential for many aquatic species, particularly shrimp. Besides nutritional values to aquaculture, fish oil has the ability to improve human health and help prevent diseases such as cardiovascular disease (Kris-Etherton *et al.*, 2002; Rennie *et al.*, 2003), and brain disease (Sun *et al.*, 2018) due to high levels of n-3 fatty acids, and in particular HUFA (Rennie *et al.*, 2003). The abundant levels of n-3 HUFAs that are available in seafood have made it one of the important elements to human health, and, as such, stimulates the expansion of aquaculture (Nasopoulou and Zabetakis, 2012).

Essential fatty acids (EFAs) are PUFAs that have at least two double bonds and are converted from monosaturated fatty acids de novo (Das, 2006). EFAs are mainly n-3, n-6 (Das, 2006). EFAs play an important part in supporting the immune system and preventing diseases such as inflammatory autoimmune disease through actions of antibodies (Das, 2006). EFAs, especially DHA and n-3 HUFA, also are the key elements to reach maximum growth in *Litopenaeus vannamei* (Gonzalez-Felix *et al.*, 2002b). According to Kanazawa and Teshima (1977), crustaceans, particularly shrimp, cannot themselves convert EFAs from monosaturated fatty acids (MUFAs), thus, they have a specific requirement for long chain n-3 and n-6 HUFA in their diet in order to present good growth performance and high survival rate (Das, 2006; Gonzalez-Felix *et al.*, 2003a; NRC, 2011). This led to the later studies of fatty acid function

and dietary requirements for shrimp. In *L. vannamei,* juveniles EFA requirement of at least 0.5% for both EPA and DHA (Gonzalez-Felix *et al.,* 2003a) has been reported to result in good growth rate and high survival rate.

Fish oil has served as the primary lipid resource used in commercial diet to meet the requirement of the essential fatty acid, in the aquaculture industry (Leaver *et al.,* 2008). Fish oil production has fluctuated, and trended to decline over the years due to a decrease in raw material consumption, which also leads to a fluctuation in fish oil price (Leaver *et al.,* 2008). Furthermore, aquaculture is the primary user of 90% of total fish oil production, (Nasopoulou and Zabetakis, 2012); so, the production and price of fish oil are challenges for the aquaculture industry. In order for the aquaculture industry to further develop, a cost-effective, expandable, and nutritionally-adequate alternative solution to fish oil is necessary. This is why plant oils are currently being studied as possible replacements.

Canola oil has been proposed as a possible substitute for fish oil (Bowyer *et al.,* 2012) in fish and shrimp feeds. It is completely refined, edible, highly available with sustainable production, and is cheaper than fish oil (Turchini *et al.,* 2009). While fish oil production is predicted to decrease (Tacon and Metian, 2008), canola oil production has been increasing approximately 35 million tons in the period of 1975-2007 and expanding through the countries in Asia, Europe, and America (Rosillo-Calle *et al.,* 2009). However, canola oil has a high percentage of MUFAs and n-6 PUFAs while LC-HUFAs, EPA, and DHA which are required for marine shrimp (Higgs *et al.,* 2006) are not available. Canola oil lacks these EFAs, so it'-s inclusion is limited but it can still be used to replace up to 60% of dietary fish oil in marine feeds without showing any deficiencies in growth rate, survival, and health (Izquierdoa *et al.,* 2005; Mourente *et al.,* 2005b). The shift from fish oil to canola oil is primarily limited due to changes in fatty acid profiles, particularly the availabilities of EFAs. In order to have both a high survival rate and growth rate, we need to consider the possible utilization of modifying/

improving canola oil as a replacement because of its necessary properties. By genetically modifying plant oils, the essential fatty acids that are not typically available in the plant oils such as DHA, EPD, DPA (Docosapentaenoic acid, C22: 5n-3), and ARA could be enriched which means it would then be able to fulfil the requirements.

If higher levels of canola oil are to be used in commercial feeds without causing negative effects to the target species, the fatty acid profile has to be modified to include EFAs. With the development of technology, fatty acid profiles of plant oils can be modified with the desired fatty acids by directing the fatty acid profile (Yuan and Knauf, 1997; Zou *et al.*, 1997) through metabolic engineering. In order to induce metabolic engineering, traditional plant breeding and biotechnology are needed. The purposes of metabolic engineering can be to redirect the enzymatic reactions to improve the existing compounds, produce new compounds, or degrade the unwanted compounds (Newell-McGloughlin, 2008). Thus, modified canola oil can be a promising alternative by being enriched with LC-HUFAs such as EPA, DHA, and ARA, which meets the EFA requirements for the target species through metabolic engineering. Nutritional value of modified canola oil has made it one of the reasons that modified canola oil is a promising alternative besides its availability, and market price. However, there is limited information and research on this alternative and its effectiveness in feed for various aquatic species, particularly Pacific white shrimp. For all the reasons stated above, the objectives of this study were to 1) estimate the efficacy of modified canola oil used to replace fish oil, and 2) determine what levels of replacement would not cause impacts to growth performance, survival, and feed conversion ratio (FCR) of *L. vannamei*.

References

Bowyer, J.N., Qin, J.G., Smullen, R.P., Stone, D.A.J., 2012. Replacement of fish oil by poultry oil and canola oil in yellowtail kingfish (*Seriola lalandi*) at optimal and suboptimal temperatures. Aquaculture. 356-357, 211-222.

Das, U., 2006. Essential Fatty Acids - A Review. Current Pharmaceutical Biotechnology. 7, 467 - 482.

Gonzalez-Felix, M.L., III, D.M.G., Lawrence, A.L., Perez-Velazquez, M., 2002b. Effect of dietary phospholipid on essential fatty acid requirements and tissue lipid composition of *Litopenaeus vannamei* juveniles. Aquaculture. 207, 151-167.

Gonzalez-Felix, M.L., D.M., G.I., Lawrance, A.L., Perez-Velazqez, M., 2003a. Nutritional evaluation of fatty acids for the open thelycum shrimp, *Litopenaeus vannamei*: II. Effect of dietary n-3 and n-6 polyunsaturated and highly unsaturated fatty acids on juvenile shrimp growth, survival, and fatty acid composition. Aquaculture Nutrition. 9, 115-122.

González-Félix, M.L., Silva, F.S.D.d., Davis, D.A., Samocha, T.M., Morris, T.C., Wilkenfeld, J.S., Perez-Velazquez, M., 2010. Replacement of fish oil in plant based diets for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture. 309, 152-158.

Higgs, D.A., Balfry, S.K., Oakes.J.D., M., R., B.J., S., G., D., 2006. Efficacy of an equal blend of canola oil and poultry fat as an alternative dietary lipid sources for Atlantic salmon (*Salmo salar L.*) in sea water. I: effects on growth performance, and whole body and fillet proximate and lipid composition. Aquaculture Research. 37, 180-191.

Izquierdoa, M.S., Monteroa, D., Robainaa, L., Caballeroa, M.J., Rosenlund, G., Ginesa, R., 2005. Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. Aquaculture. 250, 431-444.

Kanazawa, A., Teshima, S.-i., 1977. Biosynthesis of Fatty Acids from Acetate in the Prawn, *Penaeus japonicus*. Mem. Fac. Fish., Kagoshima Univ. 26.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E., Etherton, T.D., 2002. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. Am. J. Med. 113, 71-88.

Leaver, M.J., Bautista, J.M., Björnsson, B.r.T., Jönsson, E., Krey, G., Tocher, D.R., Torstensen, B.E., 2008. Towards Fish Lipid Nutrigenomics: Current State and Prospects for Fin-Fish Aquaculture. Reviews in Fisheries Science. 16:S1, 73-94.

Mourente, G., Dick, J.R., Bell, J.G., Tocher, D.R., 2005b. Effect of partial substitution of dietary fish oil by vegetable oils on desaturation and h-oxidation of [1-14C]18:3n-3 (LNA) and [1-14C]20:5n-3 (EPA) in hepatocytes and enterocytes

of European sea bass (*Dicentrarchus labrax L*.). Aquaculture. 248, 173-186.

Nasopoulou, C., Zabetakis, I., 2012. Benefits of fish oil replacement by plant originated oils in compounded fish feeds. A review. LWT-Food Science and Technology. 47, 217-224.

Newell-McGloughlin, M., 2008. Nutritionally improved agricultural crops. Plant Physiol. 147, 939-953.

NRC, 2011. Nutrient requirements of fish and shrimp. National Academic Press.

Rainuzzo, J.R., Reitan, K.I., Olsen, Y., 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture. 155, 103-115.

Rennie, K.L., Hughes, J., Lang, R., Jebb, S.A., 2003. Nutritional management of rheumatoid arthritis: a review of the evidence. J. Hum. Nutr. Diet. 16, 97-109.

Rosillo-Calle, F., Pelkmans, L., Walter, A., 2009. A global overview of vegetable oils, with reference to biodiesel. A report for the IEA Bioenergy Task. 40.

Silva, S.S.D., Francis, D.S., Tacon, A.C.J., 2011. Fish oil in aquaculture : in retrospect, in Fish oil replacement and alternative lipid sources in aquaculture feeds. CRC Press, Boca Raton, Flo, 1-20.

Soller, F., Rhodes, M.A., Davis, D.A., 2017. Replacement of Fish Oil with Alternative Lipid Sources in Plant-based Practical Feed Formulations for Marine Shrimp (*Litopenaeus vannamei*) Reared in Outdoor Ponds and Tanks. Aquaculture Nutrition. 23, 63-75.

Sun, G.Y., Simonyi, A., Fritsche, K.L., Chuang, D.Y., Hannink, M., Gu, Z.Z., Greenlie, C.M., Yao, J.K., Lee, J.C., Beversdorf, D.Q., 2018. Docosahexaenoic acid (DHA): An essential nutrient and a nutraceutical for brain health and diseases. Prostaglandins Leukot. Essent. Fatty Acids. 136, 3-13.

Tacon, A.G.J., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture. 285, 146-158.

Tocher, D.R., 2003. Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish. Reviews in Fisheries Science. 11, 107-184.

Turchini, G.M., Torstensen, B.E., Ng, W.-K., 2009. Fish oil replacement in finfish nutrition. Reviews in Aquaculture. 1, 10-57.

Yuan, L., Knauf, V.C., 1997. Modification of plant components. Curr. Opin. Biotechnol. 8, 227-233.

Zou, J.T., Katavic, V., Giblin, E.M., Barton, D.L., MacKenzie, S.L., Keller, W.A., Hu, X., Taylor, D.C., 1997. Modification of seed oil content and acyl composition in the brassicaceae by expression of a yeast sn-2 acyltransferase gene. Plant Cell. 9, 909-923.

CHAPTER II

THE EFFICACY OF USING MODIFIED CANOLA OIL AS AN ALTERNATIVE OF MENHADEN FISH OIL IN PRACTICAL DIET OF PACIFIC WHITE SHRIMP, *Litopenaeus vannamei*

Abstract

In order to expand and maintain the shrimp industry, marine oils must be replaced with oils that are both sustainable and capable of meeting the animal essential fatty acid requirements. Modified canola oil which contains enhanced levels of omega-3 fatty acids, particularly DHA (Docosahexaenoic acid, C22:6n-3), and EPA (Eicosapentaenoic acid, C20:5n-3) could serve this purpose. Hence, this experiment was designed to evaluate the efficiency of modified canola oil (MCO) serving as a replacement for menhaden fish oil (MFO) in practical diet of Pacific white shrimp. A series of nine fishmeal-free based diets were formulated to contain 36% protein and 8% lipid. The basal diet was supplemented with MFO which was then incrementally replaced with MCO) at 0, 25%, 50%, 75%, and 100% or standard canola oil (SCO) at 50%, 75%, and 100%. Juvenile shrimp (average 0.19g/individual) were cultured in an indoor recirculating aquaculture system over a 6-week culture period. Each dietary treatment was offered to 6 replicate groups of 10 shrimp/tank. The results indicated that the mean final weight of shrimp generally decreased as a consequence of the decrease in MFO in the diets. However, there was no significant difference in mean final weight, survival, and FCR among shrimp offered the basal diet (100% MFO) and the treatments of 25%, 50%, and 75% MCO-1, 50%, and 75% SCO. The results suggested that MCO-1 could be used to replace fish oil in diet for Pacific white shrimp up to 75% without inhibiting growth, feed efficiency

or survival. The significant reduction of growth could be possibly caused by the decrease of EFAs, which was found in DHA levels from the 100% MCO-1 supplemented diet.

1. Introduction

With capture fishery production assuming a relatively static stance since the late 1800s, aquaculture has taken responsibility for the continuation of the expansive growth in terms of the supply of fish for human consumption (FAO, 2018). Aquaculture plays an important part in world seafood production. Total production of seafood from aquaculture has increased from 25.7% in 2000 to 46.8% in 2016 (FAO, 2018), and is continuing to expand presently accounting for over 50% of world seafood supply. Crustacean contributed 7.9 million tons to the 80 million tons of total aquaculture and fisheries production. The Pacific white leg shrimp, *Litopenaeus vannamei*, contributed 53% in total production of crustacean in 2016 (FAO, 2018). In the past two decades, *L. vannemei* production has been dominant in total penaeids production in Asia, particularly China, Thailand, Taiwan, Indonesia, and Vietnam (Liao and Chien, 2010) as well as the America's (Cuzona *et al.*, 2004; Roy and Davis, 2010). *L. vannamei* has become one of the largest cultured species for its advantages which are high larval survival, fast growth rate, and abilities to adapt to wide range of temperatures and salinities (Liao and Chien, 2010). As for the production advantages, Pacific white shrimp have increased benefits when compared to *P. monodon* which has been the most popular culture species. This includes lower fry cost, higher larval survival, lower feed cost, lower dietary protein requirements as well as more efficient consumption of plant proteins in formulated diets (Cuzona *et al.*, 2004; Roy and Davis, 2010; Shiau, 1998).

The trend of increasing aquaculture production has led to the high demand for commercial feed (Hasan, 2001). The advantages of commercial feed are that the nutritional values of feed for a target species contain specific levels of required nutrients such as proteins, lipids, vitamins, and minerals. Commercial feeds are also stable and available in comparison to natural food. Fish oil, aside from minerals, vitamins, protein resources, and carbohydrate

resources etc, is one of the key elements that plays an important role in the production of formulated feed for aquaculture (Silva *et al.*, 2011). In the traditional feed industry, lipid has been provided by fish oil from marine species such as menhaden, anchovy, mackerel, and sand eel (Silva *et al.*, 2011). Fish oil has been used as the common lipid resource because of its fatty acid profile which meets the essential fatty acid (EFA) requirements for marine species. Consequently, the demand for lipid sources, especially from fish oil, has increased. However, fish oil production cannot be expanded due to the raw material base, and it's price fluctuation and trends of increase (Silva *et al.*, 2011). To maintain and expand a sustainable aquaculture industry we cannot rely on fish oil or marine-origin lipid sources, which is unfortunately due to the over-fishing and seasonal availability of fish. Thus, we are not able to use this as a sustainable long-term option.

An alternative for fish oil is necessary. The alternative must be qualified in terms of stable production, economic affordability, and nutrition. There have been many studies on replacing fish oil by plant oils in diets of marine and freshwater species (Caballero *et al.*, 2002; González-Félix *et al.*, 2010; Izquierdoa *et al.*, 2005; Mourente and Bell, 2006; Mourente *et al.*, 2005a; Mourente *et al.*, 2005b; Panserat *et al.*, 2009). The use of plant oils leads to an improvement in production cost due to the cheaper price and higher available production as well as more stable to access of plant oils (Nasopoulou and Zabetakis, 2012). With the trend of fish oil being replaced by plant oils in commercial feeds, we then have the opportunity to establish a similar improvement with Pacific white shrimp feed formulations.

With the opportunity to establish an improvement in Pacific white shrimp feed formulations, we then have to give priority to cost reduction while meeting the nutritional requirements. If we are to reduce the cost of shrimp feeds and improve the sustainability, we need to replace fish oil and identify less expensive lipid sources that meet the EFA requirements of shrimp. Pacific white shrimp requires a certain amount of EFAs in their diet in order to

support growth and normal development. Essential fatty acids which are required for Pacific white shrimp are the highly unsaturated fatty acids (HUFAs) such as Eicosapentaenoic acid (20:5n-3, EPA), Docosahexaenoic acid (22:6n-3, DHA), and Arachidonic acid (20:4n-6, ARA; (NRC, 2011). These fatty acids are considered essential in the diet because marine species have a limited ability to elongate long chain highly unsaturated fatty acids (LC-HUFAs) from polyunsaturated fatty acids (PUFAs) such as α-linolenic acid (LNA, C18:3n3) and cis-linoleic acid (LOA, C18:2n-6; (Kanazawa *et al.*, 1979a; Kanazawa *et al.*, 1979b). Different species have different optimal ranges for EFAs that are required to support growth and survival. The Pacific White Shrimp is reported to require 0.5% of DHA and EPA in their diet (Gonzalez-Felix *et al.*, 2003a)

World canola or rape seed production was around 27.8 million metric ton in 2018 and represents the third largest vegetable oil source. Production from current varieties is stable and the market price in inexpensive (Nasopoulou and Zabetakis, 2012; Turchini *et al.*, 2009). It contains high energy, and is rich in monounsaturated fatty acids (MUFAs) and PUFAs although traditional canola oil lacks LC-HUFAs (Higgs *et al.*, 2006) thus limiting inclusion levels. Newer varieties of canola oil or modified canola oil (MCO) are being produced that have higher level of n-3 fatty acids and may serve as a source of EFAs. Given the modified fatty acid profile, this could be a promising alternative source of lipid that can satisfy both the demands of quality and quantity. In this research, modified canola oil was the canola oil that was applied metabolic engineering to produce long chain highly unsaturated fatty acids (LC-HUFAs), specifically focus on DHA, DPA, EPA, and ARA, into their fatty acid composition. Thus, the objectives of this study were to evaluate the nutritional values and the efficacy of modified canola oil as well as its level of replacement to fish oil on growth, survival, and FRC of Pacific white shrimp. The hypothesis of this study was that if the modified canola oil provided EFAs at the amount that as effective as menhaden fish oil (MFO), the growth rate, survival, and feed

conversion ratio (FCR) of the shrimp offered the treatments of diets replaced fish oil by different levels of modified canola oil would not be significantly different.

2. Materials and methods

2.1 Experimental diets

In order to estimate the effects of fish oil replaced by canola oil -based diets, a series of fishmeal-free based diets were formulated to contain 36% protein and 8% lipid. Soybean meal, and corn protein concentrate were used as the primary protein sources and poultry meal was added as the only animal protein. Menhaden fish oil (MFO) served as the primary lipid source (supplemented at 5.45 g/100g diet) and was systematically replaced with MCO's (MCO-1, MCO-2) and standard canola oil (SCO) based on the experimental design. MFO and the oils used to replace MFO were analysed their FA profiles (Table 1). A total of 9 test diets were evaluated using MCO's or SCO replacing MFO at incremental levels (0, 25, 50, 75 and 100%; Table 2).

The test diets were prepared in the feed laboratory of Auburn University, Auburn, AL, USA using standard practices. Pre-ground dry ingredients were mixed in a food mixer (Hobart Corporation, Troy, OH, USA) until the ingredients were fully mixed. Then, the oil was added and mixed followed by boiling water which was added at sufficient quantities to produce a mash of appropriate consistency for pelleting. The mash was forced through meat grinder with 3-mm die to make pellets under pressure. The diets were placed on trays to dry in a forced air dryer at under 45°C overnight to reach a moisture of less than 10%. After drying, the diets were crumbled and sieved to a uniform size. The diets were stored in zip lock bags in a freezer (at -20°C) for later uses. Proximate composition (g $100g^{-1}$ sample as is) and fatty acid profile (g $100g^{-1}$ sample as is) of the diets were analysed at the Midwest Laboratories, Omaha, Nebraska, USA, and Cargill's oil division, Colorado, USA, respectively.

Table 1. Fatty acid profile (% fatty acids) of two modified canola oils (MCO-1, MCO-2), standard canola oil (SCO), and menhaden fish oil (MFO) that were used in the tested diets.

Fatty Acids	MCO-1	MCO-2	SCO	MFO
C14:0	0.05	0.08	0.06	6.93
C15:0	0.07	0.06	-	0.64
C16:0	4.42	4.60	4.03	14.05
C16:1n7	0.15	0.15	0.15	10.01
C17:0	0.05	0.05	0.04	2.11
C18:0	2.70	2.66	2.12	2.72
C18:1n9	25.71	37.87	64.85	5.16
C18:1n7	2.86	2.31	3.28	2.73
C18:2n6	28.56	26.38	20.73	1.51
C18:3n6	2.45	1.94	-	0.34
C18:3n3	4.68	2.45	1.95	1.47
C20:0	0.61	0.59	0.6	0.24
C20:1n9	0.73	0.65	1.11	0.85
C20:2n6	0.17	0.26	0.05	0.32
C20:3n6	4.95	1.65	-	0.32
C20:4n6 (ARA)	3.37	2.26	-	1.28
C20:3n3 (11,14,17)	-*	-	-	0.27
C20:5n3 (EPA)	7.33	8.82	-	16.45
C22:0	0.32	0.29	0.26	-
C22:1n9	-	-	0.09	-
C23:0	-	-	0.12	-
C22:4n6	0.85	0.30	-	-
C22:3n6	-	0.08	-	0.74
C22:5n3 (DPA)	3.61	1.95	-	2.54
C22:6n3 (DHA)	0.81	0.70	-	19.56
C24:0	-	0.10	0.15	-
C24:1n9	0.32	0.26	0.13	0.42
TT n3	16.43	13.92	1.95	40.29
TT n6	40.35	32.87	20.78	4.51
n3/n6	0.41	0.42	0.09	8.93
n6/n3	2.46	2.36	10.66	0.11

*Not detected

Table 2. Formulations (g/100 g "as-is") of test diets designed to contain varying levels of modified canola oils (MCO-1, MCO-2) and standard canola oil (SCO) as a replacement for the supplemented menhaden fish oil (MFO). All diets were formulated to contain 36% protein and 8% lipids on an "as is" basis.

Ingredient	Basal	MCO-1				MCO-2	SCO		
	0	25	50	75	100	100	50	75	100
Poultry by product meal [1]	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Soybean meal [2]	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00
Corn protein concentrate [3]	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Menhaden fish oil [4]	5.45	4.09	2.73	1.36			2.73	1.36	
MCO-1 [5]		1.36	2.73	4.09	5.45				
MCO-2 [5]						5.45			
SCO [5]							2.73	4.09	5.45
Lecithin [6]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol [7]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Corn Starch [7]	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Whole wheat [8]	20.50	20.50	20.50	20.50	20.50	20.50	20.50	20.50	20.50
Mineral premix [9]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix [10]	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Choline chloride [11]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin C[12]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-dibasic [11]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00

[1] Tyson Foods, Inc., Springdale, AR, USA. **[2]**De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA. **[3]**CPC - Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA. **[4]**Omega Protein Inc., Houston, TX, USA. **[5]**Cargills, Fort Collins, CO, USA. **[6]**The Solae Company, St. Louis, MO, USA. **[7]**MP Biomedicals Inc., Solon, OH, USA. **[8]**Bob's red mill, Milwaukie, OR, USA. **[9]**Trace mineral premix (g/100g 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. **[10]**Vitamin premix (g/kg premix): Thiamin HCl, 4.95; Riboflavin, 3.83; Pyridoxine HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Biotin, 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. **[11]**VWR Amresco, Suwanee, GA, USA
[12]Stay-C® (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA

2.2 Growth trial

The growth trial was conducted at E. W. Shell Fisheries Center, Auburn, Alabama. Post larval shrimp were received from Shrimp Improvement Systems (Islamorada, Florida, USA) were nursed using Zeigler's commercial feeds until appropriate size was reached. Juvenile shrimp with the mean weight of 0.19g ± 0.02 g were size sorted to uniform size and randomly stocked into 75-L rectangle tanks, which was a component of a 5,500 L indoor recirculating aquaculture system (RAS). Treatments were randomly assigned to the RAS, stocked with 10 shrimp per tank. The daily amount of feed offered to the shrimp was calculated according to a feed conversion ratio (FCR) of 1.8 and the predicted growth of 0.19, 0.38, 0.76, 0.85, 0.9, and 1.0g/week over a 6-week growth trial. Daily feed was divided into four feedings per day. The amount of feed offered was adjusted each week based on predicted growth, survival, as well as observation of the feeding response. At the end of the growth trial, shrimp were counted, and group weighed to determine weight gain, survival rate (%), and FCR. After weighing, the shrimp were frozen and stored (a short-term storage) in a freezer at -20°C for proximate analysis.

The culture system consists of a series of culture tanks, a sump tank, two circulation pumps, bead filter and fluidized bed biological filter. Water temperature was maintained at around 27°C using a submerged 3,600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA). Dissolved oxygen was maintained near saturation using air stones in each culture tank and the fluidized bed biological filter using a common airline connected to a regenerative blower. Dissolved oxygen, salinity, and water temperature were measured twice a day using a YSI-55 digital oxygen/temperature meter (YSI corporation, Yellow Springs, Ohio, USA). pH was measured twice a week by Sper Scientific 850050 large display pH pen. Water samples were collected and kept frozen to test total ammonia nitrogen (TAN) and nitrite-N two times

per week by using methods that are described by Solorzano (1969) and Spotte (1979), respectively. Ammonia was monitored daily by TAN disk maintaining in the sump tank. Water inlets and outlets were checked every day to maintain proper water exchange.

2.3 Lipid extraction from diets, and whole shrimp body

Fatty acid compositions in diets and in shrimp body were analyzed. Frozen shrimp were thawed and five shrimp from each tank were pooled into a sample and ground. From each sample of shrimp and diets, two sub-samples were taken with an approximate weight of 2g/each from shrimp bodies and an approximate weight of 0.5g/sub-sample of diet. These sub-samples were extracted using the methods of Folch *et al.* (1957). In short, weighed flesh or feed was homogenized in 20 mL of chloroform/methanol (2:1) for 1 minute. 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 minute and filtered through 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) 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, flushed with nitrogen gas, and the vials were sealed with parafilm paper. 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.

2.4 Statistical analysis

Feed conversion ratio was calculated by feed offered/ (final weight-initial weight), and percentage weight gain was calculated as (final weight-initial weight)/initial weight × 100. One-way ANOVA was used to test if there were any significant differences ($P<0.05$) in final mean weights, growth, survival, percent weight gain, and FCR among treatment means. Linear regression analysis was tested to present the relationship of the target EFAs in diet, and in shrimp and the replacement of MFO. Multi-comparison test (Tukey) and Student-Newman-Keuls test were used to identify differences among treatment means of animal responses and fatty acid compositions in shrimp tissues, respectively. All statistical analyses were conducted using SAS system for Windows (SAS Institute, Cary, NC).

3. Results

3.1 Fatty acid profiles of tested oils, and experimental diets

Fatty acid analysis of the tested oils is presented in Table 1, MFO had the highest level of EPA (16.45%) and DHA (19.56%) while SCO had 0% in ARA, EPA, DPA, and DHA level. The EPA level in the modified canola oil MCO-2 was 8.82% and MCO-1 was 7.33%. While EPA level in MCO's were quite high, the DHA levels were low. The DHA levels were 0.81% (MCO-1), and 0.70% (MCO-2). On the other hand, DPA levels was found highest in MCO-1 with 3.61%, followed by MFO with 2.54%, and MCO-2 with 1.95%. ARA level in the MCO-1 were found highest (3.37%), followed by MCO-2 with 2.26%, and MFO with 1.28%.

Fatty acid analysis of experimental diets is presented in Table 3. In general, total n-3 fatty acids decreased and total n-6 fatty acid increased as a result of the increase in MCO's and SCO supplementation. As expected, the diet containing 100% of SCO had the lowest level of total n-3 fatty acids and the diet of 100% MCO-1 had the highest level of total n-6 fatty acids. Particularly, the percent of EPA and DHA in the tested diets declined when the percent of MCO-1 and SCO replacement increased. The highest level of EPA and DHA was in the diet of 100% MFO. Although the level of EPA and DHA decreased with the increase of MCO-1, it was in the range of 4.77-8.67% and 0.57-7.01%, respectively. The EPA level showed the highest value (9.79%) in the diet of 100% MFO and the lowest value in the diet of 100% SCO with 0.15%. The diets of 100% MCO-1, and 100% MCO-2 had an average EPA value of 5.06% and 6.15%, respectively. The DHA levels dropped from 9.04% in the diet of 100% MFO to 0.57% in the diets of 100% MCO-1 and 100% MCO-2 to 0.16% in the diet of 100% SCO. On the other hand, DPA and ARA levels increased when the replacement of MCO increased. It reached the highest value of 2.54% (DPA) and 2.44% (ARA) when the replacement of the MCO-1 reached 100%.

Table 3. Proximate composition of tested diets using modified canola oils (MCO-1, MCO-2), and standard canola oil (SCO) to replace menhaden fish oil (MFO)

	Basal	MCO-1			MCO-2		SCO		
g/100g as is	0*	25	50	75	100	100	50	75	100
Crude protein	38.20	38.30	37.40	38.20	38.70	39.00	39.10	39.30	38.90
Crude lipid	8.15	8.14	8.21	8.27	8.42	8.36	8.47	7.74	8.20
Moisture	9.43	8.73	9.54	9.13	7.22	6.91	7.06	6.45	6.51
Ash	6.77	6.81	6.67	6.76	6.74	6.81	6.75	7.15	6.92
Crude Fiber	2.57	2.74	2.28	1.39	2.26	2.78	2.50	1.86	2.71
Fatty acids (%)									
C12:0	0.09	0.08	0.06	0.04	0.03	0.03	0.07	0.05	0.03
C12:1n3	0.01	0.01	0.03	0.02	0.02	0.03	0.01	0.01	0.02
C13:1	0.04	0.03	0.02	-**	-	-	0.01	-	-
C14:0	5.52	4.41	3.06	1.64	0.34	0.30	2.86	1.64	0.33
C14:1n5	0.04	0.04	0.03	0.02	-	-	0.03	0.02	-
C15:0	0.47	0.39	0.27	0.15	0.05	0.06	0.28	0.15	0.04
C16:0	16.09	14.47	11.91	10.19	7.94	8.40	11.95	10.09	8.03
C16:1n7	8.29	6.62	4.53	2.54	0.57	0.52	4.45	2.67	0.64
C17:0	1.70	1.34	0.91	0.51	0.09	0.08	0.88	0.51	0.10
C18:0	3.44	3.42	3.32	3.34	3.27	3.30	3.16	3.03	2.92
C18:1n9	9.90	13.18	17.34	20.60	24.57	32.15	31.70	41.16	51.04
C18:1n7	2.46	2.31	2.26	2.15	2.29	1.76	2.25	2.15	2.18
C18:2n6	15.82	19.91	24.13	29.18	33.32	32.57	22.21	25.11	28.53
C18:3n6	0.31	0.62	1.03	1.37	1.76	1.40	0.18	0.10	-
C18:3n3	2.48	2.99	3.41	4.01	4.52	3.03	2.58	2.63	2.72
C20:0	0.19	0.27	0.37	0.45	0.54	0.51	0.36	0.43	0.50
C20:1n9	0.50	0.56	0.64	0.62	0.66	0.57	0.75	0.80	0.90
C20:2n6	0.18	0.17	0.17	0.15	0.16	0.22	0.13	0.10	0.07
C20:3n6	0.21	0.98	1.91	2.69	3.58	1.18	0.15	0.07	-
C20:4n6 (ARA)	0.96	1.31	1.72	2.06	2.44	1.73	0.54	0.34	0.14
C20:3n3	0.14	0.12	0.10	0.07	-	0.03	0.07	0.01	-
C20:5n3 (EPA)	9.79	8.67	7.57	6.25	5.06	6.15	4.77	2.53	0.15
C22:0	0.21	0.21	0.22	0.27	0.27	0.29	0.24	0.26	0.28
C22:1n9	0.05	0.04	0.01	-	-	-	0.02	0.02	0.02
C22:2n6	0.56	0.45	0.34	0.16	-	-	0.27	0.20	0.00
C22:4n6	0.29	0.39	0.49	0.60	0.71	0.35	0.25	0.20	0.17
C22:3n6	0.36	0.30	0.22	0.15	0.03	0.03	0.17	0.10	-
C22:5n3 (DPA)	1.71	1.90	2.15	2.32	2.54	1.40	0.84	0.44	-
C22:6n3 (DHA)	9.04	7.01	4.85	2.72	0.57	0.57	4.32	2.33	0.16
C24:0	-	-	-	-	0.05	0.09	0.05	0.03	0.06
C24:1n9	0.12	-	0.04	-	-	-	-	0.03	-
TTn3	23.17	20.70	18.11	15.38	12.70	11.20	12.58	7.95	3.03
TTn6	18.70	24.13	30.00	36.37	41.99	37.48	23.89	26.22	28.91
n3/n6	1.24	0.86	0.60	0.42	0.30	0.30	0.53	0.30	0.10
n6/n3	0.81	1.17	1.66	2.36	3.31	3.35	1.90	3.30	9.53

*The percent of the replacement

**Not detected

This value was also higher than the level of DPA (1.71%) and ARA (0.96%) in the diet of 100% MFO as well as the diet of 100% MCO-2 (DPA, 1.40%; ARA, 1.73%). DPA and ARA levels presented a negative trend when the replacement of SCO and reached 0% in the diet of 100% SCO. In addition to individual EFA being evaluated, the n3/n6 ratio was also calculated. The highest n3/n6 ratio was 1.24 in the basal diet with 100% MFO and steadily decreased when the percent of MCO-1 increased in the diets. The ratio dropped down to 0.3, 0.3 and 0.1 when 100% MFO was replaced by 100% MCO-1, 100% MCO-2, and 100% SCO, respectively. In addition, the replacement of MFO led to an impact in oxidization of the diets, which was reflected by peroxide value. Peroxide value of the diet that contained 100% fish oil was 6.3 meq/kg, 100% MCO-1 was 21.7 meq/kg, 100% MCO-2 was 16.2 meq/kg, and 100% SCO was not detected.

3.2 Water quality

Dissolved oxygen, temperature, salinity, pH, TAN and nitrite-N were maintained at 6.50± 0.35mg/L, 27.6 ± 1.05°C, 8.0 ± 0.69 ppt, 8.5 ± 0.16, 0.1 ± 0.03 mg/L and 0.1 ± 0.12 mg/L respectively (Mean ± *SD*). Water quality conditions were maintained within typical value for these types of systems and within the range that would support normal growth and survival of Pacific white shrimp, *Litopenaeus vannamei* (Table 4).

3.3 Growth trial

The growth performances of Pacific white shrimp that were offered with the set of nine experimental diets over a 6-weeks period are presented in Table 5. Overall, mean final weights of the shrimp decreased as the MFO replacement percentage increased. Mean final weight decreased from 4.29g (shrimp offered the basal diet) to 3.16g, 3.20g, and 3.10g in the treatment of 100% MCO-1, 100% SCO, and 100% MCO-2 replacement. This resulted in a reduction of percent weight gain from 2252% to 1478%, respectively.

Table 4. Water quality data for the experiment that shrimp were offered diets using modified canola oil (MCO-1, MCO-2), and standard canola oil (SCO) to replace menhaden fish oil (MFO). The data were presented by Mean ± *SD*, and the range of the values (n=6).

Water parameters	Mean ± *SD*	Range
Dissolved Oxygen (mg/L)	6.50 ± 0.35	5.51- 7.37
Temperature (°C)	27.6 ± 1.05	25 - 29.4
Salinity (ppt)	8.0 ± 0.69	6.4 - 9.2
pH	8.5 ± 0.16	8.3 - 8.7
Ammonia (mg/L)	0.1 ± 0.03	0.02 0.11
Nitrite (mg/L)	0.1 ± 0.12	0.01 - 0.36

Table 5. Growth performance of Pacific white shrimp juvenile (0.19g) offered tested diets containing various level of modified or standard canola oil (MCO-1, MCO-2 SCO, respectively) as a replacement for menhaden fish oil (MFO) (n=6).

Treatment	Mean Final Weight (g)	Survival rate (%)	Weight Gain (g)	Percent Weight Gain (%)	FCR*	Whole Body Lipid (%)	TGC**
Basal	4.29a	88a	4.10a	2252.2a	1.90c	1.99ab	0.05a
25MCO-1***	4.13ab	92a	3.95a	2154.8ab	1.93bc	2.26ab	0.05a
50MCO-1	3.82ab	87a	3.65abc	2159.9ab	2.11abc	2.13ab	0.05a
75MCO-1	3.88ab	83a	3.68ab	1929.5abc	2.14abc	2.17ab	0.05ab
100MCO-1	3.16c	92a	2.98cd	1660.3bc	2.57a	2.30ab	0.05bc
100MCO-2	3.10c	88a	2.90d	1477.5c	2.70a	1.76b	0.04c
50SCO	3.63bc	93a	3.44abcd	1844.2abc	2.21ab	2.41a	0.05abc
75SCO	3.96ab	95a	3.76a	1980.4abc	2.03bc	2.34ab	0.05a
100SCO	3.20c	90a	3.00bcd	1567.3c	2.54a	1.94ab	0.05bc
P-Value	<0.0001	0.3674	<0.0001	<0.0001	<0.0001	0.0160	<0.0001
*PSE*****	0.150	3.38	0.149	11.35	0.107	0.130	0.001

*FCR: feed conversion ratio

**Thermal growth coefficient

***Percent replacement of enhanced canola oil in each diet.

***Pooled standard error (PSE) = $\sqrt{(Mean\ square\ error/n)}$

However, there were no significant differences in mean final weight between shrimp maintained on diets with 100% MCO's, and SCO replacement. Besides, there were no statistically significant differences presented in the mean final weights of the shrimp maintained on diets containing 0%, 25%, 50% and, 75% of MCO-1 replacement (diets 1 to 4). Mean final weight of the treatments of 50% and 75% SCO replacement did not show statistical difference, and the same result was found in the treatment of 50% and 100% SCO replacement. However, when the replacement of SCO increased from 75% to 100%, it resulted in a significant loss in mean final weight *(P= 0.02).*

In terms of the effectiveness of fish oil replaced by modified canola oil in practical diets for Pacific white shrimp, FCR tended to increase when the level of the replacement went up. There were no significant differences between shrimp offered the basal diet (100% MFO) and the treatments of MCO-1 replacement at 25% *(t (45)= -0,85, P = 0.99), 50% (t (45)= -2.51, P = 0.25), 75% (t(45)= -2.58, P = 0.22),* and SCO at 75% *(t(45)=-1.31, P = 0.92).* However, growth rate showed a decrease when FCR increased in the treatments of 100% MCO-1, 50% SCO, 100% SCO, and 100% MCO-2. FCR values in these treatments were significantly lower than the basal treatment with *t(45)= -5.68, P = < 0.0001* (100% MCO-1), *t(45)= -3.50, P = 0.03* (50% SCO), *t(45)= -5.54, P = < 0.0001* (100% SCO), *t(45)= -5.57, P = < 0.0001*(100% MCO-2). FCR values were not significantly affected in the treatments with the lower percentages of the replacement of MFO, particularly 25%, 50%, 75% of MCO-1.

Survival is also one of the important variables that was considered in this study. Although survival showed the highest in the treatment of 75% SCO, it was not statistically different among the treatments *(P=0.37,* Table 5*).*

3.4 Fatty acid profile in whole shrimp bodies

Whole shrimp were analyzed for lipid content and the fatty acid profiles determined (Table 6). Fatty acid profiles of the shrimp reflected the profiles of the diets

Table 6. Fatty acid profile (%) of lipids extracted from whole shrimp after being reared on various test diets containing increasing levels of using modified canola oil (MCO-1, MCO-2), or standard canola oil (SCO) (n=6). The results were presented Mean ± *SE*.

Fatty acids (%)	Basal	25MCO-1	50MCO-1	75MCO-1	100MCO-1	100MCO-2	50SCO	75SCO	100SCO	P-value
C14:0	0.99 ± 0.04a*	0.91 ± 0.03b	0.59 ± 0.02c	0.36 ± 0.01d	0.15 ± 0.01e	0.07 ± 0.02f	0.57 ± 0.02c	0.4 ± 0.02d	0.15 ± 0.01e	<.0001
C15:0	0.36 ± 0.01a	0.33 ± 0.00**b	0.27 ± 0.00c	0.23 ± 0.00d	0.18 ± 0.01e	0.19 ± 0.00e	0.26 ± 0.00c	0.22 ± 0.00d	0.17 ± 0.00f	<.0001
C16:0	18.9 ± 0.11a	17.86 ± 0.10b	16.53 ± 0.2c	15.22 ± 0.11d	13.63 ± 0.18f	14.27 ± 0.21e	16.21 ± 0.17c	15.08 ± 0.17d	13.27 ± 0.07f	<.0001
C16:1n7	2.39 ± 0.06a	2.13 ± 0.07b	1.42 ± 0.04c	0.94 ± 0.02d	0.5 ± 0.02e	0.38 ± 0.01f	1.39 ± 0.04c	1.02 ± 0.04d	0.54 ± 0.02e	<.0001
C17:0	1.01 ± 0.01a	0.89 ± 0.03b	0.81 ± 0.02c	0.71 ± 0.02d	0.6 ± 0.03f	0.68 ± 0.03de	0.75 ± 0.02cd	0.62 ± 0.02ef	0.51 ± 0.02g	<.0001
C18:0	8.54 ± 0.11a	7.9 ± 0.17cb	7.84 ± 0.13bc	7.77 ± 0.12bc	7.31 ± 0.25cd	8.03 ± 0.24b	7.08 ± 0.17d	6.24 ± 0.15e	5.53 ± 0.09f	<.0001
C18:1n9	11.22 ± 0.08i	13.26 ± 0.14h	15.26 ± 0.19g	17.04 ± 0.22f	19.45 ± 0.38e	21.41 ± 0.54d	23.21 ± 0.41c	28.69 ± 0.38b	35.34 ± 0.2a	<.0001
C18:1n7	3.52 ± 0.04a	3.37 ± 0.05b	3.24 ± 0.03c	3.12 ± 0.01cd	3.03 ± 0.04d	2.62 ± 0.05f	3.12 ± 0.03cd	2.92 ± 0.01e	2.71 ± 0.04f	<.0001
C18:2n6	14.67 ± 0.09e	17.07 ± 0.28d	18.17 ± 0.32d	20.16 ± 0.32c	22.35 ± 0.66b	20.04 ± 0.63c	18.24 ± 0.32d	21.56 ± 0.27b	26.7 ± 0.22a	<.0001
C18:3n6	0.26 ± 0.01f	0.33 ± 0.00e	0.37 ± 0.01d	0.42 ± 0.01b	0.50 ± 0.01a	0.40 ± 0.01c	0.21 ± 0.00g	0.18 ± 0.00h	0.14 ± 0.00i	<.0001
C18:3n3	1.02 ± 0.03e	1.21 ± 0.05d	1.25 ± 0.05d	1.38 ± 0.04c	1.64 ± 0.06b	1.10 ± 0.04de	1.15 ± 0.03de	1.40 ± 0.03c	1.89 ± 0.04a	<.0001
C20:0	0.28 ± 0.01d	0.3 ± 0.01cd	0.33 ± 0.01c	0.37 ± 0.00b	0.4 ± 0.01a	0.39 ± 0.01a	0.31 ± 0.00cd	0.30 ± 0.01cd	0.3 ± 0.01cd	<.0001
C20:1n9	0.7 ± 0.01g	0.78 ± 0.01f	0.84 ± 0.00e	0.93 ± 0.02d	0.99 ± 0.02c	1.06 ± 0.02b	1.05 ± 0.02b	1.12 ± 0.02a	1.09 ± 0.01ab	<.0001
C20:2n6	1.92 ± 0.05e	2.11 ± 0.02d	2.36 ± 0.04c	2.72 ± 0.02b	2.85 ± 0.07b	3.08 ± 0.06a	2.11 ± 0.03d	2.13 ± 0.07d	2.22 ± 0.05d	<.0001
C20:3n6	0.31 ± 0.01f	0.84 ± 0.01e	1.39 ± 0.02c	1.91 ± 0.04b	2.44 ± 0.07a	0.98 ± 0.01d	0.29 ± 0.00f	0.25 ± 0.00f	0.25 ± 0.01f	<.0001
C20:4n6 ARA	2.26 ± 0.06e	2.63 ± 0.05d	3.36 ± 0.10c	4.02 ± 0.13b	4.73 ± 0.20a	4.45 ± 0.18a	1.91 ± 0.06f	1.67 ± 0.07f	1.6 ± 0.06f	<.0001
C20:3n3	0.36 ± 0.01ab	0.36 ± 0.01ab	0.34 ± 0.01abc	0.36 ± 0.01ab	0.39 ± 0.02a	0.33 ± 0.01bc	0.3 ± 0.02c	0.3 ± 0.01c	0.35 ± 0.01ab	<.0001
C20:5n3 EPA	12.64 ± 0.2a	11.3 ± 0.21bc	10.64 ± 0.22c	9.7 ± 0.22d	8.74 ± 0.33e	11.86 ± 0.45b	8.93 ± 0.27e	6.29 ± 0.19f	2.27 ± 0.12g	<.0001
C22:0	0.21 ± 0.01a	0.2 ± 0.01a	0.2 ± 0.01a	0.2 ± 0.01a	0.19 ± 0.01a	0.13 ± 0.03b	0.17 ± 0.01ab	0.16 ± 0.01ab	0.16 ± 0.01ab	<.0001
C22:1n9	0.35 ± 0.35a	0.01 ± 0.01a	0.04 ± 0.04a	nd	0.02 ± 0.01a	0.03 ± 0.02a	0.04 ± 0.02a	0.02 ± 0.02a	0.02 ± 0.01a	0.05499
C23:0	0.38 ± 0.03a	0.34 ± 0.01ab	0.26 ± 0.01c	0.19 ± 0.01d	0.01 ± 0.01e	nd	0.3 ± 0.02bc	0.21 ± 0.03d	nd	<.0001
C22:4n6	0.15 ± 0.02d	0.2 ± 0.03d	0.28 ± 0.00c	0.34 ± 0.00b	0.43 ± 0.01a	0.29 ± 0.01c	0.17 ± 0.01d	0.15 ± 0.01d	0.19 ± 0.02d	<.0001
C22:3n6	0.36 ± 0.01a	0.34 ± 0.01ab	0.31 ± 0.01abc	0.28 ± 0.01bc	0.27 ± 0.02c	0.32 ± 0.03abc	0.3 ± 0.01abc	0.28 ± 0.01bc	0.28 ± 0.03bc	0.0019
C22:5n3 DPA	1.46 ± 0.02e	1.73 ± 0.03d	2.12 ± 0.03c	2.59 ± 0.02b	3.32 ± 0.09a	2.58 ± 0.08b	1.14 ± 0.02f	0.85 ± 0.03g	0.42 ± 0.02h	<.0001
C24:0	0.14 ± 0.01a	0.12 ± 0.01ab	0.13 ± 0.01a	0.13 ± 0.01a	0.14 ± 0.01a	0.13 ± 0.01a	0.11 ± 0.01ab	0.1 ± 0.01b	0.1 ± 0.01b	0.0003
C22:6n3 DHA	11.35 ± 0.18a	9.4 ± 0.20b	7.59 ± 0.18c	5.27 ± 0.13d	2.12 ± 0.13f	2.71 ± 0.19e	7.46 ± 0.16c	5.34 ± 0.14d	1.97 ± 0.10f	<.0001
C24:1n9	0.16 ± 0.02b	0.15 ± 0.01b	0.17 ± 0.01b	0.18 ± 0.01b	0.2 ± 0.02ab	0.25 ± 0.02a	0.20 ± 0.01ab	0.20 ± 0.01ab	0.25 ± 0.02a	<.0001

*The values across a row superscripted with the same letter indicates there was no significant difference (P>0.05). ** SE ≤ 0.005. nd: not detected

EPA and DHA levels decreased as MFO was replaced by MCO's and SCO in the diets. These fatty acids were lowest in shrimp offered diets with 100% SCO with the resulting EPA level being 2.27± 0.12 and DHA being 1.97 ± 0.1. These results were significantly below that of shrimp maintained on the basal diet. DPA level of the shrimp lipids increased when MCO-1 replacement increased constantly from 25% to 100% in the diet. On the other hand, incremental increases in SCO resulted in significant reductions in DPA level in the fatty acid profile of lipids extracted from the shrimp. ARA levels in the shrimp lipids were found to show a significant increase from 2.26% to 4.73% when the replacement of MCO-1 increased from 0% to 100%. Briefly, the result of EPA, DHA, DPA, and ARA levels in the whole shrimp body lipid followed the levels found in the diets as modified and standard canola oils levels shifted in the diet

Figure 1 showed the shift of total n-3 and n-6 in oils and diets. The decrease of n-3 and increase of n-6 in the diets correlated to the replacement of MCO-1. To allow for visualization of the response of growth to shifts in fatty acid profiles of the diets Figures 2 and 3 were developed. Figure 2 demonstrates that the response of the shrimp is not likely due to EPA levels of the diet whereas the lowest levels of dietary DHA seem to be related to poor performance. Similarly, DPA and ARA levels of the diet do not follow that of the growth (Figure 3).

Figures 4 to 7, were developed to help visualize the response of tissue deposition and that of the diet. The linear regression data were analysed based on the data of all the samples of each treatment. The regressions of EPA and DHA levels in diets had MCO-1 and SCO presence, and the regressions of lipids extracted from whole shrimp bodies offered MCO-1, and SCO showed a trend of a significant decline when the level of MCO's, and SCO increased, and it reached the lowest point (0%) when MFO was completely replaced by 100% of SCO. According to Figure 4, the regression analysis of EPA level in MCO-1 and SCO supplemented diets reported $R^2 = 0.999$, $P < 0.0001$, and $R^2 = 1.000$, $P = 0.0002$, respectively.

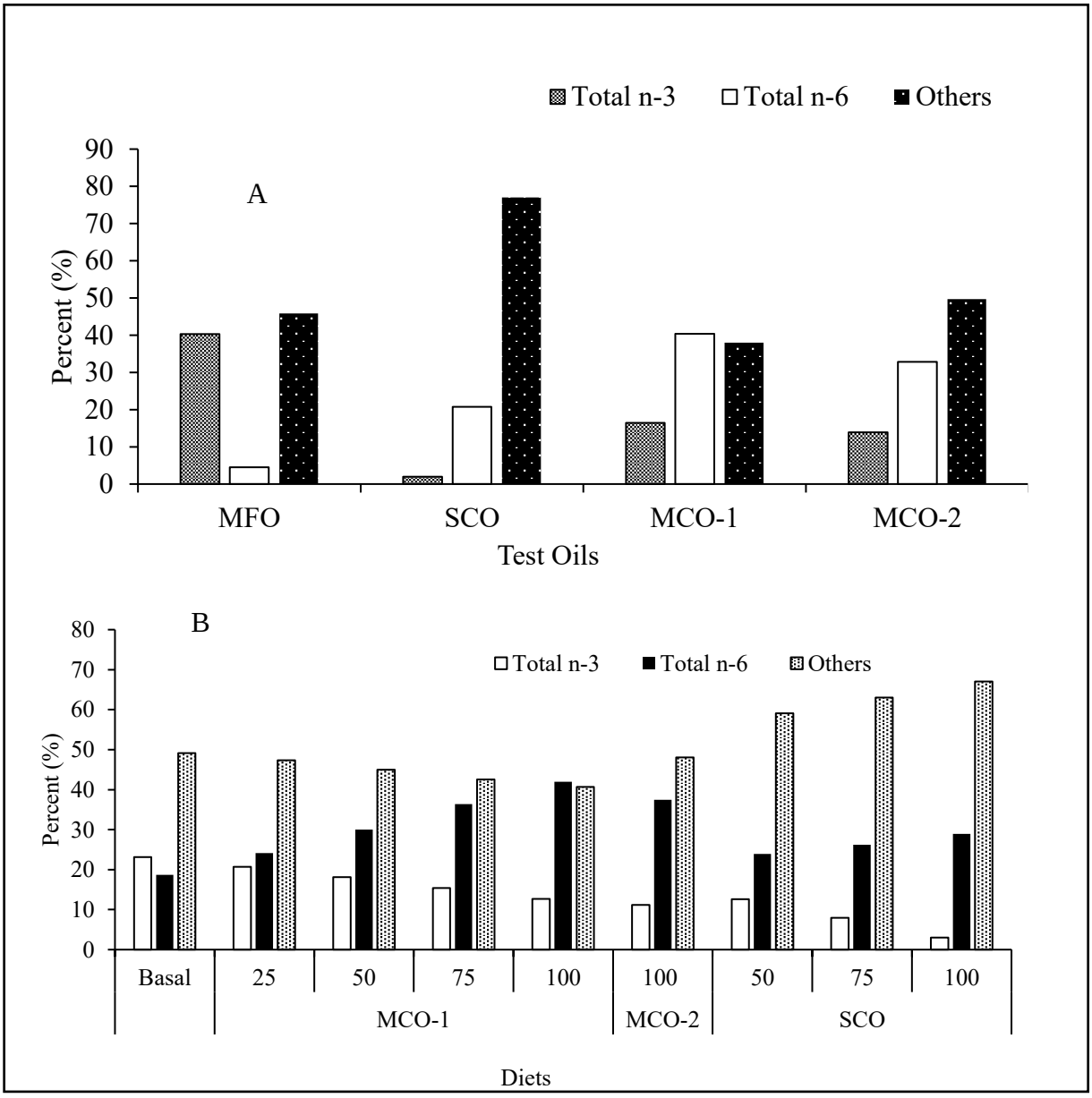

Figure 1. Percentage of total n-3 fatty acid, and total n-6 fatty acids in total fatty acids in test oils (A; menhaden fish oil, MFO; modified canola oils, MCO-1, MCO-2; standard canola oil , SCO) and in diets (B; basal diet, 100% MFO, supplemented diets MCO-1, MCO-2, and SCO.

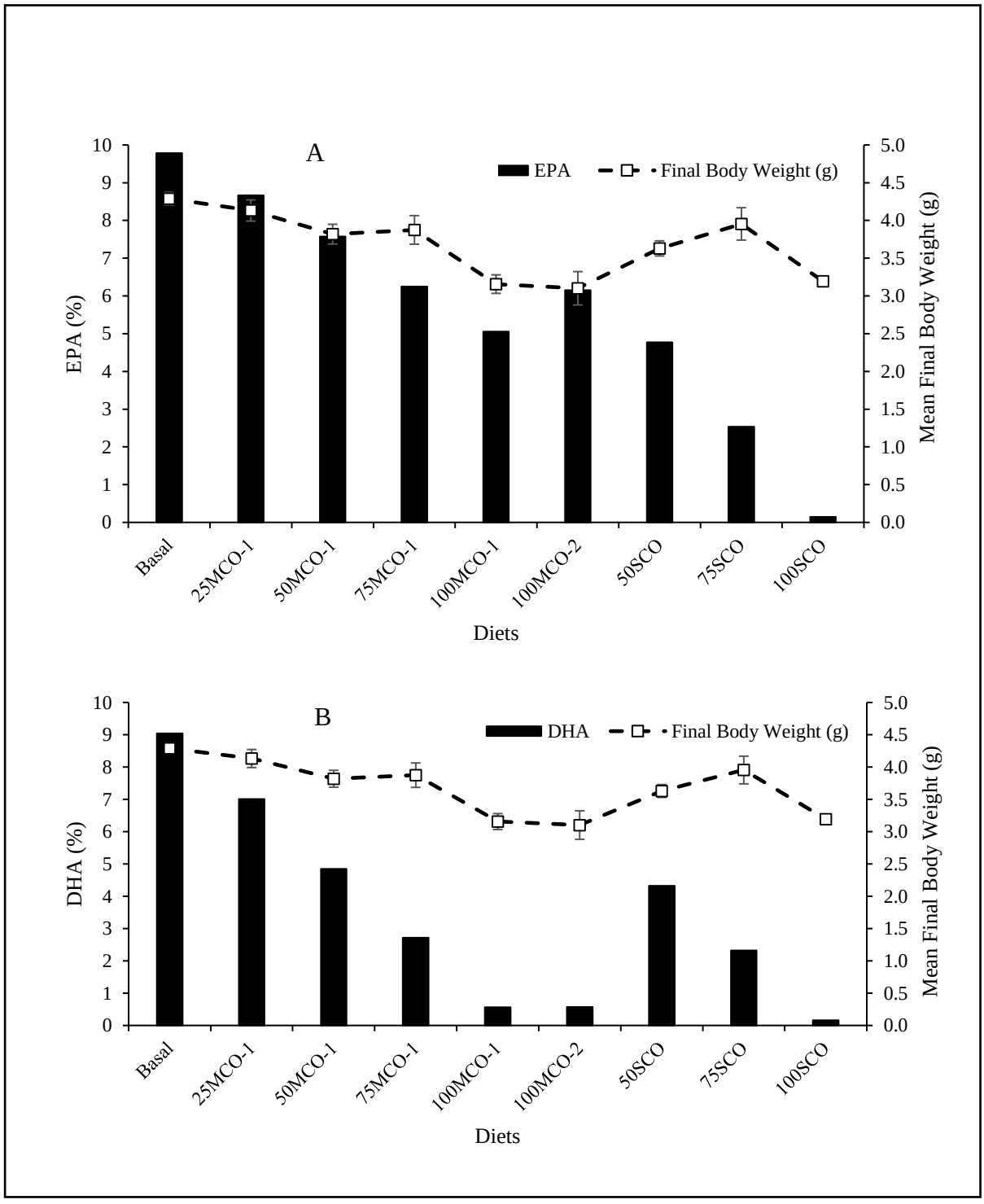

Figure 2. The relationship between final body weight (g) and EPA (Eicosapentaenoic acid, C20:5n-3) level (%) (A), and DHA (Docosahexaenoic acid, C22:6n-3) level (%) (B) in tested diets using modified canola oils (MCO-1, MCO-2) and standard canola oil (SCO) to replace menhaden fish oil (MFO).

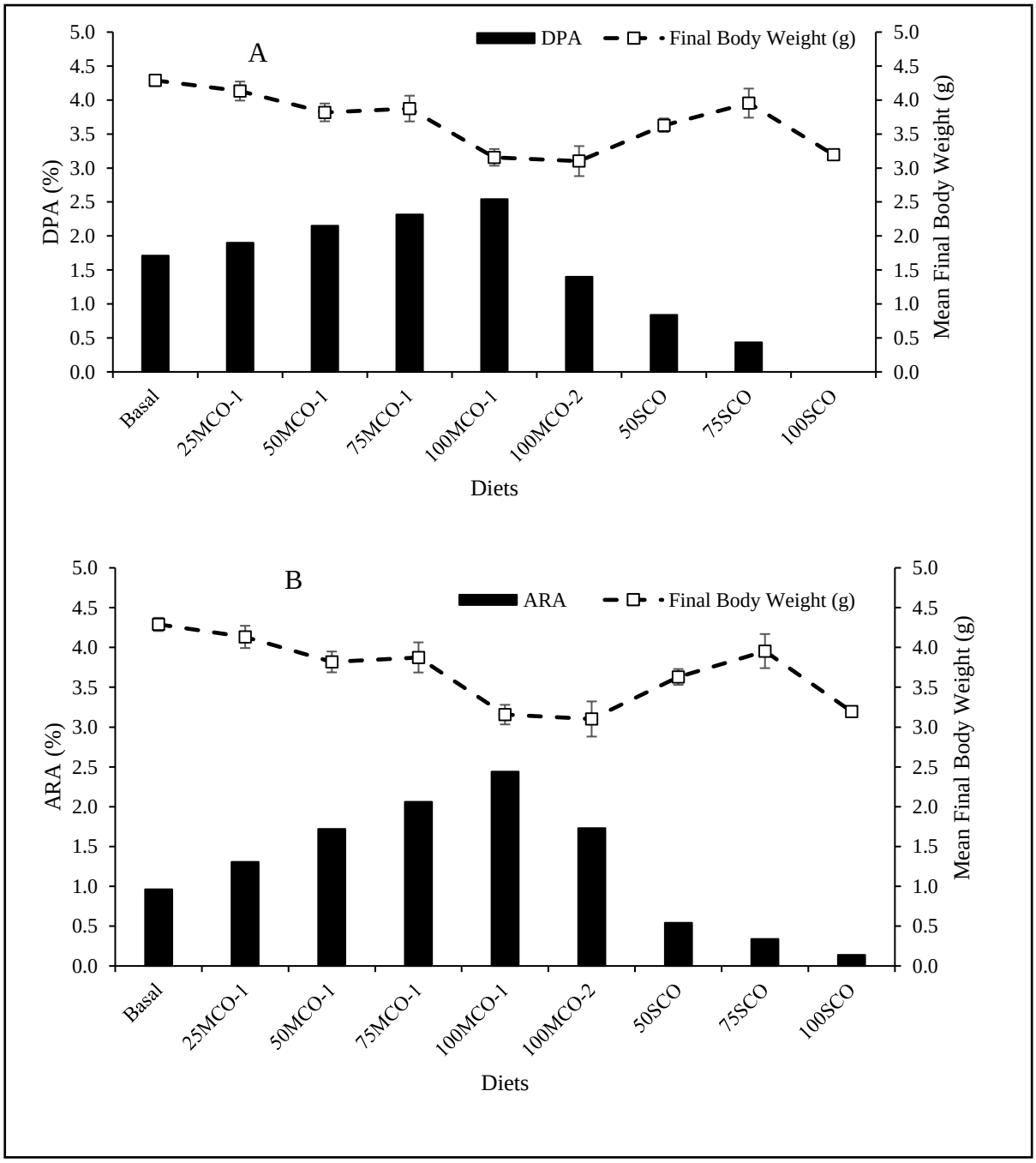

Figure 3. The relationship between final body weight (g) and DPA (Docosapentaenoic acid, C22: 5n-3) level (%) (A) and ARA (Arachidonic acid, C20:4n-6) level (%) (B) in test diets using modified canola oils (MCO-1, MCO-2), and standard canola oil (SCO) to replace menhaden fish oil (MFO).

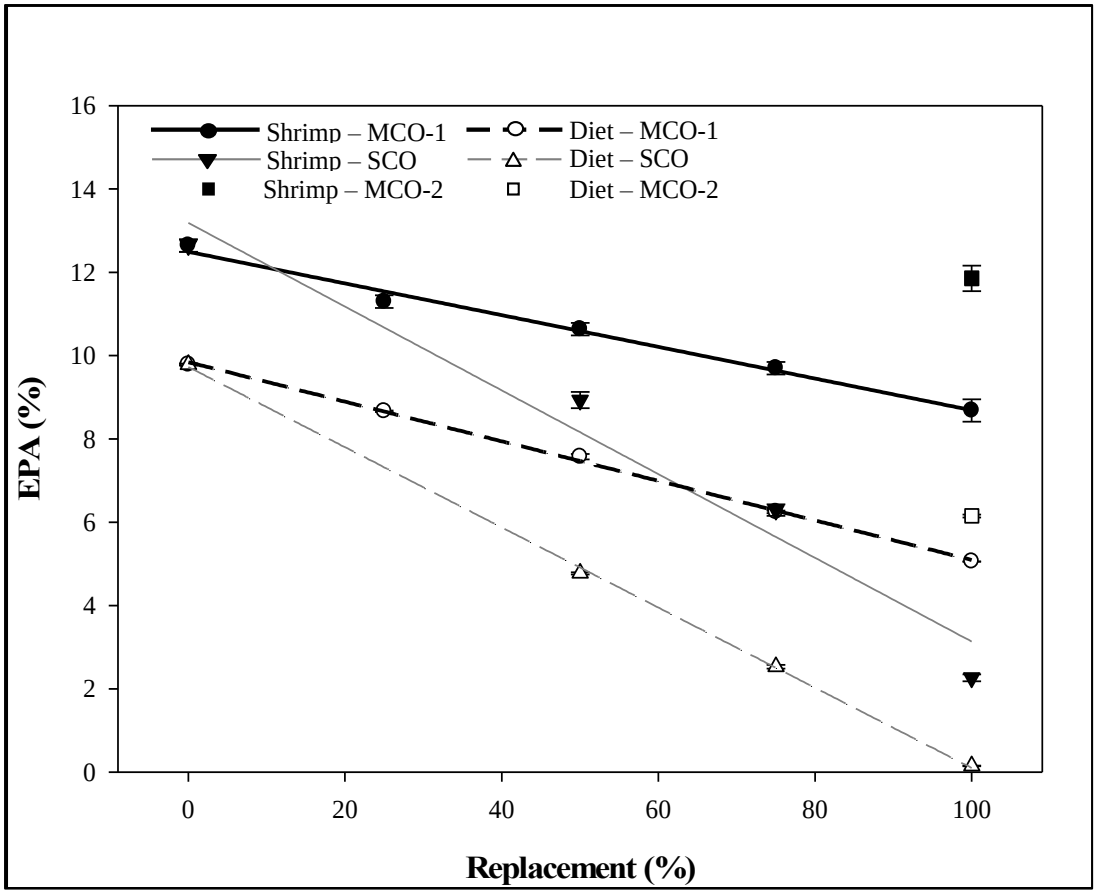

Figure 4. Relationship between levels of modified canola oil (MCO-1) replacement and EPA (Eicosapentaenoic acid, C20:5n-3) level (%) in diets and in shrimp. The regression line of EPA level for the diets with the MCO-1 is y = 9.842 - 0.0475x (R^2 = *0.999, P <0.0001)*, and in shrimp offered the respective diets is y = 12.4937 - 0.0381x (R^2 = *0.990, P <0.0001*). The regression line of EPA level for diets with the SCO is y = 9.7244 - 0.0963x (R^2 = *1.000, P = 0.0002*), and in shrimp offered the respective diets is y = 13.1871 - 0.1005x (R^2 =*0.964, P= 0.0072*).

The regression data of shrimp bodies offered MCO-1 diets showed $R^2 = 0.990$, $P < 0.0001$, and shrimp bodies offered SCO diets presented $R^2 = 0.9640$, $P = 0.0072$. With the results of regression analysis of DHA level (Figure 5) in diets of MCO-1 ($R^2 = 1.000$, $P < 0.0001$), of SCO ($R^2 = 0.999$, $P = 0.0005$), and in shrimp offered MCO-1 supplemented diets ($R^2 = 0.986$, $P < 0.0001$), in shrimp offered SCO diets ($R^2 = 0.982$, $P = 0.0038$) the trend was a negative correlation to the increase in MCO-1 replacement in the diets.

In contrast, the results of DPA and ARA levels (Figure 6 and 7) in diets that contained MCO-1 and SCO, and in shrimp bodies offered diets of MCO-1 and SCO were positively correlated to the increases of MCO-1 level in diets and negatively correlated to the increases of SCO in diets.

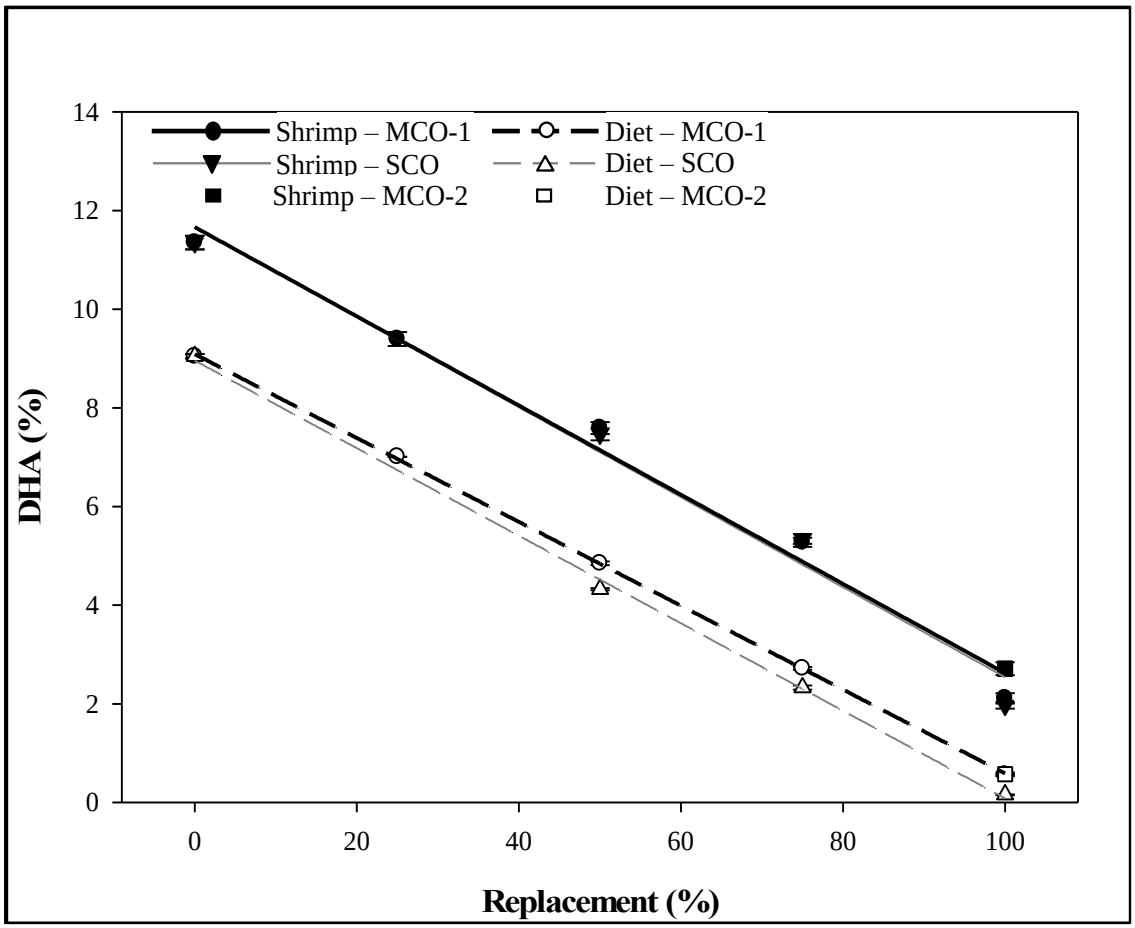

Figure 5. Relationship between levels of modified canola oil (MCO-1) replacement and DHA (Docosahexaenoic acid, C22:6n-3) level (%) in diets and in shrimp. The regression line of DHA level for the diets the MCO-1 is y = 9.0853 - 0.085x (R^2 = *1, P < 0.0001*), and in shrimp offered respective the diets is y = 11.6621 - 0.0904x (R^2 = *0.986, P= 0.0001*). The regression line of DHA level for the diets with the SCO is y = 8.9534 - 0.0887x (R^2 = *0.999, P = 0.0005*), and in shrimp offered the respective diets is y = 11.659 - 0.0912x (R^2 = *0.982, P = 0.0038*).

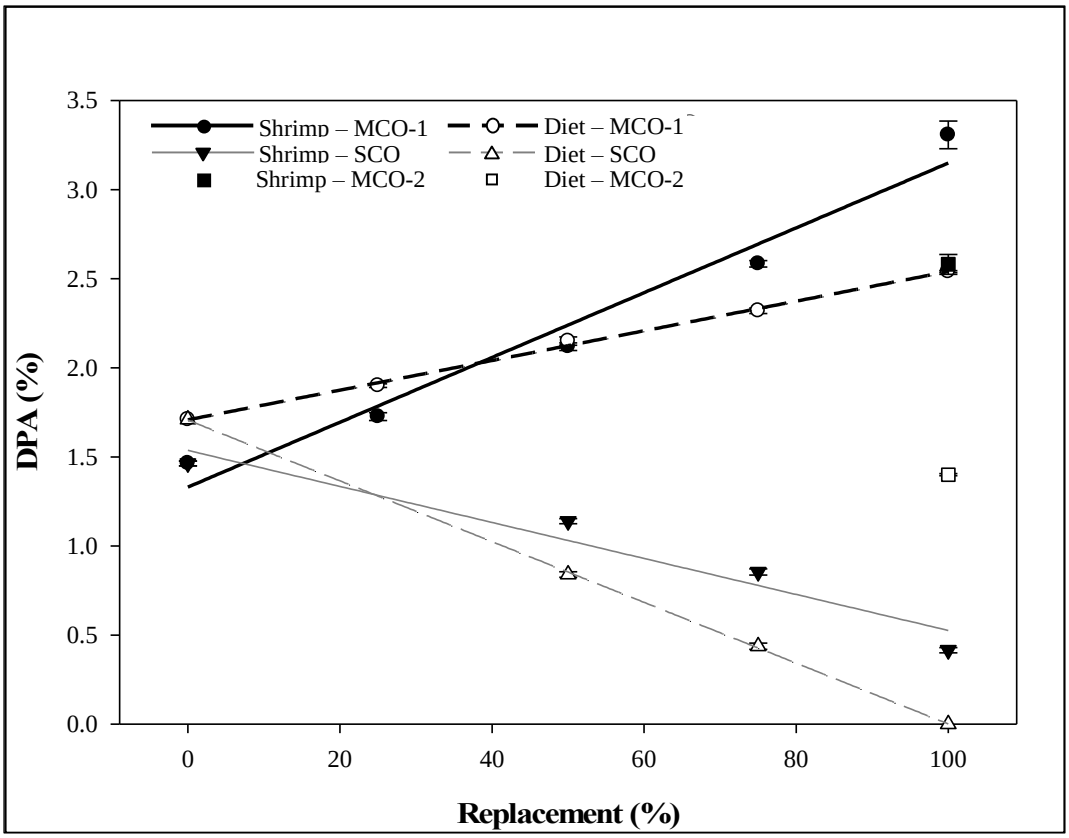

Figure 6. Relationship between levels of modified canola oil (MCO-1) replacement and DPA (Docosapentaenoic acid, C22: 5n-3) level (%) in diets and in shrimp. The regression line of DPA level in diets with the MCO-1 is y = 1.708 + 0.0083x (R^2 = *0.998, P<0.0001*), and in shrimp offered the respective diets is y = 1.3304 + 0.0182x (R^2 = *0.966, P< 0.0001*). The regression line of DPA level in diets with the SCO is y = 1.7058 - 0.0171x (R^2 = *1.000, P <0.0001*), and in shrimp offered respective the diets is y = 1.536 - 0.0101x (R^2 = *0.941, P = 0.008*).

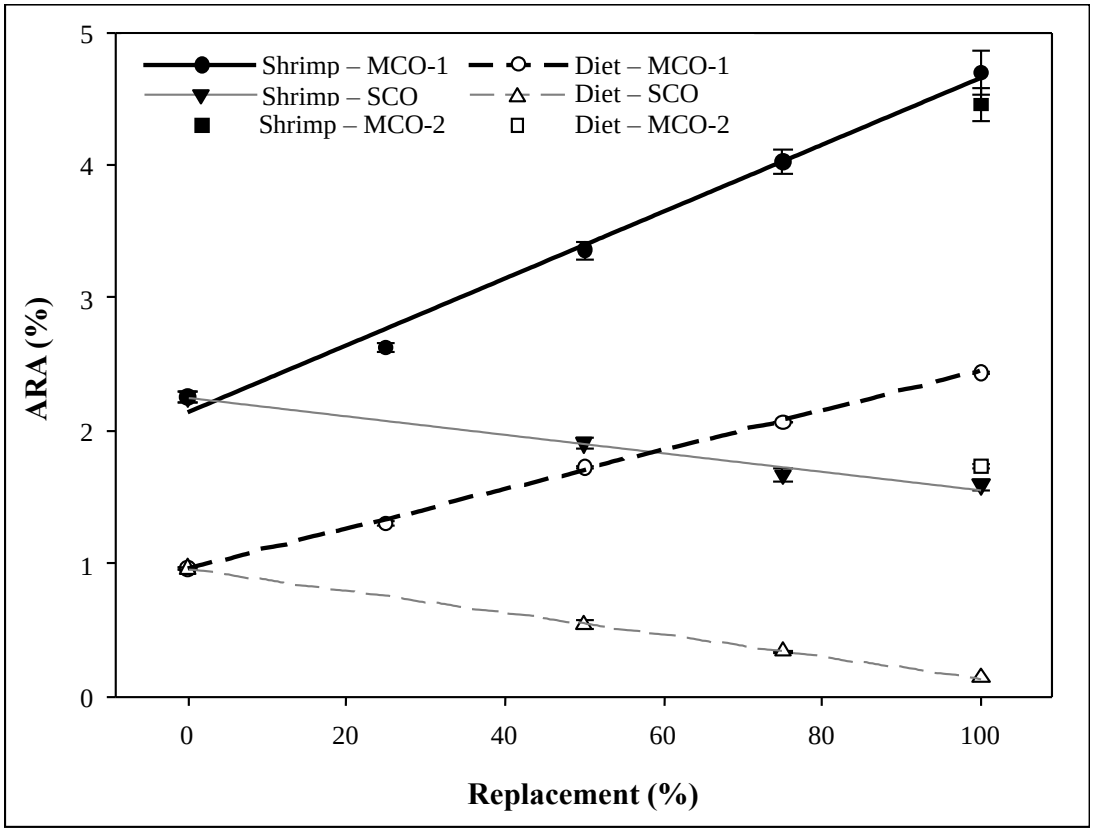

Figure 7. Relationship between levels of modified canola oil (MCO-1) replacement and ARA (Arachidonic acid, C20:4n-6) level (%) in diets and in shrimp. The regression line of ARA level in diets with the MCO-1 is y=0.9573 + 0.0148 (R^2 = *0.999, P < 0.0001*), and in shrimp offered the respective diets is y = 2.1369 + 0.0251x (R^2 = *0.991, P < 0.0001*). The regression line of ARA level in diets with the SCO is y = 0.9593 - 0.0082x (R^2 = *1.000, P =<0.0001*), and in shrimp offered the respective diets is y=2.2428 - 0.0069x (*R2 = 0.977, P = 0.0004*).

4. Discussion

Lipid is one of the most important nutrient components of the feed. Fish oil has been used in shrimp feed for its nutritional values of omega-3 and 6, long chain polyunsaturated fatty acids such as EPA, DHA as well as ARA, which are required by many marine species (Olsen and Hasan, 2012) as chain elongation from monounsaturated fatty acids such as α-linolenic acid (LNA, C18:3n3) and cis-linoleic acid (LOA, C18:2n-6) are inefficient (NRC, 2011). Because of the inability or limited ability of marine species to convert LC-HUFAs from PUFAs, these fatty acids have become an essential consideration in meeting the dietary requirements. Most vertebrates and crustaceans cannot convert C18 to LC-HUFA, so provision of C20 and C22 from the diet is required (NRC, 2011). A study by Soller *et al.* (2017) also demonstrated that Pacific white shrimp could not synthesize LC-HUFA from C18 by analysing fatty acids in the tissue of shrimp. The shrimp that were offered plant-based diets reported a large amount of α-linolenic acids present in the tissue, however, no significant increase in the amount of EPA, DHA, and DPA were found. Numerous studies have been conducted with the purpose of using plant oils to replace fish oil in different species such as seabass (Mourente and Bell, 2006), salmon (Rosenlund *et al.*, 2001), rainbow trout (Caballero *et al.*, 2002), and Pacific white shrimp (González-Félix *et al.*, 2010). In such species, fish oil could not be completely replaced due to abundance of n-6 fatty acids and absence of LC-HUFAs.

If plant oils are to be utilized, they must either be used as partial replacements, or the fatty acid profile must be adjusted. The MCO-1 utilized in this work contained enhanced levels of EPA (7.33%) whereas SCO did not have significant levels of EFA (Table 1). In the diet series where MCO-1 replaced MFO, EPA decreased from 9.97 to 5.06%, and DHA dropped from 9.04 % to 0.57% when MCO-1 increased from 0% to 100% in the diets. All the MCO tested diets were expected to show non-significant differences in growth, survival, and FCR if

the EFAs in MCO's reached the requirements of EFAs in Pacific white shrimp. The DHA levels of the diets using MCO's were designed to just meet the requirement whereas diets containing 100% MCO's (MCO-1, MCO-2) would have likely been deficient.

The mentioned studies have indicated that different shrimp species have different dietary requirements for EFA for proper development and growth. For example, *M. rosenbergii* requires DHA at 0.075% (D'Abramo and Sheen, 1993), *P. japonicus* requires EPA and DHA at 1.0% each (Kanazawa *et al.*, 1978; Kanazawa *et al.*, 1979c), *P. monodon* needs 0.9% EPA and DHA each (Glencross and Smith, 2001). In *L. vannamei,* juveniles requirements of at least 0.5% both EPA and DHA has been recommended (Gonzalez-Felix *et al.*, 2003a). In the results of fatty acid analysis of tested diets of this study, only the 100% SCO diet had EPA and DHA levels below this threshold. However, diets containing 100% MCO's (both sources) had the levels of DHA at 0.57% which was very close to the theoretical requirements for DHA. Hence, all three of these diets with 100% replacement of MFO resulted in significant depressions in growth which may indicate a limitation of DHA in the diet.

Other fatty acids of concern would be ARA. Xu *et al.* (1993), reported that the influence of ARA bon growth was better than that of both linoleic (LOA, 18:2n-6) and linolenic (LNA, 18:3n-3). However, when there was a presence of DHA, DHA played an essential role in growth performance which was better in regard to the influence of ARA. In regard to this study, ARA levels were only low in diets with SCO as the supplement and actually increased as MCO-1 was included in the diets. This would indicate that ARA was not limiting in these diets which is in agreement with other studies.

The fatty acid components of the lipids extracted from whole shrimp reflected the respective levels in the diets (Figures 4 - 7), demonstrated highly correlated responses of the various fatty acids. These reults showed that dietary lipids regulated carcass lipids by reflecting the change in lipid compositions. The increase of DPA and ARA and the decrease of EPA and

DHA due to the high percent of MCO-1 replacement in diets were followed by the same trend in shrimp tissues. This result was supported by the the results of Gonzalez-Felix *et al*. (2003b) and Gonzalez-Felix *et al*. (2010); where the authours reported that the increase of n-3 HUFAs in diet was followed by the increase of n-3 HUFAs in shrimp tissues. Additionally, higher level of EFAs were found in shrimp tissues in comparision to their repsective diatary levels, which was in agreement with the study of Gonzalez-Felix *et al.* (2002a). The accumulation of fatty acid compositions in the shrimp tissues was probably due to the limitation of the matabolic rate or the imbalances between fatty acid concentrations and enzyme specificities in shrimp (NRC, 2011). An example of the imbalance is DHA which resistants to its enzyme resulting in DHA retained in tissues (NRC, 2011), which was found to match with the result of DHA in fatty acid profile in shrimp tissues of this study.

According to the study of Kanazawa (1985), besides EPA, 20:5n-3 and DHA, 22:6n-3, C18, linoleic (LOA, 18:2n-6) and linolenic (LNA, 18:3n-3) were also considered as EFAs in the penaeid prawn, *Penaeus japonicus*. On the other hand, Lim *et al.* (1997) concluded that *L. vannamei* offered diets containing LC-HUFAs such as EPA (20:5n-3) and DHA (22:6n-3) which showed better growth performance than those of LNA(18:3n-3) and LOA(18:2n-6). The study also stated that among terrestrial oils which had higher n-3 that presented a better result of growth and feed efficiency than those higher in n-6 even though both n-3 and n-6 were essential for shrimp growth, and survival. Thus, to successfully replace fish oil with canola oil without causing deficiency in growth, development, and survival of the animals, these requirements of EFAs have to be met.

A number of studies have demonstrated EFA deficiencies in practical diets. Samocha *et al.* (2010) reported that marine oil could be completely replaced by plant oils combined with fermented products in order to provide the EFAs. Another study of Samocha *et al*. (2011) found that algae supplements could be a source of ARA and fermented heterotrophic algae oils were

viable sources of EFAs. However, EFA deficiencies occurred even in green water conditions where natural foods were present in that study. The replacement of fish oil that could not be successful at the level of 100% in these two studies could be due to the refinement of diet formulations that used HUFA supplementals (Samocha *et al.*, 2010). Soller *et al.* (2017) worked with *L. vannamei* in outdoor ponds and was also able to replace a portion of the fish oil with plant-based oils possibly because the required level of EFAs were provided by low levels of marine oils. Gonzalez-Felix *et al.* (2010) carried out a similar experiment in outdoor tanks using soybean oil and linseed oil combined with MFO in Pacific white shrimp diets. There were no deficiencies found in FCR, growth, or survival within the treatments of soybean oil which replaced fish oil at different levels with up to 90% of the replacement with the presence of natural food. These results suggested that lipid from plant oil sources could be used as long as EFAs were reached through combination of oil sources. However, with regards to the current study, the complete replacement of MFO was not successful even though DHA level in the diet was 0.57%. Hence, the response could be due to other factors.

In addition to a specific deficiency of EFAs, the ration of major fatty acids has been suggested to cause problem with lipid metabolism. For example, Lim *et al.* (1997) suggested that oils high in n-3 fatty acids resulted in better performance of *Penaeus vannamei*. In this current study, n3/n6 was found significantly lower than the basal when 100% MFO was replaced by 100% MCO-1, MCO-2, and SCO with the value of 0.3, 0.3, and 0.1, respectively. According to the results of Gonzalez-Felix *et al.* (2010), the lowest n3/n6 ratio was 0.15 and it was found in the diet of 90% MFO replaced by plant oil; however, no significant difference in mean final weight was found in the treatment compared to the commercial diet which had 0.71 n3/n6 ratio. From another study of Gonzalez-Felix *et al.* (2009), it was reported that there was not a difference in mean final weight of shrimp that offered a different source of lipid that led to different n3/n6 ratio. According to the authors, the best mean final weight was found in the

diet had the n3/n6 ratio of 0.56; however, the result did not statistically differ from the result of the diet that had the n3/n6 ratio of 0.21. Different experiments showed different ranges of the n3/n6 ratio that maintained the optimal range of growth performance in *L. vannamei*. The effects of n3/n6 ratio are not conclusive in production diets but it appears shrimp can tolerate a wide range. Thus, the n3/n6 ratio in diet seems not to be a likely reason for poor performance of the shrimp in this study.

A high level of HUFAs in diets can cause issues of toxicity and contamination due to oxidization products, which will lead to a negative effect on growth performance and survival (Gonzalez-Felix *et al.*, 2002b; Kanazawa *et al.*, 1985). As the test oils were produced under research conditions, one possible explanation of the poor response at high levels could be due to oxidation. The maximum peroxide level of the EPA and DHA-rich oil such as fish oil is 10 meq/kg and the same limit is also applicable for plant oils (Ismail *et al.*, 2016). To evaluate this possibility, peroxide values of the tested diets that contained 100% of a given oil source were evaluated. The diet supplemented with 100% MCO-1 and 100% MCO-2 were analysed for peroxide values with levels of 21.7 meq/kg, and 16.2 meq/kg, respectively. Hence, the reduction of growth performance in the treatment offered the 100% MCO's supplement diet could be in part due to oxidation and possible reductions in palatability. As rancidity was not detected in the SCO diet, this is not the primary factor resulting in poor growth, although it could be a contribution. There may be other reasons causing poor growth performance and FCR in shrimp that offered a high percent of canola oil diet. These included palatability, and contaminants of oxidization processes. It is difficult to determine palatability because it is species specific in terms of variabilities of physical structures and feeding behaviours (Lamb, 2001). Geurden *et al.* (2005) reported that rainbow trout could distinguished diets made with different oil sources by reflecting different demands in different oil based diets. Particularly, diet made with fish oil had the highest demand in comparison to the diets made with the other

plant oils (linseed oil, sunflower oil, and rapeseed oil). Another study of Geurden *et al*.(2007) demontrated reduction in feed consumption of plant oil-based diets in rainbow trout, which was significantly lower than the demand for fish oil-based diet. Hence, the authors concluded that dietary oil affected feeding behaviour of rainbow trout.

In the current study, no direct testing of feed palatability was incuded, however, according to the results of FCR, it increased when modified canola oil replacement increased from 0% to 100%, and it was significantly different from the basal diet treatment and the treatment of 100% MCO-1. The difference was also found between the basal diet treatment and the treatments of 100% MCO-2, and 100% SCO. Samocha *et al.* (2010) conducted an experiment using fermentation products as a highly unsaturated fatty acid source to compare the difference between HUFA supplemented, non-HUFA, and MFO diets on *L. vannamei.* In the results of FCR, it showed that there was no significant difference between the treatments of HUFA supplemented, and non HUFA supplemented, as well as the treatments of MFO and HUFA supplemented diet. However, there was a significant difference between the treatments of the diets that contained MFO and the diet that did not contain MFO. This may be indicating that fish oil can improve performance due to factors other than EFAs. Another experiment of Soller *et al.* (2017), used plant oil to replace fish oil in practical diets for marine shrimp. The results of FCR found that there was a significant difference between the diets that contained MFO and the diets that did not contain MFO. However, in all the treatments of the study, MFO was sprayed on top of the feed, so it was hard to determine whether feed palatability was improved by MFO. There is very little information of palatability of fish oil and plant oil affecting the quality of practical diet for shrimp, *L. vannamei.* Given the limited information, the effects of palatability and digestibility would be worth pursuing in future studies on *L. vannamei*

5. Conclusion

From the results of this study, the modified canola oil MCO-1 can be used to replace fish oil up to 75% in practical diet for *L. vannamei* in recirculating system. Under the reported conditions 100% replacement was not successful most likely due to EFA deficiencies which occurred at 100% replacement when using MCO and SCO as the lipid source. However, potential effects of palatability of the oil source or oxidation by products cannot be eliminated as MCO-1 showed signs of oxidation. The results indicate that EFAs reduced in diets as well as in shrimp bodies when the replacement of MFO increased, which consequently caused poor growth performance. The significant depression of growth could be caused by the decrease of EFAs, which was found in DHA levels. However, a portion of the response could also be caused by the oxidization products decreasing palatability in diets containing MCO-1. Additional studies should be conducted to confirm possible DHA deficiencies as well as possible palatability issues with the oils.

6. References

Caballero, M., Obach, A., Rosenlund, G., Montero, D., Gisvold, M., Izquierdo, M., 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 214, 253-271.

Cuzona, G.r., Lawrenceb, A., Gaxiolac, G., Rosasc, C., Guillaumed, J., 2004. Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. Aquaculture. 235, 513-551.

D'Abramo, L.R., Sheen, S.S., 1993. Polyunsaturated fatty acid nutrition in juvenile freshwater prawn *Macrobrachium rosenbergii*. Aquaculture. 115, 63-86.

FAO, 2018. The State of World Fisheries and Aquaculture- Meeting The Sustainable Development Goals.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497-509.

Geurden, I., Corraze, G., Boujard, T., 2007. Self-feeding behaviour of rainbow trout, *Oncorhynchus mykiss*, offered diets with distinct feed oils. Applied animal behaviour science. 108, 313-326.

Geurden, I., Cuvier, A., Gondouin, E., Olsen, R., Ruohonen, K., Kaushik, S., Boujard, T., 2005. Rainbow trout can discriminate between feeds with different oil sources. Physiology & behavior. 85, 107-114.

Glencross, B.D., Smith, D.M., 2001. Optimizing the essential fatty acids, eicosapentaenoic and docosahexaenoic acid, in the diet of the prawn, *Penaeus monodon*. Aquaculture Nutrition. 7, 101-112.

Gonzalez-Felix, M., III, D.G., Lawrence, A.L., Perez-Velazquez, M., 2002a. Effect of various dietary lipid levels on quantitative essential fatty acid requirements of juvenile Pacific

White Shrimp *Litopenaeus vannamei*. Journal of the World Aquaculture Society. 33, 330-340.

Gonzalez-Felix, M.L., III, D.M.G., Lawrence, A.L., Perez-Velazquez, M., 2002b. Effect of dietary phospholipid on essential fatty acid requirements and tissue lipid composition of *Litopenaeus vannamei* juveniles. Aquaculture. 207, 151-167.

Gonzalez-Felix, M.L., D.M., G.I., Lawrance, A.L., Perez-Velazqez, M., 2003a. Nutritional evaluation of fatty acids for the open thelycum shrimp, *Litopenaeus vannamei*: II. Effect of dietary n-3 and n-6 polyunsaturated and highly unsaturated fatty acids on juvenile shrimp growth, survival, and fatty acid composition. Aquaculture Nutrition. 9, 115-122.

Gonzalez-Felix, M.L., Lawrence, A.L., Gatlin, D.M., Perez-Velazquez, M., 2003b. Nutritional evaluation of fatty acids for the open thelycum shrimp, *Litopenaeus vannamei*: I. Effect of dietary linoleic and linolenic acids at different concentrations and ratios on juvenile shrimp growth, survival and fatty acid composition. Aquaculture Nutrition. 9, 105-113.

González-Félix, M.L., Perez-Velazquez, M., Quintero-alvarez, J.S.M., 2009. Effect of Various Dietary Levels of Docosahexaenoic and Arachidonic Acids and Different n-3/n-6 Ratios on Biological Performance of Pacific White Shrimp, *Litopenaeus vannamei*, Raised in Low Salinity. Journal of the World Aquaculture Society. 40, 194-206.

González-Félix, M.L., Silva, F.S.D.d., Davis, D.A., Samocha, T.M., Morris, T.C., Wilkenfeld, J.S., Perez-Velazquez, M., 2010. Replacement of fish oil in plant based diets for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture. 309, 152-158.

Hasan, M.R., 2001. Nutrition and Feeding for Sustainable Aquaculture Development in the Third Millennium. In R.P. Subasinghe, P. Bueno, M.J. Phillips, C. Hough, S.E. McGladdery & J.R. Arthur, eds. Aquaculture in the Third Millennium. Technical

Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20-25 February 2000., 193-219.

Higgs, D.A., Balfry, S.K., Oakes.J.D., M., R., B.J., S., G., D., 2006. Efficacy of an equal blend of canola oil and poultry fat as an alternative dietary lipid sources for Atlantic salmon (*Salmo salar L.*) in sea water. I: effects on growth performance, and whole body and fillet proximate and lipid composition. Aquaculture Research. 37, 180-191.

Ismail, A., Bannenberg, G., Rice, H.B., Schutt, E., MacKay, D., 2016. Oxidation in EPA- and DHA-rich oils: an overview. Lipid Technology published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. 28, 55-59.

Izquierdoa, M.S., Monteroa, D., Robainaa, L., Caballeroa, M.J., Rosenlund, G., Ginesa, R., 2005. Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. Aquaculture. 250, 431-444.

Kanazawa, A., 1985. Nutrition of penaeid prawns and shrimps. In Taki Y., Primavera J.H. and Llobrera J.A. (Eds.). Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps, 4-7 December 1984, Iloilo City, Philippines, Iloilo City, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center., 123-130.

Kanazawa, A., Teshima, S., Tokiwa, S., 1979a. Biosynthesis of fatty acids from palmitic acid in the prawn, *Penaeus japonicus.* Memoirs Fac Fisheries, Kagoshima University. 28.

Kanazawa, A., Teshima, S., Sakamoto, M., 1985. Effects of dietary lipids, fatty acids, and phospholipids on growth and survival of prawn (*Penaeus japonicus*) larvae. Aquaculture, 50: 39-49.

Kanazawa, A., Teshima, S.-i., Endo, M., Kayama, M., 1978. Effects of Eicosapentaenoic Acid on Growth and Fatty Acid Composition of the Prawn, *Penaeus japonicus*. Mem. Fac. Fish., Kagoshima Univ. 27, 35-40.

Kanazawa, A., Teshima, S., Ono, K., Chalayondeja, K., 1979b. Biosynthesis of fatty acids from acetate in the prawns, *Penaeus monodon* and *Penaeus merguiensis*. Memoirs Fac Fisheries, Kagoshima University. 28.

Kanazawa, A., Shin-ichinTeshima, Tokiwa, S., Kayama, M., Hirata, M., 1979c. Essential Fatty Acids in the Diet of Prawn-II Effectof Docosahexaenoic Acid on Growth. Bulletin of the Japanese Society of Scientific Fisheries. 45, 1151-1153.

Lamb, C.F., 2001. Gustation and feeding behaviour. Food intake in fish, 108-130.

Liao, I.C., Chien, Y.-H., 2010. The Pacific White Shrimp, *Litopenaeus vannamei*, in Asia: The World's Most Widely Cultured Alien Crustacean. Invading Nature - Springer Series in Invasion Ecology. 6, 489-520.

Lim, C., Ako, H., Brown, C.L., Hahn, K., 1997. Growth response and fatty acid composition of juvenile *Penaeus vannamei* fed different sources of dietary lipid. Aquaculture. 151, 143-153.

Mourente, G., Bell, J.G., 2006. Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax L*.) over a long term growth study: effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 145, 389-399.

Mourente, G., Good, J., Bell, J., 2005a. Partial substitution of fish oil with rapeseed, linseed and olive oils in diets for European sea bass (*Dicentrarchus labrax L*.): effects on flesh fatty acid composition, plasma prostaglandins E2 and F2α, immune function and effectiveness of a fish oil finishing diet. Aquaculture Nutrition. 11, 25-40.

Mourente, G., Dick, J.R., Bell, J.G., Tocher, D.R., 2005b. Effect of partial substitution of dietary fish oil by vegetable oils on desaturation and h-oxidation of [1-14C]18:3n-3 (LNA) and [1-14C]20:5n-3 (EPA) in hepatocytes and enterocytes

of European sea bass (*Dicentrarchus labrax L*.). Aquaculture. 248, 173-186.

Nasopoulou, C., Zabetakis, I., 2012. Benefits of fish oil replacement by plant originated oils in compounded fish feeds. A review. LWT-Food Science and Technology. 47, 217-224.

NRC, 2011. Nutrient requirements of fish and shrimp. National Academic Press.

Olsen, R.L., Hasan, M.R., 2012. A limited supply of fishmeal: Impact on future increases in global aquaculture production. Trends in Food Science & Technology. 27, 120-128.

Panserat, S., Hortopan, G., Plagnes-Juan, E., Kolditz, C., Lansard, M., Skiba-Cassy, S., Esquerre, D., Geurden, I., Medale, F., Kaushik, S., 2009. Differential gene expression after total replacement of dietary fish meal and fish oil by plant products in rainbow trout (*Oncorhynchus mykiss*) liver. Aquaculture. 294, 123-131.

Rosenlund, G., Obach, A., Sandberg, M., Standal, H., Tveit, K., 2001. Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (*Salmo salar L*.). Aquaculture Research. 32, 323-328.

Roy, L.A., Davis, D.A., 2010. Requirements for the culture of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters: water modification and nutritional strategies for improving production. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D.A., Gamboa-Delgado, J. (Eds), Avances en Nutrición Acuicola X-Memorias del Décimo Simposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N.L.; Mexico. ISBN 978-607-433-546-0. Universidad Autonoma de Nuevo Autonoma de Nuevo Leon, Monterrey, Mexico, pp. 61-78.

Samocha, T.M., Patnaik, S., Davis, D.A., Bullis, R.A., Browdy, C.L., 2010. Use of commercial fermentation products as a highly unsaturated fatty acid source in practical diets for the Pacific white shrimp *Litopenaeus vannamei*. Aquaculture Research. 41, 961-967.

Samocha, T.M., Davis, D.A., Roy, L.A., Carpenter, B., BULLIS, R.A., 2011. The effect of non-marine HUFA supplementation with fish oil removal on growth and survival of the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture Nutrition. 17, 518-525.

Shiau, S.-Y., 1998. Nutrient requirements of penaeid shrimps. Aquaculture. 164, 77-93.

Silva, S.S.D., Francis, D.S., Tacon, A.C.J., 2011. Fish oil in aquaculture : in retrospect, in Fish oil replacement and alternative lipid sources in aquaculture feeds. CRC Press, Boca Raton, Flo, 1-20.

Soller, F., Rhodes, M.A., Davis, D.A., 2017. Replacement of Fish Oil with Alternative Lipid Sources in Plant-based Practical Feed Formulations for Marine Shrimp (*Litopenaeus vannamei*) Reared in Outdoor Ponds and Tanks. Aquaculture Nutrition. 23, 63-75.

Solorzano, L., 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. . Limnology and Oceanography. 14, 799-801.

Spotte, S., 1979. Fish and invertebrate culture: Water managment in closed systems, 2nd edition. Wiley, New York.

Turchini, G.M., Torstensen, B.E., Ng, W.-K., 2009. Fish oil replacement in finfish nutrition. Reviews in Aquaculture. 1, 10-57.

Xu, X., Ji, W., Castell, J.D., O'Dor, R., 1993. The nutritional value of dietary n-3 and n-6 fatty acids for the Chinese prawn (*Penaeus chinensis*). Aquaculture. 118, 277-285.

CHAPTER III

THE EFFICIENCIES OF USING MODIFIED CANOLA OIL AS AN ALTERNATIVE OF MENHADEN FISH OIL WITH FISHMEAL SUPPLEMENTAL COMPARING TO POULTRY MEAL COMPLEMENTAL IN PRACTICAL DIETS OF PACIFIC WHITE SHRIMP

Abstract

With the trend of seeking an alternative to fish oil in the shrimp feed industry, many studies using different types of lipid sources have been conducted using different species. In this study, modified canola oil (MCO-3) was applied in practical diet for Pacific white shrimp with the purpose of replacing fish oil without affecting the shrimp growth performance. A 6-weeks growth trial was conducted to examine the efficiency of MCO-3 which was enriched with essential fatty acids such as DHA (docosahexaenoic acid, C22:6n-3), EPA (Eicosapentaenoic acid, C20:5n-3), DPA (Docosapentaenoic acid, C22: 5n-3), and ARA (Arachidonic acid, C20:4n-6) on growth, survival, and feed conversion of Pacific white shrimp. The tested diets were divided into two series including fishmeal based and poultry meal based diets. 100% menhaden fish oil (MFO) in the series of the fishmeal based diets was replaced by 0, 25%, 50%, 75%, and 100% MCO-3. In the second series of the fishmeal free based diets, 100% MFO was replaced by 75%, 100%, and 75% with 4% hydrolysed salmon by product meal (AS) which served as an attractant. Under the reported conditions there were no significant differences in term of growth, survival as well as feed conversion among the dietary treatments. The result confirmed that the MCO-3 can replace fish oil by 100% without causing deficiency in growth of Pacific white shrimp.

1. Introduction

Fish oil has been a critical component of fish and shrimp feed with it's expanded use paralleling the development of the aquaculture industry (Bell and Waagbø, 2008). Fish oil has been used as the major source of lipid because it provides not only necessary energy but also essential fatty acids such as long chain poly unsaturated fatty acids (LC-PUFAs) (Silva *et al.*, 2011). In general, marine species have a limited ability of converting α-linolenic acid (LNA, C18:3n3) and *cis*-linoleic acid (LOA, C18:2n-6) to LC-HUFA such as DHA (docosahexaenoic acid, C22:6n-3), EPA (Eicosapentaenoic acid, C20:5n-3), DPA (Docosapentaenoic acid, C22 : 5n-3), and ARA (Arachidonic acid, C20:4n-6) (NRC, 2011) which are required for proper development. With and increasing market demand and limited production supply of fish oil, there has been a shift from marine lipid sources to plant derived lipid sources. This started in freshwater and anadromous fish which in general require lower levels of LC-HUFA (Bell and Waagbø, 2008).

When the lipid source is shifted from fish oils to plant oils, the fatty acid profile will also be changed. This means that the essential fatty acids which are present in fish oil but are typically absent in plant oils will be reduced (Silva *et al.*, 2011). Although in recent studies partial replacement has been successful in Pacific white shrimp (Soller *et al.*, 2017; Zhou *et al.*, 2007), yellowtail kingfish (Bowyer *et al.*, 2012), Atlantic salmon (Bell *et al.*, 2001; Dosanjh *et al.*, 1998; Wonnacott *et al.*, 2004), sunshine bass (Wonnacott *et al.*, 2004), etc. However, most studies indicate that you cannot completely replace marine oils as the replacement oils do not contain essential fatty acids (EFAs).

Although plant oils, specifically canola oils used in this study, are better in terms of available production and price, its disadvantage is the lack of LC-HUFAs in the fatty acid profile. Canola oil is rich in α-linolenic acids (18:3n-3, LNA), *cis*-linoleic acids (18:2n-6, LOA) but lack EPA, DHA, and ARA which are required in many crustacean species (Das,

2006). In order to use canola oil in practical diet for Pacific white shrimp without causing deficiency in growth and survival, it has to be proposed that the level of EFAs in the diet must meets the optimal level of EFAs for the development of the species. According to Gonzalez-Felix *et al.* (2002a), Pacific white shrimp require a diet with 0.5% of LC-HUFA in diet to maximise growth. Fortunately, with the development of technology and genetics, modified canola oil which is enriched in LC-HUFAs, especially DHA and EPA, is promising in terms of meeting EFA requirements.

Hence, the objective of the current study was to evaluate the efficiency of modified canola oil as a replacement for fish oil in practical diets for the Pacific white shrimp.

2. Materials and methods

2.1 Experimental diets

A series of eight diets were designed to contain 35% protein and 8% lipid to evaluate the efficacy of a newly developed modified canola oil (MCO-3). MFO and MCO-3 used to replace MFO were analysed their FA profiles (Table 1). The first five diets contained fishmeal and soybean meal as the primary protein sources. MFO served as the primary lipid source (supplemented at 4.73 g/100g diet). The other three diets were formulated using a basal diet without fishmeal and utilized poultry by product meal, soybean meal and corn protein concentrate as the primary protein sources. MFO also served as the primary lipid source and was supplemented at 5.12 g/100g diet. MFO in the fishmeal basal diet (FMB) was incrementally replaced with the MCO-3 at 0%, 75%, and 100% replacement. Additionally, the final diet included 75% replacement of MFO in combination with 4% hydrolysed salmon (AS) by product meal which served as an added attractant (Table 2).

Table 1. Fatty acid profile of modified canola oil (MCO-3), and menhaden fish oil (MFO) that used in the tested diets.

Fatty acids (%)	MFO	MCO-3
C14:0	6.93	0.09
C15:0	0.64	0.09
C16:0	14.05	4.65
C16:1n7	10.01	0.17
C17:0	2.11	0.13
C18:0	2.72	2.76
C18:1n9	5.16	27.17
C18:1n7	2.73	2.92
C18:2n6	1.51	28.82
C18:3n6	0.34	2.38
C18:3n3	1.47	4.44
C20:0	0.24	0.66
C20:1n9	0.85	0.78
C20:2n6	0.32	0.23
C20:3n6	0.32	4.69
C20:4n6 (ARA)	1.28	3.02
C20:3n3 (11,14,17)	0.27	-
C20:5n3 (EPA)	16.45	6.26
C22:0	-*	0.27
C23:0	-	0.07
C22:4n6	-	0.75
C22:3n6	0.74	0.14
C22:5n3 (DPA)	2.54	3.12
C22:6n3 (DHA)	19.56	0.58
C24:0	-	0.12
C24:1n9	0.42	0.08
TTn3	40.29	14.40
TTn6	4.51	40.03
n3/n6	8.93	0.36
n6/n3	0.11	2.78

*not detected

Table 2. Contents of tested diets using modified canola oil (MCO-3) to replace menhaden fish oil (MFO).

Results are expressed on an "as is" basis unless otherwise indicated.

Diet number	Fishmeal based diets					Poultry meal based diets		
g/100g as is	Basal	25MCO-3	50MCO-3	75MCO-3	100MCO-3	PMBasal	PM75	PM75+4AS
Menhaden fishmeal[1]	15.00	15.00	15.00	15.00	15.00			
Hydrolyzed salmon meal [2]								4.00
Poultry by product meal[3]						6.00	6.00	6.00
Soybean meal[4]	48.50	48.50	48.50	48.50	48.50	51.30	51.30	45.60
Corn Protein Concentrate[5]						7.00	7.00	7.00
Menhaden fish oil[6]	4.73	3.55	2.37	1.18		5.12	1.28	1.21
MCO-3 [7]		1.18	2.37	3.55	4.73		3.84	3.84
Lecithin [8]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol [9]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Corn Starch [9]	1.07	1.07	1.07	1.07	1.07	5.88	5.88	7.65
Whole wheat [10]	26.00	26.00	26.00	26.00	26.00	18.00	18.00	18.00
Mineral premix[11]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[12]	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Choline chloride[13]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Rovimix Stay-C 35%[14]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-dibasic[13]	1.00	1.00	1.00	1.00	1.00	3.00	3.00	3.00

*The percent of the replacement.

[1] Omega Protein Inc., Huston TX, USA. [2] Empresas Fiordo Austral S.A. [3] Tyson Foods, Inc., Springdale, AR, USA. [4] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA. [5] Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA. [6] Menhaden fish oil. Omega Protein Inc., Houston, TX, USA. [7] Cargills, Fort Collins, CO, USA. [8] The Solae Company, St. Louis, MO, USA. [9] MP Biomedicals Inc., Solon, OH, USA. [10] Bob's red mill, Milwaukie, OR, USA. [11] Trace mineral premix (g/100g 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.

[12] Vitamin premix (g/kg premix): Thiamin HCl, 4.95; Riboflavin, 3.83; Pyridoxine HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Biotin, 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.

[13] VWR Amresco, Suwanee, GA, USA

[14] Stay-C® (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA

The test diets were prepared in the feed laboratory of Auburn University, Auburn, Alabama, USA using standard practices. Pre-ground dry ingredients followed by oil and then boiling water were mixed in a food mixer (Hobart Corporation, Troy, OH, USA) Water was added until the mash was of suitable consistency for pelleting. The mash was then formed through a meat grinder equipped with 3-mm die to make pellets under pressure. The diets were then placed on trays in a forced air-drying oven at a temperature < 45°C overnight to reach a moisture of less than 10%. After drying, the diets were crumbled and sieved to a uniform size and stored in zip lock bags in a freezer for later uses. Proximate composition (g 100g-1 sample as is) and fatty acid profile (g 100g-1 sample as is) of the diets were analysed at the Midwest Laboratories, Omaha, Nebraska, USA, and Cargill's oil division, Colorado, USA, respectively.

2.2 Growth trial

Experimental system

The growth trial was conducted at E. W. Shell Fisheries Center, Auburn, Alabama. Post larval shrimp which were received from Shrimp Improvement Systems (Islamorada, Florida, USA) were nursed using commercial feeds until they reach an appropriate size for research. Juvenile Pacific white shrimp with the mean weight of 0.16g ±0.01g were size sorted and randomly stocked into 75-L rectangle tanks, which was a component of a 4,000 L indoor recirculating aquaculture system (RAS) at 10 shrimp per tank. This system consists of a series of culture tanks, sump tank, two circulation pumps, bead filter and fluidized bed biological filter. Each diet was offered to 5 replicate tanks over a 6-week growth period.

Water temperature was maintained at around 28.5°C using a submerged 3,600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA). Dissolved oxygen was maintained near saturation using air stones in each culture tank using a common airline connected to a regenerative blower. Dissolved oxygen and water temperature were measured twice a day using a YSI-55 digital oxygen/temperature meter (YSI corporation, Yellow Springs, Ohio,

USA) while pH were measured twice per week by Sper Scientific 850050 large display pH pen. These parameters were measured in the sump tank of the cultured system. Water samples were collected and kept frozen to test total ammonia nitrogen (TAN) and nitrite-N two times per week by using methods that are described by Solorzano (1969) and Spotte (1979), respectively. Ammonia was monitored daily by TAN disk maintaining in the sump tank. Water inlets and outlets were checked every day to maintain proper water exchange.

The daily amount of diets offered to the shrimp was calculated according to a feed conversion ratio of 1.8 and the predicted growth of 0.16, 0.32, 0.48, 0.64, 0.75, and 1.35 g/week over a 6-weeks growth trial. Daily feed was divided into four feedings per day. The amount of feed offered was adjusted each week based on predicted growth, survival, as well as observation of the feeding response. At the end of the growth trial, shrimp were counted, and group weighed to determine weight gain, survival rate and feed conversion ratio. After weighing, the shrimp were frozen and stored (a short-term storage) at -20C for proximate analysis. Ammonia was monitored daily by TAN disk maintaining in the sump tank. Water inlets and outlets were checked every day to maintain proper water exchange

2.3 Lipid extraction from diets and from whole shrimp bodies

Fatty acid compositions in diets and in shrimp body were analysed. Frozen shrimp were thawed and five shrimp from each tank were pooled into a sample and ground. From each sample of shrimp and diets, two sub-samples were taken with an approximate weight of 2g/each from shrimp bodies and an approximate weight of 0.5g/sub-sample of diet. These sub-samples were extracted using the methods of Folch *et al.* (1957). In short, weighed flesh or feed was homogenized in 20 mL of chloroform/methanol (2:1) for 1 minute. 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 minute and filtered through 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) 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 % 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, flushed with nitrogen gas, and the vials were sealed with parafilm paper. 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 analysed 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.

2.4 Statistical analysis

Feed conversion ratio was calculated by feed offered/ (final weight-initial weight), and percentage weight gain was calculated by (final weight-initial weight)/initial weight × 100. The Shapiro–Wilk test was utilized to check for the normality assumption. For non-normally distributed variables, two-way ANOVA was conducted on the Log-transformed response variable. Two-way ANOVA was used to test if there were any significant differences ($P<0.05$) in final mean weights, growth, survival, percent weight gain, and FCR among the treatment means. Multi-comparison test (Tukey) and Student-Newman-Keuls test were used to identify differences among treatment means ($P <0.05$). Linear regression analysis was tested to present the relationship of the target EFAs in diet, and in shrimp and the replacement of MFO. All statistical analysis was conducted using SAS system for windows (SAS Institute, Cary, NC).

3. Results

3.1 Fatty acid profiles of tested oils, and experimental diets

The fatty acid profile of MFO and the MCO-3 is presented in Table 1. Primary fatty acid differences include EPA which was 16.45% in MFO while it was only 6.26 % in the MCO-3. MFO also showed a dominance in the availability of DHA with 19.56% while the MCO-3 contained 0.58%. On the other hand, the tested MCO-3 showed a higher availability of ARA (3.02%) and DPA (3.12%) than those of MFO (1.28 and 2.54%, respectively). With regards to possible oxidation of the oils the peroxide value of MFO was 4.1 meq/kg and of MCO-3 was 4.6 meq/kg, respectively.

Fatty acid profiles of the diets are presented in Table 3. There was a negative trend between EPA, DHA and levels of MFO replacement by MCO-3 while DPA and ARA showed a positive correlation with the replacement of MFO by MCO-3 in both the groups of fishmeal based and poultry meal based diets. These trends also reflected by the reduction of total n-3 fatty acids and the increase of total n-6 fatty acids. Particularly, the highest value of EPA (9.87%) and DHA (11.11%) were found in the diet formulated with fishmeal and 100% MFO. These levels were sequentially reduced with the increased MCO-3 in the diet. On the other hand, DPA values tended to increase when the level of MCO replacement went up. It reached the highest level of 2.09% in the diet of 100% MCO-3 within the fishmeal based group. ARA levels of the diets increased as MFO was replaced with MCO-3 irrespective of protein source. The diet of 100% MFO (fishmeal based group) had the lowest level of ARA with 0.85% while the diet of 100% MCO-3 (fishmeal based group) took the highest rank with 1.93%. Besides the evaluation of EPA, DHA, and DPA levels in the tested diets, n3/n6 and n6/n3 ratios were also calculated (Table 3). The n3/n6 ratio declined from 1.25 to 0.41, in parallel with the increase of MCO-3 replacement level from 0% to 100%, in the fishmeal based group. The coefficient of the decline was not constant, although the percentages of the replacement was constant (0, 25, 50, and 100%) within the fishmeal based diet group. The n3/n6 ratio was 0.38 and 0.37 in the diets of PM75, and PM75+4AS, respectively. In the fishmeal based diet group, the n6/n3 ratio rose up and reached the peak in the diet of 100% MCO-3. In the group poultry meal based diets, the value of n6/n3 in the diet of 100% MFO was lower than that of the diet contained 75% of MCO-3. The diet of PM75+4AS had the highest ratio within all the diet treatments with the value of 2.74.

Table 3. Proximate composition of tested diets using modified canola oil (MCO-3) to replace menhaden fish oil (MFO), and using fishmeal or poultry meal as the only animal protein source, and hydrolyzed salmon meal (AS) as an attractant.

Diets	Fishmeal based					Poultry meal based		
g/100g as is	Basal	25* MCO-3	50 MCO-3	75 MCO-3	100 MCO-3	PMBasal	PM75	PM75+ 4AS
Crude protein	36.4	36.9	36.8	37.1	36.6	36.3	36.4	36.8
Crude fat	8.55	8.56	8.10	8.00	8.14	8.27	8.23	8.29
Crude fiber	4.40	5.60	4.20	3.70	4.10	5.30	6.10	4.90
Ash	7.50	7.35	7.26	7.28	7.25	6.58	6.56	6.88
Fatty acids (%)								
C12:0	0.08	0.06	0.05	0.02	-	0.07	-	-
C13:1n1	0.04	0.03	-**	-	-	-	-	-
C14:0	4.97	4.08	3.1	2.1	1.16	4.39	1.39	1.23
C14:1n5	0.26	0.21	0.08	0.05	-	0.02	-	-
C15:0	0.49	0.43	0.36	0.26	0.17	0.41	0.16	0.15
C16:0	16.54	15.17	14	12.6	11.01	16.64	12.23	11.96
C16:1n7	6.32	5.17	3.87	2.63	1.39	6.2	2.33	2.13
C17:0	0.52	0.49	0.41	0.32	0.23	0.45	0.19	0.18
C18:0	3.34	3.33	3.33	3.33	3.30	3.56	3.56	3.55
C18:1n9	7.69	10.36	12.97	15.86	18.74	11.42	20.05	21.20
C18:1n7	2.27	2.37	2.37	2.39	2.41	2.24	2.28	2.31
C18:2n6	18.54	21.85	25.41	29.21	32.45	20.27	31.48	31.61
C18:3n6	0.19	0.45	0.74	1.02	1.33	0.23	1.09	1.16
C18:3n3	3.08	3.45	3.78	4.22	4.59	2.99	4.25	4.32
C20:0	0.2	0.25	0.30	0.37	0.43	0.21	0.38	0.39
C20:1n9	0.56	0.56	0.54	0.54	0.54	0.56	0.53	0.58
C20:2n6	0.24	0.23	0.21	0.20	0.18	0.24	0.19	0.21
C20:3n6	0.19	0.74	1.29	1.90	2.51	0.28	2.07	2.13
C20:4n6 (ARA)	0.85	1.11	1.35	1.63	1.93	0.82	1.63	1.67
C20:3n3	0.16	0.14	0.11	0.04	-	0.07	-	-
C20:5n3 (EPA)	9.87	8.84	7.71	6.61	5.52	8.78	5.32	5.07
C22:0	0.26	0.27	0.29	0.30	0.31	0.25	0.29	0.29
C22:4n6	0.19	0.33	0.40	0.47	0.46	0.16	0.52	0.52
C22:3n6	0.48	0.41	0.36	0.32	0.27	0.38	0.22	0.21
C22:5n3 (DPA)	1.62	1.71	1.79	1.90	2.09	1.68	1.80	1.76
C24:0	0.13	0.07	0.05	0.05	0.15	0.02	0.09	0.05
C22:6n3 (DHA)	11.11	9.29	7.50	5.65	3.79	8.52	2.80	2.55
C24:1n9	0.26	0.23	0.21	0.15	0.12	0.21	0.09	0.12
TTn3	25.84	23.43	20.90	18.43	15.99	22.04	14.17	13.70
TTn6	20.70	25.12	29.76	34.75	39.14	22.38	37.20	37.52
n3/n6	1.25	0.93	0.7	0.53	0.41	0.98	0.38	0.37
n6/n3	0.80	1.07	1.42	1.89	2.45	1.02	2.63	2.74

*Percentage of the replacement of the oils.

** Not detected

3.2 Water quality

Dissolve oxygen, temperature, salinity, pH, TAN and nitrite-N were maintained at 6.7 ± 0.45 mg/L, 28.5 ± 0.50°C, 8.3 ± 0.16 ppt, 8.1 ± 0.4, 0.2 ± 0.17 mg/L and 0.0 ± 0.04 mg/L, respectively (Mean ± *SD*). Water quality conditions were maintained to stay stable within the range of normal growth and survival of Pacific white shrimp, *Litopenaeus vannamei* (Table 4)

3.3 Growth trial

The results of performance growth of Pacific white shrimp offered the eight tested diets are presented in Table 5. Although the results showed that the mean final weights, as well as % gain, of shrimp maintained on the various dietary treatments decreased with the increase of MCO-3 replacement levels, there was no statistical difference in mean final weight, percent weight gain, survival, and FCR between the treatments and the control. The mean final weight ranged from 2.93-3.68g/individual. The lowest value belonged to shrimp offered the diet of 75% MCO-3 combined with 4% of AS. The highest final mean weight was presented at the diet with 100% MFO with the fish meal based diet set. Survival was found to be highest (96%) in the treatment of the diet with 75% MCO-3 within the group of poultry meal based diets. With the treatment of 25% MCO-3 replacement within the fishmeal based diet group, the survival was reported as the second highest (94%) and followed by the treatment of 100% MCO-3 in the poultry meal based diet (92%). The treatment of 100% MCO-3 in the fishmeal based diet set also had a high survival result with 90%.

3.4 Fatty acid profiles in shrimp whole bodies

The results of EPA, DHA, DPA, and ARA levels in whole body shrimp were similar to the results found in diets (Table 6). EPA and DHA levels in lipid extracted from whole shrimp body showed an inverse correlation with the percent of MCO-3 in the diet treatments that were offered to the shrimp.

Table 4. Water quality of the experiment that shrimp were offered tested diets using modified canola oil (MCO-3) to replace menhaden fish oil (MFO), and using fishmeal or poultry meal as the only animal protein source, and hydrolyzed salmon meal (AS) as an attractant. The data were presented by Mean ± *SD*, and the range of the values (n=5).

Water parameters	Mean ± *SD*	Range
Dissolved Oxygen (mg/L)	6.7 ± 0.45	5.18-7.79
Temperature (°C)	28.5 ± 0.50	27.1-29.5
Salinity (ppt)	8.3 ± 0.16	7.9-8.6
pH	8.1 ± 0.4	7.5-8.6
Ammonia (mg/L)	0.20 ± 0.17	0.00-0.43
Nitrite (mg/L)	0.00 ± 0.04	0.00-0.09

Table 5. Growth performance of Pacific white shrimp juvenile (0.16g) offered tested diets using modified canola oil (MCO-3) to replace menhaden fish oil (MFO), fishmeal or poultry meal as the only animal protein source, and hydrolyzed salmon meal (AS) as an attractant (n=5).

Treatment		Mean Final Weight (g)	Survival Rate (%)	Weight Gain(g)	Weight Gain Percent (%)	FCR*	Whole Body Lipid (%)	TGC**
Fish-meal based	Basal	3.68	86	3.52	2238.7	2.02	2.32	0.09
	25MCO-3***	3.61	94	3.45	2181.7	1.95	2.74	0.08
	50MCO-3	3.48	80	3.33	2208.5	2.15	2.74	0.08
	75MCO-3	3.48	84	3.32	2068.7	2.16	2.62	0.08
	100MCO-3	3.06	90	2.9	1777.6	2.38	2.46	0.08
Poultry meal based	PMBasal	3.44	92	3.28	2132.7	2.07	2.87	0.08
	PM75	3.31	96	3.16	2014.4	2.26	2.70	0.08
	PM75 +4AS	2.93	92	2.83	1746.3	2.48	2.41	0.08
One-way ANOVA								
P-Value		0.1544	0.2807	0.2101	0.1534	0.1654	0.6772	0.1175
*PSE*****		0.202	4.770	0.203	147.326	0.140	0.228	0.003
P-Value from 2-way ANOVA								
$P_{\text{Base source}}$		0.086	0.064	0.084	0.144	0.239	0.311	0.085
$P_{\text{Oil-replacement (\%)}}$		0.132	0.283	0.119	0.045	0.098	0.649	0.066

*FCR: feed conversion ratio
** Thermal growth coefficient (TGC)
***Percent replacement of modified canola oil in each diet.
****Pooled standard error (PSE) = $\sqrt{(Mean\ square\ error/n)}$

Table 6. Fatty acid profile in whole shrimp body (Mean ± SE) offered tested diets using modified canola oil (MCO-3) to replace menhaden fish oil (MFO), and using fishmeal or poultry meal as the only animal protein source, and hydrolyzed salmon meal (AS) as an attractant (n=5).

Fatty acids (%)	Fishmeal based					Poultry meal based			P-value
	Basal	25MCO-3	50MCO-3	75MCO-3	100MCO-3	PMBasal	PM75	PM75+4AS	
C12:0	0.02 ± 0.01a*	0.02 ± 0.00a	0.01 ± 0.00ab	0.01 ± 0.00ab	nd	0.02 ± 0.00ab	0.02 ± 0.00ab	0.01 ± 0.00ab	0.0153
C12:1	0.03 ± 0.00**a	0.03 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.00a	0.04 ± 0a	0.03 ± 0.00a	0.03 ± 0.01a	0.03 ± 0.00a	0.0683
C13:1	0.01 ± 0.01a	0.02 ± 0.00a	0.02 ± 0.00a	0.02 ± 0.01a	0.02 ± 0a	0.02 ± 0.00a	0.01 ± 0.00a	0.02 ± 0.01a	0.7706
C14:0	1.12 ± 0.11a	1.12 ± 0.06a	0.89 ± 0.02b	0.6 ± 0.05c	0.33 ± 0.02d	1.1 ± 0.05a	0.44 ± 0.02d	0.32 ± 0.02d	<0.0001
C15:0	0.37 ± 0.01a	0.34 ± 0.01b	0.32 ± 0.00c	0.27 ± 0.00d	0.22 ± 0.01e	0.33 ± 0.01bc	0.21 ± 0.01ef	0.19 ± 0.01f	<0.0001
C16:0	19.54 ± 0.07a	18.41 ± 0.09b	17.63 ± 0.17c	16.3 ± 0.27d	15.52 ± 0.18e	19.11 ± 0.17a	15.64 ± 0.22e	15.63 ± 0.16e	<0.0001
C16:1	2.36 ± 0.12a	2.06 ± 0.07b	1.62 ± 0.05c	1.13 ± 0.06d	0.68 ± 0.03e	2.21 ± 0.07ab	1.02 ± 0.04d	0.76 ± 0.03e	<0.0001
C17:0	1.17 ± 0.02a	1.04 ± 0.01b	0.91 ± 0.01c	0.8 ± 0.03d	0.72 ± 0.02e	0.94 ± 0.02c	0.63 ± 0.01f	0.64 ± 0.01f	<0.0001
C18:0	8.69 ± 0.25a	7.98 ± 0.2ab	7.52 ± 0.21b	7.5 ± 0.31b	7.7 ± 0.12b	8.05 ± 0.18ab	7.43 ± 0.25b	8.02 ± 0.23ab	0.0071
C18:1	9.35 ± 0.08f	11.09 ± 0.02e	13.01 ± 0.23d	14.3 ± 0.33c	15.72 ± 0.16b	12.81 ± 0.15d	17.87 ± 0.32a	17.93 ± 0.31a	<0.0001
C18:1	3.56 ± 0.03a	3.41 ± 0.03b	3.28 ± 0.03c	3.13 ± 0.02d	3.15 ± 0.08cd	3.26 ± 0.03cd	2.87 ± 0.02e	2.89 ± 0.02e	<0.0001
C18:2	13.74 ± 0.14f	15.67 ± 0.21e	17.3 ± 0.31d	18.6 ± 0.65c	18.83 ± 0.36c	15.9 ± 0.26e	21.46 ± 0.5a	19.97 ± 0.4b	<0.0001
C18:3 (GAM)	0.23 ± 0.00c	0.13 ± 0.01d	0.22 ± 0.03c	0.41 ± 0.01ab	0.43 ± 0.02a	0.24 ± 0.01c	0.41 ± 0.01ab	0.36 ± 0.01b	<0.0001
C18:3 (ALP)	1.08 ± 0.03d	1.28 ± 0.03c	1.34 ± 0.02bc	1.47 ± 0.06b	1.45 ± 0.06bc	1.12 ± 0.03d	1.62 ± 0.06a	1.41 ± 0.05bc	<0.0001
C20:0	0.26 ± 0.00e	0.28 ± 0.01d	0.33 ± 0.01c	0.34 ± 0.00bc	0.4 ± 0.00a	0.25 ± 0.01e	0.34 ± 0.00bc	0.35 ± 0.01b	<0.0001
C20:1	0.74 ± 0.02c	0.81 ± 0.01c	0.88 ± 0.01abc	0.88 ± 0.04abc	1.08 ± 0.13a	0.83 ± 0.01bc	0.96 ± 0.02abc	1.03 ± 0.01ab	0.0008
C20:2	1.6 ± 0.02e	1.82 ± 0.03d	2.07 ± 0.03c	2.34 ± 0.05b	2.61 ± 0.01a	1.81 ± 0.01d	2.39 ± 0.05b	2.52 ± 0.05a	<0.0001
C20:3	0.27 ± 0.01g	0.79 ± 0.01e	1.22 ± 0.04d	1.57 ± 0.07c	1.94 ± 0.04a	0.43 ± 0.02f	1.76 ± 0.05b	1.84 ± 0.04ab	<0.0001
C20:4n6 (ARA)	2.14 ± 0.08de	2.37 ± 0.06d	2.75 ± 0.09c	3.33 ± 0.16b	4.1 ± 0.09a	2.01 ± 0.04e	3.32 ± 0.12b	3.82 ± 0.12a	<0.0001
C20:3	0.37 ± 0.01a	0.35 ± 0.01ab	0.37 ± 0.00a	0.38 ± 0.01a	0.38 ± 0.01a	0.32 ± 0.01c	0.34 ± 0.01bc	0.34 ± 0.01bc	<0.0001
C20:5n3 (EPA)	12.63 ± 0.28a	11.3 ± 0.15b	10.3 ± 0.27c	10.14 ± 0.40c	9.66 ± 0.09cd	11.12 ± 0.31b	8.6 ± 0.28e	9.08 ± 0.21de	<0.0001
C22:0	0.21 ± 0.01a	0.19 ± 0.00a	0.22 ± 0.01a	0.19 ± 0.01a	0.2 ± 0.01a	0.21 ± 0.01a	0.19 ± 0.01a	0.18 ± 0.01a	0.2082
C22:1	0.07 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.01a	0.05 ± 0.01a	0.16 ± 0.10a	0.07 ± 0.01a	0.07 ± 0.01a	0.07 ± 0.01a	0.5508
C23:0	0.08 ± 0.00a	0.08 ± 0.00a	0.09 ± 0.00a	0.05 ± 0.02a	0.06 ± 0.01a	0.07 ± 0.01a	0.08 ± 0.00a	0.06 ± 0.02a	0.3144
C22:4	0.15 ± 0.01c	0.19 ± 0.01c	0.24 ± 0.01b	0.26 ± 0.02b	0.3 ± 0.01a	0.17 ± 0.02c	0.32 ± 0.01a	0.32 ± 0.01a	<0.0001
C22:3	0.34 ± 0.00a	0.33 ± 0.00ab	0.31 ± 0.00bc	0.31 ± 0.01bc	0.29 ± 0.01c	0.31 ± 0.01bc	0.23 ± 0.01e	0.25 ± 0.01d	<0.0001
C22:5n3 (DPA)	1.43 ± 0.04g	1.65 ± 0.02f	1.79 ± 0.02e	2.01 ± 0.03d	2.31 ± 0.02b	1.51 ± 0.02g	2.2 ± 0.04c	2.4 ± 0.03a	<0.0001
C24:0	0.13 ± 0.01a	0.12 ± 0.00a	0.13 ± 0.00a	0.13 ± 0.01a	0.13 ± 0.01a	0.1 ± 0.01a	0.11 ± 0.01a	0.13 ± 0.01a	0.1857
C22:6n3 (DHA)	13.03 ± 0.17a	11.57 ± 0.10b	9.94 ± 0.15c	8.78 ± 0.37d	7.14 ± 0.11e	10.44 ± 0.17c	5.01 ± 0.19f	5.44 ± 0.13f	<0.0001
C24:1	0.16 ± 0.01a	0.14 ± 0.01ab	0.14 ± 0.01ab	0.14 ± 0.01ab	0.17 ± 0.01a	0.11 ± 0.02b	0.14 ± 0.01ab	0.14 ± 0.01ab	0.0442

* The values in the same row superscripted with the same letter indicates there was no significant difference (P>0.05). ** SE ≤ 0.005. nd: Not detected

EPA level was highest in the diet of 100% MFO within the fishmeal based diet group (12.63 ± 0.28%) and lowest in the diet of 75% MCO-3 of the poultry based diet group (8.6 ± 0.28%). EPA in the diet of 100% MFO (12.63 ± 0.28%) was significantly lower than that in the diet of 100% MCO-3 (9.66 ± 0.09%). Besides, DHA levels decreased in the body of shrimp that were offered a higher percent of MCO-3 replacement. The lowest value was in the treatment of 75% MCO-3 (5.01± 0.19%) in the poultry based diet group. On the other hand, DPA level remarkably increased when the replacement of MCO-3 increased.

The highest DPA was presented in the shrimp that offered the diet of 75% MCO-3 combined with 4% AS in the poultry meal based group with 2.40 ± 0.03 % while the lowest value was 1.43 ± 0.04% in the treatment diet of 100% MFO in the fishmeal based group. ARA level showed the same trend as DPA level, which was significantly risen by the increment of MCO-3 level.

To allow for visualization of the response of growth to shifts in fatty acid profiles of the diets Figures 1, 2 and 3 were developed. Figure 1 showed the shift of total n-3 and n-6 in oils and diets. The decrease of n-3 and increase of n-6 in the diets correlated to the replacement of MCO-3. According to Figure 2A and 2B, EPA and DHA levels in diets and mean final weights decreased when the percent of the MCO-3 replacements increased in both fishmeal based and poultry meal based diet groups. On the other hand, the relationship of ARA level (Figure 3B) and the increases of MCO-3 in the diets of both groups was found positive while mean final weight showed a negative correlation with the replacement. The result of DPA level (Figure 3A) remained stable through different levels of the replacement. In the group fishmeal based diets, mean final weight and the n3/n6 ratio were relevant with the reduction of MFO in diets (Figure 4A). These values were slightly decreased when shrimp were offered the diets by 25, 50, 75% MCO-3 replacement and it remarkably dropped when the replacement of MCO-3 rose from 75% to 100%.

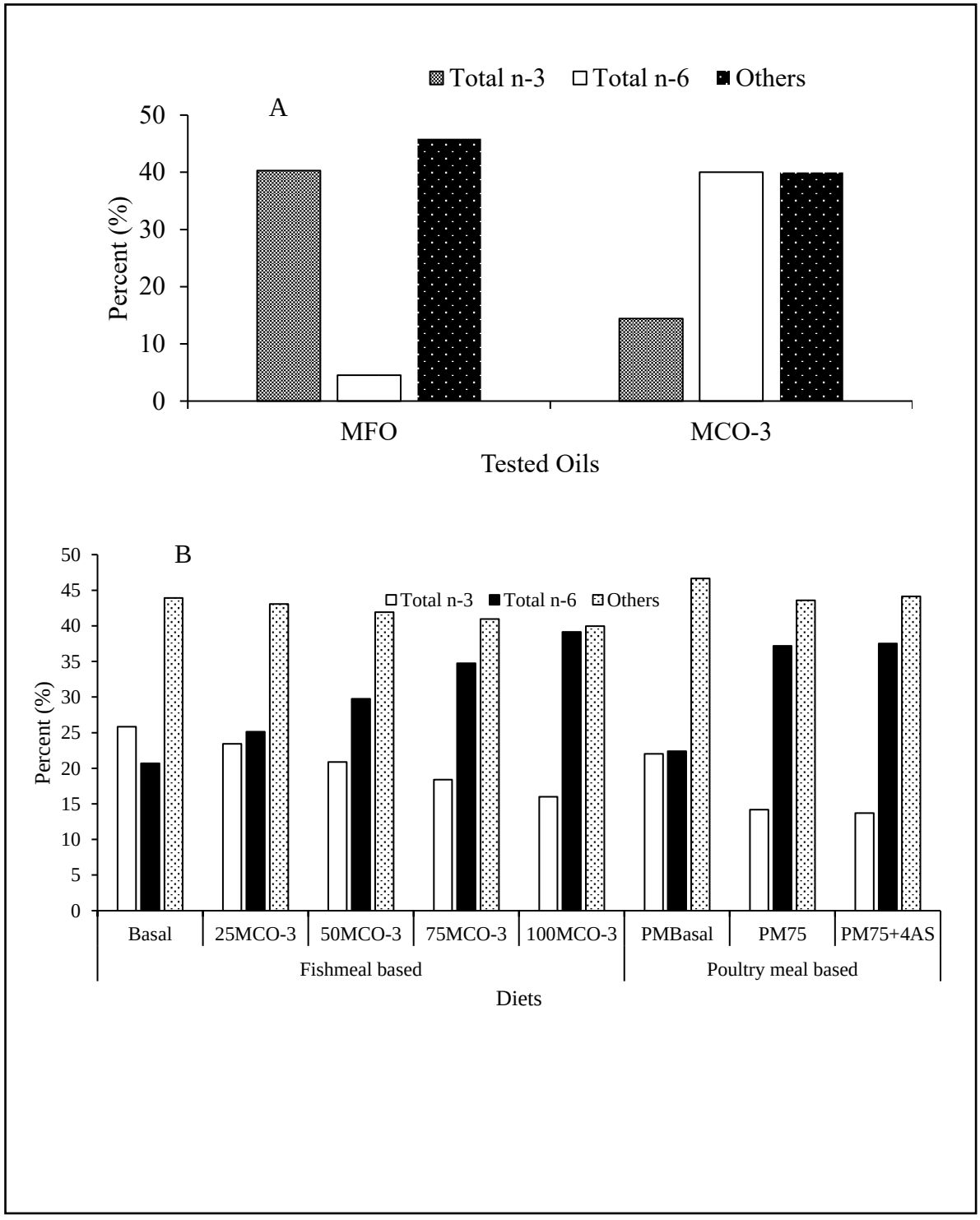

Figure 1. Percentage of total n-3 fatty acid (Ttn3), total n-6 fatty acids (Ttn6) in the test oils (menhaden fish oil, MFO; modified canola oil, MCO-3 and test diets (basal diets, 100% MFO; MCO-3 supplemented diets)

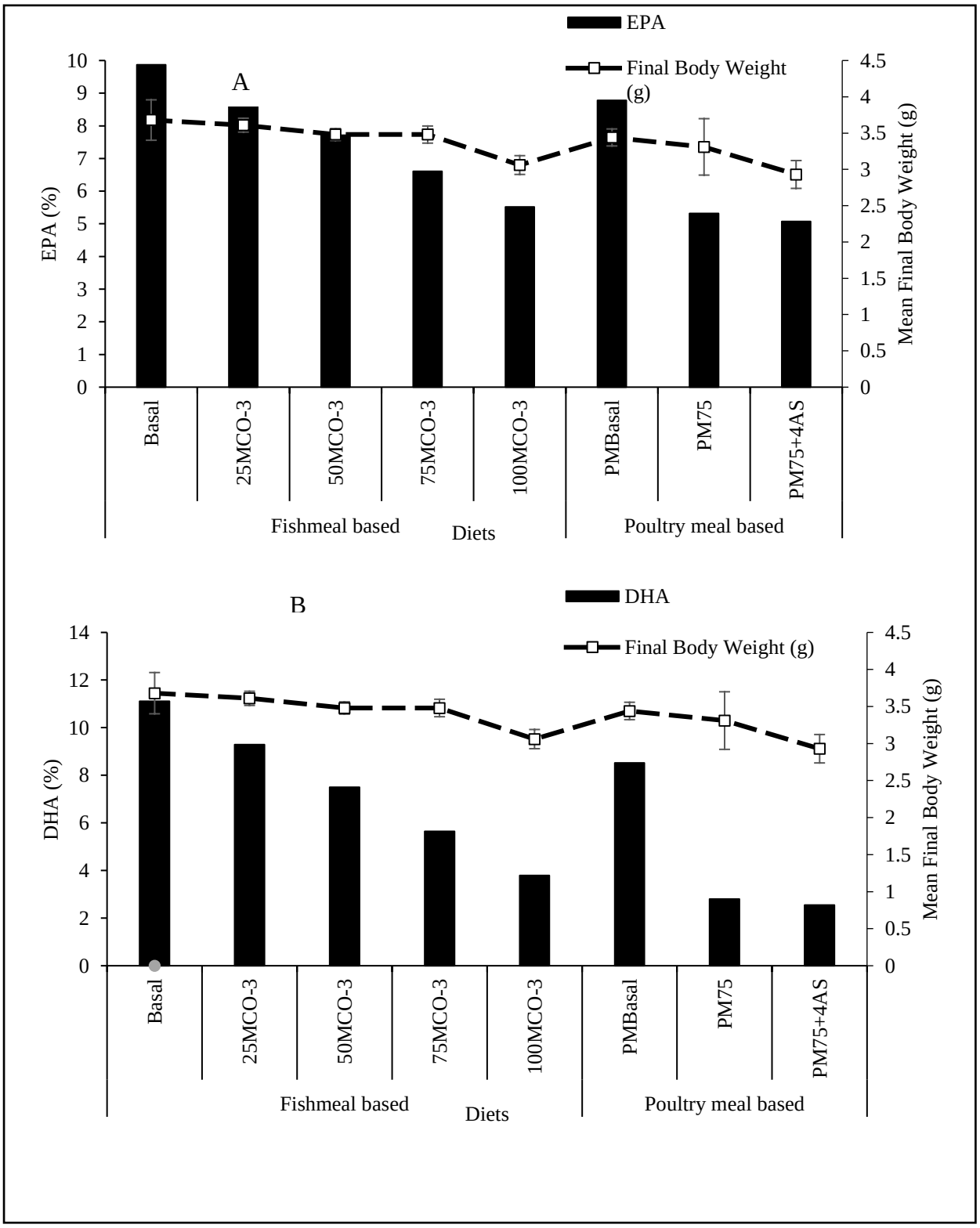

Figure 2. The relationship between mean final body weight (g) and EPA (Eicosapentaenoic acid, C20:5n-3) level (%) (A) and DHA (Docosahexaenoic acid, C22:6n-3) level (%) (B) in diets using modified canola oil (MCO-3) to replace menhaden fish oil (MFO), and using fishmeal or poultry

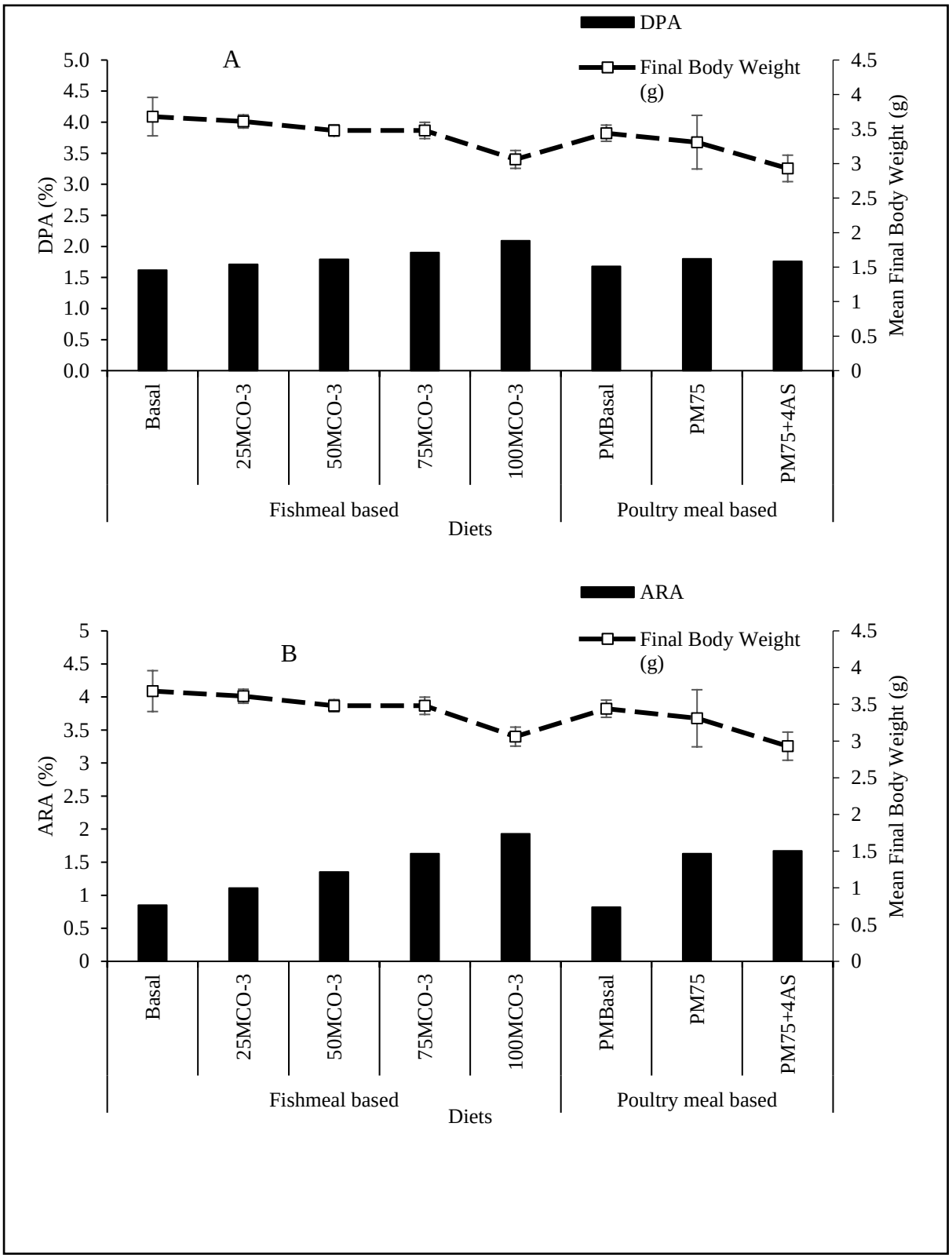

Figure 3. The relationship between mean final body weight (g) and DPA (Docosapentaenoic acid, C22 : 5n-3) level (%) (A) and ARA (Arachidonic acid, C20:4n-6) level (%) (B) in diets using modified canola oil (MCO-3) to replace menhaden fish oil (MFO), and using fishmeal

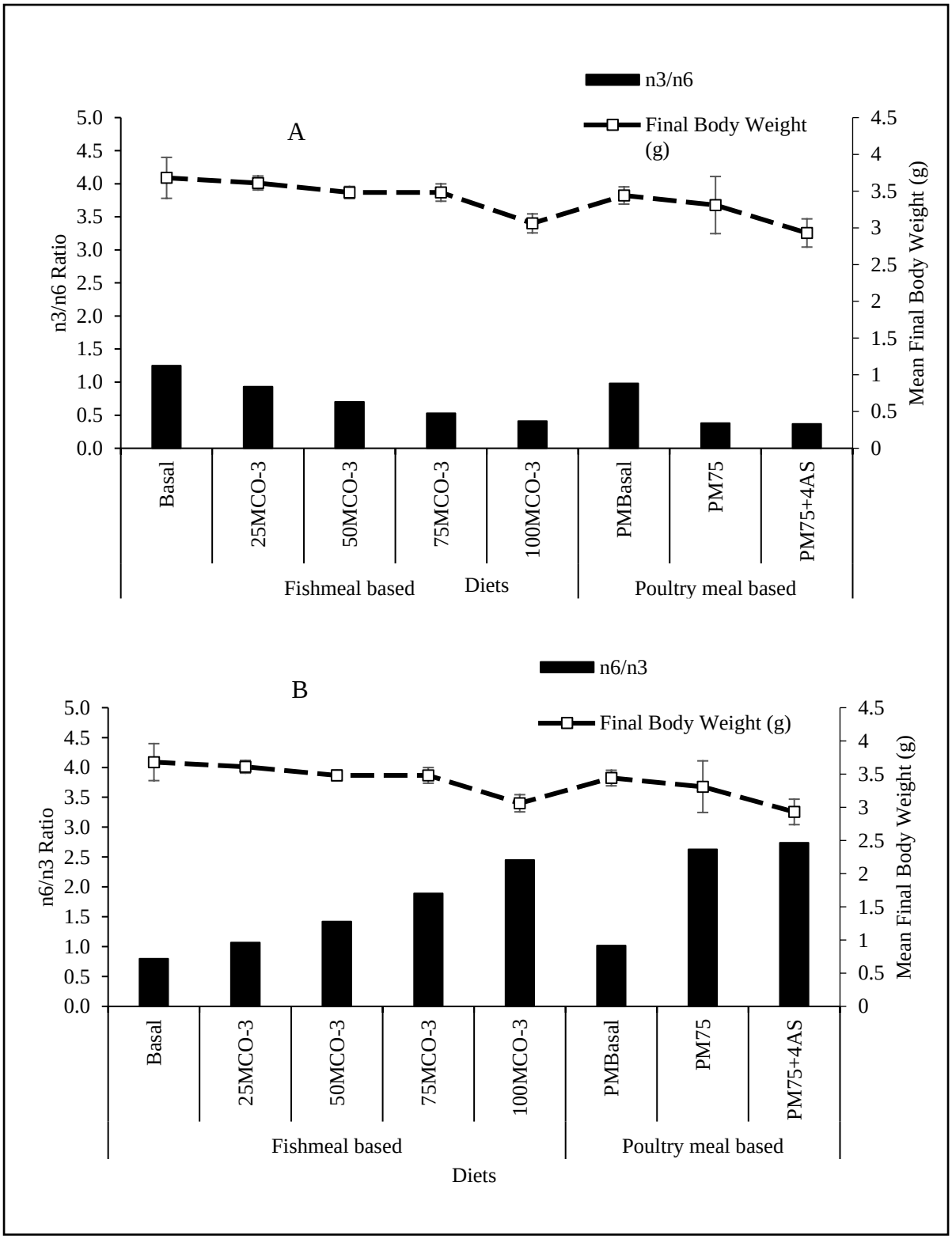

Figure 4. The relationship between mean final body weight (g) and n3/n6 ration (A), n6/n3 ratio (B) in diets using modified canola oil (MCO-3) to replace menhaden fish oil (MFO), and using fishmeal or poultry meal as the only animal protein source, and hydrolyzed salmon meal (AS) as an attractant.

The n6/n3 ratio showed the opposite results to the n3/n6 ratio (Figure 4B). The n6/n3 ratio were positively correlated with the percent of MCO-3 replacement while final mean weight showed a negative trend.

The linear regression data of the essential fatty acids in the diets and shrimp tissues of the fishmeal based group presented were analysed based on the data of all the samples of each treatment. The regression analysis of EPA level in diets ($R^2 = 0.903, P < 0.0001$) and in shrimp bodies ($R^2 = 0.999, P < 0.0001$) (Figure 5) showed a trend of significant decline when the percent of MCO-3 replacement increased, and EPA level in whole shrimp bodies were found to be higher than that in the diets. The regression of DHA level in diets ($R^2 = 1.000, P < 0.0001$) and in whole shrimp bodies ($R^2 = 0.998, P < 0.0001$) were again found to present a negative trend (Figure 6) with the increasement of MCO-3.

In contrast, DPA and ARA levels in diets and in shrimp bodies were reported to have significantly rose with the increase of MCO-3 replacement (Figure 7 and 8). The regression analysis of DPA level showed $R^2 = 0.9635, P < 0.0001$ in diets, and $R^2 = 0.9837, P < 0.0001$ in shrimp. The trend of DPA levels in diets and in shrimp were remarkably positively correlated with the percent of MCO-3 replacement. The regression analysis of ARA level in diets and in shrimp were found to be a positive relationship with the replacement of MCO-3 with $R^2 = 0.998, P < 0.0001$ in diets, and $R^2 = 0.952, P = 0.0001$ in shrimp. Instead of the MCO-3 supplemented diets giving the same value of fatty acid components in diets and in shrimp bodies as the fish oil, it seemed like the essential fatty acids were influenced (decreased in EPA and DHA levels, increased in DPA and ARE levels) by the replacement of MCO-3, although this negative influence did not cause a significant difference in mean final weights, FCR, or the survival of shrimp.

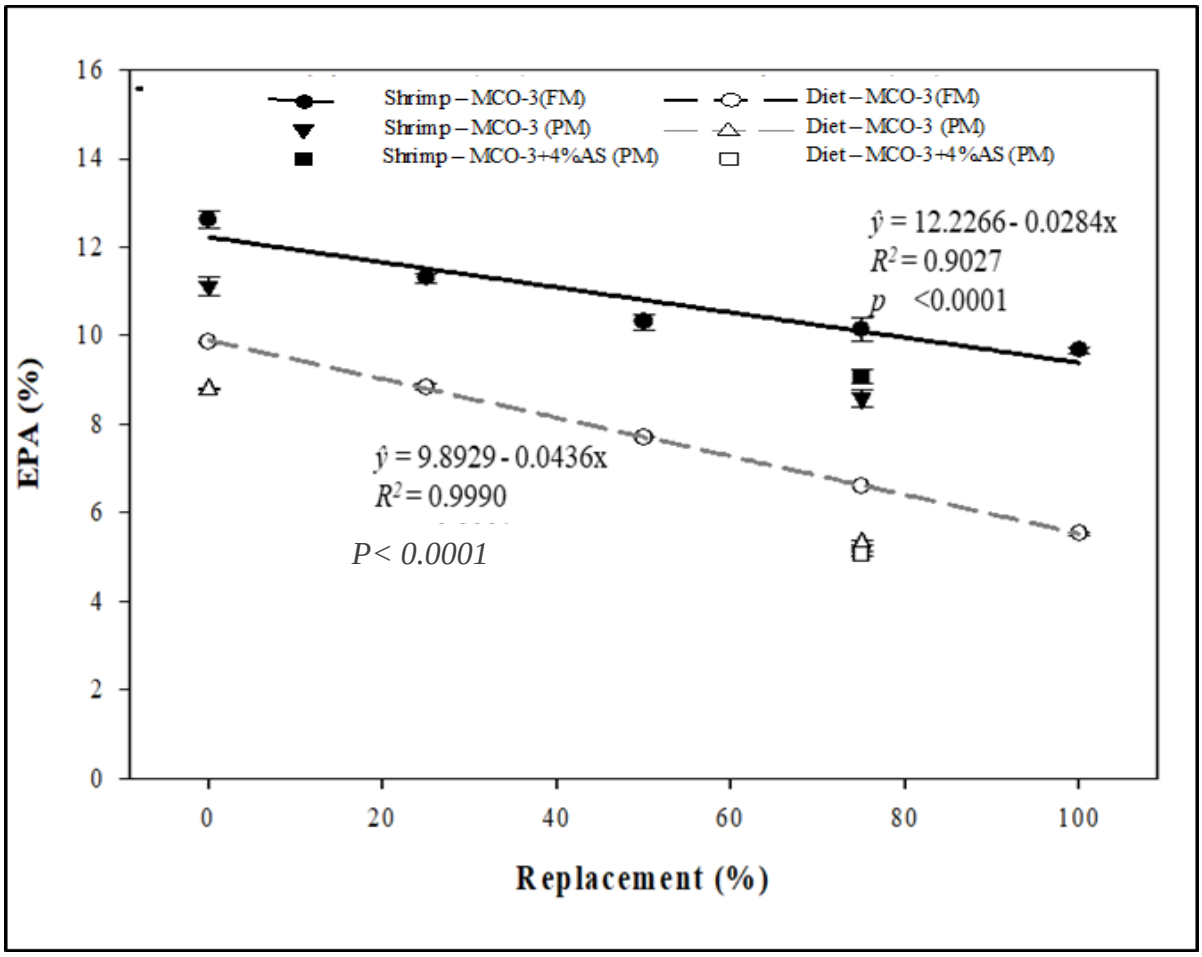

Figure 5. Relationship between levels of modified canola oil (MCO-3) replacement and EPA (Eicosapentaenoic acid, C20:5n-3) level (%) in diets and in shrimp (within the fishmeal based diet treatments). The regression line for EPA level for diets with the MCO-3 is y = 9.8929 - 0.0436x (R^2 = *0.9990, P< 0.0001*), and in shrimp offered the respective diets is y = 12.2266 - 0.0284x (R^2 = *0.9027, P< 0.0001*).

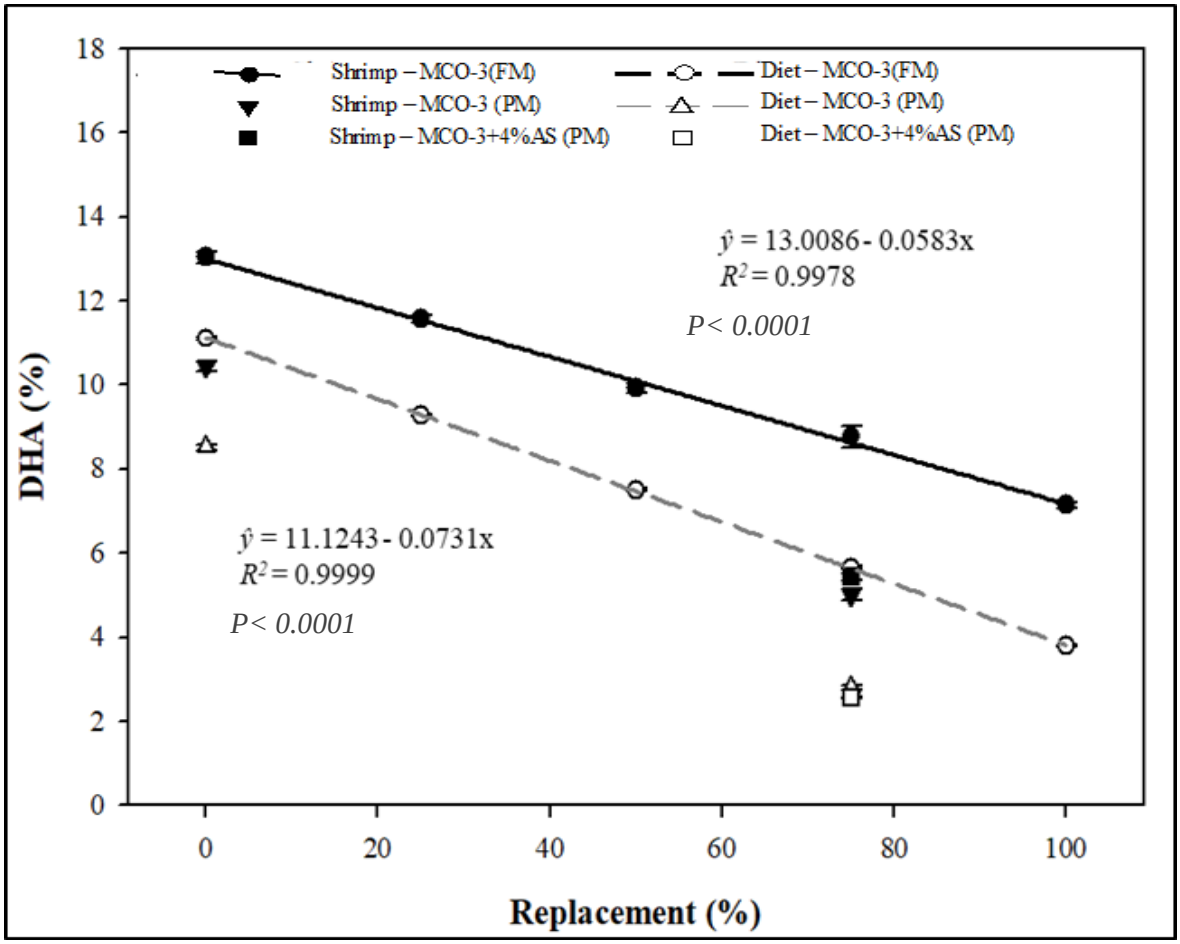

Figure 6. Relationship between levels of modified canola oil (MCO-3) replacement and DHA (Docosahexaenoic acid, C22:6n-3) level (%) in diets and in shrimp (within the fishmeal based diet treatments). The regression line for DHA level for with the MCO-3 is y = 11.1243 - 0.0731x (R^2 = *0.9999, P<0.0001*), and in shrimp offered the respective diets y = 13.0086 - 0.0583x (R^2 = *0.9978, P<0.0001*).

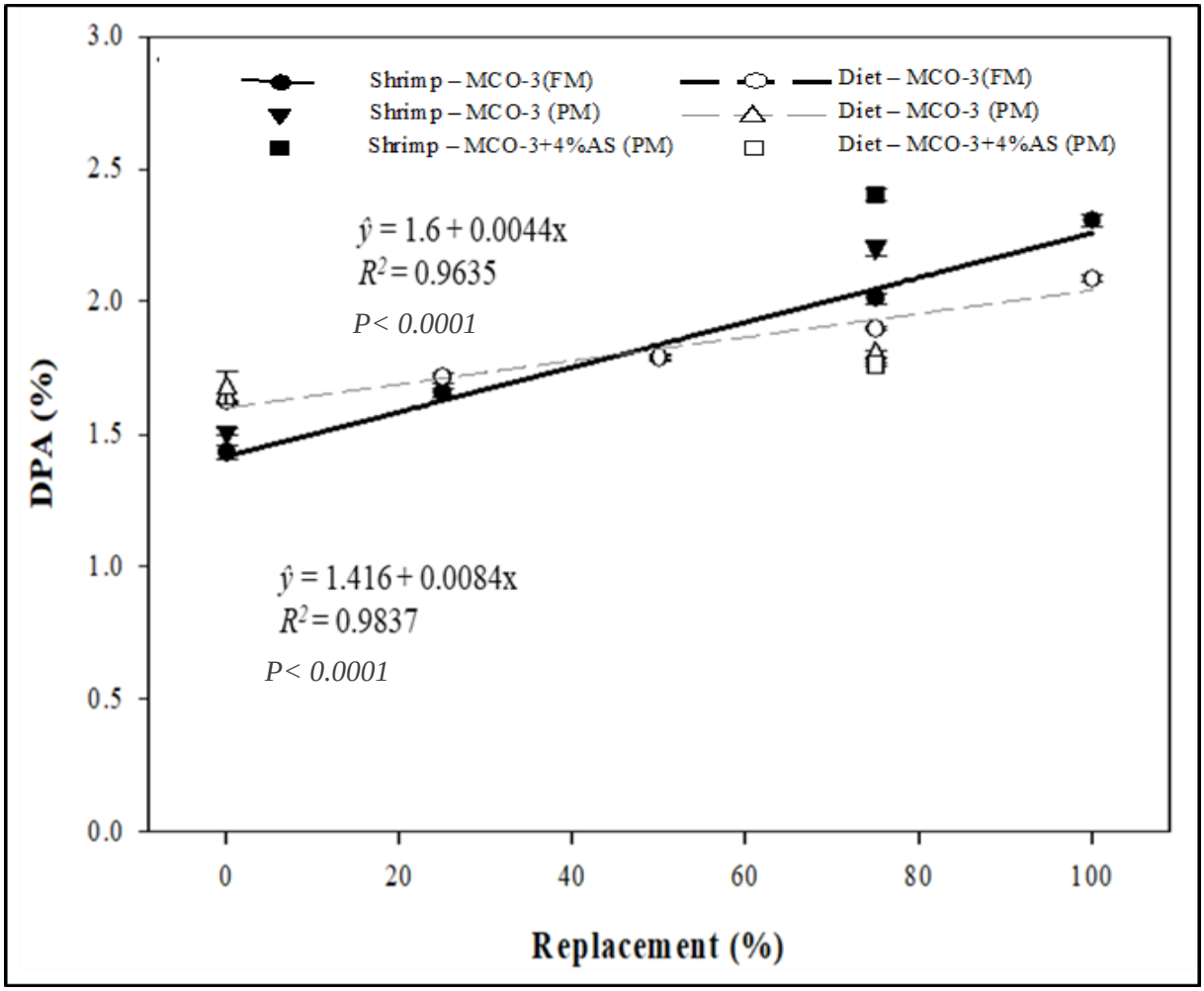

Figure 7. Relationship between levels of modified canola oil (MCO-3) replacement and DPA (Docosapentaenoic acid, C22 : 5n-3) level (%) in diets and in shrimp (within the fishmeal based diet treatments). The regression line for DPA level for the diets with the MCO-3 is y = 1.6 + 0.0044x (R^2 = *0.9635*, *P<0.0001*), and in shrimp offered the respective diets is y = 1.416 + 0.0084x (R^2 = *0.9837*, *P <0.0001*).

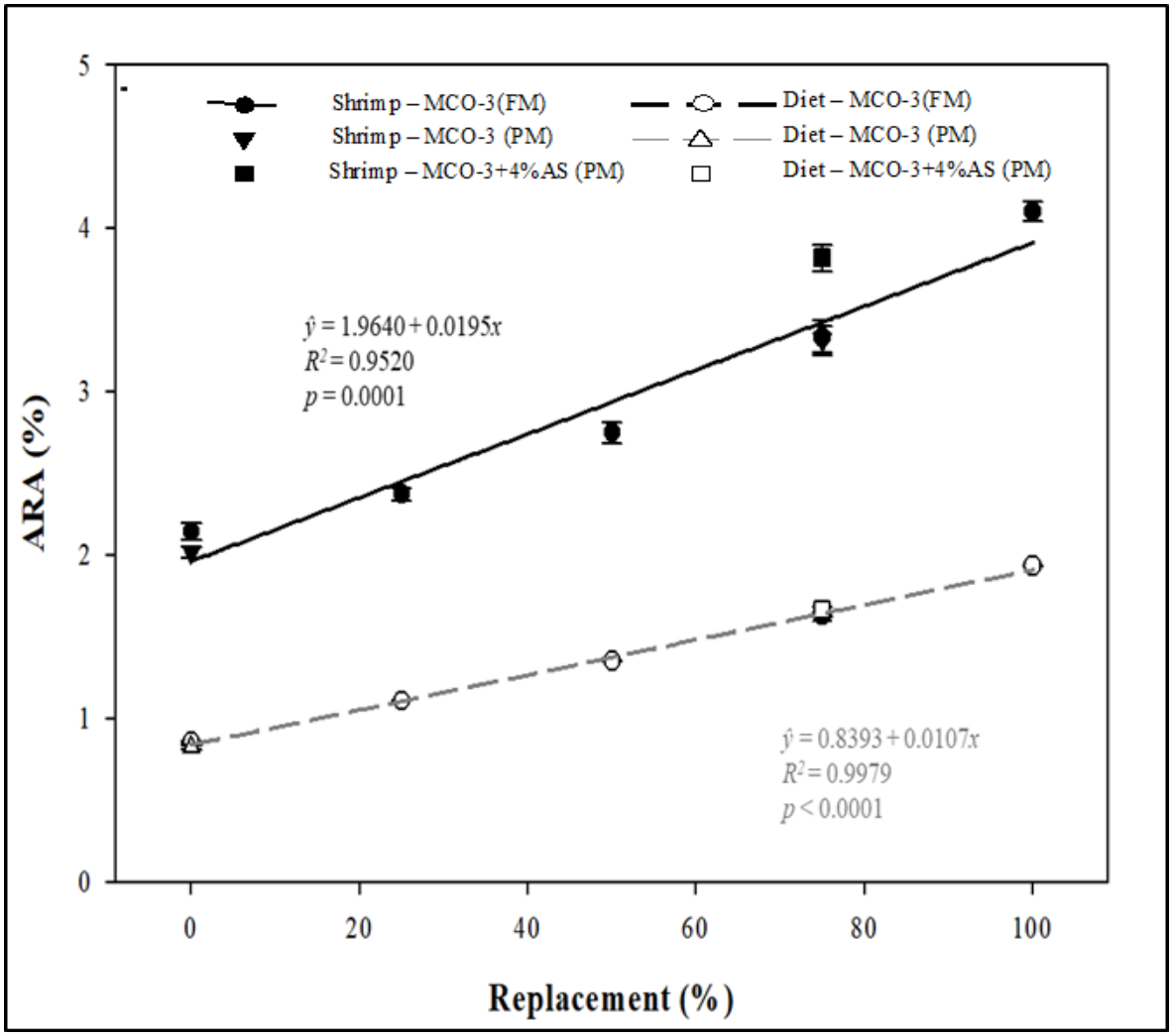

Figure 8. Relationship between levels of modified canola oil (MCO-3) replacement and ARA (Arachidonic acid, C20:4n-6) level (%) in diets and in shrimp (within the fishmeal based diet treatments). The regression line for ARA level for diets with the MCO-3 is y = 0.8393 + 0.0107x (R^2 = *0.9979*, *P<0.0001*), and in shrimp offered the respective diets is y = 1.964 + 0.0195x (R^2 = *0.9520*, *P=0.0001*).

The fluctuation of EPA, DHA, DPA, and ARA led to the decrease/increase of n-3, and n-6, which was performed by the linear regressions of the n3/n6 ratio (Figure 9) and the n6/n3 ratio (Figure 10). The regressions of the n3/n6 ratio in diets ($R^2 = 0.968, P = 0.0003$) and in shrimp bodies ($R^2 = 0.955, P = 0.0002$) significantly declined with the increase of MCO-3 replacement from 0% to 100%. On the other hand, the regressions of the n6/n3 ration in diets with $R^2 = 0.980, P = 0.0001$ and in shrimp bodies with $R^2 = 0.999, P = < 0.0001$ showed a trend of increase, which was statistically and significantly different.

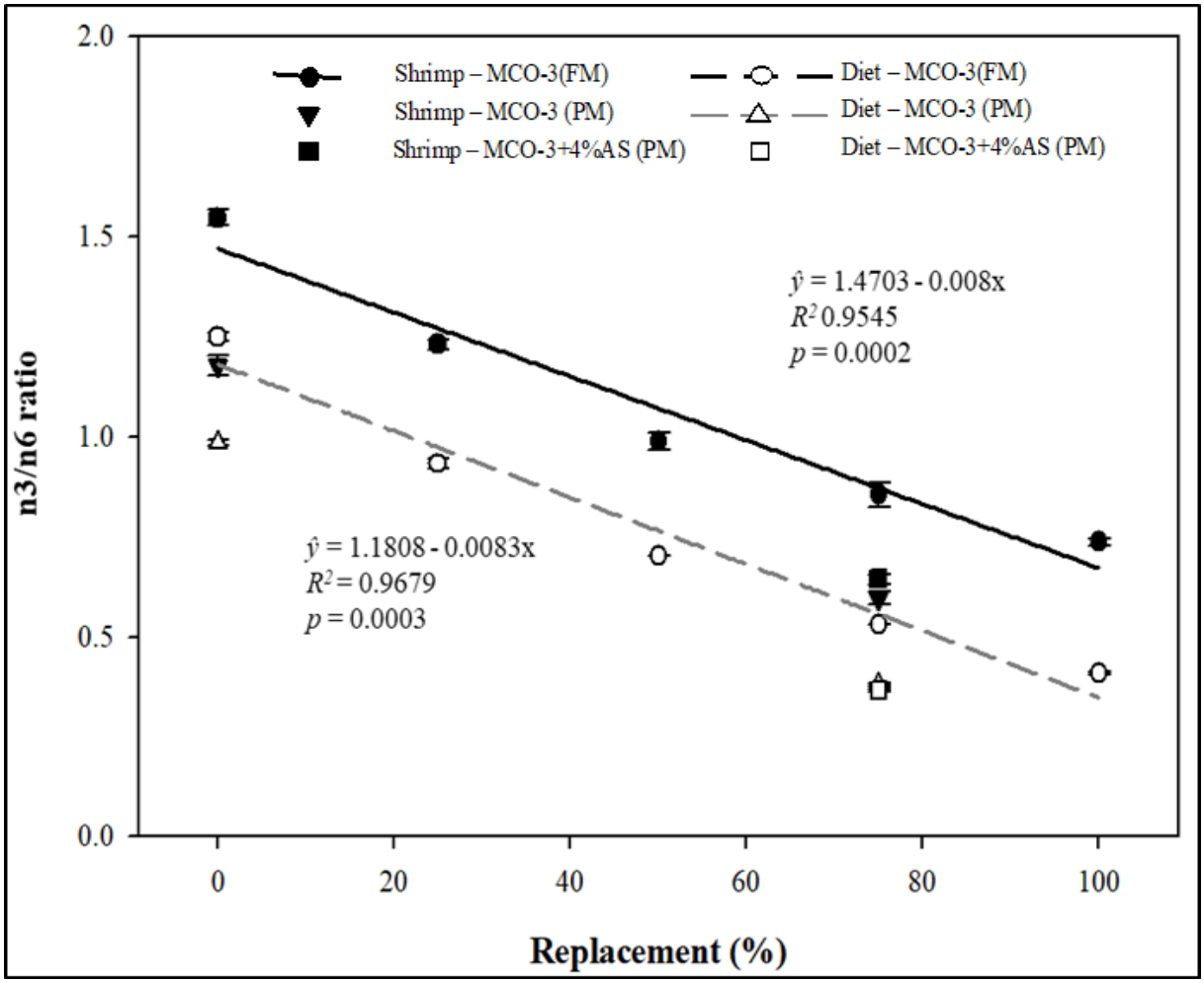

Figure 9. Relationship between levels of modified canola oil (MCO-3) replacement and the n3/n6 ratio in diets and in shrimp (within the fishmeal based diet treatments). The regression line for the n3/n6 ratio level in diets with the MCO-3 is y = 1.1808 - 0.0083x (R^2 = *0.9679, P= 0.0003*), and in shrimp offered the respective diets is y = 1.4703 - 0.008x (R^2 = *0.9545, P= 0.0002*).

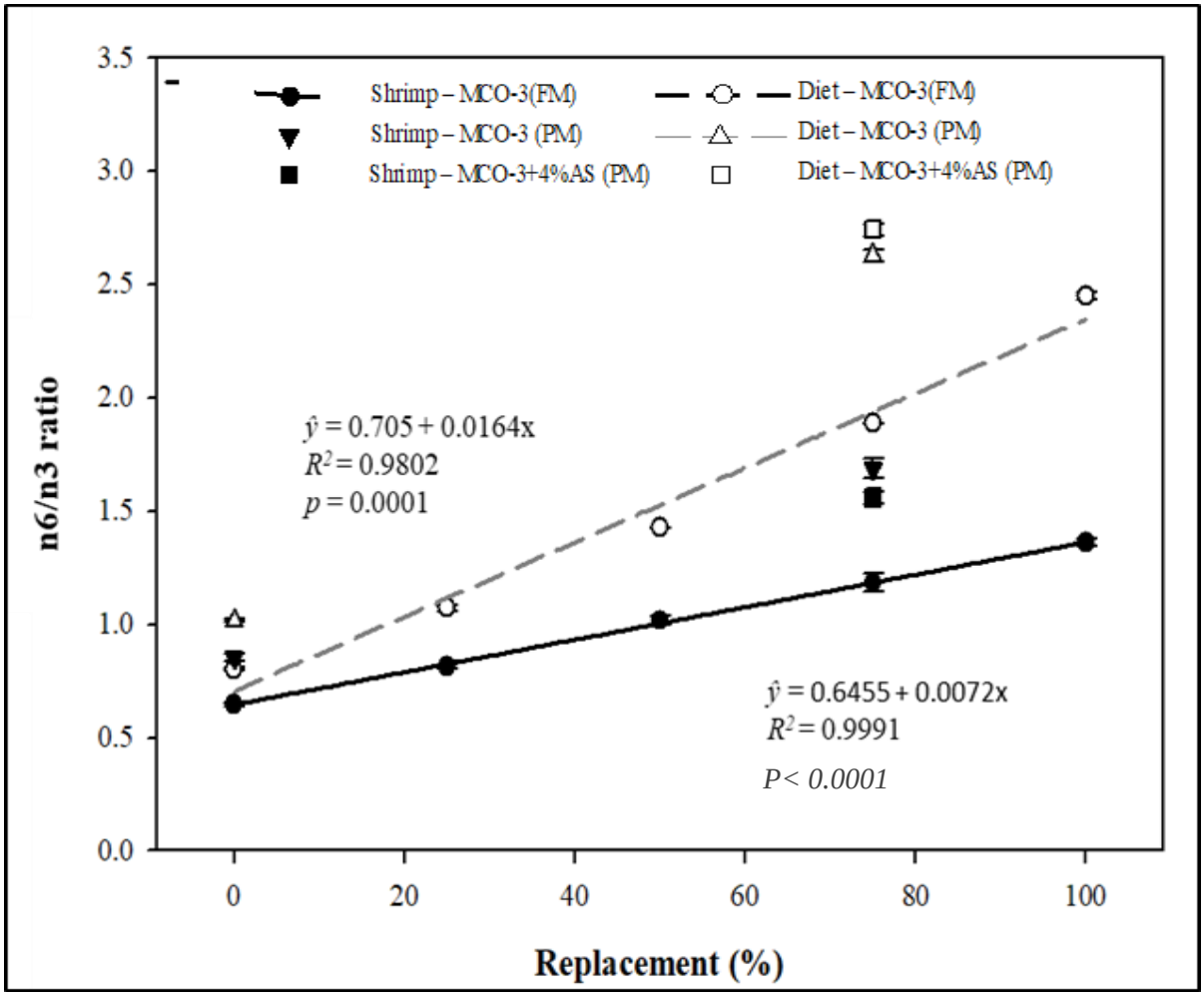

Figure 10. Relationship between levels of modified canola oil (MCO-3) replacement and the n6/n3 ratio in diets and in shrimp (within the fishmeal based diet treatments). The regression line for the n6/n3 ratio level in diets with the MCO-3 is y = 0.705 + 0.0164x (R^2 = 0.9802, P= *0.0001*), and in shrimp offered the respective diets is y = 0.6455 + 0.0072x (R^2 = *0.9991, P< 0.0001*).

4. Discussion

The continued development and expansion of aquaculture naturally leads to an increased need for feed and consequently feed ingredients that are cost effective and sustainable (Silva *et al.*, 2011). The demand of formulated feed has increased periodically and dramatically as a result of aquaculture production growth (Tacon and Metian, 2015) for its advantages of long-term storage, nutrient content, and various sizes and formulations for different physical stages of lifecycle, and nutritional species specific (Juell, 1995; Tacon and Metian, 2015). Particularly, commercial feed becomes more needy for its ability to provide the specific elements such as amino acids, fatty acids, as well as minerals and vitamins at a specific level that reach each specific target species requirements (NRC, 2011). Crustaceans and fish have been the largest consumers of non-food fish captured production (Naylor *et al.*, 2009). The components of crustacean production has shifted from *P. monodon* to *L. vannamei* in Asia (Naylor *et al.*, 2009), which makes feed ingredient demands change due to the different nutrition requirements for diet. Fish oil has been historically used in aquaculture feeds (Bell and Waagbø, 2008) because of its abundance of LC-HUFAs, such as EPA; 20:5n-3, and DHA; 22:6n-3 (Das, 2006) which are required in marine species diets, particularly Pacific white shrimp. However, with the general increase of fish oil price and high demand for consumption for both human and aquaculture, fish oil will not be a sustainable and expandable source of lipids over the long term (Tacon and Metian, 2009). Thus, an alternative that is qualified in economic as well as nutritional value is needed. In the last decade, plant oils have been experimented with to replace fish oil in diets of many freshwater and marine water species (Bell and Waagbø, 2008) with positive results. However, a complete replacement of fish oil has been less than complete especially with regards to marine species. In order to replace fish

oil in diets of marine species, a good understanding of EFA requirements of the target species has to be established and the essential fatty acids have to be present in the alternative.

The n3/n6 ratio of the lipids in the test diets showed a decrease which parallels the replacement of MFO with MCO-3. The highest ratio was 1.25 at the treatment of 100% MFO down to 0.41 at 100% MCO-3. In the series of poultry meal based diets, the n3/n6 ratio of the 100% MFO diet was 0.98 which was lower than that of the 100% MFO in fishmeal based group. With the diet of 75% MCO-3, and the diet of PM75 + 4AS, the n3/n6 ratio was 0.38 and 0.37, respectively. Although the n3/n6 ratio of the diet treatments were lower than 1 and lower than the basal diet treatment (100% MFO), there was no significant difference in mean final weights of *L. vannamei* among the treatments. The diets were applied to the animals from the stocked size of 0.19g to an average of 3.68g/shrimp (the treatment of 100% MFO) and to 3.06g/shrimp (the treatment of 100% MCO-3). This resulted in percent weight gain of 2239% to 1778% in the mentioned examples, respectively. This level of tissue replacement is similar to that reported in chapter 2 where significant differences were observed.

In a study of Xu *et al.* (1993) induced in Chinese prawn (*Penaeus chinensis*), n-3 fatty acids had better values in promoting growth performance than n-6 fatty acids. In regard to this this study, under the tested experiments, there was no significant difference in mean final weight when the tested diets had total n-3 fatty acids decreased and total n-6 fatty acids increased, so it was not a clear evidence that the n-3 fatty acid family was more beneficial than the n-6 fatty acid family. This result is also similar with the results of Gonzalez-Felix *et al.* (2003a), which showed no differences in growth was found due to the effects of dietary DHA, EPA, ARA, or n-3 HUFA mixture. The authors also concluded that *L. vannamei* needed to have HUFAs either n-3 (DHA, EPA) or n-6 (ARA), so chain length and degree of saturation were more essential. In this study, the level of ARA was found higher than 0.5% (in all diets) which was determined as the recommended level for *L. vannamei* growth by (Gonzalez-Felix

et al., 2003a). According to Gonzalez-Felix *et al.* (2003a) study, shrimp increased in growth when they were offered 0.5% ARA while the treatment of 0.5 LNA (18:3n-3) and 0.5% LOA (18:2n-6) showed a lower percent of weight gain. However, the weight gain of the 0.5% ARA diet was lower than 0.5% EPA, and 0.5% DHA diet. Combining with the results of this study, it could be included that ARA is important when there is no EPA or DHA available. In this study, both EPA and DHA value in diet was higher than 0.5% in all diets, this could be the reason that 100% MFO could be replaced by 100% MCO-3 without causing deficiency in growth performance although 100% MCO-3 had lower percent weight gain in comparison to the other treatments.

A decline in shrimp growth or survival rate is normally caused by the lack of nutrients, such as EFAs. However, overdose of EFAs is also acknowledged as a reason of growth deficiency or poor survival. A study of Kanazawa *et al.* (1985) in prawn (*Penaeus japonicus*) suggested the optimal level of n-3 HUFA was lower than 0%, from 2% or above would cause low survival due to toxicity of oxidation products. The optimal level of LNA in *Penaeus aztecus* was suggested from 1%-2%, over 5% would cause a decrease in growth (Shewbart and Mies, 1973). Gonzalez-Felix *et al.* (2002b) conducted a research in *L. vannamei* with three dietary levels (0%, 0.25% or 0.5% of diet) of DHA or an n-3 HUFA mixture containing EPA 416 mg /g and DHA 237 mg /g. The study reported the optimal range of DHA or n-3 HUFA for growth was 0.25%, over 0.5%. A high level of HUFAs in diets can cause issues of toxicity and contamination due to oxidization products, which will be the reason causing the negative effect on growth performance and survival (Gonzalez-Felix *et al.*, 2002b; Kanazawa *et al.*, 1985). In this recent study, the lowest level of DHA was 2.55% and the highest DHA levels were in the range of 8.52 - 11.11 % in the diet of 100% MFO (poultry meal based and fishmeal based diets). Although the level of DHA or n-3 HUFAs in this study was much higher than in the previous studies, there was no significant decrease in growth found between the treatments.

The essential fatty acid components in shrimp tissues were in an agreement with their respective dietary levels. These reults showed that dietary lipids regulated carcass lipis by reflecting the change in lipid compositions. The increase of DPA and ARA and the decrease of EPA and DHA due to the high percent of modified canola oil replacement in diets were follwed by the same trend in shrimp tissues. This result was supported by the the results of Gonzalez-Felix *et al*. (2003b) and Gonzalez-Felix *et al*. (2010), the authours particularly reported that the increase of n-3 HUFAs in diet was follwed by the increase of n-3 HUFAs in shrimp tissues. Besides, a higher level of the essental fatty acids were found in shrimp tissues in comparision to the respective elements in the diets. This result was in agreement with the study of Gonzalez-Felix *et al.* (2002a).

According to a study of Yu and Sinnhuber (1979) in coho salmon, the optimum of n-3 was from 1% to 2.5% and n-6 was around 1%. The authors suggested that too high or too low EFAs would inhibit growth, which was similar with the other study in shrimp mentioned above. Although the authors did not conclude a specific ratio of n3/n6, they concluded that n-3 fatty acids would not be inhibited by a dominant available level of n-6 fatty acids. There are few studies designed to specifically evaluate n3/n6 ratio. In this study, n3/n6 ratio ranged from 0.41 to 1.25 when MCO-3 replaced MFO from 100% to 0% within the fishmeal based group. Although the n3/n6 ratio steadily dropped down when the percent of modified canola oil increased, there was no significant difference in mean final weight and survival of the treatment offered the lowest n3/n6 ratio of 0.41. This could explain the essential roles of DHA, and EPA rather than the n3/n6 ratio.

To evaluate if palatability may be part of the issue of poor performance when fish oil is reduced, the poultry meal diet containing 75% MCO-3 was also supplemented with a salmon by product silage which would be expected to improve palatability of the diets. However,

shrimp maintained on this diet had the lowest mean final weight. This could be interpreted as there is no palatability issue or that salmon silage was not an effective in improving feed intake.

In this study, mean final weight was decreased with an increase of modified canola oil replacement, however, the decrease was not statistically different. As the decrease in performance is seen across studies as well as diet type this is likely to be biologically relevant. Possible, reasons for reduced performance could be due to palatability as previously described or due to digestibility of the oil source as well as other unknown factors attributed to fish oil. In studies conducted by Davis *et al.* (2004), algae oils and low levels of MFO served as the EFA source. When fish oil was reduced there were also numerical reductions in growth again indicating a biological effect.

In this research, the tested canola oil which is modified with the essential fatty acids that is absent in the original oil but required in Pacific white shrimp successfully replaced fish oil completely in diets for Pacific white shrimp in the laboratory scale. However, as there appeared to be some reductions in growth performance further experiments should be conducted for longer periods of time and to investigate digestibility and palatability issues that could occur. Then of course, these trials should also be extended to human health and taste testing of the shrimp to ensure a quality produce.

5. Conclusion

As the development of aquaculture as well as the increase of demand in seafood productions, commercial feed plays a key role in order to develop and maintain a sustainable aquaculture industry. The tested canola oil successfully replaced fish oil in diet of Pacific white shrimp at 100% of replacement. Although the higher percent of the replacement caused a slight decrease in mean final weight, it was not a significant difference. As laboratory tests were positive longer term trials under more practical conditions are warranted. In addition, the

application of this MCO in formulated diets should be evaluated for different species that have similar requirements of fatty acids with Pacific white shrimp.

6. References

Bell, J.G., Waagbø, R., 2008. Safe and nutritious aquaculture produce: benefits and risks of alternative sustainable aquafeeds. In: Holmer M., Black K., Duarte C.M., Marbà N., Karakassis I. (eds) Aquaculture in the Ecosystem. Springer, Dordrecht, 185-225.

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., Sargent, J.R., 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. The Journal of Nutrition. 131, 1535-1543.

Bowyer, J.N., Qin, J.G., Smullen, R.P., Stone, D.A.J., 2012. Replacement of fish oil by poultry oil and canola oil in yellowtail kingfish (*Seriola lalandi*) at optimal and suboptimal temperatures. Aquaculture. 356-357, 211-222.

Das, U., 2006. Essential Fatty Acids - A Review. Current Pharmaceutical Biotechnology. 7, 467 - 482.

Davis, D.A., Samocha, T.M., Bullis, R.A., Patnaik, S., Browdy, C.L., Stokes, A.D., Atwood, H.L., 2004. Practical diets for *Litopenaeus vannamei* (Boone, 1931): working towards organic and/or all plant production diets.

Dosanjh, B.S., Higgs, D.A., McKenzie, D.J., Randall, D.J., Eales, J.G., Rowshandeli, N., Rowshandeli, M., Deacon, G., 1998. Influence of dietary blends of menhaden oil and canola oil on growth, muscle lipid composition, and thyroidal status of Atlantic salmon (*Salmo salar*) in sea water. Fish Physiology and Biochemistry. 19, 123-134.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497-509.

Gonzalez-Felix, M., III, D.G., Lawrence, A.L., Perez-Velazquez, M., 2002a. Effect of various dietary lipid levels on quantitative essential fatty acid requirements of juvenile Pacific

White Shrimp *Litopenaeus vannamei*. Journal of the World Aquaculture Society. 33, 330-340.

Gonzalez-Felix, M.L., III, D.M.G., Lawrence, A.L., Perez-Velazquez, M., 2002b. Effect of dietary phospholipid on essential fatty acid requirements and tissue lipid composition of *Litopenaeus vannamei* juveniles. Aquaculture. 207, 151-167.

Gonzalez-Felix, M.L., D.M., G.I., Lawrance, A.L., Perez-Velazqez, M., 2003a. Nutritional evaluation of fatty acids for the open thelycum shrimp, *Litopenaeus vannamei*: II. Effect of dietary n-3 and n-6 polyunsaturated and highly unsaturated fatty acids on juvenile shrimp growth, survival, and fatty acid composition. Aquaculture Nutrition. 9, 115-122.

Gonzalez-Felix, M.L., Lawrence, A.L., Gatlin, D.M., Perez-Velazquez, M., 2003b. Nutritional evaluation of fatty acids for the open thelycum shrimp, *Litopenaeus vannamei*: I. Effect of dietary linoleic and linolenic acids at different concentrations and ratios on juvenile shrimp growth, survival and fatty acid composition. Aquaculture Nutrition. 9, 105-113.

González-Félix, M.L., Silva, F.S.D.d., Davis, D.A., Samocha, T.M., Morris, T.C., Wilkenfeld, J.S., Perez-Velazquez, M., 2010. Replacement of fish oil in plant based diets for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture. 309, 152-158.

Juell, J.-E., 1995. The behaviour of Atlantic salmon in relation to efficient cage-rearing. Reviews in fish biology and fisheries. 5, 320-335.

Kanazawa, A., Teshima, S., Sakamoto, M., 1985. Effects of dietary lipids, fatty acids, and phospholipids on growth and survival of prawn (*Penaeus japonicus*) larvae. Aquaculture, 50: 39-49.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J., Hua, K., 2009. Feeding aquaculture in an era of finite resources. Proceedings of the National Academy of Sciences, pnas. 0905235106.

NRC, 2011. Nutrient requirements of fish and shrimp. National Academic Press.

Shewbart, K., Mies, W., 1973. Studies on nutritional requirements of brown shrimp-the effect of linolenic acid on growth of *Penaeus aztecus*, Proceedings of the annual workshop-World Mariculture Society. Wiley Online Library, pp. 277-287.

Silva, S.S.D., Francis, D.S., Tacon, A.C.J., 2011. Fish oil in aquaculture : in retrospect, in Fish oil replacement and alternative lipid sources in aquaculture feeds. CRC Press, Boca Raton, Flo, 1-20.

Soller, F., Rhodes, M.A., Davis, D.A., 2017. Replacement of Fish Oil with Alternative Lipid Sources in Plant-based Practical Feed Formulations for Marine Shrimp (*Litopenaeus vannamei*) Reared in Outdoor Ponds and Tanks. Aquaculture Nutrition. 23, 63-75.

Solorzano, L., 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. . Limnology and Oceanography. 14, 799-801.

Spotte, S., 1979. Fish and invertebrate culture: Water managment in closed systems, 2nd edition. Wiley, New York.

Tacon, A.G., Metian, M., 2009. Fishing for aquaculture: non-food use of small pelagic forage fish—a global perspective. Reviews in Fisheries Science. 17, 305-317.

Tacon, A.G.J., Metian, M., 2015. Feed Matters: Satisfying the Feed Demand of Aquaculture. Reviews in Fisheries Science & Aquaculture. 23, 1-10.

Wonnacott, E.J., Lane, R.L., Kohler, C.C., 2004. Influence of Dietary Replacement of Menhaden Oil with Canola Oil on Fatty Acid Composition of Sunshine Bass. North American Journal of Aquaculture. 66, 243-250.

Xu, X., Ji, W., Castell, J.D., O'Dor, R., 1993. The nutritional value of dietary n-3 and n-6 fatty acids for the Chinese prawn (*Penaeus chinensis*). Aquaculture. 118, 277-285.

Yu, T., Sinnhuber, R., 1979. Effect of dietary ω3 and ω6 fatty acids on growth and feed conversion efficiency of coho salmon (*Oncorhynchus kisutch*). Aquaculture. 16, 31-38.

Zhou, Q.C., Li, C.C., Liu, C.W., Chi, S.Y., Yang, Q.H., 2007. Effects of dietary lipid sources on growth and fatty acid composition of juvenile shrimp, *Litopenaeus vannamei*. Aquaculture Nutrition. 13, 222-229.

CHAPTER IV

SUMMARY AND CONCLUSION

Lipid is one of the primary factors contributing to the development, and survival of Pacific white shrimp as well as the other species. It plays roles in membrane structures, and as a source of energy for maintenance and growth of a body. Fish oil has been used as the major source of lipid among other oils such as plant oils due to its fatty acid profile which meets the essential fatty acid requirement for Pacific white shrimp. The availability of omega-3 in fish oil makes this source of lipid one of the most needed in aquaculture as well as in human consumption. Fish oil is rich in long chain highly unsaturated fatty acids (LC-HUFAs) such as DHA, and EPA which are required in many marine species due to their limit ability to convert C22 from C18 and Pacific white shrimp is one of them. Because the nutritional requirements of different species vary, commercial feed that contains the nutrients required to fulfil the necessary dietary needs of the target species is increasing in terms of production. This leads to a high demand of lipid sources that provide the important elements such as the essential fatty acids DHA, and EPA. Marine originated oils qualify these requirements of nutrition; however, they are not always available in nature due to overexploitation and high demand for different purposes.

In order to promote a sustainable industry and expand its production, the long-term goal of commercial producers is to completely replace fish oil in practical diet for Pacific white shrimp without causing deficiencies in its production. In order to provide the essential fatty acids required by many species, plant-based oils will have to be modified to include HUFAs. In the case of canola oil, such modifications have been successfully made and have resulted in

enhanced oil with contains high levels of EPA, DHA, DPA, and ARA. As these oils become available we need to evaluate efficacy of their use.

In the first experiment, the objectives were to evaluate the nutritional values of the test modified canola oils and the effects of their levels of replacement for fish oil on growth, survival, and FCR of Pacific white shrimp. A series of nine diets were formulated with 36% of protein and 8% of lipid. 100% of fish oil as a basal diet was formulated. In the other diets, fish oil was sequentially decreased, and those decreases were replaced by modified canola oils and standard canola oil to reach 8% of total lipid in diet. The replacement at different levels was to estimate the ability of modified canola oil to provide the optimal level of essential fatty acids that Pacific white shrimp require in their diet. The results of growth showed the response of a decrease rather than an increase. The trend of the decline in growth positively correlated to the increase of the modified canola oil and it was statistically different when MCO's were replaced MFO at 100%. DHA was suspected as the limiting factor due to its level being close to the threshold in the diet of 100% MCO's supplemented. Besides the low level of DHA in the diet, peroxide value of the diet of 100MCO-1 was over the safety range due to high level of HUFA, which might lead to the reduction of palatability due to oxidization products. This result rose questions about what the exactly reason was that the responses of animals offered modified canola oil treatments was not as good as the 100% menhaden fish oil treatment. The questions could be whether the essential fatty acid levels were not qualified, or the peroxide levels of the modified canola oils were the reason causing the deficiencies due to toxicity.

Thus, the second experiment was to evaluate the efficiency of modified canola oil replacing menhaden fish oil at different levels and up to 100% of the replacement by evaluating the fatty acid profile particularly DHA, and EPA levels of the newly modified oil for growth, survival, and FCR for Pacific white shrimp. Besides, palatability of the modified canola oil using in this experiment was tested by adding 4% of hydrolysed salmon meal as an attractant

in the diet of 75% modified canola oil. In the results of this study, the level of DHA and EPA in the diets decreased with the increase of MCO-3 replacement; however, these levels were not close to the threshold when MFO was completely replaced. It showed that the lowest value of the n3/n6 ratio was 0.37 at the treatment, and the highest value was 1.25 at the control. However, growth performances of the control and the treatments were not significantly different. Also, the peroxide value of the modified canola was in the safe range (less than 10meq/Kg). The result suggested that this modified canola oil can be applied to replace fish oil at the level of 100% replacement. The modified canola oil used in the second study showed a succeed of usage of this oil to replace fish oil at 100% and it is a positive potential to use in practical diet for Pacific white shrimp in farming scale.

In comparison to diets of the first experiment, EFAs levels in the diets of the second experiment were higher than the threshold level which is 0.5%. Particularly, in the diet contained 100% of MCO-3, DHA level was 3.79% while it was found to be 0.57% in the both diets contained 100% of MCO-1, and 100% of MCO-2.

Thus, further studies which are possibly induced in pond systems, or other outdoor systems are needed in order to improve the decrease in final mean weight which was caused possibly by the physiological effects. Moreover, formulated feed for different aquatic species that have the similar requirements of essential fatty acids with Pacific white shrimp should be experimented with this modified canola oil. This modified canola oil comes with a tremendous benefit that may be able to further support the feed industry, which is not just in Pacific white shrimp but also in the other marine species. The availability of modified canola oil is significantly higher than the predicted future of fish oil production, and its quality has met the requirements of Pacific white shrimp, which means it has the possibility of becoming a multispecies ingredient of formulated feed if approved.

Reference

Bell, J.G., Waagbø, R., 2008. Safe and nutritious aquaculture produce: benefits and risks of alternative sustainable aquafeeds. In: Holmer M., Black K., Duarte C.M., Marbà N., Karakassis I. (eds) Aquaculture in the Ecosystem. Springer, Dordrecht, 185-225.

Das, U., 2006. Essential Fatty Acids - A Review. Current Pharmaceutical Biotechnology. 7, 467 - 482.

Davis, D.A., Samocha, T.M., Bullis, R.A., Patnaik, S., Browdy, C.L., Stokes, A.D., Atwood, H.L., 2004. Practical diets for *Litopenaeus vannamei* (Boone, 1931): working towards organic and/or all plant production diets.

Gonzalez-Felix, M., III, D.G., Lawrence, A.L., Perez-Velazquez, M., 2002a. Effect of various dietary lipid levels on quantitative essential fatty acid requirements of juvenile Pacific White Shrimp *Litopenaeus vannamei*. Journal of the World Aquaculture Society. 33, 330-340.

Gonzalez-Felix, M.L., III, D.M.G., Lawrence, A.L., Perez-Velazquez, M., 2002b. Effect of dietary phospholipid on essential fatty acid requirements and tissue lipid composition of *Litopenaeus vannamei* juveniles. Aquaculture. 207, 151-167.

Gonzalez-Felix, M.L., D.M., G.I., Lawrance, A.L., Perez-Velazqez, M., 2003a. Nutritional evaluation of fatty acids for the open thelycum shrimp, *Litopenaeus vannamei*: II. Effect of dietary n-3 and n-6 polyunsaturated and highly unsaturated fatty acids on juvenile shrimp growth, survival, and fatty acid composition. Aquaculture Nutrition. 9, 115-122.

Gonzalez-Felix, M.L., Lawrence, A.L., Gatlin, D.M., Perez-Velazquez, M., 2003b. Nutritional evaluation of fatty acids for the open thelycum shrimp, *Litopenaeus vannamei*: I. Effect of dietary linoleic and linolenic acids at different concentrations

and ratios on juvenile shrimp growth, survival and fatty acid composition. Aquaculture Nutrition. 9, 105-113.

González-Félix, M.L., Silva, F.S.D.d., Davis, D.A., Samocha, T.M., Morris, T.C., Wilkenfeld, J.S., Perez-Velazquez, M., 2010. Replacement of fish oil in plant based diets for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture. 309, 152-158.

Juell, J.-E., 1995. The behaviour of Atlantic salmon in relation to efficient cage-rearing. Reviews in fish biology and fisheries. 5, 320-335.

Kanazawa, A., Teshima, S., Sakamoto, M., 1985. Effects of dietary lipids, fatty acids, and phospholipids on growth and survival of prawn (*Penaeus japonicus*) larvae. Aquaculture, 50: 39-49.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J., Hua, K., 2009. Feeding aquaculture in an era of finite resources. Proceedings of the National Academy of Sciences, pnas. 0905235106.

NRC, 2011. Nutrient requirements of fish and shrimp. National Academic Press.

Shewbart, K., Mies, W., 1973. Studies on nutritional requirements of brown shrimp-the effect of linolenic acid on growth of *Penaeus aztecus*, Proceedings of the annual workshop-World Mariculture Society. Wiley Online Library, pp. 277-287.

Silva, S.S.D., Francis, D.S., Tacon, A.C.J., 2011. Fish oil in aquaculture : in retrospect, in Fish oil replacement and alternative lipid sources in aquaculture feeds. CRC Press, Boca Raton, Flo, 1-20.

Tacon, A.G., Metian, M., 2009. Fishing for aquaculture: non-food use of small pelagic forage fish—a global perspective. Reviews in Fisheries Science. 17, 305-317.

Tacon, A.G.J., Metian, M., 2015. Feed Matters: Satisfying the Feed Demand of Aquaculture. Reviews in Fisheries Science & Aquaculture. 23, 1-10.

Xu, X., Ji, W., Castell, J.D., O'Dor, R., 1993. The nutritional value of dietary n-3 and n-6 fatty acids for the Chinese prawn (*Penaeus chinensis*). Aquaculture. 118, 277-285.

Yu, T., Sinnhuber, R., 1979. Effect of dietary ω3 and ω6 fatty acids on growth and feed conversion efficiency of coho salmon (*Oncorhynchus kisutch*). Aquaculture. 16, 31-38.

CHAPTER V

LITERATURE CITED

Bell, J.G., Waagbø, R., 2008. Safe and nutritious aquaculture produce: benefits and risks of alternative sustainable aquafeeds. In: Holmer M., Black K., Duarte C.M., Marbà N., Karakassis I. (eds) Aquaculture in the Ecosystem. Springer, Dordrecht, 185-225.

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., Sargent, J.R., 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. The Journal of Nutrition. 131, 1535-1543.

Bowyer, J.N., Qin, J.G., Smullen, R.P., Stone, D.A.J., 2012. Replacement of fish oil by poultry oil and canola oil in yellowtail kingfish (*Seriola lalandi*) at optimal and suboptimal temperatures. Aquaculture. 356-357, 211-222.

Caballero, M., Obach, A., Rosenlund, G., Montero, D., Gisvold, M., Izquierdo, M., 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 214, 253-271.

Cuzona, G.r., Lawrenceb, A., Gaxiolac, G., Rosasc, C., Guillaumed, J., 2004. Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. Aquaculture. 235, 513-551.

D'Abramo, L.R., Sheen, S.S., 1993. Polyunsaturated fatty acid nutrition in juvenile freshwater prawn *Macrobrachium rosenbergii*. Aquaculture. 115, 63-86.

Das, U., 2006. Essential Fatty Acids - A Review. Current Pharmaceutical Biotechnology. 7, 467 - 482.

Davis, D.A., Samocha, T.M., Bullis, R.A., Patnaik, S., Browdy, C.L., Stokes, A.D., Atwood, H.L., 2004. Practical diets for *Litopenaeus vannamei* (Boone, 1931): working towards organic and/or all plant production diets.

Dosanjh, B.S., Higgs, D.A., McKenzie, D.J., Randall, D.J., Eales, J.G., Rowshandeli, N., Rowshandeli, M., Deacon, G., 1998. Influence of dietary blends of menhaden oil and canola oil on growth, muscle lipid composition, and thyroidal status of Atlantic salmon (*Salmo salar*) in sea water. Fish Physiology and Biochemistry. 19, 123-134.

FAO, 2018. The State of World Fisheries and Aquaculture- Meeting The Sustainable Development Goals.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497-509.

Geurden, I., Corraze, G., Boujard, T., 2007. Self-feeding behaviour of rainbow trout, *Oncorhynchus mykiss*, offered diets with distinct feed oils. Applied animal behaviour science. 108, 313-326.

Geurden, I., Cuvier, A., Gondouin, E., Olsen, R., Ruohonen, K., Kaushik, S., Boujard, T., 2005. Rainbow trout can discriminate between feeds with different oil sources. Physiology & behavior. 85, 107-114.

Glencross, B.D., Smith, D.M., 2001. Optimizing the essential fatty acids, eicosapentaenoic and docosahexaenoic acid, in the diet of the prawn, *Penaeus monodon*. Aquaculture Nutrition. 7, 101-112.

Gonzalez-Felix, M., III, D.G., Lawrence, A.L., Perez-Velazquez, M., 2002a. Effect of various dietary lipid levels on quantitative essential fatty acid requirements of juvenile Pacific White Shrimp *Litopenaeus vannamei*. Journal of the World Aquaculture Society. 33, 330-340.

Gonzalez-Felix, M.L., III, D.M.G., Lawrence, A.L., Perez-Velazquez, M., 2002b. Effect of dietary phospholipid on essential fatty acid requirements and tissue lipid composition of *Litopenaeus vannamei* juveniles. Aquaculture. 207, 151-167.

Gonzalez-Felix, M.L., D.M., G.I., Lawrance, A.L., Perez-Velazqez, M., 2003a. Nutritional evaluation of fatty acids for the open thelycum shrimp, *Litopenaeus vannamei*: II. Effect of dietary n-3 and n-6 polyunsaturated and highly unsaturated fatty acids on juvenile shrimp growth, survival, and fatty acid composition. Aquaculture Nutrition. 9, 115-122.

Gonzalez-Felix, M.L., Lawrence, A.L., Gatlin, D.M., Perez-Velazquez, M., 2003b. Nutritional evaluation of fatty acids for the open thelycum shrimp, *Litopenaeus vannamei*: I. Effect of dietary linoleic and linolenic acids at different concentrations and ratios on juvenile shrimp growth, survival and fatty acid composition. Aquaculture Nutrition. 9, 105-113.

González-Félix, M.L., Perez-Velazquez, M., Quintero-alvarez, J.S.M., 2009. Effect of Various Dietary Levels of Docosahexaenoic and Arachidonic Acids and Different n-3/n-6 Ratios on Biological Performance of Pacific White Shrimp, *Litopenaeus vannamei*, Raised in Low Salinity. Journal of the World Aquaculture Society. 40, 194-206.

González-Félix, M.L., Silva, F.S.D.d., Davis, D.A., Samocha, T.M., Morris, T.C., Wilkenfeld, J.S., Perez-Velazquez, M., 2010. Replacement of fish oil in plant based diets for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture. 309, 152-158.

Hasan, M.R., 2001. Nutrition and Feeding for Sustainable Aquaculture Development in the Third Millennium. In R.P. Subasinghe, P. Bueno, M.J. Phillips, C. Hough, S.E. McGladdery & J.R. Arthur, eds. Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20-25 February 2000., 193-219.

Higgs, D.A., Balfry, S.K., Oakes.J.D., M., R., B.J., S., G., D., 2006. Efficacy of an equal blend of canola oil and poultry fat as an alternative dietary lipid sources for Atlantic salmon (*Salmo salar L.*) in sea water. I: effects on growth performance, and whole body and fillet proximate and lipid composition. Aquaculture Research. 37, 180-191.

Ismail, A., Bannenberg, G., Rice, H.B., Schutt, E., MacKay, D., 2016. Oxidation in EPA- and DHA-rich oils: an overview. Lipid Technology published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. 28, 55-59.

Izquierdoa, M.S., Monteroa, D., Robainaa, L., Caballeroa, M.J., Rosenlund, G., Ginesa, R., 2005. Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. Aquaculture. 250, 431-444.

Juell, J.-E., 1995. The behaviour of Atlantic salmon in relation to efficient cage-rearing. Reviews in fish biology and fisheries. 5, 320-335.

Kanazawa, A., 1985. Nutrition of penaeid prawns and shrimps. In Taki Y., Primavera J.H. and Llobrera J.A. (Eds.). Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps, 4-7 December 1984, Iloilo City, Philippines, Iloilo City, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center., 123-130.

Kanazawa, A., Teshima, S.-i., 1977. Biosynthesis of Fatty Acids from Acetate in the Prawn, *Penaeus japonicus*. Mem. Fac. Fish., Kagoshima Univ. 26.

Kanazawa, A., Teshima, S., Tokiwa, S., 1979a. Biosynthesis of fatty acids from palmitic acid in the prawn, *Penaeus japonicus.* Memoirs Fac Fisheries, Kagoshima University. 28.

Kanazawa, A., Teshima, S., Sakamoto, M., 1985. Effects of dietary lipids, fatty acids, and phospholipids on growth and survival of prawn (*Penaeus japonicus*) larvae. Aquaculture, 50: 39-49.

Kanazawa, A., Teshima, S.-i., Endo, M., Kayama, M., 1978. Effects of Eicosapentaenoic Acid on Growth and Fatty Acid Composition of the Prawn, *Penaeus japonicus*. Mem. Fac. Fish., Kagoshima Univ. 27, 35-40.

Kanazawa, A., Teshima, S., Ono, K., Chalayondeja, K., 1979b. Biosynthesis of fatty acids from acetate in the prawns, *Penaeus monodon* and *Penaeus merguiensis*. Memoirs Fac Fisheries, Kagoshima University. 28.

Kanazawa, A., Shin-ichinTeshima, Tokiwa, S., Kayama, M., Hirata, M., 1979c. Essential Fatty Acids in the Diet of Prawn-II Effectof Docosahexaenoic Acid on Growth. Bulletin of the Japanese Society of Scientific Fisheries. 45, 1151-1153.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E., Etherton, T.D., 2002. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. Am. J. Med. 113, 71-88.

Lamb, C.F., 2001. Gustation and feeding behaviour. Food intake in fish, 108-130.

Leaver, M.J., Bautista, J.M., Björnsson, B.r.T., Jönsson, E., Krey, G., Tocher, D.R., Torstensen, B.E., 2008. Towards Fish Lipid Nutrigenomics: Current State and Prospects for Fin-Fish Aquaculture. Reviews in Fisheries Science. 16:S1, 73-94.

Liao, I.C., Chien, Y.-H., 2010. The Pacific White Shrimp, *Litopenaeus vannamei*, in Asia: The World's Most Widely Cultured Alien Crustacean. Invading Nature - Springer Series in Invasion Ecology. 6, 489-520.

Lim, C., Ako, H., Brown, C.L., Hahn, K., 1997. Growth response and fatty acid composition of juvenile *Penaeus vannamei* fed different sources of dietary lipid. Aquaculture. 151, 143-153.

Mourente, G., Bell, J.G., 2006. Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax L*.) over a long term growth study: effects on muscle and liver fatty acid

composition and effectiveness of a fish oil finishing diet. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 145, 389-399.

Mourente, G., Good, J., Bell, J., 2005a. Partial substitution of fish oil with rapeseed, linseed and olive oils in diets for European sea bass (*Dicentrarchus labrax L*.): effects on flesh fatty acid composition, plasma prostaglandins E2 and F2α, immune function and effectiveness of a fish oil finishing diet. Aquaculture Nutrition. 11, 25-40.

Mourente, G., Dick, J.R., Bell, J.G., Tocher, D.R., 2005b. Effect of partial substitution of dietary fish oil by vegetable oils on desaturation and h-oxidation of [1-14C]18:3n-3 (LNA) and [1-14C]20:5n-3 (EPA) in hepatocytes and enterocytes

of European sea bass (*Dicentrarchus labrax L*.). Aquaculture. 248, 173-186.

Nasopoulou, C., Zabetakis, I., 2012. Benefits of fish oil replacement by plant originated oils in compounded fish feeds. A review. LWT-Food Science and Technology. 47, 217-224.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J., Hua, K., 2009. Feeding aquaculture in an era of finite resources. Proceedings of the National Academy of Sciences, pnas. 0905235106.

Newell-McGloughlin, M., 2008. Nutritionally improved agricultural crops. Plant Physiol. 147, 939-953.

NRC, 2011. Nutrient requirements of fish and shrimp. National Academic Press.

Olsen, R.L., Hasan, M.R., 2012. A limited supply of fishmeal: Impact on future increases in global aquaculture production. Trends in Food Science & Technology. 27, 120-128.

Panserat, S., Hortopan, G., Plagnes-Juan, E., Kolditz, C., Lansard, M., Skiba-Cassy, S., Esquerre, D., Geurden, I., Medale, F., Kaushik, S., 2009. Differential gene expression after total replacement of dietary fish meal and fish oil by plant products in rainbow trout (*Oncorhynchus mykiss*) liver. Aquaculture. 294, 123-131.

Rainuzzo, J.R., Reitan, K.I., Olsen, Y., 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture. 155, 103-115.

Rennie, K.L., Hughes, J., Lang, R., Jebb, S.A., 2003. Nutritional management of rheumatoid arthritis: a review of the evidence. J. Hum. Nutr. Diet. 16, 97-109.

Rosenlund, G., Obach, A., Sandberg, M., Standal, H., Tveit, K., 2001. Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (*Salmo salar L.*). Aquaculture Research. 32, 323-328.

Rosillo-Calle, F., Pelkmans, L., Walter, A., 2009. A global overview of vegetable oils, with reference to biodiesel. A report for the IEA Bioenergy Task. 40.

Roy, L.A., Davis, D.A., 2010. Requirements for the culture of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters: water modification and nutritional strategies for improving production. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D.A., Gamboa-Delgado, J. (Eds), Avances en Nutrición Acuicola X-Memorias del Décimo Simposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N.L.; Mexico. ISBN 978-607-433-546-0. Universidad Autonoma de Nuevo Autonoma de Nuevo Leon, Monterrey, Mexico, pp. 61-78.

Samocha, T.M., Patnaik, S., Davis, D.A., Bullis, R.A., Browdy, C.L., 2010. Use of commercial fermentation products as a highly unsaturated fatty acid source in practical diets for the Pacific white shrimp *Litopenaeus vannamei*. Aquaculture Research. 41, 961-967.

Samocha, T.M., Davis, D.A., Roy, L.A., Carpenter, B., BULLIS, R.A., 2011. The effect of non-marine HUFA supplementation with fish oil removal on growth and survival of the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture Nutrition. 17, 518-525.

Shewbart, K., Mies, W., 1973. Studies on nutritional requirements of brown shrimp-the effect of linolenic acid on growth of *Penaeus aztecus*, Proceedings of the annual workshop-World Mariculture Society. Wiley Online Library, pp. 277-287.

Shiau, S.-Y., 1998. Nutrient requirements of penaeid shrimps. Aquaculture. 164, 77-93.

Silva, S.S.D., Francis, D.S., Tacon, A.C.J., 2011. Fish oil in aquaculture : in retrospect, in Fish oil replacement and alternative lipid sources in aquaculture feeds. CRC Press, Boca Raton, Flo, 1-20.

Soller, F., Rhodes, M.A., Davis, D.A., 2017. Replacement of Fish Oil with Alternative Lipid Sources in Plant-based Practical Feed Formulations for Marine Shrimp (*Litopenaeus vannamei*) Reared in Outdoor Ponds and Tanks. Aquaculture Nutrition. 23, 63-75.

Solorzano, L., 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. . Limnology and Oceanography. 14, 799-801.

Spotte, S., 1979. Fish and invertebrate culture: Water managment in closed systems, 2nd edition. Wiley, New York.

Sun, G.Y., Simonyi, A., Fritsche, K.L., Chuang, D.Y., Hannink, M., Gu, Z.Z., Greenlie, C.M., Yao, J.K., Lee, J.C., Beversdorf, D.Q., 2018. Docosahexaenoic acid (DHA): An essential nutrient and a nutraceutical for brain health and diseases. Prostaglandins Leukot. Essent. Fatty Acids. 136, 3-13.

Tacon, A.G., Metian, M., 2009. Fishing for aquaculture: non-food use of small pelagic forage fish—a global perspective. Reviews in Fisheries Science. 17, 305-317.

Tacon, A.G.J., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture. 285, 146-158.

Tacon, A.G.J., Metian, M., 2015. Feed Matters: Satisfying the Feed Demand of Aquaculture. Reviews in Fisheries Science & Aquaculture. 23, 1-10.

Tocher, D.R., 2003. Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish. Reviews in Fisheries Science. 11, 107-184.

Turchini, G.M., Torstensen, B.E., Ng, W.-K., 2009. Fish oil replacement in finfish nutrition. Reviews in Aquaculture. 1, 10-57.

Wonnacott, E.J., Lane, R.L., Kohler, C.C., 2004. Influence of Dietary Replacement of Menhaden Oil with Canola Oil on Fatty Acid Composition of Sunshine Bass. North American Journal of Aquaculture. 66, 243-250.

Xu, X., Ji, W., Castell, J.D., O'Dor, R., 1993. The nutritional value of dietary n-3 and n-6 fatty acids for the Chinese prawn (*Penaeus chinensis*). Aquaculture. 118, 277-285.

Yu, T., Sinnhuber, R., 1979. Effect of dietary ω3 and ω6 fatty acids on growth and feed conversion efficiency of coho salmon (*Oncorhynchus kisutch*). Aquaculture. 16, 31-38.

Yuan, L., Knauf, V.C., 1997. Modification of plant components. Curr. Opin. Biotechnol. 8, 227-233.

Zhou, Q.C., Li, C.C., Liu, C.W., Chi, S.Y., Yang, Q.H., 2007. Effects of dietary lipid sources on growth and fatty acid composition of juvenile shrimp, *Litopenaeus vannamei*. Aquaculture Nutrition. 13, 222-229.

Zou, J.T., Katavic, V., Giblin, E.M., Barton, D.L., MacKenzie, S.L., Keller, W.A., Hu, X., Taylor, D.C., 1997. Modification of seed oil content and acyl composition in the brassicaceae by expression of a yeast sn-2 acyltransferase gene. Plant Cell. 9, 909-923.