Determination of the Digestibility of a Whole-Cell DHA-Rich Algal Product and Its Effect on the Lipid Composition of Rainbow Trout and Atlantic Salmon

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By
Chuyuan Zhang

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ABSTRACT

A whole-cell DHA-rich algal product (A-DHA, provided by Evonik Industries) that is rich in DHA (125 mg DHA/g dry matter) is a possible replacement for fish oil in salmonid diets. The nutrient digestibilities of the algal product were measured in rainbow trout in freshwater and in Atlantic salmon in saltwater (32-33 ppm). In experiment 1, rainbow trout (initial weight ~ 300g) were randomly assigned to 12 x 120 L tanks (n = 10 per tank). A reference diet containing 1% Celite as an indigestible marker and three test diets with increasing percentage of A-DHA substitution (6.67%, 13.33% and 20%) were fed. Feces were collected using a settling column and feed and feces analyzed for digestible dry matter (DM), gross energy (GE), ash, crude protein (CP), essential amino acids and total lipid. The digestibility of six long-chain fatty acids including 18:1n-9 (OA), 18:2n-6 (LA), 18:3n-3 (ALA), 20:4n-6 (ARA), 20:5n-3 (EPA) and 22:6n-3 (DHA) was measured. In experiment 2, Atlantic salmon (~170g) were randomly distributed to 12 fiberglass tanks (600L) with 106 fish per tank. The fish were assigned to four diets with the same levels of A-DHA inclusion as for rainbow trout and yttrium oxide (Y_2O_3) was used as an inert marker. Feces were collected by stripping and the digestibilities of DM, CP and lipid as well as OA, LA, ALA, ARA, EPA and DHA were determined.

In experiment 1, the apparent digestibility of dietary DM, GE and lipid in rainbow trout declined significantly with increasing inclusion of A-DHA ($P < 0.01$). The inclusion of A-DHA had no effect on the digestibility of CP and ash as well as the availability of essential amino acids ($P > 0.05$). Furthermore, increased inclusion of A-DHA resulted in significantly lower digestibility of ARA, EPA and DHA ($P < 0.05$). A similar pattern was seen in the digestibility of OA, LA and ALA, although the effect of A-DHA inclusion was not statistically significant. Regression analysis revealed that nutrient contribution from A-DHA had significantly negative linear and quadratic effects on the apparent digestibility of DM, GE, and lipid. The inclusion levels of A-DHA had both significantly negative linear and quadratic effects on digestibility of LA and ALA, whereas only significantly negative linear effect was found on OA. Significantly negative linear and quadratic regressions were observed for the digestibility of ARA, EPA and DHA. The linear regression for CP was significantly negative and the regressions for the individual amino acids were not significant ($P > 0.05$).

In experiment 2, dietary inclusion of A-DHA had a significantly negative effect on lipid digestibility in Atlantic salmon, at all inclusion rates whereas the significant negative effect on

digestibilities of DM and CP was only observed in fish fed 20% A-DHA. The digestibilities of OA, LA, ALA and EPA were greater than 91%. In contrast, the apparent digestibilities of ARA and DHA decreased significantly with increasing substitution of A-DHA ($P < 0.01$). Significantly negative linear and quadratic regressions were found between nutrient contribution from A-DHA to the diets and apparent digestibility of DM, CP and lipid, so were LA, EPA and DHA. However, there were only significant quadratic regressions for OA, ALA and ARA, but not significant linear effects.

Subsequently, a twelve-week feeding trial in rainbow trout was conducted to investigate the impact of replacing fish oil with A-DHA in canola-oil-based diets on the growth performance and fatty acid composition and retention. Four experimental diets containing only canola oil (CO; 13.5%), fish oil (FO; 13.5%), canola oil and fish oil (C+F; 7.4% and 6.1%, respectively) or canola oil and A-DHA (C+A; 15.5% and 6%, respectively) were formulated to contain 386.2 g/kg digestible crude protein and 17.58 MJ/kg digestible energy. In addition, the C+A diet was formulated to have the same DHA concentration as in the C+F diet. Each diet was fed to three tanks of rainbow trout (average initial weight of 70g; n = 17/tank) and the fish were fed to apparent satiation 2 times daily.

At the end of the growth trial, all fish approximately tripled their weight. No significant differences were noted between the dietary treatments in growth performance as measured by final weight, average weight gain, feed intake, specific growth rate (SGR) and feed conversion ratio (FCR). Although FO and C+A fed fish tended to accumulate more lipids, final whole body lipid content did not differ significantly between dietary treatments ($P = 0.11$). The concentrations of EPA, DHA as well as total n-3 fatty acid were significantly higher in fish fed the FO diet than fish fed the other 3 diets. The C+A fed fish had lower EPA and higher DHA concentrations compared with the CO and C+F fed fish; however, the differences were not significant.

Apparent retention of total lipid in the trout was not significantly influenced by treatments ($P > 0.05$). Similarly, dietary treatments had no significant effect on the apparent retention of total saturated fatty acids, total mono-unsaturated fatty acids, n-3 polyunsaturated fatty acids and n-6 polyunsaturated fatty acids. The retention of 18:4n-3 (SDA) was significantly higher (> 100%) in fish fed CO and C+A compared with fish fed FO and C+F (< 51%), indicating greater bioconversion of ALA to SDA in the CO and C+A fed fish than in FO and

C+F fed fish. The retention of EPA in the CO and C+A fed fish was over 100%, suggesting a net synthesis of EPA in these treatment groups. In contrast, the EPA retention in the FO and C+F fed fish was 55 and 21%, respectively, which showed a tendency to be significantly lower than that in the other two groups ($P = 0.09$). The CO fed fish had significantly higher DHA retention than fish fed the other 3 diets. The DHA retention in the FO fed fish (112%) was numerically but not significantly higher than in the C+F (66%) and C+A fed fish (73%). Thus, feeding the C+A to rainbow trout resulted in DHA retention equal to feeding the C+F.

Keywords: Whole-cell DHA-rich algae (A-DHA), Fish oil replacement, Digestibility, Growth performance, Fatty acid retention, Rainbow trout, Atlantic salmon

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LIST OF ABBREVIATIONS

ADC = Apparent Digestibility Coefficient

A-DHA = Whole-Cell DHA-Rich Algal Product

ALA = Alpha-Linolenic Acid, 18:3n-3

ARA = Arachidonic Acid, 20: 4n-6

C+A = Canola oil and A-DHA

C+F = Canola Oil and Fish Oil

CO = Canola Oil

CP = Crude Protein

CTH = Canola Oil and Thraustochytrids

DAG = Diacylglycerol

DE = Digestible Energy

DHA = Docosahexaenoic Acid, 22:6n-3

DM = Dry Matter

DP = Digestible Protein

DPA = Docosapentaenoic Acid, 22:5n-3

EFA = Essential Fatty Acid

EPA = Eicosapentaenoic Acid, 20:5n-3

FCR = Feed Conversion Ratio

FO = Fish oil

GE = Gross Energy

GLA = Gamma-Linolenic Acid, 18:3n-6

LA = Linoleic Acid, 18:2n-6

LC-PUFAs = Long-chain Polyunsaturated Fatty Acids

MAGs = Monoacylglycerols

OA = Oleic Acid, 18:1n-9

SDA = Stearidonic acid, 18:4n-3

SGR = Specific Growth Rate

GC = Gas Chromatography

GLA = Gamma- Linolenic Acid, 18:3n-6

PUFAs = Polyunsaturated Fatty Acids (Fatty acids have chain length ≥ 18C)

MUFAs = Monounsaturated Fatty Acids

OSI = Oxidative Stability Index

PLs = Phospholipids

SEM = Standard Error of the Mean

SFA = Saturated Fatty Acids

TAGs = Triacylglycerols

WSI = Water Stability Index

1. Introduction

Fish are a critical source of protein and essential fatty acids in human diets. In 2009, 16.6% of animal protein consumed by the world population was derived from fish (FAO, 2012a). In addition to being a good source of protein, many fish species, such as salmon, trout, sardines, mackerel and tuna, are rich sources of omega-3 fatty acids, particularly eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA). Consumption of n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) has been found to have beneficial effects on human health, including lowered risk of cardiovascular disease, stroke, autoimmune and inflammatory diseases. There is also evidence that they may reduce the risk of cancer, dementia and Alzheimer's disease (Kris-Etherton et al., 2003; Hooper et al., 2006; Singer et al., 2008). Increasing population and the desirability of high-quality protein, as well as health benefits from consumption of n-3 LC-PUFAs attributed to fish, has increased the demand for fish products. Global fish consumption was 17.1 kilos per person in 2008, and is expected to increase to approximately 20 kilos per person by 2030 (FAO, 2012b). However, fish production from capture fisheries has plateaued at approximately 90 million tonnes and most fisheries are fully exploited (FAO, 2012a). Increases in the supply of fish for human consumption will have to come primarily from aquaculture. According to FAO's annual report, world aquaculture yield (excluding aquatic plants and non-food products) has increased from 51.7 million tonnes in 2006 (FAO, 2009), to 60 million tonnes in 2010 (FAO, 2012a). Currently, aquaculture has expanded by almost 12 times with an average annual growth rate of 8.8% in the last three decades (FAO, 2012a). It is estimated that in order to meet the growing demand of fish products, aquaculture production will have to produce 80 million tonnes by the year 2050 (FAO, 2006).

The rapid expansion of aquaculture has led to a concomitant increase in aquafeed production (Lunger et al., 2007). Fish oil is the primary lipid source in aquafeed, which derives exclusively from wild small pelagic fish, such as anchovy, herring, sardine, capelin and others (Péron et al., 2010). Currently, the heavy dependence on fish oil is one of the major issues confronted by the aquaculture industry, especially salmonid production, which, uses almost 50% of the world fish oil production (FAO,

2009). Fish oil from capture fisheries has stagnated and the world's demand for fish oil has already exceeded supplies (Naylor et al., 2009). Consequently, it is necessary to find suitable and high quality sources of alternative oils for the aquaculture industry to maintain its high growth rate and economic viability.

Vegetable oils offer the potential to be fish oil substitutes as supplies of vegetables oils are about 100 times higher than fish oil, and their prices are less volatile than for fish oil (Diaz-Lopez et al., 2009). However, the fatty acid composition of aquaculture fish is highly dependent on the composition of the fatty acids they consume in their diets (Guillou et al., 1995; Bell et al., 2004; Torstensen et al., 2004). Fish oil-based diets have a unique fatty acid composition, which are rich in LC-PUFAs, such as EPA and DHA, whereas vegetable-based oils contain relatively high levels of linoleic acid (18:2n-6), oleic acid (18:1n-9) and alpha linolenic acid (ALA; 18:3n-3) and contain no EPA and DHA (Bharadwaj et al., 2010). Salmonids are able to convert ALA to EPA and DHA through a series of desaturation and elongation reactions (Bharadwaj et al., 2010). However, the conversion from ALA to EPA and DHA in fish is limited (Bell and Dick, 2004). Many previous studies have shown that fish oil replacement with vegetable oils, either partially or totally, in fish feeds results in significantly lower tissue concentrations of EPA and DHA compared to fish fed diets containing fish oil as the sole oil source (Bell et al., 2001b; Bell et al., 2002; Bell et al., 2004; Rinchard et al., 2006; Østbye et al., 2011). However, it is important to maintain high levels of EPA and DHA in fish products since health-conscious consumers desire these beneficial PUFAs in their diets (Schmidt et al., 2005). Therefore, replacing fish oil with vegetable oil while maintaining the composition of LC-PUFAs in fish products remains a significant challenge for the aquaculture industry.

Microalgae have been used in aquaculture since early times. They are primarily used as feed for zooplankton (rotifers and shrimps), which can then be fed to fish larvae and fry (Dallaire et al., 2007). Microalgae are also used as live feed for larvae of bivalves, crustaceans and marine fish (Patil et al., 2006). Dried microalgae biomasses can also be directly incorporated in fish feeds (Bennemann, 1992). The studies by Atalah et al. (2007) and Ganuza et al. (2008) revealed that using microalgae biomass (*Schizochytrium* and *Cypthecodinium cohnii*) to replace fish oil in gilthead sea bream had no deleterious

effects. Many species of microalgae have the ability to produce EPA and DHA and considerable evidence has revealed that n3-PUFAs in fish oils are derived via the marine food chain from zooplankton that feeds on algae (Yongmanichai and Ward, 1989; Miller et al., 2007). Moreover, the PUFAs in algae seem to have high stability, which is due to their natural antioxidant component: carotenoids and vitamins, and the lipids are protected by algal cell wall (Patil et al., 2006). Therefore, it might be feasible to use microalgae as potential sources of n-3 PUFAs to replace fish oil in aquaculture feeds.

The objectives of this thesis were to determine the digestibility of a whole-cell DHA-rich algal product (A-DHA) in rainbow trout and Atlantic salmon, and to evaluate the effects of dietary fish oil replacement with canola oil and A-DHA on the growth performance and fatty acid composition of rainbow trout.

2. Literature Review

2.1 Lipids in Fish

2.1.1 General

Lipids together with proteins and carbohydrates are the major macronutrient groups of aquafeeds supplying the energy required for physiological processes and physical activities (NRC, 1993; Wilson, 1994; Trushenski et al., 2006; Bell and Koppe, 2010; Gatlin, 2010). The ability of fish species to metabolize these nutrients for energy production is variable. Cold-water and marine fish have limited ability to use carbohydrates for energy, therefore, have higher dietary requirement of lipid and protein compared to warm-water fish (NRC, 1993; Wilson 1994; Trushenski et al., 2006). Lipids in feeds are the densest source of metabolic energy as they supply about 2.25 times the digestible energy in an equal weight of protein or carbohydrates (Lofgreen, 1951; NRC, 2001; Bauer et al., 2005). Aquafeeds typically contain 15% lipids (NRC, 1993). Nowadays, higher levels of lipids tend to be included in the diets to partially spare the use of protein for energy in aquafeeds, which reduces the high costs of diets and also minimizes pollution from nitrogen losses (Vergara et al., 1996; Van der Meer et al., 1997; McGoogan and Gatlin III, 2000). However, the increased dietary lipid content should be carefully evaluated and determined since too much dietary lipid may lead to excessive fat deposition in fish, adversely affecting health, yield and the market quality of fish (Peres and Oliva-Teles, 1999). Besides being an important energy source, the lipid content of feed is also required for absorption of fat-soluble vitamins A, D, E and K (NRC, 1993). Additionally, dietary lipids are also fundamental for the supply of essential fatty acids (EFAs; Sargent et al., 2002).

2.1.2 Essential Fatty Acids

Linoleic (LA, 18:2n-6) and α-linolenic (ALA, 18:3n-3) are considered the two basic EFAs since fish (as well as all other vertebrates) cannot synthesize these two polyunsaturated fatty acids (PUFAs) *de novo*. Therefore LA and ALA must be provided in the diet (Trushenski et al., 2006; Rinchard et al., 2007). Almost all fish have the

potential to bioconvert LA and ALA to the primary longer chain PUFAs (LC-PUFAs) such as ARA (20:4n-6), EPA (20:5n-3) and DHA (22:6n-3) (Nakamura and Nara, 2004; Trushenski et al., 2006). However, EFA requirements vary between fish species (NRC, 1993; Tocher, 2003; Turchini et al., 2009). Fish with a 'freshwater' pattern are able to endogenously synthesize LC-PUFAs, thus have a requirement of dietary LA and ALA. By contrast, fish with a 'marine' pattern either are not capable or have very poor ability to bioconvert LA and ALA, which is possibly due to the adaption of fish to the LC-PUFA-rich environment as well as the lack of efficiency in Δ5 fatty acid desaturase and/or the C18-20 elongase (Steffens, 1997; Sargent et al., 1993; Mourente et al., 2005; Turchini et al., 2009). Therefore, ARA, EPA and DHA are considered as EFA for marine fish (Sargent et al., 1999; Sargent et al., 2002; Mourente et al., 2005; Tocher et al., 2006b). Interestingly, even in species that can synthesize LC-PUFA, C20 and C22 are all termed EFAs, due to their higher nutritional values than C18 counterparts (Tocher, 2010).

EFAs are component of phospholipids that play an important role in maintaining the fluidity of biomembranes (Steffen, 1997; Strijbosch et al., 2008; Ratnayake and Galli, 2009). Moreover, EFAs are precursors of eicosanoids and docosanoids, which are hormone-like signaling molecules with a host of bioactivities, including regulation of inflammation and immune responses (Ratnayake and Galli, 2009; Gogus and Smith, 2010).

Eicosanoids are derived from the oxidation of the twenty-carbon fatty acids ARA and EPA (Figure 2.1). Eicosanoids formed from ARA are generally more inflammatory than those derived from EPA (Tocher, 2003; Ratnayake and Galli, 2009; Gogus and Smith, 2010). Furthermore, eicosanoids formed from EPA are less biologically active than those formed from ARA and competitively inhibit the actions of ARA-derived eicosanoids (Bell et al., 1997; Tocher, 2003; Hamre et al., 2005; Ratnayake and Galli, 2009). Docosanoids are produced from twenty-two carbon EFAs, especially DHA. Docosanoids are known to possess potent anti-inflammatory and neuroprotective bioactivity (Hong et al., 2003; Serhan, 2006; Zhao et al., 2011). In addition to the functional role in inflammatory and immunoregulatory process, eicosanoids and docosanoids are also involved in reproductive function, hormone release, stress coping,

etc. (Stanley-Samuelson, 1994; Trushenski et al., 2006). Therefore, the ratio of n-3 to n-6 fatty acids is as important as the concentration of individual fatty acid in the diet.

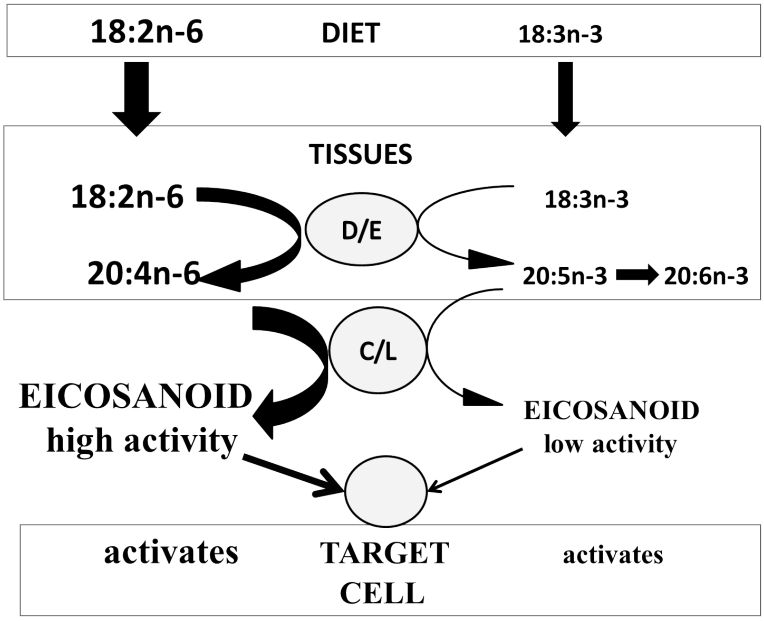

Figure 2.1 The link between dietary PUFAs, tissue PUFAs and eicosanoid production (Adapted from Tocher, 2003). D/E: desaturation and elongation; C/L: cyclo-oxygenase and lipoxygenase enzymes.

2.1.3 Lipid Metabolism in Fish

Lipids, fatty acids and their metabolic derivatives play important roles in growth, reproduction, health, etc. Different species tend to have different lipid storing and utilizing mechanisms due to the variety of environmental conditions such as different levels of temperature and salinity. Salmonid lipid metabolism will be extensively described in the following sections since rainbow trout and Atlantic salmon are used as experimental fish in this study.

2.1.3.1 Synthesis of LC-PUFAs

All fish species can synthesize 16:0 and 18:0 *de novo* (Sargent et al., 2002) by FA synthase in the cytoplasm, which can then be converted to 16:1n-9 and 18:1n-9 (OA) via the action of microsomal Δ9 desaturase (Δ9; Figure 2.2). However, it is not well

known to what extent fish can further elongate these two n-9 monounsaturated fatty acids (MUFAs) to longer chain MUFA (Sargent et al., 2002). A lack of the capability to convert 18:1n-9 to 18:2n-6 (LA) and 18:2n-6 to 18:3n-3(ALA) is due to the absence of Δ12 desaturase and Δ15 desaturase in fish (Wallis et al., 2002; Tocher et al., 2006b). Therefore, LA and ALA are defined as EFAs. However, their biological value as precursors to LC-PUFA is less than their derivative products (ARA, EPA and DHA; Tocher, 2010).

Plant oils are a rich source of C18 PUFAs as plants possess the enzymes needed to convert OA to LA and ALA. Plant oils can therefore act as substrates for fish and other vertebrates to produce LC-PUFAs (Wallis et al., 2002). Biosynthesis of LC-PUFAs from plant oils requires desaturation and elongation enzymes (Cook, 1996; Tocher, 2003) and freshwater fish have all these enzymes (Henderson, 1987; Tocher, 2003). In freshwater fish, synthesis of ARA requires Δ6 desaturation of 18:2n-6 to produce 18:3n-6 which, is elongated to 20:3n-6 followed by Δ5 desaturation to ARA. In freshwater fish, synthesis of EPA requires the same enzymes and pathway as for ARA (Figure 2.2). DHA synthesis requires two further elongation steps and a second Δ6 desaturation followed by a chain-shortening step (β-oxidation in the peroxisome) (Wallis, 2002; Sprecher, 2000). In humans (De Antueno et al., 2001) and mice (D'Andrea et al., 2002), it has been reported that the same Δ6 desaturase enzyme is involved in both reactions in the pathways, however, it is still unknown if it is the same enzyme or not in fish (Sargent et al., 2002). It should be noted that most fish do not have Δ4 desaturase that directly converts 22:5n-3 to 22:6n-3 or 22:4n-6 to 22:5n-6 (Wallis et al., 2002), with the exception of *Siganus canaliculatus* (while-spotted spinefoot or rabbitfish; Li et al., 2010) and *Solea senegalensis* (flatfish; Morais et al., 2012).

2.1.3.2 Factors Affect LC-PUFAs Metabolism

2.1.3.2.1 Species

Different fish species have considerable differences in their abilities to convert LA and ALA to LC-PUFAs. These differences are due to species, nutritional and environmental factors (Zheng et al., 2005a), among which, species differences are a

major determinant of the ability to synthesize LC-PUFAs in fish. Freshwater fish require only dietary 18:2n-6 and 18:3n-3 as precursors for synthesizing ARA, EPA and DHA, while carnivores and most marine fish require dietary EPA and DHA because of low or non-existent activities of one or more of the enzymes required for LC-PUFAs synthesis (Ghioni et al., 1999; Tocher and Ghioni, 1999, Hansen et al., 2008). This is probably due to the evolutionary adaptation to the different fatty acids sources (availabilities) in the environment (Tocher, 2010). EPA and DHA are abundant in the marine food chain, which markedly reduces the requirement for Δ5 desaturase activity in marine fish (Sargent et al., 1993). For the freshwater fish, although C18 PUFAs (LA and ALA) are rich in the food chain, the lack of EPA and DHA requires fish to convert C18 fatty acids to the physiologically more important C20 and C22 PUFAs (Bell et al., 1994).

Salmonids possess all of the enzymes required to accomplish these conversions and produce the primary LC-PUFAs, ARA (n-6) and EPA and DHA (n-3) (Ruyter et al., 1999; Ruyter et al., 2000; Tocher, 2003). Although an appreciable amount of EPA can be produced from ALA in freshwater fish, the final steps in the n-3 synthetic pathway to produce DHA are inefficient (Ruyter et. al., 1999, 2000; Sargent et al., 2001; Tocher, 2003). Interestingly, similar effects have been reported in humans, where only 2 to 5% of ALA can be converted to LC-PUFAs (Goyens et al., 2005; Hussein et al., 2005). According to Bell and Dick (2004), salmonids have limited ability to produce DHA, thus, dietary inclusion of EPA and DHA is essential to maintain normal growth and development.

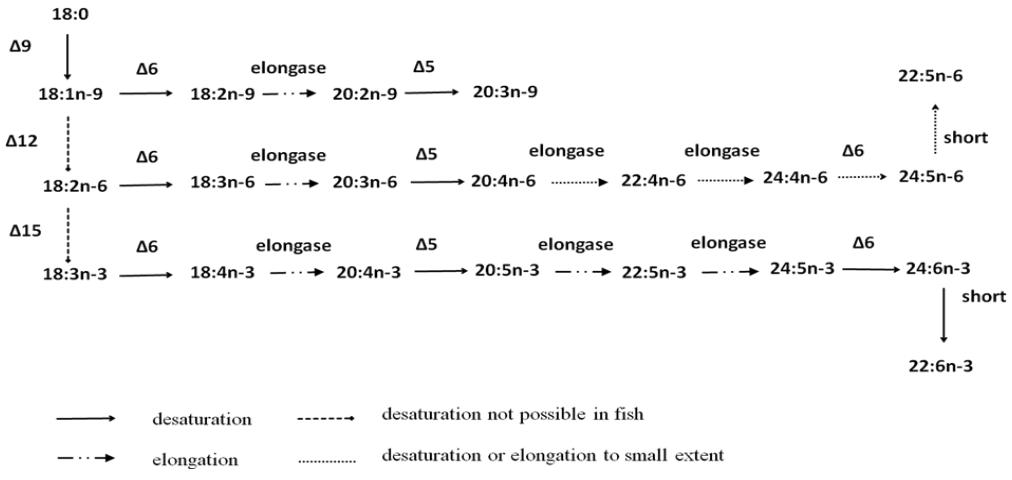

Figure 2.2 Biosynthesis of LC-PUFAs in fish from C18 fatty acids (After Tocher, 2003).

2.1.3.2.2 Environment (Salinity and Temperature)

The ability to convert C18 fatty acids to LC-PUFAs in salmonids is also influenced by water salinity (Bell et al., 1997; Tocher et al., 2000; Fonseca-Madrigal et al., 2006). Salmonids are anadromous fish, which, are hatched in freshwater and migrate to sea after parr-smolt transformation (also termed smoltification or smolting) to spend most of their adult life, returning to freshwater only to breed (Bell et al., 1997; Ojima and Iwata, 2007). During smoltification, the 'parr', a juvenile fish, undergoes a complex series of morphological, behavioral and physiological changes, developing into 'smolt', and then begin the trip to the ocean (Hoar, 1976; Hoar, 1988). Smoltification is an important process which helps fish to preadapt to marine life, allowing them to live and grow in seawater.

In Atlantic salmon, LC-PUFA biosynthesis activity increases during smoltification and is maximal around the transfer of fish to seawater (Zheng et al., 2005a). This is followed by a lowered enzyme activity during the seawater phase of production (Zheng et al., 2005a), which is likely not caused solely by the transfer to seawater but also higher levels of tissue DHA as the fish increase in size (Kjær et al., 2008). Bell et al. (1997) found that regardless of diets, ARA, EPA and DHA seemed to increase to a genetically predetermined level prior to seawater entry in salmonids. This preadaptive response, which happens in freshwater, allows fish to prepare themselves for life in a marine environment (Bell et al., 1997). In addition, fish undergo desmoltification (kept in freshwater while must be transferred to seawater) have low LC-PUFA content in the body, indicating that ambient salinity changes can lead to changes in lipid metabolism (Li and Yamada, 1992). Although fatty acid metabolism can be affected by smoltification, the mechanism is still unclear (Tocher et al., 2000).

The ambient temperature of water is inversely correlated with conversion efficiency of ALA to DHA (Tocher and Sargent, 1990). Many previous studies have shown that increased desaturase activity caused by reduced water temperature results in higher proportion of unsaturated fatty acids in phospholipids in fish, which aids in maintaining membrane fluidity (Farkas et al., 1994; Ruyter et al., 2003; Calabretti et al., 2003; Moya-Falcon et al., 2006). For example, increased Δ6 desaturase activity was found in rainbow trout reared at 5^{o}C or 7^{o}C compared with fish reared at 20^{o}C or 15^{o}C,

respectively (Hagar and Hazel, 1985; Tocher et al., 2004), while higher Δ9 desaturase activity was found in endoplasmic membranes of carp liver kept at lower temperature (Wodtke and Cossin, 1991). The phenomenon of higher level of LC-PUFAs in fish at lower temperatures is defined as homeoviscous regulation, which preserves the membrane functionality during temperature changes (Robertson and Hazel, 1999). However, Sargent et al. (2002) suggested that the abundance of DHA in fish phospholipid, is independent to environmental variables, becasue the intrinsic structure of DHA is resistant to the change of temperature.

2.1.3.2.3 Dietary Fatty Acid Compositions

The fatty acid content of the diet is a major determinant in LC-PUFA synthesis in rainbow trout and Atlantic salmon (Bell et al., 2001b; Bell et al., 2002, Kjær et al., 2008). Both Δ6 desaturase and Δ5 desaturase gene expressions are generally higher in fish fed diets containing high levels of LA and ALA (vegetable oil-based diets) compared with fish fed diets containing high levels of EPA, DHA and ARA (fish oil-based diets). The replacement of 75% fish oil by equal amount of blend vegetable oils (rapeseed, palm and linseed oils in a ratio of 3.7: 2: 1) in diets for Atlantic salmon induced higher Δ6 desaturase mRNA levels in both liver and red muscle compared with fish fed fish oil control diet (Zheng at al., 2005a,b). Similar results were obtained in rainbow trout by replacing fish oil with linseed oil (Seiliez et al., 2001). Increased gene expression of fatty acid desaturase and elongase involved in the LC-PUFAs biosynthetic pathway was found in livers of Atlantic salmon fed diets when fish oil was replaced by linseed oil (Zheng et al., 2005b). Moreover, Bell and Dick (2004) reported that the rate of synthesis of DHA from ALA was 5 to10 fold higher in rainbow trout fed vegetable oil diets compared to fish fed fish oil diets, and Ruyter et al. (2000) demonstrated that the desaturation and elongation products formed from 18:2n-6 were twice as high in hepatocytes from salmon fed a fish oil diet as a salmon fed a linseed oil diet. Similarly, many studies on salmonids kept in freshwater have shown Δ6 desaturase activities were about 2.5 times (Bell et al., 1997), 2.8 times (Tocher et al., 2001) and 4 times (Tocher et al., 2003) higher in fish fed vegetable oil diets than fish fed only fish oil diet. The lower desaturase gene expression or enzyme activities in fish fed fish oil diet have been interpreted as a feedback inhibition

process via increased n-3 LC-PUFAs and reduced C18 PUFA substrates (Tocher et al., 2003).

2.1.4 Lipid Digestion and Absorption

Triacylglycerols (TAGs) are a primary lipid class in both marine and freshwater fish diets (Tocher, 2003). In mammals, both the pancreatic lipase-colipase system and bile salt-activated lipases (less specific) are involved in the TAG digestive process (Tocher, 2003). However, digestion of TAGs in fish is mainly activated by bile salt and the role of pancreatic lipase is still uncertain since most fish species lack a discrete pancreas (Tocher, 2003). The digestive mechanisms of phospholipids (PL) are relatively unstudied in fish and are assumed to be similar to that in mammals, which are activated by pancreatic or intestinal phospholipases A_2 (Tocher, 2003; Tocher et al., 2008).

Since lipids are hydrophobic, they must be emulsified with bile salts, which, makes digestion of lipids easier (Glencross, 2009). This emulsification step is then followed by lipolytic hydrolysis (Smith et al., 1983). Different species of fish have various digestive processes due to the complexity of the intestinal tract and anatomical differences, such as different tract length and numbers of pyloric caeca (Tocher, 2003). In general, the digestion of lipids in fish is via extracellular hydrolysis by bile salt-activated lipases in the stomach, pyloric caecum and intestines (Glencross, 2009). In salmonids, pyloric caecum and anterior intestine seem to be the major sites of lipid hydrolysis (Denstadli et al., 2004). Once cleaved by lipases, TAG mainly forms sn-2 monoacylglycerol and two free fatty acids, while PL produces 1-acyl lyso-phospholipids and free fatty acids. Free short-chain and possibly some medium-chain fatty acids with chain length of less than 12C as well as glycerol are directly absorbed by enterocytes. In contrast, long-chain fatty acids, monoacylglycerols (MAGs), diacylglycerols (DAGs) and 1-acyl lyso-phospholipids are emulsified by bile salts to form micelles (Figure 2.3). These micelles diffuse into the intestinal mucosa and then are absorbed into enterocytes (Tocher, 2003; Tocher et al., 2008), where MAGs, DAGs and free fatty acids are re-esterified to TAGs while 1-aycl-lyso-phspholiopids and free fatty acids are incorporated in resynthesized PLs. sn-2 MAGs are the preferred substrates for resynthesizing TAGs and phosphatidylcholines in salmonids (Oxley et al., 2007). The reformed TAGs and PLs

along with cholesterols are packaged together with a protein-rich "shell" to form chylomicrons, which are then transported via lymphatic system to the circulatory system and/or delivered directly to the liver via the hepatic portal system as lipoproteins (Babin and Vernier, 1989; Tocher, 2003). TAGs are primarily stored in adipose tissue and secondary processing of TAGs and PLs (such as modulation of chain length and degree of unsaturation of fatty acids) occurs in liver (Sargent et al., 1993). Salmonids tend to accumulate most of TAGs in the flesh whereas marine fish store the lipids in the liver or viscera, such as Atlantic cod and sharks (Glencross, 2009).

The ability to digest lipids differs not only between fish species, but also can be influenced by fish age and temperature (Morais et al., 2005; Morais et al., 2007; Bogevik et al., 2008). Younger fish, especially larval fish, appear to have a poorer capability to digest higher levels of dietary lipids (Morais et al., 2005; Morais et al., 2007) and better lipid digestibility in higher water temperature (Hepher, 1988). Notably, carnivorous fish generally can digest higher amount of dietary lipids (Peres and Oliva-Teles, 1999; Williams et al., 2003; Denstadli et al., 2004). For example, salmonids have the ability to digest dietary lipids at the level of greater than 300 g/kg (Hevroy et al., 2004). Lipid digestion in fish is also highly related to the dietary fatty acid composition.

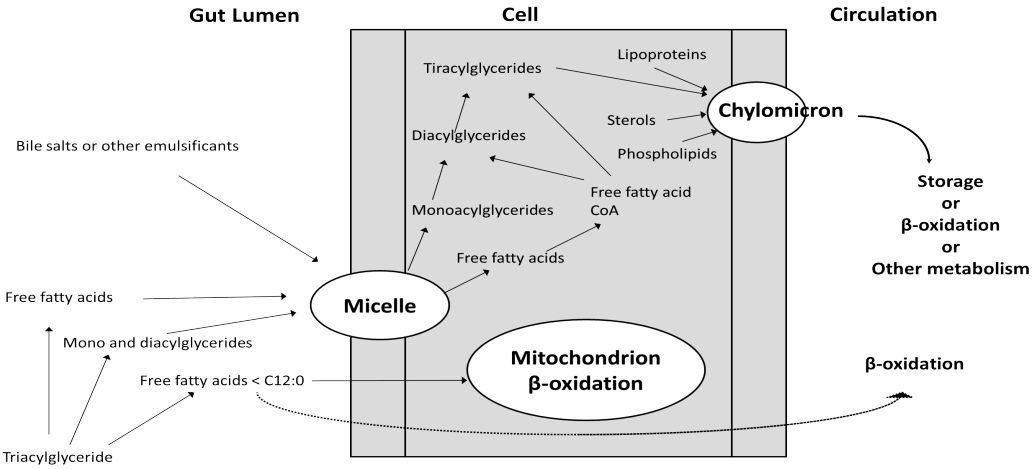

Figure 2.3 Triacylglycerides (TAGs) absorption and metabolism (Adpated from Glencross, 2009).

2.1.5 β-oxidation of Fatty Acids

As mentioned previously, one of the primarily functions of lipids, in particular their constituent fatty acids is to provide energy. Briefly, the process of energy production is accomplished by the catabolism of ingested or stored fatty acids by β-oxidation, which sequentially removes two carbons by oxidation at the β-carbon position of the fatty acyl-CoA molecule. This reaction mainly takes place in the inner space of mitochondria (matrix) and/or in the peroxisomes (Karalazos, 2007). The products of β-oxidation are one mole of NADH, one mole of $FADH_2$ and one mole of acetyl-CoA. Acetyl-CoA is further oxidized to CO_2 in the tricarboxylic acid cycle, generating three moles of NADH, one mole of $FADH_2$ and one mole of ATP. The highest β-oxidation activities are generally known to occur in the red muscle, liver and heart (Henderson and Tocher, 1987). However, white muscle, although least dense in mitochondria, is also considered an important tissue in energy production as fish have high amount of while muscle (Froyland et al., 2000).

It is well documented that fish have special preference to utilize certain fatty acids for β-oxidation. Studies have shown that short- and medium-chain SFAs and MUFAs are preferentially oxidized over LC-PUFAs, with the former mainly oxidized in mitochondrial and the latter generally catabolized in peroxisomes (Wander et al., 2001). In addition, studies have reported that 16:0, 18:1n-9, 20:1n-9 and 22:1n-11 are readily metabolized for energy production in salmonids. In contrast, EPA is generally oxidized to a lesser extent and DHA is usually retained in tissues instead of being catabolized (Kiessling and Kiessling, 1993; Sargent et al., 2002; Tocher, 2003). However, when EPA and DHA present in excess in the diet, such as fish oil diets, they can also be significant substrates for β-oxidation (Stubhaug et al., 2005a; 2005b). Therefore, replacing fish oil with vegetable oils in diets for fish may alter their β-oxidation activity, while other factors, such as fish size, maturation and water temperature are also of importance (Torstensen and Stubhaug, 2004; Stubhaug et al., 2005a; Stubhaug et al., 2005b).

2.1.6 Fish Oil Replacement in Salmonids

2.1.6.1 Effect on Digestion of Lipid and Fatty Acids

In generally, over 90% of lipids are digested by fish regardless of the oil sources; however, the absorption rates of various fatty acids are different (Turchini et al., 2009). Fish appear to absorb n-3 fatty acids at a higher rate than n-6 and n-9 fatty acids (Francis et al., 2007b). Also, digestion of fatty acids improves as degree of unsaturation increases and chain length of fatty acids decreases (Hepher, 1988; Olsen et al., 1998; Morais et al., 2005; Francis et al., 2007b). As such, the lipid melting point of a given oil is considered a good indicator of potential lipid digestibility in fish (Morais et al, 2005). Since higher digestibility is associated with lower melting point of the fatty acids, vegetable oils and other oils and fats containing greater amount of SFAs with relatively higher melting point compared to fish oil can lead to a reduced lipid digestibility, which, could be a concern when fish oil is replaced with more saturated oil sources, particularly for salmonids during winter period (Torstensen et al., 2000; Ng et al., 2007).

2.1.6.2 Effect on Fatty Acid Composition of Fillets

Vegetable oils are the most viable alternative sources of lipids for fish oil in aquafeeds due to their large supplies and relatively stable prices (Diaz-Lopez et al., 2009). However, most vegetable oils are relatively poor sources of n-3 fatty acids and devoid of LC-PUFAs such as EPA and DHA. Replacing fish oil with vegetable oils results in markedly different fatty acid composition of fish tissues and lowered nutritional quality of fish products for human consumption (Caballero et al., 2002; Bell et al., 2004; Grisdale-Helland et al., 2002; Rørå et al., 2005; Rinchard et al., 2007; Østbye et al., 2011). The feeding of diets containing vegetable oils to Atlantic salmon or rainbow trout significantly reduces the levels of EPA and DHA in all the diets in comparison with the levels found in fish fed fish oil (Table 2.1).

2.1.6.3 Strategies for Improving the Fatty Acid Composition

Since replacement of fish oil with vegetable oil in diets for salmonids lead to lowered EPA and DHA in fish fillets, efforts have been focused on finding new ways to

improve fatty acid composition of salmonids fed diets with reduced concentrations of fish oil.

One strategy is to switch the fish to fish oil-based diets during the finishing phase (washout). Several studies have shown that the decreased levels of EPA and DHA in fish fed vegetable oil-based diets could be restored by feeding fish oil-based diet for 16-24 weeks prior to slaughter (Bell et al., 2003b; Bell et al., 2004; Torstensen et al., 2005). Although, this strategy improves the fatty acid composition of vegetable oil fed fish, it does not completely eliminate reliance on fish oil and the resultant levels of EPA and DHA may still be lower than fish fed fish oil diet throughout the production cycle.

Since Δ6 desaturase is the rate limiting step in the production of LC-PUFAs, plant oils that contain fatty acids following Δ6 Desaturase, might be a possible replacement for fish oil in diets for salmonids. For example, oil from *Echium plantagineum* contains approximately 14% stearidonic acid (18:4n-3; SDA), which is presumed to be a superior fatty acid substrate for *de novo* biosynthesis of LC-PUFAs as it bypasses Δ6 desaturase. In support of this notion, human (Harris et al., 2008) and dog (Harris et al., 2007) studies have reported that the efficiency of conversion of SDA to EPA ranged from 17-30% of the efficiency of feeding EPA directly compared to less than 10% for ALA. This suggests that bypassing Δ6 desaturase by feeding SDA is a feasible strategy for increasing the production of EPA and DHA in salmonids. However, studies in Arctic charr (Tocher et al., 2006a), Atlantic cod (Bell et al., 2006) and Atlantic salmon smolts have reported that substitution of fish oil with echium oil results in reduced flesh EPA and DHA compared to fish fed fish oil (Miller et al., 2008). Although the inclusion of echium oil (SDA) did not seem to improve EPA and DHA in fish flesh, increased levels of SDA itself is believed to have potential benefits in human nutrition (Whelan, 2009).

Other than fish oil, there are oil sources that contain n-3 LC-PUFAs, such as oils derived from microalgae. Thus, microalgae are a potential replacement for fish oil in salmonid diets.

Table 2.1 Effect of replacing fish oil with vegetable oils on the DHA and EPA contents of rainbow trout and Atlantic salmon. Values are % of total lipid.

Study	DHA+ EPA	Fish oil replacement used in test group	DHA+EPA test group
Rainbow trout			
Caballero et al., 2002	6.9	20% FO + 80% Canola/palm oil	3.1
Bell and Dick, 2004	5.3	Linseed oil/Soybean	2.4
Rinchard et al., 2006	9.2	Linseed oil	1.9
Drew et al., 2007	21.2	Linseed/Canola	4.4
Nilson, 2008	14.5	Linseed oil	8.9
Ng et al., 2010	19.9	25% FO + 75% palm oil	5.1
Atlantic salmon-fresh			
WingKeong et al., 2007	22.0	Palm oil	8.3
Bell et al., 2001b	20.6	Canola oil	8.3
Tocher et al., 2002	28.1	Linseed oil	6.4
Brandsen et al., 2003	23.8	Sunflower oil	10.8
Grisdale-Helland et al.,	15.0	Soybean oil	9.2
Østbye et al., 2011	48.7	Rapeseed oil	43.2
Atlantic salmon- seawater			
Bell et al., 2003a	21.7	Canola oil	10.4
Bell et al., 2003b	18.4	Linseed oil	5.8
Bell et al., 2004	12.4	Linseed oil	4.4
Bell and Dick, 2004	12.4	Canola oil	6.6
Berge et al., 2009	14.4	Soybean oil	5.7
Rørå et al., 2005	21.0	Soybean oil	4.5

2.2 Microalgae

2.2.1 General

Microalgae are single-celled organisms with size ranging from a few micrometers (μm) to a few hundreds of micrometres. They exist as discrete individuals alone, as well as in chains or groups (Ryckebosch et al., 2012). The majority of

microalgae are photoautotrophic and only a few species are heterotrophic in nature (Hemaiswarya et al., 2011). In addition, there are some microalgal species (known as mixtrophic) which are capable to utilize both inorganic and organic carbon concurrently, while some species can metabolically switch between phototropic and heterotrophic metabolism under different cultivation conditions (Mata, 2010). Furthermore, microalgae can live in sea, brackish or fresh water (Ryckebosch et al., 2012), thus, utilizing environments not presently used for food production.

It is estimated that there are approximately 30,000 species of microalgae. However, only a few hundred have been investigated for their chemical composition and just a few are currently of commercial significance (Olaizola, 2003; Gouveia et al., 2008). Commercial large-scale microalgal production started in the early 1960s in Japan and then spread to other countries (Spolaore et al., 2006). According to recent data, the annual production of microalgae is about 10,000 tons (Becker, 2007).

Many microalgae species, such as *Chlorella vulgaris*, *Haematococcus pluvialis*, *Dunaliella salina* and the Cyanobacteria *Spirulina maxima*, are already commercially available, and are used primarily as additives in animal feed and as nutritional supplements for humans (Gouveia et al., 2008). Microalgae are also commercially used in cosmetic industries and are believed to have the potential to be used to produce biofuels (Mata et al., 2010) and hydrogen (Dutta et al., 2005). More recently, the n-3 LC-PUFA content of microalgae has been of particular interest as a potential replacement for fish oil.

2.2.2 Production

Microalgae can be cultured photoautotrophically or heterotrophically depend on the species. Photoautotrophic production of microalgae refers to the photosynthetic ability, which converts inorganic matter (CO_2) into organic compounds in the presence of light (Perez-Garcia et al., 2011; Ryckebosch et al., 2012). Today, photoautotrophic production is the most common method of microalgal cultivation. Cultivation is performed in two ways: open-culture systems, such as lake and raceway ponds or close-culture system, such as photobioreactors (Mata et al., 2010; Perez-Garcia et al., 2011). Open-culture systems are cheaper as natural light is used and construction costs are

relatively less. However, this method of cultivation is more susceptible to contamination and the growth parameters which, rely on weather conditions are hard to control (Perez-Garcia et al., 2011). In contrast, closed-culture system are more resistant to contamination and have higher yields, however, the requirement of artificial light markedly increases the cost for large-scale production (Perez-Garcia et al., 2011). Moreover, poor efficiency of light diffusion is a problem in both open and close-culture systems (Perez-Garcia et al., 2011). Heterotrophic microalgae can grow in the absence of light; however, have a requirement for organic carbon (such as acetate and glucose; Day et al., 1991; Ryckebosch et al., 2012). Heterotrophic production (usually performed in closed fermenters) is limited to a few species (Perez-Garcia et al., 2011). This method has many advantages over photoautotrophic culture, such as the capability to produce higher cell densities. Furthermore, there is considerably more knowledge for the large-scale cultivation of single cells such as yeast and bacteria in fermenters and most importantly, a significantly lowered cost by elimination of artificial light (Brown, 2002; Ryckebosch et al., 2012). Therefore, heterotrophic cultivation of microalgae seems to be the most economically viable way for large-scale production. Methods for large-scale heterotrophic cultivation of microalgae have been developed in the last two decades (Khozin-Goldberg et al., 2011). Microalgal-DHA produced from *Schizochytrium* species by heterotrophic cultivation is already commercially available as dietary supplement and has also been used in health foods, infant food, animal feed and aquaculture (Ryckebosch et al., 2012). It is estimated that the value of microalgal LC-PUFAs produced by heterotrophic cultivation was about $195 million in 2004 (Khozin-Goldberg et al., 2011).

According to Ryckebosch et al. (2012), microalgae are harvested from their suspension (liquid concentrate), followed by centrifugation. The microalgal paste is then freeze or spray dried in order to protect the valuable nutrition compounds. The algal powder then can either be used to produce algal oil by solvent extraction or be consumed as the whole microalgal biomass. The later method omits the expensive extraction process, but the bioaccessibility of the nutrients (such as LC-PUFAs) is unclear because of the complexity of the algal cell wall (Ryckebosch et al., 2012).

2.2.3 DHA Synthesis in Microalgae

Unlike vertebrates, microalgae contain Δ12 desaturase and Δ15 desaturase and hence, they are capable to bio-convert OA to LA via Δ12 desaturation and LA can be further desaturated to ALA by Δ15 desaturase (Figure 2.4). LA and ALA are further converted to longer-chain and more unsaturated fatty acids via the same n-6 and n-3 biosynthetic pathways as found in vertebrates. The pathway for DHA biosynthesis from EPA in microalgae, however, is different from that in vertebrates. In vertebrate, the bioconversion of EPA to DHA involves two steps of elongation and a retro-conversion process (chain-shortening) in peroxisomes. However, in DHA-producing microalgae, EPA is converted to DPA (22:5 n-3) by one step of elongation, which is further desaturated to DHA by Δ4 desaturase (Figure 2.4). This pathway has been identified in many microalgae species (Qiu et al., 2001; Meyer et al., 2003; Pereira et al., 2004; Zhou et al., 2007). DHA synthesis via this elongation and desaturation pathway is known to have a requirement of molecular oxygen as a cofactor and thus it is also named the aerobic DHA biosynthetic pathway (Qiu, 2003).

There is another alternative DHA synthesis pathway called the polyketide synthase (PKS) pathway, which is an anaerobic pathway discovered in some heterotrophic marine microalgae (Metz et al., 2001). The PKS pathway does not require aerobic desaturation while introducing the double bonds to the acyl chain during the process of fatty acid synthesis (Qiu, 2003; Khozin-Goldberg et al., 2011). The full cycle of fatty acid synthesis involves four steps: 1) condensation of an acyl-ACP and a malonyl-ACP, producing a ketoacyl-ACP, 2) ketoreduction of ketoacyl-ACP to produce hydroxyacyl-ACP, 3) dehydration to produce unsaturated enoyl-ACP by removing a water molecule from hydroxyacyl-ACP, and 4) reduction to convert enoyl-ACP to a saturated acyl-chain (Figure 2.5). However, the PKS pathway omits the steps of dehydration and reduction, resulting in the products with various structures, which generally contain keto and hydroxyl groups with double bonds (Qiu, 2003). In most microalgae, LC-PUFAs are generally incorporated in polar lipids, while very few species store LC-PUFAs in TAGs (Cohen and Khozin-Goldberg, 2005). Interestingly, some heterotrophic species can store LC-PUFAs in both PLs and TAGs, such as *Pythium*, *Crypthecodinium* and *Nitzschia* (Ward and Singh, 2005).

2.2.4 Microalgae in Aquaculture

Algae are the base of aquatic food web and have been used diversely in aquaculture since very early times. Currently, the primary application of microalgae is in aquaculture, as a source of n-3 LC-PUFAs in larval or pigments for salmoinds and shrimps ((Muller-Feuga, 2000; Gouveia et al., 2008). Microalgae are primarily used for raising mollusks (62%), shrimps (21%) and fish (16%). The usage form is predominantly as total biomass (either dried or in slurry form) and live algae (Muller-Feuga, 2000). The following are some examples of the application of microalgae in aquaculture.

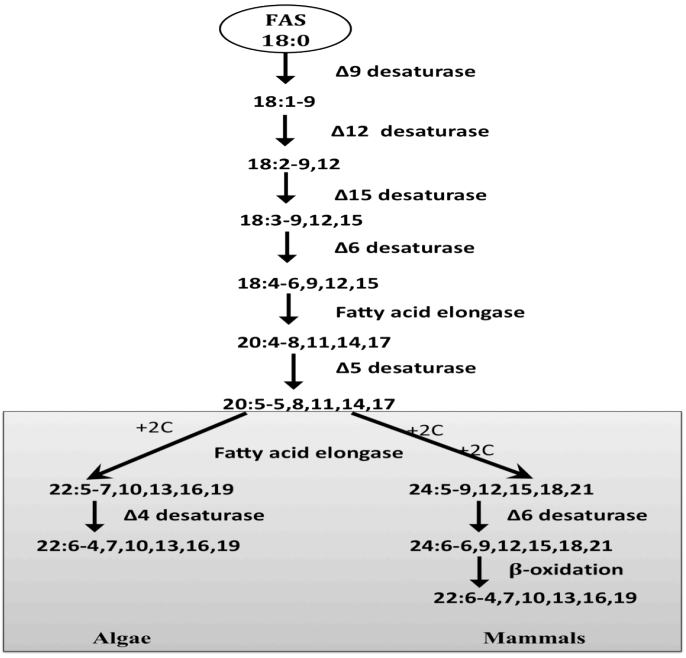

Figure 2.4 The aerobic pathway of DHA biosynthesis in microalgae and mammals (After Qiu, 2003).

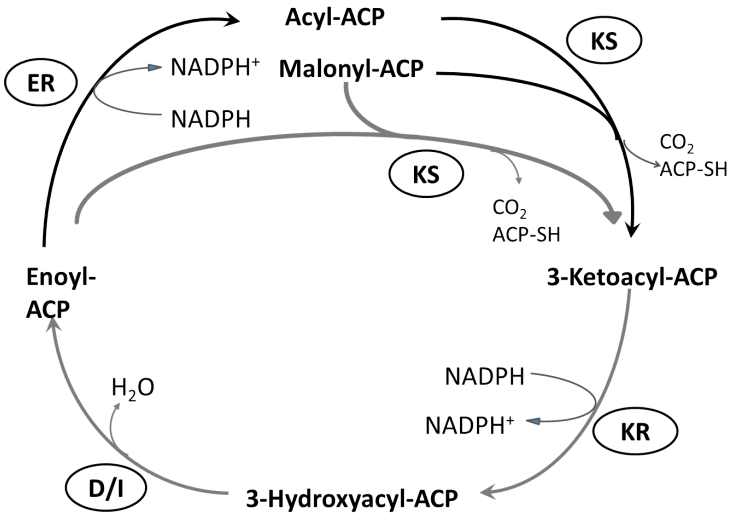

Figure 2.5 The anaerobic polyketide synthase (PKS) pathway of DHA biosynthesis in microalgae (Adapted from Qiu, 2003). ACP, acyl carrier protein; KS, 3-ketoacyl-ACP synthase; KR, 3-hydroxyacyl-ACP reductase; D/I, bifunctional dehydrase/isomerase; ER, enoyl-ACP reductase.

2.2.4.1 Microalgae as a Source of PUFAs

Isochrysis galbana and *Diacronema vlkianum* (Haptophyceae) are known marine microalgae species with high content of EPA and DHA, which have been used as a food source for larval and juvenile molluscs, crustacean and fish species (Fidalgo et al., 1998). AlgaMac2000 and Docosa God (products derived from microalgae) containing 5-15% DHA of the dry weight can be used to enrich zooplankton with DHA, which are then fed to fish larval (Hemaiswarya et al., 2011). Thraustochytrids (heterotrophic microalgae) can produce oils with several n-3 LC-PUFAs, particularly DHA (up to 60% DHA) and are low in n-6 fatty acids (Nichols et al., 2004). Because of this, thraustochytrids were used to produce DHA GOLD (Martek Biosciences Corporation, Columbia, Maryland, United States), a whole-celled alga containing 42% DHA of the total lipids, which is commercially available and has been widely applied in animal nutrition, including aquaculture (Miller et al., 2010). For example, dried algae *Schizochytrium sp.* (a Thraustochytrid) has been used for the enrichment of DHA in

Artemia *nauplii* and rotifers as well as in brookstock diets of marine fish (Barclay and Zeller, 1996; Harel et al., 2002; Yamasaki et al., 2007).

Recently, thraustochytrids have been investigated as a replacement for fish oil in salmonids. In the study by Carter et al. (2003), three experimental diets including canola oil (CO), canola oil and fish oil (CFO), or canola oil and the thraustochytrids (a dried whole-celled product; CTH) were fed to Atlantic salmon (37g). No significant differences were observed within different dietary treatments and fish fed CTH showed the highest DHA in PL fraction in liver, white muscle and eye. However, it should be noted that dietary DHA in CTH was much higher than that in CFO (9.92 vs. 4.90 % of total lipids). Miller et al. (2007) fed Atlantic salmon diets containing 100% thraustochytrid oil, 100% palm oil or a 4:1 palm:thraustochytrid mix. Fish fed the 100% thraustochytrid oil had significantly higher EPA + DHA levels than fish fed fish oil diets (25.6 vs. 16.1% of total fatty acids). The fish fed the palm:thraustochytrid diets had 13.2% EPA + DHA, which was significantly lower than fish fed fish oil but since palm oil is a poor source. Further, the ARA levels of fish fed the palm:thraustochytrid or thraustochytrid diets were equal and significantly lower than fish fed fish oil. It should be noted that palm oil is almost devoid of ALA and this would significantly reduce the synthesis of HUFAs.

2.2.4.2 Microalgae as Sources of other Nutrients

Most microalgae contain essential amino acids such as lysine, methionine, threonine and valine (Becker, 2007) and several feeding trials have been carried out to evaluate the potential of using algal meal as a partial or complete replacement for fish meal in aquafeeds. Nile tilapia (*Oreochromis niloticus)* and Redbelly tilapia (*Tilapia zillii)* fed diet containing 19.2% algal meal (*Hydrodictyon reticulatum*) had specific growth rates (SGR) similar to those fed fishmeal control diet, however, SGR markedly decreased when inclusion levels were increased above this level (Appler, 1985). Similarly, the growth and feeding performance of tilapia fed diets containing 20% and 40% algal meal (*Spirulina*) was not significantly different from fish fed the control diet. Further increases in the inclusion of algal meal resulted in adverse growth performance (Olvera-Novoa et al., 1998). A recent study by Kiron et al. (2012) reported that the replacement of fish meal with algal meal at 5 or 10% levels did not significantly affect

the growth and feed performance as well as whole body proximate compositions of Atlantic salmon.

Microalgae have also been used as a source of pigments in salmonid diets. Many microalgae species contain astaxanthin; a pigment which, is currently used as an addictive in commercial aquafeeds for salmon, trout and shrimp in order to enhance the colour of the muscles (Lorenz and Cysewski, 2000).

2.2.5 Microalgae as a Source of PUFAs in other Animals

2.2.5.1 Ruminants

In recent years, PUFA-rich microalgae have been used in ruminant diets for increasing the levels of PUFAs in meat and dairy products. Milk fat with higher amount of DHA and lower level of SFAs were found in dairy cows fed diets containing n-3 fatty acid-rich microalgae (*Schizochytrium sp.*) (Franklin et al., 1999). In another study, increased levels of milk fat and protein as well as higher amount of EPA and DHA in milk fat were observed in ewes fed a diet supplemented with algae (*Schizochytrium sp.)* containing high amount of PUFAs (Papadopoulos et al., 2002). This study also reported an increase in PUFA concentration in goat dairy products (such as yogurt and feta cheese) in goats fed microalgae compared with controls. In addition, the fatty acid composition was not affected after a five-month storage period (Papadopoulos et al., 2002). Moreover, lambs fed diets containing DHA-enriched microalgae resulted in higher level of DHA in the phospholipid fraction in the lamb meat (Cooper et al., 2004).

2.2.5.2 Monogastric Animals

Supplementation of DHA-rich algae into feed of pigs resulted in an increase of DHA in pork meat and subcutaneous fat, while no significant effects were observed in body weight and carcass yield (Marriot et al., 2002; Sardi et al., 2006). Furthermore, laying hens fed diets containing rapeseed oil with 20% supplementation of *Nannochloropsis oculata* (microalgae rich in ALA and EPA, but containing no DHA) led to increased level of DHA in both PL and TAG of egg yolk, compared with hens fed diets based on maize oil with or without the addition of *N. oculata* (Fredriksson et al., 2006).

Cheng et al. (2006) reported that egg-yolk is enhanced in DHA by the addition of algal DHA oil to the diet for laying Tsaiya duck, while the increased DHA was also found in plasma, liver and skeletal muscle of ducks fed the diet with addition of algal DHA oil. Thus microalgae have the potential to increase the LC-PUFA concentrations present in animal products in a large number of species.

2.3 Hypothesis

Overall, the replacement of fish oil with vegetable oil results in reduced tissue levels of EPA and DHA in salmonids. Of the possible methods of improving the fatty acid composition of salmoninds fed vegetable oil-based diets, the use of LC-PUFA-rich single cell oil appears promising. Based on these observations, it was hypothesized that feeding a DHA-rich algal product (provided by Evonik Industries) combined with vegetable oil to rainbow trout or Atlantic salmon will result in tissue levels of HUFAs equal to those found in fish fed fish oil-based diets.

3. Determination of the Chemical Composition and Digestibility of a Whole-Cell DHA-Rich Algal Product in Rainbow Trout in Freshwater and in Atlantic Salmon in Saltwater

3.1 Introduction

Rainbow trout and Atlantic salmon are the dominant salmonid species of major economic interest in aquaculture industry worldwide (Azevedo et al., 2004). Salmonids are a rich source of n-3 PUFAs, especially EPA and DHA, and the nutritional and health benefits from the consumption of these fatty acids have been well established (Simopoulos, 1999; Connor, 2000). Increasing human populations and the desirability n-3 LC-PUFA-rich products have increased the demand for fish and led to a rapid expansion of aquaculture production (Bell et al., 2004; FAO, 2006). With the rapid increase in aquaculture industry, there has been a parallel increase in aquafeed production. This has resulted in hugely increased demands on the primary raw ingredients for salmonid diets-fish oil and fish meal. However, these two ingredients have limited supplies. The global demand for fish oil for aquafeeds already exceeded total available supplies (Shepherd et al., 2005) and by the year 2020, fish meal supplies may also run out (New and Wijkstroem, 2002). The current scarcity of fish oil highlights the necessity of finding a suitable replacement for the aquaculture industry to maintain its high growth rate and economic viability.

The fatty acid composition of farmed fish is a reflection of dietary fatty acid composition (Robin et al., 2003, Berge et al., 2009, Østbye et al., 2011) and presently, LC-PUFAs present in farmed salmonids are derived from dietary fish oil, which is an excellent source of n-3 LC-PUFAs (Bell et al., 2004; Torstensen et al., 2004). Vegetable oils have been intensively investigated as the potential alternatives for fish oil in salmonid production. However, the replacement of fish oil is problematic as markedly lowered tissue concentration of EPA+DHA was observed in fish fed diets with vegetable oil replacement, due to the absence of EPA and DHA in vegetable oils (Caballero et al., 2002; Bell et al., 2004; Rørå et al., 2005; Rinchard et al., 2007; Østbye et al., 2011). Some vegetable oils, including canola and flax oils, are rich in ALA (18:3n-3) and

salmonids are able to convert ALA to EPA and DHA through a series of desaturation and elongation reactions. However, the conversion is inefficient (Zheng et al., 2009). Therefore, in order to produce farmed fish with high content of n-3 LC-PUFAs, a direct supply of dietary n-3 LC-PUFA is required (Turchini and Francis, 2009).

Many species of microalgae, diatoms and thraustochytrids have the ability to produce EPA and DHA and considerable evidence has indicated that microalgae are the base of the marine food chain, which provide the n-3 LC-PUFAs for fish (Yongmanichai and Ward, 1989). Moreover, the PUFAs in algae have high stability owning to their natural antioxidant component-carotenoids and the lipids are protected by algal cell wall (Patil et al., 2006). Therefore, n-3 LC-PUFA-rich microalgae seem to have potential as fish oil replacements in aquafeeds (Lewis et al., 1999; 2001).

Determination of the nutrient digestibility of microalgae is the first step to evaluate the feasibility of the utilization of the algal product in aquafeeds (Allan et al., 2000; Tibbetts et al., 2006; Guedes and Malcata, 2012). Therefore, these studies aimed at investigating the effect of feeding a whole-cell DHA-rich algal product (A-DHA) at different inclusion levels on the nutrient digestibility in rainbow trout and Atlantic salmon.

3.2 Materials and Methods

3.2.1 Diets

3.2.1.1 Diets for Rainbow Trout

Diet formulations for rainbow trout studies are shown in Table 3.1. The reference diet was formulated according to Bureau and Cho (1994). Celite was added to the reference diet at 10g/kg as a nonabsorbable inert marker for determination of digestibility. The tested diets were formulated by replacing the reference diet with 6.77, 13.33 and 20% of A-DHA. The experimental diets were processed at the University of Saskatchewan, Saskatoon, Saskatchewan. All dry ingredients were weighed and well mixed for 15 mins in a Hobart legacy floor mixer (Hobart Corporation, Troy, OH). Canola oil and cold water were added to the dry mix to achieve a stiff dough and mixed for a further 15 mins. The doughs were then cold extruded using a 4822 Hobart Food

Grinder (Hobar Corporation, Troy, OH) with a 3mm die. Lastly, the diets were then dried in a forced air oven (55°C, 12 h), chopped and screened to obtain the appropriate pellet size. Samples of pelleted feed were taken for chemical analysis.

Table 3.1 Composition of the diets used in digestibility trial (experiment 1) for rainbow trout (g/kg).

	A-DHA inclusion (%)			
Ingredient	0	6.67	13.33	20.00
Fishmeal[1]	300.00	279.99	260.01	240.00
Soybean protein concentrate	170.00	158.66	147.34	136.00
Corn gluten meal	130.00	121.33	112.67	104.00
Wheat flour	280.00	261.39	242.65	224.00
A-DHA	0.00	66.67	133.33	200.00
Vitamin mineral premix[2]	10.00	9.33	8.67	8.00
Celite[3]	10.00	9.33	8.67	8.00
Canola oil	100.00	93.30	86.67	80.00

[1]South American Aquagrade; EWOS Canada Ltd.

[2]The vitamin/mineral premix was a commercial premix (EWOS; closed formulation) formulated to meet the requirements of juvenile rainbow trout.

[3]Celite 545, <125μm; Celite Corporation, World Minerals Co., Lompoc, CA, USA.

3.2.1.2 Diets for Atlantic Salmon

Diet formulations are shown in Table 3.2. The reference diet was formulated to contain similar amount of digestible crude protein (45.5% vs. 45.8%) and crude fat (15.2% vs. 15.7%) as that used in the experiment with rainbow trout. The experimental diets were processed at the Norwegian University of Life Sciences, Ås, Norway. All dry ingredients were mixed in a Kenwood mixer (MX270, Hampshire, UK). Propyl gallate (0.15g/kg oil, Paradigmox Blue Dry, Kemim, Eupropa N.V.) was added as an antioxidant to the mixed ingredients to protect oils from oxidation. Warm water (60°C) was added to the dry mix to achieve a firm dough, which was cold-pelleted through, an Italgi pasta extruder (P35A) equipped with a 2 mm die. The pellet was dried at 45°C for 12 hours and frozen-stored at -20°C prior to feeding. Yttrium oxide (Y_2O_3) was used as an inert marker

for determination of digestibility (Austreng et al., 2000). Samples of pelleted feed were taken for chemical analysis.

Table 3.2 Composition of experimental diets (g/kg) used in the digestibility trial (experiment 2) for Atlantic salmon.

	A-DHA inclusion (%)			
Ingredient	0	6.67	13.33	20.00
Fishmeal[1]	320.00	298.67	277.34	256.00
Soybean protein concentrate	150.00	140.00	130.01	120.00
Corn gluten meal	130.00	121.33	112.67	104.00
Wheat flour	279.00	260.39	241.81	223.20
A-DHA	0.00	66.67	133.33	200.00
Vitamin mineral premix[2]	10.00	9.33	8.67	8.00
Yttrium oxide[3]	1.00	0.93	0.87	0.80
Rapeseed oil	110.00	102.66	95.34	88.00

[1] Norse LT-94, low temperature dried fish meal, Norsildmel, Bergen, Norway;

[2] Vitamin Mineral premix (mg/kg dry diet unless otherwise stated), vitamin A (as acetate), 7500 IU kg^{-1} dry diet; vitamin D_3 (as cholecalcipherol), 6000 IU kg^{-1} dry diet; vitamin E (as dl-a-tocopheryl-acetate), 150 IU kg^{-1} dry diet; vitamin K (as menadione Na-bisulfate) 3; vitamin B_{12} (as cyanocobalamin), 0.06; Ascorbic acid (as ascorbyl polyphosphate), 150; d-biotin, 42; choline (as chloride), 3000; folic acid, 3; niacin (as nicotinic acid), 30; pantothenic acid, 60; pyridoxine, 15; riboflavin, 18; thiamin, 3; NaCl, 6.12; ferrous sulfate, 0.13; copper sulfate, 0.06; manganese sulfate, 0.18; potassium iodide, 0.02; zinc sulfate, 0.3; carrier (wheat middling or starch).

[3] Yttrium oxide was used as an inert marker.

3.2.2 Water Stability

Diets for Atlantic salmon were tested for water stability according to Baeverfjord et al. (2006). Three parallel samples of each diet with 10 g per sample were weighed into netting baskets with 2 mm mesh size and a diameter of 8 cm. The baskets with feed samples were placed in 600 ml beakers containing 300 ml dd H_2O, and then the beakers were incubated in a water bath (SBK 25 D, Salvis AG, Reussbühl, Switzerland) at 25^{o}C

and 110 shakings per min for 120 mins. The baskets were weighed again after drying at 105^{o}C for 18h. Water stability index (WSI) was calculated using the following equation: **WSI** = (Net sample mass after oven drying (g) / Net sample mass placed on baskets (g)) / % dry matter of feed * 100

3.2.3 Fish Management

In experiment 1, rainbow trout (initial weight ~300g/fish) were housed at the Prairie Aquaculture Research Centre (PARC) in 120 L tanks filtered via a biological filtration system. Photoperiod for these fish was a 14 h light/10 h dark cycle. Water temperature was maintained at 15 ± 1° C and dissolved oxygen, pH and temperature were measured daily. Chlorine, nitrate, nitrite and ammonia were monitored on a weekly basis or more often if deemed necessary. A total of twelve experimental units (10 fish per tank) with three replicates per treatment were utilized. Fish were fed twice daily to visual satiation over a 5-week period. The fish were maintained in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993; CCAC, 2005).

In experiment 2, Atlantic salmon *(Salmo salar)* with approximately 170 g initial weights were used in the digestibility experiment. The experiment was carried out at Nofima, Sunndalsøra, Norway. Fish were randomly allocated to 12 tanks. The fish were kept in fiberglass tanks (600L) with 106 fish per tank. The fish were kept in full salinity saltwater (32-33ppm) maintained at 14±1° C under artificial illumination 24 h^{-1} day. The four experimental diets were fed to triplicate groups of fish over a period of 12 days. The fish were fed with electrically driven disc feeders, for 10 s, every 10 min 24 h d^{-1}. The feeding was 20% in excess, based on 2.5% of biomass in each tank. Fish were weighed initially and at the end of the experiment. Oxygen and temperature was measured every three days. Mortalities and unusual incidents were reported daily according to standard routines.

3.2.4 Sample Collection

Rainbow trout were adapted to the experimental diets for 7 days and fecal material was collected over a 5-week period using a settling column, which separated the feces from the effluent water (Randall and Drew, 2010). The collected feces were

centrifuged at 3000 rpm for 10 minutes (Beckman Coulter J6-MC Centrifuge, Mississauga, ON) frozen and freeze dried prior to analysis.

For the Atlantic salmon study, approximately 20 kg biomass per tank was used. At the termination of the feeding period fish were stripped for feces (applying gentle hand pressure to the abdomen, moving from ventral fins to the anus) as described by Austreng (1978). Feces was immediately frozen at -80ºC in liquid nitrogen after the stripping and freeze-dried and ground to a powdery consistency before analyses.

3.2.5 Analytical Methods

3.2.5.1 Chemical Analyses for Samples from Rainbow Trout Study

The A-DHA, experimental diets, and feces were ground using a ZM 100 Retsch Mill (Retsch GmbH, Haan, Germany) with 1 and 0.5 mm screens. Analysis of all samples was conducted in duplicate using the following methods, moisture by oven at 135ºC for 2 hours (AOAC, 1990; method, 934.01), crude protein as 6.25 times total nitrogen determined using a Leco analyzer (model FP-528, Leco Corporation, St. Joseph, MI), lipid (acid ether extract) (AOAC, 1995; method, 954.02). Determination of ash and acid insoluble ash was performed according to the following procedure. Samples (quadruple replicates) were charred (250°C, 18 h), followed by a gradual increase in temperature to 500°C (3 h), and then ashed (48 hours). Four mL of 4N HCl was added and the samples were heated at 120°C for a minimum of one hour. Samples were then centrifuged (3000 x g, 10 min) followed by aspiration of the supernatant. Five mL of water was added, vortexed, centrifuged and aspirated two times. The samples were then dried overnight (80°C) followed by ashing (500°C, 24 h). Gross energy was determined using a 1281 bomb calorimeter (Parr Adiabatic Calorimeter, Model 1200, Moline, Illinois). The Oxidative Stability Index (OSI) assay (AOCS, 1998; method cd 12b-92) was used to measure the oxidative stability of the A-DHA prior to incorporation into feed (Sun West Laboratories, Saskatoon, Saskatchewan). Amino acids were determined using chromatographic analysis at Evonik Industries, Essen, Germany.

The fatty acids analyzed in this study were: 18:2n-6, 18:1n-9, 18:3n-3, 20:4n-6, 20:5n-3 and 22:6n-3. These fatty acids were selected as they are the six major fatty acids

found in plant and fish oils. Direct fatty acid methylation was then performed according to the procedure described by O'Fallon et al. (2007), with a few minor differences. The samples were vortex-mixed using a single tube vortex instead of a multi-tube vortex, and samples were centrifuged (Beckman Coulter J6-MC Centrifuge, Mississauga, ON) for 5 minutes at 1500 rpm. Non-methylated C13:0 (Sigma-Aldrich, Inc., St. Louis, MO) was used as the internal standard, and all other chemicals used were of GC grade, and obtained from Sigma-Aldrich, Inc. (St. Louis, MO). Pressurized helium, air and hydrogen were purchased from Praxair Canada Inc. (Mississauga, ON). Analysis of fatty acid composition was performed on a GC Agilent 6890 system (Hewlett Packard 6890 GC, Agilent Technologies, Inc. Santa Clara, CA, USA) with a flame ionization detector and a fused-silica capillary column (SP-2560; Supelco Park, Bellefonte, PA, USA). Individual FA methyl esters were identified by comparing retention times to a Supelco 37 FAME mix (Cat# 47885) and expressed as mg/g sample on dry matter basis.

3.2.5.2 Chemical Analyses for Samples from Atlantic Salmon Study

Diets were ground through 1.0 and 0.5 mm screens and feces were crushed in a porcelain mortar prior to analyses for crude protein (Kjeldahl-N x 6.25; EU Dir 93/28), crude fat (pre-extraction with diethylether and hydrolysis with 4M HCl hydrolysis prior to petroleum ether extraction; method B in EU Dir 98764/EC), DM (EU Dir 71/393), and ash (EU Dir 71/250). Total lipids were extracted using the Folch method (Folch et al., 1957). The chloroform phase was dried under N_2 and the residual lipid extract was redissolved in benzene, and then transmethylated overnight with 2,2-dimethoxypropane and methanolic HCl at room temperature, as described by Mason and Waller (1964) and Hoshi et al. (1973). The fatty acid methyl esters thus formed were separated in a gas chromatograph (Hewlett Packard 6890) with a split injector, SGE BPX70 capillary column (length 60 m, internal diameter 0.25 mm and thickness of the film 0.25 mm) flame ionisation detector and the results analyzed using HP Chem Station software. The carrier gas was helium. The injector and detector temperatures were 280°C. The oven temperature was raised from 50°C to 180°C at the rate of 10°C/min, and then raised to 240°C at the rate of 0.7°C/min. The relative quantity of each FA present was determined by measuring the area under the peak corresponding to that FA. Yttrium concentration in

diets and feces was determined by inductively coupled plasma mass spectroscopy (ICP-MS) after complete digestion of the homogenized and dried samples in HNO_3 after cooking in a microwave oven for 1 h.

3.2.6 Digestibility Determination

Apparent digestibility coefficient (ADC, %) of diets was measured using an indirect method with diets containing Celite or Yttrium oxide as a nonabsorbable indicator. The ADC for the individual diets was calculated using the following equation (Bureau and Cho 1994):

ADC = 1 – (F/D x Di/Fi)

Where: D = % nutrient in the diet (dry matter (DM) basis)

F = % nutrient in the feces (DM basis)

Di = % indicator in the diet (DM basis)

Fi = % indicator in the feces (DM basis)

The ADC of chemical components in the A-DHA was estimated by regression analysis based on the individual nutrient contribution from algae to total dietary nutrient intake.

3.2.7 Statistical Analysis

Apparent digestibility coefficients (ADC) were analysed as a completely randomized design (CRD) using the General Linear Model procedure of SPSS (Version 18, SPSS Inc., Chicago, IL, USA). Mean values were separated using the Tukey's test with the accepted level of significancee at $P \leq 0.05$. Linear and quadratic regression models were calculated for the ADC of chemical component in the algal product to determine a best-fit equation using the Regression procedure of SPSS. Regressions were considered significant when $P \leq 0.05$.

3.3 Results

3.3.1 Digestibility in Rainbow Trout

The proximate, amino acid and fatty acid composition of A-DHA and experimental diets are reported in Table 3.3. The algae product used in the present study contained 14.6% CP and 62.1% lipid. The concentration of ALA in the A-DHA was very low with only 0.3 mg/g DM, whereas the DHA concentration was 125.6 mg/g. The A-DHA had low amino acid content, ranging from 2.3 to 8.0 g/kg DM. As the inclusion of A-DHA increased from 0 to 20%, dietary lipids increased from 16% to 24% and DHA concentrations increased from 1.9 to 25.3 mg/g, while dietary protein decreased from 47% to 40%.

Table 3.4 shows that the substitution of canola oil with increasing levels of A-DHA significantly decreased the digestibilities of DM and GE but no significant effects on ash, CP or individual essential amino acids were observed ($P < 0.05$). Also, increasing inclusion rates of A-DHA significantly decreased lipid digestibility and digestibility of ARA (20:4n-6), EPA (20:5n-3) and DHA (22:6n-3) but no significant effect on OA (18:1n-9), LA (18:2n-6) and ALA (18:3n-3) ($P > 0.05$; Table 3.4).

Significantly negative linear and quadratic regressions were observed for digestibilities of DM, GE and lipids, but no significant regression was seen for ash (Table 3.5). There were significant negative linear relationships between the A-DHA inclusion and apparent digestibility of OA, LA and ALA. Quadratic regressions for the digestibility of LA and ALA were significant, but were not for OA. The A-DHA inclusion had both significantly negative linear and quadratic relationships with the digestibility of ARA, EPA and DHA (Table 3.5). The linear regression for CP was significantly negative and the quadratic effect was not significant. For the individual amino acids, the regressions were not significant (Table 3.5).

Table 3.3 Composition of proximate, fatty acid and amino acids (g/kg dry matter) of the algal product (A-DHA) and experiment diets for rainbow trout in experiment 1.

		A-DHA inclusion (%)			
Component	A-DHA	0	6.67	13.33	20.00
Proximate composition (g/kg dry matter)					
Dry matter	963.19	927.26	927.99	930.42	935.13
Crude protein	145.66	470.74	438.91	405.73	401.98
Gross energy (MJ/Kg)	30.15	21.77	22.21	22.56	22.74
Crude fat	620.59	159.05	186.01	216.56	240.15
Ash	81.87	94.47	94.10	98.37	93.69
Fatty acids (mg/g dry matter)					
18:1n-9	3.8	39.7	41.1	43.0	43.6
18:2n-6	0.8	30.1	32.7	34.4	35.7
18:3n-3	0.3	9.6	12.4	13.3	13.3
20:4n-6	3.6	0.9	1.0	1.2	1.1
20:5n-3	1.6	3.6	4.3	4.9	5.3
22:6n-3	125.6	1.9	8.5	17.1	25.3
Essential amino acids(mg/g dry matter)					
Lysine	5.7	20.8	21.3	20.1	18.7
Threonine	5.7	17.8	16.1	15.9	15.4
Methionine	2.3	10.8	9.6	9.5	8.9
Valine	5.9	21.8	20.4	19.8	19.1
Isoleucine	4.4	19.2	18.1	17.4	15.6
Leucine	7.5	48.4	40.0	38.9	39.4
Phenylalanine	4.5	23.6	21.0	20.1	19.9
Histidine	2.4	11.3	10.3	9.7	7.8
Arginine	8.0	25.2	25.0	23.8	22.2
Non-essential amino acids(mg/g dry matter)					
Alanine	7.5	31.0	26.6	25.7	25.9
Aspartic Acid + Asparagine	12.3	38.3	37.0	35.7	33.6
Cysteine + Cystine	2.1	6.5	5.8	5.7	5.6
Glutamic Acid + Glutamine	21.3	86.2	78.5	75.7	73.9
Glycine	5.7	26.4	25.5	23.9	22.6
Proline	4.9	34.0	29.3	28.4	26.2
Serine	5.5	22.3	19.8	18.9	19.2

Table 3.4 Apparent digestibility coefficients (ADC, %) of dry matter, crude protein (N × 6.25), lipid, six long chain fatty acids and essential amino acids in rainbow trout fed four inclusion levels of the algal product (A-DHA) in experiment 1.

	A-DHA inclusion (%)					
Component	0	6.67	13.33	20.00	SEM	*P*-Value
Proximate composition						
Dry matter	68.4[a]	60.8[ab]	58.3[b]	57.2[b]	1.52	<0.01
Crude protein	90.9	90.3	88.6	87.3	0.68	0.23
Gross energy	77.6[a]	68.5[ab]	64.2[b]	60.1[b]	2.15	<0.01
Lipid	88.0[a]	70.5[b]	62.0[bc]	53.0[c]	4.17	<0.01
Ash	40.7	38.5	40.9	41.1	0.90	0.79
Fatty acids						
18:1n-9	86.6	71.3	69.5	57.2	4.42	0.11
18:2n-6	87.2	78.5	75.6	66.8	2.95	0.08
18:3n-3	91.1	85.3	83.6	73.9	2.62	0.12
20:4n-6	82.5[a]	54.2[b]	53.8[b]	42.0[b]	5.20	<0.01
20:5n-3	87.6[a]	77.6[b]	72.7[c]	65.9[d]	2.58	<0.01
22:6n-3	83.0[a]	70.4[b]	50.2[c]	46.8[d]	4.48	<0.01
Essential amino acids						
Lysine	91.1	92.6	92.2	91.9	0.50	0.82
Threonine	89.3	88.3	88.0	87.0	0.62	0.68
Methionine	90.2	88.8	86.5	87.6	0.78	0.41
Valine	89.8	89.1	90.5	88.0	0.81	0.79
Isoleucine	90.0	89.6	85.8	87.8	0.93	0.40
Leucine	91.2	89.2	92.1	88.2	1.16	0.68
Phenylalanine	90.9	89.7	89.4	88.6	0.63	0.71
Histidine	91.3	91.6	91.0	89.5	0.60	0.69
Arginine	92.4	93.2	92.9	91.8	0.48	0.78

[ab] Means in the same row with different superscripts are significantly different ($P < 0.05$)

SEM=Standard error of the mean

Table 3.5 Linear and quadratic regression parameters for the relation between apparent digestibility of dry matter, crude protein, gross energy, lipid, ash, six long-chain fatty acids and essential amino acids in algae and % of nutrient contribution from algae to total diet (X) in rainbow trout in experiment 1.

		Unstandardized coefficients				
		Constant	X	X^2	r^2	*P*-Value
Proximate composition						
Dry matter	Linear	66.647	-0.530		0.651	<0.01
	Quadratic	68.255	-1.234	0.034	0.753	<0.01
Crude protein	Linear	91.143	-0.521		0.393	0.03
	Quadratic	90.993	-0.333	-0.026	0.397	0.10
Gross energy	Linear	76.168	-0.642		0.792	<0.01
	Quadratic	77.370	-1.051	0.015	0.820	<0.01
Lipid	Linear	87.088	-0.668		0.866	<0.01
	Quadratic	87.793	-0.803	0.003	0.869	<0.01
Ash	Linear	39.736	0.064		0.019	0.67
	Quadratic	40.304	-0.219	0.016	0.054	0.79
Fatty acids						
18:1n-9	Linear	84.930	-15.395		0.474	0.01
	Quadratic	85.553	-18.585	1.812	0.476	0.06
18:2n-6	Linear	86.885	-42.515		0.534	<0.01
	Quadratic	86.679	-38.316	-9.345	0.534	0.03
18:3n-3	Linear	91.594	-3.915		0.470	0.01
	Quadratic	90.561	-1.665	-0.549	0.484	0.05
20:4n-6	Linear	76.950	-0.573		0.648	<0.01
	Quadratic	81.287	-1.121	0.008	0.710	<0.01
20:5n-3	Linear	87.277	-3.606		0.845	<0.01
	Quadratic	87.431	-3.857	0.043	0.846	<0.01
22:6n-3	Linear	84.193	-0.573		0.709	<0.01
	Quadratic	82.998	3.654	-0.083	0.987	<0.01
Essential amino acids						
Lysine	Linear	91.675	0.094		0.016	0.69
	Quadratic	91.238	-0.102	0.724	0.081	0.68
Threonine	Linear	89.227	-0.298		0.158	0.20
	Quadratic	89.249	-0.324	0.003	0.158	0.46
Methionine	Linear	89.686	-0.568		0.178	0.17
	Quadratic	90.430	-1.821	0.241	0.257	0.26
Valine	Linear	89.921	-0.190		0.027	0.61
	Quadratic	89.452	0.483	-0.108	0.057	0.77
Isoleucine	Linear	89.686	0.528		0.128	0.25
	Quadratic	90.522	-1.805	0.223	0.194	0.38
Leucine	Linear	91.030	-0.450		0.028	0.60
	Quadratic	90.549	0.702	-0.303	0.044	0.82
Phenylalanine	Linear	90.700	-0.467		0.142	0.23
	Quadratic	90.812	-0.692	0.049	0.145	0.49
Histidine	Linear	91.693	-0.310		0.127	0.26
	Quadratic	91.325	0.196	-0.080	0.156	0.47
Arginine	Linear	92.886	-0.097		0.027	0.61
	Quadratic	92.391	0.505	-0.083	0.119	0.57

3.3.2 Digestibility in Atlantic Salmon

The water stability index (WSI) of experimental diets from 1-4 was 77.05%, 85.74%, 81.87% and 84.32% respectively. The proximate and fatty acid compositions of A-DHA and experimental diets are reported in Table 3.6. The A-DHA contains 15.8% CP and 59.5% lipid. The DHA concentration of A-DHA was 125.9 mg/g DM, while the concentrations of ALA and EPA were only 0.3 and 1.5 mg/g, respectively. When the inclusion rate of A-DHA increased from 0 to 20%, dietary lipids increased from 16.3 to 25.8% and DHA concentrations increased from 2.1 to 32.5 mg/g, while the CP levels decreased from 46.1 to 39.5%.

The apparent digestibility of DM, CP, lipid, and fatty acids for the four experimental diets are reported in Table 3.7. Increasing inclusion of A-DHA significantly lowered the apparent lipid digestibility of the diets even at the inclusion of 6.67% A-DHA, but a significant negative effect on the apparent DM and CP digestibility was only found at the highest inclusion with 20% A-DHA. The inclusion of A-DHA had significantly adverse effect on the digestibility of 16:0. No effects were found on apparent OA digestibility, but significantly lowered LA digestibility was found at 20% A-DHA. Interestingly, both OA and LA were well digested in all dietary treatments, with the digestibility greater than 92.7%. In contrast, the apparent digestibility of ARA was significantly decreased to 39.6% when the inclusion of A-DHA increased to 20%. ALA and EPA were all well digested (>90%) regardless of the A-DHA inclusion. However, a significant adverse effect was found on the apparent DHA digestibility, which decreased from 72% to 39%. It should be noticed that, even in the reference diet, the apparent DHA digestibility (72%) was much lower compare to other fatty acids that have been measured. This could possibly due to the low concentration of DHA in the reference diet.

There were significant negative linear and quadratic relationships between nutrient contribution from A-DHA to the diets and apparent digestibilities of DM, CP and lipid (Table 3.8.). In terms of individual fatty acids, significant quadratic effects of A-DHA inclusion were observed on the digestibility of OA, ALA and ARA and the linear regressions were not significant. By contrast, both significantly negative linear and quadratic regressions for 16:0, LA, EPA and DHA were found (Table 3.8).

Table 3.6 Analyzed proximate and lipid composition of the algal product (A-DHA) and experiment diets for Atlantic salmon in experiment 2.

		A-DHA inclusion (%)			
	A-DHA	0	6.67	13.33	20.00
Chemical composition (g/kg dry matter)					
Dry matter	963.8	907.6	939.7	923.9	929.9
Crude protein	158.2	460.7	444.9	428.9	395.2
Gross energy (MJ/Kg)	29.8	20.9	22.1	22.2	22.9
Crude fat	594.5	163.1	194.7	215.4	248.4
Ash	108.7	70.6	65.6	65.8	68.1
Yttrium oxide	0	0.8	0.8	0.7	0.7
Fatty acids *(mg/g dry matter)*					
16:0	370.9	12.1	34.2	53.1	67.4
18:1n-9	1.0	81.3	75.8	67.3	70.3
18:2n-6	0.2	30.4	27.3	24.5	11.8
18:3n-3	0.3	4.5	9.4	8.4	8.8
20:4n-6	2.6	0.1	0.4	0.7	1.0
20:5n-3	1.5	2.9	3.1	3.0	3.2
22:6n-3	125.9	2.1	15.3	25.2	32.5

Table 3.7 Apparent digestibility coefficients (ADC, %) of dry matter, crude protein (N × 6.25), lipid and different fatty acid fractions in Atlantic salmon fed four inclusion levels of the algal product (A-DHA) in experiment 2.

	Ingredient inclusion (%)					
Component	0	6.67	13.33	20.00	SEM	*P*-Value
Proximate composition						
Dry matter	53.3[a]	53.0[a]	51.3[a]	46.3[b]	0.90	<0.01
Crude protein	83.7[a]	83.1[a]	82.6[a]	79.6[b]	0.53	<0.01
Lipid	95.8[a]	85.3[b]	76.2[c]	63.4[d]	3.60	<0.01
Fatty acids						
16:0	87.7[a]	49.3[b]	40.3[c]	19.5[d]	7.48	<0.01
18:1n-9	98.1	98.8	98.7	98.1	0.13	0.09
18:2n-6	96.5[a]	97.4[a]	97.6[a]	92.7[b]	0.62	<0.01
18:3n-3	97.2[b]	99.4[a]	99.1[a]	98.1[ab]	0.28	<0.01
20:4n-6	83.5[a]	91.4[a]	62.9[b]	39.6[c]	6.20	<0.01
20:5n-3	94.9[a]	96.3[a]	94.9[a]	91.0[b]	0.65	<0.01
22:6n-3	72.2[a]	66.3[ab]	61.1[b]	39.0[c]	3.87	<0.01

[ab] Means in the same row with different superscripts are significantly different ($P < 0.05$)

SEM= Pooled standard error of the mean

Table 3.8 Linear and quadratic regression parameters for the relation between apparent digestibility of dry matter, crude protein, lipid and different fatty acid fractions in algae and % of nutrient contribution from algae to total diet (X) in experiment 2.

		Unstandardized coefficients				
		Constant	X	X^2	r^2	*P*-Value
Proximate composition						
Dry Matter	Linear	54.336	-0.322		0.727	<0.01
	Quadratic	53.190	0.159	-0.023	0.870	<0.01
Crude Protein	Linear	84.185	-0.495		0.689	<0.01
	Quadratic	83.628	0.110	-0.075	0.781	<0.01
Lipid	Linear	97.178	-0.648		0.959	<0.01
	Quadratic	95.447	-0.281	-0.008	0.986	<0.01
Fatty acids						
16:0	Linear	89.210	-0.582		0.964	<0.01
	Quadratic	87.490	-0.287	-0.003	0.980	<0.01
18:1n-9	Linear	98.469	-0.357		0.008	0.78
	Quadratic	98.152	9.855	-34.928	0.533	0.03
18:2n-6	Linear	97.663	-11.681		0.690	<0.01
	Quadratic	96.488	18.500	-74.415	0.907	<0.01
18:3n-3	Linear	98.086	0.106		0.086	0.36
	Quadratic	97.309	1.178	-0.157	0.771	<0.01
20:4n-6	Linear	86.651	-0.475		0.241	0.11
	Quadratic	83.415	3.799	-0.086	0.644	<0.01
20:5n-3	Linear	96.195	-0.411		0.443	0.02
	Quadratic	94.841	0.969	-0.150	0.853	<0.01
22:6n-3	Linear	75.575	-0.321		0.555	0.01
	Quadratic	72.032	0.777	-0.015	0.909	<0.01

3.4 Discussion

In the present studies, increasing inclusion of the A-DHA (0-20%) led to a significant decrease in lipid digestibility in both rainbow trout and Atlantic salmon, from 88 to 53% and 96 to 63%, respectively. A previous study also demonstrated that Atlantic salmon fed diets containing 1:1 canola oil and thraustochytrid mixture had significantly lower lipid digestibility compared to those fed 100% canola oil, however, the digestibility was still greater than 94% (Carter et al., 2003). Regarding fatty acids, the present Atlantic salmon study showed general high digestibility at over 91% regardless of dietary inclusion level of algae, except for 16:0, ARA and DHA. By contrast, the findings by Carter et al. (2003) showed good digestibility of all fatty acids at over 99%. The finding of protein digestibility in Atlantic salmon from the current studies is different from Carter et al. (2003), who illustrated no significantly adverse effect of algae inclusion on protein digestibility. Decreasing protein and lipid digestibility with increasing inclusion of algae was also found in mink, where it has been reported that the poor digestibility of lipids (as well as fatty acids) and poor amino acid availability might be due to the resistance of the complex cellulosic algal cell wall structure to the digestive enzymes, which may inhibit lipid and protein digestion (Skrede et al., 2011). In addition, the complex cell wall structure of algae may not only affect the digestibility of inherent algae lipids, but also other lipids in the diets (Skrede et al., 2011).

The present studies showed systematically higher CP and lower lipid digestibility in rainbow trout compared with that in Atlantic salmon. To our knowledge, comparative studies with these two species have not been investigated in terms of using algae as a source of fish oil replacement. A comparative study by Refstie et al. (2000) examined the effect of feeding soybean meal on nutritional responses in Atlantic salmon and rainbow trout and indicated that rainbow trout have higher digestibility of lipid and protein than Atlantic salmon when fed soybean meal. Krogdahl et al. (2004) reported the same effects of replacing fishmeal with two different levels of precooked maize starch in rainbow trout and Atlantic salmon. It should be noted that although experimental diets used in the present studies for Atlantic salmon and rainbow trout were formulated to contained equal amounts of digestible CP and lipid, the body size of fish and rearing environments were different (Talbot and Hole, 1994; Hossain, 2011). In addition,

variability in fish management practices, analytical methods, specific ingredient composition and fecal collection methods are also a problem for such inter-study comparisons (Vandenberg and de la Noue, 2001).

It has to be pointed out that, in the studies on rainbow trout, the feces were collected using a modified Guelph system (settling column), but in the Atlantic salmon study, feces were collected by stripping. Belal (2005) reported that using a settling column to collect samples results in overestimation of digestibility due to the leaching problem while the stripping method causes an underestimation of the digestibility because of contamination of striped fecal samples with body fluid and intestinal epithelium. This could be one of the reasons that the digestibility of DHA in diets for rainbow trout was higher than that for Atlantic salmon. In diets for Atlantic salmon, EPA digestibility was very high (> 91%), whereas EPA digestibility in rainbow trout ranged from 66-88%. The reason for the difference in EPA digestibility between rainbow trout and Atlantic salmon is unknown and needs further investigation. Marine algae have been reported to contain pancreatic lipase inhibitors (Bitou et al., 1999) and phospholipase A2 inhibitors (Mayer et al., 1993), which negatively affect the absorption of dietary triacylglycerol and phospholipids. In both experiments, the diets were prepared during low temperatures, thus the presence of lipase inhibitors and phospholipid inhibitors cannot be ruled out. In diets for Atlantic salmon, EPA digestibility was very high (> 91%). Conversely, DHA digestibility declined dramatically with the increasing inclusion of A-DHA. This might be partially explained by the different positions in algal phospholipids: DHA binds to position 1 and EPA binds to position 2 (the fatty acid attached to position 2 is always hydrolyzed before position 1). Furthermore, fatty acids, especially the DHA is prone to oxidation, and especially during the drying process. DHA is more unstable than the EPA, and might partially explain the differences in the digestibility between the two n-3 PUFAs. During oxidation there might be formation of polymers that are not digestible, reducing the digestibility of the fatty acids (Borsting et al, 1994) and further compromise growth (Zhong et al., 2007).

In conclusion, the inclusion of A-DHA had adverse effects on nutrient digestibility, hence it is essential to improve the digestibility before including the A-DHA in the compound feed. Suppressing the lipase inhibitors by thermal treatment during feed

processing might be useful for enhancing nutrient digestibility of the A-DHA in rainbow trout. Moreover, free the lipids from algae by rupturing the rigid algal cell wall might further improve the digestibility. Since DHA is very unstable (can be easily oxidized or degraded), an adequate processing technique is required not only to free the lipids, but also make sure DHA is well protected from oxidation.

4. Effects of Dietary Fish Oil Replacement with Canola Oil and DHA-Rich Algal Product on Growth Performance and Fatty Acid Composition of Rainbow Trout

4.1 Introduction

The fatty acid composition of farmed fish is a reflection of dietary fatty acid composition (Robin et al., 2003, Berge et al., 2009, Rørå et al., 2005, Østbye et al., 2011) and presently, n-3 LC-PUFAs present in farmed salmonids are derived from dietary fish oil. The rapid growth in aquaculture production has put pressure on the requirement of raw feed ingredients of marine origin, particularly fish oil. The total production of fish oil is static at 0.8-1.5 million tonnes per year (Tacon and Mettian, 2008) and the global demand for fish oil for aquafeeds already exceeded total available supplies (Shepherd et al., 2005). The stagnant supply of fish oil from wild capture and increasing demand of fish oil for aquaculture production has resulted in a 130% increase in fish oil prices from 2005-2008 (Naylor et al., 2009). Therefore, the replacement of fish oil in aquafeeds, in particular salmonid diets is of central importance to the sustainability of the aquaculture industry.

Vegetable oils appear to be a logical replacement for fish oil since supplies of vegetables oils are about 100 times higher than fish oil, and their prices remain constant (Diaz-Lopez et al., 2009). However, while fish oil is an excellent source of n-3 LC-PUFAs, most vegetable oils are relatively poor sources of n-3 fatty acids and are devoid of EPA and DHA. Although salmonids can bioconvert ALA (rich in some vegetable oils, such as canola and flax oils) to EPA and DHA *de novo* via series of desaturation and elongation reactions, the conversion is inefficient (Zheng et al., 2009). It has been reported that replacing fish oil with vegetable oils has the effect of significantly lowering the composition of EPA and DHA in fish tissues, which compromises nutritional and health benefits of fish products in the human diet (Rinchard et al., 2007, Berge et al., 2009, Østbye et al., 2011). It is important to maintain high levels of EPA and DHA in fish since health-conscious consumers have a dietary requirement of these PUFAs (Nislon, 2008). Therefore, the replacement of fish oil with vegetable oils while maintaining the levels of EPA and DHA in fish products remains a significant challenge for the industry.

Many species of microalgae, diatoms and thraustochytrids have the ability to produce EPA and DHA and are the primary sources of these lipids in the marine environment (Miller et al., 2008). Thraustochytrids have been investigated as a replacement for fish oil in salmonids. These organisms produce oil with up to 60% DHA and are low in n-6 fatty acids (Nichols et al., 2004). Miller et al. (2007) fed Atlantic salmon diets containing 100% thraustochytrid oil, 100% palm oil or a 4:1 palm:thraustochytrid mix. Fish fed the 100% thraustochytrid oil had significantly higher (25.6 vs. 16.1% of total fatty acids) EPA + DHA levels than fish fed fish oil diets. The fish fed the palm:thraustochytrid diets had 13.2% EPA + DHA, which, was significantly lower than fish fed fish oil but since palm oil is a poor source. Further, the ARA levels of fish fed the palm:thraustochytrid or thraustochytrid diets were equal and significantly lower than fish fed fish oil. It should be noted that palm oil is almost devoid of ALA and this would significantly reduce the synthesis of LC-PUFAs. Based on these observations we hypothesize that using an ALA-rich vegetable oil, such as canola oil, in combination with a DHA-rich algae oil will increase the concentration of LC-PUFA in the tissues of rainbow trout.

4.2 Materials and Methods

4.2.1 Fish Management

Rainbow trout (female triploid) were housed at the Prairie Aquaculture Research Centre at the University of Saskatchewan. The fish were housed in a recirculating aquaculture system, which was filtered biologically. Water temperature was maintained at 14 ± 1°C. Daily, dissolved oxygen, pH and temperature were monitored. Chlorine, nitrate, nitrite and ammonia were monitored on a weekly basis. Photoperiod was a 14 h light/10 h dark cycle. The fish were maintained in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993; CCAC, 2005).

Before the experiment, 6 fish were selected at random from the whole population to determine initial whole body fatty acids of the fish. Then the fish were randomly allocated to 12 x 360 L tanks with 17 fish per tank (~70g mean start weight). Treatments were randomly assigned to tanks with three replicates per treatment. During

the 12-week experiment, the fish were fed twice daily to apparent satiation and feed consumption was recorded weekly. The tanks of fish were weighed on d 0 and 84. After the last day of feeding, 3 fish from each tank were randomly collected, killed and stored at -80 °C before chemical analysis. The 3 fish from each tank were pooled and homogenized.

4.2.2 Experimental Design

The experiment used a completely randomized design with 4 treatments and 3 experimental units (tanks) per treatment. The diets were formulated to contain 386.2 g/kg digestible crude protein and 17.58 MJ/kg DE and met all other nutrient requirements of rainbow trout (Table 4.1). The control diet (CO) used canola oil as the source of dietary fatty acids. Diets containing 100% fish oil (FO) and a diet containing a blend of canola and fish oils (C+F) differed only in the oil source. A reference diet containing the algal product (C+A) was formulated by adding the product (A-DHA) to give the same DHA concentration as in the C+F diet. Canola oil was added to balance the diet for DE. All diets were cold extruded on a Hobart Food Grinder (Hobart Corporation, Troy, OH) with a 3mm die, dried in a forced air oven (55°C, 12 h), chopped and screened to obtain the appropriate pellet size (see 3.2.1.1).

4.2.3 Analytical Methods

Prior to analysis, samples from all four diets were ground in a Retsch mill (Brinkmann Corp) with 1.0 and 0.5 mm screens. Proximate analysis was conducted on all experimental diets, which was analyzed for dry matter content, energy, lipids, ash and protein as described in Chapter 3. Total lipids of diets and fish samples were extracted using the Folch method (Folch et al., 1957). Fatty acid profiles were analyzed by Sun West Lab (Sun West Laboratories, Saskatoon, Saskatchewan).

4.2.4 Growth Performance

Growth performance of the fish was assessed using the following measures:

Weight gain =final weight - initial weight

Specific growth rate = [ln final weight – ln initial weight] / time (days) x 100

Feed conversion ratio = feed intake (as fed) / wet weight gain

Total feed intake (as fed) =the amount of feed consumed throughout the feeding period

Table 4.1 Composition of the diets used in growth trial for rainbow trout (g/kg).

	Diets			
Ingredient	CO	FO	C+F	C+A
Blood meal	20.00	20.00	20.00	20.00
Poultry by-product meal	200.00	200.00	200.00	200.00
Soy protein concentrate	213.98	213.98	213.98	215.31
Corn gluten meal	200.00	200.00	200.00	200.00
Wheat flour	200.00	200.00	200.00	119.12
Dicalcium phosphate	18.46	18.46	18.46	20.13
DL-methionine	1.49	1.49	1.49	-
Vitamin mineral premix[1]	6.73	6.73	6.73	6.73
Vit C	0.05	0.05	0.05	0.05
Choline Cl	4.00	4.00	4.00	4.00
A-DHA	-	-	-	60.00
Canola oil	135.28	-	74.28	154.67
Fish oil	-	135.28	61	-
Digestible crude protein	386.20	386.20	386.20	386.20
Digestible energy (MJ/kg)	17.58	17.58	17.58	17.58

[1] The vitamin/mineral premix was a commercial premix (EWOS; closed formulation) formulated to meet the requirements of juvenile rainbow trout.

4.2.5 Apparent Retention of Fatty Acids

The apparent retention of fatty acids (FAs) for the individual diets was calculated using the following equation (Modified from Aas et al., 2006):

FA retention (% of digested FA intake) = 100 * (amount of FA deposited in fish (g) / amount of FA digested by the fish (g))

Where: FA deposited in fish = FA in fish at the end (g) - FA in fish at the start (g)

FA digested by fish = FA consumed (g) * FA digestibility

*FA digestibility data was obtained from previous digestibility studies in rainbow trout performed at the U of S.

* Calculations were based on total biomass increase and total feed consumption in each tank.

4.2.6 Statistical analysis

Analyses of the results used the General Linear Model procedure of SPSS (Version 19, SPSS Inc., Chicago, IL, USA). Differences between dietary treatments were separated using the Tukey's test and were considered significantly different when $P < 0.05$.

4.3 Results

4.3.1 Dietary Composition

The proximate and fatty acid composition of the algae product (A-DHA) and experimental diets are shown in Table 4.2. The algae product used in present study contained 15.9% CP and 53.6% lipid, of which, 56% of total lipid was 16:0 and 23% was DHA. Dietary fatty acid composition is a direct result of the inclusion of oil sources since no fishmeal was used in the experimental diets. CO and C+A diet had similar fatty acid composition with the exception of 16:0 and DHA, which was mostly contributed from A-DHA. In general, canola oil has a high concentration of OA, moderate concentration of LA and relatively lower concentration of ALA. Dietary OA decreased from about 97 mg/g in the CO and C+A diets to 30.26 mg/g in FO diet. Similar, dietary LA decreased from 34 to 10.9 mg/g. ALA in the FO diet was 4 times lower than the C+F diet and 8 times lower than the CO and C+A diets. The CO and C+A diet had very small amount of EPA (≤ 0.5 mg/g diet) while the diet containing fish oil had a greater concnetration (>13 mg/g diet). Total DHA in C+F and C+A was similar, with the concentration of 8.33 and 9.02 mg/g diet, respectively. However, based on DHA digestibility, the concentrations were equal (ADC of DHA was 91.5 for C+F and 85.94% for C+A).

4.3.2 Growth Results

The mortality was very low in the experiment. Only two fish died during the experiment. After feeding the experimental diets for 84 days, all fish approximately

tripled their weight (Table 4.3). There were no significant differences between the dietary treatments in growth performance as measured by final weight, average weight gain, feed intake, SGR and FCR (Table 4.3).

4.3.3 Whole Body Lipid and Fatty Acid Composition

The fatty acid composition of fish fed the experimental diets is shown in Table 4.4 (expressed as per unit of wet tissue). Over 84 days, fish had higher final total lipid content (ranged from 117.0 to 150.9 g/kg) compared to the initial level of 101.6 g/kg. Oil sources did not have significant impact on the final lipid content of fish ($P = 0.11$). The total n-3 fatty acid composition of the fish fed the FO diet was significantly higher than fish fed the other three diets. This was generally true for all n-3 fatty acids with the exception of 18:3n-3 where concentrations were highest in the C+A fed fish. The EPA concentrations of the fish were significantly higher in the FO fed fish compared to the other three treatments. There were no significant differences in EPA concentration between the CO, C+F and C+A-fed fish, however, the C+A fed fish had the lowest concentrations of EPA in both the diets and fish tissues. Similarly, the FO-fed fish had the significantly higher concentrations of DHA than fish in the other three treatment groups. Fish fed the C+A diet had higher DHA concentrations than the CO and C+F fed fish, however, the differences were not significant ($P > 0.05$).

Table 4.2 Analyzed proximate and lipid composition of the algal product (A-DHA) and experimental diets used in growth trial for rainbow trout.

		Diets			
	A-DHA	CO	FO	C+F	C+A
Chemical composition (g/kg dry matter)					
Dry matter	966.16	925.24	964.77	908.39	936.12
Crude protein	159.39	482.04	479.13	483.27	483.00
Gross energy (MJ/Kg)	29.76	24.00	23.21	23.46	24.15
Crude fat	536.07	196.26	193.47	203.42	224.54
Ash	90.22	66.63	64.60	65.61	70.12
Fatty acids (mg/g dry matter)					
14:0	25.09	0.52	11.92	5.95	1.95
16:0	298.77	15.56	34.19	24.41	31.71
18:0	7.83	5.07	9.14	7.25	5.19
20:0	0.56	0.17	0.22	0.48	0.08
22:0	0.08	0.03	0.09	0.05	0.02
24:0	0.71	0.20	0.09	0.18	0.22
Total SFAs	344.58	22.27	58.12	39.77	40.48
14:1n-5	1.46	0.10	0.67	0.38	0.18
16:1n-7	1.01	2.78	15.03	8.53	2.40
17:1n-7	0.03	0.36	2.23	1.26	0.34
18:1n-9	0.29	97.79	30.26	67.57	96.83
18:1n-7	0.71	5.50	5.36	5.51	5.43
20:1n-11	0.00	2.23	1.18	1.78	2.23
20:1n-9	0.11	0.06	0.55	0.31	0.05
22:1n-11	0.35	0.03	0.55	0.29	0.03
22:1n-9	0.06	0.05	0.18	0.13	0.07
Total MUFAs	4.45	109.19	56.73	86.32	107.86
18:3n-3	0.24	19.03	2.23	11.24	19.32
18:4n-3	0.39	0.12	3.79	2.11	0.09
20:4n-3	4.30	0.03	0.07	0.04	0.31
20:5n-3	1.56	0.50	24.27	13.47	0.35
21:5n-3	1.69	0.02	1.00	0.55	0.14
22:5n-3	0.51	0.11	3.09	1.75	0.09
22:6n-3	125.58	0.34	14.56	8.33	9.02
Total n-3 PUFAs	134.59	20.65	49.19	37.86	29.91
18:2n-6[a]	0.14	34.39	10.90	24.62	33.43
18:3n-6	0.10	0.10	0.35	1.14	0.07
20:3n-6	0.32	0.04	0.23	0.15	0.06
20:4n-6	0.29	0.17	1.59	0.97	0.19
22:4n-6	0.94	0.05	1.30	0.75	0.04
22:5n-6	22.97	0.04	0.48	0.28	1.65
Total n-6 PUFAs	24.89	34.99	15.24	28.16	35.65
Total others[b]	14.81	2.42	10.28	5.65	3.07
Total PUFAs	159.48	55.64	64.43	66.02	65.56

[a] Total of isomers t10, c12 and c9, t11 of 18:2

[b] Total of others represent 19 detected fatty acids of small quantity

Table 4.3 Growth performance data of rainbow trout fed four different diets. Data are presented as mean (n=3).

	Diets					
	CO	FO	C+F	C+A	SEM	*P*-Value
Initial Weight (g)	68.50	70.63	66.67	70.50	2.837	0.73
Final Weight (g)	216.87	230.00	209.70	220.63	10.529	0.61
Average Daily Gain (g)	1.77	1.90	1.70	1.79	0.132	0.78
Feed intake (g/fish)	198.46	201.09	181.61	204.59	5.340	0.60
SGR (% /day)	1.37	1.41	1.36	1.36	0.078	0.97
FCR (feed /wt gain)	1.35	1.26	1.23	1.36	0.085	0.62

SEM = Standard error of the mean

Table 4.4 Total lipid (g/kg) and fatty acid contents (mg/g) in fish sampled at the start of the experiment and after feeding the experimental diets for 12 weeks (Data are presented as mean, n=3).

		Diets					
	Start	CO	FO	C + F	C + A	SEM	*P*-Value
Total lipid (g/kg)	101.60	122.28	150.93	117.04	146.34	6.121	0.11
Fatty acids (mg/g)							
14:0	3.93	1.71^{a}	7.08^{c}	2.75^{b}	1.78^{a}	0.667	<0.01
16:0	18.53	14.37^{a}	25.49^{b}	14.08^{a}	18.16^{a}	1.518	<0.01
18:0	4.22	4.30^{a}	6.91^{b}	3.91^{a}	4.38^{a}	0.393	<0.01
20:0	0.12	0.57	0.16	0.19	0.29	0.079	0.26
22:0	0.15	0.14^{a}	0.12^{a}	0.18^{a}	0.31^{b}	0.024	<0.01
24:0	0.10	0.07	0.10	0.07	0.10	0.006	0.15
Total SFAs	28.12	22.36^{a}	41.50^{b}	21.95^{a}	25.82^{a}	2.557	<0.01
14:1n-5	0.11	0.10^{a}	0.39^{c}	0.16^{b}	0.11^{a}	0.358	<0.01
16:1n-7	6.29	4.31^{a}	10.66^{b}	4.79^{a}	3.77^{a}	5.886	<0.01
17:1n-7	0.60	0.35^{a}	1.31^{c}	0.53^{b}	0.32^{a}	0.123	<0.01
18:1n-9	19.75	49.13^{bc}	27.64^{a}	35.61^{ab}	57.76^{c}	3.884	<0.01
18:1n-7	2.99	3.59^{ab}	4.81^{b}	3.35^{a}	4.01^{ab}	0.204	<0.01
20:1n-11	1.16	2.20^{ab}	1.54^{a}	1.70^{a}	2.67^{b}	0.153	<0.01
20:1n-9	0.17	0.14^{ab}	0.39^{b}	0.18^{c}	0.13^{a}	0.031	<0.01
22:1n-11	0.37	0.16	0.37	0.19	2.59	0.607	0.47
22:1n-9	0.17	0.26^{ab}	0.21^{ab}	0.19^{a}	0.32^{b}	0.019	0.04
Total MUFAs	31.94	60.49^{ab}	47.74^{a}	46.99^{a}	72.01^{b}	3.796	0.03
18:3n-3	1.72	4.03^{b}	1.39^{a}	4.09^{b}	7.23^{c}	0.664	<0.01
18:4n-3	0.73	1.48^{bc}	1.84^{c}	0.94^{a}	1.14^{ab}	0.110	<0.01
20:4n-3	0.07	0.03^{a}	0.10^{b}	0.03^{a}	0.14^{b}	0.014	<0.01
20:5n-3	5.38	2.09^{a}	11.63^{b}	3.43^{a}	1.92^{a}	1.221	<0.01
21:5n-3	0.53	0.35^{a}	0.92^{b}	0.44^{a}	0.44^{a}	0.069	<0.01
22:5n-3	1.85	0.87^{a}	3.48^{c}	1.26^{b}	0.78^{a}	0.333	<0.01
22:6n-3	10.58	5.26^{a}	16.44^{b}	7.50^{a}	8.68^{a}	1.249	<0.01
Total n-3	23.81	14.28^{a}	37.89^{b}	17.81^{a}	20.53^{a}	2.870	<0.01
18:2n-6	7.85	14.69^{ab}	9.77^{a}	12.35^{a}	18.56^{b}	1.092	<0.01
18:3n-6	0.19	0.97^{c}	0.27^{a}	0.29^{ab}	0.56^{b}	0.090	<0.01
20:4n-6	0.31	0.74^{b}	0.38^{a}	0.39^{a}	0.68^{b}	0.056	<0.01
22:4n-6	0.53	0.26^{a}	0.98^{c}	0.38^{b}	0.23^{a}	0.093	<0.01
22:5n-6	23.81	14.28^{a}	37.89^{b}	17.81^{a}	20.53^{a}	2.870	<0.01
Total n-6	11.28	18.98^{ab}	13.94^{a}	15.16^{a}	23.06^{b}	1.223	<0.01
Total PUFAs	35.08	33.26^{a}	51.83^{b}	32.96^{a}	43.59^{ab}	2.719	<0.01
Sum others	4.58	1.02^{a}	2.97^{b}	1.20^{a}	0.79^{a}	0.266	<0.01

^{abc} Values with different superscripts within rows are significantly different ($P < 0.05$)

4.3.4 Lipid and Fatty Acid Retention

There were no significant differences in total lipid retention between dietary treatments. Apparent retention of total lipid ranged from 67.8 to 77.7%, suggesting that ~22-32% of lipid was used for energy. Similarly, there were no significant differences in the apparent retention of SFA, MUFA, n-3 PUFA and n-6 PUFA between treatments. There were no significant differences in the retention of 18:3n-3 between treatments. However, the retention of 18:4n-3 was significantly higher in the CO and C+A fed fish compared to the FO and C+F fed fish. The same patterns were also found on the retention of 18:2n-6 and 18:3n-6. This suggests that 18:3n-3 and 18:2n-6 were bioconverted to 18:4n-3 and 18:3n-6 respectively to a much greater extent in the CO and C+A fed fish than in the FO or C+F fed fish, indicating the activity of Δ6 desaturase might be inhibited by dietary fish oil. There was a trend (P = 0.09) towards higher retention of EPA in the CO and C+A fed fish compared to the other two groups. Furthermore, the retention of EPA in the CO and C+A fed fish was over 100%. This suggests a net synthesis of EPA in these treatments groups. DHA was synthesized in the CO-fed fish, which had significantly higher DHA retention than fish fed the other three diets. The level of DHA in the FO fed fish was numerically but not significantly higher than in the C+F and C+A fed fish.

Table 4.5 Apparent retention (%) of the fatty acids in rainbow trout fed four different experimental diets after 84 days (Data are presented as mean, n=3).

	Diets					
	CO	FO	C + F	C + A	SEM	*P*-Value
Total lipid	67.83	77.73	68.76	77.27	3.821	0.76
Fatty acids						
14:0	120.74[a]	60.44[b]	33.95[b]	33.60[b]	9.611	<0.01
16:0	66.11	70.46	45.17	48.07	6.189	0.45
18:0	70.51	74.91	47.62	72.69	5.985	0.37
20:0	174.90[b]	65.11[c]	42.39[c]	417.02[a]	53.110	<0.01
22:0	406.55[b]	101.62[b]	346.32[b]	1393.36[a]	159.260	<0.01
Total SFAs	72.95	68.90	44.05	51.79	6.457	0.36
14:1n-5	82.76	65.14	43.05	51.45	6.836	0.14
16:1n-7	131.79[a]	71.99[ab]	43.94[b]	104.3[a]	11.360	0.02
17:1n-7	74.04	62.07	35.94	47.49	6.784	0.23
18:1n-7	57.84	89.39	58.53	70.66	6.152	0.22
18:1n-9	52.72	89.58	58.58	66.70	5.540	0.12
20:1n-11	99.11	128.56	101.13	129.52	8.187	0.43
20:1n-9	180.26[ab]	75.14[bc]	56.72[c]	193.84[a]	17.804	<0.01
22:1n-9	510.96[a]	113.03[b]	143.71[b]	511.86[a]	65.641	<0.01
22:1n-11	161.98	55.35	33.48	71.97	26.525	0.34
24:1n-9	64.74	64.84	49.59	93.95	7.545	0.17
Total MUFAs	55.51	83.54	57.54	71.94	5.504	0.25
18:3n-3	22.15	48.35	42.72	43.49	4.337	0.12
18:4n-3	1280.73[a]	50.37[b]	45.42[b]	1285.33[a]	200.650	<0.01
20:3n-3	31.69[b]	33.92[a]	31.35[b]	36.59[b]	2.650	0.91
20:4n-3	42.71[b]	144.98[a]	32.45[b]	45.46[b]	15.890	0.02
20:5n-3	145.10	55.28	21.09	109.30	19.413	0.09
21:5n-3	796.47[a]	93.17[c]	65.71[c]	241.50[b]	41.782	<0.01
22:5n-3	307.04[a]	127.46[ab]	51.53[b]	248.81[ab]	38.224	0.04
22:6n-3	688.95[a]	111.56[b]	66.56[b]	72.77[b]	101.155	0.02
Total n-3	39.48	69.27	36.51	53.92	5.427	0.16
18:2n-6	42.65	83.16	53.97	60.34	5.466	0.07
18:3n-6	1090.96[a]	67.94[b]	27.23[b]	916.45[a]	158.300	<0.01
20:2n-6	482.81[b]	210.97[c]	362.20[bc]	860.28[a]	78.459	<0.01
20:3n-6	1799.46[a]	139.68[b]	267.76[b]	1169.17[a]	226.313	<0.01
20:4n-6	388.31[a]	68.93[b]	45.83[b]	382.49[a]	60.293	0.02
22:2n-6	120.17	54.29	113.13	76.62	21.516	0.77
22:4n-6	208.34	76.74	36.92	158.01	29.520	0.12
22:5n-6	756.21[a]	71.50[b]	43.48[b]	54.72[b]	93.890	<0.01
Total n-6	52.98	83.26	55.56	68.63	5.012	0.16
Total PUFAs	47.97	72.58	44.63	61.92	4.821	0.18

[abc] Values with different superscripts within rows are significantly different ($P < 0.05$)

4.4 Discussion

Since all diets contained equal amounts of digestible crude protein and digestible energy, the growth response of fish to different oil sources was not expected to be significantly different. This result is in accordance with previous studies, which have shown that fish oil could be partially or completely replaced by dried algae or algae derived oil in diets for Atlantic salmon parr (Carter et al., 2003; Miller et al., 2007) and for sea bream larvae (Ganuza et al., 2008) without compromising fish growth.

The present study indicated that the final whole body fatty acid composition of fish generally reflected the fatty acid composition of diets. This finding has been well documented for many species, such as Murray cod (Francis et al., 2006), gilthead sea bream (Menoyo et al., 2004), Atlantic salmon (Torstensen et al., 2004) and rainbow trout (Thanuthong et al., 2011). In addition, the fatty acid composition in fish was also affected by *in vivo* fatty acid metabolism, including β-oxidation, biosynthesis of longer and/or more unsaturated fatty acids (Thanuthong et al., 2011). The DHA concentration in final whole fish body was lower in fish fed all experimental diets expect for the FO-fed fish, compared with DHA level in initial fish samples. Although the diet CO was almost void of DHA (0.34 mg/g), fish fed CO had final DHA content of 5.26 mg/g tissue, which, however, was still significantly lower than fish fed FO with 16.44 DHA mg/g tissue. This finding is similar to the study by Francis et al. (2007a) which indicated that replacing fish oil with vegetable oil resulted in increased synthesis of LC-PUFAs, but the synthesized amounts were still lower than LC-PUFAs consumed from FO-based diets. It should be pointed out that the amount of DHA in the FO diet (14.56 mg/g) was significantly higher than that in C+O and C+A (8.33 and 9.02 mg/g, respectively). This could partially explain the much higher final DHA level in FO fed fish compared to fish fed blended oils.

In general, the retention of SFA and MUFA with 18 carbons or less had retention below 100%, indicating these fatty acids were either absorbed at rates less than the rate of growth or oxidized for energy production, as suggested by Kiessling and Kiessling (1993). In contrast, the retention of 22:0 in the present study was greater than 100% in all treatments, confirming a study by Stubhaug et al. (2007), who reported increased

retention of SFAs was associated with increasing fatty acid chain length. Furthermore, this result was also supported by previous studies where it was reported that increased chain length of fatty acids led to decreased β-oxidation specificity in rainbow trout liver (Henderson and Sargent 1985; Kiessling and Kiessling, 1993). Turchini and Francis (2009) indicated that high oxidation rate of 20:1n-11 and 22:1n-11 was found in rainbow trout regardless of the low dietary concentration. However, current results showed high retention (>99%) of 20:1n-11 in all groups and the retention of 22:1n-11 was higher in fish fed the CO and C+A diets (162% and 72%, respectively) compared to fish fed diets containing fish oil (< 55%). Previous studies demonstrated that β-oxidation of fatty acids is proportional to the dietary supply and fatty acids supplied in surplus are more readily oxidized (Stughaug et al., 2006; 2007). In the present study, dietary composition of 20:1n-11 and 22:1n-11 was much lower than SFAs and other MUFAs with chain length shorter than 20C, therefore, these two fatty acids tended to accumulate to a greater extent and SFAs and other MUFAs seemed to be more readily oxidized for energy production. This result is also in accordance with a previous study reported that 18:1n-9 and 22:1n-11 tended to accumulate to a higher extent in fish body than in diets when dietary supply was low. Interestingly, the retention of all fatty acids was generally similar in CO and C+A groups except for significantly greater retention of DHA and 22:5n-6 in CO compared to other groups. This is due to very similar dietary fatty acid composition in both diets CO and C+A with the exception of the present of DHA in C+A. Bell et al. (2003a) and Torstensen et al. (2004) revealed that OA, LA and ALA are all readily β-oxidized when a large amount is supplied in diet and the degree of retention of these fatty acids followed the order: OA > LA > ALA. The results from the present study showed the retention of these three fatty acids followed the same order although retention of each fatty acid varied between dietary treatments.

Biosynthesis of n-3 and n-6 LC-PUFAs uses the same enzymes. Δ-6 desaturase is the first enzyme in the pathway to convert LA and ALA to 18:3n-6 and 18:4n-3, respectively. The retention of ALA was low in all treatments (<50%) indicating these fatty acids were oxidized for energy production and/or desaturated by Δ-6 desaturase (Bell et al., 2001a; Stubhaug et al., 2007). The retention of 18:4n-3 in both CO and C+A groups was very high (>1000%) and this supports the notion that the LA and ALA were

desaturated by Δ-6 desaturase. In contrast, retention of 18:4n-3 and 18:3n-6 was significantly lower in FO and C+F groups (50% and 45.2% for 18:4n-3 and 68% and 27% for 18:3n-6, respectively). This suggests that Δ6 desaturase activity was decreased by feeding diets containing fish oil relative to fish fed the CO and C+F diets. This is supported by previous studies that reported that higher dietary levels of C_{18} fatty acids resulted in an increased Δ6 desaturase activity (Francis et al., 2009) and both the Δ5 and Δ6 desaturase genes were up-regulated in response to feeding diets where fish oil was replaced by vegetable oil (Zheng et al., 2005b). There is controversy as to whether dietary EPA or DHA reduces the activity of Δ6 desaturase (Jump, 2004; Zheng et al., 2005b). In the current study, the C+A fed fish received diets with high concentrations of DHA and low EPA concentrations. In contrast, the C+F diets contained higher concentrations of EPA and apparent bioconversion of ALA to 18:4n-3 was decreased. This suggests that it is EPA, but not DHA, that decreases the activity of Δ6-desaturase. Thus, feeding a mixture of canola oil and the algae product might result in higher rates of biosynthesis of LC-PUFA from ALA and LA compared to a canola/fish oil blend. Further studies should determine the effects on hepatic enzyme activities in terms of fatty acyl desaturases and elongases.

The retention of EPA was greater than 100% in fish fed CO and C+A, which suggests increased biosynthesis of EPA from ALA. This agrees with the findings of Stubhaug et al. (2007). By contrast, low retention of EPA was found in FO and C+F groups (55 and 21 %, respectively). Stubhaug et al. (2007) reported that EPA is readily β-oxidized when present in high dietary content. In addition, the retention of EPA in all dietary groups was lower than DHA retention. Similar results were found by previous research reporting that EPA is selectively catabolized over DHA for energy production and/or a possible chain elongation to DHA (Hansen et al., 2008; Torstense et al., 2004), The high retention of DHA in CO group (689%) indicates a higher rate of synthesis of DHA. This is consistent with a previous study in Atlantic salmon, showing higher retention of DHA in white muscle with lowered DHA supply in diets (Torstensen et al., 2004). Fish fed FO had DHA retention greater than 100%, which could possibly be attributed to high dietary concentrations and possibly conversion from EPA. The retention of DHA in rainbow trout fed FO diet has been observed in previous studies

(Thanuthong et al., 2011; Turchini and Francis, 2009), however, no previous studies have addressed whether the synthesis of DHA in fish fed FO diet is from the conversion of EPA or from other possible precursors. Fish fed C+F and C+A had similar DHA retention of 67 and 73%, respectively, which was not statistically significantly different from fish fed FO. The retention values of less than 100% are probably due to a combination of rates of absorption of DHA less than the rate of growth and a relatively low rate of biosynthesis. However, the magnitude of these two factors in fish fed the C+F and the C+A diets cannot be determined.

In conclusion, although feeding C+A diet resulted in lower DHA content and poorer DHA retention in fish compared with fish oil diet, A-DHA has the potential to replace fish oil in canola-oil-based diets since fish fed C+A had final DHA content and apparent DHA retention similar to fish fed C+F while no compromised growth performance was observed. It is believed that increasing lipid digestibility of A-DHA could further enhance DHA retention and DHA content in fish products. Therefore, in order to increase lipid digestibility of the algal product, extracting oil from the product could be the possible approach; however, this increases the costs of using algae oil significantly. Since extrusion of fish feed can increase digestibility of nutrients, a future recommendation would be to determine the optimal extrusion parameters required to maximize the digestibility of the algae/canola oil blend feed.

5. General Conclusion and Future Perspectives

The therapeutic significance of consuming n-3 LC-PUFAs especially EPA and DHA been well established. Fish including Atlantic salmon and rainbow trout have traditionally been unique sources of these health beneficial fatty acids for consumers. Today, these fatty acids can also be obtained from n-3 LC-PUFA-enriched terrestrial animal sources, such as omega-3 eggs and milk (Lewis et al., 2000; Simopoulos, 2002; Dawczynski et al., 2010). However, consumption of products from terrestrial animals has become controversial due to concomitant increases in blood cholesterol levels (Kritchevsky and Kritchevsky, 2000; Nakamura et al., 2004; Tholstrup et al., 2004). In addition, n-3 oils and capsules are a widely available and important source of n-3 LC-PUFAs in human diets. However, consumers have preferences for fish over capsules or functional foods (Cox et al., 2008a; 2008b). Hence, the fish remain as the primary source of n-3 LC-PUFAs in human diets.

Farmed salmonids are rich sources of n-3 LC-PUFAs, which is primarily due to the inclusion of fish oil aquafeeds. The global shortage of fish oil supplies has led to an increasing demand for high quality, stable and affordable alternatives for aquafeed production. Vegetables oils, such as linseed and rapeseed (canola) oil are the most readily available replacement for fish oil and have been successfully utilized in terms of fish growth. Over the past 10 years, fish oil blended with vegetable and animal oil has been used in the manufactured aquafeeds in the aquaculture industry (Miller et al., 2008). However, this results in altered fatty acid composition in fish with decreased levels of EPA and DHA, which is undesirable. As a consequence, it is necessary to examine new lipid sources of n-3 LC-PUFAs.

Unlike terrestrial crops, microalgae are the primary natural producers of LC-PUFAs: *Crypthecodinium* and *Schizochytrium* can directly produce high amount of DHA while *Nannochloropsis* and *Isochrysis* are the natural sources of EPA (Shields and Lupatsch, 2012). There is evidence that n-3 LC-PUFAs in fish actually originate from microalgae and since most of the microalgae are not suitable for direct human consumption, it might be logical to add the microalgae to fish feeds to indirectly boost their nutritional values for humans. However, relatively few studies have been carried out to date to determine the use of microalgal lipids in feeds for farmed fish (Atalah et al.,

2007; Ganuza et al., 2008). This thesis evaluated a whole-cell DHA-rich algal product (A-DHA) as a replacement for fish oil in diets for Atlantic salmon and rainbow trout. Due to the high concentration of DHA in the A-DHA, the present study used a algae/canola oil blend feed, giving a fatty acid profile closer to that of their natural diet, which was in accordance with Miller et al., 2010. Unfortunately, the inclusion of the A-DHA significantly lowered lipid digestibility, constraining the application of microalgae in aquafeeds. However, the 12-week growth experiment in this thesis showed the potential of using the algae product in the combination of canola oil for rainbow trout, since C+A fed fish had similar final body DHA concentration and DHA retention as C+F fed fish, while no deleterious growth performance was observed between dietary treatments. Improving the digestibility of A-DHA in fish is believed to further enhance the concentration of DHA in fish tissues, resulting in similar DHA content in both C+A and fish oil-fed fish. Therefore, strategies need to be developed to increase the nutrient digestibility of microalgae in fish.

A possible method to increase the nutritional value of algal ingredients would be to extract oils from microalgae by mechanically rupturing cell walls, such as pressing and bead milling (Shields and Lupatsch, 2012). However, these processing steps would be prohibitively expensive. One possible approach to this problem is feeding algal oils as finishing diets in the last weeks prior to harvest, while the majority of the production cycle could be fed on less expensive vegetable oil-based diets. This approach may increase n-3 LC-PUFAs in the fillet, as opposed to that of fish fed only vegetable oil. However, using finishing diet containing algal oil would still not be cost-effective and is only assumed to be feasible when consumers are willing to pay a premium for n-3 LC-PUFAs-rich fish products (Miller et al., 2010). To date, it seems to be more practical to use the whole-cell algal biomass rather than algal oils in fish feeds.

Extrusion is the main method for dry feed processing for fish farming, which provides moisture, pressure, heat, shear and expansion to manufactured aquafeeds and has the effect of increasing the digestibility of nutrients (Cheng and Hardy, 2003; Barrows et al., 2007). Hence, future studies should investigate the optimal extrusion parameters (such as barrel temperature, shear, pressure and moisture content of the mash) to maximize the digestibility of the algae/vegetable oil blend feed. Furthermore, the

potential effect of feeding the A-DHA to rainbow trout and Atlantic salmon on growth performance should be examined over longer periods of the production cycle, in order to ensure that feed efficiency, fatty acid profiles and fatty acid retention are not compromised. Temperature is also an important factor that influences the fatty acid composition of fish, thus, the effect of varying water temperatures together with the dietary treatments should be further determined. Since it was clearly shown by the results of the present study that oil sources have significant effects on fatty acid retention, it would be informative to determine influences of feeding the A-DHA to rainbow trout and Atlantic salmon on LC-PUFA biosynthesis and hepatic enzyme activities in terms of fatty acyl desaturases and elongases.

There is currently ample evidence that most fish stocks have been overexploited from the world's oceans as a result of overfishing. However, humans are not really harnessing the true productivity of the oceans since 99% of the marine primary production is lost. This is due to the fact that humans harvest food from higher trophic levels in the ocean than on land (Olsen and Endal, 2002). In the agricultural food web, humans consume the primary producers (e.g. vegetables and grains) and animals (e.g. pigs, cows, and sheep) that feed on primary producers. By contrast, in the marine food web, carnivorous animals that feed on zooplankton (e.g. herring and capelin) and species even higher in the food web (e.g. halibut, salmon and tuna) are the main commercial resources harvested from the ocean. Thus, there is huge potential to capture and culture more organisms at lower trophic levels in the ocean for direct human consumption or as feed for animals. Actually, the production of microalgae has many advantages over conventional agriculture, such as higher yield per hectare compared with traditional crops and growing on land unsuitable for food crops, which releases pressure on arable land (Christaki et al., 2011). It is predicted that the global population will reach 9 to 10 billion by 2050 and mariculture (cultivation of marine organisms for food) will be a major contributor to the required increases in world food production. Algae that contain high levels of proteins and lipids (in particularly n-3 LC-PUFAs) as well as vitamins, minerals and pigments appear to be a promising source to feed the future.

Currently, small- to medium- scale production systems are used for the commercial production of n-3 LC-PUFA-rich microalgae oil, which are primarily

focusing on human nutrition, in particular infant formulas (Miller et al., 2010). At present, the cultivation capacity of microalgae is relatively small and is too expensive and inefficient to be used as a replacement for fish oil in aquafeeds (Browdy et al., 2006). In addition, it has to be pointed out that microalgae only produce high amount of lipids under suboptimal or stress conditions, such as nitrogen deprivation (Chiu et al., 2009; Rodolfi et al., 2009). This indicates that lipid productivity in microalgae is inversely related to overall growth. Therefore, in order to use microalgae as a LC-PUFA source to substitute fish oil, future efforts should be focusing on the development of large-scale and cost-effective production (Wolkers et al., 2011), while finding strains that have the combination of high biomass and lipid productivity (Rodolfi et al., 2009).

In conclusion, the use of n-3 LC-PUFA-rich microalgae from the marine environment as an alternative to replace fish oil is a logical approach. However, to date, this approach is still not economically feasible due to the high cost and productivity limitations. The current microalgal biotechnology research on the use of microalgae as biofuel sources may lead to higher availability and lower price of microalgae in the near future. The combination of price increases for fish oil and improved cost effectiveness and availability of n-3 LC-PUFA-rich microalgae will help maintain the beneficial LC-PUFA content of aquaculture fish in the future.

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