EVALUATION OF TRANSGENIC *CAMELINA SATIVA* OIL AND *SCHIZOCHYTRIUM* SP. OIL AS A SOURCE OF DIETARY LIPID FOR JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*).

by

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DEDICATION PAGE

I would like to dedicate this thesis to my mother, Katrina Anne Dennis. This wouldn't be possible without your overwhelming support, love, and dedication. You were always and will be my biggest cheerleader and inspiration. You brought me up in a household full love for nature, science, art, love, and light. You encouraged me to ask why, to be strong, to have courage, to never be afraid, to look at the bigger picture, to stay curious and above all, to be kind. This thesis is for you.

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ABSTRACT

Two experimental feeding trials examined Genetically engineered *Camelina sativa* and high DHA *Schizochytrium* sp. oils as dietary lipid alternatives for rainbow trout. Fish fed high camelina oil diets (HCO) had no differences in DHA within muscle tissue of fish fed HCO compared to the fish oil control (FO) ($p=0.000$). Trout fillets from the HCO treatment were firmer in texture and had higher orange intensity compared to FO ($p=0.001$; $p=0.012$). In muscle, DHA was highest in trout fed the microbial oil (MO), and the microbial oil camelina blend (MO/CO) diets than trout fed the FO diet ($p=0.000$). Muscle EPA was highest in trout fed the FO diet than trout fed the MO and MO/CO diets ($p=0.000$). Dietary inclusion of transgenic camelina and high and low inclusion levels of *Schizochytrium* sp.(T-18) oil can be used as an effective dietary lipid alternative for juvenile rainbow trout.

LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Percent
AFI	Apparent feed intake
ALA	α-linolenic acid, 18:3n-3
ANOVA	Analysis of variance
ARA	arachidonic acid, 20:4n-6
CF	Condition factor
CO	Camelina Oil
CSIA	Compound specific stable isotope analysis
DHA	Docosahexaenoic acid 22:6n-3
EPA	Eicosapentaenoic acid, 20:5n-3
FA	Fatty acid
FCR	Feed conversion ratio
FO	Fish oil
HCO	High Camelina oil
LCO	Low Camelina oil
LC-PUFA	Long chain polyunsaturated fatty acid
LNA	Linoleic acid, 18:2n-6
MDS	Multi-dimensional scaling
MO	Microbial oils
MO/CO	Microbial oil and camelina oil blend
MS-222	Tricaine methanesulfonate
MUFA	Mono-unsaturated fatty acids

PCoA	Principal Coordinates Analysis
PCR	polymerase chain reaction
PUFA	Polyunsaturated fatty acids
QDA	Quantitative descriptive analysis
qPCR	Quantitative polymerase chain reaction
SD	Standard deviation
SFA	Saturated fatty acids
SGR	Specific growth rate
VSI	Visceral somatic index
WG	Weight gain

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CHAPTER 1 INTRODUCTION

1.1 RATIONALE

The continued growth of the global population is leading to an unprecedented appetite for marine-based products. Total fish consumption has increased by 122% from 1990 to 2018 as fish and seafood-based products contribute to 20% of the average per capita intake of animal-based protein (FAO, 2020). Projections indicate that aquaculture will continue to provide a vital source of seafood, providing >60% for human consumption by 2030, with further increases likely as the global population swells to an estimated 9 billion by 2050 (FAO, 2020; Béné et al., 2015). Specific population development related to urbanization in North America has also led to increased demands for sustainable seafood production as population growth is estimated to increase by ~39% by 2050 (UN, 2019). To meet these growing demands, the development of sustainable aquaculture practices is crucial to the growth of enhanced seafood production with reduced reliance on wild fish stocks (Béné et al., 2015). As seafood consumption continues to grow, aquaculture will have a crucial role to play in meeting global demand for the production of finfish species, such as rainbow trout (*Oncorhynchus mykiss*). Rainbow trout production is relevant within North American markets, although it is relatively small within Canada reaching 8,771 tonnes in 2019 (FAO, 2021). Rainbow trout are native to the Pacific coasts of North America and Asia and belong to the family *Salmonidae* which include numerous trout species (*Salvelinus* sp.), Atlantic salmon (*Salmo salar*), Arctic char (*Salvelinus alpinus*), whitefish (*Coregonus* sp.) and species such as Artic grayling (*Thymallus arcticus*) (Hardy, 2002). Due to its hardiness, ease of manipulation and spawning along with rapid growth rate and high tolerance of varying production and environmental conditions, rainbow trout have been introduced to

over 82 countries as a finfish in demand within global aquaculture production with production values reaching 848,100 tonnes in 2018 (DFO, 2021; FAO, 2020). Rainbow trout are often used as a model salmonid species due its ease of accessibility, production, and tolerance of environmental change (Bobe et al., 2016). The potential for transgenesis and the generation of all female rainbow trout strains also contributes to the application of rainbow trout within laboratory settings (Bobe et al., 2016). Further, the existing and extensive body of knowledge on biochemical, biological, molecular, and genetic information highlights their value as a model salmonid species with applications within aquaculture research (Lincoln & Scott., 1983; Ribas et al., 2014; Schartl, 2014; DFO, 2018).

1.2 RAINBOW TROUT BIOLOGY AND CULTURE

Several species belonging to the family *Salmonidae* are currently cultured, including Atlantic salmon, Arctic charr and various trout species (*Salvelinus* sp.) such as rainbow trout (Williams et al., 2015). Rainbow trout are identified by a slender and elongated body type, are typically silvery on their sides with an occasional green tint and display a pink or red iridescent lateral line (DFO, 2016a). Rainbow trout inhabit both freshwater and saltwater habitats as anadromous fish migrating from the ocean to freshwater rivers and streams for spawning; however, when explicitly reared in saltwater, they are referred to and marketed as steelhead trout (Hardy, 2002; Cowx, 2006). Although rainbow trout can withstand a broad range of temperatures temperature within fresh water (0-27 °C), optimal growth and spawning typically occur between 9-14 °C (Cowx, 2006). Monoculture systems and practices are generally adopted in the cultivation of rainbow trout for their optimal

production processes (FAO, 2011). These systems allow for the calibration of factors such as food resource availability and environmental temperature, which can impact growth and development, including the incubation time from egg to fry and the onset of sexual maturity (FAO, 2011). Fertilized eggs are placed into hatching trays; hatching rate is temperature dependent and as such eggs can take up to 80 days to hatch at 4.5 °C , 31 days at 10 °C and 19 days at 15 °C (Hardy, 2002; Jobling et al., 2010; FAO, 2011). During hatching, larvae are referred to as yolk-sac fry or alevins. Following hatching, the fry are collected and placed into circular fiberglass or concrete rearing tanks. Fry can be reared in both indoor enclosed recirculating aquaculture systems (RAS) and outdoor grow-out facilities, comprised of cages, flow-through ponds or concrete raceways (FAO, 2011). Fish are grown typically for 9 months until they reach a marketable size of 30-40 cm (FAO, 2011). The farming and cultivation of rainbow trout species has been effectively developed over decades and can efficiently produce this species in well-established systems (FAO, 2020). Rainbow trout (freshwater/anadromous species) are one of Canada's most relevant commercial species, with production reaching 7,000 tonnes of farmed trout between 2012 and 2015 (DFO, 2017). Global farmed rainbow trout production has also risen substantially from 752K tonnes in 2010 to 848K tonnes in 2018, contributing to 1.6% of significant species cultivated in global aquaculture production (FAO, 2020). Despite the expansion in production, challenges in the production efficiency of farmed trout remain. Current efforts focus on the sustainability of farmed trout production and how practices might be improved upon while simultaneously reducing feed costs and ensuring the nutritional needs of trout are being met (Bélanger-Lamonde et al., 2018; Sarker et al., 2020).

1.3 OVERVIEW OF FISH NUTRITION IN AQUACULTURE

Defining the nutritional requirements of farmed fish is crucial to gain insight on the impact of digestible nutrients on the growth, health, and developmental processes of fish species (Sargent et al., 2002). The production of nutritionally inadequate feeds can result in the onset of nutrient deficiency, resulting in reduced metabolic rate, compromised immunity, stunted growth and death (NRC, 2011). Providing fish with correctly formulated and nutritious diets is crucial in meeting their physiological needs (NRC, 2011). Dietary formulation is typically tailored based on several contributing factors such as a unique species type and stage of development (NRC, 2011). The production of rainbow trout, and their essential dietary nutrient requirements have been well documented (Webster & Lim, 2002; NRC, 2011). Essential nutrients required for growth and development in formulated diets for salmonids include a combination of protein, carbohydrates, lipid, vitamins, minerals, amino acids and fatty acids (FA), all of which vary in inclusion rate based on species, stage of growth, sexual maturation as well as freshwater vs seawater rearing (Webster & Lim., 2002; Hixson, 2014). Protein in fish diets readily supplies amino acids, which are digested and used to synthesize new protein and energy for growth and reproduction (Sargent et al., 2002; Hixson, 2014). Protein requirements can be influenced by several factors such as age and size as well as water temperature. Protein requirement for trout reared within fresh water are also slightly different than those reared in marine with marine fish needing a slightly higher protein requirement of dietary formulation than those reared within fresh water (Webster & Lim., 2002). Acceptable diets for juvenile rainbow trout have a protein composition of ~40% relative to brood stock inclusion of ~38% protein inclusion (Sargent et al., 2002; Cleveland et al., 2020).

The ability to use carbohydrates as an additional energy source in fish, is dependent on factors such as species, physiological, metabolic and enzymatic constraints as carnivorous and omnivorous fish species differ in their capacity to digest and metabolize carbohydrates relative to herbivorous fish (Wilson, 1994; Sargent et al., 2002; Hixson, 2014; Azaza et al., 2020). The increase in potentially indigestible dietary carbohydrate through the incorporation of plant materials may lead to increased fat storage within the fish viscera, instead of fat deposition, as previously observed within tilapia (*Oreochromis niloticus)* (Ighwela et al., 2014) Fat deposition occurring within the viscera instead of in muscular tissue could impact both the health and nutritional quality of the fillet product for human consumption (Ighwela et al., 2014). Deposition of fat within the viscera is important to consider as the ability of fish to metabolize carbohydrates varies based on species and diet type (Ighwela et al., 2014). The evaluation of visceral fat through visceral somatic index is important as a reflection of metabolic capability to effectively digest and absorb dietary carbohydrates through enzyme synthesis within fish (Ighwela et al., 2014).

Rainbow trout can grow and remain healthy when provided with diets containing limited dietary carbohydrate inclusion dependent on molecular complexity (Song et al., 2018). Increased carbohydrate concentrations in formulated rainbow trout diets can have epigenetic impacts, impairments on growth performance and starch digestibility (Kamalam et al., 2017). As the threshold limit for carbohydrate inclusion is one which ensures the reduction of growth or developmental impairment, the maximum recommended levels of

dietary carbohydrate inclusion for rainbow trout are within 18-27% of dietary inclusion (NRC, 2011; Kamalam et al., 2017).

Dietary lipids are important energy resources for fish as they supply essential FA required for growth, maturation, and aid in membrane fluidity (Sargent et al., 2002; De Carvalho & Caramujo, 2018). Long-chain polyunsaturated fatty acids (LC-PUFA) such as α-linolenic acid (ALA, 18:3n-3) and linoleic acid (LNA, 18:2n-6) are essential dietary FA as they can be used as metabolic precursors to produce primarily marine-derived eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (De Carvalho & Caramujo, 2018). The inclusion of ALA and LNA is essential as most vertebrates, including teleosts, have limited ability to produce these FA through biosynthetic means (Gladyshev et al., 2013). Vitamins are organic compounds that must be supplemented in trace amounts for normal growth, development, and health (Webster & Lim., 2002). Finally, minerals are required for maintenance of skeletal structures, osmotic pressure, nerve impulses, muscle contraction, components of enzymes and hormone function, along with cellular respiration and can be absorbed via dietary means and through gills and skin (Sargent et al., 2002; Webster & Lim., 2002).

1.4 LIPIDS AND FATTY ACIDS

Lipids are a significant source of metabolic energy for fish and provide a source of essential FA for growth and development (Sargent et al., 2002). Lipids refer to a wide range of organic compounds made up of FA containing carbon, hydrogen and oxygen atoms linked together via covalent bonds typically esterified to alcohol groups (Sargent et al., 2002).

These FA groups may also contain phosphorous, oxygen, sulfur, or nitrogen atoms (Sargent et al., 2002). Lipids are insoluble in water but can be dissolved in organic solvents (Turchini et al., 2009). This insolubility distinguishes lipids from protein and carbohydrate structures (Turchini et al., 2009). Lipid structure can also influence its relative polarity. Lipids can either be polar, based on their phospholipid composition, or neutral (non-polar), based on the presence of compounds such as sterol and triacylglycerol (Tocher, 2003). Based on structure and composition, lipids can be further categorized as either simple or complex. Lipids in their simplest form are neutral (or non-polar), hydrophobic, contain a FA esterified to an alcohol molecule and hydrolysis of the FA results in the production of alcohol molecules (Tocher, 2003). Alternatively, complex lipids have polar properties and are slightly more hydrophilic. Complex lipids contribute to cellular membrane structure and function, acting as an environmental barrier between intra and extracellular spaces (Tocher, 2003). Based on their structure, lipids can be further categorized into five distinct lipid classes: triacylglycerols, wax esters, sterols, phospholipids and sphingolipids (Sargent et al., 2002).

Triacylglycerols also referred to as triglycerides, make up a major component of the simplest of lipid classes (Xin et al., 2019). Triacylglycerol molecules consist of a glycerol backbone esterified to three FA groups (Nelson et al., 2008; Xin et al., 2019). These FA groups are non-polar and hydrophobic, making them insoluble in water (Nelson et al., 2008; Xin et al., 2019). Triacylglycerol molecules are found commonly in vertebrates stored within fat cells and as lipids and within plant seed tissue such as those found within oilseed crops. They are commonly metabolized as a metabolic energy resource (Nelson et al., 2008;

Xin et al., 2019). Wax esters are another form of simple lipid in which the FA molecule is esterified with alcohols and are particularly abundant within marine zooplankton and serve as long-term energy reserve and aid in buoyancy (Sargent et al., 2002; Lee et al., 2006; Schots et al., 2020). Sterols, such as cholesterol, are lipid classes that aid in membrane function and signaling, commonly made up of a steroid nucleus that can be esterified to a FA (Sargent et al., 2002). Phospholipids are complex lipids characterized by the presence of a hydrophilic phosphate "head," which is linked to a hydrophobic FA-derived tail made up of acyl chains joined by an alcohol group (Li et al., 2015). This lipid class also contributes to cell membrane function as a major lipid component (Li et al., 2015). As a lipid class, sphingolipids contain an amino acid base, sphingosine, in which the primary alcohol group is linked via esterification to phosphocholine and has an amino group joined to a long-chain FA via an amide linkage (Sargent et al., 2002; Vance & Vance, 2008). Sphingolipids contribute significantly to cellular membrane's structural components and act as metabolic precursors to eicosanoids, impact hormone function, enzyme cofactors and can aid in the transport of fat-soluble vitamins and cell signaling (Turchini et al., 2008). As lipids, both simple and complex, play a major role within membrane structure and function, and as a biological energy resource, the understanding of the metabolic breakdown and utilization of these lipids is highly important.

Within biological systems, FA's are typically affiliated with cellular membranes structure (Casares et al., 2019). As such, FA themselves are structurally diverse depending on the presence or absence of double bonds within the hydrocarbon chain (Sikorski et al., 2010). Single covalent bonds occur within molecules when atoms share a single pair of electrons

(a bond) with adjacent atoms (Lodish et al., 2000). Double bonds occur when two adjacent atoms share two or more pairs of electrons resulting in a double covalent bond (Lodish et al., 2000). When no double bonds are present between carbon atoms within the hydrocarbon chain, a FA is considered to be saturated (Sargent et al., 2002). Alternatively, the presence of one or more double bonds elicits a mono (singular double bond) or poly (more than one double bond) unsaturated FA (Sargent et al., 2002).

Regarding FA nomenclature, hydrocarbon chain composition, length, the presence and position of double bonds, and additional functional groups must be considered. FA are typically referred to in numerical shorthand format within literature, which reads as follows: (the number of carbons within the FA chain length) : (position of double bonds and the degree of unsaturation) (Williams & Buck, 2010).). Numerical shorthand such as 18:0 and 20:0 indicate FA containing chain lengths with 18 and 20 carbon atoms and no double bonds (Williams & Buck, 2010). In contrast, FA such as 20:1n-9 indicates a FA with 20 carbons with a single bond are respectively 9 carbon atoms away from the methyl (or omega; w) end of the carbon chain (Gunstone, 2012). The omega prefix (ω) and the prefix n (i.e. n-3) are used interchangeably. Omega-3 (ω−3 or n-3) LC-PUFA (≥ 20 carbons in length) are characterized by the presence of a double bond located 3 atoms from the terminal methyl end of a FA hydrocarbon chain (Sargent et al., 2002). Alternatively, omega-6 LC-PUFA are distinguished by a double bond located 6 atoms from the terminal methyl end of a FA hydrocarbon chain (Gunstone, 2012).

Rainbow trout require specific n-3 LC-PUFA inclusion at 1.0% based on diet formulation and life stage (Hardy, 2002). FA requirements must also be met to facilitate several biological processes, including movement, growth, physiological development, and cellular lipid membranes from derived phospholipids (Tocher, 2003). As FA's play a key role in the maintenance of fish health, not all required FA's can be produced in sufficient quantities via biosynthetic means and thus must be supplemented into the diet (Gladyshev et al., 2013). Salmonids require the supplementation of dietary n-3 LC-PUFA, namely EPA and DHA at inclusion rates of 13-15% as well as ALA at 0.7-1.0% to meet necessary lipid dietary requirements (NRC, 2011; Sargent et al., 2002). As EPA and DHA are synthesized primarily within aquatic environments by marine and freshwater protists, fish typically obtain these nutrients as they are transferred via aquatic food web interactions (Sargent et al., 2002).

1.5 USE OF FISH OIL (FO) AND FISH MEAL IN AQUACULTURE FEEDS

FO is currently viewed as one of the richest available sources of essential n-3 LC-PUFA in formulated aquafeeds (Sprague et al., 2017). With global fish production estimated to have reached ~179 million tonnes in 2018 and total global capture fisheries production reaching 96.4 million tonnes, increased fishing pressures have left lasting impacts on wild fish stocks (FAO, 2020). Within a 27-year span, biologically stable fish stocks have estimated to have dropped from 90% to 65.8% from 1990 to 2017 (FAO, 2020). Within aquaculture production, the industry is highly reliant on wild capture fisheries in the production of feed ingredients such as FO (Sprague et al., 2017; Osmond & Colombo, 2019). The production and application of FO for use within aquafeeds is relied upon as the main dietary lipid

source for farmed carnivorous and omnivorous fish, such as salmonid species (Sprague et al., 2017; Osmond & Colombo, 2019). With 114.5 million tonnes of live weight being produced within 2018 alone, a notable proportion of the global fisheries production is allocated to the production of fish meal and FO (FAO, 2020). However, a significant proportion of aquaculture production is deemed unusable or lost, accounting for 35% of the global harvest (FAO, 2020). Economic and population pressures on the global fishery are increasing, FO production costs are rising, and global fish consumption has increased 2-fold since the 1960s (FAO, 2020). Increasing costs of FO production have shown considerable growth from 1982 from less than 500 US $ a tonne to upwards of 1,700 US$ a tonne in 2020 (O'Higgings, 2014). Increasing costs of FO production which have doubled since the 2000's also have a direct impact fish feed production costs for specific finfish aquaculture species such as salmonids. The price of fish oil is projected to increase by 7.1% by 2029 (OECD/FAO, 2020). This, in turn, may result in a shift towards specific production preference for aquaculture species with reduced requirements for dietary FO incorporation and consumption as a cost-effective production measure (Turchini et al., 2019). Increased pressures on wild fish indicate that the continued reliance on FO as a primary lipid source for farmed salmonids is both economically and environmentally unsustainable (Colombo et al., 2020; FAO, 2020). Aquaculture must explore effective and viable alternatives to FO and fish meal usage to ensure it can meet growing production demands, maintain sustainable production for its future growth and development while continuing to provide safe and nutritious seafood for human consumption (FAO, 2020).

1.6 DIETARY LIPID ALTERNATIVES

Extensive research into viable alternatives to the use of FO as a source of dietary lipid is crucial to the improvement of aquaculture sustainability and the evaluation of FO alternative's relative to fish growth performance, tissue FA composition and further n-3 LC-PUFA synthesis and storage. Recent research identified potential dietary lipid alternatives such as terrestrial plant-based oils like *Camelina sativa,* lipids derived from animal by-products, fish production by-products, marine microbial oil derived from species such as *Schizochytrium s*p., krill and copepod oils, and insect by-products as potential candidates. With the inclusion of dietary FO alternatives, there are still a number of challenges to be met relative to their application and feasibility.

Terrestrial plant oils are commonly derived from oilseed crops grown specifically to produce consumable oils such as canola, soybean, linseed oil, and flax (Turchini et al., 2009; Rahman & de Jiménez., 2016). These oilseed crops have been explored as potentially viable alternatives to FO usage in fish in part due to their high abundance and low cost of production relative to FO (Turchini et al., 2009; Rahman & de Jiménez., 2016). Although terrestrial plant oils are naturally rich in omega-6 FA, such as linolenic acid (LNA; 18:2(n - 6), they do not contain n-3 LC-PUFA that fish physiologically require for healthy growth and development, namely EPA and DHA (Turchini et al., 2009; Hixson, 2014). Most fish species do not produce these FA from omega-3 precursors efficiently enough to meet their physiological requirements (Hixson, 2014). Typically, the replacement of FO with terrestrial plant oils in fish feeds resulted in reduced concentrations of LC-PUFA in fish muscle tissue, directly lowering their nutritional value as food and negatively impacting

fish health (Turchini et al., 2009; Sprague et al., 2017). Ideally, terrestrial plant-based oils, when used in fish feeds should provide an energy source but also provide appropriate levels of n-3 LC-PUFA's to meet the dietary needs of finfish, such as salmonid species (Sprague et al., 2017). As the production of n-3 LC-PUFA is absent among higher fully terrestrial plants, selective breeding will not result in n-3 LC-PUFA production; as such, the incorporation of genetic engineering technologies is the only viable option to alter the FA profile of these oilseeds to produce novel n-3 LC-PUFA (Sprague et al., 2017).

Terrestrial plant products may also contain anti-nutritional factors which can impede the digestive process of various fish species as well result in toxicity or changes in metabolic function and nutrient uptake (Hixon et al., 2014; Kokou & Fountoulaki, 2018). Anti-nutritional factors produced by plants are forms of secondary metabolites biosynthetically produced as means of protection from herbaceous or omnivorous predators, insects, potential viral or bacterial pathogens or adverse environmental changes (Bora, 2014). In the application of genetic engineering, there is great potential to alter or remove naturally synthesized antinutritional factors within oilseed crops for use within aquaculture feeds (Osmond & Colombo, 2019). Further challenges may also be related to the modification of these naturally produced protective compounds within plants, which may negatively impact their natural defences to insects or predatory consumption (Osmond & Colombo, 2019). The potential for antinutrient interactions may occur, which could also result in affecting tolerance levels in fish and subsequent health effects (Osmond & Colombo, 2019). At present, the most effective method if antinutritional factor reduction is through mechanical and chemical processing to enhance digestibility (Osmond & Colombo, 2019).

Animal fats have also been explored as dietary lipid alternatives to FO usage in fish feed. Animal fats derived as a by-product from poultry and cattle agricultural production tend to be high in saturated and monounsaturated FA, trace amounts of n-3 LC-PUFA and moderate concentrations of n-6 PUFA (Alhazzaa et al., 2019). Lipid sources derived from these groups have been previously examined within their application as dietary lipids within aquafeeds (Turchini et al., 2009; Glencross et al., 2020). Animal-derived lipids have relatively stable production levels, reduced costs associated with production (Gause & Trushenski, 2013, Pérez et al., 2014). The replacement of fish oil at a 75% inclusion rate has also been observed to have no negative effects on growth, nutrient metabolism and feed conversion ratio when supplemented into diets of carnivorous fish species species such as European seabass (*Dicentrarchus labrax*) juveniles (Monteiro et al., 2018). Although animal by-product lipids, such as poultry oil, can have positive impacts on growth performance, the inclusion of these lipids greatly reduces EPA and DHA availability containing 0.1% EPA and 0.2% DHA respectively (Bowyer et al., 2012). The application of these dietary lipid alternatives as a source of omega-3 FA would be insufficient in meeting the nutritional needs of fish and as such, would compromise fillet nutritional composition and fish health (Turchini et al., 2009).

As part of the search for sustainable lipid alternatives, aquatic by-products from seafood production have gained attention (Turchini et al., 2009; Tocher, 2015). Fish byproducts such as heads, skin, viscera, liver, carcass and blood have traditionally been deemed low-

value by-products due to additional processing and associated production cost of refinement and nutrient recovery (Turchini et al., 2009). Although the processing of these products can produce significant quantities of fish meal, research has explored the application of production of fish offal oil as a dietary lipid alternative (Tocher, 2015). Offal oil, as an extracted fish by-product oil, could serve as a means of supplementing n-3 LC-PUFA such as EPA and DHA into the diet of fish while, simultaneously, reducing fish feed production costs (Turchini et al., 2009). As the quantity of by-product-derived oils is dictated by species, the application of offal oil may also increase the risk of infectious zoonotic disease transfer or contaminant uptake (Tocher, 2015). Regulatory constraints along with the reduced capacity for cost-effective refinement processes in marine by-product recycling further reduce their applicability within aquafeed usage (Tocher, 2015).

Krill and copepod by-products have been evaluated as replacements for FO in fish feeds (Sprague et al., 2017). Extracted oils from marine zooplankton such as *Calanus finmarchicus* have increased concentrations of n-3 LC-PUFA at 50-70 % of their dried body weight, making them a viable alternative as a dietary lipid source within aquafeed production (Sprague et al., 2017). One challenge is that copepod derived lipids are primarily made up of wax esters with increased levels of monounsaturated fatty alcohols along with saturated FA, which are typically more difficult to metabolize at reduced temperatures (Bogevik et al., 2010). Salmon fed formulated diets containing *Calanus* oil as a lipid alternative high in wax esters (50% of dietary lipid) had reduced digestibility of saturated fatty acids and alcohols at reduced temperatures of 3°C relative to those fed at 12°C (Bogevik et al., 2010; Bogevik, 2011). Including copepod meals within formulated

diets for juvenile Atlantic halibut showed improved growth performance (Hixon et al., 2013). However, primary challenges faced the harvesting of zooplankton for aquaculture production are related to cost effectiveness and quantity of production (Tocher, 2015). Concerns have also been raised regarding harvesting lower trophic organisms and the potential impact it may have on higher vertebrate species and food source availability making it a less economically viable alternative (Sprague et al., 2017).

Insect derived by-products have been evaluated as potentially sustainable alternatives to FO and FM inclusion in formulated aquaculture diets due to nutrient composition, reduced land, energy and water requirements (Oonincx & de Boer, 2012; Henry et al., 2015). Insects are naturally incorporated as food resources into diets of both marine and freshwater finfish species as they are notably rich in nutrients and dietary fatty acids (Henry et al., 2015). A broad range of insect species are currently used within aquaculture feed such as black solider flies (*Hermetia illucens*), and mealworms (*Tenebrio molitor*) as FM and FO alternatives due to their increased protein content and FA profile. Various studies have reported positive results from the inclusion of insect meals and oils as FO alternatives within species such as juvenile Jian carp (*Cyprinus carpio* var. Jian), Atlantic salmon, yellow cat fish (*Pelteobagrus fulvidraco*), European seabass (*Dicentrarchus labrax*) and rainbow trout (Li et al., 2016; Magalhães et al., 2017; Xiao et al., 2018; Belghit et al., 2019; Chemello et al., 2020). Carp fed diets including 100% inclusion of black solider fly oil had increased concentrations of DHA within muscular tissue relative to the dietary control (Li et al., 2016). Atlantic salmon fed diets containing black soldier fly meal as a FM replacement showed no significant differences within specific growth rate ($p = 0.80$) or

sensory qualities of fish (Belghit et al., 2019). With the incorporation of insect-by products, a primary challenge faced is the processing and indigestibility of exoskeleton material due to the presence of chitin as a polysaccharide (Tschirner et al., 2017; Bandara, 2018). The use of insect meals within aquaculture diets have also reported the accumulation of pesticides within fish tissue, and although they contain PUFA, reduced concentrations further reduce their applicability as a dietary lipid alternative (Bandara, 2018).

With the incorporation of lipid alternatives, their impact on fillet sensory characteristics must also be evaluated. The evaluation of sensory properties of food products within aquaculture production focuses on gaining in-depth understanding of consumer's preferences through the collection of sensometric data (Calanche et al., 2020). This is particularly important with the inclusion of novel feed ingredients within aqua feed production which not only impact nutritional feed quality but also influence muscular fillet sensory components such as color, texture, odor and palatability. Changes in specific consumer preferences based on dietary ingredient inclusion must be taken into account as they may be a contributing factor in the influence of future ingredient supplementation decision making and consumer preferences for fillet product (Cejudo-Bastante et al.2013; Calanche et al., 2020). The comprehensive evaluation of sensometrics has been readily used within aquaculture (Calanche et al., 2020). Implementation of the use of sensory panels have been done to assit in the assurance of product quality to incorporate both human sensory perception as well as statistical and mechanical evaluation of sensory components (Calanche et al., 2020). This is often completed through specific sensory evaluations such as triangle test in which three samples, often two control and one experimental, are

compared to identify visible differences within the samples (Calanche et al., 2020). Qualitative descriptive analysis (QDA) and hedonic testing are also used as well as through mechano-sensory techniques for both color and texture analysis (Calanche et al., 2020).

1.7 NOVEL LIPID ALTERNATIVES

1.7.1 TRANSGENIC PLANT OILS

Flax and canola have been evaluated as dietary lipid alternatives to reduce reliance on FO as a primary lipid source in fish feed. One of the primary challenges with terrestrial plant seed oils is their unique FA profiles (Osmond & Colombo, 2019). Terrestrial plant-based oils are naturally rich in ALA and LNA but do not naturally contain n-3 LC-PUFA. As terrestrial plant based oils do not contain EPA and DHA, the inclusion of these oils within aquafeeds cannot replace FO as a primary source of n-3 LC-PUFA as these FA are required by fish for the maintenance of health, growth and development (NRC, 2011). In the production of commercial aquafeeds, a minimum concentration of FO incorporation is necessary to meet dietary n-3 LC-PUFA requirement. For salmonid species, such as rainbow trout, an inclusion rate of 10% is recommended for dietary feed formulation (NRC, 2011). Notably, additional energy and lipid requirements are further met through the addition of terrestrially sourced feed additives (NRC, 2011). Unfortunately, selective breeding of terrestrial plants would be ineffective as the natural synthesis of n-3 LC-PUFA has not been documented in nature. Thus, genetic engineering has been explored as a viable option to alter fatty acid profiles of terrestrial oilseed bearing plants for the production of n-3 LC-PUFA (Sprague et al., 2017).

Within aquatic primary producers such as microalgae, n-3 LC-PUFA's are biosynthetically produced through an interaction pathway of elongation and desaturation reactions. The genes responsible for these reaction pathways have all been identified and characterized in a number of microalgae species and, as such, have enabled the potential for their incorporation within hosts such as oilseed plants (Napier et al., 2015; Ruiz-Lopez et al., 2014). Qi et al. (2004) demonstrated, for the first time, that a transgenic plant was capable of EPA production, therefore expressing algal components of the alternative pathway in *Arabidopsis*. Using *Arabidopsis* as a model species, this work led to genetic engineering of commercial terrestrial-based crops such as canola (*Brassica napus*) and camelina (*Camelina sativa*; Napier et al., 2015). Previous work demonstrated that EPA could be expressed in the seed only, using a seed-specific promoter, which would enhance the yield of EPA from the oil (Abbadi et al., 2004). These initial first steps formed the basis for further iterations to drive up the accumulation of target EPA and DHA and reduce the production of undesirable biosynthetic intermediates (Napier et al., 2015).

The production process of transgenic plants occurs through editing of their genome via the addition or deletion of existing innate genes within the plant's genome. Alternatively, non-host genes, can be incorporated into the plant's genome through DNA splicing. Transgenic EPA and DHA oilseeds such as camelina was achieved by incorporating a specific set of non-host gene segments into the plant's genome to produce the n-3 LC-PUFA synthesis pathway. The incorporation of non-host genes expressed the phenotype necessary for EPA and DHA production resulting in n-3 LC-PUFA accumulation within the seed (Ruiz-Lopez et al., 2014). This included the Δ6-desaturase from *Ostococcus tauri* (OtΔ6), Δ6 fatty acid

elongase from *Physcomitrella patens* (PSE1), a Δ5-desaturase from *Thraustochytrium* sp. (TcΔ5), Δ12-desaturase from *Phytophthora sojae* (PsΔ12), n-3-desaturase from *Phytophthora infestans* (Pi-n-3), Δ5-elongase from *O. tauri*, and Δ4-desaturase from *Emiliania Huxley* (Ruiz-Lopez et al., 2014; Osmond & Colombo, 2019). These genes were selected based on their abilities to optimize and efficiently synthesize and accumulate EPA and DHA in camelina (Ruiz-Lopez et al., 2014; Osmond & Colombo, 2019). Upon isolation, the aforementioned genes were cloned and incorporated into a singular t-DNA transformation vector within agrobacterium with a promoter sequence which was seed-specific (Ruiz-Lopez et al., 2014). The use of fluorescent protein markers (DsRED) were included to assist both with the visual selection as a marker and the screening and location of successfully engineered seeds (Lu & Kang, 2008). As the plants sexually matured, seeds were rescreened and those which fluoresced from the incorporated genetic marker indicated the presence of transgenic DNA and were subsequently screened through polymerase chain reaction (PCR) (Lu & Kang, 2008). Finally, fatty acid analysis was used to confirm the level of EPA and DHA accumulation in the seed.

Two oilseed crops have since been engineered to produce EPA and DHA: camelina (*Camelina sativa*) and canola (*Brassica napus*). The primary research and development of these oils have been completed by Rothamsted research (United Kingdom, Ruiz-Lopez et al., 2014) and the Commonwealth Scientific and Industrial Research Organization (CSIRO, Australia, e.g., Petrie et al., 2014) (Osmond & Colombo, 2019). Canadian companies such as Cargill Limited, a subsidiary of the Minneapolis-based Cargill in the US, and BASF Canada have also focused on producing genetically engineered canola oils enriched with

EPA and DHA (Tocher et al., 2019). Commercial availability is still unknown but could potentially be used in aquaculture within the next decade (Napier et al., 2015). The main hurdles faced with the commercial production of these oils are related to public perception, transparency, industrial acceptance, and approval along with the researching of ecological and environmental impacts to wild species (Napier et al., 2015).

Transgenic camelina was created using a gene construct of marine microbial genes enabling the promotion of FADSD6 and ELOVL-5 genes and subsequently, the biosynthesis of EPA and DHA (Ruiz-Lopez et al., 2014; Petrie et al., 2014). Based on this work, several transgenic strains were developed, including a high EPA and DHA variety (12% EPA and 14% DHA; Ruiz-Lopez et al., 2014).

1.7.2 MICROBIAL AND MICROALGAL OILS

Microalgae, along with protists and single-celled microbes, are primary producers of high value nutrients such as n-3 LC-PUFA, amino acids, carotenoids, polysaccharides, vitamins, and minerals. These single-celled primary producers are also involved with transfers of nutritional compounds in freshwater and marine food webs and have been investigated as a potentially viable source of n-3 LC-PUFA production (Sprague et al., 2017; Tibbetts, 2018; da Silva et al., 2019; Jacob-Lopes et al., 2020). Various marine microalgae species have been cultivated using is either photoautotrophic, heterotrophic or mixotrophic production processes. Photoautotrophic production of marine microalgae species includes the supplementation of inorganic CO_2, nutrients and light. Alternatively, heterotrophic production includes the additional supplementation of nutrients and organic carbon.

Mixotrophic production processes incorporate both photoautotrophic and heterotrophic practices in algal biorefinery systems to boost microalgal cultivation (Tibbetts, 2018; Wei et al., 2021; Hart et al., 2021).

Recalcitrant algal cell walls can also be a metabolic barrier for sufficient nutrient extraction and further, EPA and DHA synthesis and assimilation (Tibbetts, 2018). Physiological and anatomical constraints in salmonid species, such as relatively short digestive tract length, require subsequent processing of algal cells prior to their incorporation into diets in order for sufficient digestion of nutrients to occur (Tibbetts, 2018). The highly diluted algal cells need to be concentrated through the process of by centrifugation, followed by chemical, enzymatic or mechanical processes to rupture algal cell walls (Tibbetts, 2018). The cultivation of micro-algal species under highly controlled fermentation conditions also results in final algal nutrient compounds which are often free of contaminants such as PCBs or heavy metals, which may occur in traditional dietary FO production (Tibbetts, 2018; Wei et al., 2021; Hart et al., 2021). The successful production and optimization of microalgal oil use is reliant upon the cost efficiency of species production and the extent of downstream processing to produce economically viable and sustainable algal by-products with increased nutrient composition. As such, innovative strategies such as the application of biotechnological processes have been investigated (Spicer & Molnar, 2018).

The first documented case of successful genetic engineering of microalgae was achieved with the green alga *Chlamydomonas reinhardii*, which incorporated a plasmid containing the yeast arg4 locus gene (Rochaix & Van Dillewijn, 1982). This early work pioneered

developments toward genetically engineered microalgae lines that produce novel levels of n-3 LC-PUFA, on par or higher than FO (Hamilton et al., 2014, 2015). The first established transgenic microalgae for enhanced n-3 LC-PUFA production was the marine diatom *Phaeodactylum tricornutum* (Hamilton et al., 2014). *Phaeodactylum tricornutum* contains up to 30% EPA, but only trace levels of DHA; therefore, this species represented an opportunity to produce higher DHA levels through genetic engineering (Hamilton et al., 2014). In addition, it sustains rapid rates of reproduction and is easy to genetically transform (Zaslavskaia et al., 2000), and has a fully sequenced genome (Bowler et al., 2008), making it an attractive system for further study (Hamilton et al., 2014). The successful transformation was completed by using particle-mediated (ballistic) gene transfer in which DNA fragments are "shot" into cells through the use of a gene gun (Hamilton et al., 2014). Algal cells were exposed to particles containing gene sequences from algal species *O. tauri* coding for Δ5-elongase and Δ6-desaturase. The resulting genetically engineered *Phaeodactylum tricornutum* showed successful increases in DHA production and accumulation producing DHA at levels substantially higher (7.4-10.4%) than the wild-type species (1.3-1.9%) (Hamilton et al., 2014; Table 2). Subsequently *Phaeodactylum tricornutum* was used to investigate the potential of genetic modification to improve the accumulation of both EPA and DHA in algal cells at rates of 8.7% and 12.3 % respectively and increasing omega-3 content up to 25.5% of total lipid (Hamilton et al., 2015). About 20 microalgae and microbial species have been genetically engineered to optimize production and synthesis of n-3 LC-PUFA (Spicer & Molnar, 2018; Osmond and Colombo, 2019). This includes developing approaches to modify microalgal strains for

potential improvements in productivity, harvestability, processibility, nutritional composition, and application (Spicer & Molnar, 2018; Osmond and Colombo, 2019).

In recent research, microorganisms such as thraustochytrids, including the genera *Schizochytrium*, have been evaluated for their production and application as novel dietary lipid sources within formulated grow out diets (Tibbetts et al., 2020). These unicellular eukaryotic organisms are often misidentified as microalgae since they do not have photosynthetic capabilities as they lack a cellular plastid. Thraustochytrids have been further taxonomically identified as marine or brackish fungal protists included within subsequent generas of *Aplanochytrium, Thraustochytrium, Japonochytrium, Ulkenia*, *Japonochytrium and Schizochytrium* (Burja et al., 2006; Leyland et al., 2017; Tibbetts et al., 2020). Thraustochytrids, such as *Schizochytrium* sp., under optimal conditions can produce high lipid concentrations (>70%) while also being rich in essential LC-PUFA such as DHA (>50% total FA) (Lewis et al., 1999; Tibbetts, 2018). *Schizochytrium* sp. can accumulate DHA at 30-40% of total FA, albeit with limited EPA production, making them a promising candidate for industrial n-3 substitution of FO through the application of SC DHA-enriched products such as Pureone®, DHAgold® and Algamac® which are commercially available within a number of ground powder, dietary oil, and supplement forms for feed applications (Ratledge et al., 2010; Adarme-Vega et al., 2012, Marchan et al., 2018; Hart et al., 2021). The substitution of *Schizochytrium* sp.as a dietary FO replacement has been evaluated in rainbow trout, Atlantic salmon (*Salmo salar),* Channel catfish (*Ictalurus punctatus*) and Nile tilapia (*Oreochromis niloticus*) as noted

further in chapter 3 and section 1.9 (Kousoulaki et al., 2015; Sarker et al., 2016; Bélanger-Lamonde et al., 2018; Kousoulaki et al., 2020; Tibbetts et al., 2020; Hart et al., 2021).

1.8 APPLICATIONS OF TRANSGENIC CAMELINA OIL IN AQUACULTURE

The natural variety of *Camelina sativa* has been evaluated as a lipid source in aquafeeds for Atlantic salmon, rainbow trout, Atlantic cod (*Gadus morhua)* as well as gilthead seabream (*Sparus aurata*) and tilapia (Hixson et al. 2014; Ofori-Mensah et al. 2020; Toyes-Vargas et al. 2020). The replacement of FO with camelina oil did not negatively impact the growth performance or sensory qualities of the fish, however, it did reduce EPA and DHA levels in muscle tissue. This was particularly observed within gilthead seabream fed diets containing camelina seed oil in which a 48% reduction in initial EPA content of whole body analysis was observed relative to fish fed a FO control (Ofori-Mensah et al. 2020). Reduced EPA and DHA concentration through the inclusion of *Camelina sativa* seed oil suggests that a minimum amount of EPA and DHA should be supplied for long-term production in aquaculture (Hixson et al. 2017).

Transgenic camelina oil has been evaluated as a lipid source in aquafeeds for Atlantic salmon and gilthead seabream. Post-smolt Atlantic salmon offered three experimental diets for 11 weeks were produced where the primary dietary lipid source (20% of the diet) was supplied by either FO, non-transgenic (wild type) camelina oil, or transgenic camelina oil with high EPA and DHA (Betancor et al., 2016a). Growth performance, digestibility, tissue FA profile and metabolic impact from liver transcriptome analysis were determined after 11 weeks. Fish fed diets containing transgenic camelina oil showed no signs of negative

impacts to fish health or growth performance compared to fish fed diets containing wild type camelina seed oil. No significant differences were found in evaluated growth or performance parameters between fish fed experimental dietary treatments including camelina oil relative to the FO control (Betancor et al., 2016a). Diets containing transgenic camelina oil also showed increased apparent digestibility of n-3 LC-PUFA. The apparent digestibility of EPA and DHA within fillet tissue of fish fed diets containing transgenic camelina (98.1 ± 0.5, EPA; 98.6 ± 0.1 DHA) was increased to a level similar to that of fish fed the FO control. Total levels of n-3 LC-PUFA in fish fed transgenic camelina oil diets were comparable to fish fed diets containing FO as a lipid source, and both were higher than fish fed non-transgenic camelina oil (30.9 ± 1.8, FO; 15.5 ± 1.1, wild camelina; 21.6 ± 0.7 transgenic camelina respectively) (Betancor et al., 2016a).

Four experimental diets were formulated containing two different transgenic strains of camelina oil, one producing EPA and DHA, and the other producing EPA only, a FO control and a wild type camelina oil as the primary lipid sources. After 11 weeks of feeding, transgenic varieties of camelina oil had no apparent negative impacts on fish growth performance; however, final weights of fish fed EPA and DHA enhanced camelina (126.9 ± 20.0[a] g) were higher than fish fed EPA enriched camelina (117.3 ± 13.6[b]) but were similar to those fed the FO control (129.9 ± 10.8[a] g) (Betancor et al., 2016b). Fish fed wild type camelina had no differences in body weight compared to EPA and EPA/DHA enhanced camelina (Betancor et al., 2016[b]). However, limited biosynthesis of these FA was observed, and the authors suggested that the n-3 LC-PUFA was accumulated through dietary sources (Betancor et al., 2016[b]). These studies suggest that transgenic camelina oil is a promising

lipid source that can replace FO in aquaculture. The evaluation of transgenic EPA and DHA camelina oil has yet to be evaluated in other species, particularly freshwater species, including rainbow trout.

1.9 APPLICATIONS OF *SCHIZOCHYTRIUM* SP. OIL IN AQUACULTURE

Within aquaculture feed production, microalgae have been established as a means of improving dietary nutrient enrichment of larval fish due in part due to their rich FA profiles and presence of biosynthetically produced EPA and DHA. A key challenge to improving their applications and usage within aquaculture feed production as a potential dietary FO replacement lies in increasing production volumes and reducing production costs to meet the large feed requirements of fish during the grow-out stage to market size (Sprague et al., 2017; Tibbetts, 2018; de Carvalho et al., 2020). Previous usage of microbial oils such as *Schizochytrium* sp. have also been successfully substituted for FO in diets from Nile tilapia; parr and post- smolt Atlantic salmon and rainbow trout (Miller et al., 2007; Sarker et al., 2016; Santigosa et al., 2020; Tibbetts et al., 2020, Wei et al., 2021).

Nile tilapia diets replaced 100% of FO with *Schizochytrium* sp. oil and tilapia were fed for a period of 12 weeks (Sarker et al., 2016). The substitution of 100% *Schizochytrium* sp. oil showed increased weight gain and protein efficiency ratio with better feed conversion ratio compared to diets containing the FO (Sarker et al., 2016). As *Schizochytrium* sp. oil also had increased DHA content (43.2 % of total fatty acids) relative to the FO control (13% of total fatty acids), DHA content in the muscle tissue of fish fed the high *Schizochytrium* sp.

oil diet at 100% inclusion had increased concentrations (261.8 ± 19.3 mg/100g) relative to the FO control (143.5 ± 12.2 mg/100g) (Sarker et al., 2016).

Atlantic salmon parr (38.5-40.0 g initial weight) were also examined in the replacement of FO with a high DHA *Schizochytrium* sp. oil (Miller et al., 2007). In this trial, four experimental diets were formulated to include *Schizochytrium* sp. oil at 100% dietary oil inclusion, 100% palm oil, and 4:1 ratio of palm oil and *Schizochytrium* sp. oil mixture in the replacement of FO and was fed to the parr over 9 weeks (Miller et al., 2007). There were no significant differences in the apparent digestibility of n-3 and n-6 PUFA, feed consumption, or salmon growth (Miller et al., 2007). DHA content (% of total) was significantly higher in muscle of salmon fed diets containing 100% replacement of FO with *Schizochytrium* sp. oil (23.4 ± 0.4 %) relative to fish fed the fish oil control (12.8 ± 0.2%) (Miller et al., 2007).

In its transgenic form, a novel strain of *Schizochytrium* sp. (T18) oil was initially evaluated for its use in salmonid aquaculture as a microbial oil alternative in FO in diets formulated for juvenile Atlantic salmon (Tibbetts et al., 2020). Four experimental diets were formulated to replace 0, 33, 66 and 100% of FO with *Schizochytrium* sp. (T18) oil containing 50% LC-PUFA, of which 82% was made up of DHA (Tibbetts et al., 2020). Apparent digestibility of key essential dietary nutrients such as lipid and protein was independent of Schizo inclusion rate, with an increase in DHA digestibility relative to the control (Tibbetts et al., 2020). Since this experiment was the first to evaluate the effectiveness of *Schizochytrium* sp. (T18) as a lipid alternative within salmonids, this

transgenic oil has yet to be evaluated within rainbow trout as a source of dietary lipid. Therefore, further research is necessary to evaluate impacts on growth and developmental processes in alternative salmonid species such as rainbow trout and determine if promising results would be applicable and repeatable within this freshwater species.

Recent research has evaluated the application of the T-18 strain oil in diets for Atlantic salmon parr (24 g initial weight) (Wei et al., 2021). Four experimental diets were fed to Atlantic salmon for 16 weeks: a reference diet (20% FO), a commercial-type control diet (10% FO + 10% vegetable oil), a low microbial oil diet (5% MO) and a high microbial oil diet (10% MO) (Wei et al., 2021). Overall, the inclusion of the T-18 strain oil resulted in no differences in weight gain, growth rate, condition factor or feed conversion ratio. Muscle fatty acid (FA) content was also noted to reflect dietary composition (Wei et al., 2021).

Previous trials also have examined the application of a different strain of *Schizochytrium* sp. which contains both EPA (15.7%) and DHA (39.8%) in diets for rainbow trout (331 ± 1.0 g initial weight) and its effect on digestibility, n-3 LC-PUFA concentration within muscle tissue, growth, and palatability (Santigosa et al., 2020). Three experimental trials were conducted to evaluate the dietary inclusion of Veramaris® algal oil (isolated from *Schizochytrium* sp.) at rates of 2.5, 3.0, 4.5, 5.0, 6.0 and 10% (Santigosa et al., 2020). Trout were fed over 39 d in trial 1, 26 d in trial 2, and 96 d in trial 3. Overall, dietary inclusion of *Schizochytrium sp.* oil showed high digestibility as apparent digestibility of EPA and DHA was above 99% in diets containing Schizochytrium sp. oil when compared to fish fed the FO control ($p < 0.0001$). FA profiles of trout fillets were

reflective of experimental diets. Growth, blood parameters, and feed conversion ratio were independent of diet (Santigosa et al., 2020).

Incorporating sustainable micro-algae production systems has high potential in its applications to aid in sustainable sea food production and assist in the "blue revolution" (Tibbetts, 2018). The exploration of implementing eco-friendly, sustainable, and nutritionally complete aquaculture products via micro-algae introduction is necessary as these organisms can be grown in tandem with industrial waste by-product output (Tibbetts et al., 2018; Yarnold et al., 2019; Hart et al., 2021). Nutrient production, excess heat and CO2 can be used by several microalgae species for increased growth and production of biomass for further cultivation of nutrient by-products (Tibbetts et al., 2018; Yarnold et al., 2019; Hart et al., 2021). The production of microalgae species also does not have the same requirement for land and aquatic resources and therefore can produced within a variety of locations with reduced limitations to biomass harvesting as it can be made available year-round (Tibbetts et al., 2018; Wei et al., 2021). Species, strain section, growth rate and n-3 LC-PUFA production capabilities and metabolic rates are key components and ideal characteristics for an effective feed additive (Sarker et al., 2016; Sprague et al., 2017). These components should be considered in order for micro-algae oil to be feasible for usage in large scale aquaculture feed production sustainability (Sarker et al., 2016; Sprague et al., 2017).

1.10 OBJECTIVES

The main objectives of this thesis were to evaluate the effects of both transgenic *Camelina sativa* oil (EPA and DHA) (Chapter 2) and high DHA *Schizochytrium* sp. oil (strain T-18) (Chapter 3) as a complete replacement of FO in diets for farmed rainbow trout. The potential of these lipid alternatives was based on their effects on fish growth, tissue-specific FA composition, n-3 LC-PUFA storage and synthesis, and fillet sensory properties. Two experiments were conducted over separate periods; 12 weeks in experiment 1 using transgenic *Camelina sativa* and 8 weeks in experiment 2 using a high DHA *Schizochytrium* sp. oil to determine the effectiveness of transgenic camelina oil and high DHA-*Schizochytrium* sp. oil as a lipid source for juvenile rainbow trout and the following objectives:

1) Evaluate transgenic camelina in diets for rainbow trout to determine the impact on growth performance, tissue FA composition, and sensory properties of the fillets.
2) Quantify the effect of transgenic *Schizochytrium* sp. oil (T-18) in diets for rainbow trout on growth performance, tissue FA composition, and n-3 LC-PUFA synthesis and storage within muscle and liver tissue.

The results of this thesis have been submitted as two independent manuscripts and accepted for publishing. Chapter 2 addresses objective 1 and the results were submitted to the *Journal of the World Aquaculture Society* and published on March 30th, 2021. Chapter 3 addresses objective 2 and the results were submitted to the *Journal of Animals; Special*

Issue "Recent Advances in Fish Nutrition: Insights on the Nutritional Implications of Modern Formulations" and was published on April 21st, 2021.

CHAPTER 2.0
TRANSGENIC CAMELINA OIL IS A SUITABLE SOURCE OF EPA AND DHA IN DIETS FOR RAINBOW TROUT, IN TERMS OF GROWTH, TISSUE FATTY ACID CONTENT, AND FILLET SENSORY PROPERTIES

2.1 ABSTRACT

The oilseed *Camelina sativa* was genetically engineered to produce EPA and DHA at levels similar to fish oil, which was tested as a lipid source in diets for rainbow trout. Three experimental diets were tested, a fish oil control (FO; 100 g/kg fish oil), a low-level transgenic camelina oil (LCO) diet (no FO with 12.5 g/kg camelina) and a high-level transgenic camelina oil (HCO) diet (no FO with 130 g/kg transgenic camelina). Trout (initial mean weight 49.8 ± 11 SD g) were fed for 12 weeks in freshwater at 12 °C, and were evaluated for growth performance, fatty acid content (muscle, liver, brain, and eye), and sensory properties of fillets. Final mean fork length and body weight of fish fed LCO and HCO diets were significantly higher compared to fish fed FO ($p = 0.008$; $p = 0.001$). There were no differences in weight gain, condition factor, specific growth rate, feed intake, and feed conversion ratio. EPA in muscular fillet tissue was higher in fish fed HCO and FO diets compared to the LCO diet ($p = 0.014$). Compound specific isotope analysis revealed trout fed the FO diet stored isotopically-enriched DHA compared to the lighter DHA in fish fed HCO. Trout fillets from the HCO treatment were firmer in texture ($p = 0.001$) and had a higher orange intensity compared to the FO group ($p = 0.012$).

2.2. INTRODUCTION

Omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA) play a key role in physiological functions in all vertebrate organisms, where they contribute to cardiovascular health, growth, reproduction, and immune system function (Brenna et al. 2009; Swanson

et al. 2012; Calder 2015). N-3 LC-PUFA also supports and maintains neurological development, function and visual acuity in both brain and eye tissue these areas are specified retention sites of n-3 LC-PUFA due to the presence of neurological tissue (Brenna et al. 2009; Swanson et al. 2012; Calder 2015).

As many vertebrates are generally unable to efficiently synthesize the n-3 LC-PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in sufficient quantities to meet nutritional needs, these FA are typically obtained via dietary consumption (Arts et al. 2001; Calder 2015). EPA and DHA are primarily produced by microalgae and wild fish obtain these FA as they are trophically transferred through food webs. The most common dietary source of EPA and DHA is directly consumed from seafood, particularly from fish, as the natural production of these FA, on a global scale, is primarily aquatic-based (Colombo et al. 2020).

Culturing salmonid fishes requires a dietary source of EPA and DHA at levels up to 1% of the dry diet for normal growth and development, though specific inclusion rates vary depending on species and life stage (NRC, 2011). Wild-sourced fish oil is the main commercial source of dietary EPA and DHA, however, increases in aquaculture production and continued depletion of wild stocks demands the identification and validation of new dietary sources of EPA and DHA. For the most part, commercial aquaculture feeds have reduced FO levels by increased inclusion of other terrestrial-based lipid sources (Turchini et al. 2018). For example, nearly 70% of the oil fraction in feeds for Atlantic salmon in Norway consists of canola oil (Ruyter et al. 2019). However, some FO and fish meal must

remain to supply EPA and DHA, as terrestrial oilseed plants do not naturally produce n-3 LC-PUFA. This not only reduces the EPA and DHA in the feed, but also directly impacts the lipid profile of the final fish fillet for human consumption (Sprague et al., 2016). Consequently, the reliance on FO as a source of dietary EPA and DHA has become a major constraint for further growth of the global aquaculture industry.

The use of genetically engineered products in aquafeeds is emerging (Osmond and Colombo, 2019). The crucifer oilseed, *Camelina sativa*, in its wild form, is naturally high in the n-3 LC-PUFA precursor, alpha-linolenic acid (ALA, 18:3n-3), but does not produce either EPA or DHA. Transgenic camelina, however, produces EPA and DHA at levels comparable to that of FO (Napier et al. 2015). A specific set of seven non-host gene segments, mainly from marine microalgae, were used to produce the n-3 LC-PUFA synthesis pathway, with the expressed phenotype of EPA and DHA accumulation in the seeds of camelina (Ruiz-Lopez et al. 2014). The oil, extracted from this transgenic camelina, has been tested as a lipid source in diets for Atlantic salmon (*Salmo salar*) and gilthead seabream (*Sparus aurata*) (Betancor et al., 2015; Betancor et al., 2016a; Betancor et al., 2016b; Betancor et al., 2017). However, it has not yet been tested on rainbow trout.

In post-smolt Atlantic salmon, transgenic camelina oil was highly digestible overall, and had high apparent digestibility values for EPA and DHA (Betancor et al. 2016a). In that study, salmon fed transgenic camelina oil (20% of the diet) with EPA and DHA showed no signs of negative impacts to fish health or growth performance compared to salmon fed diets with FO or non-transgenic camelina seed oil over the 11-week study (Betancor et al. 2016a). Total levels of n-3 LC-PUFA in Atlantic salmon fed transgenic camelina oil diets

were comparable to fish fed diets containing FO as a lipid source, and both were significantly higher compared to fish fed non-transgenic camelina oil (Betancor et al., 2016a). Similar results were observed in gilthead seabream (Betancor et al. 2016b). In the study with gilthead seabream, another variety of transgenic camelina was tested that contained EPA only, in addition to the transgenic camelina oil with EPA and DHA. After 11 weeks of feeding, the transgenic varieties of camelina oil had no apparent negative impacts on fish growth performance; however, final weight of fish fed diets containing transgenic camelina with EPA only were slightly lower in comparison to fish fed transgenic camelina with both EPA and DHA, and to fish fed the control diet (Betancor et al., 2016a). It should be noted that as limited biosynthesis of these FA was observed, the n-3 LC-PUFA were said to be accumulated through dietary sources (Betancor et al., 2016a, b). Another transgenic oilseed has also been tested as a dietary lipid source for Atlantic salmon. Fingerling salmon fed diets with transgenic canola oil (with high DHA) for up to 83 days had approximately the same EPA and DHA content in whole body as salmon fed FO diets (Ruyter et al. 2019). Gene expression, lipid composition and oxidative stress-related enzyme activities showed only minor differences between the dietary groups. Collectively, these studies suggest that transgenic oilseeds represent promising lipid sources that could completely replace FO in aquafeeds.

With the application of these novel oils, interest has also grown on the source identification of FA from dietary feed ingredients through the evaluation of compound specific stable isotope analysis (CSIA) (Phillips, D., Newsome, S., & Gregg, J., 2005). CSIA works on the premise of measuring carbon ($^{13}C/^{12}C$) and the ability to identify specific isotopic signatures from carbon within FA as environmental tracers to determine FA origin and its

specific contribution to tissues through nutrient transfer (Phillips, D., Newsome, S., & Gregg, J., 2005; Hixson et al., 2014a). Understanding and tracing carbon origin through these means is highly important with the introduction and inclusion of transgenic plant-based oils, to determine whether stored FA are accumulated from a transgenic terrestrial oil source, or selectively retained (Phillips, D., Newsome, S., & Gregg, J., 2005). The application of CSIA FA analysis has previously been examined within finfish species such as juvenile tilapia (*Oreochromis niloticus*), rainbow trout and Atlantic Salmon fingerlings (Hixson et al., 2014; Toyes-Vargas et al., 2020; Lee Chang et al., 2020).

The evaluation of sensory properties is also particularly important with the inclusion of novel feed ingredients within aqua feed production, which not only impact nutritional feed quality, but also influence muscular fillet sensory components such as color, texture, odor, and palatability. This is crucial within aquaculture production of food products to gain an in-depth understanding of consumer's preferences through the collection of sensometric data (Calanche et al., 2020). Changes in specific consumer preferences based on dietary ingredient inclusion must be taken in to account as they may be a contributing factor in the influence of future ingredient supplementation decision making and consumer preferences for fillet product (Cejudo-Bastante et al.2013; Calanche et al., 2020) The comprehensive evaluation of sensometrics has been readily used within aquaculture through the implementation of the use of sensory panel in the assurance of product quality to incorporate both human sensory perception as well as statistical and mechanical evaluation of sensory components (Calanche et al., 2020). This is often completed through specific sensory evaluations such as a sensory panel visual or taste evaluation accompanied by the

completion of sensory evaluation tests such as triangle, QDA and Hedonic testing as well as through mechano-sensory techniques for the statistical analysis and comparison of both fillet colour and texture (Calanche et al., 2020). The application of transgenic camelina has yet to be evaluated in another commercially relevant salmonid species, such as rainbow trout (*Oncorhynchus mykiss*).

Rainbow trout is of particular interest in North America, as it is one of the most widely produced commercial species. In Canada, production was over 9,000 tonnes and revenue from fish sales totaled $56.8 million CAD in 2018 (DFO, 2018). In the US, production was over 21,000 tonnes and revenue from fish sales totaled $95.6 million USD in 2019 (USDA, 2020; FAO, 2020). Although rainbow trout do have the ability (albeit limited) to synthesize n-3 LC-PUFA when provided with essential dietary ALA, they still require EPA and DHA supplementation in intensive aquaculture production for optimal growth and development (Hixson et al. 2014a) and final product quality (Yildiz et al. 2018; Thanuthong et al. 2011). In this study, we evaluated the efficacy of transgenic camelina as a dietary lipid source for juvenile rainbow trout, in terms of growth performance, as well as n-3 LC-PUFA storage and synthesis. Three experimental diets were formulated with a low camelina oil inclusion (LCO) diet representing a practical, commercial diet without FO to determine if a minimum inclusion of transgenic camelina would be sufficient in providing n-3 LC-PUFA for acceptable growth performance (Table 2.3). The (high camelina oil) HCO diet uses transgenic camelina as a complete FO replacement, while also providing the majority of dietary lipid (Table 2.3). A fish oil (FO) control was also used to represent a commercial diet formulation. We also used compound specific stable isotope analysis (CSIA) as a tool to discriminate the origin of DHA in the muscle tissue of the rainbow trout. With the

introduction of transgenic plant-based oils, it is essential to determine how EPA and DHA, obtained from a plant-based source, is metabolized, and used in comparison to EPA and DHA from a marine-based oil. The overall aim of this study was to determine the impact of transgenic camelina oil on fish growth performance, tissue-specific fatty acid composition and sensory properties of juvenile rainbow trout. We hypothesize that transgenic camelina oil can fully replace fish oil in rainbow trout diets at commercially relevant inclusion levels (i.e., 100 g/kg).

2.3 MATERIALS AND METHODS

2.3.1 TEST INGREDIENT

The transgenic camelina seed oil (DHA2015.1) was produced by Rothamsted Research (West Commons, UK; Han et al. 2020). The oil contained 9.5% EPA and 7.8% DHA, for a total n-3 LC-PUFA proportion of 17.3% (Table 2.1). It also included ALA (12.2%), linoleic acid (LNA; 18:2n-6; 21.0%) and oleic acid (18:1n-9; 5.8%). The sum of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA were found in the following proportions, 14.9%, 15.8%, and 69.4%, respectively (Table 2.1). Total n-3 and total n-6 were 40.1% and 29.3%, respectively. The n-3/n-6 ratio was 1.4.

2.3.2 EXPERIMENTAL DIET FORMULATION AND COMPOSITION

All diets were formulated as isonitrogenous and isolipidic to meet the requirements of rainbow trout (NRC 2011). Diets were produced at the Chute Animal Nutrition Centre, Faculty of Agriculture, Dalhousie University (Truro, NS, Canada). Three experimental

treatments were produced as follows: a control diet containing a fish (herring) oil (FO; 100 g/kg) and canola oil (100 g/kg) blend, a low transgenic camelina oil inclusion (LCO; no FO with 12.5 g/kg) transgenic camelina) and canola oil (70 g/kg) blend, and a high transgenic camelina oil inclusion (HCO; no FO with 130 g/kg transgenic camelina) canola oil (70 g/kg) blend (Table 2.2). In addition to the transgenic camelina oil, FO, and canola oil lipid sources, fish meal (10% lipid), ground wheat (1.8% lipid), poultry by product meal (14% lipid), corn protein concentrate (2% lipid), and soybean meal (1% lipid) all contributed dietary lipid; however, remained consistent across all treatments. Diet mash was steam pelleted through a California Steam Pellet Mill (San Francisco, USA) with a 3 mm or 5 mm die plate. Pellets were dried in a JWP ST series industrial cabinet oven at 60°C for 4h. Excessive fines were sifted using a 3 mm sieve. Diets were stored at -20°C in airtight and darkened containers to reduce oxidization of fats until needed. Diets were only exposed to room temperature during periods of feeding. Fish were fed 3.0 mm pellets at the beginning of the experiment; however, pellet size was increased to 5.0 mm as fish increased in size.

2.3.3 EXPERIMENTAL FISH

Juvenile rainbow trout (n=225; 49.8 ± 10.9 g · fish^{-1} initial body weight ± SD averaged from initial week 0 sampling of n=45 trout) were obtained from Fraser's Mills Hatchery (Antigonish, NS, Canada) and transported to the Aquaculture Centre, Faculty of Agriculture, Dalhousie University in July, 2018 (Truro, NS, Canada). Guidelines for ethical treatment of fish were followed by the Canadian Council of Animal Care (Dalhousie, approved protocol #2017-101). The rainbow trout were pooled in a rearing tank at 12°C were acclimated for two week and fed EWOS Vita 3mm; complete fee for salmonids, prior

to being fed the experimental diets. The rainbow trout were randomly distributed into nine 203 L circular fiberglass experimental tanks, with each tank containing 25 fish. Fish were not tagged for identification purposes. Each experimental diet was fed to three replicate tanks, with tank as the experimental unit. Each tank was supplied with a flow-through system of freshwater at a rate of 2-3 L $\cdot min^{-1}$, with water supplied from the local aquifer on campus (ambient pH of 7.8 and alkalinity of 120 ppm as CaCO3). The dissolved oxygen concentration (10.77mg$\cdot$ L^{-1}, 100%) and target water temperature (12°C) were monitored and recorded daily. Fish were reared on a simulated natural photoperiod cycle at Latitude 45°N over the 12-week trial period starting on July 6th 2018 (15 h light: 9 h dark) and ending on October 4th 2018 (with 13 h light : 11 h dark). Fish were fed twice daily at 9:00 and 15:00h to apparent satiation and the amount offered to each tank recorded. There were no observed mortalities throughout the trial.

2.3.4 TISSUE SAMPLING

Fish sampling occurred initially at week 0, the day before feeding the experimental diets and final sampling occurred after 12 weeks of feeding. Five fish per tank were randomly netted and euthanized with an overdose of anesthetic (tricaine methane sulfonate; MS-222). The five fish sampled from each tank were weighed using a tarred weight scale and initial body weights at week 0 (to 0.1g) were recorded only for these fish. Fork length (to 0.1cm) was measured and recorded. The whole viscera was removed, weighed (to 0.1g), and recorded. Liver tissue samples were taken for lipid analysis. Skin was removed from the left side of the fish under the dorsal fin, above the lateral line and skin-less dorsal muscle tissue was sampled from this location for lipid and protein analysis. At week 12, the same

sampling regime was conducted, however brain and eye tissue were also sampled at this time. Brain tissue was carefully removed through a vertical incision made at the top of the cranium at its center using scissors. Tweezers were then used to carefully remove and sample brain tissue. The eye was removed through the insertion of small surgical scissors into the ocular cavity to snip connective tissue to and the use of tweezers extract the eye tissue. All tissue samples were placed in 2 ml cryogenic vials, flash frozen in liquid nitrogen, and stored at −80 °C until analysis.

2.4 ANALYTICAL METHODS

2.4.1 GROWTH PERFORMANCE

Growth performance was assessed at Week 0 (initial) and Week 12 (final) sampling. Initial mean body weight was determined from initial sampling of rainbow trout weights recorded at week 0. Final body weight, final fork length, weight gain, specific growth rate, condition factor, apparent feed intake, feed conversion ratio and visceral somatic index was determined based on the following equations:

$\text{Weight gain (g per fish)} = (\text{final weight} - \text{initial weight})$, (calculated by individual fish)

$\text{Condition factor} = \frac{\text{(Body mass g)}}{\text{(Fork length cm)}^{\wedge}3} * 100$ (calculated by individual fish)

$\text{Visceral somatic index}(\%) = 100 * (\frac{\text{Viscera mass (g)}}{\text{body mass (g)}})$, (calculated by individual fish)

$\text{Specific Growth Rate } (\%\ \text{day}^{-1}) = \frac{(\ln(\text{final mean body weight}) - \ln(\text{initial mean body weight}))}{\text{number of days in period}} * 100$, (calculated by tank)

$$\text{Apparent feed intake (g fish}-^{1}) = \frac{\text{(feed offered,g)}}{\text{(number of fish per tank)}}$$, (calculated by tank)

$$\text{Feed conversion ratio (g fish}-^{1}\text{)} = \frac{\text{(feed intake,fish}-1)}{\text{(weight gain,g fish}-1)}$$, (calculated by tank)

2.4.2 DIET AND TISSUE NUTRIENT COMPOSITION

Dry matter and nutritional composition of the diets was analyzed at the Department of Agriculture Laboratory Services (Truro, NS, Canada). Crude protein of rainbow trout muscle tissue was analyzed using a LECO FP-528 Nitrogen analyzer (Model FP-528, Leco Coperation, St. Joseph, MI, USA) using the conversion of % nitrogen x 6.25.

2.4.3 TOTAL LIPID AND FATTY ACIDS

Total lipid and fatty acid content were analyzed from liver and muscle tissue sampled from fish at both weeks 0 and 12, as well as whole brain and single eye tissue at week 12. Tissue samples were placed into 2 ml micro centrifuge tubes, flash frozen in liquid nitrogen and were stored in a -80°C freezer until they were shipped to Ryerson University (Toronto, ON, Canada) for fatty acid analysis. Muscle and liver tissues were freeze-dried and individually ground to a fine powder in liquid nitrogen using a ceramic mortar and pestle (which were washed with soap and water and lipid-cleaned three times with 2 mL chloroform:methanol (2:1; v/v) between each sample), and the resulting powder was subsampled and weighed to the nearest microgram. Total lipid was extracted using a modified Folch method (Folch et al., 1957). Each sample was extracted three times, using 2 mL of chloroform/methanol (2:1; v/v) and then pooled (total 6 mL). Polar impurities were removed by adding 1.6 mL of KCl solution (0.9% w/v). The organic layer was removed using a lipid-cleaned glass pipette and

pooled. The resulting lipid-containing solvent was concentrated to 2 mL by evaporating with nitrogen gas. The lipid extract was then prepared for gas chromatography (GC) by derivatizing into fatty acid methyl esters (FAME) using the Hildich reagent (1.5 H_2SO_4: 100 anhydrous MeOH) as the catalyst (Christie et al., 2003). Reagents were added in the proportion of 1.5 mL reagent per 4–16 mg of lipid. Samples were heated at 90°C for 90 min and vortexed halfway through the derivatization reaction. The FAME were extracted twice using hexane: diethyl ether (1:1; v/v), then dried under a gentle stream of nitrogen. The dry FAME extract was re-dissolved in hexane and individual FAME were separated using a GC Shimadzu-2010 Plus, (Nakagyo-ku, Kyoto, Japan) equipped with an SP-2560 column (Sigma-Aldrich, St. Louis, Missouri). All solvents used in the extraction and FAME derivatization procedures were of high purity HPLC grade (>99%). FAME in samples were identified by comparison of their retention times with a known standard (GLC-463 reference standard; Nu-chek Prep, Inc., Waterville, Minnesota) and quantified with a 5-point calibration curve using this same standard. A known concentration of 5 alpha-cholestane (C8003, Sigma-Aldrich, St. Louis, MI, USA) was added to each sample prior to extraction to act as the internal standard to estimate extraction and instrument recovery efficiency. Fatty acid methyl esters (FAME) were sent from Ryerson University to the University of Toronto (Toronto, ON, Canada) for compound specific stable isotope analysis.

2.4.4 COMPOUND SPECIFIC STABLE ISOTOPE ANALYSIS

The $\delta^{13}C$ values of DHA were analyzed by GC-combustion-isotope mass spectrometry (GC-C-IRMS) following methods by Lacombe et al. (2017). FAME from transgenic

camelina oil, FO, and rainbow trout muscle tissue from FO and HCO groups were analyzed. All $\delta^{13}C$ values were reported relative to the Vienna Pee Dee Belemnite (VPDB) standard. An aliquot of the methanol used during methylation of FA was analyzed for the $\delta^{13}C$ composition. The average $\delta^{13}C$ of methanol was used to correct for the additional methyl group added to FA during transesterification, by subtracting the proportional contribution of methanol to the $\delta^{13}C$ of FAME.

2.4.5 SENSORY PROPERTIES

Texture and color analyses were completed at the Faculty of Agriculture, Dalhousie University with laboratory equipment made available through Dr. Rathgeber's laboratory (Truro, NS, Canada). Texture and color analyses were performed on fillet samples (n=9 per treatment) that were previously frozen at -18° C in October 2018, for a period of three months and thawed in January 2019 prior to testing. Fillets were transferred to a refrigerator (4°C) 24 h prior to sampling to thaw before analysis was undertaken (FAO, 2018). Each fillet was analyzed mid-loin, below the dorsal fin and above the lateral line. Each fillet sample was analyzed twice, and the average was used for data analysis.

Texture of the fillets was evaluated utilizing a TA.XT texture analyzer (Texture Technologies Corp., Scarsdale, New York, US) equipped with a 9.5 mm cylindrical probe and data was recorded using Exponent stable micro systems software. The probe was pressed into the fillet at a pre-test speed of 5.0 mm $\cdot$ s^{-1}. The probe penetration depth was 50% of the fillet thickness. The fillet thickness (mm) and the necessary force required to

penetrate the surface (the breakpoint force) along with the maximum force (max force) reached during compression were recorded generating a fillet texture average.

The instrumental color analysis of the fillets was completed using a Hunter Lab MiniScan EZ 45/0 LAV (Hunter Associates Lab, Inc., Reston, VA, US) colorimeter with EZMQC-OPT EasyMatch Quality Control Software. Color analysis was completed on the same fillet samples used for texture analysis. The Hunter color scale was used to determine lightness (ΔL), redness (Δa), and yellowness (Δb). Values were generated using daylight setting (D65, CIE L, a, b).

Fish were sampled for the sensory evaluation panel one day after the week 12 final sampling period. The HCO and FO treatments only were presented to the sensory evaluation panel as the most distinct treatments that potentially could have detectable differences to untrained panelists. No taste testing occurred as sensory evaluation was completed by visual analysis only. Prior to sampling, three fish from each dietary group were euthanized via submersion in tricaine methanesulfonate (MS-222). Fish were filleted and portioned into ~3 cm x 3 cm squares from the mid-loin region, below the dorsal fin and above the lateral line, and placed into clear plastic sampling cups. The sensory evaluation was completed in a lab with standard lighting at the Aquaculture Centre. An untrained panel (n=24) of volunteers was recruited that consisted of faculty, staff, and students at the Faculty of Agriculture, Dalhousie University. Panelists were briefed in the testing procedure and fillet evaluation techniques. Board approval was not necessary as this particular test was not a test in which consumption of fish fillets was being utilized as a sensory measure. Untrained panelists were used in this study to evaluate consumer

preferences, to complement the instrumental analyses for color and texture. Panelists were introduced to different evaluation techniques such as smelling and evaluating texture and color of the fillet tissue. Evaluation of the different testing procedures was discussed. The panelists completed a triangle test, a hedonic test, and a quantitative descriptive analysis (QDA) as in Hixson et al. (2014b, 2017) to evaluate fillet samples in terms of appearance, texture, odor, and color. Participants were given specific instructions on how to evaluate the given samples for appearance, texture, odor, and color, prior to beginning the test. Three fillet samples were provided to each volunteer: two fillet samples from the FO control group and one was from the HCO diet group. The triangle test required panelists to determine which one of the three provided samples appeared to be different in terms of texture, odor, color, and appearance. For 24 panelists, 13 assessors in a triangle test are required to give correct judgments at the 5% level. In the hedonic test, panelists were asked to rate fillets on a two anchored linear scale for odor, texture, and appearance of individual samples as per (Hixson et al., 2014b, 2017). Panelists were also required to complete an objective QDA test in which they were asked to rate fillet samples on a scale from 1-7 (1 = no intensity; 7= distinct intensity) for a number of traits (such as brightness and orange intensity) as per (Hixson et al., 2014b, 2017).

2.5 STATISTICAL ANALYSIS

Growth performance results were analyzed by ANOVA using the general linear model in Minitab 18 Statistical Software. For individual measurements such as body weight, fork length, viscera somatic index, condition factor, a two-level nested ANOVA was used to analyze growth data. This model was designed to test the effect of diet treatment (fixed

factor) on the growth performance (response variable) and nested fish individuals (random factor) within tanks, to remove variability among fish within tanks, while also testing for effects of individual tanks (Ruohonen, 1998). For measurements that were based on tank means, fish individuals were not independent for measurements such as weight gain, specific growth rate, feed intake, feed conversion ratio, a one-way ANOVA was conducted to test the effect of diet. Tukey HSD post-hoc tests ($p < 0.05$ significance level) were applied to assess differences among treatments.

For lipid and fatty acid content of the muscle, liver, brain, and eye tissue, as well as instrumental analyses of color and texture, a one-way ANOVA was used to detect treatment differences, followed by a Tukey post-hoc test for multiple comparisons ($p < 0.05$ significance level). To determine whole fatty acid profile changes in individual fish tissues among treatments, multivariate analyses, including permutational multivariate analysis of variance (PERMANOVA) and Principal Coordinates Analysis (PCoA) were used. The non-metric Bray–Curtis dissimilarity statistic was used to quantify the compositional dissimilarity between samples in the PCO plot (Bray and Curtis 1957). Multivariate statistics were determined using PRIMER (PRIMER-E, version 7.0.13, Plymouth, UK).

2.6 RESULTS

2.6.1 GROWTH PERFORMANCE

Final mean body weight of trout fed the HCO and LCO was significantly greater than trout fed the FO diet ($p = 0.001$; Table 2.4). Fork length was increased in trout fed the HCO diet than fish fed the FO diets ($p = 0.008$). Final VSI of fish fed the LCO diet was significantly

higher in comparison to fish fed the FO diet ($p = 0.014$). Final VSI of fish fed LCO and HCO diets and FO and HCO diets were not significantly different. There were no significant differences in the initial weight ($p = 0.328$), initial length ($p = 0.062$), initial condition factor ($p = 0.422$), initial VSI ($p = 0.640$), final condition factor ($p = 0.099$), SGR ($p = 0.092$), weight gain ($p = 0.115$), FCR ($p = 0.145$) and apparent feed intake ($p = 0.144$) among treatments.

2.6.2 PROTEIN AND DRY MATTER COMPOSITION OF MUSCLE AND LIVER

There was no difference in protein content of muscle tissue among rainbow trout depending on treatment in either week 0 or week 12 ($p > 0.05$) (Table 2.5). Muscle tissue dry weight showed no differences among treatments (Table 2.5). Liver tissue at week 0 from trout fed FO diets had higher dry matter content than trout that were fed the LCO diet ($p = 0.021$) (Table 2.6). At week 12, liver tissue showed no differences in dry matter content in fish fed any of the experimental diets ($p = 0.099$).

2.6.3 FATTY ACID CONTENT OF MUSCLE TISSUE

Overall, the fatty acid content ($\mu g \cdot mg^{-1}$, dry weight) of rainbow trout muscle tissue showed notable differences in fish fed diets containing transgenic camelina oil in comparison to the FO diet (Table 2.5). ALA was significantly higher in fish fed the LCO (23.6 ± 8.9^{a} μg/mg) diet when compared to HCO (10.9 ± 2.7^{c}) and FO diets (6.2 ± 2.4^{b}) ($p < 0.0001$). EPA accumulated in higher amounts in trout fed the FO diet (5.8 ± 4.8^{a}) compared to trout fed the LCO diet (2.1 ± 1.3^{b}); however, no differences were found between fish fed FO and

HCO diets (3.5 ± 2.7^{ab}) ($p = 0.014$). DHA was also stored in higher amounts in trout fed both FO (27.6 ± 7.9^{a}) and HCO diets (22.7 ± 4.8^{a}) in comparison to trout fed LCO diets (15.4 ± 2.8^{b}) but did not differ between trout fed FO and HCO ($p < 0.0001$). Erucic acid was found to be highest in muscle tissue of trout fed the LCO diet in comparison to fish fed the HCO and the FO control ($p = 0.000$). Total MUFA in muscle tissue was higher in fish fed FO diets than in fish fed HCO diets ($p = 0.030$). The n-3/n-6 ratio was higher in trout fed the FO diet than trout fed the LCO and HCO diets ($p < 0.0001$). No differences were found in LNA ($p = 0.089$), total PUFA ($p = 0.312$), total n-3 or total n-6 in the muscle tissue. Total lipid (wet weight and dry weight) in trout muscle was independent of treatments; however, total lipid in muscle tissue increased from week 0 to week 12, regardless of dietary treatment.

2.6.4 FATTY ACID CONTENT OF LIVER TISSUE

The fatty acid content of liver tissue differed based on dietary treatment (Table 2.6). ALA was higher in trout fed the LCO and HCO diets in comparison to FO diets ($p < 0.001$). LNA was higher in trout fed LCO and HCO diets in comparison to trout fed FO diets ($p = 0.003$). Erucic acid was found to be highest in liver tissue of trout fed the HCO diet in comparison to fish fed the LCO and the FO control ($p = 0.031$). Total n-6 stored was higher in trout fed LCO and HCO diets compared to trout fed FO diets ($p < 0.0001$). The n-3/n-6 ratio was highest in trout fed FO diets in comparison to trout fed LCO and HCO diets ($p < 0.0001$). There were no differences in LNA ($p = 0.295$), EPA ($p = 0.722$), DHA ($p = 0.087$), total SFA ($p = 0.052$), total MUFA ($p = 0.765$), and total PUFA (p value) among treatments.

Total lipid (wet weight and dry weight) of the liver was not different among treatments; however, increased from week 0 to week 12, regardless of dietary treatment.

2.6.5 FATTY ACID CONTENT OF BRAIN TISSUE

There were significant differences in brain FA based on dietary treatment (Table 2.7). LNA was higher in trout fed LCO diets in comparison to trout fed HCO or FO diets ($p = 0.007$). ALA was highest in trout fed the LCO diet compared to trout fed either the HCO or FO diets ($p < 0.0001$). Arachidonic acid (ARA; 20:4n-6) was higher in trout fed LCO and HCO compared with trout fed FO ($p < 0.001$). Total MUFA was highest in trout fed LCO diets in comparison to HCO diets; however, total MUFA in fish fed both LCO and HCO diets was not different than FO ($p = 0.024$). Total PUFA was found to be highest in trout fed LCO diets in comparison to FO and HCO diets ($p = 0.012$). Total n-3 in trout fed LCO diets was higher than for fish fed HCO diets; however, total n-3 in fish fed both LCO and HCO diets was not different than fish fed the FO diet ($p = 0.039$). Total n-6 was highest in trout fed LCO diets in comparison to FO and HCO ($p = 0.001$). Trout fed FO diets had a higher n-3/n-6 ratio in the brain in comparison to trout fed the LCO and HCO diets ($p < 0.001$). There were no differences depending on treatment in EPA ($p = 0.280$), DHA ($p = 0.207$), and total SFA ($p = 0.093$).

2.6.6 FATTY ACID CONTENT OF EYE TISSUE

Eye tissue FA content was significantly affected by diet (Table 2.8). LNA was significantly higher in eye tissue in fish fed HCO (200.9 ± 33.5^a µg/mg) and LCO (200.9 ± 33.5^aµg/mg) diets in comparison to the FO control (127.1 ± 34.4^b µg/mg) ($p < 0.001$). ALA was higher

in eye tissue of fish fed LCO (136.7 ± 24.9[a] µg/mg) diets in comparison to fish fed HCO (71.0 ± 15.8[b] µg/mg) and FO (30.6 ± 8.6[c] µg/mg) diets ($p < 0.001$). EPA was higher in trout fed HCO 39.8 ± 9.2[a] µg/mg) and FO (34.7 ± 9.0[a] µg/mg) diets in comparison to LCO (15.3 ± 3.0[b]) ($p < 0.0001$). DHA was higher in eye (74.0 ± 15.1[a] µg/mg) tissue of fish fed HCO diets in comparison to fish fed the FO (60.7 ± 12.3[b] µg/mg) and LCO (40.3 ± 7.6[c]) diets ($p < 0.001$). Total PUFA was higher in trout fed the HCO and LCO diets when compared to fish fed FO ($p < 0.001$). Total SFA ($p = 0.365$), n-3/n-6 ratios ($p = 0.264$) and MUFA ($p = 0.385$) were not different among treatments.

2.6.7 MULTIVARIATE ANALYSES OF FATTY ACID DATA

PERMANOVA results indicated the difference in FA profiles was dependent on both diet ($F = 5.93$; $p = 0.002$) and tissue type ($F = 2.94$; $p = 0.001$). This was apparent in the PCoA plot (Figure 2.1), with 91.6% of the variation was accounted for, mainly in PCO1 (74.6%), where strong distinction among tissue type was evident along the PCO1 axis. PCO2 (17%) also shows distinction among treatments, although not as distinctly separated as tissue type.

2.6.8 COMPOUND SPECIFIC STABLE ISOTOPE ANALYSIS

The $\delta^{13}C$ value for DHA in FO (-27.3) was isotopically different than the transgenic camelina oil (-34.10). Consequently, the $\delta^{13}C$ values for DHA for the HCO diet (-32.85) and FO diet (-28.91) were also isotopically different. The $\delta^{13}C$ values for DHA in muscle tissue were significantly different between the trout fed the HCO diet compared with the FO diet (Figure 2.2). The $\delta^{13}C$ for DHA in muscle tissue of trout fed HCO (-30.8 ± 1.12) were isotopically lighter when compared to trout fed FO (-26.75 ± 0.84; $p = 0.001$).

2.6.9 SENSORY PROPERTIES

Fillet texture was independent of treatments (p = 0.377; Table 2.9). Similarly, fillet colour was independent of treatment no differences in the Hunter lab scale parameters L (p = 0.066), a (p = 0.895) or b scores (p = 0.581) depending on treatment. Considering the results of the sensory panel, tor the triangle test, 9 out of 24 panelists chose the correct sample as the “odd” fillet compared with the other two, which is not significant (13/24 assessors are required to give correct judgement at the 5% level). For the hedonic test, participants detected a significant difference in fillet texture, where HCO fillets were more elastic in comparison to FO fillets, which was noted to be slightly firmer (Table 2.9). There was no significant difference in appearance or odor based on the hedonic test. For the QDA, fillets from the HCO treatment were found to be more orange in color (p = 0.012) and firm in texture (p = 0.001) compared with fillets from the FO control (Figure 2.3). Fillet surface moistness, marine odor, vegetable odor, rancid odor, texture, and brightness were not significantly different between rainbow trout fed the HCO and FO diets.

2.7 DISCUSSION

2.7.1 IMPACT ON FATTY ACID CONTENT ON GROWTH PERFORMANCE

Overall, rainbow trout in this study responded positively to the transgenic camelina oil treatments and growth performance was significantly better as final body weight was greater than trout fed FO diets, indicating that this oil was digested and utilized. These findings agree with studies showing that both a high-EPA oil and an EPA+DHA oil from transgenic camelina included in feeds for post-smolt Atlantic salmon and gilthead seabream

had no detrimental effects on fish performance, metabolic responses or the nutritional quality of fillet (Betancor et al. 2016a, b).

In the present study, juvenile rainbow trout fed diets with transgenic camelina oil (high or low inclusion) had a higher final weight and were longer in fork length, with the same FCR as trout fed FO. Weight gain was observed to not be significantly different within fish fed the experimental treatments however the values, although not statistically different, were notably higher in trout fed LCO and HCO diets relative to trout fed the FO control. As weight gain calculations were based on tank means and the initial weight and final weight were not linked to the same individual fish; therefore, as the n is only 3, the power of the test is lower contributing to the obtained values. It is possible that n-3 LC-PUFA in transgenic camelina oil may be more bioavailable, more digestible, and better utilized than in FO, which may explain increased growth performance in rainbow trout fed LCO and HCO compared to trout fed FO. Also, since total PUFA were higher in the CO diets, fish may have metabolized the FA more efficiently compared to fish fed the FO diet, which was higher in total SFA. Notably, the final VSI was greater in fish fed LCO diets compared to those fed the FO and HCO diets, indicating additional fat accumulation in the viscera. Increased fat deposition in fish tissues appears to be a common effect of dietary plant oil inclusion (Torstensen et al., 2011; Morais et al., 2012; Hixson et al., 2014; Betancor et al., 2016a). This increase in VSI may be attributed to elevated quantities of plant-associated FA (ALA and LNA) in LCO diets. If ALA and LNA are not used as substrates toward n-3 and n-6 LC-PUFA, and if not oxidized, the FA are stored in adipose tissue.

Similarly, in salmon fed non-transgenic camelina oil, more lipid was stored surrounding the viscera than salmon fed a FO-based diet (Torstensen et al., 2011). As it is not advantageous, from an aquaculture perspective, to accumulate visceral fat as opposed to somatic growth, HCO diets may offer an advantage over LCO diets as a result of higher n-3 LC-PUFA and lower ALA and LNA, particularly when fish are fed for longer periods of time.

2.7.2 IMPACT ON TISSUE FATTY ACID CONTENT

When FO was replaced by transgenic camelina oil (both high and low inclusion levels), significant changes were observed in the quantities of individual FA in trout muscle. After 12 weeks, EPA and DHA stored in muscle tissue of rainbow trout fed the HCO diet was similar to that of fish fed the FO diet. In previous research, Atlantic salmon fed transgenic camelina had been found to have increased concentrations of EPA and DHA within fillet tissue when compared to salmon fed diets with non-transgenic camelina and fish oil respectively (Betancor et al. 2017). This is an important result, since generally when plant-based lipid alternatives to FO are used, a major concern is reduced levels of n-3 LC-PUFA in the fillet tissue which reduces the nutritional benefits consumers seek (Turchini et al., 2018; Sprague et al., 2016). Rainbow trout appear to have a high n-3 bioconversion capability and the ability to maintain high stored EPA and DHA within the fillet when fed a diet that is high in ALA (Turchini et al., 2018; Hixson et al. 2014a). In the present study, trout fed the LCO diet stored significantly less EPA and DHA in muscle tissue, and instead accumulated ALA without further bioconversion. This suggests the LCO diet provided sufficient dietary levels of EPA and DHA for health and growth, but the amounts of EPA

and DHA were not low enough to trigger biosynthetic action toward production of surplus EPA and DHA, even if high amounts of ALA were supplied. Despite the accumulation of ALA, there was no difference total lipid stored in the fillet among treatments. The n-3/n-6 ratio was lower in muscle tissue of trout fed diets containing transgenic camelina oil. Changes to n-3/n-6 fatty acid ratios could directly impact eicosanoid production; however, the quantity of total n-3 FA's stored within the muscle tissue in trout fed HCO were similar to trout fed diets containing FO. This is an important consideration from both a consumer health and fish health perspective as the consumption of increased n-3 LC-PUFA assists in the production of anti-inflammatory eicosanoids. Trout fed the HCO diet also had an increased quantity of ARA in their muscle tissue, which could be attributed to the presence of ARA (and LNA) in the transgenic camelina oil used in this study (Table 1; as in Han et al. 2020) and the HCO diet.

With the inclusion of GE *Camelina sativa*, it is also important to be aware of the subsequent inclusion of fatty acids such erucic acid (22:1n-9). Erucic acid is a monounsaturated omega-9 fatty acid which is naturally occurring in increased concentrations within plant seeds from the Brassicacea family (Sissener et al., 2018). Reduced concentrations of erucic acid are also naturally present in reduced quantities within fish, however, concentrations can vary based on factors such as species and tissue type (Sissener et al., 2018).

The inclusion of erucic acid has been noted to have negative effects on the health of cardiac tissue when exposed to increased dietary concentrations (Sissener et al., 2018). Previous research within terrestrial livestock, such as pigs and chickens, have noted that increased concentrations of dietary erucic acid have result in the onset of lipidosis, or triacylglycerol

accumulation, within the heart tissue resulting in reduced heart contractions and subsequent damage to cardiac muscular tissue (Sissener et al., 2018).

The transgenic *Camelina sativa* oil used within this study was found to have concentrations of erucic acid of 0.7% or 14.9 μg/mg present within the experimental oil and was found to be in increased concentrations within LCO and HCO diets with the highest concentration being within the LCO diet relative to the fish oil control (Table 2.3). This increase in erucic acid could also attributed to the inclusion of non-transgenic camelina oil within the LCO diet formulation. Within trout muscle tissue, trout fed the LCO diet had significantly higher concentrations of erucic acid relative to those fed the HCO and the FO control. Regarding human safety, the European Food and Safety Authority (EFSA) noted that a tolerable daily intake of 7 mg/kg per body weight per day of erucic acid was allowed for human consumption (ESFA, 2016; Sissener et al., 2018). This report was issued relative to the aforementioned instances of cardiac lipidosis documented within research trials conducted with terrestrial livestock (ESFA, 2016; Sissener et al., 2018). Relative to food safety and to assurance of health within both humans and fish, further research is necessary relative to the inclusion of oilseeds rich in erucic acid in diets of farmed fish relative to fish health and concentrations present within fillet tissue for human consumption.

For trout fillets from both HCO and LCO treatments, the quantitative amount of EPA+DHA meets the human nutritional requirement for these essential FA's. According to the World Health Organization (WHO), the daily requirement for EPA+DHA is 250 mg (WHO, 2008); although other regulatory bodies suggest higher intake levels. The recommended

serving of fish is 100 g of cooked fish (American Heart Association, 2017). The sum of EPA+DHA (wet weight) in one serving of rainbow trout muscle for fish fed FO is 823 mg, 652 mg, and 433 mg in trout fed FO, HCO, and LCO, respectively. Therefore, consuming one serving of rainbow trout fillet that was fed either HCO or LCO is more than sufficient to meet the daily requirement (250 mg) as recommended by the WHO.

Liver tissue is a primary site of lipid metabolism, oxidation, and LC-PUFA synthesis (Caballero et al., 2004). As imbalances or changes in dietary FA's could influence the functioning of the liver, it is important to consider the impact on this organ as an indicator of changes in lipid and fatty acid metabolism (Caballero et al., 2004) in the fish. Overall, the inclusion of dietary transgenic camelina oil significantly influenced profiles of individual FA's in the liver; however, the liver appeared to be more resistant to changes in dietary FA profile compared to muscle tissue (see Figure 2.1). EPA and DHA in the liver of fish fed transgenic camelina oil was found to be similar to that of fish fed FO. This result agrees with previous findings in which transgenic camelina oil fed to Atlantic salmon resulted in high levels of EPA and DHA in the liver in comparison to the FO diet (Betancor et al., 2015, 2016, 2017). The conservation of DHA in the liver in fish fed the HCO diet compared to those fed the FO diet was reflective of diet as the HCO diet contained transgenic camelina oil with EPA and DHA proportions that are equivalent to that of FO. ALA and LNA stored in the liver tissue were also greater in fish fed LCO and HCO diets compared to the FO diet. In fingerling salmon fed diets with transgenic canola oil (high DHA, but low EPA), the liver stored elevated levels of ALA and LNA (Ruyter et al., 2019).

Because DHA is required for neural development in vertebrates, brain and eye tissues are areas of DHA localization and it has been consequently been proposed that these tissues may be relatively resistant to changes in the fatty acid profile of the diet (Brodtkorb et al., 1997; Stoknes et al., 2004). Nevertheless, it is proposed that marked changes in diet FA composition diet (in particular, with respect to the n-3 PUFA content) has the potential to change FA profiles in these tissues. Brain and eye tissue assays were completed to check if there were changes in FA profiles of these tissues as a result of changes in diet. Not surprisingly, the brain appeared to be most resistant to dietary change, compared to the other tissues in this study (see Figure 2.8.1). For example, despite different amounts of dietary EPA and DHA among diets in this study, after 12 weeks of feeding, the brain still showed similar amounts of EPA and DHA among treatments, which likely suggests selective retention. It could also be possible that trout in the LCO group were metabolizing ALA toward EPA and DHA synthesis, and subsequently transporting these n-3 LC-PUFA to the brain. However, excess amounts of ALA stored in the muscle and liver suggest that this may not be the case. Higher ALA (and LNA) amounts were found stored in the brain tissue of rainbow trout fed transgenic camelina diets compared to trout fed the FO control diet. Higher amounts of ARA were stored in the brain in trout fed LCO and HCO diets compared to the FO treatment, despite the fact that ARA levels in the feed were similar for FO and LCO diets (although about 4 times higher in the HCO diet). Increased ARA concentrations have also been observed within brain tissue of Atlantic salmon fed diets containing transgenic camelina in comparison to those fed wild camelina and a FO control (Betancor et al., 2017). The ARA in transgenic camelina oil may perhaps be more bioavailable and therefore, more accessible for efficient digestion and utilization than in

FO. Approximately 5% of ARA is stored in phosphatidylcholine in transgenic camelina (Ruiz-Lopez et al. 2014), which is highly digestible and bioavailable to fish. It is also possible that the surplus LNA in the LCO diet went toward ARA production, which was stored in the brain, compared to the trout fed FO. Feeding Atlantic salmon oils rich in LNA has resulted in elevated ARA in membrane phospholipids (Bell et al. 1993). These findings are relevant as ARA along with DHA makes up ~ 20% of FA's within the mammalian brain and both play important functions in FA brain make up, function and structure and infant brain development (Rapoport, 2008; Forsyth et al., 2017).

Since plant-based oils are typically devoid of ARA, bioavailability of ARA is important for brain function in fish that are fed plant-based diets (Oxley et al. 2010). Major groups of FA's remained the same among treatments, notably total SFA, MUFA, PUFA, n-3 and n-6, suggesting FA conservation in the brain, to prioritize critical maintenance of brain function. Similar findings have been reported by Betancor et al. (2016a; 2016b; 2017) in which both Atlantic salmon and gilthead seabream fed diets containing transgenic camelina oil were found to have less dietary influence in fatty acid composition of brain tissue sampled from fish as there were no differences in total lipid contents of brain tissue among fed dietary treatments. The robustness of brain tissue, in its ability to maintain levels of n-3 LC-PUFA, regardless of dietary source, has also been reported in gilthead sea bream, meagre (*Argyrosomus regius*) and silver barb fingerlings (*Puntius gonionotus*) (Benedito-Palos et al., 2010; Silva-Brito et al., 2016; Nayak et al., 2017).

The presence of neural tissue within the eye prioritizes the specific retention of n-3 LC-PUFA such as DHA, to assist in optimizing the integrity of the retina and maintaining visual acuity. In the vertebrate eye, DHA contributes to 50-60% of the total FA content found within the rod outer segment of photoreceptors (Querques et al., 2011). As such, retinal tissue within the eye is used as an indicator for specific changes in n-3 FA storage related to dietary influences or deficiencies. Compared to the brain, the eye tissue was more reflective of the diet, especially for juvenile rainbow trout fed the LCO treatment. The most distinct examples of this are higher amounts of ALA and LNA and lower amounts of EPA and DHA stored in eye tissue of trout fed LCO vs. HCO and FO diet groups. It is unknown whether this level of change in eye tissue FA profile can eventually impact vision of trout fed LCO diets due to reduced EPA and DHA. Interestingly, DHA in eye tissue of trout fed HCO was higher than in the FO group, despite having similar amounts of DHA in the diet. Again, this may perhaps suggest superior bioavailability of LC-PUFA in transgenic camelina. Navarro et al. (1997) noted that various dietary inclusion levels of DHA fed to seabass larvae significantly influenced the composition of lipids within eye tissue. In contrast, changes in dietary DHA inclusion when fed to Atlantic salmon had no significant impacts on DHA levels in eye tissue (Brodtkorb et al., 1997). Clearly, factors such as the species, diet, duration of feeding, life stage of the fish, can influence the outcome of FA storage in tissues. However, few studies have evaluated the effect of dietary on eye tissue in general. Thus, we suggest that more research needs to be done to better understand the relationship between dietary DHA and its effect on neural tissues in fish.

The PCoA provides a visualization of the effect of diet and tissue type on the FA outcome of this study (see Figure 2.1). Each tissue type is clustered, regardless of diet, which was

confirmed by PERMANOVA, for example: tissue type is a significant factor in the variation of FA profiles. Most of the variation is explained by PC1 (~75%), which clearly separated groups of tissues, rather than dietary groups. This indicates that tissue type is more of a defining factor in determining FA composition than the impact of diet. The clustering of individual samples is obvious for brain tissue, but treatment effect is virtually undistinguishable within the cluster of brain data points, again, indicating that brain tissue has a distinct FA profile, that is largely independent of dietary FA's. In contrast, muscle tissue in salmonids is known to be quite plastic with respect to its fatty acid profile in relation to FA supplied in the diet, and as such, retains the FA signature of the diet. There are clearly are two separate groups along PC2 dividing the muscle tissue data points (and eye tissue), clearly separating FO groups from HCO and LCO groups. Since only muscle and eye are distinctly separated along PC2 (and less of the variation explained, ~17%), diet is less of an explanatory factor considering rainbow trout tissues together.

2.7.3 δ^{13} VALUES FOR DHA

With the inclusion of novel dietary oils, such as transgenic camelina, it is important to observe whether stored FA's are accumulated from the new transgenic oil source, or selectively retained from the fish meal that was also provided in each of the diets. CSIA was used to determine the DHA isotopic signature in the muscle tissue. With CSIA, the origin of carbon and its specific contribution to tissues can be determined, as $^{13}C/^{12}C$ ratios stay relatively unchanged as they pass through the food web, they can be used to evaluate nutrient transfer (Hixson et al., 2014a). As such, it can be determined if the DHA stored in muscle is from fish meal origin or stored directly from GE camelina. The DHA in

transgenic camelina oil and FO were isotopically distinct, with DHA in FO being more isotopically enriched (-27.3‰) than in camelina oil (-34.1‰), as camelina is a C3 plant and has $\delta^{13}C$ values between -25 and -35‰ (O'Leary, 1988) and marine origins are isotopically heavier and enriched with ^{13}C (Phillips et al. 2005). This was reflected in the muscle tissue of juvenile rainbow trout in our study. Different $\delta^{13}C$ values for DHA in muscle of trout fed HCO likely indicate direct storage of DHA from transgenic camelina, which resulted in isotopically lighter DHA (-30.8‰) compared to trout fed FO (-26.75‰), a difference of 4.05‰. The $\delta^{13}C$ of a consumer is assumed to be equivalent to the weight proportion of the $\delta^{13}C$ of all dietary components, therefore, the significant difference in DHA $\delta^{13}C$ represents the difference in terrestrial vs. marine isotopic signatures, and therefore suggests that the DHA stored in muscle tissue in trout was directly from dietary transgenic camelina, rather than selectively retained from the DHA in fish meal only. Although, since there was a slight shift, it is likely that some DHA is from marine origin in trout fed HCO.

2.7.4 SENSORY PROPERTIES OF FILLETS

Applied aquaculture research should, ideally, be complemented with the findings offered by sensometrics (Calanche et al. 2019). There were minor, yet significant, differences in the sensory properties of rainbow trout fed diets containing high levels of transgenic camelina compared with traditional fish oil. Instrumental analyses of color and texture revealed no significant difference in fillets between HCO and FO treatments; however, untrained consumers reported a difference in color and texture based on their perception. Untrained panelists are "consumers", who represent a valuable source of information with regards to collecting sensory information based on consumer preference, which has gained

increasing significance and relevance in aquaculture and seafood assessment (Calanche et al. 2019). The triangle test indicated that panelists could not distinguish between fillets from trout that were fed diets containing transgenic camelina vs. fillets from trout that were fed a typical commercial (FO-based) diet, but when asked to rate fillets according to attributes such as fillet color, texture and odour in the QDA and hedonic test, panelists found slight, but significant differences. The observed higher orange intensity in trout fed HCO could be attributed to the pigment in transgenic camelina oil. Non-transgenic camelina can contain up to 112 mg of β-carotene/kg oil (Raczyk et al. 2016). Astaxanthin is the major carotenoid pigment responsible for the pink color in the flesh of wild rainbow trout, while both astaxanthin and canthaxanthin are used for pigmenting farmed trout (Storebakken and No, 1992). β-carotene is also one of the carotenoids responsible for the orange and red pigmentation of fish (Keleştemur and Çoban, 2016). For example, β-carotene supplementation in juvenile rainbow trout diets has been shown to improve growth performance and skin carotene concentration at 70 m β-carotene (Keleştemur and Çoban, 2016). However, it is important to note that the color differences observed by the sensory panel in the present study were not detected by instrumental analysis (l, a, b color scores; see Table 9), which highlights a discrepancy between the sensory panel and analytical methods. An increase in firmness may be attributed to the incorporation of a vegetable oil lipid substitute as these can impact the sensory characteristic of fish (Martínez-Llorens et al., 2007). 40% of studies that investigated plant product inclusion in aquafeeds stated that texture was significantly affected, particularly in larger or higher quantity substitutions (Gatlin et al., 2007). Also, fillet texture is multifactorial, with complex biological interactions (Mørkøre et al. 2020). Plant-based oils have been found to increase lipid levels

stored within the fillet, leading to changes within the sensory properties of the fillet. However, in this study, total lipid, crude protein, and dry matter were not different between fillets from trout fed FO vs. HCO diets, so this likely did not impact the texture. These results have often been found to be contradictory to each other. For example, in previous studies, in which non- transgenic camelina oil or FO was fed to Atlantic salmon there were no significant differences in sensory properties including brightness and texture of raw and cooked fillets (Hixson et al., 2014b; Hixson et al. 2017). Perception of raw fish fillets (which is influenced by color and texture) is recognized as an important factor related to consumer satisfaction (Veiseth-Kent et al., 2010). Because our sensory analysis represents preliminary results (raw fillets of juvenile trout, and therefore not necessarily directly market-applicable), we suggest that a sensory analysis on cooked fillets of market size fish would be pertinent prior to using transgenic camelina commercially in the grow out phase.

2.7.5 CONCLUSIONS

Transgenic camelina oil, at a high inclusion level (>50% of the total dietary oil) and as a full replacement of FO, was found to be an effective substitute for FO as a dietary lipid source of n-3 LC-PUFA in diets for rainbow trout. Fish fed high levels of transgenic camelina oil enriched with EPA and DHA (HCO diet) had FA profiles that were generally similar to those of fish fed FO. Inclusion of lower levels of transgenic camelina (LCO diet) resulted in similar growth performance as trout fed FO; however, the FA profile was more impacted, particularly in the muscle tissue. This evidence informs that greater quantities of transgenic camelina oil in the feed are warranted in order to effectively replace FO in aquafeed (full replacement of FO and >50% of the total dietary oil). Dietary inclusion of

transgenic camelina oil is effective in meeting the nutritional requirements of juvenile rainbow trout as their growth and development were not affected by the addition of this oil to the feed. Relative to fish health, the inclusion of transgenic plant-based oil must be further examined to evaluate possible impacts of novel oils on both fish health and lipid profiles, which could also further impact fillet product quality and consumer health. Future research is needed to evaluate transgenic camelina at grow out stages closer to market size, applications in finishing diets or for a full production cycle.

2.8 TABLES AND FIGURES

Table 2.1 Fatty acid composition of transgenic camelina oil.

Fatty acid	Transgenic Camelina oil (%)	Transgenic Camelina oil (μg/mg)
14:0	0.1	1.9
15:0	<0.1	0.8
16:0	6.5	138.8
16:1n-7c	0.1	2.6
16:1n-7t	<0.1	0.7
17:0	0.1	1.3
17:1n-7	<0.1	0.2
18:0	4.8	102.4
18:1n-9c	5.8	123.8
18:1n-9t	0.2	3.1
18:1n-12c	0.3	6.1
18:1n-7c	1.3	28.8
18:1n-7t	<0.1	0.3
19:0	<0.1	0.6
18:2n-6 (LNA)	21.0	446.8
20:0	2.5	52.3
18:3n-6	2.2	46.0
20:1n-15	0.1	2.1
20:1	0.1	1.9
20:1n-9	6.7	142.4
18:3n-3 (ALA)	12.2	260.3
18:2n-6t	< 0.1	0.3
18:4n-3	1.4	30.5
20:2n-6	1.2	24.7
22:3n-3	0.1	2.4
22:0	0.8	16.6
20:3n-6	0.8	17.5
22:1n-9	0.7	14.9
20:3n-3	1.2	24.9
20:4n-6 (ARA)	3.1	65.4
20:4n-3	2.4	50.1
22:2n-6	0.2	3.4
24:0	0.1	1.6
20:5n-3 (EPA)	9.5	202.9
24:1n-9	0.3	5.8
22:4n-6	0.8	16.9
22:5n-6	0.1	1.6
22:5n-3	5.6	118.4

Fatty acid	Transgenic Camelina oil (%)	Transgenic Camelina oil (μg/mg)
22:6n-3 (DHA)	7.8	165.8
ΣSFA[1]	14.9	316.5
ΣMUFA[2]	15.8	335.7
ΣPUFA[3]	69.4	1475.5
ΣMUFA≥18C	15.6	332.1
ΣMUFA>18C	7.9	169.9
ΣC18 PUFA	36.8	783.9
ΣC20 PUFA	18.1	385.6
ΣC22 PUFA	14.4	306.0
ΣEPA & DHA	17.3	368.7
Σn-6	29.3	622.5
Σn-3	40.1	853.0
ΣOdd chain	0.2	3.2
n-3/n-6	1.4	1.4

[1] Saturated fatty acid
[2] Monounsaturated fatty acid
[3] Polyunsaturated fatty acid

Table 2.2 Diet formulation and composition (g kg^{-1} as fed basis) of experimental diets fed to rainbow trout.

Ingredient[1] (g /kg)	**FO**	**LCO**	**HCO**
Fish meal	150	150	150
Fish (herring) oil	100	0	0
Transgenic camelina oil[2]	0	12.5	130
Camelina oil[3]	0	117.5	0
Ground wheat	117.5	117.5	117.5
Empyreal (corn protein concentrate)	250	250	250
Canola oil	100	70	70
Poultry by-product meal	170	170	170
Soybean meal	80	80	80
Vitamin and mineral mix	2	2	2
Dicalcium phosphate	20	20	20
Pigment mix[5]	2.5	2.5	2.5
Lysine HCl	5	5	5
Choline chloride	3	3	3
Chemical composition (as fed, g/kg)			
Dry matter	943	937	925
Crude protein	472	489	477
Total lipid	314	260	299

[1]All ingredients were supplied and donated by Northeast Nutrition (Truro, Nova Scotia, Canada)
[2]Produced by Rothamsted Research (West Commons, UK)
[3]Commercial grade Camelina oil, produced by Smart Earth Seeds (Saskatoon, SK, Canada)
[4]Vitamin and mineral premix contains (/kg): zinc, 77.5 mg; manganese, 125 mg; iron, 84 mg; copper, 2.5 mg; iodine, 7.5 mg; vitamin A, 5000 IU; vitamin D, 4000 IU; vitamin K, 2 mg; vitamin B12, 4 µg; thiamine, 8 mg; riboflavin, 18 mg; pantothenic acid, 40 mg; niacin, 100 mg; folic acid, 4 mg; biotin, 0.6 mg; pyridoxine, 15 mg; inositol, 100 mg; ethoxyquin, 42 mg; wheat shorts, 1372 mg.
[5]Pigment mix contains (/kg): selenium, 0.220 mg; vitamin E, 250 IU; vitamin C, 200 mg; astaxanthin, 60 mg; wheat shorts, 1988 mg.

Table 2.3 Fatty acid content (μg/mg dry weight) of the three experimental diets; FO, LCO and HCO fed to juvenile rainbow trout over a 12-week period[1].

FA	FO	LCO	HCO
14:0	7.8	0.8	1.1
16:0	33.6	21.7	23.0
16:1n-7	10.0	2.8	3.1
18:0	8.1	7.5	9.9
18:1n-9	78.9	73.0	63.8
18:2n-6 (LNA)	37.0	55.1	56.2
18:3n-3 (ALA)	9.7	48.3	20.8
20:1n-9	4.8	19.7	10.9
20:4n-6 (ARA)	1.5	1.0	4.1
20:5n-3 (EPA)	16.6	2.3	12.9
22:1n-9	0.6	3.3	1.2
22:5n-3	2.0	0.8	6.7
22:6n-3 (DHA)	11.1	2.6	10.8
24:1n-9	0.7	0.9	0.6
∑SFA[2]	52.7	33.7	39.0
∑MUFA[3]	111.1	108.9	89.2
∑PUFA[4]	83.1	116.1	124.2
∑n-3	42.6	56.5	57.3
∑n-6	40.5	59.6	66.9
n-3/n-6	1.0	0.9	0.9
Total	246.9	258.7	252.37

[1]Data expressed as μg/mg dry weight, values are means (n=3) ± standard deviation. Means with different superscripts indicate significant differences based on Tukey's post-hoc test following a one-way ANOVA. FO, fish oil; LCO, low camelina oil; HCO, high camelina oil.
[2]Saturated fatty acid
[3]Monounsaturated fatty acid
[4]Polyunsaturated fatty acid

Table 2.4 Growth performance of rainbow trout fed three experimental diets: a fish oil control (FO), low camelina oil inclusion diet (LCO) and high camelina oil inclusion diet (HCO) over a 12-week experimental period[1].

Parameters	FO	LCO	HCO	P-value
Initial body weight[2]	50.8 ± 7.9	46.4 ± 12.9	52.1 ± 11.2	0.328
Final weight[3]	178.5 ± 30.7[b]	193.3 ± 25.0[a]	197.6 ± 25.0[a]	0.001
Weight gain[4]	127.5 ± 8.8	146.9 ± 12.1	145.5 ± 13.4	0.115
Initial Length	15.7 ± 0.8	14.8 ± 0.9	15.6 ± 0.9	0.062
Final Length	23.5 ± 1.3[b]	23.9 ± 1.1[ab]	24.2 ± 1.4[a]	0.008
Initial CF[5]	1.33 ± 0.2	1.39 ± 0.12	1.35 ± 0.1	0.422
Final CF	1.37 ± 0.1	1.41 ± 0.10	1.39 ± 0.1	0.099
Initial VSI[6]	12.47 ± 2.9	12.52 ± 2.9	11.77 ± 1.1	0.640
Final VSI	10.6 ± 1.0 [b]	11.7 ± 1.2 [a]	10.9 ± 1.0 [ab]	0.014
SGR[7]	1.44 ± 0.3	1.75 ± 0.5	1.59 ± 0.4	0.092
AFI[8]	143.3 ± 2.2	139.3 ± 1.2	145.4 ± 4.2	0.095
FCR[9]	1.13 ± 0.1	0.95 ± 0.1	1.01 ± 0.1	0.145

[1]Means with different superscripts indicate significant differences among treatments (P > 0.05).
[2]Initial measurements are mean ± standard deviation, body weight (g fish^{-1}), fork length (cm fish^{-1}); n = 45 per treatment.
[3]Final measurements are mean ± standard deviation, body weight (g fish^{-1}), fork length (cm fish-^{1}). Weight, length, and condition factor are calculated from individual fish; *n* = 58 (FO); *n* = 60 (LCO); *n* = 58 (HCO) per treatment.
[4]Weight gain (g fish^{-1}) = final weight - initial weight (calculated by tank means; n=3).
[5]Condition factor = body mass/length^3 (calculated by individual fish).
[6]Visceral somatic index (%) = 100 * (viscera mass/body mass).
[7]Specific Growth Rate = (ln (final body weight) – ln ((initial body weight)) / number of days in period * 100.
[8]Apparent feed intake (g fish^{-1}) = (feed consumed, g)/(number of fish per tank) (calculated by tank means).
[9]Feed conversion ratio (g fish^{-1}) = (feed intake, g fish^{-1})/(weight gain, g fish^{-1}) (calculated by tank means).

Table 2.5 Fatty acid content (μg/mg dry weight), total fat, protein and dry matter of rainbow trout muscle tissue fed experimental FO, LCO and HCO diets from week 0 (initial) and week 12[1].

Fatty acid	Initial	FO	LCO	HCO	F-value	P-value
14:0	8.9 ± 4.9	5.8 ± 2.3[a]	1.6 ± 0.7[b]	1.4 ± 0.4[b]	46.45	0.000
14:1n-5	0.2 ± 0.1	0.1 ± 0.0[a]	0.0 ± 0.0[b]	0.0 ± 0.0[b]	8.65	0.001
15:0	0.7 ± 0.4	0.5 ± 0.2[a]	0.2 ± 0.1[b]	0.2 ± 0.0[b]	38.87	0.000
16:0	51.7 ± 26.7	32.3 ± 11.2[a]	24.7 ± 7.9[b]	22.0 ± 4.2[b]	6.15	0.005
16:1n-7c	15.8 ± 8.8	8.0 ± 3.1[a]	3.7 ± 1.5[b]	3.2 ± 0.8[b]	24.57	0.000
16:1n-7t	0.3 ± 0.2	0.2 ± 0.2[a]	0.1 ±0.1[b]	0.1 ± 0.1[b]	8.21	0.001
17:0	0.6 ± 0.3	0.5 ± 0.2[a]	0.2 ± 0.1[b]	0.2 ± 0.1[b]	29.78	0.000
17:1n-7	0.6 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	2.18	0.126
18:0	11.4 ± 5.8	8.2 ± 2.8	7.6 ± 2.6	7.8 ± 1.8	0.19	0.831
18:1n-9c	57.6 ± 31.8	61.2 ± 23.5[a]	56.6 ±20.9[ab]	43.4 ± 11.4[b]	3.43	0.042
18:1n-9t	0.6 ± 0.3	0.2 ± 0.1[a]	0.1 ± 0.0[b]	0.2 ± 0.1[ab]	6.81	0.003
18:1n-12c	4.1 ± 2.1	0.9 ± 0.3	0.8 ± 0.2	0.8 ± 0.2	0.88	0.421
18:1n-7c	8.5 ± 4.4	5.9 ± 2.2[a]	4.1 ± 1.5[b]	3.8 ± 1.0[b]	6.60	0.003
18:1n-7t	0.0 ± 0.0	0.2 ± 0.3[a]	0.0 ± 0.0[b]	0.0 ± 0.0[b]	9.58	0.000
19:1n-12	0.4 ± 0.3	0.5 ± 0.2[a]	0.1 ± 0.1[b]	0.1 ± 0.0[b]	42.87	0.000
18:2n-6 (LNA)	22.5 ± 11.8	25.2 ± 9.61	33.5 ± 12.8	31.9 ± 9.0	2.56	0.089
20:0	0.4 ± 0.2	0.5 ± 0.2[b]	1.1 ± 0.5[a]	1.3 ± 0.4[a]	18.51	0.000
18:3n-6	0.4 ± 0.3	0.3 ± 0.1[c]	0.9 ± 0.3[b]	1.1 ± 0.3[a]	35.36	0.000
20:1n-15	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.32	0.728
20:1	9.5 ± 5.0	1.8 ± 0.7[a]	0.8 ± 0.7[b]	0.7 ± 0.6[b]	12.01	0.000
20:1n-9	5.2 ± 2.8	4.2 ± 1.6[b]	10.6 ± 4.4[a]	6.21 ± 1.6[b]	20.32	0.000
18:3n-3 (ALA)	4.3 ± 2.3	6.2 ± 2.4[b]	23.6 ± 8.9[a]	10.9 ± 2.7[c]	39.77	0.000
18:4n-3	3.0 ± 1.6	1.4 ± 0.5[b]	2.7 ± 1.0[a]	1.3 ± 0.4[b]	22.12	0.000
20:2n-6	2.1 ± 1.1	1.8 ± 0.7[b]	2.9 ± 1.0[a]	2.0 ± 0.5[b]	8.61	0.001
22:3n-3	0.0 ± 0.0	0.0 ± 0.0[b]	0.2 ± 0.1[a]	0.1 ± 0.0[b]	40.75	0.000
22:0	0.9 ± 0.5	0.2 ± 0.09	0.3 ± 0.14	0.3 ± 0.11	3.33	0.046
20:3n-6	1.1 ± 0.6	0.7 ± 0.3	1.5 ± 0.5	1.6 ± 0.4	22.13	0.000
22:1n-11	8.9 ± 5.5	2.9 ± 1.0[a]	2.4 ± 1.0[ab]	2.1 ± 0.6[b]	3.37	0.044
22:1n-9	2.5 ± 1.1	0.6 ± 0.2[b]	1.8 ± 0.7[a]	0.8 ± 0.2[b]	32.74	0.000
20:3n-3	0.2 ± 0.1	0.4 ± 0.2[c]	1.8 ± 0.6[a]	1.1 ± 0.2[b]	47.10	0.000
20:4n-6 (ARA)	0.1 ± 0.1	1.6 ± 0.5[b]	1.7 ± 0.4[b]	3.0 ± 0.6[a]	38.19	0.000
22:2n-6	13.3 ± 6.3	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.05	0.948
24:0	8.9 ± 4.9	2.6 ± 3.4	1.4 ± 1.3	2.9 ± 2.7	1.40	0.258
20:5n-3 (EPA)	0.7 ± 0.4	5.8 ± 4.8[a]	2.1 ± 1.3[b]	3.5 ± 2.7[ab]	4.74	0.014
24:1n-9	1.0 ± 0.5	0.7 ± 0.2[ab]	0.8 ± 0.3[a]	0.5 ± 0.1[b]	5.52	0.007
22:4n-6	0.3 ± 0.1	0.2 ± 0.1[b]	0.2 ± 0.1[b]	0.6 ± 0.2[a]	81.22	0.000
22:5n-3	3.6 ± 1.8	3.0 ± 0.9[b]	1.3 ± 0.4[c]	3.7 ± 0.8[a]	44.28	0.000
22:6n-3 (DHA)	44.0 ± 19.9	27.6 ± 7.9[a]	15.4 ± 2.8[b]	22.7 ± 4.8[a]	18.28	0.000
ΣSFA[2]	74.1 ± 38.4	50.6 ± 17.5[a]	37.2 ± 12.2[b]	36.2 ± 8.7[b]	5.48	0.008
ΣMUFA[3]	113.6 ± 61.8	87.6 ± 32.9[a]	82.3 ± 28.0[ab]	62.2 ± 16.1[b]	3.80	0.030
ΣMUFA≥C18	96.7 ± 52.5	79.1 ± 29.6	78.2 ± 26.6	58.7 ± 15.2	3.29	0.047
ΣMUFA<C18	25.9 ± 14.2	10.7 ± 3.6[b]	16.7 ± 5.6[a]	10.5 ± 2.6[b]	10.92	0.000
ΣPUFA[4]	101.0 ± 48.0	74.5 ± 24.8	88.0 ± 28.7	83.8 ± 18.8	1.20	0.312
ΣC18 PUFA	30.3 ± 15.9	33.2 ± 12.6[b]	60.7 ± 22.8[a]	45.2 ± 12.4[b]	10.32	0.000
ΣC20 PUFA	21.8 ± 10.5	10.2 ± 5.7	10.0 ± 3.3	11.3 ± 2.7	0.45	0.640
ΣC22 PUFA	48.9 ± 22.2	31.1 ± 8.8[a]	17.3 ± 3.3[b]	27.3 ± 5.7[a]	18.97	0.000
ΣEPA+DHA	3.7 ± 1.9	33.4 ± 10.8[a]	17.6 ± 3.2[c]	26.2 ± 4.9[b]	18.62	0.000
Σn-3	70.9 ± 32.8	44.4 ± 14.3	46.9 ± 13.6	43.3 ± 8.28	0.35	0.707
Σn-6	30.2 ± 15.5	30.1 ± 11.2[a]	40.9 ± 15.0[b]	40.4 ± 11.0[b]	3.56	0.037

Fatty acid	Initial	FO	LCO	HCO	F-value	P-value
Σodd chain	2.5 ± 1.3	1.8 ± 0.7[a]	0.7 ± 0.2[b]	0.6 ± 0.2[b]	32.32	0.000
n-3/n-6	2.4 ± 0.4	1.5 ± 0.2[a]	1.2 ± 0.1[b]	1.1 ± 0.1[b]	27.14	0.000
Total lipid, protein, dry matter (g/kg)						
Lipid ww	34 ± 10	48 ± 18	52 ± 17	45 ± 11	0.81	0.452
Lipid dw	150 ± 40	205 ± 73	218 ± 66	191 ± 44	0.67	0.515
Protein dw	765 ± 117	714 ± 41	692 ± 42	717 ± 39	1.67	0.200
Dry matter	226 ± 9.0	234 ± 11	239 ± 8.0	234 ± 7.3	1.14	0.330

[1]Data expressed as μg FAME/mg (dry weight), values are means (n=3 per treatment) ± standard deviation.

Means with different superscripts indicate significant differences based on Tukey's post-hoc test following a one-way ANOVA. FO, fish oil; LCO, low camelina oil; HCO, high camelina oil. Reported fatty acids are > 1 μg/mg

[2]Saturated fatty acid

[3]Monounsaturated fatty acid

[4]Polyunsaturated fatty acid

Table 2.6 Fatty acid content (μg/mg dry weight), total lipid, protein and dry matter of rainbow trout liver tissue fed experimental FO, LCO and HCO diets from week 0 (initial) and week 12[1].

Fatty acid	Initial	FO	LCO	HCO	F-value	P-value
14:0	3.0 ± 2.0	1.1 ± 0.2[a]	0.4 ± 0.1[b]	0.5 ± 0.2[b]	52.19	0.000
14:1n-5	0.0 ± 0.1	0.2 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[b]	68.51	0.000
15:0	0.3 ± 0.3	0.2 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[b]	68.51	0.000
16:0	41.3 ± 22.4	18.5 ± 2.2[a]	15.8 ± 2.1[b]	16.6 ± 2.9[ab]	4.89	0.012
16:1n-7c	7.7 ± 5.5	1.1 ± 0.3[a]	0.6 ± 0.2[b]	0.8 ± 0.3[b]	13.50	0.000
16:1n-7t	0.3 ± 0.4	0.1 ± 0.0[a]	0.0 ± 0.0[b]	0.1 ± 0.0[b]	6.54	0.003
17:0	0.4 ± 0.4	0.3 ± 0.1[a]	0.2 ± 0.0[b]	0.2 ± 0.0[b]	57.34	0.000
17:1n-7	0.3 ± 0.3	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.19	0.825
18:0	14.7 ± 7.3	7.3 ± 1.4	7.2 ± 1.2	7.0 ± 1.4	0.09	0.916
18:1n-9c	37.3 ± 21.8	11.7 ± 2.9	12.3 ± 2.3	12.8 ± 4.3	0.46	0.637
18:1n-9t	0.6 ± 0.3	0.1 ± 0.1[a]	0.1 ± 0.0[b]	0.1 ± 0.0[ab]	4.21	0.022
18:1n-12c	2.1 ± 1.3	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.43	0.654
18:1n-7c	6.4 ± 3.5	1.5 ± 0.4[a]	1.2 ± 0.2[b]	1.3 ± 0.4[ab]	4.05	0.025
19:0	0.3 ± 0.4	0.1 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[b]	13.65	0.000
19:1n-12	0.1 ± 0.2	0.0 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	2.48	0.096
18:2n-6 (LNA)	6.8 ± 4.1	5.4 ± 1.6[b]	7.3 ± 1.5[a]	7.6 ± 2.3[a]	6.54	0.003
20:0	0.4 ± 0.4	0.1 ± 0.1[b]	0.4 ± 0.1[a]	0.4 ± 0.1[a]	12.72	0.000
18:3n-6	0.1 ± 0.2	0.1 ± 0.0[b]	0.2 ± 0.1[a]	0.2 ± 0.1[a]	17.96	0.000
20:1n-15	0.0 ± 0.1	0.0 ± 0.0[a]	0.0 ± 0.0[b]	0.0 ± 0.0[b]	4.18	0.022
20:1	2.9 ± 1.8	0.3 ± 0.1[a]	0.2 ± 0.1[b]	0.2 ± 0.1[b]	11.17	0.000
20:1n-9	4.7 ± 2.7	0.1 ± 0.0[b]	0.1 ±0.1[ab]	0.1 ± 0.2[a]	3.78	0.031
18:3n-3 (ALA)	0.9 ± 0.6	1.1 ± 0.5[b]	2.7 ± 1.0[a]	2.7 ± 1.7[a]	9.16	0.000
18:4n-3	0.3 ± 0.3	0.2 ± 0.1[b]	0.6 ± 0.3[a]	0.4 ± 0.2[b]	12.82	0.000
20:2n-6	2.2 ± 1.2	0.8 ± 0.3[b]	1.2 ± 0.3[ab]	1.4 ± 0.8[a]	6.57	0.003
22:3n-3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.87	0.068
22:0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.3	1.59	0.216
20:3n-6	2.6 ± 1.0	0.6 ± 0.2[b]	1.6 ± 0.5[a]	1.4 ± 0.6[a]	15.29	0.000
22:1n-11	1.3 ± 1.0	0.1 ± 0.1	0.2 ± 0.2	0.1± 0.1	0.5	0.612
22:1n-9	0.4 ± 0.3	0.1 ± 0.0[b]	0.1 ± 0.1[ab]	0.1 ± 0.2[a]	3.78	0.031
20:3n-3	0.2 ± 0.1	0.1 ± 0.1[b]	0.5 ± 0.1[a]	0.5 ± 0.3[a]	17.97	0.000
20:4n-6 (ARA)	7.8 ± 4.4	4.2 ± 1.4[b]	5.5 ± 1.1[a]	5.7 ± 1.4[a]	5.75	0.006
22:2n-6	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	1.22	0.305
24:0	0.0 ± 0.1	1.8 ± 2.4	1.3 ± 17	1.0 ± 1.8	0.64	0.530
20:5n-3 (EPA)	11.8 ± 6.7	4.4 ± 2.6	3.8 ±2.3	3.8 ±2.4	0.33	0.722
24:1n-9	2.3 ± 1.3	1.0 ± 0.4	0.9 ± 0.2	0.8 ± 0.2	2.29	0.114
22:4n-6	1.2 ± 1.3	0.3 ± 0.4	0.3 ± 0.1	0.3 ± 0.1	0.13	0.881
22:5n-3	4.0 ± 2.2	2.0 ± 0.4	1.8 ± 0.5	2.1 ± 0.7	0.96	0.390
22:6n-3 (DHA)	100.0 ± 54.0	32.1 ± 7.9	27.0 ± 4.6	29.0 ± 5.6	2.59	0.087
ΣSFA[1]	60.5 ± 31.6	29.6 ± 5.3	25.5 ± 3.9	26.1 ± 5.1	3.17	0.052
ΣMUFA[2]	66.4 ± 38.5	17.3 ± 4.2	17.9 ± 3.3	18.7 ± 6.8	0.27	0.761
ΣMUFA≥18	58.0 ± 33.0	16.0 ± 3.9	17.2 ± 3.2	17.8 ± 6.5	0.51	0.604
ΣMUFA<18	11.6 ± 6.7	2.4 ± 0.9	3.4 ± 0.8	3.3 ± 2.0	2.53	0.092
ΣPUFA[3]	140.9 ± 75.9	51.5 ± 11.7	52.6 ± 8.2	55.1 ± 10.3	0.51	0.605
ΣC18 PUFA[3]	8.2 ± 5.0	6.8 ± 2.1[b]	10.9 ± 2.6[a]	10.9 ± 4.1[a]	8.78	0.001

Fatty acid	Initial	FO	LCO	HCO	F-value	P-value
ΣC20 PUFA[3]	25.5 ± 14.2	10.2 ± 3.0[b]	12.5 ± 2.7[ab]	12.8 ± 3.0[a]	3.72	0.033
ΣC22 PUFA[3]	107.2 ± 57.6	34.5 ± 8.0	29.1 ± 5.0	31.4 ± 6.2	2.52	0.093
ΣEPA & DHA	4.1 ± 2.2	36.6 ± 8.4	30.8 ± 5.2	32.8 ± 6.3	2.80	0.072
Σn-3	118.1 ± 63.9	40.1 ± 9.1	36.5 ± 5.8	38.6 ± 7.0	0.90	0.415
Σn-6	22.8 ±12.4	11.4 ± 3.1[b]	16.1 ± 2.8[a]	16.5 ± 4.3[a]	10.29	0.000
Σodd chain	1.4 ± 1.3	0.7 ± 0.2[a]	0.5 ± 0.1[b]	0.4 ± 0.1[b]	19.96	0.000
n-3/n-6	5.2 ± 0.7	3.6 ± 0.5[a]	2.3 ± 0.2[b]	2.4 ± 0.5[b]	37.08	0.000
Total lipid, protein, dry matter (g/kg))						
Lipid ww	4.4 ± 1.3	3.4 ± 0.8	3.4 ± 1.0	3.1 ± 0.8	0.70	0.501
Lipid dw	18.6 ± 5.8	13.6 ± 2.0	14.0 ± 3.9	12.6 ± 2.4	0.92	0.406
Dry matter	23.6 ± 3.5	25.2 ± 4.3	24.5 ± 3.1	24.5 ± 0.8	0.24	0.786

[1]Data expressed as μg FAME/mg (dry weight), values are means (n=3 per treatment) ± standard deviation. Final means with different superscripts indicate significant differences based on Tukey's post-hoc test following a one-way ANOVA. FO, fish oil; LCO, low camelina oil; HCO, high camelina oil. Reported fatty acids are > 1 μg/mg

[2]Saturated fatty acid

[3]Monounsaturated fatty acid

[4]Polyunsaturated fatty acid

Table 2.7 Fatty acid content (μg/mg dry weight), total lipid and dry matter of rainbow trout brain tissue fed experimental FO, LCO and HCO diets after 12 weeks of feeding[1].

Fatty acid	FO	LCO	HCO	F-value	P-value
14:0	3.5 ± 1.6[a]	2.0 ± 0.8[b]	1.4 ± 0.2[b]	15.03	0.000
15:0	0.2 ± 0.2[a]	0.0 ± 0.1[b]	0.0 ± 0.0[b]	17.55	0.000
16:0	72.3 ± 21.2	80.7 ± 22.5	64.7 ± 13.2	2.36	0.107
16:1n-7c	7.3 ± 2.5[a]	6.9 ± 1.7[ab]	5.3 ± 0.9[b]	4.49	0.017
16:1n-7t	0.4 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	1.34	0.273
17:0	0.6 ± 0.5[a]	0.2 ± 0.2[b]	0.2 ± 0.3[b]	4.73	0.014
18:0	30.3 ± 7.3	34.0 ± 8.7	27.5 ± 5.2	2.81	0.072
18:1n-9c	104.6 ± 26.6[ab]	123.3 ± 32.5[a]	95.7 ± 15.9[b]	4.13	0.023
18:1n-9t	0.8 ± 1.5	0.1 ± 0.1	0.1 ± 0.2	3.65	0.035
18:1n-12c	0.8 ± 0.4	0.9 ± 0.3	0.6 ± 0.2	2.34	0.109
18:1n-7c	9.9 ± 2.4	10.4 ± 2.3	8.8 ± 1.5	2.05	0.142
19:1n-12	0.1 ± 0.2[a]	0.0 ± 0.0[b]	0.0 ± 0.0[b]	5.52	0.008
18:2n-6 (LNA)	11.3 ± 5.5[b]	17.5 ± 8.4[a]	10.4 ± 3.3[b]	5.57	0.007
20:0	0.5 ± 0.2[b]	0.8 ± 0.3[a]	0.6 ± 0.2[b]	6.50	0.004
18:3n-6	0.1 ± 0.1 [b]	0.5 ± 0.3[a]	0.3 ± 0.1[a]	14.04	0.000
20:1n-15	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	2.15	0.130
20:1n-9	7.6 ± 2.1[b]	12.3 ± 3.0[a]	8.5 ± 1.9 [b]	15.50	0.000
18:3n-3 (ALA)	3.6 ± 1.4[b]	11.9 ± 5.3[a]	4.5 ± 0.9[b]	29.40	0.000
18:4n-3	0.8 ± 0.6[b]	1.7 ± 1.2[a]	0.6 ± 0.4[b]	7.38	0.002
20:2n-6	1.6 ± 0.5[b]	2.7 ± 0.7[a]	1.7 ± 0.4[b]	18.15	0.000
22:3n-3	2.3 ± 0.6	2.8 ± 0.7	2.5 ± 0.7	2.21	0.123
22:0	0.9 ± 0.7	0.8 ± 0.6	0.7 ± 0.7	0.38	0.687
20:3n-6	0.9 ± 0.3[c]	2.5 ± 0.7[a]	1.3 ± 0.1[b]	49.19	0.000
22:1n-9	1.9 ± 0.4[b]	2.7 ± 0.6[a]	2.0 ± 0.5[b]	9.71	0.000
20:3n-3	1.0 ± 0.3[c]	2.8 ± 0.7 [a]	1.6 ± 0.3[b]	65.93	0.000
20:4n-6 (ARA)	4.9 ± 1.1 [b]	8.0 ± 2.0 [a]	7.2 ± 1.4 [a]	16.46	0.000
22:2n-6	0.6 ± 0.1[b]	0.9 ± 0.2[a]	0.6 ± 0.1[b]	11.83	0.000
24:0	1.1 ± 0.3	1.2 ± 0.3	1.0 ± 0.2	1.77	0.183
20:5n-3 (EPA)	19.2 ± 4.3	19.6 ± 4.9	17.3 ± 2.7	1.31	0.280
24:1n-9	22.1 ± 5.1	24.5 ± 7.3	20.9 ± 5.2	1.29	0.286
22:4n-6	2.6 ± 3.3	3.8 ± 5.2	4.4 ± 5.2	0.55	0.583
22:5n-3	8.2 ± 2.2	8.2 ± 1.8	7.9 ± 1.9	0.11	0.896
22:6n-3 (DHA)	85.0 ± 20.7	94.9 ± 25.7	80.0 ± 19.8	1.64	0.207
ΣSFA[1]	110.3 ± 29.8	120.4 ± 31.8	97.1 ± 19.1	2.53	0.093
ΣMUFA[2]	162.5 ± 40.3[ab]	189.0 ± 47.4[a]	147.7 ± 24.6[b]	4.11	0.024
ΣPUFA[3]	142.2 ± 33.7[b]	177.8 ± 43.4[a]	140.3 ± 28.3[b]	4.97	0.012
ΣC18 PUFA[3]	15.9 ± 7.6[b]	31.6 ± 14.9[a]	15.8 ± 14.6[b]	11.77	0.000
ΣC20 PUFA[3]	27.6 ± 6.2[b]	35.5 ± 8.4[a]	29.1 ± 4.6[b]	5.85	0.006
ΣC22 PUFA[3]	98.7 ± 23.3	110.6 ± 30.3	95.3 ± 26.0	1.28	0.288
ΣEPA & DHA	104.2 ± 24.6	114.5 ± 30.3	97.2 ± 22.2	1.57	0.221
Σn-3	117.8 ± 27.6[ab]	139.1 ± 33.7[a]	111.8 ± 24.1[b]	3.52	0.039
Σn-6	22.0 ± 8.1[b]	35.8 ± 13.0[a]	26.0 ± 4.8[b]	8.55	0.001

Fatty acid	FO	LCO	HCO	F-value	P-value
n-3/n-6	5.7 ± 1.2[a]	4.1 ± 1.0[b]	4.3 ± 0.7[b]	11.32	0.000
Total lipid and dry matter (g/kg)					
Lipid ww	79 ± 27	62 ± 26	79 ± 9.0	2.97	0.062
Lipid dw	404 ± 132	329 ± 151	418 ± 42	2.42	0.102
Dry matter	195 ± 9.0	194 ± 19	191 ± 11	0.31	0.733

[1]Data expressed as μg FAME/mg (dry weight), values are means (n=3 per treatment) ± standard deviation. Means with different superscripts indicate significant differences based on Tukey's post-hoc test following a one-way ANOVA. FO, fish oil; LCO, low camelina oil; HCO, high camelina oil. Reported fatty acids are > 1 μg/mg

[2]Saturated fatty acid

[3]Monounsaturated fatty acid

[4]Polyunsaturated fatty acid

Table 2.8 Fatty acid content (μg/mg dry weight), total lipid and dry matter of rainbow trout eye tissue fed experimental FO, LCO and HCO diets after 12 weeks of feeding[1].

Fatty acid	FO	LCO	HCO	F-value	P-value
14:0	29.4 ± 7.8[a]	12.7 ± 2.8[b]	13.1 ± 3.1[b]	50.67	0.000
15:0	2.1 ± 0.5[a]	1.1 ± 0.2[b]	1.2 ± 0.2[b]	39.18	0.000
16:0	133.9 ± 38.1	121.3 ± 25.9	120.5 ± 29.6	0.83	0.445
16:1n-7c	42.7 ± 10.3[a]	27.0 ± 6.0[b]	27.5 ± 6.6[b]	18.72	0.000
16:1n-7t	0.7 ± 0.2[a]	0.5 ± 0.6[ab]	0.3 ± 0.1[b]	3.41	0.043
17:0	2.0 ± 0.5[a]	1.1 ± 0.3[b]	1.3 ± 0.2[b]	29.16	0.000
18:0	31.0 ± 7.6[b]	35.2 ± 5.9[ab]	39.8 ± 8.7[a]	5.19	0.010
18:1n-9c	323.3 ± 91.0	358.3 ± 74.6	319.1 ± 77.1	1.00	0.375
18:1n-9t	1.2 ± 0.5[ab]	0.9 ± 0.1[b]	1.3 ± 0.3[a]	5.23	0.009
18:1n-12c	3.2 ± 1.1	8.1 ± 14.1	4.8 ± 1.1	1.39	0.262
18:1n-7c	27.3 ± 6.0	24.1 ± 3.4	25.1 ± 4.9	1.61	0.212
19:1n-12	2.8 ± 0.7[a]	0.6 ± 0.2[b]	0.7 ± 0.2[b]	123.16	0.000
18:2n-6 (LNA)	127.1 ± 34.4[b]	200.9 ± 33.5[a]	215.2 ± 47.9[a]	21.61	0.000
20:0	2.1 ± 0.5[c]	5.1 ± 0.8[b]	7.0 ± 1.6[a]	76.04	0.000
18:3n-6	1.7 ± 0.5[c]	4.9 ± 1.7[b]	7.6 ± 2.1[a]	54.66	0.000
20:1n-15	0.3 ± 0.2[b]	0.7 ± 0.3[a]	0.9 ± 0.3[a]	20.73	0.000
20:1n-9	19.7 ± 6.2	30.2 ± 28.3	30.0 ± 17.1	1.46	0.245
18:3n-3 (ALA)	30.6 ± 8.6[c]	136.7 ± 24.9[a]	71.0 ± 15.8[b]	134.38	0.000
18:4n-3	7.8 ± 2.0[b]	16.6 ± 3.5[a]	10.2 ± 2.6[b]	40.48	0.000
20:2n-6	8.3 ± 2.0[c]	15.5 ± 2.2[a]	12.1 ± 2.5[b]	37.57	0.000
22:3n-3	0.3 ± 0.1[c]	1.0 ± 0.2[a]	0.6 ± 0.1[b]	73.92	0.000
22:0	1.0 ± 0.2[b]	1.4 ± 0.3[a]	1.6 ± 0.4[a]	15.57	0.000
20:3n-6	3.0 ± 0.6[c]	6.5 ±0.9[b]	8.9 ± 2.0[a]	76.81	0.000
22:1n-9	2.7 ± 0.5[c]	8.2 ± 2.7[a]	4.5 ± 0.9[b]	42.64	0.000
20:3n-3	2.0 ± 0.7[c]	9.2 ± 1.2[a]	6.8 ± 1.3[b]	156.80	0.000
20:4n-6 (ARA)	4.9 ± 1.1[b]	5.3 ± 0.9[b]	14.6 ± 3.1[a]	112.13	0.000
22:2n-6	0.9 ± 0.2[b]	1.1 ± 0.2[a]	1.2 ± 0.3[a]	9.41	0.000
24:0	0.5 ± 0.1[b]	0.7 ± 0.1[a]	0.6 ± 0.1[b]	12.52	0.000
20:5n-3 (EPA)	34.7 ± 9.0[a]	15.3 ± 3.0[b]	39.8 ± 9.2[a]	40.67	0.000
24:1n-9	2.4 ± 0.6[b]	3.7 ± 0.6[a]	2.8 ± 0.6[b]	16.93	0.000
22:4n-6	1.3 ± 0.8[b]	0.9 ± 0.5[b]	3.1 ± 1.4[a]	21.52	0.000
22:5n-3	9.4 ± 1.8[b]	4.7 ± 0.9[c]	16.5 ± 3.1[a]	109.42	0.000
22:6n-3 (DHA)	60.7 ± 12.3[b]	40.3 ± 7.6[c]	74.0 ± 15.1[a]	28.07	0.000
ΣSFA[1]	202.5 ± 55.2	179.1 ± 35.3	185.4 ± 43.4	1.03	0.365
ΣMUFA[2]	450.3 ± 123.1	496.0 ± 109.7	439.4 ± 111.6	0.98	0.385
ΣPUFA[3]	292.9 ± 72.4[b]	444.7 ± 94.5[a]	481.7 ± 101.6[a]	18.35	0.000
ΣC18 PUFA[3]	167.4 ± 45.2[b]	345.0 ± 82.2[a]	304.1 ± 67.9[a]	28.76	0.000
ΣC20 PUFA[3]	53.0 ± 13.2[b]	51.8 ± 7.4[b]	82.2 ± 17.3[a]	24.66	0.000
ΣC22 PUFA[3]	72.5 ± 14.3[b]	48.0 ± 8.8[c]	95.4 ± 19.3[a]	36.89	0.000
ΣEPA & DHA	95.4 ± 21.2[b]	55.6 ± 10.1[c]	113.9 ± 23.9[a]	33.51	0.000
Σn-3	145.2 ± 34.0[b]	222.7 ± 35.7[a]	218.3 ± 44.6[a]	18.96	0.000
Σn-6	147.3 ± 38.4[b]	221.0 ± 67.0[a]	262.8 ± 57.7[a]	16.72	0.000

Fatty acid	FO	LCO	HCO	F-value	P-value
n-3/n-6	1.0 ± 0.0	1.4 ± 1.7	0.8 ± 0.0	1.30	0.264
Total lipid and dry matter (g/kg)					
Lipid ww	235 ± 69	227 ± 55	199 ± 25	1.90	0.162
Lipid dw	739 ± 192	744 ± 131	694 ± 67	0.60	0.553
Dry matter	315 ± 26	302 ± 52	286 ± 23	2.28	0.115

[1]Data expressed as μg FAME/mg (dry weight), values are means (n=3 per treatment) ± standard deviation. Means with different superscripts indicate significant differences based on Tukey's post-hoc test following a one-way ANOVA. FO, fish oil; LCO, low camelina oil; HCO, high camelina oil. Reported fatty acids are > 1 μg/mg

[2]Saturated fatty acid

[3]Monounsaturated fatty acid

[4]Polyunsaturated fatty acid

Table 2.9 color and texture and sensory evaluation of rainbow trout muscle tissue after 12 weeks feeding experimental FO, LCO and HCO diets.

Sensory parameter	FO	LCO	HCO	F- or T- value	P-value
Instrumental analysis[1]					
Color score, l	52.86 ± 3.3	56.29 ± 3.7	55.45 ± 2.0	3.06	0.066
Color score, a	21.2 ± 2.5	20.5 ± 3.6	21.2 ± 3.7	0.11	0.895
Color score, b	31.7 ± 1.9	30.9 ± 3.1	30.5 ± 2.5	0.56	0.581
Texture (Force)	215.9 ± 58.9	200.3 ± 29.6	184.8 ± 45.8	1.02	0.377
Qualitative descriptive analysis[2]					
Brightness	5.729 ± 2.533	-	6.125 ± 1.825	-0.76	0.451
Orange intensity	5.729 ± 2.430b	-	7.167 ± 2.099a	-2.60	0.012
Surface moistness	6.063 ± 2.254	-	5.917 ± 1.909	0.29	0.775
Firmness	5.354 ± 1.995b	-	7.188 ± 1.774a	-3.96	0.001
Marine odour	4.271 ± 2.727	-	3.458 ± 2.963	1.13	0.267
Vegetable odour	1.458 ± 2.010	-	1.292 ± 2.010	0.33	0.742
Rancid odour	0.375 ± 1.104	-	0.250 ± 0.532	0.65	0.519
Hedonic test[2]					
Appearance	3.688 ± 1.764	-	3.604 ± 1.700	0.20	0.846
Texture	2.375 ± 0.809a	-	1.771 ± 1.063b	2.45	0.019
Odour	2.229 ± 0.893	-	2.563 ± 0.681	-1.76	0.083

[1]Values are means ± standard deviation (n=9). Means with different superscripts indicate significant difference as determined by one-way ANOVA (F-value in table).
[2]Values are means ± standard deviation (FO, n=48; HCO, n=24). Sensory evaluation conducted with untrained panelists on FO (fish oil) and HCO (high camelina oil) treatments only. Means with different superscripts indicate significant difference as determined by t-test (T-value in table).

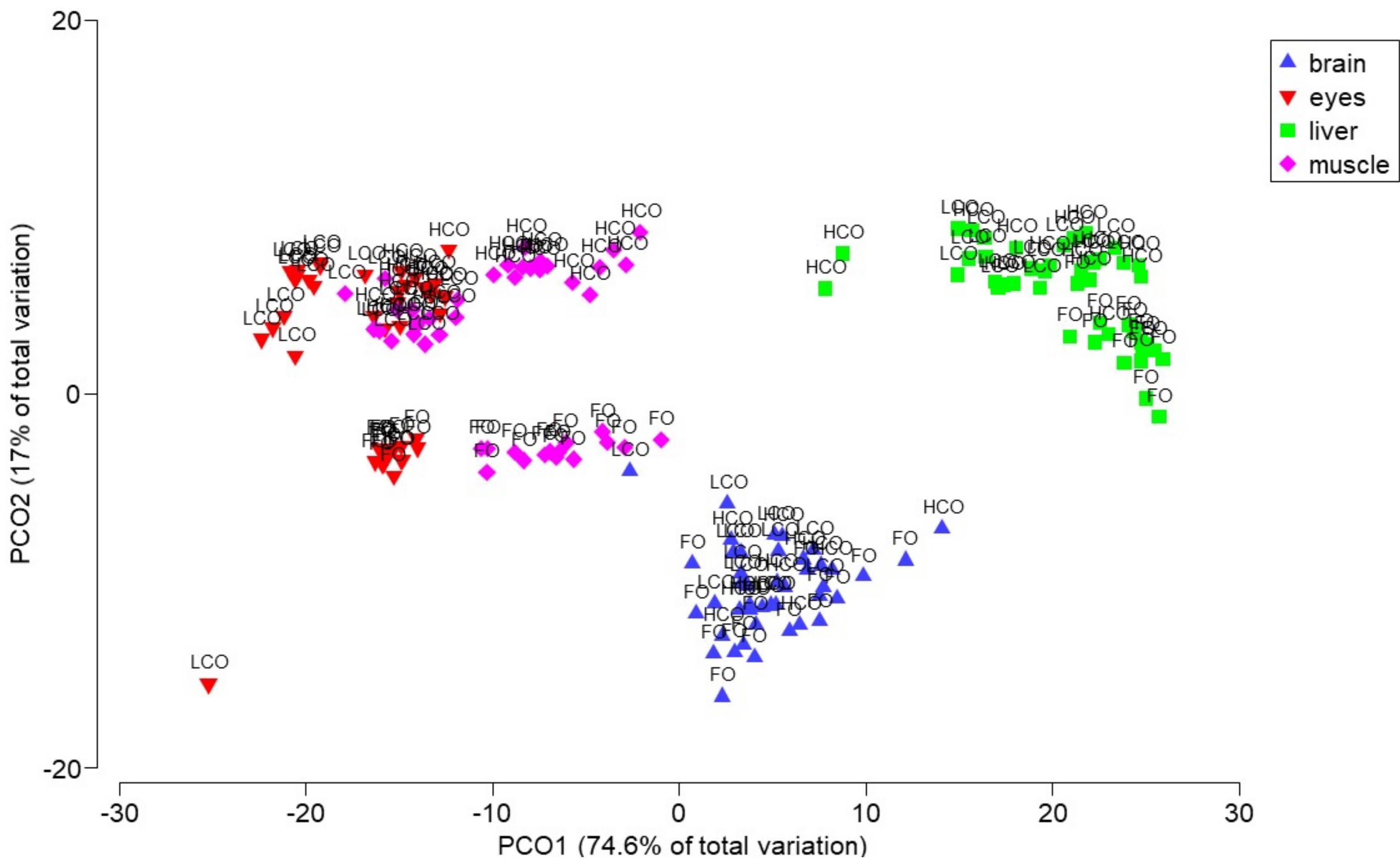

Figure 2.1 Principal co-ordinate ordination plot of fatty acid profiles of individual rainbow trout tissues (brain, eye, liver, muscle) using a Bray–Curtis similarity matrix, where three dietary treatments are represented (FO, HCO, LCO), with n=9 per treatment.

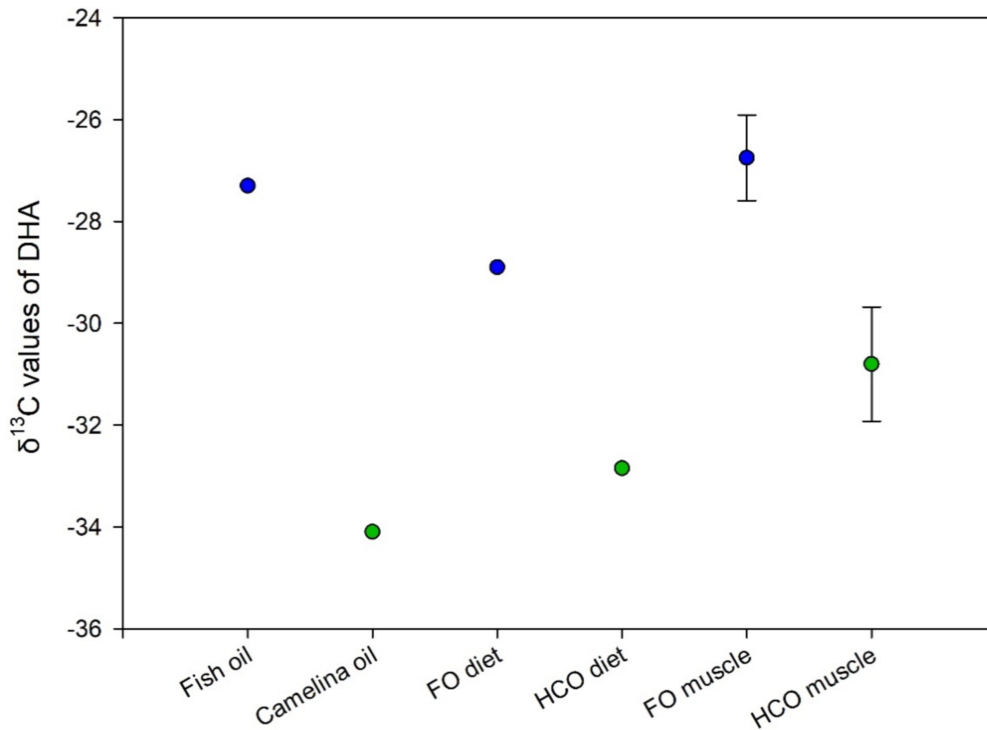

Figure 2.2 δ13C values of DHA in fish oil (‰), transgenic camelina oil, and rainbow trout muscle tissue fed fish oil (FO) and high camelina oil (HCO) diets. Values for muscle samples are means ± standard deviation (n=9) and were significantly different (p = 0.001). The value for fish oil was obtained from Hixson et al. (2014a). Data points in blue are from fish oil sources and data points in green are from camelina sources.

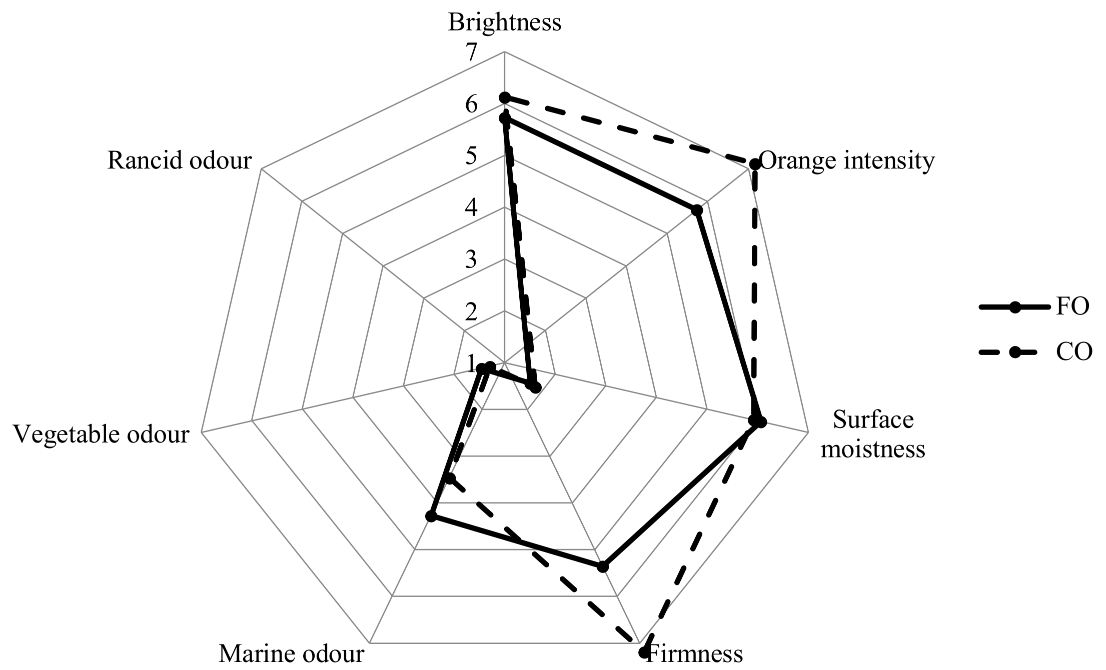

Figure 2.3 Radial plot of QDA sensory comparative analysis of week 12 rainbow trout muscle tissue sampled from trout fed FO and HCO diets.

CHAPTER 3.0
SCHIZOCHYTRIUM SP. (T-18) OIL AS A FISH OIL REPLACEMENT IN DIETS FOR JUVENILE RAINBOW TROUT: EFFECTS ON GROWTH PERFORMANCE AND TISSUE FATTY ACID CONTENT.

3.1 ABSTRACT

Oil, with high levels of DHA (40%), was extracted from isolates of the marine microbe *Schizochytrium* sp. (strain T-18; MO), and combined with camelina oil. To determine if this oil could completely replace fish oil (FO), three formulations were tested: fish oil diet (100 g/kg FO and 50 g/kg camelina oil; CO); Low microbial oil (MO) and Camelina oil (CO) blend (MO/CO; no FO, with 75 g/kg each of MO and CO); High MO diet (MO; no fish oil, with 100 g/kg MO and 50 g/kg CO). DHA content was highest in the MO/CO (28.6 ± 0.8^{b}) and MO (48.9 ± 4.9^{a}) diets relative to FO (13.2 ± 0.2^{c}), while EPA content was higher in the FO diet (19.0 ± 0.8^{a}) relative to MO/CO (1.8 ± 0.1^{b}) and MO (2.1 ± 0.2^{b}) diets. After feeding for eight weeks (14°C), rainbow trout (18.8 ± 2.9 g fish^{-1} initial weight ± SD) exhibited no significant differences in weight gain, final weight, condition factor, visceral somatic index, specific growth rate, apparent feed intake, and feed conversion ratio. Fatty acid profiles, by comparison, differed highly significantly depending on both diet ($p < 0.001$) and tissue type ($p < 0.001$). Liver, EPA significantly higher in trout fed the FO diet compared to trout fed the MO/CO and MO diets ($p < 0.001$). Liver DHA was similar between trout fed the FO and MO diets; however, DHA was highest in trout fed the MO/CO diet. In muscle, DHA was highest in trout fed the MO and MO/CO diets compared to trout fed the FO diet ($p < 0.001$). Muscle EPA was highest in trout fed the FO diet compared to trout fed the MO and MO/CO diets. ALA was higher in muscle of trout fed the MO/CO diet compared to trout fed the FO and MO diets. Overall, *Schizochytrium* sp. (T-18) oil was an effective substitute for FO as a dietary source of DHA for juvenile rainbow trout.

3.2 INTRODUCTION

One of the central concerns in aquaculture is the overreliance on wild-sourced fish oil (FO) as a primary source of dietary omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA) (Tocher et al., 2019). Unlike FO, which is a primary source of marine-derived LC-PUFA, namely eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), the inclusion of alternative terrestrially-sourced oils in formulated aquaculture diets typically do not contain these essential n-3 LC-PUFA(Colombo, 2020). Both EPA and DHA contribute substantially to optimal fish growth, development, inflammatory response, and neural and ocular tissue function (Tocher, 2003). Inclusion of n-3 LC-PUFA in salmonid diets such as rainbow trout (*Oncorhynchus mykiss*) is recommended at a rate of 0.5 to 1.0% however this value can vary based on species type and stage of development (NRC, 2011). For humans, consumption of fish and fish-based products remains the primary source of FO containing essential n-3 LC-PUFA necessary for optimum health (da Silva et al., 2019). The n-3 LC-PUFA contributes substantially to human growth and development, affecting immune system function, cardiovascular and neurological health, retinal health, membrane fluidity, cellular metabolism, and inflammation (Tocher, 2015; da Silva et al., 2019). Dietary DHA, in particular, has also been closely linked to healthy infant development during pregnancy, infant behaviour, mood, and cognitive function (Ryan et al., 2010; Bazan et al., 2011; Lauritzen et al., 2016).

In aquatic ecosystems, marine algae play an important role in producing several high-value nutritional compounds such as EPA and DHA (Nichols., 2003; da Silva et al., 2019; Yarnold et al., 2019; Jacob-Lopes et al., 2020). The active biosynthesis of compounds such

as n-3 LC-PUFA by microorganisms is an endogenous response to growth, development, and changing environmental conditions such as temperature (Adarme-Vega et al., 2012). Fish typically rely on dietary n-3 LC-PUFA, such as EPA and DHA, due to their reduced capacity to biosynthesize these compounds (Nichols., 2003; Jacob-Lopes et al., 2020). As such, fish obtain these nutrients as they are transferred from primary producers (and other microbes) along the food chain (Nichols., 2003; Jacob-Lopes et al., 2020). Derived microbial oils (MO) from microorganisms have become of great interest as sustainable and advantageous alternatives to FO use in aquaculture and other industries (Tibbetts et al., 2020).

Recent research has focused on the incorporation of microbially-derived n-3 LC-PUFA for aquafeeds and other industrial applications due to their capacity to be produced through efficient and sustainable means (Desvilettes & Bec, 2009; Adarme-Vega et al., 2012; Gong & Gong, 2014; Patel et al., 2020). In this application, microbially-sourced n-3 LC-PUFA provide an opportunity to bypass food chain nutrient transfers and instead provide fish with a dietary source n-3 LC-PUFA from microbial species reared within a closed system (Cohen & Ratledge, 2015). This has the added benefit of reducing exposure to environmental contaminants, such as heavy metals, PCBs, pharmaceuticals, etc., that can be present in fish-derived feed products (Sprague et al., 2015; Wei et al., 2021). Many microbes produce high amounts of total lipids, contributing to 30-70% of their total dry weight (10-50% wet weight) content (Adarme-Vega et al., 2012). In the past 5 years, various strains of *Schizochytrium* sp. have been evaluated for their applications in aquafeed production (Leyland et al., 2017; Tibbetts et al. 2020). Members of the genus

Schizochytrium are unicellular eukaryotic organisms that belong to the family *Thraustochytriacea* in the kingdom *Stramenopila* (Leyland et al., 2017). Often mistaken for microalgae, these non-photosynthetic organisms lack cellular plastids and are taxonomically identified as marine or brackish water fungal protists (Leyland et al., 2017; Tibbetts et al., 2020).

A novel strain of *Schizochytrium* sp. (T-18) has gained attention as a potential alternative and sustainable source of LC-PUFA (Damare, 2019; Tibbetts et al. 2020; Wei et al. 2021). When provided with adequate nutrient supplementation from nitrogen and carbon sources and under ideal growth conditions, thraustichytrids, such as *Schizochytrium* sp. produce high concentrations of lipid through the use of both the Type 1 fatty acid synthase or the polyketide synthase pathway (Metz et al., 2001; Desvilettes & Bec, 2009; Morabito et al., 2019). Biosynthetic production of lipids in thraustichytrids has been observed at >70% of their cellular mass (dry weight) and their overall lipid profile is naturally high in n-3 LC-PUFA (Tibbetts et al., 2020). *Schizochytrium* sp. can accumulate DHA at 30-40% of total FA's, making them a potential candidate for industrial production of DHA as a substitute for DHA in FO (Ratledge et al., 2010; Adarme-Vega et al., 2012). The specific non-transgenic strain, *Schizochytrium* sp. (T-18), used in our study has been employed effectively in several industry markets, including infant milk formula, and has been established for commercial DHA production (Winwood, 2013; Mathimani & Pugazhendhi, 2019). Relative to its natural counterpart, the transgenic T-18 strain has been genetically modified to have enhanced capacity for cellulosic sugar uptake and the efficient use of by-product sugars for growth and development (Tibbetts et al., 2020).

The extracted oil and dried biomass of *Schizochytrium* sp. have previously been tested as a lipid source in diets for juvenile Atlantic salmon (*Salmo salar*). A complete replacement of FO with T-18 oil showed high apparent digestibility (Tibbetts et al., 2020). In that study, juvenile salmon (32 g initial weight) were fed the T-18 strain oil at 0, 33, 66, and 100% rate of inclusion for 11 d for a digestibility trial. After 11 d of feeding, the inclusion of the T-18 strain oil showed no signs of negative impacts on fish health or growth performance in comparison to salmon fed the FO control (Tibbetts et al., 2020). Apparent digestibility of saturated FA's and DHA appeared to improve with the increasing replacement of FO with T-18 oil. Replacement of FO with a high DHA *Schizochytrium* sp. oil was evaluated in the diets for Atlantic salmon parr (38.5-40.0 g initial weight) (Miller et al., 2007). In this trial, four experimental diets were formulated to include *Schizochytrium* sp. oil at 100% dietary oil inclusion, 100% palm oil, and 4:1 ratio of palm oil and *Schizochytrium* sp. oil mixture in the replacement of FO and were fed to the parr over 9 weeks (Miller et al., 2007). There were no significant differences in the apparent digestibility of n-3 and n-6 PUFA, feed consumption, or salmon growth (Miller et al., 2007). DHA content was significantly higher in the muscle of salmon fed diets containing 100% replacement of FO with *Schizochytrium* sp. oil (Miller et al., 2007).

Recent research has evaluated the application of the T-18 strain oil in diets for Atlantic salmon parr (24 g initial weight) (Wei et al., 2021). Four experimental diets were fed to Atlantic salmon for 16 weeks: a reference diet (20% FO), a commercial-type control diet (10% FO + 10% vegetable oil), a low microbial oil diet (5% MO) and a high microbial oil diet (10% MO) (Wei et al., 2021). Overall, the inclusion of the T-18 strain oil resulted in

no differences in weight gain, growth rate, condition factor or feed conversion ratio. Muscle fatty acid (FA) content was also noted to reflect dietary composition (Wei et al., 2021). Previous trials also have examined the application of a different strain of *Schizochytrium sp.* which contains both EPA (15.7%) and DHA (39.8%) in diets for rainbow trout (99.9 ± 0.1 g to 331 ± 1.0 g initial weight) and its effect on digestibility, n-3 LC-PUFA concentration within muscle tissue, growth, and palatability (Santigosa et al., 2020). Three experimental trials were conducted to evaluate the dietary inclusion of Veramaris® algal oil (isolated from *Schizochytrium* sp.) at rates of 2.5, 3.0, 4.5, 5.0, 6.0 and 10% (Santigosa et al., 2020). Trout were fed over 39 d in trial 1, 26 d in trial 2, and 96 d in trial 3. Overall, dietary inclusion of *Schizochytrium* sp., oil showed high digestibility, and the FA profiles of trout fillets were reflective of experimental diets (Santigosa et al., 2020). No adverse effects were observed in trout growth, blood parameters, or feed conversion ratio relative to the control treatment. Although this was the first study to evaluate *Schizochytrium* sp. in diets for rainbow trout, the strain evaluated in Santigosa et al. (2020) differed from the one used within this study as it contained relatively high levels of EPA. However, *Schizochytrium* sp. T-18 oil, which is high in DHA (43.4% total FA) and low in EPA (0.7% total FA) has yet to be evaluated in diets for rainbow trout.

Rainbow trout are one of the most widely farmed aquaculture species in North America. Globally, the production of farmed rainbow trout has risen from 752,000 tonnes in 2010 to 848,000 tonnes in 2018 and now contributes 1.6% to the total tonnage of aquaculture species globally (FAO, 2020). In Canada, production exceeded 9,000 tonnes, and revenue from fish sales totalled $56.8 million CAD in 2018 (DFO, 2018). In the US, > 21,000 tonnes

were produced with revenue from fish sales totalling $95.6 million USD in 2019 (USDA, 2020; FAO, 2020). Rainbow trout require dietary supplementation of n-3 LC-PUFA of up to 1% of the dry diet (NRC, 2011). Although rainbow trout have the ability to synthesize n-3 LC-PUFA when provided with ALA and LNA, their capacity to do so is limited (Hixson et al. 2014a). Therefore n-3 LC-PUFA must be supplied in the diet for intensive aquaculture purposes.

In the current study, we evaluated a novel strain of *Schizochytrium* sp. (T-18) as a lipid source to completely replace FO in diets for juvenile rainbow trout relative to growth performance, muscle and liver FA content. With the application of novel microbial oils solely containing high DHA and no EPA, it is essential to understand how it is metabolized and used compared to FO (the industry standard). As such, the overall aim of this study is to evaluate the impact of this novel high DHA MO on the growth, metabolism, and fatty acid content (nutritional quality) of farmed juvenile rainbow trout.

3.3 MATERIALS AND METHODS

3.3.1 MICROBIAL OIL

The extracted microbial oil (MO) was produced heterotrophically from a thraustochytrid strain identified as *Schizochytrium* sp. produced by Mara Renewables (Dartmouth, NS, Canada). The variety used was DHA-02122016-C3, the same as two recent studies (Tibbetts et al. 2020, Wei et al. 2021). The FA component of the oil contained 0.7% EPA and 43.4% DHA, for a total n-3 LC-PUFA proportion of 44.1% (presented as % and μg/mg in Table 3.1). The MO also included low levels of alpha-linolenic acid (ALA; 18:3n-3;

0.1%) and linoleic acid (LNA; 18:2n-6; 0.3%) and oleic acid (18:1n-9: 0.6%). The sum of saturated FA (SFA), monounsaturated FA (MUFA) and PUFA were identified in the following proportions 46.7%, 8.2% and 45.2%, respectively. Total n-3 and total n-6 were 44.5 % and 0.7%, respectively. The n-3/n-6 ratio for the oil was 4.5 (Table 3.1).

3.3.2 EXPERIMENTAL DIET FORMULATION AND COMPOSITION

All diets were formulated to be isonitrogenous and isolipidic to meet the nutritional requirements of rainbow trout (NRC 2011) (Table 3.4). Diets were produced at the Chute Animal Nutrition Centre, Faculty of Agriculture, Dalhousie University (Truro, NS, Canada). All three experimental diets contained 150 g of oil/kg in the following ratios: a control diet with fish (herring) oil (FO diet; 100 g/kg [1]FO and 50 g/kg camelina oil; CO), a lower-level MO and camelina oil (CO) blend (MO/CO; no FO, with 75 g/kg each of MO and CO) and a higher MO inclusion diet (MO; no fish oil, with 100 g/kg MO and 50 g/kg CO; Table 3.2). Diet mash was extruded using a laboratory extruder (AMANDUS KAHL GmbH & Co. KG, Dieselstraße 5-9, D- 21465, Reinbek, Germany) with a 2.0 mm die. Pellets were dried in a JWP ST series industrial cabinet oven at 65°C for 4-5 hours. Excessive fines were sifted using both a 2.5 mm and 3 mm sieve. Final pellet length was 2.8 mm. Diets were vacuumed coated at room temperature and pre heated at 70°C. Pellets were exposed to a pressure of -0.9 bar at 70°C with added experimental oil blends at 150 g/kg of the diet. Pellets were held for 5 minutes at -0.9 bar, then slowly released over a 5-min period until pellets reach atmospheric pressure to ensure optimum absorption of fats into pellets. Diets were stored at -20°C in airtight containers to reduce oxidization of fats until needed. Diets were only exposed to room temperature (20–22 °C) during periods of

feeding. Diets were analyzed for nutritional composition and fatty acid analysis at Nova Scotia Department of Agriculture Laboratory Services (Truro, NS, Canada).

3.3.3 EXPERIMENTAL FISH

Juvenile rainbow trout (n=225; 18.8 ± 2.9 g $fish^{-1}$ initial weight ± SD) which were all-female triploids from Riverence, were obtained from North River Fish Farms (North River, NS, Canada) and transported to the Aquaculture lab, Faculty of Agriculture, Dalhousie University (Truro, NS, Canada). Fish were acclimated for a period of two weeks prior to random distribution and fed EWOS Vita 3mm; complete fee for salmonids, prior to being fed the experimental diets. Guidelines for ethical treatment of fish was followed in accordance with the Canadian Council of Animal Care (Dalhousie University approved protocol 2019-101). Twenty-five trout were randomly distributed into each of nine conical fiberglass experimental tanks (203 L per tank). Conical tank dimensions were 70 cm diameter and 42 cm. Each experimental diet was fed to triplicate tanks, with tank as the experimental unit (n=3). A flow-through supply of freshwater from a local aquifer was supplied to the tank system at a rate of 2-3 L per min^1 (ambient pH of 7.8 and alkalinity of 120 ppm as CaCO3). The dissolved oxygen (10.29 mg L^{-1}; 100% saturation) and approximate water temperature (14°C) were monitored and recorded daily. Fish were reared on a non-fixed simulated natural photoperiod cycle at Latitude 45°N over the 8-week trial period starting on October 11th 2019 13 h light : 11 h dark and ending on December 8th 2019 with a 10 h light : 14 h dark. Fish were fed twice daily at 9:00 and 15:00h to apparent satiation and feed intake was recorded weekly. Any observed mortalities were weighed and recorded throughout the experimental trial.

3.3.4 TISSUE SAMPLING

Fish were sampled at week 0, the day before feeding the experimental diets, and at week 8. At week 0, all fish in each of the experimental tanks (n= 25 fish per tank) were weighed using a tarred balance scale and initial body weight (g) for each individual fish was recorded to observe for any initial variance in weight of fish. Fish were also measured to obtain fork length (cm). At week 0, four fish per tank were randomly netted and euthanized with an overdose of anesthetic (tricaine methane sulfonate; MS-222, Syndel, Nanaimo, BC, Canada). Three fish per tank were sampled for tissues. The whole viscera was removed, weighed, and recorded. Liver samples were taken for FA analysis. Skin was removed in the left side of the fish under the dorsal fin, above the lateral line and dorsal muscle was sampled for FA analysis. The fourth fish was sampled as a full specimen for whole body protein and total lipid analysis (see Chapter 2 sections 2.4.2 and 2.4.3). At week 8, the same sampling regime was conducted.

3.3.5 GROWTH PERFORMANCE

Growth performance was assessed at week 0 (initial) and week 8 (final) sampling. Batch weights were recorded at week 4 to ensure fish would reach nearly 300% their initial weight within the 8-week trial period. Three mortalities were observed during this experiment. Mortalities of fish occurred during the last week of the trial period in which fluctuations in temperature resulted in a temperature drop over an hourly period from 14.0 to 11°C. During this time, the oxygen concentration in the tank system dropped from 100% saturation to 70%. This may have resulted in unanticipated stress on fish resulting in mortality. Fish

weight and length were measured on individual fish. Weight gain, condition factor (CF), visceral somatic index (VSI), specific growth rate (SGR), apparent feed intake (AFI) and feed conversion ratio (FCR) were calculated based on the following:

Weight gain (g/fish) = (final weight – initial weight)

CF = body mass / fork length^3

VSI (%) = 100 * (viscera weight / body weight)

SGR (%) = 100 * [ln(final body weight) – ln(initial body weight)]/ 56 days *100

AFI (g/fish) = feed consumed per tank (g) / number of fish per tank

FCR = AFI / weight gain

3.3.6 WHOLE BODY NUTRIENT COMPOSITION

Whole rainbow trout were partially thawed and coarsely ground using a bench-top meat grinder (Paderno, Pardinox Inc., Toronto, Ontario, Canada) and mixed for homogeneity. The sample was frozen and freeze-dried using Thermo Fisher Scientific, modulyoD thermo electron corporation, FR-Drying Digital Unit, Asheville, NC; Edwards Freeze Dryer for 72 h and ground into a fine powder using a bench-top homogenizer Nutribullet: Nutri Ninjablender, imported by Shark Ninja operating LLC Quebec, Canada. Nutrient analysis was completed at the Nova Scotia Department of Agriculture Laboratory Services (Truro, NS, Canada).

3.3.7 TOTAL LIPID AND FA CONTENT OF DIETS AND TISSUES

Total lipid and FA content were analyzed on liver and muscle sampled from trout at both weeks 0 and 8. Tissue samples were placed into 2 ml micro centrifuge tubes, flash frozen

in liquid nitrogen and were stored in a -80°C freezer until they could be shipped to Ryerson University (Toronto, ON) for FA analysis. Tissues were freeze-dried and individually ground to a fine powder in liquid nitrogen using a ceramic mortar and pestle (which were washed with soap and water and lipid-cleaned three times with 2 mL chloroform:methanol (2:1; v/v) between each sample), and the resulting powder was subsampled and weighed to the nearest microgram. Total lipid was extracted using a modified Folch method (Folch et al., 1957). Briefly, each sample was extracted three times, using 2 mL of chloroform/methanol (2:1; v/v) and then pooled (total 6 mL). Polar impurities were removed by adding 1.6 mL of an aqueous KCl solution (0.9% w/v). The organic layer was removed using a lipid-cleaned glass pipette and pooled. The resulting lipid-containing solvent was concentrated to 2 mL by evaporating with nitrogen gas. The lipid extract was then prepared for gas chromatography (GC) by derivatizing into fatty acid methyl esters (FAME) using the Hildich reagent (1.5 H_2SO_4: 100 anhydrous MeOH) as the catalyst (Christie et al., 2003). Reagents were added in the proportion of 1.5 mL reagent per 4–16 mg of lipid. Samples were heated at 90°C for 90 min and vortexed halfway through the derivatization reaction. The FAME were extracted twice using hexane: diethyl ether (1:1; v/v), then dried under a gentle stream of nitrogen. The dry FAME extract was re-dissolved in hexane and individual FAME were separated using a GC (Shimadzu-2010 Plus, Nakagyo-ku, Kyoto, Japan) equipped with an SP-2560 column (Sigma-Aldrich, St. Louis, Missouri). All solvents used in the extraction and FAME derivatization procedures were of high purity HPLC grade (>99%). FAME in samples were identified by comparison of their retention times with a known standard (GLC-463 reference standard; Nu-chek Prep, Inc., Waterville, Minnesota) and quantified with a 5-point calibration curve using this same

standard. A known concentration of 5 alpha-cholestane (C8003, Sigma-Aldrich, St. Louis, MI, USA) was added to each sample prior to extraction to act as the internal standard to estimate extraction and instrument recovery efficiency.

3.3.8. STATISTICAL ANALYSIS

Growth performance results were analyzed by ANOVA using the general linear model in Minitab 18 Statistical Software. For measurements on individual fish (e.g., weight, length, VSI, CF), a two-level nested ANOVA was used to analyze growth data. This model was designed to test the effect of diet treatment (fixed factor) on the growth performance (response variable) and nested fish individuals (random factor) within tanks, to remove variability among fish within tanks, while also testing for effects of individual tanks (Ruohonen, 1998). For measurements that were based on tank means, fish individuals were not independent (e.g., weight gain, SGR, AFI, FCR), a one-way ANOVA was conducted to test the effect of diet. Tukey HSD post-hoc tests ($p < 0.05$ significance level) were applied to assess differences among treatments.

For lipid and FA content of the muscle and liver, a one-way ANOVA was used to detect treatment differences, followed by a Tukey post-hoc test for multiple comparisons ($p < 0.05$ significance level). Multivariate analyses, including permutational multivariate analysis of variance (PERMANOVA) and Principal Coordinates Analysis (PCoA) were used to determine whole fatty acid profile changes in individual fish tissues among treatments. Vectors were included in the plot that showed correlations > 0.75 (Pearson correlation). The non-metric Bray–Curtis dissimilarity statistic was used to quantify the compositional

dissimilarity between samples in the PCO plot (Bray and Curtis 1957). PERMANOVA analyses were performed on resemblance matrices, which were built upon the Bray-Curtis similarity matrix. Multivariate statistics (i.e., PCoA and PERMANOVA) were performed using Primer 7 with the PERMANOVA+ add on package (Primer-E version 7, Auckland, New Zealand).

3.4 RESULTS

3.4.1 GROWTH PERFORMANCE

Dietary MO inclusion had a significant impact on growth performance (Table 3.4). Trout fed the FO diet had a longer fork length than trout fed the MO/CO diet ($p = 0.009$; Table 3.4). Final mean body weight ranged from 75.6 to 82.1 g (Table 3.4). There were no significant differences among treatments for the following measurements (Table 3.4): initial weight ($p = 0.419$), final weight ($p = 0.064$), the initial length ($p = 0.356$), final CF ($p = 0.623$), initial VSI ($p = 0.552$), SGR ($p = 0.225$), final VSI ($p = 0.\ 169$), weight gain ($p = 0.135$), SGR ($p = 0.225$), AFI ($p = 0.107$), and FCR ($p = 0.467$).Initial CF was higher in trout in the FO treatment compared to trout in the MO/CO treatment; however, this measurement was taken prior to feeding experimental diets, so a treatment effect was not observed at this time. There were no significant differences in the whole body nutrient content of rainbow trout among treatments for crude protein, total fat, ash, calcium, potassium, magnesium, phosphorus, sodium, and zinc (Table 3.4).

3.4.2 FATTY ACID CONTENT OF LIVER TISSUE

LNA was higher in trout fed the MO/CO diet (4.5 ± 0.6^{a}) compared to trout fed either the FO (3.4 ± 0.5^{b}) or MO (3.0 ± 0.6^{b}) diet ($p < 0.001$; Table 3.5). ALA was highest in trout fed the MO/CO diet (2.6 ± 0.6^{a}) compared to trout fed either the FO (1.6 ± 0.3^{b}) or MO (1.5 ± 0.3^{b}) diet ($p < 0.001$). EPA was higher in trout fed the FO diet (6.2 ± 0.9^{a}) compared to trout fed the MO (2.0 ± 0.3^{b}) and MO/CO (2.4 ± 0.5^{b}) diets ($p < 0.001$). DHA was higher in trout fed the MO/CO (53.6 ± 6.9^{a}) diet compared to the FO (40.7 ± 5.8^{b}) and MO (44.7 ± 8.6^{b}) diet ($p < 0.001$). Total MUFA was highest in liver of trout fed the MO/CO diet (21.2 ± 3.1^{a}) compared to the MO (17.8 ± 3.3^{b}) diet; however, there was no difference in total MUFA between trout fed the MO/CO diet and the FO diet, or between trout fed the MO diet and FO diet ($p = 0.034$). Total PUFA was highest in trout fed the MO/CO diet compared to those fed the FO and the MO diet ($p < 0.001$). Total n-3 was higher in trout fed MO/CO diets (60.3 ± 8.0^{a}) compared to trout fed the FO (50.9 ± 7.0^{b}) and MO (49.2 ± 9.0^{b}) diets ($p < 0.001$). Total n-6 was higher in trout fed the MO/CO diet compared to trout fed the FO and MO diets ($p < 0.001$). The n-3/n-6 ratio was highest in trout fed both the FO and MO diets compared to trout fed the MO/CO diet ($p = 0.003$). The sum of EPA and DHA was highest in trout fed MO/CO diets compared to trout fed the FO and MO diets ($p < 0.001$). Total lipid (wet and dry %) was not different among treatments in liver; however, total lipid (wet and dry weight) was significantly higher in trout fed the MO/CO diet when compared to trout fed either the FO or MO diet ($p < 0.001$). After 8 weeks, total lipid stored in liver decreased compared to the initial measurement (Table 3.5).

3.4.3 FATTY ACID CONTENT OF MUSCLE TISSUE

In muscle, LNA was significantly higher in trout fed the MO/CO diet compared to trout fed the FO diet, but did was not different to the trout fed the MO diet ($p = 0.036$; Table 3.6). ALA was significantly higher in trout fed the MO/CO diet compared to trout fed the FO and MO diet ($p < 0.001$). EPA was higher trout fed the FO diet than in trout fed both the MO and MO/CO diets ($p < 0.001$). DHA was highest in trout fed the MO diet, followed by the MO/CO diet, and was lowest in the FO diet compared to all treatments ($p < 0.001$). Total PUFA was higher in trout fed the MO/CO diet compared to the FO treatment; however, there was no difference between trout fed the FO and MO diet or between the MO and MO/CO treatments ($p = 0.018$). Total n-3 was higher in trout fed the MO and MO/CO diets compared to trout fed the FO diet ($p= 0.004$). The n-3/n-6 ratio was highest in trout fed the MO diet compared to trout fed either the FO or MO/CO diet ($p< 0.001$). The sum of EPA and DHA was higher in muscle of trout fed the MO diet, followed by trout fed the MO/CO diet, and was lowest in trout fed the FO control ($p< 0.001$). Trout fed the MO diet were highest in C_{22} PUFA, followed by trout fed the MO/CO diet, and lowest in trout fed the FO diet ($p<0.001$); however, trout fed the FO diet were highest in C_{20} PUFA than the other treatments. The EPA/ARA ratio was highest in trout fed the FO diet, followed by trout fed the MO diet, and lowest in trout fed the MO/CO diet. No differences were found in total MUFA ($p = 0.632$) and total n-6 ($p= 0.058$) in the muscle. Total lipid (wet and dry weight) in trout muscle was not different among treatments; however, total lipid accumulated in the muscle tissue increased after 8 weeks when compared to the initial measurement, regardless of dietary treatment.

3.4.4 MULTIVARIATE ANALYSES OF FATTY ACID DATA

PERMANOVA results indicated that the spatial dispersion of groups was not equivalent, indicating a significant difference in FA content depending on both diet treatment (p(perm) < 0.001) and tissue type (p(perm) < 0.001); however, diet and tissue factors did not interact (p(perm) = 0.324). This is apparent in the PCoA plot (Figure. 3.1), where 94.1% of variation was accounted for, mainly in PCO1 where strong distinction among tissue type is present along the PCO1 axis and PCO2 (9.5%) shows distinction among dietary treatments. The FA vectors indicate correlations among liver and DHA, ARA and higher n-3/n-6 ratio. The FA vectors also indicate correlations among muscle and EPA, EPA+DHA, MUFA, n-6 and SFA. The vectors also indicate differences in FA distribution due to treatment. DHA, SFA and MUFA were more strongly correlated with the MO treatment compared to the FO treatment. DHA, n-3, PUFA, and SFA were more correlated with the MO treatment, whereas n-6, MUFA, ALA and LNA were more correlated with the MO/CO treatment. EPA, EPA+DHA, and 22:5n-3 were more correlated with the FO treatment.

3.5 DISCUSSION

3.5.1 IMPACT ON GROWTH PERFORMANCE AND WHOLE BODY NUTRIENT CONTENT

Overall, the substitution of FO with MO in diets for juvenile rainbow trout had no detrimental effect on growth performance, FCR, or whole body nutrient content. Trout fed the MO/CO diet were slightly shorter in fork length compared to trout fed the FO diet, though there was no difference in terms of weight or CF due to this difference in length.

The results of the present study were also consistent with previous findings reported by Santigosa et al., (2020), where juvenile rainbow trout were provided with *Schizochytrium* sp. oil at inclusion rates ranging from 2.5, 3, 5, 10% of the diet over a period of 96 days, showed no adverse effects on growth performance. It should be noted that these experimental diets did not contain camelina sativa oil as reflected in our diet formulation. *Schizochytrium* sp. oil was also included at 13% of the diet for Atlantic salmon and showed with no significant difference to the FO control treatment in terms growth performance (Miller et al. 2007). Similar findings were reported for juvenile Atlantic salmon, with *Schizochytrium* sp. (T-18) oil as a complete replacement of FO did not result in significant treatment effects in growth performance compared to the FO control (Tibbetts et al., 2020). Together, these findings strongly indicate *Schizochytrium* sp. in diets for rainbow trout and Atlantic salmon (both parr and post-smolts) show no impacts on growth performance in comparison to diets containing FO as the primary lipid source (Miller et al., 2007; Santigosa et al., 2019; Tibbetts et al., 2020; Wei et al., 2020).

Dietary inclusion of dried whole cell *Schizochytrium* sp. has also been examined in its applicability as a FM and FO replacement and its effect on growth performance. Sarker et al., (2016) evaluated the inclusion of dried whole cell *Schizochytrium* sp. in replacing FO at 25%, 50%, 75%, and 100% inclusion in diets for Nile tilapia (*Oreochromis niloticus*) over 84 days. Tilapia fed diets containing dried whole cell *Schizochytrium* sp. were found to have increased final weight, increased weight gain, reduced FCR (Sarker et al., 2016). In contrast, the application of dried whole cell *Schizochytrium* sp. in diets of Atlantic salmon had no significant impact on growth performance (Kousoulaki et al., 2016). Dried

whole cell *Schizochytrium* sp. was also found to be highly digestible when included at 30% of the diet, with PUFA and DHA >95% digestible and were significantly more digested in the *Schizochytrium* sp. diet compared to the reference diet (Hart et al., 2021). Another study tested the digestibility of whole cell *Schizochytrium* sp. by juvenile rainbow trout and found that dry matter, crude protein, total fat, energy, and FAs were all >85% digestible (Belanger et al. 2021). Longer-term growth performance and fish health studies would be required to evaluate the relative merits of *Schizochytrium* sp. whole cell biomass vs extracted oil.

3.5.2 IMPACT ON LIVER FA CONTENT

The liver is a primary site of lipid metabolism and involves activities such as lipogenesis, oxidation, and biosynthesis of LC-PUFA (Jordal et al. 2005). As such, the liver is considered to be a biological indicator of change for lipid and FA metabolism. In the present study, inclusion of high DHA *Schizochytrium* sp. oil in diets for trout significantly affected liver FA profiles as EPA in liver was three times lower in trout that were fed the MO and MO/CO diets compared to the FO diet (Table 3.5). Considering the low levels of EPA in *Schizochytrium* sp. oil used in this study, the reduced amount of EPA stored in the liver in these treatments is expected. DHA was highest in trout fed the MO/CO diet compared to trout fed either the FO or MO diet. This is an interesting result as it contrasts increased DHA concentrations observed in the muscle from fish fed MO diets and does not match dietary DHA levels. While the conservation of DHA in the liver was expected, as liver is relatively high in PL compared to muscle (which is high in TAG) and is more resistant to dietary change, it is unexpected that trout fed the MO/CO diet stored the most DHA. This may be due to selective retention of DHA to optimize circulated levels in the plasma, as a

result of higher levels of dietary C_{18} PUFA in the MO/CO diet. Selective retention of DHA has been previously documented in salmon and rainbow trout that were fed vegetable oil-based diets (e.g., flaxseed, sunflower, camelina oils) with high levels of dietary C_{18} PUFA (Colombo et al. 2018). Also, the lack of evidence of additional EPA storage in the liver, suggests that retro-conversion of DHA to EPA did not occur to a significant extent, (probably because it was not required given dietary levels). This finding was also suggested in the study by Wei et al. (2021) with Atlantic salmon fed diets containing oil from *Schizochytrium* sp. (T-18). It is unknown whether low reserves of EPA in the liver could eventually have consequences for fish health if biosynthesis from ALA or retro-conversion from DHA cannot keep up with demand.

3.5.3 IMPACT ON MUSCLE FA CONTENT

The replacement of FO by high-DHA Schizochytrium sp. (T-18) oil, at both lower and higher inclusion levels, significantly influenced FA content in trout muscle, and generally, muscle was reflective of the FA composition of the diet due to MO inclusion. Similarly, in the Miller et al. (2007) study, the major difference between treatments was in the FA profile of the salmon, particularly the n-3 and n-6 LC-PUFA. Notably, the DHA content in muscle tissue of trout fed diets containing MO was found to be increased when compared to those fed FO control diet. This result was also reported by Wei et al. (2021) in Atlantic salmon. Higher amounts of DHA stored in muscle is expected due to the significantly higher content of DHA in the MO and MO/CO diets compared to the FO diet. On the other hand, EPA was much lower in the MO and MO/CO diets compared to the FO diet, and consequently, significantly lower levels of EPA were stored in trout muscle that were fed the MO-

containing diets compared to trout fed the MO diet. Previous studies have reported similar findings when *Schizochytrium* sp. oil was tested as a FO alternative in diets for rainbow trout and Atlantic salmon (Miller at al. 2007; Betiku et al., 2016; Santigosa et al. 2019; Wei et al. 2021), as well as *Schizochytrium* sp. whole cell biomass in rainbow trout (Bélanger-Lamonde et al., 2018).

ALA and LNA stored in muscle were highest in trout fed the MO/CO diet compared to trout fed either the FO or MO diet. The increase in these C_{18} PUFA in trout fed the MO/CO diet is attributed to the dietary CO inclusion, which is characteristically high in these FA, and is also known to accumulate in muscle of fish when fed diets containing CO, such as rainbow trout (Hixson et al. 2014a), Atlantic salmon (Hixson et al. 2014b), tilapia (Toyes-Vargas et al. 2020), and seabream (Huyben et al., 2021). Lower dietary EPA content was reflected in the muscle tissue, with EPA ~3x lower in trout fed the MO and MO/CO diets compared to the FO diet. Given the lower EPA amount in the MO diets, it was hypothesized that this may induce biosynthetic production of EPA from ALA to compensate for lower dietary EPA. However, it is likely that the MO diets still supplied enough EPA, since the diet provided at least 1.5 µg/mg EPA (from FM) and EPA in muscle was stored at >3 µg/mg. Despite differences in ALA, EPA, and DHA accumulation, no significant differences were noted in total lipid within trout fed any of the experimental diets. However, since EPA is a precursor to anti-inflammatory classes of eicosanoids, which contributes to the immune response and fish health, subsequent research must be completed to observe the impact on reduced dietary EPA availably within fish relative to growth and subsequent potential impacts on immune system function and response.

Aside from EPA and DHA, Docosapentaneoic acid (DPA, 22:5n-3), is known to be an elongated metabolite of EPA and an intermediate fatty acid product between EPA and DHA synthesis (Kaur et al., 2011). Previous research has noted that DPA may have significant contributions in health benefits affiliated with anti-inflammatory eicosanoid production, and enhanced endothelial cell migration capabilities relative to wound healing (Kaur et al., 2011). The (T18) *Schizochitrum* sp. oil used within this study contained 0.1% or 1.8 ug/mg of DPA and concentrations of DPA were noted to be highest within the FO diet formulation relative to the MO and MO/CO experimental diets ($p = 0.000$). Trout muscle tissue of fish fed the FO diet were also noted to have significantly higher concentrations of DPA relative to the MO/CO diet and the MO diet and similar findings were also noted within Wei et al., (2021) in feeding trials with *Schichytrium* sp. and Atlantic Salmon. Future research is necessary to further evaluate health implications of DPA within salmonids relative to anti-inflammatory eicosanoid production.

Total n-3 content was higher in trout fed the MO and the MO/CO diet compared to the FO control. As a result, the n-3/n-6 ratio was highest in trout fed the MO diet compared to any dietary treatment. Although these findings contrast Wei et al. (2021), where total n-3 amounts were higher in muscle of salmon fed the FO diet compared to trout fed the fed both low and high concentrations of *Schizochytrium* sp. (T-18) oil, it depends on the FO inclusion level. In the Wei at al., (2020) study, FO was included at 20% of the diet, compared to 10% inclusion in the current study. From a human health perspective, consuming rainbow trout fillets with a higher total n-3 content and higher n-3/n-6 ratio is

beneficial. Further, rainbow trout fillets from both the MO and MO/CO treatments contain EPA+DHA amounts that meet the recommended daily requirement for EPA+DHA intake of 250 mg, as suggested by both the World Health Organization (WHO, 2008) and the Global Organization for EPA and DHA (GOED, 2014). The recommended serving of fish is 100 g of cooked fish (American Heart Association, 2017). The sum of EPA+DHA (wet weight) in 100 g fillet of trout fed FO, MO/CO, and MO is 799 mg, 1008 mg, and 1252 mg, respectively. As such, the consumption of one-100 g serving of fillets from rainbow trout that were fed either MO or MO/CO is more than sufficient to meet the daily requirement (250 mg). In fact, for trout fed the MO diet, consumers need only eat 1.4 servings (140 g fillet) per week to achieve the total weekly EPA+DHA requirement.

3.5.4 MULTIVARIATE ANALYSIS OF FA PROFILES

Based on spatial dispersion of data points representing the FA profile of each trout (see Figure. 3.1), both tissue type and diet contributed to the variation of FA profiles of trout in this study, although they were not interacting factors. Most of the variation is attributed to PCO1 (84.6%) with a clear separation among tissue types, indicating that the FA difference in liver vs. muscle, regardless of treatment, was a more defining factor in FA composition than diet. Muscle tissue in salmonids species is known to be receptive to dietary change and therefore can emulate specific FA's under dietary influence. Along PCO2, there are two clear separate groups dividing muscle and liver data points, where FO is clearly separated from MO and MO/CO diets. This visual representation clearly shows the influence of the MO on the muscle tissue compared to traditional FO as the lipid source,

regardless of the MO inclusion level. In liver, FO is separate from MO/CO, however; this division is less distinct.

3.5.5 CONCLUSIONS

High DHA *Schizochytrium* sp. (T-18) oil was an effective dietary DHA supplement to fully replace FO in diets for rainbow trout. Inclusion of *Schizochytrium* sp. (T-18) oil at high or low levels in the diet (MO and MO/CO diets) resulted in similar growth performance as seen in trout fed FO; however, muscle and liver FA profiles were affected due to the diet. Trout fed the MO diets had greater amounts of DHA in their liver and muscle tissues than trout fed the FO diet, and contained lower EPA, but total n-3 was highest in trout fed the highest inclusion level of *Schizochytrium* sp. oil. Both high and low inclusion levels of *Schizochytrium* sp. (T-18) oil can appropriately replace FO in aquafeeds as a source of DHA but does not supply ample EPA. Reduced dietary EPA concentrations would require further investigation to evaluate possible impacts on fish health; however, this did not impact fish health or growth over the 8-week study. Future research is necessary to consider *Schizochytrium* sp. oil use in rainbow trout diets for a longer production period, including trout that are closer to market production size.

3.6 TABLES AND FIGURES

Table 3.1 FA composition of the microbial oil (MO; *Schizochytrium* sp. T-18) used in the study.

FA	MO (%)	MO (μg/mg)
14:0	13.1	193.0
15:0	1.5	21.9
16:0	30.7	453.5
16:1n-7c	4.5	66.5
16:1n-7t	<0.1	0.3
17:0	0.3	4.2
17:1n-7	<0.1	0.2
18:0	0.9	13.6
18:1n-9c	0.6	9.0
18:1n-9t	<0.1	0.0
18:1n-12	<0.1	0.0
18:1n-7c	2.8	41.0
18:1n-7t	<0.1	0.0
19:0	<0.1	0.2
18:2n-6	0.3	4.4
20:0	0.1	0.8
18:3n-6	0.1	1.6
20:1n-15	<0.1	0.0
20:1	<0.1	0.0
20:1n-9	<0.1	0.3
18:3n-3	0.1	1.2
18:2n-6t	0.1	0.8
18:4n-3	0.2	2.6
20:2n-6	0.0	0.0
22:3n-3	0.1	0.8
22:0	<0.1	0.3
20:3n-6	0.1	1.1
22:1n-9	<0.1	0.5
20:3n-3	<0.1	0.0
20:4n-6	0.1	1.8
22:2n-6	<0.1	0.0
24:0	0.1	1.6
20:5n-3 (EPA)	0.7	10.5
24:1n-9	<0.1	0.0
22:4n-6	<0.1	0.2
22:5n-3	0.1	1.8
22:6n-3 (DHA)	43.4	640.7

FA	MO (%)	MO (μg/mg)
ΣSFA[1]	46.7	689.1
ΣMUFA[2]	8.2	120.6
ΣPUFA[3]	45.2	666.7
ΣMUFA≥18C	3.5	51.6
ΣMUFA>18C	0.1	1.6
ΣC18 PUFA	0.7	10.5
ΣC20 PUFA	0.9	13.4
ΣC22 PUFA	43.6	642.8
ΣEPA & DHA	0.2	651.2
Σn-6	0.7	9.9
Σn-3	44.5	656.8
ΣOdd chain	1.9	28.0
n-3/n-6	67.0	67.0

[1] Saturated fatty acid
[2] Monounsaturated fatty acid
[3] Polyunsaturated fatty acid

Table 3.2 Diet formulation and composition (g/kg, as fed basis) of experimental FO, MO/CO and MO diets fed to rainbow trout.

Ingredient[1] (g/kg)	FO	MO/CO	MO
Fish meal	150	150	150
Fish (herring) oil	100	0	0
Microbial oil (MO)[2]	0	75	100
Camelina oil[3]	50	75	50
Ground wheat	170	170	170
Empyreal (corn protein concentrate)	120	120	120
Poultry by-product meal	210	210	210
Blood meal	160	160	160
Vitamin and mineral mix	2	2	2
Dicalcium phosphate	20	20	20
Pigment mix[5]	2.5	2.5	2.5
Lysine HCl	5	5	5
Choline chloride	10.5	10.5	10.5
Chemical composition (as fed, g/kg)			
Dry matter	94.7	95.1	94.9
Crude protein	44.2	44.5	44.6
Total lipid	23.9	24.1	24.2

[1]All ingredients were supplied and donated by Northeast Nutrition (Truro, NS, Canada)
[2] MO (*Schizochytrium* sp. T-18) was supplied by Mara Renewables (Dartmouth, NS, Canada)
[3]Commercial grade camelina oil, produced by Smart Earth Seeds (Saskatoon, SK, Canada)
[4]Vitamin and mineral premix contains (/kg): zinc, 77.5 mg; manganese, 125 mg; iron, 84 mg; copper, 2.5 mg; iodine, 7.5 mg; vitamin A, 5000 IU; vitamin D, 4000 IU; vitamin K, 2 mg; vitamin B12, 4 µg; thiamine, 8 mg; riboflavin, 18 mg; pantothenic acid, 40 mg; niacin, 100 mg; folic acid, 4 mg; biotin, 0.6 mg; pyridoxine, 15 mg; inositol, 100 mg; ethoxyquin, 42 mg; wheat shorts, 1372 mg.
[5]Pigment mix contains (/kg): selenium, 0.220 mg; vitamin E, 250 IU; vitamin C, 200 mg; astaxanthin, 60 mg; wheat shorts, 1988 mg.

Table 3.3 FA content (µg/mg, dry weight) of experimental FO, MO/CO and MO diets fed to rainbow trout[1].

FA	FO	MO/CO	MO	F-value	P-value
14:0	10.8 ± 0.5[b]	12.3 ± 0.9[b]	18.3 ± 1.6[a]	40.25	0.000
16:0	45.7 ± 3.0[b]	49.3 ± 5.0[b]	61.3 ± 4.2[a]	11.77	0.008
16:1n-7	13.2 ± 0.5[a]	7.8 ± 0.7[c]	9.5 ± 0.8[b]	49.08	0.000
18:0	9.8 ± 0.5[a]	6.8 ± 0.6[b]	6.6 ± 0.5[b]	30.76	0.001
18:1n-9	41.3 ± 1.4[a]	36.0 ± 3.5[ab]	31.5 ± 2.6 [b]	10.28	0.012
18:2n-6 (LNA)	27.3 ± 0.9	31.9 ± 2.8	29.0 ± 2.4	3.33	0.107
18:3n-3 (ALA)	25.0 ± 0.8[b]	31.1 ± 1.5[a]	25.1 ± 2.5[b]	11.74	0.008
20:1n-9	13.0 ± 0.3[b]	15.6 ± 1.1[a]	12.6 ± 1.1[b]	9.61	0.013
20:4n-6	1.3 ± 0.0[a]	0.6 ± 0.0[b]	0.7 ± 0.1[b]	190.94	0.000
20:5n-3 (EPA)	19.0 ± 0.8[a]	1.8 ± 0.1[b]	2.1 ± 0.2[b]	1229.24	0.000
22:1n-9	2.2 ± 0.0[b]	2.8 ± 0.2[a]	2.3 ± 0.2[b]	9.83	0.013
22:5n-3	2.2 ± 0.1[a]	0.3 ± 0.0[b]	0.4 ± 0.0[b]	1950.49	0.000
22:6n-3 (DHA)	13.2 ± 0.2[c]	28.6 ± 0.8[b]	48.9 ± 4.9[a]	118.78	0.000
24:1n-9	1.1 ± 0.0[a]	0.8 ± 0.1[b]	0.7 ± 0.1[c]	34.65	0.001
∑SFA[2]	70.1 ± 4.0[b]	72.3 ± 6.7[b]	90.8 ± 6.7[a]	10.95	0.010
∑MUFA[3]	86.3 ± 2.4[a]	73.6 ± 6.5[ab]	68.0 ± 5.9[b]	9.72	0.013
∑PUFA[4]	95.3 ± 2.9	98.3 ± 5.4	109.9 ± 10.2	3.80	0.086
∑n-3	63.6 ± 1.9[b]	63.4 ± 2.5[b]	78.0 ± 7.8[a]	8.97	0.016
∑n-6	31.6 ± 1.0	34.9 ± 3.0	31.9 ± 2.6	1.74	0.254
n-3/n-6	2.0 ± 0.0[b]	1.8 ± 0.1[b]	2.4 ± 0.1[a]	42.82	0.000
Total	251.7 ± 9.1	244.2 ±18.5	268.7 ± 22.5	1.51	0.295

[1]Data expressed as µg/mg dry weight, values are means (n=3) ± standard deviation. Means with different superscripts indicate significant differences based on Tukey's post-hoc test following a one-way ANOVA. FO, fish oil; MO/CO, microbial oil/camelina oil blend; MO, microbial oil (*Schizochytrium* sp. T-18).
[2]Saturated fatty acid
[3]Monounsaturated fatty acid
[4]Polyunsaturated fatty acid

Table 3.4 Growth performance and whole body analysis of rainbow trout fed experimental FO, MO/CO and MO diets for 8 weeks[1].

Growth Parameters	FO	MO/CO	MO	P-value
Initial weight	19.2 ± 2.9	18.7 ± 3.1	18.7 ± 2.8	0.419
Final weight	82.1 ± 14.4	75.6 ± 17.3	80.3 ± 15.8	0.064
Weight gain	62.7 ± 4.6	56.9 ± 2.0	61.6 ± 2.2	0.135
Initial length	11.7 ± 0.7	11.8 ± 0.8	11.7 ± 0.6	0.356
Final length	18.5 ± 1.0^{a}	17.9 ± 1.2^{b}	18.3 ± 1.2^{ab}	0.009
Initial CF	1.2 ± 0.1^{a}	1.1 ± 0.1^{b}	1.2 ± 0.1^{ab}	0.011
Final CF	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	0.623
Initial VSI	12.3 ± 1.6	13.0 ± 1.1	12.9 ± 1.7	0.552
Final VSI	11.6 ± 1.3	11.2 ± 1.1	10.7 ± 1.4	0.169
SGR	2.6 ± 0.4	2.9 ± 0.3	2.5 ± 0.2	0.225
AFI	48.1 ± 2.5	43.5 ± 3.5	50.1 ± 3.6	0.107
FCR	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.467
Whole body analysis				
Crude protein (%)	52.5 ± 2.1	51.7 ± 2.2	51.9 ± 2.0	0.897
Total fat (%)	32.5 ± 2.6	33.4 ± 3.2	31.9 ± 1.7	0.773
Ash (%)	7.2 ± 0.7	6.9 ± 0.4	6.9 ± 0.5	0.700
Calcium (%)	1.43 ± 0.18	1.37 ± 0.11	1.43 ± 0.16	0.848
Potassium (%)	1.10 ± 0.04	1.07 ± 0.05	1.05 ± 0.05	0.483
Magnesium (%)	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.688
Phosphorus (%)	1.38 ± 0.11	1.30 ± 0.08	1.34 ± 0.07	0.598
Sodium (%)	0.27 ± 0.01	0.25 ± 0.03	0.22 ± 0.05	0.351
Zinc (ppm)	67.3 ± 7.1	61.8 ± 3.2	62.5 ± 5.3	0.462

[1] Initial measurements are mean ± standard deviation, body weight (g fish^{-1}), fork length (cm fish^{-1}) n= 5

[2] Final measurements are mean ± standard deviation, body weight (g fish^{-1}), fork length (cm fish^{-1}) n = 5

[3] Means with different superscripts indicate significant differences among treatments ($P > 0.05$)

[4] Weight gain (g fish^{-1}) = final weight - initial weight (calculated by tank means).

[5] CF (Condition factor) = body mass/length^3 (calculated by individual fish).

[6] VSI (Visceral somatic index, %) = 100 * (viscera mass/body mass).

[7] SGR (Specific Growth Rate) = (ln (final body weight) – ln ((initial body weight))/ 56 days*100.

[8] AFI (Apparent feed intake, g/fish) = (feed consumed, g)/(number of fish per tank) (calculated by tank means).

[9] FCR (Feed conversion ratio) = (feed intake, g fish^{-1})/(weight gain, g fish^{-1}) (calculated by tank means).

Table 3.5 Fatty acid content (μg/mg dry weight) and total lipid of rainbow trout liver, experimental FO, MO/CO and MO from week 0 (initial) and week 8[1].

Fatty acid	Initial	FO	MO/CO	MO	F-value	P-value
14:0	2.6 ± 0.7	1.3 ± 0.2[b]	1.8 ± 0.3[a]	1.8 ± 0.5[a]	8.87	0.001
14:1n-5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.38	0.683
15:0	0.3 ± 0.1	0.2 ± 0.0[b]	0.4 ± 0.1[a]	0.3 ± 0.1[a]	39.99	0.000
16:0	31.0 ± 5.6	20.3 ± 3.4[b]	26.0 ± 4.3[a]	21.9 ± 3.2[b]	9.94	0.000
16:1n-7c	4.4 ± 2.4	1.7 ± 0.4	1.6 ± 0.6	1.7 ± 0.5	0.26	0.774
16:1n-7t	0.1 ± 0.0	0.1 ± 0.0[a]	0.0 ± 0.0[b]	0.0 ± 0.0[b]	9.56	0.000
17:0	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	3.13	0.054
17:1n-7	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.24	0.119
18:0	10.0 ± 1.7	8.0 ± 1.3[ab]	8.8 ± 1.3[a]	7.3 ± 1.9[b]	3.52	0.039
18:1n-9c	18.8 ± 9.0	10.7 ± 2.4[ab]	11.8 ± 1.9[a]	9.6 ± 1.7[b]	4.12	0.023
18:1n-9t	0.1 ± 0.0	0.1 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[b]	17.71	0.000
18:1n-12	0.3 ± 0.1	3.0 ± 0.1[a]	0.3 ± 0.1[a]	0.2 ± 0.0[b]	10.48	0.000
18:1n-7c	4.0 ± 1.0	1.8 ± 0.4	1.9 ± 0.4	2.0 ± 0.3	1.14	0.329
19:0	0.2 ± 0.0	0.1 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[b]	37.67	0.000
19:1n-12	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.02	0.982
18:2n-6 (LNA)	3.4± 1.0	3.4 ± 0.5[b]	4.5 ± 0.6[a]	3.0 ± 0.6[b]	27.33	0.000
20:0	0.2 ± 0.1	0.3 ± 0.0[b]	0.3 ± 0.0[a]	0.2 ± 0.1[c]	23.95	0.000
18:3n-6	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.45	0.245
20:1n-15	0.1 ± 0.0	0.0 ± 0.0[a]	0.0 ± 0.0[b]	0.0 ± 0.0[b]	78.17	0.000
20:1	0.8 ± 0.3	0.3 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[b]	124.72	0.000
20:1n-9	2.2 ± 0.7	3.1 ± 0.6[b]	3.8 ± 0.7[a]	2.6 ± 0.9[b]	9.88	0.000
18:3n-3 (ALA)	0.5 ± 0.2	1.6 ± 0.3[b]	2.6 ± 0.6[a]	1.5 ± 0.3[b]	34.95	0.000
18:4n-3	0.2 ± 0.1	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.1 ± 0.0[b]	13.12	0.000
20:2n-6	1.0 ± 0.3	1.7 ± 0.3[b]	2.1 ± 0.4[a]	1.3 ± 0.4[c]	17.72	0.000
22:3n-3	0.0 ± 0.0	0.0 ± 0.0[ab]	0.0 ± 0.0[a]	0.0 ± 0.0[b]	6.75	0.003
22:0	0.1 ± 0.1	0.1 ± 0.0[a]	0.0 ± 0.0[b]	0.0 ± 0.0[b]	9.90	0.000
20:3n-6	0.9 ± 0.2	0.8 ± 0.1[a]	0.8 ± 0.2[a]	0.5 ± 0.2[b]	14.69	0.000
22:1n-11	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	1.02	0.371
22:1n-9	0.1 ± 0.1	0.2 ± 0.1[ab]	0.2 ± 0.0[a]	0.1 ± 0.0[b]	8.36	0.001
20:3n-3	0.1 ± 0.1	0.7 ± 0.1[b]	1.1 ± 0.3[a]	0.6 ± 0.2[b]	23.08	0.000
20:4n-6 (ARA)	6.2 ± 1.1	4.2 ± 0.7[b]	5.7 ± 0.9[a]	4.8 ± 0.9[b]	11.31	0.000
22:2n-6	0.1 ± 0.0	0.1 ± 0.0[a]	0.0 ± 0.0[b]	0.0 ± 0.0[c]	55.59	0.000
24:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.94	0.400
20:5n-3 (EPA)	9.3 ± 1.5	6.2 ± 0.9[a]	2.4 ± 0.5[b]	2.0 ± 0.3[b]	191.76	0.000
24:1n-9	1.7 ± 0.3	1.1 ± 0.3	1.1 ± 0.4	1.0 ± 0.3	0.41	0.665
22:4n-6	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.3 ± 0.3	1.00	0.375
22:5n-3	2.2 ± 0.4	1.5 ± 0.2[a]	0.5 ± 0.2[a]	0.5 ± 0.1[b]	203.28	0.000
22:6n-3 (DHA)	66.4 ± 9.1	40.7 ± 5.8[b]	53.6 ± 6.9[a]	44.7 ± 8.6[b]	12.71	0.000
ΣSFA[1]	44.7 ± 7.5	30.5 ± 4.8[b]	37.6 ± 5.1[a]	31.8 ± 4.9[b]	8.92	0.001
ΣMUFA[2]	33.3 ± 13.8	19.6 ± 3.9[ab]	21.2 ± 3.1[a]	17.8 ± 3.3[b]	3.67	0.034
ΣMUFA≥18	28.5 ± 11.3	17.7 ± 3.5[ab]	19.5 ± 2.7[a]	15.9 ± 3.0[b]	4.83	0.013
ΣMUFA<18	5.3 ± 1.3	4.8 ± 0.8[ab]	5.4 ± 1.0[a]	4.0 ± 1.1[b]	7.80	0.001
ΣPUFA[3]	90.7 ± 12.2	61.2 ± 8.2[b]	73.6 ± 9.6[a]	59.2 ± 10.8[b]	9.85	0.000
ΣC18 PUFA[3]	4.2 ± 1.3	5.1 ± 0.8[b]	7.2 ± 1.1[a]	4.6 ± 0.8[b]	33.90	0.000
ΣC20 PUFA[3]	17.5 ± 2.6	13.7 ± 1.8	12.1 ± 2.0	9.1 ± 1.8	22.47	0.000
ΣC22 PUFA[3]	68.9 ± 9.4	42.4 ± 6.0[b]	54.4 ± 7.2[a]	45.4 ± 8.9[b]	10.59	0.000
ΣEPA & DHA	75.8 ± 10.3	47.0 ± 6.6[b]	50.6 ± 7.3[a]	46.7 ± 8.8[b]	7.20	0.002
Σn-3	78.8 ± 10.6	50.9 ± 7.0[b]	60.3 ± 8.0[a]	49.2 ± 9.0[b]	8.17	0.001

Fatty acid	Initial	FO	MO/CO	MO	F-value	P-value
Σn-6	11.9 ± 1.9	10.4 ± 1.4[b]	13.4 ± 1.9[a]	9.9 ± 2.0[b]	16.73	0.000
Σodd chain	1.2 ± 03	0.7 ± 0.1[b]	0.8 ± 0.1[a]	0.7 ± 0.1[ab]	6.95	0.003
n-3/n-6	6.7 ± 0.7	4.9 ± 0.3[a]	4.5 ± 0.4[b]	5.0 ± 0.4[a]	6.69	0.003
EPA/ARA	1.5 ± 0.3	1.5 ± 0.1[a]	0.4 ± 0.0[b]	0.4 ± 0.1[b]	860.23	0.000
Total lipid						
Lipid % ww	4.7 ± 0.6	4.2 ± 1.0	3.9 ± 0.5	4.2 ± 1.0	0.59	0.560
Lipid % dw	20.1 ± 2.7	17.4 ± 4.0	16.2 ± 2.3	17.5 ± 3.7	0.62	0.543
Lipid (μg/mg) ww	39.7 ± 6.9	26.9 ± 3.7[b]	32.0 ± 3.7[a]	26.2 ± 4.7[b]	8.93	0.001
Lipid (μg/mg) dw	168.7 ± 29.8	111.2 ±15.1[b]	132.5 ± 16.9[a]	108.7 ± 18.5[b]	8.85	0.001

[1]Data expressed as μg FAME/mg (dry weight), values are means (n=3 per treatment) ± standard deviation. Means with different superscripts indicate significant differences based on Tukey's post-hoc test following a one-way ANOVA. FO, fish oil; MO/CO, microbial oil/camelina oil blend; MO, microbial oil (*Schizochytrium* sp. T-18).

[2]Saturated fatty acid

[3]Monounsaturated fatty acid

[4]Polyunsaturated fatty acid

Table 3.6 Fatty acid content (μg/mg dry weight) and total lipid of rainbow trout muscle experimental FO, MO/CO and MO from week 0 (initial) and week 8[1]

Fatty acid	Initial	FO	MO/CO	MO	F-value	P-value
14:0	5.1 ± 1.5	7.1 ± 2.3^{b}	8.9 ± 3.3^{ab}	10.9 ± 2.5^{a}	7.35	0.002
14:1n-5	0.1 ± 0.0	0.1 ± 0.1^{b}	0.1 ± 0.0^{ab}	0.1 ± 0.0^{a}	3.83	0.030
15:0	0.4 ± 0.1	0.5 ± 0.2^{b}	1.0 ± 0.4^{a}	1.2 ± 0.3^{a}	25.99	0.000
16:0	26.6 ± 7.3	39.6 ± 12.1^{b}	47.7 ± 15.2^{ab}	51.8 ± 10.7^{a}	3.56	0.037
16:1n-7c	8.7 ± 2.7	11.3 ± 4.0	9.7 ± 3.9	10.8 ± 2.6	0.73	0.490
16:1n-7t	0.1 ± 0.0	0.2 ± 0.1^{a}	0.1 ± 0.0^{b}	0.0 ± 0.0^{b}	33.25	0.000
17:0	0.3 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	1.05	0.361
17:1n-7	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	2.10	0.135
18:0	5.6 ± 1.6	8.4 ± 2.7	8.7 ± 2.7	8.3 ± 1.6	0.11	0.894
18:1n-9c	31.0 ± 9.7	40.1 ± 15.0	44.5 ±17.4	39.1 ± 9.6	0.59	0.557
18:1n-9t	0.2 ± 0.1	0.2 ± 0.1^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	9.90	0.000
18:1n-12	0.9 ± 0.3	0.7 ± 0.2	0.8 ± 0.3	0.7 ± 0.1	0.38	0.684
18:1n-7c	4.7 ± 1.4	5.1 ± 1.8	5.8 ± 2.2	6.0 ± 1.3	1.08	0.350
18:1n-7t	0.3 ± 0.2	0.6 ± 0.3^{a}	0.1 ± 0.1^{b}	0.0 ± 0.0^{b}	71.09	0.000
19:1n-12	0.3 ± 0.1	0.7 ± 0.3^{a}	0.1 ± 0.0^{b}	0.0 ± 0.0^{b}	83.41	0.000
18:2n-6	11.6 ± 3.4	18.9 ± 6.5^{b}	26.4 ± 10.3^{a}	21.7 ± 5.3^{ab}	3.59	0.036
20:0	0.2 ± 0.1	0.5 ± 0.2^{b}	0.7 ± 0.3^{a}	0.5 ± 0.1^{b}	4.72	0.014
18:3n-6	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.57	0.571
20:1n-15	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	23.89	0.000
20:1	2.6 ± 0.8	1.2 ± 0.5^{a}	1.1 ± 0.4^{ab}	0.8 ± 0.2^{b}	3.69	0.033
20:1n-9	2.2 ± 0.7	7.7 ± 2.7^{b}	11.7 ± 4.4^{a}	8.8 ± 2.2^{b}	6.01	0.005
18:3n-3	2.4 ± 0.7	13.8 ± 4.5^{b}	23.1 ± 8.7^{a}	16.1 ± 4.0^{b}	9.31	0.000
18:4n-3	1.4 ± 0.4	2.2 ± 0.7^{a}	1.4 ± 0.5^{b}	1.1 ± 0.3^{b}	19.14	0.000
20:2n-6	0.9 ± 0.3	2.2 ± 0.6^{ab}	2.7 ± 1.0^{a}	2.0 ± 0.4^{b}	4.35	0.019
22:3n-3	0.0 ± 0.0	0.1 ± 0.1^{b}	0.2 ± 0.1^{a}	0.2 ± 0.1^{ab}	7.45	0.002
22:0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	1.13	0.332
20:3n-6	0.5 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.6 ± 0.1	1.09	0.347
22:1n-11	2.3 ± 0.7	2.4 ± 0.9	2.8 ± 1.1	2.6 ± 0.6	0.88	0.422
22:1n-9	0.4 ± 0.1	1.2 ± 0.4^{b}	1.8 ± 0.7^{a}	1.4 ± 0.3^{ab}	5.84	0.006
20:3n-3	0.2 ± 0.1	1.1 ± 0.3^{b}	1.9 ± 0.7^{a}	1.3 ± 0.3^{a}	11.82	0.000
20:4n-6	1.3 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.5 ± 0.2	0.64	0.534
22:2n-6	0.1 ± 0.0	0.2 ± 0.1^{a}	0.2 ± 0.1^{ab}	0.1 ± 0.0^{b}	4.34	0.019
24:0	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	1.61	0.213
20:5n-3 (EPA)	7.4 ± 1.8	9.5 ± 2.2^{a}	3.4 ± 0.8^{b}	3.0 ± 0.5^{b}	105.04	0.000
24:1n-9	0.5 ± 0.1	0.8 ± 0.3	1.0 ± 0.3	0.9 ± 0.2	1.59	0.215
22:4n-6	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.78	0.467
22:5n-3	2.0 ± 0.5	2.6 ± 0.8^{a}	1.0 ± 0.3^{b}	0.9 ± 0.2^{b}	60.54	0.000
22:6n-3 (DHA)	20.6 ± 4.6	23.3 ± 3.5^{c}	38.7 ± 8.8^{b}	47.1 ± 7.7^{a}	43.89	0.000
ΣSFA[2]	38.5 ± 10.6	56.9 ± 17.7^{a}	67.8 ± 22.1^{ab}	73.5 ± 15.3^{b}	3.10	0.056
ΣMUFA[3]	54.6 ± 16.6	72.7 ± 26.5	80.1 ± 30.8	72.0 ± 17.1	0.46	0.632
ΣMUFA≥C18	45.4 ± 13.8	61.0 ± 22.3	69.9 ± 26.9	60.7 ± 14.4	0.87	0.426
ΣMUFA<C18	8.3 ± 2.5	14.2 ± 5.1	18.7 ± 7.1	14.7 ± 3.5	3.06	0.057
ΣPUFA[4]	48.9 ± 12.0	76.5 ± 19.3^{b}	101.3 ± 31.6^{a}	95.9 ± 18.8^{ab}	4.44	0.018
ΣC18 PUFA	15.7 ± 4.6	35.3 ± 11.7^{b}	51.2 ± 19.6^{a}	39.2 ± 9.6^{ab}	5.00	0.011
ΣC20 PUFA	10.3 ± 2.6	14.8 ± 3.4^{a}	10.0 ± 2.9^{b}	8.3 ± 1.4^{b}	22.94	0.000
ΣC22 PUFA	22.9 ± 5.1	26.3 ± 4.3^{c}	40.1 ± 9.2^{b}	48.3 ± 7.9^{a}	33.51	0.000
ΣEPA & DHA	28.0 ± 6.3	32.8 ± 5.5^{c}	42.1 ± 9.5^{b}	50.1 ± 8.1^{a}	17.97	0.000
Σn-3	34.0 ± 7.9	52.6 ± 11.6^{b}	69.5 ± 19.6^{a}	69.5 ± 12.7^{a}	6.30	0.004

Fatty acid	Initial	FO	MO/CO	MO	F-value	P-value
Σn-6	14.9 ± 4.2	23.9 ± 7.7	31.8 ± 12.0	26.4 ± 6.2	3.05	0.058
ΣOdd chain	1.3 ± 0.4	2.1 ± 0.7	1.8 ± 0.7	2.0 ± 0.4	0.92	0.408
n-3/n-6	2.3 ± 0.3	2.3 ± 0.3[b]	2.3 ± 0.2[b]	2.7 ± 0.2[a]	13.36	0.000
EPA/ARA	5.9 ± 0.4	6.9 ± 0.5[a]	2.1 ± 0.2[c]	2.4 ± 0.2[b]	1039.80	0.000
EPA+DHA/100g	607.0 ± 141	799.4 ± 151.6[c]	1007.8 ± 241.2[b]	1252.3 ± 231.0[a]	17.9	0.000
Total lipid						
Lipid % ww	3.6 ± 0.7	4.6 ± 1.2	4.8 ± 1.1	5.3 ± 1.2	1.34	0.272
Lipid % dw	16.6 ± 3.2	19.0 ± 4.5	20.2 ± 4.3	21.2 ± 4.4	0.92	0.406
Lipid (μg/mg) ww	30.8 ± 8.8	50.4 ± 16.6	59.8 ± 20.9	60.4 ± 14.3	1.55	0.223
Lipid (μg/mg) dw	141.9 ± 38.8	206.1 ± 63.2	249.2 ± 84.1	241.3 ± 50.8	1.73	0.189

[1]Data expressed as μg FAME/mg (dry weight), values are means (n=3 per treatment) ± standard deviation. Means with different superscripts indicate significant differences based on Tukey's post-hoc test following a one-way ANOVA. FO, fish oil; MO/CO, microbial oil/camelina oil blend; MO, microbial oil (*Schizochytrium* sp. T-18).

[2]Saturated fatty acid

[3]Monounsaturated fatty acid

[4]Polyunsaturated fatty acid

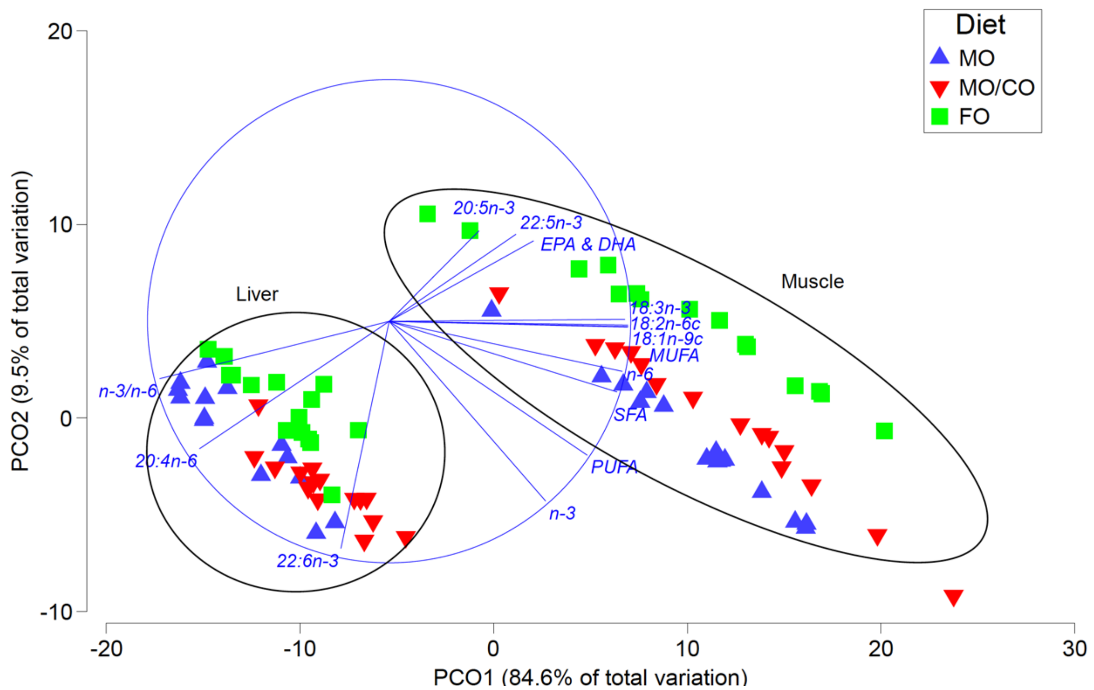

Figure 3.1 Principal co-ordinate ordination plot of fatty acid profiles of individual rainbow trout tissues (liver and muscle) using a Bray–Curtis similarity matrix, where three diet treatments are represented (FO, MO/CO, MO), with n=9 per treatment.

CHAPTER 4

CONCLUSION

The effects of the replacement of dietary fish oil with GE *Camelina sativa* and *Schizochytrium* sp. oil on growth performance, tissue FA composition, sensory properties of the fillets and n-3 LC-PUFA synthesis and storage were investigated. When supplemented into formulated diets trout at elevated rates of inclusion (>50% of the total dietary oil) juvenile rainbow trout were found to have FA profiles that were similar to those of trout fed the FO diet. The inclusion of dietary inclusion of transgenic camelina oil was effective in meeting the nutritional requirements of juvenile rainbow trout as their growth and development were not affected by the addition of this oil to the feed. Relative to fish health, the inclusion of transgenic plant-based oil must be further examined to evaluate possible impacts of novel oils on both fish health and lipid profiles, which could also further impact fillet product quality and consumer health. Fish fed high levels of transgenic camelina oil enriched with EPA and DHA (HCO diet) had FA profiles that were generally similar to those of fish fed FO. Inclusion of lower levels of transgenic camelina (LCO diet) resulted in similar growth performance as trout fed FO; however, the FA profile was more impacted, particularly in the muscle tissue.

In general, high DHA *Schizochytrium* sp. (T-18) oil was an effective dietary DHA supplement to fully replace FO at high or intermedia levels in the diet (MO and MO/CO diets) and resulted in similar growth performance as seen in trout fed FO; however, muscle and liver FA profiles were impacted due to the diet. Juvenile rainbow trout fed the reduced concentrations of *Schizochytrium* sp. (T-18) oil within MO diets were noted to have greater

amounts of DHA in their liver and muscle tissues than trout fed the FO diet, and contained lower EPA, but total n-3 was highest in trout fed the highest inclusion level of *Schizochytrium* sp. oil. Both high and intermediate inclusion levels of *Schizochytrium* sp. (T-18) oil can appropriately replace FO in aquafeeds as a source of DHA but does not supply ample EPA, however, this did not impact fish health or growth over the 8-week study.

This study clearly demonstrates that GE terrestrial plant-based oils (GE *Camelina sativa oil*) and marine microbial oil (*Schizochytrium* sp.) are a good source of dietary lipid for feed formulation of farmed rainbow trout. This conclusion is based on the overall impact on growth performance, tissue FA composition, sensory properties of the fillets and n-3 LC-PUFA synthesis and storage of rainbow trout. There are several challenges to consider prior to using these oils in commercial aquaculture feeds. As this study was completed with juvenile rainbow trout over shorter periods of 8-12 weeks, future studies on the impact of these dietary lipid alternatives over increased durations must be examined as well as their long-term impact on market size fish as dietary lipid alternatives as well as cost efficiency for sustainable production. Relative to fish health, the inclusion of transgenic plant-based oil must be further examined to evaluate possible impacts of novel oils on both fish health and lipid profiles, which could also further impact fillet product quality and consumer health. Future research is needed to evaluate transgenic camelina at grow out stages closer to market size (i.e., finishing diets), or for a full production cycle.

Future research is also necessary to consider *Schizochytrium* sp. oil use in rainbow trout diets for a longer production period, including trout that are closer to market production size. Health implications must also be further investigation to evaluate possible impacts on fish health as it does not supply ample EPA for metabolic use. Similarly, the inclusion of GE plant-based oils requires further investigation in full production cycle setting.

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APPENDIX
CHAPTER 2 SUPPLEMETNTARY RAINBOW TROUT PERCENT WEIGHT AND GRAVIMETRIC LIPID DATA

Table 1: Percent weight fatty acid composition of week 12 rainbow trout brain tissue samples[4].

Fatty Acid	FO	HCO	LCO	F Value	P-Value
14:0	0.8 ± 0.4 [a]	0.4 ± 0.1 [b]	0.4 ± 0.2 [b]	18.42	0.000
15:0	0.1 ± 0.0 [a]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	16.59	0.000
16:0	17.7 ± 16.7 [a]	16.3 ± 2.0 [ab]	0.8 ± 1.4 [b]	3.42	0.042
16:1n-7c	1.8 ± 0.5 [a]	1.4 ± 0.2 [b]	1.5 ± 0.3 [b]	5.39	0.008
17:0	0.2 ± 0.2 [a]	0.1 ± 0.1 [ab]	0.1 ± 0.0 [b]	3.89	0.028
18:0	7.5 ± 0.6 [a]	7.1 ± 0.3 [ab]	6.8 ± 0.6 [b]	5.20	0.010
18:1n-9c	23.3 ± 6.7 [a]	24.9 ± 2.0 [a]	25.4 ± 1.1 [a]	1.03	0.365
18:1n-9t	0.2 ± 0.3 [a]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	4.79	0.013
18:1n-7c	2.4 ± 0.2 [a]	2.3 ± 0.2 [ab]	2.2 ± 0.2 [b]	6.66	0.003
18:1n-7t	0.2 ± 0.4 [a]	0.0 ± 0.0 [ab]	0.0 ± 0.0 [b]	4.12	0.023
19:1n-12	0.0 ± 0.0 [a]	0.0 ± 0.0 [b]	0.0 ± 0.0 [ab]	3.99	0.026
18:2n-6c	2.8 ± 1.4 [a]	2.8 ± 1.1 [a]	4.1 ± 2.3 [a]	2.85	0.069
20:0	0.1 ± 0.0 [b]	0.2 ± 0.1 [ab]	0.2 ± 0.1 [a]	3.90	0.028
18:3n-6	0.0 ± 0.0 [b]	0.1 ± 0.0 [a]	0.1 ± 0.1 [a]	12.96	0.000
20:1n-9	1.9 ± 0.3 [b]	2.2 ± 0.2 [b]	2.7 ± 0.6 [a]	14.83	0.000
18:3n-3	0.9 ± 0.3 [b]	1.2 ± 0.3 [b]	2.8 ± 1.5 [a]	19.50	0.000
18:4n-3	0.2 ± 0.2 [b]	0.2 ± 0.1 [b]	0.4 ± 0.3 [a]	5.11	0.010
20:2n-6	0.4 ± 0.1 [b]	0.4 ± 0.1 [b]	0.6 ± 0.2 [a]	14.37	0.000
20:3n-6	0.2 ± 0.0 [c]	0.4 ± 0.0 [b]	0.5 ± 0.1 [a]	155.94	0.000
22:1n-9	0.5 ± 0.1 [b]	0.5 ± 0.1 [ab]	0.6 ± 0.1 [a]	9.41	0.000
20:3n-3	0.3 ± 0.0 [c]	0.4 ± 0.0 [b]	0.6 ± 0.0 [a]	297.24	0.000
20:4n-6	1.2 ± 0.1 [c]	1.9 ± 0.1 [a]	1.6 ± 0.2 [b]	57.61	0.000
22:2n-6	0.1 ± 0.0 [b]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	10.04	0.000
24:0	0.3 ± 0.1 [a]	0.3 ± 0.0 [ab]	0.2 ± 0.0 [b]	3.37	0.044
20:5n-3	4.8 ± 0.5 [a]	4.5 ± 0.2 [a]	3.9 ± 0.5 [b]	14.70	0.000
22:5n-3	2.0 ± 0.2 [a]	2.1 ± 0.3 [a]	1.7 ± 0.3 [b]	8.92	0.001
22:6n-3	21.0 ± 2.6 [a]	20.6 ± 2.0 [a]	18.9 ± 2.8 [a]	3.10	0.056
ΣSFA[1]	27.1 ± 2.6 [a]	25.1 ± 0.7 [b]	24.4 ± 1.5 [b]	9.05	0.001
ΣMUFA[2]	37.6 ± 6.1 [a]	38.5 ± 2.6 [a]	39.0 ± 1.7 [a]	0.45	0.642
ΣPUFA[3]	35.2 ± 4.5 [a]	36.4 ± 2.4 [a]	36.6 ± 1.5 [a]	0.85	0.433
ΣC18 PUFA[3]	3.9 ± 1.9 [b]	4.3 ± 1.6 [b]	7.4 ± 4.0 [a]	7.14	0.002
ΣC20 PUFA[3]	6.9 ± 0.7 [b]	7.6 ± 0.3 [a]	7.2 ± 0.5 [ab]	6.59	0.003
ΣEPA & DHA	25.8 ± 2.9 [b]	25.1 ± 2.0 [ab]	22.8 ± 3.2 [a]	4.88	0.013
Σ n-6	5.5 ± 2.1 [b]	6.8 ± 1.0 [ab]	7.8 ± 2.4 [a]	5.48	0.008
Σ n-3	29.2 ± 3.1 [a]	28.9 ± 2.0 [a]	28.3 ± 1.8 [a]	0.62	0.545
n-3/n-6	5.7 ± 1.2 [a]	4.3 ± 0.7 [b]	3.9 ± 1.1 [b]	11.88	0.000

Fatty Acid	FO	HCO	LCO	F Value	P-Value
Lipid wet	7.9 ± 2.7 [a]	8.2 ± 0.7 [a]	6.2 ± 2.6 [a]	*	*
Lipid dry	40.4 ± 13.3 [a]	42.7 ± 2.7 [a]	33.0 ± 15.1 [a]	*	*

Values are means ± SD. Means with different superscripts indicate significant differences

[1] Saturated fatty acid

[2] Monounsaturated fatty acid

[3] Polyunsaturated fatty acid

[4] Data expressed as % mg

Table 2: Percent weight fatty acid composition analysis of week 12 rainbow trout eye tissue samples[4]

Fatty Acid	FO	HCO	LCO	F Value	P -Value
14:0	3.6 ± 0.7 [a]	1.4 ± 0.2 [b]	1.2 ± 0.4 [b]	117.49	0.000
15:0	0.3 ± 0.1 [a]	0.1 ± 0.0 [b]	0.1 ± 0.0 [b]	70.64	0.000
16:0	16.4 ± 2.9 [a]	12.7 ± 1.6 [b]	12.4 ± 2.3 [b]	13.31	0.000
16:1n-7c	5.3 ± 0.9 [a]	2.9 ± 0.4 [b]	2.6 ± 0.9 [b]	51.11	0.000
16:1n-7t	0.1 ± 0.0 [a]	0.0 ± 0.0 [b]	0.0 ± 0.1 [b]	7.29	0.002
17:0	0.3 ± 0.1 [a]	0.1 ± 0.0 [b]	0.1 ± 0.0 [b]	50.38	0.000
18:1n-9c	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	4.3 ± 11.3 [a]	2.07	0.139
18:1n-9t	0.2 ± 0.1 [a]	0.1 ± 0.0 [ab]	0.1 ± 0.0 [b]	4.56	0.016
18:1n-7c	26.0 ± 14.2 [a]	19.0 ± 13.3 [a]	21.0 ± 15.3 [a]	0.91	0.411
19:0	0.1 ± 0.0 [a]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	14.81	0.000
18:2n-6c	15.8 ± 3.1 [b]	22.8 ± 3.4 [a]	19.3 ± 6.3 [ab]	8.87	0.001
20:0	0.3 ± 0.0 [c]	0.3 ± 0.0 [a]	0.5 ± 0.1 [b]	57.24	0.000
18:3n-6	0.2 ± 0.1 [c]	0.8 ± 0.2 [a]	0.5 ± 0.2 [b]	63.76	0.000
20:1n-15	0.0 ± 0.0 [b]	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	10.69	0.000
20:1	0.9 ± 0.9 [a]	0.5 ± 0.7 [ab]	0.2 ± 0.4 [b]	4.50	0.017
20:1n-9	2.5 ± 0.6 [a]	3.2 ± 1.8 [a]	3.0 ± 2.8 [a]	0.45	0.642
18:3n-3	3.8 ± 0.8 [c]	7.5 ± 1.2 [b]	14.0 ± 2.0 [a]	186.25	0.000
18:2n-6t	0.0 ± 0.0 [a]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	35.4	0.000
18:4n-3	1.0 ± 0.2 [a]	1.1 ± 0.2 [b]	1.6 ± 0.6 [b]	12.24	0.000
20:2n-6	1.0 ± 0.2 [a]	1.3 ± 0.3 [b]	1.6 ± 0.4 [b]	13.26	0.000
22:3n-3	0.0 ± 0.0 [c]	0.1 ± 0.0 [B]	0.1 ± 0.0 [a]	61.14	0.000
22:0	0.1 ± 0.0 [b]	0.2 ± 0.0 [a]	0.1 ± 0.0 [ab]	6.69	0.003
20:3n-6	0.4 ± 0.1 [c]	0.9 ± 0.2 [a]	0.7 ± 0.2 [b]	49.41	0.000
22:1n-11	2.0 ± 0.4 [a]	1.8 ± 0.3 [ab]	1.6 ± 0.5 [b]	3.32	0.046
22:1n-9	0.3 ± 0.1 [c]	0.5 ± 0.1 [b]	0.9 ± 0.2 [a]	68.7	0.000
20:3n-3	0.3 ± 0.1 [c]	0.7 ± 0.1 [b]	0.9 ± 0.2 [a]	82.07	0.000
20:4n-6	0.6 ± 0.1 [b]	1.6 ± 0.3 [a]	0.6 ± 0.1 [b]	120.5	0.000
24:0	0.1 ± 0.0 [ab]	0.1 ± 0.0 [b]	0.1 ± 0.0 [a]	4.00	0.026
20:5n-3	4.3 ± 0.7 [a]	4.3 ± 0.9 [a]	1.6 ± 0.3 [b]	79.35	0.000
24:1n-9	0.3 ± 0.1 [b]	0.3 ± 0.1 [b]	0.4 ± 0.1 [a]	5.98	0.005
22:4n-6	0.2 ± 0.1 [b]	0.3 ± 0.2 [a]	0.1 ± 0.0 [b]	17.26	0.000
22:5n-3	1.2 ± 0.3 [b]	1.8 ± 0.4 [a]	0.5 ± 0.1 [c]	84.22	0.000
22:6n-3	7.6 ± 1.5 [a]	7.9 ± 1.5 [a]	4.2 ± 1.4 [b]	29.72	0.000
ΣSFA[1]	25.0 ± 4.4 [a]	19.6 ± 2.6 [b]	18.3 ± 3.6 [b]	13.81	0.000
ΣMUFA[2]	38.5 ± 11.3 [a]	29.1 ± 10.5 [a]	36.0 ± 13.7 [a]	2.46	0.098
ΣPUFA[3]	36.5 ± 7.0 [b]	51.3 ± 8.2 [a]	45.7 ± 10.3 [a]	10.76	0.000
ΣC18 PUFA[3]	20.8 ± 4.1 [b]	32.3 ± 4.8 [a]	35.4 ± 8.3 [a]	23.13	0.000

Fatty Acid	FO	HCO	LCO	F Value	P -Value
ΣC20 PUFA[3]	6.6 ± 1.2 [b]	8.8 ± 1.6 [a]	5.3 ± 1.1 [c]	25.88	0.000
ΣC22 PUFA[3]	9.1 ± 1.8 [a]	10.3 ± 2.0 [a]	5.0 ± 1.5 [b]	35.4	0.000
ΣEPA & DHA	11.9 ± 2.2 [a]	12.2 ± 2.3 [a]	5.8 ± 1.6 [b]	45.25	0.000
Σn-6	18.3 ± 3.6 [b]	27.9 ± 4.3 [a]	22.8 ± 6.9 [b]	12.69	0.000
Σn-3	18.2 ± 3.4 [b]	23.3 ± 4.0 [a]	22.8 ± 4.1 [a]	7.83	0.001
n-3/n-6	1.0 ± 0.0 [a]	0.8 ± 0.0 [a]	1.3 ± 1.5 [a]	1.30	0.285
Lipid wet	23.0 ± 6.9 [a]	19.9 ± 2.5 [a]	22.7 ± 5.5 [a]	*	*
Lipid dry	72.8 ± 19.4 [a]	69.4 ± 6.7 [a]	74.4 ± 13.1[a]	*	*

Values are means ± SD. Means with different superscripts indicate significant differences

[1] Saturated fatty acid

[2] Monounsaturated fatty acid

[3] Polyunsaturated fatty acid

[4] Data expressed as % mg

Table 3: Week 0 and week 12 percent weight lipid composition of rainbow trout liver tissue samples[4].

Fatty Acid	Initial	FO	HCO	LCO	F Value	P - Value
14:0	1.1 ± 0.3	1.2 ± 0.2[a]	0.5 ± 0.2[b]	0.5 ± 0. [b]	64.86	0.000
14:1n-5	0.0 ± 0.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	1.66	0.202
15:0	0.1 ± 0.2	0.2 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[b]	80.63	0.000
15:1n-5	0.0 ± 0.0	*	*	*	*	*
16:0	15.4 ± 1.2	19.2 ± 2.6[a]	16.7 ± 1.5[b]	16.7 ± 0.8[b]	10.06	0.000
16:1n-7c	2.8 ± 0.9	1.2 ± 0.2[a]	0.8 ± 0.3[b]	0.6 ± 0.1[b]	24.92	0.000
16:1n-7t	0.1 ± 0.2	0.1 ± 0.0[a]	0.1 ± 0.0[b]	0.0 ± 0.0[b]	6.69	0.003
17:0	0.2 ± 0.2	0.3 ± 0.0[a]	0.2 ± 0.0[b]	0.2 ± 0.0[b]	80.78	0.000
17:1n-7	0.1 ± 0.1	0.1 ± 0.1[a]	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.18	0.832
18:0	5.7 ± 1.0	7.5 ± 1.1[a]	7.1 ± 0.8[a]	7.6 ± 0.6[a]	1.16	0.323
18:1n-9c	13.8 ± 2.2	11.7 ± 0.97[a]	12.7 ± 1.94[a]	12.9 ± 1.39[a]	2.57	0.089
18:1n-9t	0.2 ± 0.1	0.2 ± 0.2[a]	0.1 ± 0.0[a]	0.1 ±0.0[a]	3.53	0.038
18:1n-12c	0.8 ± 0.2	0.3 ± 0.1[a]	0.3 ± 0.0[a]	0.3 ± 0.0[a]	0.09	0.911
18:1n-7c	2.4 ± 0.2	1.5 ± 0.2[a]	1.3 ± 0.2[b]	1.3 ± 0.1[b]	15.30	0.000
18:1n-7t	0.0 ± 0.0	0.0 ± 0.1[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	1.63	0.208
19:0	0.1 ± 0.3	0.1 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[b]	28.22	0.000
19:1n-12	0.0 ± 0.0	0.0 ± 0.1[a]	0.0 ± 0.1[a]	0.1 ± 0.1[a]	2.62	0.085
18:2n-6c	2.5 ± 0.4	5.4 ± 0.8[a]	7.0 ±2.2[a]	6.5 ± 2.7[a]	2.51	0.093
20:0	0.2 ± 0.4	0.2 ± 0.1[b]	0.4 ± 0.1[a]	0.4 ± 0.1[a]	24.52	0.000
18:3n-6	0.1 ± 0.0	0.1 ± 0.0[c]	0.2 ± 0.1[b]	0.2 ± 0.1[a]	26.63	0.000
20:1n-15	0.0 ± 0.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	3.82	0.030
20:1	1.1 ± 0.2	0.3 ± 0.0[a]	0.2 ± 0.1[b]	0.2 ± 0.0[b]	27.85	0.000
20:1n-9	1.8 ± 0.3	0.9 ± 0.3[b]	1.9 ± 1.1[a]	2.1 ± 0.6[a]	12.26	0.000
18:3n-3	0.3 ± 0.1	1.1 ± 0.3[b]	2.6 ± 1.1[a]	2.8 ± 0.8[a]	19.65	0.000
18:2n-6t	0.0 ± 0.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	2.37	0.105
20:1n-11	*	*	*	*	*	*
18:4n-3	0.1 ± 0.1	0.2 ± 0.1[c]	0.4 ± 0.1[b]	0.7 ± 0.3[a]	18.82	0.000
20:2n-6	0.8 ± 0.1	0.8 ± 0.1[b]	1.4 ± 0.6[a]	1.3 ± 0.3[a]	11.45	0.000
22:3n-3	0.0 ± 0.0	0.0 ± 0.0[b]	0.0 ± 0.0[ab]	0.0 ± 0.0[a]	3.72	0.033
22:0	0.0 ± 0.1	0.1 ± 0.0[a]	0.2 ± 0.3[a]	0.1 ± 0.0[a]	1.52	0.231
20:3n-6	1.0 ± 0.2	0.6 ± 0.1[b]	1.4 ± 0.6[a]	1.7 ± 0.5[a]	19.85	0.000
22:1n-11	0.5 ± 0.2	0.1 ± 0.1[a]	0.1 ± 0.1[a]	0.1 ± 0.1[a]	0.19	0.826
22:1n-9	0.1 ± 0.1	0.1 ± 0.0[b]	0.1 ± 0.1[a]	0.1 ± 0.1[a]	7.08	0.002
20:3n-3	0.1 ± 0.0	0.1 ± 0.1[b]	0.5 ± 0.2[a]	0.5 ± 0.1[a]	33.17	0.000
20:4n-6	3.0 ± 0.7	4.2 ± 0.8[b]	5.7 ± 1.0[a]	5.7 ± 0.6[a]	17.75	0.000
20:4n-3	0.3 ± 0.1	*	*	*	*	*
22:2n-6	0.0 ± 0.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.87	0.428
24:0	0.0 ± 0.0	1.6 ± 2.2[a]	1.0 ± 1.3[a]	1.2 ± 1.6[a]	0.47	0.629
20:5n-3	4.4 ± 0.8	4.7 ± 2.5[a]	3.9 ± 2.1[a]	4.2 ± 1.9[a]	0.46	0.637
24:1n-9	0.9 ± 0.3	1.0 ± 0.3[a]	0.8 ± 0.2[a]	0.9 ± 0.3[a]	3.22	0.050
22:4n-6	0.5 ± 0.5	0.4 ± 0.6[a]	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.45	0.643
22:5n-6	0.7 ± 0.2	*	*	*	*	*

Fatty Acid	Initial	FO	HCO	LCO	F Value	P - Value
22:5n-3	1.5 ± 0.2	2.1 ± 0.3 [a]	2.1 ± 0.6 [a]	1.9 ± 0.4 [a]	0.93	0.401
22:6n-3	37.3 ± 2.3	32.4 ± 2.5 [a]	29.5 ± 2.9 [b]	28.5 ± 2.4 [b]	8.93	0.001
ΣSFA[1]	22.9 ± 1.9	30.4 ± 3.4 [a]	26.3 ± 2.1 [b]	26.8 ± 1.4 [b]	12.68	0.000
ΣMUFA[2]	24.5 ± 3.2	17.5 ± 1.2 [a]	18.4 ± 3.3 [a]	18.9 ± 2.1 [a]	1.33	0.275
ΣPUFA	52.6 ± 3.0	52.1 ± 2.7 [b]	55.2 ± 2.8 [a]	54.3 ± 2.6 [ab]	5.46	0.008
ΣMUFA≥18	21.5 ± 2.6	16.2 ± 1.0 [a]	17.5 ± 3.2 [a]	18.1 ± 2.1 [a]	2.79	0.073
ΣMUFA>18	4.3 ± 0.6	2.4 ± 0.5 [b]	3.2 ± 1.3 [ab]	3.6 ± 0.8 [a]	6.33	0.004
ΣC18 PUFA[3]	3.0 ± 0.5	6.8 ± 1.2 [b]	10.2 ± 3.0 [a]	10.2 ± 2.7 [a]	9.86	0.000
ΣC20 PUFA[3]	9.5 ± 1.6	10.4 ± 2.3 [b]	13.0 ± 1.9 [a]	13.4 ± 1. 8 [a]	9.63	0.000
ΣC22 PUFA[3]	40.1 ± 2.3	34.9 ± 2.1	32.0 ± 3.5	30.7 ± 2.6	8.78	0.001
ΣEPA & DHA	1.5 ± 0.2	37.1 ± 3.0 [a]	33.5 ± 4.4 [b]	32.7 ± 2.5 [b]	7.06	0.002
Σn-6	8.6 ± 1.1	11.4 ± 1.4 [b]	16.1 ± 2.6 [a]	15.7 ± 2.2 [a]	21.51	0.000
Σn-3	44.0 ±2.5	40.6 ± 3.0 [a]	39.2 ± 3.6 [a]	38.6 ± 1.8 [a]	1.95	0.155
ΣOdd chain	0.5 ± 0.7	*	*	*	*	*
n-3/n-6	5.2 ± 0.7	3.6 ± 0.5 [a]	2.5 ± 0.7 [b]	2.5 ± 0.5 [b]	15.95	0.000
Total	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	*	*
Lipid wet	4.4 ± 1.3	3.4 ± 0.8[a]	3.4 ± 0.8 [a]	3.4 ± 1.0 [a]	0.70	0.501
Lipid dry	18.6 ± 5.8	13.6 ± 2.0 [a]	12.6 ± 2.4 [a]	14.0 ± 3.9[a]	0.92	0.406

Values are means ± SD. Means with different superscripts indicate significant differences

[1] Saturated fatty acid

[2] Monounsaturated fatty acid

[3] Polyunsaturated fatty acid

[4] Data expressed as % mg

Table 4: Week 0 and week 12 percent weight lipid composition of rainbow trout muscle tissue samples[4].

Fatty Acid	Initial	FO	HCO	LCO	F Value	P - Value
14:0	3.0 ± 0.2	2.8 ± 0.4 ^{a}	0.8 ± 0.1 ^{b}	0.8 ± 0.1 ^{b}	252.06	0.000
14:1n-5	0.1 ± 0.0	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	24.73	0.000
15:0	0.2 ± 0.0	0.2 ± 0.0 ^{a}	0.1 ± 0.0 ^{b}	0.1 ± 0.0 ^{b}	209.37	0.000
15:1n-5	0.0 ± 0.0	*	*	*	*	*
16:0	*	16.1 ± 1.9 ^{a}	12.9 ±1.3 ^{b}	12.6 ± 1.2 ^{b}	23.78	0.000
16:1n-7c	17.8 ± 0.8	3.9 ± 0.6 ^{a}	1.9 ± 0.3 ^{b}	1.9 ± 0.3 ^{b}	116.27	0.000
16:1n-7t	5.4 ± 0.6	0.1 ± 0.1 ^{a}	0.1 ± 0.0 ^{b}	0.1 ± 0.0 ^{b}	9.39	0.000
17:0	0.1 ± 0.1	0.2 ± 0.0 ^{a}	0.1 ± 0.0 ^{b}	0.1 ± 0.0 ^{b}	143.81	0.000
17:1n-7	0.2 ± 0.0	0.1 ± 0.0 ^{a}	0.1 ± 0.0 ^{a}	0.1 ± 0.0 ^{a}	0.30	0.742
18:0	0.2 ± 0.0	4.1 ± 0.6 ^{ab}	4.6 ± 0.6 ^{a}	3.9 ± 0.5 ^{b}	6.26	0.004
18:1n-9c	4.0 ± 0.2	22.6 ± 11.8 ^{a}	18.8 ± 9.8 ^{a}	21.3 ± 11.0 ^{a}	0.47	0.627
18:1n-9t	19.5 ± 1.7	0.1 ± 0.1 ^{a}	0.1 ± 0.0 ^{a}	0.1 ± 0.0 ^{b}	8.20	0.001
18:1n-12c	0.2 ± 0.0	0.4 ± 0.1 ^{ab}	0.5 ± 0.1 ^{a}	0.4 ± 0.1 ^{b}	6.71	0.003
18:1n-7c	1.4 ± 0.1	4.6 ± 6.6 ^{a}	2.2 ± 0.3 ^{a}	4.0 ± 7.4 ^{a}	0.68	0.510
18:1n-7t	2.9 ± 0.1	0.1 ± 0.1 ^{a}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	12.87	0.000
19:0	0.0 ± 0.0	0.1 ± 0.0 ^{a}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	53.45	0.000
19:1n-12	0.1 ± 0.0	0.2 ± 0.1 ^{a}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	96.07	0.000
18:2n-6c	0.1 ± 0.0	12.5 ± 2.5 ^{b}	18.5 ±3.1 ^{a}	16.9 ± 3.0 ^{a}	17.41	0.000
20:0	7.7 ± 0.6	0.2 ± 0.1 ^{c}	0.8 ± 0.2 ^{a}	0.5 ± 0.1 ^{b}	71.25	0.000
18:3n-6	0.1 ± 0.0	0.2 ± 0.0 ^{c}	0.7 ± 0.1 ^{a}	0.4 ± 0.1 ^{b}	99.91	0.000
20:1n-15	0.2 ± 0.0	0.0 ± 0.0 ^{a}	0.1 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.59	0.558
20:1	0.0 ± 0.0	0.9 ± 0.3 ^{a}	0.4 ± 0.3 ^{b}	0.4 ± 0.3 ^{b}	13.21	0.000
20:1n-9	3.3 ± 0.3	2.1 ± 0.4 ^{c}	3.6 ± 0.5 ^{b}	5.6 ± 1.7 ^{a}	43.83	0.000
18:3n-3	1.7 ± 0.2	3.1 ± 0.6 ^{c}	6.3 ± 0.9 ^{b}	11.9 ± 1.9 ^{a}	186.82	0.000
18:2n-6t	1.5 ± 0.1	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	52.36	0.000
20:1n-11	*	*	*	*	*	*
18:4n-3	1.0 ± 0.1	0.7 ± 0.1 ^{b}	0.8 ± 0.2 ^{b}	1.4 ± 0.3 ^{a}	53.13	0.000
20:2n-6	0.7 ± 0.0	0.9 ± 0.2 ^{c}	1.2 ± 0.2 ^{b}	1.5 ± 0.2 ^{a}	36.12	0.000
22:3n-3	0.0 ± 0.0	0.0 ±0.0 ^{c}	0.0 ±0.0 ^{b}	0.1 ± 0.0 ^{a}	120.66	0.000
22:0	0.1 ± 0.0	0.1 ± 0.0 ^{b}	0.2 ± 0.0 ^{a}	0.2 ± 0.0 ^{a}	15.65	0.000
20:3n-6	0.4 ± 0.0	0.4 ± 0.0 ^{c}	0.9 ± 0.1 ^{b}	0.8 ± 0.1 ^{a}	94.87	0.000
22:1n-11	3.0 ± 0.8	1.5 ± 0.2 ^{a}	1.2 ± 0.3 ^{b}	1.2 ± 0.3 ^{b}	5.30	0.009
22:1n-9	0.3 ± 0.0	0.3 ± 0.1 ^{c}	0.5 ± 0.1 ^{b}	0.9 ± 0.2 ^{a}	142.94	0.000
20:3n-3	0.1 ± 0.0	0.2 ± 0.0 ^{c}	0.7 ± 0.0 ^{b}	0.9 ± 0.1 ^{a}	251.08	0.000
20:4n-6	0.9 ± 0.1	0.8 ± 0.1 ^{b}	1.8 ±0.20 ^{a}	0.9 ± 0.1 ^{b}	246.09	0.000
20:4n-3	0.8 ± 0.2	*	*	*	*	*
22:2n-6	0.1 ± 0.0	0.1 ± 0.0 ^{a}	0.1 ± 0.0 ^{a}	0.1 ± 0.0 ^{a}	3.56	0.037
24:0	0.0 ± 0.0	1.5 ± 1.4 ^{a}	1.1 ± 1.2 ^{a}	0.7 ± 0.6 ^{a}	2.11	0.134
20:5n-3	4.7 ± 0.5	2.7 ±1.3 ^{a}	2.8 ± 1.5 ^{a}	1.2 ± 0.5 ^{b}	8.64	0.001
24:1n-9	0.3 ± 0.0	0.3 ± 0.1 ^{b}	0.3 ± 0.1 ^{b}	0.4 ± 0.1 ^{a}	9.88	0.000
22:4n-6	0.1 ± 0.0	0.1 ± 0.1 ^{b}	0.4 ± 0.1 ^{a}	0.1 ± 0.0 ^{b}	158.13	0.000
22:5n-6	0.3 ± 0.1	*	*	*	*	*
22:5n-3	1.2 ± 0.1	1.5 ± 0.2 ^{b}	2.2 ± 0.3 ^{a}	0.7 ± 0.1 ^{a}	219.24	0.000
22:6n-3	16.0 ± 2.9	14.1 ± 2.0 ^{a}	13.3 ± 1.5 ^{a}	8.1 ± 1.1 ^{b}	64.89	0.000
ΣSFA[1]	25.5 ± 0.9	25.4 ± 3.7 ^{a}	20.6 ± 2.6 ^{b}	18.9 ± 2.1 ^{b}	20.86	0.000

Fatty Acid	Initial	FO	HCO	LCO	F Value	P - Value
ΣMUFA[2]	38.6 ± 2.9	37.4 ±8.1 [a]	29.7 ± 8.4 [ab]	36.4 ± 7.6 [b]	4.00	0.026
ΣPUFA	35.9 ± 2.8	37.2 ± 4.6 [b]	49.6 ± 6.1 [a]	44.8 ± 5.8 [a]	19.28	0.000
ΣMUFA≥18	32.9 ± 2.4	33.2 ± 8.7 [b]	27.7 ± 8.6 [b]	34.3 ± 7.8	2.96 [a]	0.080
ΣMUFA>18	8.8 ± 1.0	5.3 ± 0.9 [b]	6.1 ± 0.8 [b]	8.6 ± 1.9 [a]	25.84	0.000
ΣC18 PUFA[3]	10.4 ± 0.8	16.5 ± 3.2 [c]	26.3 ± 4.2 [b]	30.6 ± 5.1 [a]	43.36	0.000
ΣC20 PUFA[3]	7.7 ± 0.6	4.9 ± 1.3 [b]	7.4 ± 1.6 [a]	5.2 ± 0.7 [b]	17.38	0.000
ΣC22 PUFA[3]	17.7 ± 2.9	15.8 ± 2.2 [a]	16.0 ± 1.8 [a]	9.0 ± 1.1 [b]	78.30	0.000
ΣEPA & DHA	1.3 ± 0.1	16.8 ± 2.0 [a]	16.1 ± 2.1 [a]	9.3 ± 1.0 [b]	82.58	0.000
Σn-6	10.4 ± 0.5	14.9 ± 2.8 [b]	23.5 ± 3.7 [a]	20.6 ± 3.4 [a]	25.93	0.000
Σn-3	25.4 ± 3.1	22.3 ± 2.4 [b]	26.1 ± 2.9 [a]	24.2 ± 2.5 [ab]	8.07	0.001
ΣOdd chain	0.9 ± 0.1	0.9 ± 0.2 [a]	0.4 ± 0.1 [b]	0.4 ± 0.1 [b]	116.70	0.000
n-3/n-6	2.5 ± 0.4	1.5 ± 0.2 [a]	1.1 ± 0.1 [b]	1.2 ± 0.1 [b]	29.45	0.000
Total	100.0 ± 0.0	100.0 ±0.0	100.0 ± 0.0	100.0 ± 0.0	*	*
Lipid Wet	3.4 ± 1.0	4.8 ± 1.8 [a]	4.5 ± 1.1 [a]	5.2 ± 1.7 [a]	0.81	0.452
Lipid Dry	15.0 ± 4.0	20.5 ± 7.3 [a]	19.1 ± 4.4 [a]	21.8 ± 6.6 [a]	0.68	0.514

Values are means ± SD. Means with different superscripts indicate significant differences

[1] Saturated fatty acid

[2] Monounsaturated fatty acid

[3] Polyunsaturated fatty acid

[4] Data expressed as % mg

Table 5: Week 0 and week 12 gravimetric lipid analysis of average lipid percent in wet and dry rainbow trout liver tissue samples[1].

Diet	Lipid wet	Lipid dry
Initial	4.4 ± 1.3	18.6 ± 5.8
FO	3.4 ± 0.8[a]	13.6 ± 2.0 [a]
HCO	3.4 ± 0.8 [a]	12.6 ± 2.4 [a]
LCO	3.4 ± 1.0 [a]	14.0 ± 3.9[a]
F Value	0.70	0.92
P-Value	0.501	0.406

[1]Values are means ± SD. Means with different superscripts indicate significant differences

Table 6: Week 0 and week 12 gravimetric lipid analysis of average lipid percent in wet and dry rainbow trout muscle tissue samples[1].

Diet	**Lipid wet**	**Lipid dry**
Initial	3.4 ± 1.0	15.0 ± 4.0
FO	4.8 ± 1.8 [a]	20.5 ± 7.3[a]
HCO	4.5 ± 1.1[a]	19.1 ± 4.4[a]
LCO	5.2 ± 1.7[a]	21.8 ± 6.6[a]
F Value	0.81	0.68
P-Value	0.452	0.514

[1]Values are means ± SD. Means with different superscripts indicate significant differences

Table 7: Percent weight of rainbow trout liver samples from week 0.

FA Code	FO	HCO	LCO	F value	P-Value
14:0	1.2 ± 0.2 a	0.5 ± 0.2 b	0.5 ± 0.1 b	64.86	0.000
14:1n-5	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	1.66	0.202
15:0	0.2 ± 0.0 a	0.1 ± 0.0 b	0.1 ± 0.0 b	80.63	0.000
16:0	19.2 ± 2.6 a	16.7 ± 1.5 b	16.7 ± 0.8 b	10.06	0.000
16:1n-7c	1.2 ± 0.2 a	0.8 ± 0.3 b	0.6 ± 0.1 b	24.92	0.000
16:1n-7t	0.1 ± 0.0 a	0.1 ± 0.0 b	0.0 ± 0.0 b	6.69	0.003
17:0	0.3 ± 0.0 a	0.2 ± 0.0 b	0.2 ± 0.0 b	80.78	0.000
17:1n-7	0.1 ± 0.1 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.18	0.832
18:0	7.5 ± 1.1 a	7.1 ± 0.8 a	7.6 ± 0.6 a	1.16	0.323
18:1n-9c	11.7 ± 0.97 a	12.7 ± 1.94 a	12.9 ± 1.39 a	2.57	0.089
18:1n-9t	0.2 ± 0.2 a	0.1 ± 0.0 a	0.1 ± 0.0 a	3.53	0.038
18:1n-12c	0.3 ± 0.1 a	0.3 ± 0.0 a	0.3 ± 0.0 a	0.09	0.911
18:1n-7c	1.5 ± 0.2 a	1.3 ± 0.2 b	1.3 ± 0.1 b	15.30	0.000
18:1n-7t	0.0 ± 0.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	1.63	0.208
19:0	0.1 ± 0.0 a	0.1 ± 0.0 b	0.1 ± 0.0 b	28.22	0.000
19:1n-12	0.0 ± 0.1 a	0.0 ± 0.1 a	0.1 ± 0.1 a	2.62	0.085
18:2n-6c	5.4 ± 0.8 a	7.0 ±2.2 a	6.5 ± 2.7 a	2.51	0.093
20:0	0.2 ± 0.1 b	0.4 ± 0.1 a	0.4 ± 0.1 a	24.52	0.000
18:3n-6	0.1 ± 0.0 c	0.2 ± 0.1 b	0.2 ± 0.1 a	26.63	0.000
20:1n-15	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	3.82	0.030
20:1	0.3 ± 0.0 a	0.2 ± 0.1 b	0.2 ± 0.0 b	27.85	0.000
20:1n-9	0.9 ± 0.3 b	1.9 ± 1.1 a	2.1 ± 0.6 a	12.26	0.000
18:3n-3	1.1 ± 0.3 b	2.6 ± 1.1 a	2.8 ± 0.8 a	19.65	0.000
18:2n-6t	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	2.37	0.105
20:1n-11	*	*	*	*	*
18:4n-3	0.2 ± 0.1 c	0.4 ± 0.1 b	0.7 ± 0.3 a	18.82	0.000
20:2n-6	0.8 ± 0.1 b	1.4 ± 0.6 a	1.3 ± 0.3 a	11.45	0.000
22:3n-3	0.0 ± 0.0 b	0.0 ± 0.0 ab	0.0 ± 0.0 a	3.72	0.033
22:0	0.1 ± 0.0 a	0.2 ± 0.3 a	0.1 ± 0.0 a	1.52	0.231
20:3n-6	0.6 ± 0.1 b	1.4 ± 0.6 a	1.7 ± 0.5 a	19.85	0.000
22:1n-11	0.1 ± 0.1 a	0.1 ± 0.1 a	0.1 ± 0.1 a	0.19	0.826
22:1n-9	0.1 ± 0.0 b	0.1 ± 0.1 a	0.1 ± 0.1 a	7.08	0.002
20:3n-3	0.1 ± 0.1 b	0.5 ± 0.2 a	0.5 ± 0.1 a	33.17	0.000
20:4n-6	4.2 ± 0.8 b	5.7 ± 1.0 a	5.7 ± 0.6 a	17.75	0.000
22:2n-6	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.87	0.428
24:0	1.6 ± 2.2 a	1.0 ± 1.3 a	1.2 ± 1.6 a	0.47	0.629
20:5n-3	4.7 ± 2.5 a	3.9 ± 2.1 a	4.2 ± 1.9 a	0.46	0.637
24:1n-9	1.0 ± 0.3 a	0.8 ± 0.2 a	0.9 ± 0.3 a	3.22	0.050
22:4n-6	0.4 ± 0.6 a	0.3 ± 0.1 a	0.3 ± 0.1 a	0.45	0.643
22:5n-3	2.1 ± 0.3 a	2.1 ± 0.6 a	1.9 ± 0.4 a	0.93	0.401
22:6n-3	32.4 ± 2.5 a	29.5 ± 2.9 b	28.5 ± 2.4 b	8.93	0.001
Sum SFA	30.4 ± 3.4 a	26.3 ± 2.1 b	26.8 ± 1.4 b	12.68	0.000
Sum MUFA	17.5 ± 1.2 a	18.4 ± 3.3 a	18.9 ± 2.1 a	1.33	0.275
Sum PUFA	52.1 ± 2.7 b	55.2 ± 2.8 a	54.3 ± 2.6 ab	5.46	0.008

FA Code	FO	HCO	LCO	F value	P-Value
Sum EPA & DHA	37.1 ± 3.0 [a]	33.5 ± 4.4 [b]	32.7 ± 2.5 [b]	7.06	0.002
Sum n-6	11.4 ± 1.4 [b]	16.1 ± 2.6 [a]	15.7 ± 2.2 [a]	21.51	0.000
Sum n-3	40.6 ± 3.0 [a]	39.2 ± 3.6 [a]	38.6 ± 1.8 [a]	1.95	0.155
n-3/n-6	3.6 ± 0.5 [a]	2.5 ± 0.7 [b]	2.5 ± 0.5 [b]	15.95	0.000

[1]Values are means ± SD. Means with different superscripts indicate significant differences

Table 8: Percent weight of rainbow trout muscle week 0 analysis

Fatty Acid	FO	HCO	LCO	F value	P-Value
14:0	2.8 ± 0.4 [a]	0.8 ± 0.1 [b]	0.8 ± 0.1 [b]	252.06	0.000
14:1n-5	0.0 ± 0.0 [a]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	24.73	0.000
15:0	0.2 ± 0.0 [a]	0.1 ± 0.0 [b]	0.1 ± 0.0 [b]	209.37	0.000
16:0	16.1 ± 1.9 [a]	12.9 ± 1.3 [b]	12.6 ± 1.2 [b]	23.78	0.000
16:1n-7c	3.9 ± 0.6 [a]	1.9 ± 0.3 [b]	1.9 ± 0.3 [b]	116.27	0.000
16:1n-7t	0.1 ± 0.1 [a]	0.1 ± 0.0 [b]	0.1 ± 0.0 [b]	9.39	0.000
17:0	0.2 ± 0.0 [a]	0.1 ± 0.0 [b]	0.1 ± 0.0 [b]	143.81	0.000
17:1n-7	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	0.30	0.742
18:0	4.1 ± 0.6 [ab]	4.6 ± 0.6 [a]	3.9 ± 0.5 [b]	6.26	0.004
18:1n-9c	22.6 ± 11.8 [a]	18.8 ± 9.8 [a]	21.3 ± 11.0 [a]	0.47	0.627
18:1n-9t	0.1 ± 0.1 [a]	0.1 ± 0.0 [a]	0.1 ± 0.0 [b]	8.20	0.001
18:1n-12c	0.4 ± 0.1 [ab]	0.5 ± 0.1 [a]	0.4 ± 0.1 [b]	6.71	0.003
18:1n-7c	4.6 ± 6.6 [a]	2.2 ± 0.3 [a]	4.0 ± 7.4 [a]	0.68	0.510
18:1n-7t	0.1 ± 0.1 [a]	0.0 ± 0.0[b]	0.0 ± 0.0 [b]	12.87	0.000
19:0	0.1 ± 0.0 [a]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	53.45	0.000
19:1n-12	0.2 ± 0.1 [a]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	96.07	0.000
18:2n-6c	12.5 ± 2.5 [b]	18.5 ± 3.1 [a]	16.9 ± 3.0 [a]	17.41	0.000
20:0	0.2 ± 0.1 [c]	0.8 ± 0.2 [a]	0.5 ± 0.1 [b]	71.25	0.000
18:3n-6	0.2 ± 0.0 [c]	0.7 ± 0.1 [a]	0.4 ± 0.1 [b]	99.91	0.000
20:1n-15	0.0 ± 0.0 [a]	0.1 ± 0.0 [a]	0.0 ± 0.0 [a]	0.59	0.558
20:1	0.9 ± 0.3 [a]	0.4 ± 0.3 [b]	0.4 ± 0.3 [b]	13.21	0.000
20:1n-9	2.1 ± 0.4 [c]	3.6 ± 0.5 [b]	5.6 ± 1.7 [a]	43.83	0.000
18:3n-3	3.1 ± 0.6 [c]	6.3 ± 0.9 [b]	11.9 ± 1.9 [a]	186.82	0.000
18:2n-6t	0.0 ± 0.0 [a]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	52.36	0.000
20:1n-11	*	*	*	*	*
18:4n-3	0.7 ± 0.1 [b]	0.8 ± 0.2 [b]	1.4 ± 0.3 [a]	53.13	0.000
20:2n-6	0.9 ± 0.2 [c]	1.2 ± 0.2 [b]	1.5 ± 0.2 [a]	36.12	0.000
22:3n-3	0.0 ±0.0 [c]	0.0 ±0.0 [b]	0.1 ± 0.0 [a]	120.66	0.000
22:0	0.1 ± 0.0 [b]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	15.65	0.000
20:3n-6	0.4 ± 0.0 [c]	0.9 ± 0.1 [b]	0.8 ± 0.1 [a]	94.87	0.000
22:1n-11	1.5 ± 0.2 [a]	1.2 ± 0.3 [b]	1.2 ± 0.3 [b]	5.30	0.009
22:1n-9	0.3 ± 0.1 [c]	0.5 ± 0.1 [b]	0.9 ± 0.2 [a]	142.94	0.000
20:3n-3	0.2 ± 0.0 [c]	0.7 ± 0.0 [b]	0.9 ± 0.1 0.1	251.08	0.000
20:4n-6	0.8 ± 0.1 [b]	1.8 ± 0.20 [a]	0.9 ± 0.1 [b]	246.09	0.000
22:2n-6	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	3.56	0.037
24:0	1.5 ± 1.4 [a]	1.1 ± 1.2 [a]	0.7 ± 0.6 [a]	2.11	0.134
20:5n-3	2.7 ±1.3 [a]	2.8 ± 1.5 [a]	1.2 ± 0.5 [b]	8.64	0.001
24:1n-9	0.3 ± 0.1 [b]	0.3 ± 0.1 [b]	0.4 ± 0.1 [a]	9.88	0.000
22:4n-6	0.1 ± 0.1 [b]	0.4 ± 0.1 [a]	0.1 ± 0.0 [b]	158.13	0.000
22:5n-3	1.5 ± 0.2 [b]	2.2 ± 0.3 [a]	0.7 ± 0.1 [c]	219.24	0.000
22:6n-3	14.1 ± 2.0 [a]	13.3 ± 1.5 [a]	8.1 ± 1.1 [b]	64.89	0.000
Sum SFA	25.4 ± 3.7 [a]	20.6 ± 2.6 [b]	18.9 ± 2.1 [b]	20.86	0.000
Sum MUFA	37.4 ±8.1 [a]	29.7 ± 8.4 [ab]	36.4 ± 7.6 [b]	4.00	0.026
Sum PUFA	37.2 ± 4.6 [b]	49.6 ± 6.1 [a]	44.8 ± 5.8 [a]	19.28	0.000

Fatty Acid	FO	HCO	LCO	F value	P-Value
Sum EPA & DHA	16.8 ± 2.0 [a]	16.1 ± 2.1 [a]	9.3 ± 1.0 [b]	82.58	0.000
Sum n-6	14.9 ± 2.8 [b]	23.5 ± 3.7 [a]	20.6 ± 3.4 [a]	25.93	0.000
Sum n-3	22.3 ± 2.4 [b]	26.1 ± 2.9 [a]	24.2 ± 2.5 [ab]	8.07	0.001
n-3/n-6	1.5 ± 0.2 [a]	1.1 ± 0.1 [b]	1.2 ± 0.1 [b]	29.45	0.000

[1]Values are means ± SD. Means with different superscripts indicate significant difference