USE OF ω3 RICH OILSEED CAMELINA (*CAMELINA SATIVA*) AS A FISH OIL REPLACEMENT IN AQUACULTURE FEEDS: IMPLICATIONS FOR GROWTH AND LIPID BIOCHEMISTRY OF FARMED ATLANTIC COD (*GADUS MORHUA*), RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND ATLANTIC SALMON (*SALMO SALAR*)

by

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ABSTRACT

Camelina oil (CO) is a potential lipid replacement for fish oil (FO) in aquaculture feeds due to its high lipid content (40%), high levels of α-linolenic acid (ALA, 18:3ω3) (30%), antioxidants, and low levels of saturated fatty acids. Five feeding experiments were conducted to determine the effectiveness of CO as a FO substitute for three farmed fish species relevant to Canadian aquaculture: Atlantic cod (*Gadus morhua*), rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*).

Three experiments were conducted with Atlantic cod. In the first experiment, a fish oil control (FO), 40% (CO40) and 80% (CO80) replacement of FO with CO were fed to cod for 9 weeks. There was no effect of replacing FO with CO on growth performance. The second study tested diets with 100% replacement of FO with CO (100CO), solvent extracted fish meal (100COSEFM) and partially substituted fish meal (FM) with 15% inclusion of camelina meal (CM) (100CO15CM) for 13 weeks. Cod fed CO had a lower final weight than cod fed FO, while cod fed 100CO15CM had a lower final weight than all other groups. Cod tissue lipid and fatty acid profiles were significantly affected by CO inclusion. In the third experiment, cod were unintentionally exposed to the parasite *Loma morhua,* which was a significant factor that affected growth in this experiment.

In the rainbow trout experiment, CO replaced 50% and 100% of FO. Growth was not affected after the 12 week feeding trial. Tissue lipid and fatty acid profiles were significantly affected by the addition of CO. Compound-specific stable isotope analysis indicated that 27% of docosahexaenoic acid (DHA, 22:6ω3) was synthesized *de novo* from the CO diet. The Atlantic salmon study tested diets with 100% CO, SEFM and 10%

inclusion of CM, in a 16 week feeding trial. Growth was not affected by using 100% CO; however, it was lower in groups fed SEFM and 10% CM diets. Total lipid in salmon flesh fed a diet with CO, SEFM and CM was significantly higher than FO. Amounts of DHA in salmon fed any CO diet were similar to FO-fed salmon due to increased flesh lipid. The sensory quality of salmon fillets was not affected by CO.

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Δ: delta

ω: omega

AFI: apparent feed intake

ALA: alpha linolenic acid

AMPL: acetone mobile polar lipid

ANOSIM: analysis of similarities

ANOVA: analysis of variance

ARA: arachidonic acid

BF_3: boron trifluoride

CF: condition factor

$CHCl_3$: chloroform

CO: camelina oil

CM: camelina meal

CSIA: compound specific stable isotope analysis

DHA: docosahexaenoic acid

EFA: essential fatty acid

EPA: eicosapentaenoic acid

FAME: fatty acid methyl ester(s)

FCR: feed conversion ratio

FFA: free fatty acid

FM: fish meal

FO: fish oil

GC: gas chromatography

GC/IRMS: gas chromatography isotope ratio mass spectrometry

H_2SO_4: sulfuric acid

HSI: hepatosomatic index

LC: long chain

LNA: linoleic acid

MeOH: methanol

MDS: multidimensional scaling

mmt: million metric tonnes

MUFA: monounsaturated fatty acid

NL: neutral lipid

PL: polar lipid

PIT: passive integrated transponder

PUFA: polyunsaturated fatty acid(s)

SEFM: solvent extracted fish meal

SFA: saturated fatty acid

SGR: specific growth rate

SIMPER: similarity percentages

ST: sterol

TAG: triacylglycerol

TLC/FID: thin layer chromatography with flame ionization detector

VO: vegetable oil

VSI: visceral somatic index

ww: wet weight

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Chapter 1. General Introduction

1.1 Rationale

Aquaculture has become the world's fastest growing food production industry in the past two decades (FAO, 2009). After the increase in capture of both wild marine and inland fish in the 1950-1960's, global fisheries production has leveled off since the 1970's (Pickova and Morkore, 2007). It is estimated that 75% of the major marine fish stocks are either depleted, overexploited or being fished at their biological limit (FAO, 2009). Based on the current trends, scientists have predicted collapse of all species of wild seafood that are currently fished by the year 2048 (Worm et al., 2006). Global population growth and increases in fish consumption have led to rapid increases in global fish demand. Since the wild-capture fishery can only provide a maximum of 100 mmt annually (Watanabe, 2002; FAO, 2009), the world would face a global seafood shortage of 50-80 mmt by 2030, if it were not for aquaculture. Farmed fish accounts for nearly half of all fish consumed globally, and this is predicted to increase in the coming years (Turchini et al., 2009). There are numerous benefits of aquaculture being the main supply of seafood for the world, from a nutritional, economic and social standpoint; however achieving a balance between providing safe, nutritious and good quality food while maintaining environmental sustainability may be challenging in the future.

There is considerable interest in Atlantic cod (*Gadus morhua*), and especially Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) aquaculture in Canada, particularly Newfoundland. Newfoundland is one of the top three provincial aquaculture producers, with revenues that are significant at the community, provincial

and national level. Salmonid farming has been more established than Atlantic cod in the past three decades and is more commercially relevant; although interest in farming alternative marine species such as cod has grown, partly due to the collapse of the wild cod fishery in Newfoundland in the early 1990's. Further research in aspects related to the farming of these species can help develop the aquaculture industry in this part of the world.

1.2 Salmonid biology and culture

There are numerous species and strains of salmon and trout in the family Salmonidae, and the most commercially relevant belong to the genera *Salmo* and *Oncorhynchus*. They are either anadromous (ascending rivers from the sea to breed) or undertake their entire life cycle in freshwater. The life cycle of Atlantic salmon is anadromous, although some landlocked strains exist. Mature fish return to freshwater during the 12 months before spawning, which usually takes place in late autumn and early winter. Smoltification is a process in which fish in freshwater undergo a series of morphological, physiological and behavioural changes that enable them to migrate, survive and grow in marine conditions. These changes include: obscured lateral parr marks, darkened fin margins, silvering of scales, decline in condition factor, and tolerance of high salinities. In the final stages of smoltification, there are increases in cortisol, growth hormone and Na, K, ATPase, which aid in the osmoregulatory and ionic regulatory process in seawater, and a decrease in prolactin, which is involved in freshwater osmoregulation (Lucas and Southgate, 2003).

Culture of Atlantic salmon involves production under a natural thermal regime, which gives a bimodal distribution in length in parr populations; the upper mode completing smolting during the first spring and lower mode becoming smolts one year later. Production can be promoted by manipulating light, temperature and feeding to optimise growth during the early stages of development (Purser and Forteath, 2003). Seasonally changing temperature regulates metabolism, which affects appetite, consumption of food and growth rate, and photoperiod affects seasonal cycle. After the smoltification process, salmon are transferred to seawater cages from freshwater at a particular size and at suitable times of the year, usually in fall or spring. The average size of salmonids at harvest is determined by market demand balanced with the cost of production. For example, freshwater trout may be harvested at about 200-400 g, whereas marine Atlantic salmon may occupy a different market niche at 2-6 kg. Before harvest, samples may be taken to determine the quality of the fish in terms of lipid content, flesh colour, and average size (Lucas and Southgate, 2003).

1.3 Cod biology and culture

Atlantic cod are a marine finfish species inhabiting North Atlantic waters with temperatures in the range of 1-20°C. The natural diet of wild cod off Eastern Canada is piscivorous, but also includes crustaceans and echinoderms, so this species has an inherent capacity to utilize benthic foods, unlike more pelagic species like salmonids (Lall and Nanton, 2002).

Atlantic cod has traditionally been an important species for fisheries and processors in Newfoundland. Wild cod stocks declined and eventually the fishery

collapsed, especially on the Grand Banks, which prompted the federal government to enforce a moratorium on the cod fishery in Newfoundland in the early 1990s (deYoung and Rose, 1993; Myers et al., 1997; Rose et al., 2000). Cod farming was initiated in Newfoundland by acquisition of undersized cod from the inshore trap fishery and transferred to holding pens where they were fed intensively (Shahidi and Dunajski, 1994). High growth rates were obtained due to higher water temperature and intense feeding regimes. Cod farming has been a focus of government and industry since then, with advances in hatchery technologies, larval development, feed development and health management. However, the progress in commercial cod farming has slowed in recent years due to several factors, including disease, nutrition and economics (Booman et al., 2011), although increased demand has renewed interest in further advancement of the industry (Bolton-Warberg and Fitzgerald, 2012). The cod farming industry in the North Atlantic is expected to expand within the next 20 years (Birt et al., 2009; Standal and Utne, 2007).

As a marine fish, the entire production cycle for cod culture occurs in seawater. The spawning period is manipulated through photoperiod and occurs mid-winter. Incubation occurs in total darkness in upwelling conical tanks. The weaning period between live feed and the introduction to formulated feeds is the predominant bottleneck for producing high numbers of juveniles for commercial culture. After weaning, the fish are grown in tanks or deep raceways and fed commercially manufactured extruded marine feeds until the fish grow to approximately 5 g. The juveniles are transferred to sea in modified salmon cages for on-growing to market weight (2-3 kg) (Tibbetts, 2012).

1.4 Fish nutrition in aquaculture

The essential nutrients for animals, including fish, are: amino acids, fatty acids, vitamins, minerals and energy-yielding macronutrients (protein, lipid and carbohydrate). Diets for fish must supply all essential nutrients and energy required to meet the physiological needs of growing animals. Guidelines for nutrient adequacy for some farmed fish species suggest the minimum nutrient requirement to promote growth and prevent signs of nutrient deficiency (NRC, 2011). Nutritional requirements depend on the nutrient, as well as species and life stage of the fish. Protein is required in the diet to obtain amino acids, which are utilized to synthesize new proteins or maintain existing proteins in tissues; while excess protein is converted to energy (Wilson, 2002). Generally, the protein requirements of fish decrease with increasing size and age and higher protein levels are required for carnivorous fish than herbivorous fish (Lall and Tibbetts, 2009). Lipids supply essential fatty acids and energy in the diet. The requirement of essential fatty acids can only be met by supplying long chain (LC) polyunsaturated fatty acids (PUFA) in the diet, specifically α-linolenic acid (ALA, 18:3ω3) and linoleic acid (LNA, 18:2ω6). Marine and salmonid fish have a high dietary requirement for both eicosapentaenoic acid (EPA, 20:5ω3), docosahexaenoic acid (DHA, 22:6ω3) and arachidonic acid (ARA, 20:4ω6) because of the limited ability to biosynthesize them, whereas freshwater fish have been shown to meet their requirements for these fatty acids through synthesis from ALA and LNA (Sargent et al., 2002). Dietary lipids are also important structural components of membranes, and act as precursors of steroid hormones and prostaglandins in fish. Dietary carbohydrates can be a source of energy for fish.

However, their ability to utilize dietary carbohydrate for energy varies depending on the species and their natural diet; for example most carnivorous species are more limited in their ability to digest carbohydrates than herbivorous and omnivorous species (Lall and Tibbetts, 2009). Therefore, depending on species, protein and lipid are the main source of energy for fish. Vitamins are organic compounds that act as substrates in some metabolic reactions (De Silva and Anderson, 1995). Minerals are required for skeletal structure maintenance, cellular respiration, oxygen transport, immune function and are also important components of certain hormones and enzyme activators (Lall, 2002). Fish have unique physiological mechanisms to absorb and retain minerals from both their diet and from the aqueous environment (Oliva-Teles, 2012). Feeds in aquaculture are formulated with a balance of nutrients in order to meet specific nutrient requirements for different species, life stages and other purposes, such as medicated feeds.

1.5 Lipid nutrition

Lipids can be defined as compounds soluble in organic solvents. Acyl lipids are those containing fatty acids, and these are the ones that usually dominate in biological systems. Animal lipids are either polar (composed mainly of phospholipid) or neutral (composed mainly of triacylglycerol and sterol).

Triacylglycerol (TAG) is often the major class of neutral lipid, and it consists of three molecules of fatty acids esterified to the three hydroxy groups of glycerol. When esterified, these positions are termed *sn*-1, *sn*-2 and *sn*-3 due to the asymmetry induced by the enzymatic esterification. In fish lipids, generally saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) are preferentially located in the *sn*-1 and *sn*-3

positions, whereas PUFA are preferentially located in the *sn*-2 position (Tocher, 2003). TAG is a primary deposit of energy in fish. Phospholipids (PL) are a major class of polar lipid characterized by a common backbone of phosphatidic acid, which is L-glycerol 3-phosphate containing two esterified fatty acids. SFA and MUFA are preferentially esterified on position *sn*-1 with PUFA preferentially esterified on *sn*-2. Phosphatidic acid is esterified to the "bases" choline, ethanolamine, serine and inositol to form the major phospholipids of the animal (Sargent et al., 2002). PL is a key component in cellular membranes and is important in maintaining cell membrane fluidity. It also acts as a precursor for various biologically active mediators such as eicosanoids, platelet activating factors and inositol phosphate (Tocher, 2003). Sterols, specifically cholesterol in all animals, play an important structural role together with phospholipids.

Sterols are grouped with TAG as neutral lipids based on their polarity. Cholesterol, a tetracyclic alcohol compound, is a principal component of cell membranes that helps to maintain membrane order and fluidity (Sargent et al., 2002). With the exception of cholesterol, all of the lipid classes contain fatty acids, esterified to alcohol groups. Fatty acids are designated on the basis of their chain lengths, degree of unsaturation and position of the double bonds. Marine organisms contain many PUFA, generally of the ω3 series; however in fish, in addition to EPA and DHA the main PUFA are ARA and its metabolic precursor LNA, together with ALA the metabolic precursor of EPA and DHA (Tocher, 2003).

Fatty acids are designated on the basis of their chain lengths, degree of unsaturation (number of double bonds) and the position of their double bonds. Thus, 14:0

and 16:0 designate fatty acids with 14 and 16 carbon atoms, respectively, and with no double bonds. 18:1n-9 and 18:1n-7 designate fatty acids with 18 carbon atoms whose single double bonds are, respectively, 9 and 7 carbon atoms from the methyl end of the molecule. 18:2ω6 and 18:3ω3 also designate fatty acids with 18 carbon atoms with two and three double bonds positioned 6 and 3 carbon atoms from the methyl end of the molecule. PUFA contain two or more double bonds, with the double bonds generally interrupted by a single methylene group to generate "methylene–interrupted cis dienoic" structures. Consequently, the structure of a particular PUFA can be defined by specifying the position of the first double bond relative to the methyl end. For example, in 18:3ω3 the first double bond is situated three carbon atoms from the methyl end of the molecule, 18:3ω3 representing 18:3Δ9, 12, 15 (Tocher, 2003).

1.6 Dietary lipid requirements

Lipids and fatty acids are the major organic constituents of fish (along with proteins) and play major roles as sources of metabolic energy for growth, including movement, reproduction and migration (Tocher, 2003). Fish actually utilize feed more efficiently than other farmed animals, since fish do not expend energy to maintain a constant body temperature and have the ability to utilize lipids for energy, while saving protein for deposition and growth (Sarker et al., 2013); therefore inclusion of lipids in diets for fish is important for growth and energy purposes. Gadids and salmonids are evolutionarily divergent families of teleosts and this is evident in their life history, physiology and metabolism. The biological diversity of these families is also observed in their energy utilization and requirements. In general, salmonids have a more lipid-rich

diet than gadids, as they require more energy based on expenditure. Unexpended energy in all teleosts is stored as lipid, although metabolism and storage is different in both families. Salmonids store lipid in adipose tissue of the muscle at levels between 15-20% lipid (Polvi and Ackman, 1992; Bell, 1998), the majority of which is triacylglycerol. Gadids store most available dietary energy in the liver, with muscle accumulating between 1-2% lipid (Ackman, 1967; Dos Santos et al., 1993), the majority of which is phospholipid. Differences in their physiology and metabolism dictate different lipid requirements; therefore feed formulations must be adjusted accordingly.

Fish is a significant source of ω3 PUFA in the human diet. Long chain PUFA, particularly EPA and DHA, are important for a range of beneficial functions, and can help to prevent cardiovascular and inflammatory diseases and neurological disorders (Siriwardhana et al. 2012). Therefore, it is very important to supply the minimum PUFA requirement, for both fish and human health. All vertebrate species have absolute dietary requirements for certain PUFA which are termed essential fatty acids (EFA). Marine fish in particular require 1-2% each of EPA and DHA in the total diet (NRC, 2011). EPA and DHA have important roles in visual and neural development and are required for optimal growth, feed efficiency, health and flesh quality (Sargent et al., 2002). In addition to EPA, ARA is a precursor for eicosanoids, important bioactive compounds that are in involved in the stress and inflammatory response (Tocher, 2003). Establishing the EFA requirements for a given fish species involves determining the absolute requirements of ω3 and ω6 PUFA and also the optimal balance between the two series.

1.7 Lipid digestion

Lipid digestion, absorption and transport in fish are similar to that which occurs in mammals, although species-specific differences exist due to structural differences of the digestive tract. Lypolytic activity is highest in the proximal part of the intestine, specifically the pyloric caeca, but can extend into the lower parts of the intestine with activity decreasing (Sargent et al., 2002). TAG hydrolysis is affected by two main lipases, the pancreatic lipase-colipase system and the bile salt-activated lipase; PL is digested by pancreatic or intestinal phospholipases (Tocher, 2003). The main products of lipid digestion are free fatty acids and monoacylglycerol produced by the lipolytic action on major lipid classes. Short chain fatty acids (<10 carbons) are absorbed directly through enterocytes; longer chain fatty acids (>12 carbons) are cleaved by lipase and emulsified by bile salts to form micelles (negatively charged aggregates) (Rust, 2002). The micelles are transported from the lumen to the brush border (the enterocyte cellular membrane that borders the lumen and is highly folded into thousands of microvilli) where they dissociate, and free fatty acids diffuse across the epithelial membrane. Once inside the enterocyte, the fatty acids are re-esterified and grouped with proteins to form chylomicrons and transported via the hepatic portal vein to the liver (Rust, 2002).

Excess dietary fatty acids are exported from the liver in the form of lipoproteins and are accumulated and stored as TAG in specific lipid storage sites. Lipid storage is different in salmonids and cod. In salmonids, the primary site for lipid storage is the mesenteric adipose tissues, with significant amounts of fat within the white muscle and between skin and muscle (Tocher, 2003). Dark muscle has higher lipid content than white muscle, and contains most of the lipid as finely dispersed oil droplets within the muscle fibres. Cod, however, primarily store lipid in the liver, with very little lipid in the muscle

tissue (1-2%) (Shahidi and Dunajski, 1994). TAG is stored as a source of energy that can be used when energy requirements of the fish exceed the energy available from the diet. Catabolism of fatty acids is called β-oxidation, which involves transport of fatty acids into the mitochondria in the form of fatty acylcarnitine esters, then conversion back into fatty acyl-CoA derivatives, which then undergo a series of dehydrogenation, hydration and cleavage steps to produce acetyl-CoA and NADH, providing metabolic energy in the form of ATP through oxidative phosphorylation (Tocher, 2003). SFA and MUFA are readily catabolized by mitochondrial β-oxidation in fish, particularly 16:0, 18:1ω9, 20:1ω9 and 20:1ω11 since they are consumed in large amounts during the growth of fish. However, β-oxidation of PUFA is variable due to selective catabolism and retention of certain fatty acids. Selective catabolism leads to selective retention of PUFA such as DHA and EPA (Tocher, 2003).

1.8 Lipid biosynthesis

The saturated fatty acids 16:0 and 18:0 can be biosynthesized by all known organisms, including fish. Desaturases and elongases are the critical enzymes in the pathways for the biosynthesis of the LC PUFA from shorter-chain fatty acids to longer, more unsaturated chains. Fish can desaturate 16:0 and 18:0 to yield 16:1ω7 and 18:1ω9 by Δ9 desaturase. However, all vertebrates lack Δ12 and Δ15 desaturases, which are necessary to form ALA and LNA, so these fatty acids are considered essential (Sargent et al., 2002). These EFAs can be desaturated and elongated to form the physiologically essential EPA, DHA and ARA (Figure 1.1). However, the degree to which an animal can synthesize these fatty acids from ALA and LNA depends on the activities of the elongase

and desaturase enzymes (Δ6 and Δ5) in their tissues (Tocher, 2003). The activities of these enzymes depends on the extent to which the species obtain the end product (ARA, EPA and DHA) preformed in their natural diet. For example, carnivorous marine fish consume smaller fish that are rich in EPA and DHA derived from phytoplankton and consumed by zooplankton; therefore they do not need to convert dietary intake of ALA to EPA and DHA. Consequently, carnivorous marine fish have lost much of the capacity to synthesize these fatty acids during evolution since they remained in an environment where such a conversion is not necessary. Freshwater fish have a greater ability to biosynthesize EPA and DHA from ALA, since the natural prey of many freshwater fish is not rich in EPA and DHA, but rather ALA and LNA. Consequently, some freshwater species meet their EFA requirements solely from ALA and LNA (Sargent et al., 2002). Anadromous fish such as Atlantic salmon, spend part of their life cycle in freshwater, therefore have some limited abilities to convert ALA and LNA to DHA and EPA (Sargent et al., 2002). The fatty acid desaturation and elongation pathway has been extensively studied in fish at both the molecular and enzymatic levels (Turchini et al., 2009). Several of the genes encoding fatty acid desaturases and elongases have been cloned from a range of freshwater and marine fish. For example, cDNAs encoding the Δ6 and Δ5 desaturases and the PUFA elongase have been cloned from Atlantic salmon (Hastings et al., 2004; Zheng et al., 2005). Salmon express Δ5 and Δ6 desaturase in their tissues (at the mRNA level), but the levels of DHA and EPA produced are insufficient to meet requirements for health and growth. The Δ6 desaturase cDNA has been cloned and characterised in Atlantic cod (Tocher et al., 2006). Interestingly, the apparent inability of marine fish to convert ALA to EPA and DHA is not due to the complete absence of the required genes,

but rather to one or more of the required genes not being sufficiently expressed (Sargent et al., 2002). Although anadromous and marine species have shown expression of the elongase and Δ6 and Δ5 desaturase genes (and hence the possibility to convert LNA and ALA to ARA and EPA), the extent of fatty acid bioconversion is minimal (Turchini et al., 2009). Diet obviously influences fatty acid biosynthesis capabilities, given the natural diet and gene expression of freshwater fish compared to marine fish. Diets with low levels of EPA and DHA, but having high levels of their precursor ALA, may encourage the bioconversion of ALA to EPA and DHA in fish due to insufficient amounts in the diet that are needed for health and growth. It is possible that the fatty acid composition of the diet may influence the use of these enzymes in fish, particularly marine fish.

1.9 Lipids in aquaculture feeds

Fish oil (FO) is the main supply of lipid in diets for most aquaculture species. At least half of the world's recognized fish stocks are fully exploited and 32% are overexploited or depleted (FAO, 2010). With the production of farmed fish surpassing that of wild fish, aquaculture's share of global FO consumption has more than doubled over the past decade (Naylor et al., 2009). For the past 25 years, annual FO production has not increased beyond 1.5 million tons per annum; therefore the aquaculture industry cannot continue to rely on finite stocks of marine pelagic fish as a supply of FO (Turchini et al., 2009). According to estimates, aquaculture feeds currently use approximately 87% of the global supply of FO as a lipid source (Tacon et al., 2006). The reliance on such a finite resource raises questions about the sustainability of this practice, which may lead to

overfishing of wild fish stocks and decreased availability of FO for the aquaculture feed industry, thereby threatening global food security.

The unsustainable use of FO in aquaculture has sparked major research into alternative lipids to replace FO over the past two decades. The focus of this research has mainly been on terrestrial plant oils; and many of these studies have led to a reduction in the average inclusion of FO in commercial feeds (Olsen and Hasan, 2012). The FAO predicts that the sustainability of the aquaculture industry will likely depend on using terrestrial plant oils for aquaculture feeds rather than solely depending on FO as a lipid source (FAO, 2012). Apart from terrestrial plant oils, there are other lipid sources which may substitute for FO, for example: fishery by-products from seafood processing plants (such as fish guts, heads, blood, skin, bones, liver, etc.), single-celled microalgae oils, and zooplankton oils (such as Antarctic krill and *Calanus* copepod) (Hertrampf and Piedad-Pascual, 2000). With the reduction in FO in commercial feeds, recent calculations using the marine nutrient dependency ratio (the amount of each marine-derived nutrient used to feed salmon is divided by the amount of each nutrient produced as a result of salmon farming) estimate ratios of marine input to fish output are less than one, indicating that farmed salmon for example can be net producers of FO (Crampton et al., 2010). In fact, aquaculture is producing more than three times as much fish as it uses in the feed; therefore reducing the amount of FO in the feeds actually increases the effective global supply of fish for human consumption. This has changed from approximately a decade ago, when FM and FO were the main source of protein and lipid, and the ratio of the amount of wild fish in the diet and the amount of farmed fish produced (the fish in-fish out ratio- FIFO), was documented in the range of 1.7-5.0 for farmed salmon (Sarker et al.,

2013). A decade later, commercial feed companies commonly replace portions of FO with various plant oils, particularly soybean oil (Crampton and Carr, 2012); however it is a challenge to find feed ingredients that meet the nutritional requirements of many farmed fish species, while achieving sustainability. Therefore, some production of FO will likely remain necessary due to nutritional and industry growth constraints. However, with the predicted rise in aquaculture to meet future seafood demands and the stagnant production of wild fisheries for both food fish and fish for FM and FO, there must be other sustainable lipid sources to include in aquaculture feeds that can meet these demands.

1.10 Terrestrial plant oils in aquaculture feeds

Terrestrial plant oils are used as an energy source and as a source of essential fatty acids in feeds for farmed fish. In terms of processing of the plant ingredients prior to diet inclusion, solvent extraction is the main method for separating the oil from seeds. Initially, the oilseeds are scoured and ground, then ground material may be pressed prior to solvent extraction or is immediately solvent extracted, normally with a nonpolar solvent such as hexane. After extraction, the solvent is removed from the crude oil, followed by de-gumming, de-acidifying, bleaching and deodorising (Hertrampf and Piedad-Pascal, 2000). The use of terrestrial plant oils in practical feed formulations has some technical limitations. Most of the oils have high levels of MUFA or PUFA, thus are prone to lipid oxidation. Therefore an antioxidant is typically added during processing to prevent oxidation. Inclusion rates of plant oils in feeding trials may result in poor pellet quality. The oil level suggested for a common pelleting machine is 4-5%, although higher inclusion rates are possible if the feed is extruded. Plant oils are permitted as a component

for animal feeds according to EU and US regulations and must contain no less than 90% fatty acids, no more than 2% unsaponifiable matter and no more than 1% insoluble impurities. Maximum free fatty acids and moisture must also be guaranteed and the use of an antioxidant must be declared (Hertrampf and Piedad-Pascual, 2000).

Terrestrial plants will probably be the main choice when replacing FM and FO in aquaculture diets due to sustainability and product consistency; however they are fairly limited in their ability to fully replace FM and FO in diets for fish. Most plant oils are relatively poor sources of ω3 fatty acids in comparison to marine FO. As previously mentioned, LC ω3 PUFA are essential for the growth of healthy fish and these fatty acids are not present in terrestrial plant oils. Rather, they are rich sources of ω6 and ω9 fatty acids, mainly LNA and 18:1ω9, with the exception of some oilseeds. Several studies have shown that replacing fish oils with various terrestrial plant oils in diets for different fish species does not compromise health, growth or feed consumption and that they are readily catabolised by fish as an energy source for growth (Stubhaug et al., 2007). However, most terrestrial plant oils are composed mainly of ω6 and ω9 fatty acids and lack the critical LC ω3 PUFA that are abundant in fish oil, resulting in lower levels of DHA and EPA in tissues of fish fed plant oils (Jobling et al., 2008; Bell et al., 2010; Alhazzaa et al., 2011), which is detrimental to fish health and also compromises the health benefits associated with consuming fish. Due to high levels of ω6 fatty acids and low levels of ω3 in plant oils, the ω3/ω6 ratio is low and studies have shown that extensive replacement of FO with plant oil causes a high incidence of cardiovascular disorders in fish (Waagbo et al., 2008). Therefore, plant oils best suited as a substitute for FO should contain high levels of ω3

fatty acids (Turchini et al., 2009). Studies have also found preferential use of SFA and MUFA for energy production in the mitochondria of fish, although MUFA and especially SFA are not as well digested and absorbed as PUFA in fish (Sigurgisladottir et al., 1992; Colombo-Hixson et al., 2011). Therefore, the plant oils best suited as a substitute for FO should contain high MUFA as an energy source, with high levels of ω3 PUFA and lower amounts of LNA, in order to decreases the ω3/ω6 ratio.

Research on FO replacements often have several different purposes, including effect on growth, tissue composition, liver metabolism, fatty acid metabolism, immunological response, and sensory quality. Studies replacing fish oil with rapeseed oil, palm oil (Bell et al., 2002), linseed oil (Menoyo et al., 2005), sunflower oil (Brandensen et al., 2003) and vegetable oil blends (Jordal et al., 2007; Petropoulos et al., 2007; Tortensen et al., 2005) all used 100% of the vegetable oil to replace fish oil in diets for salmon. Generally, the results showed a significant reduction in EPA and DHA in muscle and liver, a significant increase in 18:1ω9, LNA, and ALA in muscle and liver and significant differential regulation of genes involved in lipid metabolism and transcription. Fewer studies have tested dietary vegetable oil in non-salmonid marine fish, however similar results were observed in species such as Atlantic cod, European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata* L.), and barramundi (*Lates calcarifer*), in terms of growth, increases in 18:1ω9, LNA and/or ALA and reductions in EPA and DHA in tissues.

In general, studies have found that terrestrial plant oils can replace substantial amounts of FO in the diets of many fish species without affecting growth or feed

efficiency, provided that adequate amounts of specific essential fatty acids are supplied in the diet. Ideally, FO replacements should contain high levels of lipid with high levels of ω3 PUFA, low levels of ω6 PUFA and lower levels of SFA.

1.11 Camelina (*Camelina sativa)* oil

The oilseed *Camelina sativa* (L. Crantz) is an ancient crop that originated in Germany around 600 B.C. and cultivation spread throughout central Europe as an oil crop. Although production declined in the Middle Ages (5^{th} to 15^{th} centuries), camelina continued to evolve as a weed with flax, as such camelina is also known as false flax. Camelina is a member of the Cruciferae (Brassicaceae) family, which includes mustards, rapes, canola, radish, turnip, broccoli, cabbage, collards, cauliflower, rutabaga, Brussels sprouts, kohlrabi and many weeds (Budin et al., 1995).

Camelina, also known as "Gold of Pleasure", has several unique and positive agronomic attributes. It requires minimal input for growth and grows well in semi-arid regions and in low-fertility and saline soils, which is unusual for an oilseed crop (Putnim, 1993). In comparison to other oil crops, canola, soy and sunflower have high nutrient requirements (Budin et al., 1995). Camelina also appears to be tolerant of insects and weeds. It can also be cast at low seeding rates onto frozen ground and can survive frost and freeze-thaw cycles after emergence during late winter and spring (Putnim et al., 1993). Due to its agriculturally robust nature, it has recently been reintroduced to Canadian soils, particularly in Atlantic Canada and the Prairies. Its agronomical and biochemical properties have drawn the attention of the biofuel and feed industries.

The oil content of camelina seeds is as high as 28% to 40% (Budin et al., 1995). The oilseed is particularly unique due to its high total lipid content (40 %) and unusually high amount of ALA (30%), a medium chain ω3 fatty acid which is a precursor to longer chain ω3 PUFA (Zubr, 1997; Ni Eidhin et al., 2003). Camelina oil contains higher levels of ω3 PUFA than most plant oils commonly used in aquaculture feeds, which could give it nutritional and commercial advantages over the currently available plant oils, such as canola, corn, linseed, soybean or palm oil (Table 1.1). It also contains high levels of LNA (19%), 18:1ω9 (18%) and 20:1ω9 (16%), which means that both MUFA and PUFA content are high compared to SFA content. Due to high levels of PUFA compared to other plant oils, camelina oil is susceptible to lipid oxidation in theory, however, it contains a high amount of ϒ-tocopherol, the most potent antioxidant tocopherol isomer; therefore, it is naturally protective against lipid oxidation, despite increased PUFA content (Ni Eidhin et al., 2003). It is less susceptible to oxidation than the main alternative sources of ω3 fatty acids, fish oil and linseed oil. Camelina oil is sufficiently stable to constitute a technically and economically competitive alternative to fish oil as a food ingredient source of ω3 PUFA (Ni Eidhin et al., 2003).

1.12 Objectives

Aquaculture will likely be the main supply of seafood for human consumption in the future. Therefore it is important to develop sustainable practices in the industry. The use of FO in aquaculture feeds for marine and salmonid species is not environmentally or economically sustainable. Therefore alternative lipid sources to replace FO in feeds for farmed fish must be investigated. In the past decade, several studies have reported the

effects of various terrestrial plant oils on the growth, lipid and fatty acid composition of several farmed fish species. Although growth is normally unaffected by this substitution, tissue lipid and fatty acid composition of the fish tissues are altered throughout the course of the study, which produces a fillet that is less desirable for human consumption due to increased levels of ω6 fatty acids and decreased levels of ω3 fatty acids, especially DHA and EPA. Therefore, sustainable lipid sources that are high in ω3 PUFA would be ideal lipid sources to replace FO in farmed fish diets. The oilseed *Camelina sativa* has potential as a lipid source in diets for farmed fish to replace FO. The main focus of this study was to investigate the effect of camelina oil as a replacement of fish oil in diets for farmed fish species relevant in Canada, specifically Atlantic cod, rainbow trout and Atlantic salmon, to evaluate growth and tissue lipid and fatty acid composition. In addition, this study investigated the effect of camelina oil on lipid metabolism, fatty acid biosynthesis and organoleptic properties. In order to address these issues, five research questions were posed and five experiments were conducted to determine the effectiveness of camelina oil as a lipid source for farmed fish, which were:

1) What is the effect of replacing fish oil with camelina oil on growth, lipid class and fatty acid composition of juvenile Atlantic cod?
2) What is the effect of replacing fish oil with camelina oil (with inclusion of camelina meal) in diets of Atlantic cod on growth and tissue lipid classes and fatty acids, with use of the fatty acid mass balance method, energy budget and multivariate statistics to determine these changes?

3) What is the effect of camelina oil on growth and tissue lipid and fatty acid composition in Atlantic cod with incidence of disease, specifically the natural outbreak of microsporan parasite *Loma morhua*?
4) What are the changes in tissue lipids and fatty acids of rainbow trout fed diets containing camelina oil as a full replacement of fish oil, with use of compound specific stable isotope analysis to determine the extent of LC PUFA synthesis?
5) What is the effect of full substitution of fish oil with camelina oil (with partial substitution of fish meal with camelina meal), in diets for farmed Atlantic salmon and its effect on tissue lipids and fatty acids, as well as sensory quality?

These research questions were addressed in five experiments (three experiments with Atlantic cod, one with rainbow trout, and one with Atlantic salmon) and each chapter in this thesis represents each of these studies.

1.13 Co-Authorship Statement

I am the first author on all of the manuscripts produced from this thesis. I identified the research questions. I assisted in all aspects of experimental design, including design of the diets and sampling protocols, and feeding trial planning. I sampled all tissues used in this thesis and completed all laboratory analyses (except compound specific stable isotope analysis in Chapter 5). I completed all data analyses and writing of the manuscripts.

Dr. Chris Parrish is a co-author on all of the manuscripts in this thesis. Dr. Parrish contributed to the conceptual design of the project and research questions in this thesis. He provided expertise and guidance in all aspects of experimental design. He reviewed all of the manuscripts in this thesis.

Dr. Derek Anderson is a co-author on Chapters 2, 5 and 6. He contributed to the conceptual design and formulation of the diets used in these experiments and produced the diets.

The publications produced from this thesis include:

Chapter 2: Hixson, S., Parrish, C., Anderson, D. 2013. Effect of replacement of fish oil with camelina (*Camelina sativa*) oil on growth, lipid class and fatty acid composition of farmed juvenile Atlantic cod (*Gadus morhua*). Fish Physiology and Biochemistry. Online. DOI 10.1007/s10695-013-9798-2

Chapter 3: Hixson, S., Parrish, C. 2013. Substitution of fish oil with camelina oil and inclusion of camelina meal in diets of Atlantic cod (*Gadus morhua*) and its effect on

growth and tissue lipid classes and fatty acids. Submitted to the Journal of Animal Science (September 13, 2013)

Chapter 5: Hixson, S., Parrish, C., Anderson, D. 2013. Changes in lipid biochemistry of farmed rainbow trout (*Oncorhyncus mykiss*) fed diets containing camelina oil (*Camelina sativa*) as a full replacement of fish oil. Accepted in Lipids.

Chapter 6: Hixson, S., Parrish, C., Anderson, D. 2013. Full substitution of fish oil with camelina oil, with partial substitution of fish meal with camelina meal, in diets for farmed Atlantic salmon (*Salmo salar*) and its effect on tissue lipids and sensory quality. Submitted to Food Chemistry (August 26, 2013).

Table 1.1. Fatty acid composition (%) of fish (herring) oil (FO) in comparison to different terrestrial oilseeds: camelina oil (CO) canola oil (CA), corn oil (CR), linseed oil (LO), soybean oil (SO), and palm oil (PO) that are commonly used in aquaculture feeds for commercial or research purposes[1]

Fatty acid (%)	FO	CO	CA	CR	LO	SO	PO
14:0	6.40	0.10	-	-	-	0.10	1.00
16:0	12.7	7.42	3.10	10.9	5.30	10.3	43.5
16:1	8.80	-	-	-	-	0.20	0.30
18:0	0.90	2.10	1.50	1.80	4.10	3.80	4.30
18:1	12.7	16.0	60.0	24.2	20.2	22.8	36.6
18:2ω6	1.10	23.8	20.2	58.0	12.7	51.0	9.10
18:3ω3	0.60	29.3	12.0	0.70	53.3	6.80	0.20
20:1	14.1	12.9	1.30	-	-	-	0.10
20:4ω6	0.30	-	-	-	-	-	
20:5ω3	8.40	-	-	-	-	-	
22:1	20.8	-	1.00	-	-	-	
22:5ω3	0.80	-	-	-	-	-	
22:6ω3	4.90	-	-	-	-	-	
∑ω6	1.40	30.4	20.2	58.0	12.7	51.0	9.10
∑ω3	17.8	25.7	12.0	0.70	53.3	6.80	0.20
ω3/ω6	12.7	1.18	0.59	0.01	4.20	0.13	0.02

[1]Table modified from NRC (2011), except for CO (analyzed values)

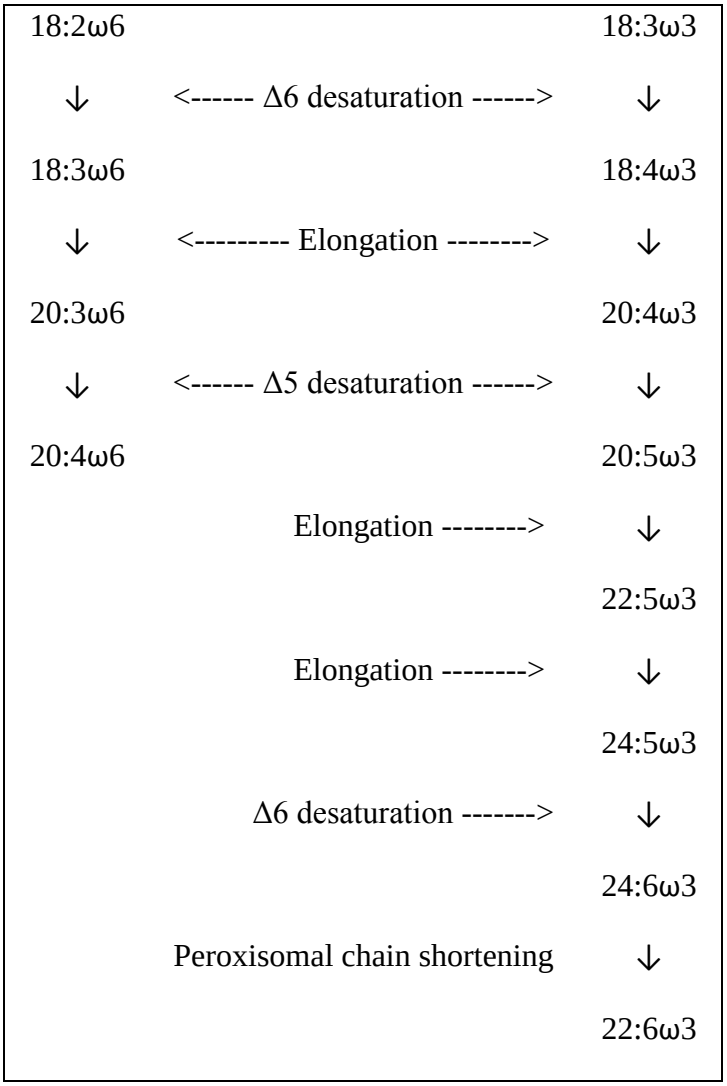

Figure 1.1. The ω3 and ω6 biosynthesis pathways from C_{18} precursors

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Chapter 2. Effect of replacement of fish oil with camelina (*Camelina sativa*) oil on growth, lipid class and fatty acid composition of farmed juvenile Atlantic cod (*Gadus morhua*)

2.1 Abstract

Camelina (*Camelina sativa*) oil was tested as a replacement for fish oil in diets for farmed Atlantic cod (*Gadus morhua*). Camelina differs from other plant oilseeds previously used in aquaculture with high lipid (40%), α-linolenic acid (30%), antioxidants, and low proportions of saturated fats. Dietary treatments were fed to cod (19 g $fish^{-1}$ initial weight) for 9 weeks and included a fish oil control (FO), 40% (CO40) and 80% (CO80) replacement of fish oil with camelina oil. There was no effect of replacing fish oil with camelina oil included at levels up to 80% on the growth performance. Cod fed CO80 stored more lipid in the liver ($p< 0.01$), including more neutral lipid ($p< 0.05$) and triacylglycerol (TAG) ($p< 0.05$). Cod fed CO80 decreased in total polyunsaturated fatty acids (PUFA) in muscle compared to CO40 and FO ($p< 0.05$); increased in monounsaturated fatty acids (MUFA) ($p< 0.01$); decreased in total ω3 fatty acids (FO> CO40> CO80; $p< 0.01$); and increased in total ω6 fatty acids (FO< CO40< CO80; $p< 0.01$). In the liver, long chain (LC) ω3 PUFA decreased when fish oil was removed from the diet ($p< 0.05$), and increased in 18-carbon fatty acids ($p< 0.01$). Camelina oil can reduce the amount of fish oil needed to meet lipid requirements; although replacing 80% of fish oil reduced LC PUFAs in both tissues. A comparison of BF_3 and H_2SO_4 as catalysts to transmethylate cod liver and muscle lipids revealed small but significant differences in some fatty acid proportions.

2.2 Introduction

Fish is a significant source of ω3 long chain polyunsaturated fatty acids (PUFA) in the human diet. Long chain PUFA, particularly eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) are important for a range of beneficial functions including cardiovascular and inflammatory diseases and neurological disorders (Siriwardhana et al., 2012; Stanley et al., 2012; De Felice et al., 2012; Gertsik et al., 2012). Pressure from commercial capture fisheries has left most fish stocks exploited, and as a result aquaculture now produces nearly half of the world's seafood (FAO, 2012). Atlantic cod (*Gadus morhua*) in Newfoundland, Canada is a clear example of a wild stock that experienced so much pressure that the fishery collapsed in the early 1990's (deYoung and Rose 1993; Myers et al., 1997; Rose et al., 2000). Since then, Atlantic cod farming has been of interest in this part of the world (Brown and Puvenandran, 2002; Brown et al., 2003; Booman et al., 2011), as well as Norway, the UK, Iceland, Spain and Chile (Rosenlund and Skretting, 2006).

The expansion of aquaculture, particularly farming of carnivorous species like cod, is placing demands on fish meal (FM) and fish oil (FO), which raises questions regarding sustainability (Naylor et al., 2000). Fish oil, produced from wild fisheries, is used more by aquaculture than any other industry (Tacon and Metian, 2008). Dependency on a limited resource like fish oil will limit growth of aquaculture in the future. Therefore research into alternative sources of lipid, especially of terrestrial origin, is necessary to both relieve pressure on wild fish stocks and to allow growth in aquaculture (Pickova and Mørkøre, 2007). Several studies have shown that replacing fish oils with various terrestrial plant oils and animal fats in diets for different marine species does not

compromise growth or overall health. However, most terrestrial plant oils are composed mainly of ω6 and ω9 fatty acids and lack the critical long chain ω3 PUFA that are abundant in fish oil, resulting in lower levels of EPA and DHA in tissues (Jobling et al., 2008; Bell et al., 2010; Alhazza et al., 2011), potentially compromising health benefits that are normally provided to humans that consume fish. Therefore, research in terrestrial plant oils with significant amounts of ω3 is of particular interest.

The oilseed *Camelina sativa* (commonly known as false flax or gold of pleasure) has recently been reintroduced to Canadian agriculture on account of its agriculturally robust nature. The plant's ability to grow in marginal land, survive frost and tolerate insects, as well as its unique fatty acid profile have drawn the attention of biofuel agronomists and aquaculturists alike. The oilseed is particularly unique due to its high total lipid content (40%) and unusually high amount of α-linolenic acid (ALA, 18:3ω3) (40%), a medium chain ω3 fatty acid which is a precursor to longer chain ω3 fatty acids (Zubr, 1997; Ni Eidhin et al., 2003). Camelina oil (CO) also contains a high amount of γ-tocopherol, the most potent antioxidant tocopherol isomer; therefore it is naturally protective against lipid oxidation, despite increased PUFA content (Ni Eidhin et al., 2003). Generally CO has more PUFA and MUFA than other terrestrial plant oils and lower saturated fatty acids (SFA), which is beneficial for both fish and humans. The combination of these biochemical characteristics potentially gives camelina a unique nutritional advantage over other plant oils that have been previously used in aquaculture.

Cod express some genes involved in the production of DHA and EPA from shorter ω3 fatty acid chains (Tocher et al., 2006). Based on this premise, we hypothesized

that cod fed camelina oil, high in ALA may be able to sustain sufficient levels of DHA and EPA in their tissues after feeding a diet with reduced fish oil. It is well known that cod liver is the primary site of lipid deposition and storage, and is lipid-rich with primarily triacylglycerol, therefore it is most frequently studied and is a tissue of interest when determining changes in fatty acid composition due to diet. Although low in total flesh lipid (1%), cod fillet contains a high proportion of phospholipid that is beneficial for membrane fatty acid function as well as being important for growth, prevention of skeletal deformities and stress resistance (Shahidi and Dunajski, 1994; Jobling et al., 2008). Therefore, changes in both liver and muscle tissue fatty acids (particularly ALA, EPA and DHA) due to dietary influence were examined.

A critical part of any experiment that measures fatty acids from lipid extracts both qualitatively and quantitatively is the method in which these lipids are isolated and derivatized from fatty acids to fatty acid methyl esters (FAMEs). The fatty acids of lipids are esterified within various lipid classes and have to be transesterified to methyl esters prior to analysis by gas chromatography. A variety of methods to transesterify lipid extracts are available based on different acidic or basic catalyst reagents (e.g. boron trifluoride, sodium methoxide, hydrochloric and sulfuric acids) (Budge and Parrish, 2003). The incomplete conversion of fatty acids esterified within lipids to fatty acid methyl esters can affect the yield and results of fatty acid analysis (Schlechtriem et al., 2008). The present study compared BF_3 and H_2SO_4 as derivatizing agents for two different sample types: one low in total lipid and triacylglycerol (cod muscle) and one high in total lipid and triacylglycerol (cod liver).

The overall objective of the study was to evaluate camelina oil as a suitable lipid resource to replace fish oil in diets for farmed Atlantic cod. Specifically, a nutritional feeding trial was conducted with diets containing camelina oil in order to analyze changes in growth performance and lipid and tissue fatty acid composition in cod.

2.3 Methods

Camelina oil

Camelina (Calena cultivar) was grown and harvested by the Department of Plant and Animal Sciences, Dalhousie University at an off-campus location (Canning, Nova Scotia, Canada). The seeds were single pressed using a KEK 0500 press at Atlantic Oilseed Processing, Ltd. (Summerside, Prince Edward Island, Canada) to extract the oil and ethoxyquin was added to the final product as an antioxidant.

Experimental diets

Three practical diets were produced at the Faculty of Agriculture Campus, Dalhousie University (Table 2.1). Diets were formulated to meet the nutritional requirements of gadoids based on previous formulations (Tibbetts et al., 2004; 2006), and were isonitrogenous and isolipidous. CO was used as the test ingredient in each of the experimental diets, and herring oil was used in the control diet (FO). Camelina oil replaced 40% (CO40) or 80% (CO80) of the total FO in two experimental diets. The maximum replacement level of 80% was chosen because marine fish have essential fatty acid requirements (NRC, 2011), which would not have been met if 100% of fish oil was replaced, therefore health and welfare of cod was considered when formulating the diets.

Experimental diets were steam pelleted using a laboratory pelleting mill (California Pellet Mill, San Francisco, USA). The initial size of pellet was 1.5 mm and was increased to 2.5 mm as the fish grew larger throughout the trial. Diets were stored at -20°C until needed.

Experimental fish

An experiment was conducted with juvenile cod (19.4 ± 3.9 g $fish^{-1}$ mean initial weight ± SD; 12.3 ± 0.8 cm mean initial length ± SD) at the Ocean Sciences Centre, Dr. Joe Brown Aquatic Research Building (St. John's, Newfoundland and Labrador, Canada) where fish were cultured and reared to initial experimental size. Fish were randomly distributed (990 total) into 9 experimental tanks (620 L capacity), each tank with 110 fish. This study was approved by the Institutional Animal Care Committee of Memorial University of Newfoundland, protocol number 10-50-CP. The fish were acclimated from a commercial diet onto the control diet for one week prior to initial sampling. Dietary treatments were fed to triplicate tanks for 67 days (> 9 weeks). A flow-through system of filtered (1µm) seawater was supplied to each tank at a rate of 8 L min^{-1} and a photoperiod of 12 hours light: 12 hours dark. The dissolved oxygen (10 mg L^{-1}) and water temperature (10°C) were monitored daily. Fish were fed 1% of body weight of feed per tank in order to ensure equal consumption of feed among treatments and were fed twice daily. Mortalities were weighed and recorded throughout the trial.

Sampling

Sampling occurred at week 0 (before experimental diets were fed), week 1, 4, and 9. Fish were starved for 24 hours prior to sampling. Ten fish per tank were randomly sampled and measured for length and weight. The whole liver was removed, weighed and

sampled for dry matter and lipid analysis. The skin was removed on the left side of the fish and muscle tissue was subsampled for dry matter and lipid analysis. Lipid samples were stored on ice during sampling of each tank and were processed within an hour. Samples were collected in 50 ml test tubes that had been rinsed three times with methanol followed by three rinses with chloroform. The tubes were allowed to dry completely before they were weighed. The tubes were weighed again following the addition of the sample. After wet weights were recorded, samples were covered with 8 ml of chloroform (HPLC-grade), the headspace in the tube was filled with nitrogen, the Teflon-lined caps were sealed with Teflon tape, and the samples were stored at -20°C.

Lipid extracts

Lipid samples were extracted according to Parrish (1999). Samples were homogenized in a 2:1 mixture of ice-cold chloroform: methanol. Samples were homogenized with a Polytron PCU-2-110 homogenizer (Brinkmann Instruments, Rexdale, Ontario, Canada). Chloroform extracted water was added to bring the ratio of cholorform:methanol:water to 8:4:3. The sample was sonicated for six min in an ice bath and centrifuged at 2688 RCF for two min at room temperature. The bottom organic layer was removed using a double pipetting technique, placing a 2 ml lipid-cleaned Pasteur pipette inside a 1 ml pipette, to remove the organic layer without disturbing the top aqueous layer. Chloroform was then added back to the extraction test tube and the entire procedure was repeated 3 times for muscle samples and 5 times for liver samples. All organic layers were pooled into a lipid-cleaned vial. The samples were concentrated using a flash-evaporator (Buchler Instruments, Fort Lee, New Jersey, USA).

Lipid class separation

Lipid class composition was determined using an Iatroscan Mark VI TLC-FID (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan), silica coated Chromarods and a three-step development method (Parrish, 1987). The lipid extracts were applied to the Chromarods and focused to a narrow band using 100% acetone. The first development system was hexane: diethyl ether: formic acid (99.95:1.0:0.05). The rods were developed for 25 min, removed from the system for 5 min and replaced for 20 min. The second development was for 40 min in hexane: diethyl ether: formic acid (79:20:1). The final development system had two steps, the first was 100% acetone for two 15 min time periods, followed by two 10 min periods in chloroform: methanol: chloroform-extracted water (5:4:1). Before using each solvent system the rods were dried in a constant humidity chamber. After each development system, the rods were partially scanned in the Iatroscan and the data were collected using Peak Simple software (version 3.67, SRI Inc). The Chromarods were calibrated using standards from Sigma Chemicals (Sigma Chemicals, St. Louis, Missouri, USA).

Fatty acid methyl ester (FAME) derivatization

For all muscle samples, lipid extracts were transesterified using 14% BF_3 in MeOH for 1.5 hours at 85°C. For all liver samples, lipid extracts were transesterified using the Hilditch reagent (1.5 H_2SO_4: 98.5 anhydrous MeOH) for 1 hour at 100°C. Reagents were added in the proportion of 1.5 ml reagent per 4-16 mg of lipid (Morrison and Smith, 1964). Samples were vortexed halfway through each derivatization reaction. To analyze the derivatization efficiency of both procedures, 18 samples of liver and

muscle were transesterified using both methods, and then the lipid class composition of the methyl ester solution was determined by TLC-FID (Flame Ionization Detector). The derivatization efficiency is calculated from the amount of underivatized acyl lipids. This value was divided by the amount of acyl lipids in the extract before transmethylation, expressed as a percentage, and subtracted from 100%.

All FAMEs were analyzed on a HP 6890 GC FID equipped with a 7683 autosampler. The GC column was a ZB wax+ (Phenomenex, Torrance, California, USA). The column length was 30 m with an internal diameter of 0.32 mm. The column temperature began at 65°C where it was held for 0.5 min. The temperature ramped to 195°C at a rate of 40°C min^{-1}, held for 15 min then ramped to a final temperature of 220°C at a rate of 2°C min^{-1}. This final temperature was held for 45 sec. The carrier gas was hydrogen flowing at 2 ml min^{-1}. The injector temperature started at 150°C and ramped to a final temperature of 250°C at 120°C min^{-1}. The detector temperature stayed at 260°C. Peaks were identified using retention times from standards purchased from Supelco (Bellefonte, Pennsylvania, USA): 37 component FAME mix (Product number 47885-U), PUFA 3 (product number 47085-U) and PUFA 1 (product number 47033-U). Chromatograms were integrated using the Varian Galaxie Chromatography Data System, version 1.9.3.2 (Agilent Technologies, Colorado, USA).

Statistical methods

Statistical analysis followed methods outlined by Sokal and Rolf (1994). The statistical approach is another critical part of any experiment. In these types of experiments, committing a type II error (accepting a false null hypothesis) may lead to

misleading conclusions regarding the use of alternative feed ingredients to fish meal/oil. In order to guard against this risk, this study tested growth data in different ways to ensure that type II errors were not committed, and that conclusions made about camelina oil truly reflected the results. For analysis of growth data, lipid class data, and fatty acid data, where individual fish were weighed, measured and sampled, a three-way nested ANOVA was performed using the General Linear Model (Minitab 16 Statistical Software, State College, Pennsylvania, USA). The model was designed to test the effect of diet on the response variable and nested fish individuals within tanks to negate variability among tanks and individuals, while also testing for tank effects. For analysis of growth data that depend on comparison to an initial measurement and thus must be pooled per tank (i.e., mean weight gain and specific growth rate), a two-way ANOVA was performed to test the effect of diet and tank variability. In both cases, where significant differences occurred, treatment means were differentiated using the Tukey HSD multiple comparison test and all residuals were evaluated for homogeneity and normality. For analysis of growth data, a *t*-test was additionally performed between FO and CO80 (highest camelina replacement) to verify results from the ANOVA. For analysis of derivatization methods, a *t*-test was used to compare the derivatization efficiency per tissue and a one-way ANOVA was used to compare tissue fatty acid proportions after derivatization by either method. To analyze differences between initial and final fatty acids in muscle and liver, a two-sample *t*-test was performed to test between the initial and final CO80 fatty acids. For each model tested, the residuals were examined to evaluate the appropriateness of the model, therefore normality, homogeneity and independence of residuals were considered. If a p-value was close to 0.05 and

residuals were not normal, a p-randomization was conducted >1,000 times to test the data empirically.

2.4 Results

Camelina oil fatty acid composition

The majority of fatty acids (%) were 18-carbon chains including ALA (35.6 ± 0.6), LNA (18.4 ± 0.3) and 18:1ω9 (11.5 ± 0.2) (Figure 2.1). The next most abundant fatty acids were over 20-carbons in length: 20:1ω9 (12.9 ± 0.5), 22:1ω9 (2.6 ± 0.4) and 20:2ω6 (2.0 ± 0.1).

Experimental diet composition

Diets ranged in moisture content from 9.3 to 10.1% and ash from 1.9 to 2.3% (Table 2.1). Protein was 49% and lipid ranged from 11.8 to 12.0%. The lipid was composed mainly of triacylglycerol (TAG) (47%) (Table 2.2). In terms of fatty acids, generally the control and CO80 diet differed significantly, mainly in terrestrial-type fatty acids (ALA, LNA, 18:1ω9). Total saturated fatty acids (SFA) were significantly lower in the CO80 diet compared to CO40 and FO. Total ω3 fatty acids did not differ among diets; although 18:3ω3 increased significantly with the inclusion of CO. Total ω6 fatty acids were significantly higher in the CO80 diet than the control diet; however, CO40 did not differ from either diet. The ω3/ω6 ratio varied significantly between CO80 and the control; but CO40 ω3/ω6 was not different from the FO and CO80 diets. There was no significant difference in total PUFA among diets.

Growth performance

The mean initial weight of the fish ranged from 18.9 to 19.5 g fish^{-1} and there was no significant difference in fish weights among the treatment groups or tanks (F= 0.41; p= 0.87) (Table 2.3). The mean initial length of the fish ranged from 12.1 to 12.3 cm fish^{-1} and did not differ significantly among treatments or tanks (F= 0.74; 0.62). After the 9 week feeding trial, the fish more than doubled their weight (126-135%), reaching a mean final mass of 45.4 g fish^{-1} (FO), 44.5 g fish^{-1} (CO40) and 44.0 g fish^{-1} (CO80). There was no significant difference in final weight among treatments or tanks (F= 1.5; p= 0.18). The fish gained 24.6- 26.0 g fish^{-1}, however the difference was not significant. Since weight gain showed the maximum change between diets, the minimum detectable difference was calculated. This determines the difference between means that could have been significantly different. Given the same variance and sample size as in the original data, a two-sample *t*-test was performed with two different means until a significant difference was observed. Each time a *t*-test was performed the difference between the two means was increased. The difference between FO mean weight gain (26.0) and CO80 weight gain (24.6) was 1.4 g. If the weight gain of FO fish was 33.0 ± 2.9 and CO80 fish was 16.6 ± 5.8 (n= 3), the difference would be 16.4 g (*t*-stat= 4.38; p= 0.048). A difference of 15.4 g between means was not significant (*t*-stat= 4.11; p= 0.054). Even tripling the sample size from 3 tanks per diet to 9 tanks per diet, there would not be a significant difference given the same mean and variance (*t*-stat= 0.65; p= 0.53). Therefore, we can conclude that the weight gain of fish is the same and the difference would have to increase 16 fold before it would become significant. The mean final length was measured at approximately 16.0 cm, but there was no significant difference among groups or tanks (F= 1.06; p= 0.39). The mean specific growth rate (SGR) did not vary significantly

among groups: it ranged from 1.23 to 1.29 % day^{-1}. The mean condition factor (CF) was 1.08 to 1.13, however, a significant difference was not detected among groups or tanks (F= 1.24; p= 0.30). The mean hepatosomatic index (HSI) ranged from 8.5 to 8.8%, but the difference was not significant and there were no significant differences among tanks (F= 1.87; p= 0.10). A two-sample *t*-test was conducted between FO and CO80 for all growth parameters, since these groups tended to be the most different; however, there were no significant differences between these two treatments for any measurement. There were 2 mortalities recorded from separate tanks during the trial.

Lipid class tissue composition

Muscle

Initial muscle tissue total lipid was 1% ww^{-1}, mainly composed of polar lipid and very little neutral lipid (Table 2.4). Lipid classes were mainly phospholipid (PL) (83%) and sterol (ST) (7%). Acetone-mobile polar lipid (AMPL) was 3% and TAG was 3% of total lipid. After the feeding trial, TAG in CO40 was significantly higher (4%) than FO (1%) and CO80 (1%). FFA, ST and PL were the same among treatments. There were no significant difference in final total lipid, neutral lipid and polar lipid among groups. There were no significant differences among tanks for any lipid classes in muscle tissue.

Liver

Initial liver total lipid was 45%, composed of neutral lipid (29-32%) and polar lipid (12-16%) (Table 2.5). Lipid classes were TAG (72%), AMPL (10%), PL (7%) and ST (3%). After 9 weeks of feeding, liver TAG was significantly higher in CO80 (82%) than CO40 (71%) and FO (76%). Liver AMPL was significantly lower in CO40 (6%) and

CO80 (4%) groups compared to the FO (9%). There was a significant difference in ST, with a decreasing trend as fish oil was removed from the diets. There was no difference in PL between CO-fed and FO-fed livers. Total lipid and neutral lipid increased significantly in CO80 compared to CO40 and FO. There were no significant differences among tanks for any of the lipid classes in liver tissue.

Fatty acid tissue composition

Muscle

Initial samples were taken after cod were fed the control diet for one week and prior to the control diet, all fish were fed a fish meal/fish oil-based commercial diet of similar composition. Initially in the muscle, PUFA was the dominant fatty acid group, accounting for 59% of total fatty acids, followed by SFA (23%) and MUFA (17%). Individual fatty acid proportions were typical for cod muscle after consuming a commercial-type diet (Shahidi and Dunajski, 1994). Terrestrial-type fatty acids were relatively low, such as 18:1ω9 (9%), LNA (4%) and ALA (<1%), whereas known marine-type fatty acids were higher, particularly EPA (19%), 22:5ω3 (3%), DHA (28%).

After 9 weeks of feeding CO diets, total PUFA decreased in CO80 compared to CO40 and FO, and MUFA increased with CO inclusion (Table 2.4). SFA remained the same among groups after feeding the CO diets. Total ω3 fatty acids decreased significantly as CO was included in the diet (FO> CO40> CO80) and ω6 fatty acids increased significantly. As a result, the ω3/ω6 ratio decreased significantly with inclusion of CO from 7 to 4. The trend of dietary fatty acid affecting the corresponding fatty acid in the flesh was found for several individual fatty acids. The total “terrestrial” fatty acids

(LNA + ALA) increased significantly in the muscle with inclusion of camelina oil. EPA and DHA, as DHA in CO80 fed cod was significantly lower than the control and CO40. Long-chain ω6 fatty acids (≥20 carbons) significantly decreased in the CO80 diet compared to the control, with the exception of 20:2ω6. 20:3ω3 significantly increased and tripled its amount in the flesh in CO80 compared to the control and doubled in CO40. There were no significant differences among tanks for any of the fatty acids in muscle tissue.

Liver

Initially, MUFA was the predominant fatty acid group (40%) in the liver, followed by PUFA (36%) and SFA (24%) (Table 2.5). The ω3 fatty acids were 25% and ω6 were 7%; which makes the ω3/ω6 ratio 7:1.

After 9 weeks of feeding, SFA was significantly lower in CO80 livers than FO from 22% to 20% (Table 2.5). There was no difference in the total MUFA, PUFA, ω3 and the ω3/ω6 ratio. However, including camelina (both CO40 and CO80) significantly increased the total amount of ω6 from 9 to 10% and terrestrial fatty acids (LNA + ALA) from 9 to 13%. 18:3ω3 and 18:2ω6 both increased significantly with the inclusion of camelina. Generally in the liver, long chain fatty acids (≥20 carbons) tended to decrease when fish oil was removed from the diet, for example 20:4ω6, EPA, 22:5ω3 and DHA. There was one exception to this trend however: 20:3ω3 was significantly higher in CO80 than the FO and CO40. There were no significant differences among tanks for any fatty acids in the liver tissue.

The difference between initial and final fatty acid compositions of cod fed CO80 in the muscle and liver for selected fatty acids was calculated by subtracting final fatty acid (%) from the mean initial fatty acid (%) (Table 2.6). All selected fatty acids in the muscle were significantly different from the initial fatty acid composition, except the sums of MUFA and PUFA. In the liver, all selected fatty acids were significantly different from the initial fatty acid composition, except DHA and total PUFA.

Derivatization efficiency

Liver and muscle samples were derivatized using both BF_3 and H_2SO_4 methods. The derivatization efficiency using BF_3 or H_2SO_4 on either liver or muscle tissue ranged from 93 to 94%, except when liver was derivatized using BF_3 and the efficiency was significantly lower (34%) than using H_2SO_4 (Table 2.7). TAG remaining in liver BF_3 derivatives caused low efficiency ratios. There were small but significant differences in fatty acid proportions in liver tissue, depending on derivatization method (Table 2.8). Total PUFA ($F= 7.4$; $p= 0.02$), 20:5ω3 ($F= 14.0$; $p= 0.002$), 22:6ω3 ($F= 5.6$; $p= 0.03$) were significantly lower in BF_3 derivatized samples than H_2SO_4 samples. Muscle tissue fatty acids proportions, however, were not significantly affected by derivatization method.

2.5 Discussion

Up to 80% of FO was replaced with CO to evaluate this new potential lipid source in diets for farmed Atlantic cod. The most abundant fatty acids in camelina oil were ALA, LNA, 18:1ω9 and 20:1ω9. The comparable PUFA and ω3 content between CO and FO

gives CO potential as a lipid source in diets for farmed cod. There was no effect of replacing FO with dietary CO included at levels up to 80% on the growth of Atlantic cod in this experiment. Several different types of vegetable oils and blends have been used to replace fish oil for Atlantic cod such as echium oil (Bell et al. 2006); soybean oil (Mørkøre et al., 2007); rapeseed, linseed, palm oil blend (Tocher et al., 2006; Jobling et al., 2008), and CO blend in salmon (Bell et al., 2010); which did not show a significant difference in growth among dietary treatments. After the present study was conducted, a study was published that showed that cod fed CO diets for 12 weeks did not show any significant differences in growth performance (Morais et al., 2012), which confirms growth results found in our experiment. Although growth performance results were similar between these two studies, the diets used in the present study were more practical in comparison. The formulations were designed to be used in a commercial setting, with a reduction in fish meal and use of alternative ingredients (Crampton and Carr, 2012), and consideration of essential fatty acid requirements of the fish without removing 100% of fish oil (NRC, 2011); compared to diets that would likely only be used in a research setting. In this study, growth rate, condition factor and hepatosomatic index of the fish were typical for farmed cod (Jobling, 1988).

The lipid class profile of the feeds varied depending on camelina inclusion. The diets were formulated to be isolipidous, so the total lipid was the same among diets (12%), composed of mainly TAG (47%) and PL (29%). Lipid storage differed in both liver and muscle tissue, depending on the lipid composition of the diet. In the liver, cod fed CO80 contained significantly more lipid, neutral lipid and TAG than CO40 and FO groups, despite all treatments being fed isolipidous diets. The HSI was the same among

groups (8-9%), which is a typical range for farmed cod (Lie et al., 1986), suggesting that the excess lipid stored in livers of CO80 did not affect the total liver weight. Cod are known to store excess dietary lipids in the form of TAG in the liver. Neutral lipids like TAG are known to more readily respond to changes in dietary lipid than polar lipids (Sargent et al., 1989; Higgs and Dong, 2002). Although the diets in this study were not significantly different in the proportion of TAG, there are differences in the ways in which the lipids in fish oil and plant oil are digested, absorbed and metabolized (Jobling et al., 2008). Fish fed different vegetable oils have been found to store more neutral lipid than fish fed diets with fish oil (Wijekoon, 2012). Salmon fed a vegetable oil blend containing 20% camelina oil had significantly higher lipid deposition and increased TAG content (Leaver et al., 2011). The results in this study suggest that high amounts of CO in the diet are stored as TAG in the liver rather than metabolized for energy compared to the control. Feeding this diet for extended time may have increased the liver weight. Fish fed CO80 had lower ST proportions compared to the fish fed FO, despite higher sterol proportions in the diet. The ST amount in CO80 was still lower than FO when considering higher total liver lipid in CO80. Cholesterol is the most common ST in animal lipid (Tocher, 2003) and is a major component of cell membranes, while some is stored together with neutral lipids. Phytosterols in plant oils like CO are considered phytoestrogens and they interfere with cholesterol synthesis. Several studies have investigated the efficiency of phytosterols in lowering cholesterol in fish (Pickova and Mørkøre, 2007). A study by Mørkøre (2006) found that cod fed soy oil had significantly lower levels of cholesterol in the liver compared to the cod fed fish oil. It is most likely that the higher ST content found in CO80 lowered levels of cholesterol in cod liver fed

CO80. Since cholesterol is a precursor of steroid hormones, the change in sterol content will impact on steroid synthesis in fish, affecting their reproductive development (Trembley and van der Kraak, 1999). Whether this mechanism can be used in farming to delay reproductive maturation of cod has not yet been studied.

The changes observed in the fatty acid profile after feeding camelina oil to cod were expected, given that we know feeding vegetable oil to marine fish increases C_{18} fatty acids in the flesh, and decreases in total ω3 fatty acids, DHA and EPA (Turchini and Mailer, 2010). In this particular study, camelina oil is quite high in 18:3ω3; therefore significant increases in this fatty acid were observed in liver and muscle, a result that was also observed in a recent study using camelina oil (Morais et al., 2012). Fatty acid profiles of the liver and muscle showed similar trends in terms of increases and decreases of particular fatty acids, however the proportions were different as is expected for different tissues (Sargant et al., 1989; Tocher, 2003). Camelina oil significantly increased C_{18} fatty acids, and the loss of fish oil decreased EPA, DHA and total ω3. Although, uptake of ALA was much greater than the loss of EPA and DHA. Bell et al., (2003) and Stubhaug et al., (2007) have shown that fish are capable of sequestering of ω3 PUFA, mainly EPA and DHA when fed diets deficient of such fatty acids. Fish are capable of utilizing surplus dietary fatty acids for β-oxidation and energy production while preserving essential and limited ω3 PUFAs in membranes. A study in red sea bream (*Pagrus auratus*) found retention of essential long chain PUFAs, like EPA and DHA, when fed rapeseed or soy oil (Glencross et al., 2003). Fish can selectively mobilize or incorporate fatty acids in response to physiological demand. Therefore, subtle, yet

significant changes in EPA and DHA are due to selective conservation, and more abundant fatty acids such as 18:1ω9 are used for energy production. However, it is unknown how long fish can sustain these levels of essential long chain PUFA if it is not provided in the diet.

Differences between initial and final fatty acid composition of CO80 fish seemed to vary depending on fatty acid rather than tissue type. Diet appeared to have equally affected muscle and liver fatty acid compositions, contrary to the idea that neutral lipids (liver) tend to respond to dietary lipids more than polar lipids (muscle) (Tocher, 2003). For example, DHA loss in the muscle was 5 times greater than in the liver; however, the proportion of DHA was over 25% in the muscle and less than 7% in the liver. Therefore the ratio change of individual fatty acids should be considered since the change is relative to the initial composition. In general, the factor by which a fatty acid changes due to camelina inclusion was similar for both liver and muscle. The results suggest that storage and sequestering of fatty acids are more dependent on the fatty acid type rather than the tissue in which it is stored. Change ratios for LNA, ALA, and ω6 were greater than change ratios for EPA, DHA, PUFA and ω3, which supports the idea that some degree of selectivity and conservation of DHA, EPA and PUFA occurred since the losses were small but still significant and the loss was smaller than the uptake of C_{18} fatty acids. Sequestering ω3 fatty acids has been studied in cold water marine fish when essential ω3 fatty acids are limited in the diet and surplus fatty acids are used for energy production (Bell et al., 2003; Stubhaug et al., 2007; Leaver et al., 2011; Wijekoon, 2012).

We find evidence that fatty acid synthesis has occurred, specifically fatty acid elongation, to produce 20:3ω3 which was not present in the diet or in initial tissue samples. In the CO80 group, muscle tissue contained 16 times more 20:3ω3 than FO tissue. The presence of this fatty acid suggests that elongation occurred from ALA to 20:3ω3 but not to EPA. It is not likely that reaching 20:3ω3 is a rate limiting step in the ω3 pathway, since 20:3ω3 does not accumulate in either tissue. This result has previously been observed with cod and salmon fed CO (Morais et al., 2012; Bell et al., 2010). Cod expressed PUFA elongase in white muscle tissue, hepatocytes and enterocytes when fed a vegetable oil diet containing camelina, however desaturase expression was low (Tocher et al,. 2006). After injection of radiolabelled LNA and ALA, radioactive 20:3ω3 and 20:2ω6 were recovered in Arctic charr (*Salvenilus alpinus*), rainbow trout, gilthead sea bream (*Sparus aurata)* and golden grey mullet (*Liza aurata*) (Olsen and Ringø, 1992; Mourente and Tocher, 1998). Elongation occurred, however desaturation did not. The DHA and EPA provided from the minimal FO and FM supplied in the CO80 was likely enough to sustain and conserve these essential fatty acids rather than encourage fatty acid synthesis to meet requirements. Bell et al. (2006) reasoned that the poor ability of marine fish to synthesize long chain PUFA is not due to lack of Δ6 desaturase, but rather to deficiencies in other parts of the biosynthetic pathway.

No single procedure is suitable for derivatization of all types of lipids (Christie, 1993) so, investigation of the results of fatty acid analysis applying different techniques to different samples is necessary. This study found using certain methods for transmethylation can be inappropriate for certain sample types and will affect the amount

of fatty acids that are actually derivatized, although it has little effect on the proportion. Using H_2SO_4 to derivatize cod liver was 94% efficient in converting acyl lipids to fatty acid methyl ester lipids. However, using BF_3 to derivatize cod liver was only 34% efficient, due to incomplete derivatization of TAG. In order to ensure equal opportunity for complete derivatization between both methods, similar amounts of acyl lipids were derivatized for each method and tissue. Also, the amount of reagent was increased from 1 ml reagent per 4-16 mg (Morrison and Smith, 1964) to 1.5 ml reagent to ensure complete transmethylation for 14 mg of lipid; so it is unlikely that the low derivatization efficiency observed using BF_3 was due to methodology. Schlechtriem et al. (2008) found that H_2SO_4 transmethylation of salmon flesh resulted in a complete derivatization of TAG and all PL classes; however, after using the BF_3 method, the fatty acid methyl ester proportion was only 33.5%. As for muscle tissue, derivatization efficiency was the same using either derivatization method. The method also did not affect the fatty acid proportions (%) of muscle tissue; however, there were significant differences between methods for liver fatty acid proportion. The BF_3 method tended to underestimate the proportion of long chain PUFA, namely EPA, DHA and total PUFA, and seemed to overestimate certain SFAs, e.g. 14:0, 16:0. The results of this study suggest that using BF_3 to derivatize samples that are lipid-rich and high in TAG is inefficient and will significantly underestimate both the amount and proportion of the same fatty acids that are in the initial lipid sample. Christie (1993) stated that certain classes of simple lipids, such TAG, are not soluble in BF_3 alone and an inert solvent must be added to ensure their solution and derivatization. This study shows that incomplete derivatization of a lipid sample significantly affects the amount of fatty acids in a sample, in this case when using BF_3 to derivatize cod liver, a sample

which is high in lipid (50% ww^{-1}) and high in TAG (80%). The use of BF_3 is appropriate for samples that are low in lipid, such as cod muscle, and has also been verified with several other low-lipid and proportionally lower TAG marine samples by Budge and Parrish (2003) such as blue mussels (*Mytilus edulis*), green algae (*Nannochloropsis sp*.) and flagellates (*Isochrysis galbana*). This study concludes that BF_3 is an inappropriate catalyst to transmethylate cod liver lipid and should be avoided for that purpose; therefore all cod livers in this study were derivatized using H_2SO_4.

Although feeding high levels of CO did not prevent reductions in EPA and DHA compared to fish fed FO, the increased levels of ALA in flesh are useful as it is an essential fatty acid for humans (Burdge, 2006; Brenna et al., 2009; Morais et al., 2012). It is a concern, however, that currently there is no regulation to include nutrition labels on farmed seafood when sold to consumers. Alternative plant oils, like camelina, reduce the amount of DHA and EPA in fish fillets, which may be deceiving to consumers that purchase fish for health purposes. Selectively breeding different camelina lines will help improve amino acid and fatty acid profiles as well as reduce antinutritional factors for improved camelina meal and oil. It may also be possible to selectively breed fish families that can efficiently utilize dietary plant oils without significant losses of DHA and EPA levels in the flesh. Using camelina oil in commercial diets for cod is an appropriate lipid source and will help to reduce the amount of fish oil needed to meet lipid requirements, particularly since growth performance is unaffected; however replacing 80% of fish oil with camelina oil will reduce essential long chain PUFAs. Long term studies are needed to determine if losses of EPA and DHA are enough to affect final amounts in fillets harvested for market.

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Table 2.1. Formulation and proximate composition of control and experimental diets[1]

Diet	FO	CO40	CO80
Ingredient (% of diet)			
Herring Oil	4.6	2.75	0.9
Camelina Oil	---	1.85	3.7
Herring meal	50.9	50.9	50.9
Wheat Gluten Meal	5.0	5.0	5.0
CPSP-G[1]	5.5	5.5	5.5
Wheat Middlings	14.6	14.6	14.6
Whey Powder	7.0	7.0	7.0
Krill Hydrolysate	2.5	2.5	2.5
Corn Starch (pre-gel)	5.6	5.6	5.6
Vitamin Mixture[2]	1.95	1.95	1.95
Mineral Mixture[3]	1.95	1.95	1.95
Choline Chloride	0.3	0.3	0.3
Proximate composition analyzed, as-fed basis (n=3)			
Moisture	10.1	9.6	9.3
Ash	2.1	1.9	2.3
Protein	48.7	48.8	49.4
Lipid	11.8	11.9	12.0

[1]Concentre proteique soluble de poisson (soluble fish protein concentrate)
[2]Northeast Nutrition, Truro, Nova Scotia, Canada. Vitamin Premix contains per kg: Vitamin A 0.9 g, Vitamin D3 8.0 g, Vitamin E 50.0 g, Vitamin K 3.0 g, Thiamin 2.8 g, Riboflavin 4.0 g, Pantothenic acid 24.0 g, Biotin 0.1 g, Folic acid 26.7 g, Vitamin B12 0.03 g, Niacin 15.1 g, Pyridixine 3.3 g, Ascorbic acid 10.8 g, Wheat middlings (carrier) 851.3 g
[3]Northeast Nutrition, Truro, Nova Scotia, Canada. Mineral Premix contains per kg: Manganese oxide 12.3 g, Zinc oxide 20.6 g, Copper sulphate 6.1 g, Iodine 15.8 g, Wheat middlings (carrier) 954.2 g.

Table 2.2. Lipid class and fatty acid composition of control and experimental diets[1]

Lipid composition	FO	CO40	CO80	F-stat	*p*-value
(% ww^{-1})					
Neutral lipid	7.0 ± 0.4	7.4 ± 4.5	7.9 ± 1.1	3.01	0.13
Polar lipid	4.5 ± 0.6	4.5 ± 0.9	4.3 ± 0.2	2.96	0.13
(*% total lipid)*					
Triacylglycerol	46.6 ± 3.9	47.0 ± 3.9	47.0 ± 4.5	2.59	0.16
Free fatty acid	3.8 ± 0.3^{a}	5.1 ± 1.0^{a}	8.4 ± 1.6^{b}	21.1	0.02
Sterol	2.9 ± 1.0^{a}	4.7 ± 1.8^{b}	7.0 ± 0.8^{c}	7.48	0.02
AMPL[2]	3.8 ± 0.6^{a}	8.4 ± 1.4^{b}	3.7 ± 0.9^{a}	10.1	0.02
Phospholipid	29.7 ± 4.1	29.6 ± 5.9	24.1 ± 1.8	1.68	0.26
Fatty acid[3]					
14:0	4.4 ± 0.6	4.4 ± 0.6	3.9 ± 0.2	2.60	0.15
16:0	20.0 ± 0.4^{a}	19.4 ± 0.5^{a}	16.9 ± 1.3^{b}	10.6	0.01
16:1ω9	5.1 ± 0.5^{a}	5.0 ± 0.5^{a}	3.5 ± 0.2^{b}	3.80	0.01
18:0	3.1 ± 0.7	2.6 ± 0.03	2.4 ± 0.06	2.41	0.17
18:1ω9	11.5 ± 0.6^{a}	12.2 ± 0.5^{ab}	13.2 ± 0.1^{b}	5.11	0.04
18:2ω6 (LNA)	10.7 ± 1.0^{a}	13.0 ± 0.7^{ab}	14.3 ± 0.2^{b}	7.67	0.01
18:3ω3 (ALA)	1.1 ± 0.8^{a}	3.7 ± 0.6^{b}	7.3 ± 1.1^{c}	47.1	<0.001
20:1ω9	5.7 ± 1.4^{a}	5.3 ± 0.3^{a}	6.6 ± 0.4^{b}	42.5	<0.001
20:4ω6	0.6 ± 0.006	0.6 ± 0.004	0.5 ± 0.002	4.86	0.06
20:5ω3 (EPA)	8.9 ± 0.2	8.6 ± 0.4	7.0 ± 0.3	5.06	0.06
22:1ω9	5.7 ± 2.4	3.8 ± 0.6	4.4 ± 1.3	1.38	0.32
22:5ω3	0.9 ± 0.007	0.7 ± 0.008	0.7 ± 0.01	5.12	0.15
22:6ω3 (DHA)	10.5 ± 0.8	10.1 ± 0.2	9.3 ± 0.1	0.34	0.72
∑SFA[4]	27.9 ± 0.3^{a}	26.7 ± 0.5^{a}	23.5 ± 0.4^{b}	14.1	0.01
∑MUFA[5]	33.5 ± 1.2	31.2 ± 1.2	33.0 ± 0.8	0.55	0.60
∑PUFA[6]	37.3 ± 2.5	40.5 ± 0.9	41.2 ± 1.5	1.68	0.30

∑ω3	23.2 ± 1.4	24.6 ± 0.5	25.0 ± 0.9	0.56	0.60
∑ω6	11.7 ± 1.7[a]	13.9 ± 0.8[ab]	15.3 ± 0.8[b]	7.08	0.03
ω3/ω6	2.0 ± 0.1[a]	1.8 ± 0.1[ab]	1.6 ± 0.2[b]	5.44	0.04

[1]Values are mean (n=9) ± SD. Means within rows with different superscripts indicate significant differences.
[2]Acetone-mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester)
[4] Saturated fatty acid
[5] Monounsaturated fatty acid
[6] Polyunsaturated fatty acid

Table 2.3. Growth performance of Atlantic cod fed experimental diets after 9 weeks[1]

	FO	CO40	CO80	Max. Difference (%)	F-stat	*p*-value	*T*-stat	*p*-value
Initial body mass (g)	19.4 ± 3.1	18.9 ± 4.4	19.5 ± 4.4	3.1	0.37	0.71	-	-
Initial length (cm)	12.3 ± 0.7	12.1 ± 0.9	12.3 ± 0.8	1.7	0.49	0.64	-	-
Final body mass (g)	45.4 ± 10.9	44.5 ± 9.7	44.0 ± 11.6	3.4	0.09	0.93	0.46	0.65
Weight gain (g fish^{-1})	26.0 ± 2.9	25.5 ± 3.2	24.6 ± 5.8	5.7	1.03	0.43	1.03	0.43
Final length (cm)	16.0 ± 1.1	15.7 ± 1.2	15.9 ± 1.2	1.9	0.40	0.69	0.40	0.68
SGR (% day^{-1})[3]	1.29 ± 0.1	1.29 ± 0.1	1.23 ± 0.2	4.9	0.91	0.48	-0.46	0.49
CF[4]	1.09 ± 0.09	1.13 ± 0.09	1.08 ± 0.1	4.6	1.90	0.22	0.36	0.72
HSI[5]	8.7 ± 1.4	8.5 ± 2.0	8.8 ± 1.4	3.5	0.23	0.80	0.23	0.80

[1]Values are mean (n=30) ± SD for measurements on individual fish. Values are mean (n=3) for measurements on tank means (weight gain, SGR).

[2] *T*-tests were conducted on final measurements between FO and CO80.

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

[4] Condition factor = body mass/ length^3

[5] Hepatosomatic index = 100 x (liver mass/ body mass)

Table 2.4. Lipid class and fatty acid composition of juvenile Atlantic cod muscle tissue after 9 weeks of growth[1]

Lipid composition	Initial	FO	CO40	CO80	F-stat	*p*-value
(% *ww*$^{-1}$)						
Total lipid	1.2 ± 0.1	0.7 ± 0.1	0.9 ± 0.3	0.7 ± 0.2	1.1	0.25
Neutral lipid	0.2 ± 0.01	0.1 ± 0.03	0.2 ± 0.1	0.1 ± 0.03	2.5	0.16
Polar lipid	1.1 ± 0.1	0.5 ± 0.01	0.6 ± 0.2	0.6 ± 0.2	1.1	0.34
(*% total lipid)*						
Steryl esters	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	0.3 ± 0.02	0.6	0.20
Triacylglycerol	3.4 ± 0.9	1.2 ± 0.5^{a}	4.0 ± 1.4^{b}	1.0 ± 0.3^{a}	7.9	0.003
Free fatty acid	0.2 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.5 ± 0.3	0.3	0.71
Sterol	7.4 ± 2.2	8.9 ± 1.5	10.0 ± 2.0	10.0 ± 1.1	0.2	0.86
AMPL[2]	3.2 ± 1.3	2.5 ± 1.7^{a}	6.1 ± 2.0^{b}	2.9 ± 0.4^{a}	6.9	0.004
Phospholipid	82.7 ± 4.8	79.4 ± 0.5	72.2 ± 4.4	80.0 ± 2.0	2.1	0.14
Fatty acid[3]						
14:0	1.2 ± 0.1	1.3 ± 0.2	1.2 ± 0.1	1.2 ± 0.4	0.3	0.73
16:0	17.1 ± 0.9	17.0 ± 0.6^{a}	16.2 ± 0.7^{b}	17.0 ±1.6^{a}	5.5	0.04
16:1ω7	2.1 ± 0.5	2.2 ± 0.3	2.1 ± 0.2	2.1 ± 0.7	2.6	0.15
18:0	3.9 ± 0.4	3.1 ± 0.3	3.3 ± 0.2	3.4 ± 0.3	1.7	0.26
18:1ω9	9.1 ± 0.8	8.7 ± 0.7^{a}	9.5 ± 0.8^{a}	11.2 ± 1.7^{b}	10.9	0.01
18:2ω6 (LNA)	4.1 ± 0.7	5.1 ± 0.7^{a}	6.3 ± 0.5^{b}	8.5 ± 1.3^{c}	54.1	<0.001
18:3ω3 (ALA)	0.6 ± 0.2	1.3 ± 0.6^{a}	2.7 ± 0.3^{b}	4.7 ± 0.7^{c}	104	<0.001
18:4ω3	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.03	0.5 ± 0.1	0.3	0.75
20:1ω9	1.6 ± 0.2	1.6 ± 0.1^{a}	1.7 ± 0.1^{b}	1.9 ± 0.2^{c}	17.3	0.003
20:4ω6	1.7 ± 0.1	1.6 ± 0.1^{a}	1.5 ± 0.1^{ab}	1.4 ± 0.1^{b}	12.1	0.001
20:2ω6	0.3 ± 0.06	0.3 ± 0.06^{a}	0.4 ± 0.06^{a}	0.6 ± 0.03^{b}	38.6	<0.001
20:3ω3	-	0.02 ± 0.06^{a}	0.1 ± 0.04^{b}	0.3 ± 0.1^{c}	64.0	<0.001
20:5ω3 (EPA)	18.8 ± 0.8	19.0 ± 0.5^{a}	17.5 ± 0.8^{b}	15.5 ± 0.8^{c}	123	<0.001

22:1ω9	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	3.4	0.1
22:5ω6	0.4 ± 0.04	0.4 ± 0.04[a]	0.4 ± 0.04[a]	0.2 ± 0.03[b]	21.9	0.001
22:5ω3	3.0 ± 0.6	0.4 ± 0.04	0.3 ± 0.04	0.3 ± 0.1	0.9	0.43
22:6ω3 (DHA)	27.7 ± 1.7	28.0 ± 2.0[a]	26.7 ± 1.2[a]	25.0 ± 2.0[b]	14.0	0.004
∑SFA[4]	22.9 ± 1.7	25.7 ± 0.8	23.7 ± 0.7	24.0 ± 1.6	0.5	0.65
∑MUFA[5]	17.2 ± 3.2	16.2 ± 1.2[a]	16.6 ± 0.8[ab]	18.5 ± 2.3[b]	14.0	<0.001
∑PUFA[6]	58.8 ± 0.8	61.3 ± 0.8[a]	61.0 ± 0.7[a]	60.5 ± 2.6[b]	5.9	0.03
∑ω3	51.7 ± 1.3	49.9 ± 1.2[a]	49.0 ± 0.6[b]	47.9 ± 1.2[c]	17.0	0.003
∑ω6	6.5 ± 0.7	7.5 ± 0.6[a]	8.5 ± 0.5[b]	10.7 ± 1.2[c]	59.7	<0.001
ω3/ ω6	8.0 ± 0.7	6.7 ± 0.7[a]	5.8 ± 0.4[b]	4.4 ± 0.9[c]	34.1	< 0.001
Terrestrial[7]	4.7 ± 0.6	6.4 ± 0.6[a]	8.7 ± 0.4[b]	10.7 ± 1.2[c]	77.7	< 0.001

[1]Values are mean (n=9) ± SD. Means within rows with different superscripts indicate significant differences at the end of the experiment.

[2]Acetone mobile polar lipid

[3]Data expressed as area percentage of FAME (fatty acid methyl ester)

[4]Saturated fatty acid

[5] Monounsaturated fatty acid

[6] Polyunsaturated fatty acid

[7] Terrestrial = 18:2ω6 + 18:3ω3

Table 2.5. Lipid class and fatty acid composition of Atlantic cod liver tissue after 9 weeks of growth[1]

Lipid composition	Initial	FO	CO40	CO80	F-stat	*p*-value
(% ww^{-1})						
Total lipid	44.8 ± 7.0	43.5 ± 16^{a}	36.0 ± 10^{a}	58.4 ± 17^{b}	5.4	0.01
Neutral lipid	31.1 ± 9.7	38.7 ± 15^{a}	32.7 ± 10^{a}	50.9 ± 12^{b}	4.1	0.02
Polar lipid	13.4 ± 4.2	4.7 ± 2.3	7.5 ± 5.1	3.4 ± 2.1	3.4	0.06
(*% total lipid*)						
Steryl esters	-	-	0.4 ± 0.1	0.1 ± 0.1	3.0	0.07
Triacylglycerol	72.1 ± 10	75.8 ± 11^{a}	71.2 ± 11^{a}	81.8 ± 4.0^{b}	3.7	0.04
Free fatty acid	-	0.3 ± 0.1	1.0 ± 0.1	1.4 ± 1.0	2.1	0.14
Sterol	3.0 ± 1.1	10.7 ± 3.0^{a}	7.8 ± 2.0^{ab}	4.2 ± 1.3^{b}	6.5	0.03
AMPL[2]	10.2 ± 4.0	8.5 ± 1.2^{a}	6.4 ± 2.2^{b}	4.0 ± 1.8^{b}	10.0	0.001
Phospholipid	6.9 ± 3.0	2.7 ± 1.3	5.4 ± 1.4	3.5 ± 1.0	1.3	0.29
Fatty acid[3]						
14:0	3.2 ± 0.5	3.2 ± 0.15^{a}	2.9 ± 0.3^{b}	2.6 ± 0.3^{c}	71.4	<0.01
16:0	14.3 ± 0.4	13.7 ± 0.7^{a}	13.3 ± 0.5^{ab}	12.6 ± 1.1^{b}	9.6	0.01
16:1ω7	6.7 ± 0.2	6.2 ± 0.3^{a}	5.6 ± 0.5^{b}	4.5 ± 1.9^{b}	27.7	<0.001
18:0	5.1 ± 0.5	4.5 ± 0.4	4.4 ± 0.6	3.9 ± 1.6	1.2	0.36
18:1ω9	18.9 ± 0.9	19.0 ± 0.6^{a}	20.6 ± 1.1^{b}	20.5 ± 1.0^{b}	9.6	0.01
18:2ω6 (LNA)	5.7 ± 0.3	7.1 ± 0.7^{a}	8.6 ± 1.2^{b}	8.5 ± 1.2^{b}	24.3	<0.001
18:3ω6	0.2 ± 0.01	0.1 ± 0.03^{a}	0.2 ± 0.02^{ab}	0.2 ± 0.06^{b}	9.4	0.01
18:3ω3 (ALA)	0.8 ± 0.3	2.3 ± 0.9^{a}	4.4 ± 1.6^{b}	4.7 ± 1.9^{c}	29.1	<0.001
18:4ω3	1.2 ± 0.1	1.2 ± 0.1^{a}	1.3 ± 0.2^{b}	1.3 ± 0.2^{b}	8.2	0.02
20:1ω9	4.4 ± 0.5	6.1 ± 0.3	6.5 ± 0.8	6.6 ± 1.0	2.4	0.17
20:2ω6	-	0.3 ± 0.1^{a}	0.4 ± 0.1^{b}	0.5 ± 0.1^{b}	7.1	0.03
20:4ω6	0.6 ± 0.1	0.5 ± 0.1^{a}	0.3 ± 0.2^{b}	0.4 ± 0.1^{c}	5.9	0.04
20:3ω3	-	0.1 ± 0.04^{a}	0.1 ± 0.1^{a}	0.3 ± 0.1^{b}	8.2	0.02
20:5ω3 (EPA)	12.3 ± 0.6	10.0 ± 0.7^{a}	8.8 ± 1.2^{b}	8.1 ± 1.5^{c}	18.1	0.002

22:1ω9	3.4 ± 0.6	4.3 ± 0.4	3.6 ± 0.6	4.0 ± 0.8	3.8	0.09
22:5ω3	1.7 ± 0.3	1.6 ± 0.1[a]	1.3 ± 0.2[b]	1.2 ± 0.2[b]	32.7	<0.001
22:6ω3 (DHA)	7.2 ± 0.5	7.4 ± 0.4[a]	6.7 ± 0.7[ab]	6.5 ± 0.9[b]	11.7	0.01
∑SFA[4]	24.1 ± 0.7	22.2 ± 1.2[a]	21.1 ± 0.9[ab]	19.7 ± 2.9[b]	7.6	0.02
∑MUFA[5]	40.0 ± 1.0	42.5 ± 0.5	42.7 ± 1.0	42.8 ± 2.5	0.2	0.8
∑PUFA[6]	35.7 ± 1.4	34.9 ± 1.5	35.7 ± 0.7	35.2 ± 0.7	2.6	0.15
∑ω3	25.0 ± 1.0	23.5 ± 0.8	23.3 ± 0.9	22.8 ± 1.2	2.2	0.2
∑ω6	6.9 ± 0.5	8.7 ± 0.4[a]	9.8 ± 1.2[b]	9.7 ± 1.3[b]	23.3	<0.001
ω3/ ω6	3.7 ± 0.3	2.8 ± 0.2[a]	2.4 ± 0.3[b]	2.4 ± 0.3[b]	22.6	0.001
Terrestrial[7]	6.6 ± 0.4	9.2 ± 1.9[a]	13.0 ± 2.8[b]	13.2 ± 1.3[b]	29.5	0.001

[1]Values are mean (n=9) ± SD. Means within rows with different superscripts indicate significant differences at the end of the experiment.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester)
[4]Saturated fatty acid
[5] Monounsaturated fatty acid
[6] Polyunsaturated fatty acid
[7] Terrestrial = 18:2ω6 + 18:3ω3

Table 2.6. Differences in fatty acid composition[1] and the change ratio[2] between initial and final samples of cod muscle and liver samples in fish fed CO80

Fatty acid	Δ Fatty acid	T-stat	*p*-value	Change ratio
Muscle				
18:1ω9	+2.2 ± 1.7	-3.7	0.002	1.2
18:2ω6 (LNA)	+4.0 ± 1.3	-8.6	<0.001	1.9
18:3ω3 (ALA)	+4.3 ± 1.0	-18	<0.001	7.8
20:4ω6	-0.2 ± 0.1	4.2	<0.001	1.4
20:5ω3 (EPA)	-3.1 ± 0.8	11.3	<0.001	1.2
22:6ω3 (DHA)	-5.0 ± 2.4	3.5	0.001	1.1
∑SFA[3]	-1.6 ± 2.0	-3.2	0.01	1.1
∑MUFA[4]	+2.2 ± 2.0	-2.0	0.06	1.1
∑PUFA[5]	-2.0 ± 1.2	-1.0	0.32	1.0
∑ω3	-5.8 ± 5.1	8.7	<0.001	1.1
∑ω6	+3.8 ± 1.2	7.1	<0.001	1.5
ω3 / ω6	-3.2 ± 0.9	6.9	<0.001	1.7
Liver				
18:1ω9	+1.8 ± 0.9	-4.8	0.001	1.1
18:2ω6 (LNA)	+2.9 ± 1.1	-7.2	<0.001	1.5
18:3ω3 (ALA)	+3.7 ± 1.9	-5.8	<0.001	4.7
20:4ω6	-0.2 ± 0.1	3.8	0.002	1.4
20:5ω3 (EPA)	-3.9 ± 1.6	7.4	0.001	1.5
22:6ω3 (DHA)	-0.7 ± 0.1	2.1	0.05	1.1
∑SFA	-4.7 ± 2.8	4.7	0.001	1.2
∑MUFA	+2.3 ± 1.5	-2.6	0.02	1.1
∑PUFA	+0.5 ± 0.1	-1.1	0.30	1.0
∑ω3	-2.6 ± 1.1	3.1	0.01	1.1
∑ω6	+2.9 ± 1.1	-6.4	<0.001	1.4
ω3 / ω6	-1.3 ± 0.4	12.3	<0.001	1.5

[1] Difference = Fatty acid% (Final) – Fatty acid% (Initial)
[2] Fatty acids with +Δ Factor = Fatty acid % (Final) / Fatty acid % (Initial); Fatty acids with –Δ Factor= Fatty acid % (Initial) / Fatty acid % (Final)
[3] Saturated fatty acid
[4] Monounsaturated fatty acid
[5] Polyunsaturated fatty acid

Table 2.7. Fatty acid methyl ester (FAME) derivatization efficiency[1] comparing H_2SO_4 and BF_3 catalysts

Catalyst	Tissue	Extract acyl lipids[2]	Derivative acyl lipids[3]	% Derivatized	*T*-stat	*p*-value
H_2SO_4	Liver	13.8 ± 0.9	0.8 ± 0.04	93.8 ± 4.0[a]	23.5	<0.001
BF_3	Liver	14.8 ± 0.6	9.8 ± 0.5	34.2 ± 10[b]		
H_2SO_4	Muscle	12.1 ± 0.4	0.8 ± 0.1	93.4 ± 10	-0.2	0.84
BF_3	Muscle	11.7 ± 0.4	0.7 ± 0.2	94.1 ± 9.0		

[1]Derivatization efficiency= [1-(Derivative acyl lipids/Extract acyl lipids)]*100 (n=18)
[2]Extract acyl lipids is the total amount of extract acyl lipids prior to derivatization
[3]Derivative acyl lipids is the remaining amount of acyl lipid that did not transmethylate after the derivatization procedure.

Table 2.8. Variation in proportions of fatty acids (%) in Atlantic cod muscle and liver tissue fed a fish oil diet using either H_2SO_4 or BF_3 as a fatty acid methyl ester (FAME) derivatization catalyst[1]

	Liver		Muscle	
Fatty acid	H_2SO_4	BF_3	H_2SO_4	BF_3
14:0	3.2 ± 0.2[a]	3.6 ± 0.6[b]	1.2 ± 0.2	1.2 ± 0.1
16:0	14.2 ± 0.4[a]	14.6 ± 0.4[b]	17.3 ± 0.3	17.1 ± 0.8
16:1ω7	6.7 ± 0.2	7.2 ± 0.9	2.4 ± 0.2	2.1 ± 0.7
18:0	5.1 ± 0.4	5.2 ± 0.4	3.8 ± 0.2	4.0 ± 0.2
18:1ω9	19.2 ± 0.7	19.1 ± 0.1	8.8 ± 0.4	8.9 ± 0.4
18:1ω7	4.7 ± 0.1	5.2 ± 0.9	2.8 ± 0.1	2.5 ± 0.1
18:2ω6(LNA)	5.8 ± 0.3	5.8 ± 0.2	4.0 ± 0.3	4.0 ± 0.3
18:3ω3(ALA)	1.0 ± 0.4	1.0 ± 0.1	0.6 ± 0.1	0.7 ± 0.03
20:1ω9	4.6 ± 0.4	4.7 ± 0.5	1.9 ± 0.1	1.6 ± 0.2
20:4ω6	0.6 ± 0.1	0.6 ± 0.04	1.7 ± 0.1	1.7 ± 0.1
20:5ω3(EPA)	12.9 ± 0.4[a]	12.1 ± 0.6[b]	19.0 ± 0.3	18.8 ± 0.7
22:1ω9	2.9 ± 0.4[a]	2.2 ± 0.4[b]	0.5 ± 0.02	0.4 ± 0.1
22:5ω3	1.7 ± 0.7	1.6 ± 0.1	3.3 ± 0.1	3.2 ± 0.1
22:6ω3(DHA)	7.3 ± 0.5[a]	6.8 ± 0.3[b]	27.6 ± 1.3	27.3 ± 1.2
∑SFA[2]	23.7 ± 0.7	22.7 ± 2.3	22.5 ± 0.5	22.7 ± 0.7
∑MUFA[3]	39.2 ± 0.9	41.3 ± 3.2	17.2 ± 0.8	18.4 ± 1.1
∑PUFA[4]	37.0 ± 0.1[a]	35.2 ± 1.6[b]	60.3 ± 0.9	59.0 ± 0.5
∑ω3	25.7 ± 1.7	24.3 ± 1.0	52.3 ± 1.2	51.9 ± 0.9
∑ω6	7.1 ± 0.6	7.0 ± 0.1	6.5 ± 0.3	6.2 ± 0.5
ω3/ ω6	3.6 ± 0.4	3.5 ± 0.2	8.1 ± 0.6	8.4 ± 0.9

[1]Data expressed as area percentage of FAME (fatty acid methyl ester), values are mean (n=10) ± SD. Means with different superscripts indicate significant differences.
[2] Saturated fatty acid
[3] Monounsaturated fatty acid
[4] Polyunsaturated fatty acid

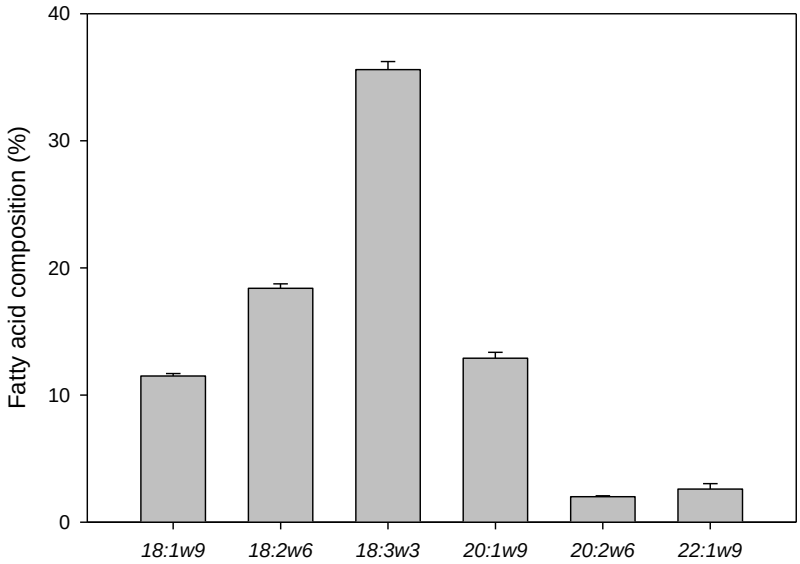

Figure 2.1. Fatty acid composition (%) of Camelina sativa oil (mean ± SD; n=3)

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Chapter 3. Substitution of fish oil with camelina oil and inclusion of camelina meal in diets of Atlantic cod (*Gadus morhua*) and its effect on growth, tissue lipid classes and fatty acids

3.1 Abstract

Developing a commercially relevant and sustainable Atlantic cod aquaculture industry will require solutions to nutritional problems such as minimizing use of fish meal (FM) and fish oil (FO) in diets. Camelina oil (CO) and meal (CM) are potential replacements of FO and FM in aquaculture feeds. CO is high in α-linolenic acid (ALA, 18:3ω3) (30%), with a ω3/ω6 ratio >1. This study tested diets with 100% replacement of FO with CO (100CO), solvent extracted FM (100COSEFM), and partially substituted FM with 15% inclusion of CM (100CO15CM) in a 13 week feeding trial with Atlantic cod (initial weight, 15 g fish^{-1}). Cod fed CO had a lower ($p<0.001$) final weight than cod fed FO (50.8 ± 10.3 g fish^{-1}), while cod fed 100CO15CM had a lower ($p<0.001$) final weight (35.0 ± 8.0 g) than those fed 100CO (43.6 ± 8.9 g) and 100COSEFM (46.7 ± 10.7 g). Cod tissues in the 100COSEFM fed group were most impacted by dietary fatty acid profile. Multivariate statistics revealed that FO and 100COSEFM tissue fatty acid profiles were 21-31% different. ALA, 18:2ω6 and 18:1ω9 were most responsible for the dissimilarity between these treatments. Removal of FO from FM and complete replacement of FO with CO had an encompassing effect on the entire fatty acid profile of the whole animal. Fatty acid mass balance calculations indicated that cod fed 100COSEFM elongated 13% of ALA to 20:3ω3 and oxidized the remaining 87%, while cod fed FO showed a much lower ($p<0.001$) elongation of ALA of 1.6%. These results suggest that excess ALA from

CO caused some fatty acid elongation, but little desaturation. Energy budget estimates indicated that cod fed 100COSEFM deposited the most energy throughout the trial (60 kJ $fish^{-1}$; p=0.02), mostly in the liver (90%). Excess CO lipids were not necessarily utilized for energy, which likely impacted growth. Feeding 100% CO to Atlantic cod impacted growth and lipid and fatty acid composition; however, additional removal of FO from FM caused the greatest change in cod lipid metabolism.

3.2 Introduction

In order for the marine finfish farming industry to be sustainable, improvements are needed in feed formulations to reduce fish oil (FO) and fish meal (FM) content. Several different terrestrial oilseeds are used in fish feeds (Turchini et al., 2009); however, they lack the long chain (LC) ω3 polyunsaturated fatty acids (PUFA) that are necessary for fish nutrition. Camelina oil (*Camelina sativa*) (CO) is especially attractive as a lipid source for fish due to its high level of α-linolenic acid (ALA, 18:3ω3) (30%), and lower levels of ω6 fatty acids, to produce a ω3/ω6 ratio >1. ALA is a LC ω3 precursor, and cod express some genes involved in the production of eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) from ALA (Tocher et al. 2006); therefore it is thought that high levels of ALA with low levels of EPA and DHA in the diet will encourage PUFA synthesis.

CO has been used as a replacement for FO in diets for Atlantic cod without significantly affecting growth compared to cod fed FO diets (Morais et al., 2012; Hixson et al., 2013, Chapter 2). However, levels of EPA and DHA in CO diets tested in both studies were enough to meet the minimum requirement for adequate growth, which was

supplied from low levels of FO and/or the residual lipid in FM. In the present study, CO fully replaced FO and all remaining FO supplied in FM was further eliminated by solvent extraction of FM (SEFM) to reduce EPA and DHA levels, in order to test growth and fatty acid synthesis capabilities of the fish. Camelina meal (CM) is also considered as a FM replacement, on account of its crude protein level (38%), inclusion of some essential amino acids and its commercial availability after oil extraction. Therefore, this study also tested a CO diet with inclusion of CM to slightly reduce FM.

The overall objective of the study was to evaluate camelina as a replacement for FO and FM in diets for farmed cod. Building on previous studies using CO, the present goals were: 1) to determine the threshold at which growth is affected by inclusion of CO; 2) to examine the effect of complete removal of FO by using SEFM; 3) to incorporate multivariate statistical analyses to compare diet and tissue fatty acid profiles; 4) to use the fatty acid mass balance method and energy budgeting to explain changes in fatty acid composition, synthesis and energy storage with diets containing CO, SEFM and CM.

3.3 Methods

Experimental diets

Camelina (Calena cultivar) was grown and harvested by the Department of Plant and Animal Sciences, Faculty of Agriculture, Dalhousie University at an off-campus location (Canning, Nova Scotia, Canada). The seeds were single pressed using a KEK 0500 press at Atlantic Oilseed Processing, Ltd. (Summerside, Prince Edward Island, Canada) to extract the oil and ethoxyquin was added to the final product as an antioxidant. The meal was pressed with a hammer mill (screen size 8 mm) into a pre-

pressed meal cake at Atlantic Oilseed Processing, then solvent extracted with petroleum ether at a concentration of 3 ml g^{-1} at the Faculty of Agriculture, Dalhousie University (Truro, Nova Scotia, Canada).

All diets were formulated as isonitrogenous, isolipidous practical diets and were produced at the Faculty of Agriculture, Dalhousie University (Table 3.1). The experimental treatments were as follows: a control diet with fish oil (FO); 100% FO replacement with camelina oil (100CO); 100% FO replacement with CO with solvent extracted FM (100COSEFM); 100% FO replacement with CO and 15% inclusion of CM (100CO15CM). SEFM was used in one of the experimental diets in order to remove all marine lipids from the diet to test the full effect of CO. The FM was solvent extracted with petroleum ether at a concentration of 3 ml g^{-1}. CM was added in one of the experimental diets in order to slightly lower the FM level, along with with 100% replacement of FO and CO. Diets were formulated to meet the nutritional requirements of gadoids based on previous formulations (Tibbetts et al. 2004; 2006). All diets were steam pelleted using a laboratory pelleting mill (California Pellet Mill, San Francisco, USA). The initial pellet size was 4.0 mm and it increased to 6.0 mm as the fish grew larger. Diets were stored at -20°C until needed.

Experimental fish

An experiment was conducted with juvenile cod (14.4 ± 1.6 g fish $^{-1}$ mean initial weight ± SD; 11.3 ± 0.4 cm mean initial length ± SD) at the Ocean Sciences Center, Memorial University of Newfoundland (St. John's, Newfoundland and Labrador, Canada) where fish were cultured from hatch and reared to initial experimental size. Fish were

randomly distributed (840 total) into 12 experimental tanks (500 L capacity), each tank with 70 fish (Memorial University Institutional Animal Care Protocol Approved 12-50-MR). The fish were acclimated on the control diet for one week prior to initial sampling. Triplicate tanks were used for each dietary treatment for 13 weeks, and fish were fed to apparent satiation to monitor feed consumption. A flow-through system of 1 µm filtered seawater was supplied to each tank at a rate of 8 L min^{-1} and the photoperiod was 12 hours. The dissolved oxygen (10 mg L^{-1}) and water temperature (10°C) was monitored daily. Mortalities were weighed and recorded throughout the trial.

Sampling methods

Sampling occurred at week 0 (the day before experimental diets were fed), week 1 (3 days of acclimation onto the test diets plus 7 days on full test diet), 6, and 13. Three fish per tank were randomly sampled and measured for length and weight; four fish per tank were sampled on week 13. The whole liver was removed, weighed, and sampled for dry matter and lipid analysis. The skin was removed on the left side and muscle tissue was sampled for dry matter and lipid analysis. At the final sampling time point, addition tissues were sampled for lipid analysis. In total this included muscle, liver, skin, brain, spleen, entire gut and the remainder of the fish (carcass). The entire gut included the esophagus to the anus. The remaining carcass (i.e., head without brain including eyes, spine, and fins) was homogenized in a blender and subsampled for lipid analysis. Lipid samples were stored on ice during sampling of each tank and processed hourly to weigh and cover with chloroform. The headspace was then flushed with nitrogen, the tube was

capped under a Teflon liner and the cap was sealed against the tube with Teflon tape. Lipid samples were stored at -20°C until analysis.

Lipid extracts

Lipid samples were extracted according to Parrish (1999). Samples were homogenized in a 2:1 mixture of ice-cold chloroform: methanol. Samples were homogenized with a Polytron PCU-2-110 homogenizer (Brinkmann Instruments, Rexdale, Ontario, Canada). Chloroform extracted water was added to bring the ratio of chloroform: methanol: water to 8:4:3. The sample was sonicated for six min in an ice bath and centrifuged at 4000 rpm for two min at room temperature. The bottom, organic layer was removed using a double pipetting technique, placing a 2 ml lipid-cleaned Pasteur pipette inside a 1 ml pipette, to remove the organic layer without disturbing the top, aqueous layer. Chloroform was then added back to the extraction test tube and the entire procedure was repeated 3 times for muscle samples and 5 times for liver samples. All organic layers were pooled into a lipid-cleaned vial. The samples were concentrated using a flash-evaporator (Buchler Instruments, Fort Lee, New Jersey, USA).

Lipid class separation

Lipid class composition was determined using an Iatroscan Mark VI TLC-FID (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan), silica coated Chromarods and a three-step development method (Parrish, 1987). The lipid extracts were applied to the Chromarods and focused to a narrow band using acetone. The first development system was hexane: diethyl ether: formic acid (99.95:1.0:0.05). The rods were developed for 25 min, removed from the system for 5 min and replaced for 20 min. The second development was for 40

min in hexane: diethyl ether: formic acid (79:20:1). The final development system had two steps, the first was 100% acetone for two 15 min time periods, followed by two 10 min periods in chloroform: methanol: chloroform-extracted water (5:4:1). Before using each solvent system, the rods were dried in a constant humidity chamber. After each development system, the rods were partially scanned in the Iatroscan and the data were collected using Peak Simple software (version 3.67, SRI Inc). The Chromarods were calibrated using standards from Sigma Chemicals (Sigma Chemicals, St. Louis, Missouri, USA).

Fatty acid methyl ester (FAME) derivatization

Lipid extracts of most tissues were transesterified using the Hilditch reagent (1.5 H_2SO_4: 98.5 anhydrous MeOH) for 1 hour at 100°C. For all muscle samples, lipid extracts were transesterified using 14% BF_3 in MeOH for 1.5 hours at 85°C, as this method efficiently derivatizes samples low in triacylglycerol (Hixson et al., 2013). Reagents were added in the proportion of 1.5 ml reagent per 4-16 mg of lipid (Morrison and Smith, 1964). Samples were vortexed half way through each derivatization reaction. To check the derivatization efficiency, samples were transesterified and then the lipid class composition of the methyl ester solution was determined by TLC-FID. The derivatization efficiency is calculated from the amount of underivatized acyl lipids compared to the amount of methyl esters in a sample. For the muscle using 14% BF_3 in MeOH, the efficiency was 93%. For the remaining tissues using the Hilditch reagent, the efficiencies were approximately 90%: liver (94%), brain (88%), skin (93%), gut (91%), spleen (89%) and the carcass (90%).

All FAMEs were analyzed on a HP 6890 GC FID equipped with a 7683 autosampler. The GC column was a ZB wax+ (Phenomenex, Torrance, California, USA). The column length was 30 m with an internal diameter of 0.32 mm. The column temperature began at 65°C where it was held for 0.5 min. The temperature ramped to 195°C at a rate of 40°C min^{-1}, held for 15 min then ramped to a final temperature of 220°C at a rate of 2°C min^{-1}. This final temperature was held for 45 sec. The carrier gas was hydrogen flowing at 2 ml min^{-1}. The injector temperature started at 150°C and ramped to a final temperature of 250°C at 120°C min^{-1}. The detector temperature stayed at 260°C. Peaks were identified using retention times from standards purchased from Supelco (Bellefonte, Pennsylvania, USA): 37 component FAME mix (Product number 47885-U), PUFA 3 (product number 47085-U) and PUFA 1 (product number 47033-U). Chromatograms were integrated using the Varian Galaxie Chromatography Data System, version 1.9.3.2 (Agilent Technologies, Colorado, USA).

Fatty Acid Mass Balance

In order to assess the level of fatty acid metabolism, the whole-body fatty acid method (Turchini et al., 2007) was applied. It was necessary to determine the partitioning of the dietary fatty acids amongst excretion, accumulation, appearance or disappearance. Fatty acid appearance/disappearance was calculated as follows (Turchini et al. 2007):

Final fatty acid body content		Fatty acid intake		
-		-		
Initial fatty acid body content		Fatty acid excretion		
=		=		
Fatty acid accumulation	**-**	**Fatty acid net intake**	**=**	**Appearance/Disappearance**

Where, fatty acid body content is the sum of fatty acid (mg) in all organs; fatty acid intake is (feed intake (g)) x (mg of fatty acid per g of feed); fatty acid excretion is (mg of fatty acid intake) x (fatty acid digestibility (%)). Digestibility values were estimated based on individual fatty acid digestibility by Atlantic cod fed a low-lipid diet (80 g kg^{-1}) (Hansen et al., 2007). Therefore, the amount of each individual fatty acid that was excreted throughout the experiment was calculated based on the digestibility of each fatty acid (Hansen et al., 2007) and the amount of the fatty acid consumed in the duration of the trial. In order to calculate final fatty acid body content, the following tissues were sampled and analyzed separately, and the sum of each fatty acid was calculated to achieve the whole body content: muscle, liver, brain, spleen, gut, skin and the carcass of the fish. Each organ was weighed on an individual basis. For example, cod were fully filleted and the fillets were weighed, then a subsample was taken for tissue analysis; cod liver was weighed, then it was subsampled. Brain, spleen and gut were weighed whole and the whole organ was used for lipid analysis. The carcass of the fish was weighed, then homogenized, then subsampled for lipid analysis of the carcass. The initial fatty acid body content was based on the mean final fatty acid body content in the control fish, since all individual tissue/organs were not sampled at the start of the experiment and since initial fatty acid profiles of muscle and liver tissues in control fish were not different between initial and final samples, it is assumed that final whole body fatty acid from the control would be representative of the initial whole body fatty acid.

The second step of the method involves the computation of the 18:3ω3 balance. The number of mmol (converted from mg fatty acid per fish) of longer chain fatty acids

that appeared is subtracted from the number of mmol of the previous fatty acid in the fatty acid elongation/ desaturation pathway. The estimation of the fate (elongation, desaturation or oxidation) of each fatty acid can therefore be computed according to its specific metabolic pathway according to the method described by Turchini et al. (2007).

Energy budget

The energy budget of cod was estimated by quantifying the amount of lipid consumed throughout the experiment [feed intake (g $fish^{-1}$) x lipid level in diet (%)] and the amount of lipid deposited into tissues at the end of the experiment (sum of total lipid content of final tissues). The difference in lipid between intake and deposition was assumed to be oxidized for energy and expressed as a percentage of intake. Lipid intake, deposition and oxidized lipid amounts were converted to kJ, on the assumption that 1 g of fat = 39.9 kJ (Bureau et al., 2002). Energy intake, energy deposited and total energy expenditure were expressed in terms of kJ per fish.

Statistical methods

Statistical analysis followed methods outlined by Sokal and Rolf (1994). For analysis of growth, lipid class, and fatty acid data, where individual fish were weighed, measured and sampled, a three-way nested ANOVA was performed using the General Linear Model (Minitab 16 Statistical Software, State College, Pennsylvania, USA). The model was designed to test the effect of diet on the response variable and nested fish individuals within tanks to negate variability among tanks and individuals, while also testing for tank effects. For analysis of growth data that depend on comparison to an initial measurement and thus must be pooled per tank (i.e., mean weight gain and specific

growth rate), a two-way ANOVA was performed to test the effect of diet and tank variability. In both cases, where significant differences occurred, treatment means were differentiated using the Tukey HSD multiple comparison. For each model tested, the residuals were examined to evaluate the appropriateness of the model, therefore normality, homogeneity and independence of residuals were considered. If a p-value was close to 0.05 and residuals were not normal, a p-randomization was conducted >5,000 times to test the data empirically. In addition, PRIMER (Plymouth Routines in Multivariate Ecological Research; PRIMER-E Ltd, Version 6.1.15, Ivybridge, UK) was used to analyze selected fatty acid data, using SIMPER (similarity of percentages analysis) and ANOSIM (analysis of similarities) to define similarities and differences among tissue and dietary fatty acid data. Fatty acids that accounted for > 0.05% of total fatty acids were included in the analyses. SIMPER and ANOSIM are multivariate analyses that use a resemblance matrix and the latter carries out an approximate analogue of ANOVA. In both cases non-parametric Bray-Curtis similarity was chosen.

3.4 Results

Experimental diets

FO was replaced by CO in all experimental diets, except the control diet, with addition of either SEFM or 15% CM (Table 3.1). Total lipid (TL) varied among diets (8.8% to 9.9% ww^{-1}) (Table 2.2), but the difference was not significant. 100CO was twice as high in polar lipid (PL) than other experimental diets. Triacylglycerol (TAG) was the predominant lipid class (85 to 91%) in all diets. Sterol (ST) was higher in the 100CO15CM diet (3.9%) than all other diets (<2.5%). Acetone-mobile polar lipid

(AMPL) was lower in 100COSEFM (1.0%) than all other diets (>2.3%). Phospholipid was higher in all CO-containing diets than the FO diet. All major fatty acids in the diets were significantly affected by the addition of CO. The addition of CO significantly decreased saturated fatty acids (SFA) and increased monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) compared to the FO diet. Notably, CO diets increased in ALA and linoleic acid (LNA, 18:2ω6) and decreased in EPA and DHA compared to FO. Total ω3 fatty acids were lower in 100CO than all other experimental diets and total ω6 fatty acids were lower in FO than all other experimental diets. The ω3/ω6 ratio was the same among CO diets, but was significantly lower in all CO diets compared to the FO diet. The sum of ALA and LNA ("terrestrial fatty acids") was over three times higher in CO diets than the FO diet.

Growth performance

Initially, cod were 14 to 15 g fish^{-1} and grew to 44 to 51 g fish^{-1} after 13 weeks of feeding experimental diets (Table 3.3). Cod fed CO had a significantly lower final weight than cod fed FO, and cod fed 100CO15CM had a lower final weight than those fed 100CO and 100COSEFM. The same result was observed in weight gain. There was no difference in final weight ($F=0.90$; $p=0.52$) or weight gain ($F=1.06$; $p=0.37$) among replicate tanks. Cod were initially 11 cm in length, however after 13 weeks of growth, cod fed FO and 100COSEFM were significantly longer than cod fed 100CO and 100CO15CM. The difference in weight and length among diets did not affect the condition factor. Cod fed 100CO15CM had a lower SGR (0.9% day^{-1}) than all other groups. The HSI was not affected by diet (6.0% to 7.6%). Cod fed FO consumed

significantly more feed throughout the duration of the trial (33.0 g $fish^{-1}$), and cod fed 100CO15CM consumed the least amount of feed (22.2 g $fish^{-1}$) and had a higher FCR (1.1) compared to all groups. There were no tank differences among replicates for any parameters.

Muscle tissue lipid and fatty acid composition

Total lipid in the muscle was not different depending on dietary group (0.7% to 1.0% ww^{-1}) (Table 3.4). Neutral lipid was higher in 100CO muscle (0.2% ww^{-1}) than other dietary groups (<0.1% ww^{-1}). TAG was higher in 100COSEFM and 100CO15CM than FO and 100CO. Free fatty acids (FFA) were higher in 100CO and 100CO15CM than FO and 100COSEFM. ST was higher in cod fed FO (11%) than CO-fed groups (<7.7%). Phospholipid, the predominant lipid class in the muscle tissue, did not vary among dietary groups (79% to 89%).

Fatty acid profiles in the muscle tissue were significantly altered when FO was replaced by CO (Table 4.4). SFA was lower in CO-fed fish (20%) than FO-fed fish (22.5%) and MUFA was higher in 100CO (20%) than FO (18%), although 100COSEFM and 100CO15CM groups did not differ from any group. PUFA did not differ among groups (59% to 61%). Total ω3 fatty acids were lower in CO-fed groups than FO and total ω6 fatty acids were about twice as high in CO-fed groups than the FO-fed group. Consequently, the ω3/ω6 ratio was twice as high in the FO-fed group as the CO-fed groups. Cod fed CO rather than FO had lower levels of EPA, and cod fed 100COSEFM had the lowest level of EPA (11%). The inclusion of CO alone did not affect levels of DHA in the muscle; but including SEFM did reduce DHA levels compared to all groups.

The DHA/EPA ratio was slightly higher in CO-fed muscle tissue than FO-fed muscle. The inclusion of CO significantly increased LNA levels in the muscle, with LNA levels highest in muscle tissue fed 100COSEFM. Similarly, ALA in CO-fed groups was over 7 times higher in CO-fed cod muscle compared to FO-fed cod muscle (0.7%), and cod fed 100COSEFM had significantly higher levels of ALA (11%) compared to all groups. The fatty acid 20:3ω3 was found in CO-fed cod muscle at the final sampling time point, however not in the initial profile and in the FO group. Other fatty acids in the ω3 pathway (e.g., 18:4ω3, 20:4ω3, 22:5ω3) did not increase with addition of CO. There was no significant difference among tank replicates for lipid class and individual fatty acids.

Liver tissue lipid and fatty acid composition

Total lipid in the liver was significantly higher in cod that were fed 100COSEFM (34.5% ww^{-1}) and 100CO15CM (34.5% ww^{-1}) compared to FO (22.2% ww^{-1}) and CO groups (21.9% ww^{-1}) (Table 3.5). Neutral lipid in the liver showed the same significant trend among dietary groups as total lipid. Polar lipid was not affected by diet. TAG was the predominant lipid class in the liver, but its proportion did not differ among dietary groups (68.8% to 77.2%). FFAs were 1% or less in the liver and did not differ among dietary groups. ST (14.5% to 16.4%) and AMPL (2.4% to 4.7%) in the liver also did not differ among dietary groups. Phospholipid was significantly lower in 100CO livers (2.5%) than FO (5.0%) and 100CO15CM (5.4%) livers.

Changes in the fatty acid profile due to the inclusion of CO were more severe in the liver than in the muscle tissue (Table 3.5). SFA was reduced with CO inclusion, with a significant decreasing trend: FO (21.8%) >100CO>100CO15CM>100COSEFM

(15.3%). MUFA did not differ among groups (40.2% to 45.8%). PUFA did not differ between FO and 100CO, but was significantly higher in livers of cod fed 100COSEFM than all other diets. Total ω3 fatty acids in the liver were significantly lower in 100CO than all other groups, but FO and 100COSEFM did not differ from each other. Total ω6 fatty acids in the liver significantly increased with CO inclusion, with significantly higher levels in 100COSEFM than in FO and CO100. The ω3/ω6 ratio in CO liver tissue was half that of the FO group. EPA was reduced by more than half by including CO in the diet; with the EPA level in 100COSEFM significantly lower than both FO and 100CO. DHA in the liver was also reduced by more than half by including CO in the diet; all CO-fed groups were equally as low in DHA compared to the FO-fed group. DHA and EPA levels in the liver drastically decreased due to CO inclusion compared to the changes observed in the muscle. The DHA/EPA level was slightly higher in 100COSEFM than all other groups. LNA levels in the liver were about twice as high in CO groups than FO, and cod fed 100COSEFM had the highest level of LNA. ALA levels showed a similar pattern, but increased up to 15-fold in 100COSEFM compared to FO. The appearance of 20:3ω3 in the liver of CO-fed groups suggests LC-PUFA biosynthesis due to the lack of this fatty acid in the initial profile and FO tissue. Cod fed 100COSEFM had the highest level of this fatty acid among the dietary groups. However, other fatty acids in the ω3 pathway (e.g., 18:4ω3, 20:4ω3, 22:5ω3) did not increase with addition of CO. There was no significant difference among tank replicates for lipid class and individual fatty acids.

Major fatty acids in selected tissues

In general, most tissues were significantly affected by the addition of CO and CM in the diet, with the greatest degree of change occurring in cod fed the 100COSEFM diet (Table 3.6).

In the skin, LNA levels was more than twice as high in 100CO and 100CO15CM and almost three times as high in 100COSEFM than in FO skin (Table 3.6). Levels of ALA in the skin were over four-fold higher in 100CO and 100CO15CM and almost 7-fold higher in 100COSEFM than FO skin. The appearance of 20:3ω3 in the skin was highest in 100COSEFM, but levels in 100CO and 100CO15CM were still significantly higher than FO. EPA was lowest in 100COSEFM, but EPA levels in 100CO and 100CO15CM were still lower than in FO skin. DHA was equally lower in all CO diets compared to FO. PUFA and total ω3 were not different among dietary groups. Total ω6 levels were highest in 100COSEFM, while ω6 in 100CO and 100CO15CM were also significantly higher than in FO.

In the brain, fatty acid profiles seemed to resist dietary change. EPA, DHA and PUFA were not different among dietary groups (Table 3.6). DHA levels were highest in the brain than all other organs/tissues. LNA and ALA were significantly higher in the brains of CO-fed groups, with significantly higher levels in 100COSEFM than in FO and 100CO. Levels of 20:3ω3 in the brain were similar as levels in the skin and were higher in all CO diets than FO, with the highest level in 100COSEFM. Total ω3 PUFA in the brain were not different between FO and 100CO, but lower in 100COSEFM and 100CO15CM than FO. Total ω6 PUFA in the brain was higher in 100COSEFM than in FO.

In the gut, all selected fatty acids and groups were significantly affected by the addition of CO (Table 6). ALA, LNA and 20:3ω3 levels were highest in 100COSEFM, with 100CO and 100CO15CM significantly higher than FO. EPA and DHA levels were lower in CO groups; DHA was equally low among CO groups compared to FO, and EPA was significantly lower in 100COSEFM than all groups, but 100CO and 100CO15CM were also lower than FO EPA levels. PUFA was lower in 100CO than all groups. Total ω3 and ω6 were lower in in all CO groups than FO, but 100CO15CM was lowest.

In the spleen, LNA and ALA were equal among CO groups and significantly higher than in FO (Table 3.6). The appearance of 20:3ω3 was significantly higher in 100COSEFM than in FO and 100CO, but 100CO15CM was not different from 100CO and 100COSEFM. Levels of EPA were not different among FO, 100CO and 100CO15CM, but were lower in 100COSEFM. DHA was significantly lower in all CO groups than FO, with levels significantly lowest in 100COSEFM. PUFA and total ω3 were not different among groups, but total ω6 was higher in all CO groups than FO.

The carcass of the fish (head without brain including eyes, spine, and fins) showed similar results to other organs and tissues (Table 3.6). ALA, LNA and 20:3ω3 levels were significantly higher in 100COSEFM carcasses than all other groups, with levels higher in 100CO and 100CO15CM than FO. Both EPA and DHA were significantly affected by CO inclusion, with the lowest levels in the 100COSEFM group. DHA levels in the carcass were similar to that found in the brain. Total PUFA was not different among treatments. Total ω3 levels were equally low in CO groups than FO and total ω6 levels

were highest in 100COSEFM, but also significantly higher in 100CO and 100CO15CM than in FO.

Multivariate statistical results

MDS and ANOSIM

Fatty acid profiles in all tissues (muscle, liver, skin, spleen, gut, and the carcass) were compared within each diet. The MDS plot revealed that organs form distinct clusters that change spatially depending on dietary composition (Figure 3.1). Regardless of diet, the liver fatty acid profile was most dissimilar compared to all other tissues, followed by the brain. The liver and brain form distinct clusters away from the main cluster of remaining tissues. DHA, EPA, ALA and 18:1ω9 mainly contributed to the patterns observed in the MDS plot among tissues for all dietary groups. ANOSIM revealed that the liver fatty acid profile was the single tissue least similar to all other tissue fatty acid profiles for all dietary groups (Table 3.7). The skin and spleen were most similar in 100CO, 100COSEFM and 100CO15CM and the brain and gut were most similar in the FO diet.

While tissues form distinct yet relatively homogenous clusters in Figure 3.1 when comparing tissues among a particular diet, comparing tissues within all diets in the same plot revealed that tissues from the FO group form a separate cluster from the CO groups. For example, the liver and muscle from all diets were compared in the same plot (Figure 3.2). The liver and muscle formed distinctly separate clusters, but liver and muscle from the FO diet were separated a distance away from the same tissues from all diets containing CO.

SIMPER

Comparisons between diet fatty acid profile and tissue fatty acid profile revealed that the brain was most dissimilar to diet fatty acid profile, for all dietary groups (Table 3.8). Comparison of FO, 100CO and 100CO15CM diet fatty acid profiles to the brain fatty acid profile showed that DHA was the major contributing fatty acid to the dissimilarity between diet and tissue profiles, while ALA was the major contributing fatty acid when comparing the brain to 100COSEFM diet fatty acid profile. After the brain, the muscle tissue had the second most dissimilar tissue fatty acid profile in comparison to that of the diet. DHA was the major contributing fatty acid to this dissimilarity for all dietary groups. The liver showed the most similar fatty acid profile in comparison to that of the diet, while 18:1ω9 was the major contributing fatty acid to the dissimilarity.

Comparison of fatty acid profiles within specific tissues across all diets revealed similarities/dissimilarities of tissue fatty acid profiles due to dietary change (Table 3.9). Overall, tissues in FO compared to 100COSEFM were the most dissimilar; 100CO and 100CO15CM were most similar. When comparing FO tissues to 100CO/100COSEFM/100CO15CM tissues, the liver was the most dissimilar tissue and ALA contributed most to this dissimilarity. However, comparing 100CO, 100COSEFM and 100CO15CM tissues amongst themselves revealed that the spleen was the most dissimilar tissue, while DHA contributed most to this dissimilarity.

Fatty acid mass balance

The results of the first step in the computation of the whole-body fatty acid balance method revealed significant differences in the intake, accumulation and

appearance/disappearance of the selected fatty acids (Table 3.10). Intake of 18:3ω3 and 18:2ω6 was significantly higher in CO fish than FO fish, with intake of these fatty acids highest in the 100COSEFM group. As excretion was a calculated value rather than based on experimental digestibility values, the result was a function of intake, therefore 100COSEFM had higher excretion values than other groups. There was no accumulation of either 18:3ω3 and 18:2ω6 in the FO group; accumulation of these fatty acids was highest in 100COSEFM. However, in all treatments, accumulation of both 18:3ω3 and 18:2ω6 was much lower than what was consumed in the diet, because all groups had negative values indicating disappearance of these fatty acids. Disappearance values were highest in 100COSEFM, followed by 100CO and 100CO15CM. Disappearance of 18:2ω6 in the FO group was much higher than 18:3ω3 in the FO group.

Since 20:3ω3 was not detected in the diet or initial body content, the accumulation of this fatty acid resulted in positive appearance values, which were highest in the 100COSEFM group, and the same between 100CO and 100CO15CM (Table 3.10). Appearance of 20:3ω3 was low in FO compared to the CO groups. This fatty acid was the only LC ω3 PUFA with positive appearance values, since both 20:5ω3 and 22:6ω3 disappeared in all treatment groups, indicating that intake was higher than accumulation in the tissues. Intake of 20:5ω3 and 22:6ω3 was significantly higher in FO > 100CO > 100COSEFM > 100CO15CM. The FO group did not accumulate either fatty acid. Accumulation of 20:5ω3 was equal amongst all CO groups, but accumulation of 22:6ω3 most negative in 100CO and 100CO15CM groups. Both 20:5ω3 and 22:6ω3 showed disappearance, and the FO group had the most negative disappearance values.

The second part of the fatty acid mass balance method could only provide information regarding the production of 20:3ω3, as it was the only elongated product to show a positive appearance value (Table 3.11). Elongation (%) of 18:3ω3 was highest in 100COSEFM > 100CO15CM > 100CO > FO. 18:3ω3 was oxidized over 87% in all groups and was not significantly different among groups, while the remaining 13% was elongated. There was no evidence of desaturation.

Energy budget

The energy budget of cod was estimated based on total lipid intake and the amount of neutral lipid stored in each organ (mg neutral lipid $fish^{-1}$; Table 3.12; Appendix I). Cod fed FO and 100COSEFM consumed the same amount of lipid (and the energy equivalent) for the duration of the experiment, but cod fed 100CO consumed less than the FO group and cod fed 100CO15CM consumed less than all other groups. Muscle, liver, gut and carcass stored significantly different amounts of neutral lipid depending on the diet, with cod fed 100COSEFM generally storing the most lipid, except in the muscle where the least amount of lipid was stored. The sum of neutral lipid stored (and energy deposited) in all organs was highest in cod fed 100COSEFM. In terms of lipid that was stored, 80-90% of the lipid was stored in the liver. Cod fed FO and 100CO oxidized more lipid for energy than cod fed 100COSEFM and 100CO15CM; cod fed 100COSEFM stored the most lipid > 100CO15CM > 100CO = FO. Cod fed FO expended more energy throughout the experiment than 100COSEFM and 100CO15CM, but no group differed in energy expenditure compared to CO.

3.5 Discussion

Atlantic cod was the most valued food fish in the North Atlantic until depletion of commercial stocks and collapse of the wild fishery which occurred over 20 years ago (deYoung and Rose, 1993; Myers et al., 1997; Rose et al., 2000). The progress in commercial cod farming has slowed in recent years due to several factors including disease, nutrition and economics (Booman et al., 2011), although increase in demand has renewed an interest in further advancement of the industry (Bolton-Warberg and Fitzgerald, 2012). Developing a commercially relevant and sustainable cod aquaculture industry will require solutions to these issues, particularly solving nutritional problems like the undesirable use of FM and FO in cod diets and replacing it with sustainable and economical alternatives.

As a carnivorous marine species, cod require high levels of protein and essential fatty acids (EFA), therefore traditional feeds for cod have been formulated to include high levels of FM and FO to satisfy these nutritional demands. However, the use of FM and FO is environmentally unsustainable and costly, so alternatives should be used in new feed formulations to ensure a viable and productive industry. Alternative oils should be highly digestible, provide high retention of ω3 PUFAs, and give high levels of precursor ω3 fatty acids for biosynthesis of LC ω3 PUFA and low levels of ω6 to maintain a high ω3/ω6 ratio which is beneficial for fish and human health (Torstensen et al., 2005; Seierstad et al., 2005). Camelina possesses many of these proposed qualities. It yields 40% total lipid, and is low in saturated fatty acids (SFA), high in monounsaturated fatty acids (MUFA) and PUFA and also high in ALA (30%) compared to other oilseeds.

Camelina meal (CM) is also considered as a FM replacement, on account of its crude protein level (38%), inclusion of methionine and phenylalanine and its availability after oil extraction. The overall objective of the study was to evaluate CO as a suitable lipid resource to replace FO, with inclusion of CM in diets for farmed Atlantic cod.

Atlantic cod were fed diets containing CO, SEFM and CM in order to reduce FO and FM levels. In this study, feeding CO to cod significantly reduced final weight and weight gain compared to cod fed a commercial type diet. Two previous experiments reported no significant difference in growth between FO and 100% CO-fed cod (Morais et al., 2013) and 80% CO-fed cod (Hixson et al., 2013, Chapter 2), which does not coincide with growth results here. However, in both previous studies, DHA and EPA were supplied in excess of the requirement for growth through FO naturally present in FM. In the Morais et al. (2012) experiment, the 100% CO diet that was tested had more DHA (6.2%) and EPA (4.2%), compared to the DHA (3.8%) and EPA (3.0%) in the 100% CO diet in this study. Therefore growth differences between these two studies might be attributed to the quantitative amount of DHA and EPA, which exceeded EFA requirements for growth in the Morais et al. (2012) trial. In terms of growth, cod fed CO and CM performed the worst, which suggests that replacing FO and including CM was more detrimental to fish growth than simply replacing 100% FO, even though the 100COSEFM diet contained nearly twice the amount of CO than any other diet. Feed intake and feed conversion were significantly lower in the group fed 15% CM. Previous studies that partially replaced FM with soybean meal and soy protein concentrate in diets fed to Atlantic cod have found reductions in feed intake and growth, although inclusion levels were >50% (Colburn et al., 2012; Lie et al., 2011; Hansen et al., 2007). Including

CM at 15% inclusion appeared to have severe consequences; plant meals with antinutritional factors, such as glucosinolates that are present in high levels in camelina meal (Matthaus and Angelini, 2005; Matthaus, 1997), can compromise the digestibility of the diet and bioavailability of nutrients (NRC, 2011), reduce feed intake and growth and inhibit normal metabolism such as thyroid function (Francis et al., 2001). However, the previously mentioned studies singly replaced FM; double replacements interfere with both digestible protein and lipid, thus reducing both the amino acid and fatty acid quality. Although including 15% CM with CO appeared to be more detrimental to feed intake and growth, removing most EFAs from the diet by using SEFM and CO appeared to be most detrimental to lipid and fatty acid composition in cod tissues.

Muscle and liver lipid composition

Lipid and fatty acid composition of tissues were significantly affected by diet. Generally, tissues lipids and fatty acids of cod fed the 100COSEFM diet were most impacted by change in the diet, since all FM lipids were removed and consequently more CO was added to the diet to compensate for lipid loss. Therefore the additional CO added in this diet compared to 100CO and 100CO15CM had significant effects on both the diet fatty acid profile and that of the tissues. In the flesh, there was no difference in total lipid, but TAG increased in the SEFM and CM diets, which is a typical result when feeding CO to fish (Leaver et al., 2011; Hixson et al., 2013, Chapter 2). Sterols in the flesh decreased with CO inclusion, despite similar levels in the diet among FO, 100CO, 100COSEFM. Studies have investigated the efficiency of phytosterols in plant oils like CO in lowering cholesterol in fish (Pickova and Mørkøre, 2007), and the same result was found in cod liver when fed 80% CO (Hixson et al., 2013, Chapter 2). Generally the fatty acid profile

of the flesh showed significant decreases in LC ω3 PUFA >C_{20}, with the exception of 20:3ω3, and significant increases in C_{18} fatty acids (ALA, LNA, 18:1ω9). This trend was accentuated in 100COSEFM compared to 100CO and 100CO15CM, since removal of FO from FM increased amounts of CO in the diet. Total PUFA, however, was the same regardless of the diet fed. Levels of PUFA and DHA were the same between FO and 100CO groups, and the ω3/ω6 ratio in all CO groups was >3, which helps prevent excess eicosanoid production associated with cardiovascular and inflammatory conditions (Sargent et al., 2002). ALA is also an EFA for humans (Brenna et al., 2009), so increased levels of ALA in cod flesh are still beneficial for human consumption.

In the liver, cod fed 100COSEFM and 100CO15CM stored more total lipid and neutral lipid compared to cod fed FO and 100CO. Neutral lipids respond more readily to changes in dietary lipid than polar lipids (Sargent et al. 2002; Higgs and Dong, 2002), and cod store excess dietary lipids as neutral lipid in the form of TAG in the liver. The TAG levels in the diets did not differ, however, there are differences in the ways in which the lipids in FO and vegetable oil (VO) are digested, absorbed and metabolized (Bureau et al., 2002; Jobling et al. 2008). Cod fed 80% CO stored more total and neutral lipid in the liver than cod fed FO diets (Hixson et al., 2013, Chapter 2). Salmon fed a VO blend containing 20% camelina oil had significantly higher lipid deposition and increased TAG content in the flesh (Leaver et al. 2011). CO in the diet (particularly in the 100COSEFM diet which contained the highest amount of CO) appeared to be stored as TAG in the liver rather than being metabolized for energy, in comparison to cod fed a FO diet. Feeding the 100COSEFM diet beyond the duration of this experiment may have eventually increased

the liver weight due to increased lipid storage, although the HSI was not different among groups after 13 weeks. Naturally, liver tissue had lower levels of DHA and EPA than muscle tissue, and had higher levels of ALA, LNA and 18:1ω9; however, it was obvious that the 100COSEFM liver fatty acid profile was more impacted by diet than those of 100CO and 100CO15CM, as observed in muscle.

Fatty acid profiles of selected cod tissues varied slightly depending on tissue type and their role in fat storage in the animal. Typically, ALA and LNA levels were lowest in FO and highest in 100COSEFM; DHA and EPA levels were typically highest in FO and lowest in 100COSEFM. Fatty acid profiles generally appeared to be similar between 100CO and 100CO15CM tissues. Certain tissues like muscle and brain changed little among treatments, although subtle changes were still significant; while other tissues like liver and gut exhibited greater differences in fatty acid composition among diets. This is a result of differential fat storage and function of the tissue. While liver is composed of neutral lipid and is the main site of fat storage in cod, the brain and muscle are composed of polar lipid; brain in particular critically requires high amounts of phospholipid for cell membranes, especially DHA for neural membrane fluidity and conformation (Sargent, 2002). Changes in brain lipid composition may have damaging consequences to fish health and metabolism; therefore this tissue is highly resistant to dietary change compared to a storage organ like the liver. Similar results were observed in sea bream brain (*Sparus aurata* L.) fed a VO diet (Benedito-Palos et al., 2010).

Relationships amongst fatty acid profiles

Multivariate statistics are a useful tool to define similarities and differences among tissue and dietary fatty acid data. Comparison of diet and tissue fatty acid profiles confirmed the observation that the brain was the most resistant tissue to dietary change. The dissimilarity between diet and brain tissue profile becomes wider with CO inclusion, with the greatest dissimilarity occurring in 100COSEFM and 100CO15CM, which were over 50% dissimilar between tissue and diet. Amongst brain tissues fed any diet, DHA was the major contributor to the dissimilarity between diet and tissue; DHA levels in the brain were higher than in the diet. There is evidence that selective incorporation of certain fatty acids in certain tissues, and selective deposition of DHA in the brain seem absolutely necessary when dietary levels of DHA were minimal. Selective incorporation of DHA and EPA into tissues after fish were fed a VO diet has been observed (Bell et al., 2004; Benedito-Palos et al., 2010; Hixson et al., 2013, Chapter 2). In this study, the muscle showed similar results as the brain, indicating resistance to dietary change. Meanwhile, the liver fatty acid profile appeared to assimilate to the diet fatty acid profile more than the muscle, with <25% dissimilarity between tissue and diet. As the main site of lipid deposition and being high in neutral lipid and TAG, it is expected that liver tissue profiles of cod would most resemble that of the diet (Nanton et al., 2003; Budge et al., 2012). It seems with VO diets in particular, excess lipid is stored and not metabolized for energy, despite diets being formulated to be isolipidous.

Assimilation of liver tissue fatty acid profile to the diet profile, especially in CO diets is a typical result in cod. The dissimilarity that did exist, however, can be attributed to 18:1ω9 as it was the major contributing fatty acid to the dissimilarity. There were

higher levels of 18:1ω9 in the liver than was provided in any CO diet, and there were higher levels of 18:1ω9 in the liver than any other tissue. Typically, 18:1ω9 is the preferred fatty acid for β-oxidation in fish, however if supply is greater than the demand, excess fatty acids not required for specific purposes or energy production will be stored (Bureau et al., 2002; Sargent et al., 2002), and fatty acids are metabolized differently according to dietary proportions (Bell et al. 2003; Torstensen et al. 2004; Budge et al., 2012). Comparison of individual tissues among all diets revealed that the FO and 100COSEFM groups demonstrated the most difference in fatty acid profiles, and this was the case for all tissues. These results confirm what was observed in the fatty acid profiles: removal of FO from FM and complete replacement of FO with CO had an encompassing effect on the entire fatty acid profile of the whole animal. Comparison of liver and muscle using MDS indicated that muscle and liver have distinct fatty acid profiles, but also both muscle and liver from the FO group form distinctly separate clusters apart from the CO fed diets. This visual representation demonstrates results from tissue comparison within diet and comparison of tissues amongst diets. Tissues behave differently from each other when incorporating dietary fatty acids, and often adjust their fatty acid profiles due to drastic changes in the diet (Budge et al., 2012). Comparison of all tissues within a single diet also demonstrates that tissues behave distinctly as seen in different clusters, and that they also assimilate diets to different degrees, mostly according to the function of the tissue.

Fatty acids have been used in ecological studies of trophic relationships based on matching tissue composition of the predator with that of potential prey. Controlled

feeding experiments as in this study where the exact diet composition and tissue composition are measured can provide quantitative estimates of diet assimilation into tissue. In a "fish-based" diet, the liver is about 25% different to the diet, muscle 27% and brain 42%. Since tissue fatty acid composition equals dietary fatty acid composition, plus fatty acid metabolism, and given these quantitative estimates, one can estimate that diet is incorporated into the tissue at 75% (liver), 73% (muscle), and 58% (brain). The biochemical processes involved may include β-oxidation or fatty acid biosynthesis.

Fatty acid synthesis

Observation of fatty acid profiles may help identify whether a fatty acid has been synthesized. For example, 20:3ω3 was not present in the diet or initial tissue composition; however, it was significantly higher in CO groups after 13 weeks of feeding CO diets. Morais et al. (2012) and Hixson et al. (2013) also reported evidence of fatty acid synthesis with the appearance of 20:3ω3, which was not present in the diet or in initial tissues, and levels were 16 times higher in cod fed CO (Hixson et al., 2013, Chapter 2). Its appearance suggests that elongation from ALA occurred to produce this fatty acid. In order to quantify the level of LC ω3 biosynthesis, the fatty acid mass balance method was employed. The fatty acid mass balance method was developed for fish by Turchini et al. (2007) in order to quantify LC ω3 fatty acid synthesis in fish to determine the level of elongation and desaturation that occurred over the course of a feeding experiment. A number of different methods can be used to measure desaturase and elongase activity; however, this method is an *in vivo* approach which provides a reliable estimation of an organism's capacity to metabolize fatty acids (Turchini et al., 2007). The model estimates

the fate (β-oxidation and/or bio-conversion towards longer and more unsaturated fatty acids) of all dietary fatty acids, which can easily be applied using the data from this experiment. Replacing FO with VO that are low in ω3 fatty acids is responsible for increased elongase and desaturase activity and transcription (mRNA) expression level to some degree (Bell et al., 2006; Turchini et al., 2009; Zheng et al., 2009; Morais et al., 2012). But this response is insufficient to compensate for the low levels of LC ω3 PUFA intake from VO diets, which results in significant reductions in LC ω3 PUFA in the tissues, particularly DHA and EPA. This was the observed result in this study when cod were fed CO, despite high levels of ALA in CO diets. Some fatty acids (18:4ω3, 20:3ω3) that were present in fatty acid profiles of cod fed CO indicate that some metabolic conversion had occurred; however, it was not enough to preserve levels of DHA and EPA in any tissue compared to cod fed FO diets.

All fatty acids in the ω3 pathway showed negative values or disappearance of the fatty acid, indicating that the fatty acid was oxidized, with the exception of 20:3ω3. Fatty acids differed in their degree of disappearance, which also depended on diet, and they were significantly different among dietary groups. The intake of ALA was significantly lower in FO than CO diets, so the level of disappearance was proportional to the amount fed; the disappearance of ALA was much greater in CO groups than the FO group. The disappearance of a fatty acid could indicate metabolic conversion to longer, more unsaturated fatty acid chains or utilization of their carbon skeleton through β-oxidation for energy production (Turchini et al., 2007). The level of disappearance of 18:4ω3 and 20:4ω3 was greater in FO, but the fact that this fatty acid disappeared in CO groups

demonstrates that this fatty acid was not synthesized by the fish and was consumed in the diet. However, 20:3ω3 showed positive values, indicating appearance of this fatty acid, and it was significantly higher in CO groups than FO and highest in 100COSEFM among CO diets. Appearance is an indication that ALA was elongated to form 20:3ω3; but disappearance of 18:4ω3 and 20:4ω3 suggests inactivity of the desaturase enzyme. The disappearance of EPA and DHA was greatest in the FO-fed group; these fatty acids disappeared because they were utilized for energy production, which indicates that more than the required amount of EPA and DHA were provided in this diet. Disappearance values were more positive in CO groups, indicating less DHA and EPA disappeared as a result of β-oxidation for energy production. It is not understood why DHA and EPA showed such negative disappearance values, particularly in CO groups, since disappearance indicates oxidation; however, tissue levels of these fatty acids in CO groups were significantly lower than FO groups. It is possible that levels of DHA and EPA in practical commercial diets are more than what is required from the observation that DHA and EPA are being oxidized in groups that were fed minimal FO.

The second step in the fatty acid mass balance method involved computation of ALA to DHA balance through the ω3 pathway. Since 20:3ω3 was the only fatty acid that did not disappear according to this method, cod in this experiment only showed signs of elongation, not desaturation. The same result was found in studies that also replaced FO with CO in diets for cod (Morais et al., 2012; Hixson et al., 2013, Chapter 2), as well as VO blends containing CO in diets for Atlantic salmon (Petropoulos et al., 2009; Bell et al., 2010). Appearance of 20:3ω3 has been documented as a dead end branch of the ω3

pathway (Turchini et al., 2007), since after elongation of ALA to 20:3ω3, further products are not formed directly from this fatty acid. Most studies in Atlantic cod gene expression have shown expression of elongase and low expression of Δ6 desaturase, perhaps associated with lower activity of Δ6 desaturase promoter (Zheng et al., 2009; Morais et al., 2012). This begs the question why evolution has allowed cod to elongate to this dead end product rather than evolve to desaturate from ALA to 18:4ω3. 20:3ω3 is not very well characterized or studied; therefore its usefulness in fish biology is relatively unknown other than being a product of a suggested dead end pathway. There have been hypotheses that synthesis of 20:3ω3 is a step in a short-cut pathway, when substrate competition between ω3 and ω6 pathways is rate limiting and the 18:4ω3 step can be by-passed by producing 20:3ω3, a potential bypass of the Δ6 desaturation, rather than the conventional Δ6 desaturation followed by an elongation (Tu et al., 2012; Park et al., 2009). However, similar results were not observed with 20:4ω3, which further suggest than cod have difficulties with desaturation rather than elongation. According to the fatty acid mass balance method by Turchini et al. (2007), cod fed 100COSEFM elongated 13% of ALA provided in the diet compared to cod fed FO which only elongated 1.6%. These results also indicate that between 87-98% of ALA was utilized for energy in this experiment. This disproves the hypothesis that providing increased amounts of ALA, the precursor ω3 fatty acid, would encourage LC ω3 synthesis to produce DHA and EPA. Rather, these results suggest that excess ALA encourages some elongation in cod, not desaturation. Even in diets with the highest level of ALA and the lowest level of DHA and EPA, over 86% of ALA was used for energy and limited fatty acid biosynthesis. The

evolution and biology of Atlantic cod explain their limited activity, in the context of their environment which is rich in LC ω3 PUFA in North Atlantic ecosystems. In comparison, rainbow trout have been shown to synthesize 27% of their own DHA from ALA in CO diets (Chapter 5). The comparison of these diverse species and their metabolic capabilities is confirmation of their different evolutionary histories, different natural diets and adaptations to their environment.

Energy deposition and expenditure

Considering the above results, we know that 87% to 98% of ALA provided in the diet was utilized for energy rather than biosynthesis of longer, more unsaturated fatty acids. A look at the bioenergetics and use of CO by cod will provide insight into the use of CO and CM in practical diets. It is well known that cod store excess dietary lipids as lipid in the liver. Cod fed 100COSEFM stored ~90% of deposited tissue lipids in the liver, greater than any other organ. Cod fed 100COSEFM also deposited more lipid in tissues than any other diet, but cod fed FO, 100CO and 100CO15CM had similar amounts of lipid deposition in all organs. Of the lipid supplied in the diet, cod fed FO stored 27% of lipid and utilized 73% for energy. In terms of energy intake, deposition and expenditure, the total deposited energy was greatest in 100COSEFM, with energy deposition relatively equal amongst the remaining groups. Total energy expenditure was highest in cod fed FO and lowest in cod fed 100COSEFM and 100CO15CM. The conversion of dietary lipids into body lipids is 96% efficient, so approximately 4% of energy from dietary lipids is dissipated as heat increment of feeding, the expenditure of energy from feeding (Bureau et al., 2002); therefore some energy loss was due to normal

metabolic processes. The results of energy expenditure correlate with the trends found in oxidation from the fatty acid mass balance method; although the mass balance method only estimated oxidation of ALA rather than total lipid energy. Cod fed 100COSEFM expended the least amount of energy, while cod fed FO expended the most. Obviously, energy intake, size and temperature all influence energy expenditure. Cod fed FO gained more weight throughout the trial, which explains the greater energy expenditure. The digestible energy requirement for maintenance for cod has been documented as approximately 53 kJ $kg^{-1}day^{-1}$ (Hatlen et al., 2007). Using this model on the initial mass and final mass and taking the average energy expenditure, the model appeared to overestimate the energy that was used over the duration of the experiment for cod fed FO (157 kJ) compared to the measured energy expenditure (83 kJ). Although dietary lipids supply most of the energy for fish, the measured energy expenditure only includes energy from lipid; protein also contributes to some of the energy used by cod, particularly considering protein was supplied as ~50% of the diet and lipid <10%. Energy expenditure appears to be lower in cod fed SEFM and CO compared to FO, and cod fed SEFM also deposited more lipid/energy than any group. Lipid deposition often indicates that it is not required for energy, or specific fatty acids are not required for certain metabolic functions. It is plausible that high amounts of certain fatty acids in CO (ALA, LNA, 18:1ω9) which were accentuated in the SEFM diet compared to the other diets that contained CO, can only be utilized to a certain extent. Fish tend to preferentially metabolize C_{18} PUFA by β-oxidation (Sargent et al., 2002); therefore fatty acids abundant in CO are often selected against in terms of flesh deposition and rather used for energy

production. Also, fatty acids present in relatively larger proportions in the diet are more readily catabolized than those present in smaller proportions (Budge et al., 2011). If most of the lipid from CO is preferentially oxidized, and maintenance energy requirements are met, excess lipids that are not useful to growth and metabolic functions of the fish will be stored in the liver. In the case of 100COSEFM diets, excess dietary CO did not significantly lend to energy production and growth, but was rather stored and some dietary lipid was wasted. Elongation of ALA at 13% could not sufficiently convert all excess ALA provided in the diet into more useful LC ω3 PUFA. These results highlight the fact that a balance of EFAs must be provided in the diet, at least through FM. For cod, increased lipid storage in the liver can lead to health issues, specifically fatty liver syndrome (Nanton et al., 2001). Meeting DE requirements by using CO and excessive replacement of FO not only reduced growth, but interfered with lipid storage and energy expenditure with very little metabolic ω3 synthesis to compensate.

Conclusion

This study replaced 100% FO with CO, removed lipids from FM, and included 15% CM in diets for Atlantic cod. This was the first study to include CM in a diet for Atlantic cod. Growth was significantly reduced when CO diets were fed for 13 weeks. Lipid storage in the liver increased when fed CO, particularly the 100COSEFM diet. Cod tissues in the 100COSEFM fed group were most impacted by change in dietary fatty acid profile, since all FM lipids were removed and consequently more CO was added to the diet to compensate for lipid loss. This was confirmed by multivariate statistics, since comparison of individual tissues among all diets revealed that the FO and 100COSEFM

groups demonstrated the most difference in fatty acid profiles, and this was the case for all tissues. The fatty acid mass balance method estimated that cod fed 100COSEFM elongated 13% of ALA, while the remaining 87% was oxidized; cod fed FO showed minimal elongation of ALA. Energy budget estimates indicated that excess CO lipids were deposited in the liver and not utilized for energy, impacting growth. Feeding 100% CO to Atlantic cod impacted growth and lipid and fatty acid composition; however, removal of FO from FM was the most detrimental to cod lipid metabolism.

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Table 3.1. Formulation and proximate composition of juvenile Atlantic cod experimental diets.

Diet	FO	100CO	100COSEFM	100CO15CM
Ingredient (% of diet)				
Herring meal	50.5	50.5	47.6	45.1
Camelina meal	-	-	-	15.0
Wheat middlings	27.1	27.1	25.7	17.0
Wheat gluten meal	5.0	5.0	5.0	5.0
Whey powder	5.0	5.0	5.0	5.0
Krill hydrolysate	2.5	2.5	2.5	2.5
Corn starch (pre-gelatinized)	0.25	0.25	0.25	0.25
Vitamin premix[1]	1.95	1.95	1.95	1.95
Mineral premix[2]	1.95	1.95	1.95	1.95
Choline chloride	0.3	0.3	0.3	0.3
Herring oil	5.43	-	-	-
Camelina oil	-	5.43	9.7	5.99
Proximate composition analyzed, as-fed basis				
Moisture	10.9 ± 0.02	10.7 ± 0.03	10.2 ± 0.02	14.7 ± 0.2
Ash	2.8 ± 0.1	2.9 ± 0.2	3.1 ± 0.6	2.9 ± 0.1
Protein	46.8 ± 0.1	47.3 ± 0.04	46.1 ± 0.01	45.1 ± 0.1

[1]Northeast Nutrition, Truro, Nova Scotia, Canada. Vitamin Premix contains per kg: Vitamin A 0.9 g, Vitamin D3 8.0 g, Vitamin E 50.0 g, Vitamin K 3.0 g, Thiamin 2.8 g, Riboflavin 4.0 g, Pantothenic acid 24.0 g, Biotin 0.1 g, Folic acid 26.7 g, Vitamin B12 0.03 g, Niacin 15.1 g, Pyridixine 3.3 g, Ascorbic acid 10.8 g, Wheat middlings (carrier) 851.3 g

[2]Northeast Nutrition, Truro, Nova Scotia, Canada. Mineral Premix contains per kg: Manganese oxide 12.3 g, Zinc oxide 20.6 g, Copper sulphate 6.1 g, Iodine 15.8 g, Wheat middlings (carrier) 954.2 g.

Table 3.2. Fatty acid and lipid composition of experimental diets[1]

Lipid composition	FO	100CO	100COSEFM	100CO15CM	F-stat	p-value
(% ww^{-1})						
Total lipid	8.8 ± 0.2	9.1 ± 1.3	9.9 ± 1.4	9.3 ± 0.2	3.1	0.09
Neutral lipid	8.5 ± 0.2	8.6 ± 1.2	9.5 ± 1.4	8.9 ± 0.1	3.0	0.10
Polar lipid	0.3 ± 0.04^{a}	0.6 ± 0.1^{b}	0.3 ± 0.02^{a}	0.3 ± 0.03^{a}	12.5	0.003
(% Total lipid)						
Triacylglycerol	85.0 ± 2.7	84.8 ± 3.2	90.9 ± 3.0	85.6 ± 3.0	1.94	0.21
Free fatty acid	4.7 ± 0.9	4.4 ± 1.1	4.4 ± 1.4	3.4 ± 1.4	0.78	0.54
Sterol	2.5 ± 1.2^{a}	2.1 ± 0.2^{a}	2.2 ± 0.2^{a}	3.9 ± 1.0^{b}	5.9	0.03
AMPL[2]	3.7 ± 0.9^{a}	2.3 ± 0.5^{ab}	1.0 ± 0.2^{b}	2.9 ± 0.7^{a}	9.5	0.01
Phospholipid	1.1 ± 0.3^{a}	4.3 ± 0.9^{b}	2.5 ± 0.5^{b}	1.6 ± 0.2^{b}	17.6	0.001
Fatty acids[3]						
14:0	5.5 ± 0.05^{a}	1.8 ± 0.09^{b}	1.0 ± 0.02^{c}	1.4 ± 0.01^{d}	2965	< 0.001
16:0	16.6 ± 0.02^{a}	10.7 ± 0.3^{b}	9.1 ± 0.2^{c}	9.6 ± 0.1^{c}	634	< 0.001
16:1ω9	8.0 ± 0.1^{a}	3.2 ± 0.1^{b}	1.8 ± 0.02^{c}	2.5 ± 0.2^{c}	3237	< 0.001
18:0	2.5 ± 0.01^{a}	1.9 ± 0.02^{b}	2.0 ± 0.03^{b}	2.4 ± 0.01^{c}	222	< 0.001
18:1ω9	9.7 ± 0.2^{a}	18.8 ± 0.1^{b}	21.1 ± 0.1^{c}	18.8 ± 0.1^{b}	2746	< 0.001
18:1ω7	3.2 ± 0.02^{a}	-	0.1 ± 0.003^{b}	0.2 ± 0.001^{b}	9608	< 0.001
18:2ω6(LNA)	6.6 ± 0.03^{a}	14.6 ± 0.1^{b}	17.1 ± 0.1^{c}	15.8 ± 0.1^{d}	6089	< 0.001
18:3ω3(ALA)	1.0 ± 0.1^{a}	13.8 ± 0.3^{b}	19.4 ± 0.1^{c}	17.3 ± 0.3^{d}	3854	< 0.001
20:1ω9	5.8 ± 0.2^{a}	11.3 ± 0.2^{b}	11.1 ± 0.2^{bc}	11.7 ± 0.1^{c}	592	< 0.001
20:4ω6	0.6 ± 0.01^{a}	0.1 ± 0.01^{b}	0.1 ± 0.02^{c}	0.1 ± 0.004^{c}	2115	< 0.001
20:5ω3 (EPA)	10.8 ± 0.2^{a}	3.0 ± 0.1^{b}	2.0 ± 0.04^{c}	2.4 ± 0.04^{d}	4720	< 0.001
22:1ω9	7.7 ± 0.02^{a}	8.1 ± 0.1^{b}	3.9 ± 0.1^{c}	6.4 ± 0.1^{d}	2128	< 0.001
22:1ω7	0.8 ± 0.2^{a}	2.2 ± 0.7^{b}	2.5 ± 0.1^{bc}	2.5 ± 0.2^{c}	160	< 0.001
22:5ω3	1.2 ± 0.1^{a}	0.3 ± 0.004^{b}	0.2 ± 0.002^{c}	0.3 ± 0.01^{bc}	524	< 0.001
22:6ω3(DHA)	9.3 ± 0.03^{a}	3.8 ± 0.1^{b}	2.8 ± 0.1^{c}	3.2 ± 0.5^{d}	1945	< 0.001

∑SFA[4]	27.1 ± 1.3[a]	15.9 ± 0.4[b]	13.7 ± 0.3[c]	14.5 ± 0.5[bc]	283	< 0.001
∑MUFA[5]	37.3 ± 0.1[a]	45.3 ± 0.1[b]	41.4 ± 0.2[c]	43.4 ± 0.1[d]	2164	< 0.001
∑PUFA[6]	35.4 ± 1.0[a]	38.5 ± 0.3[b]	44.6 ± 0.1[c]	42.0 ± 0.03[d]	257	< 0.001
∑ω3	24.3 ± 0.1[a]	22.0 ± 0.2[b]	25.5 ± 0.1[c]	24.1 ± 0.01[a]	314	< 0.001
∑ω6	7.9 ± 0.3[a]	15.7 ± 0.3[b]	18.6 ± 0.4[c]	17.2 ± 0.1[d]	1657	< 0.001
ω3/ω6	3.1 ± 0.1[a]	1.4 ± 0.03[b]	1.4 ± 0.01[b]	1.4 ± 0.01[b]	703	< 0.001
Terrestrial[7]	7.6 ± 0.1[a]	28.4 ± 0.5[b]	36.6 ± 0.04[c]	33.1 ± 0.01[d]	5648	< 0.001

[1]Values are mean (n=3) ± SD. Means with different superscripts indicate significance.
[2] Acetone-mobile polar lipid
[3] Data expressed as area percentage of FAME (Fatty Acid Methyl Ester),
[4] Saturated fatty acid
[5] Monounsaturated fatty acid
[6] Polyunsaturated fatty acid
[7]18:2ω6 + 18:3ω3

Table 3.3. Growth performance after 13 week feeding trial[1]

	FO	100CO	100COSEFM	100CO15CM	F-stat	p-value
Initial weight[1]	14.3 ± 1.2	14.6 ± 1.0	14.5 ± 1.4	15.1 ± 2.7	0.12	0.943
Final weight[1]	50.8 ± 10.3[a]	43.6 ± 8.9[b]	46.7 ± 10.7[b]	35.0 ± 8.0[c]	43.9	<0.001
Weight gain[2]	36.6 ± 1.4[a]	29.0 ± 1.2[b]	32.1 ± 1.0[b]	19.8 ± 2.3[c]	61.6	<0.001
Initial length[1]	11.3 ± 0.3	11.2 ± 0.3	11.2 ± 0.4	11.4 ± 0.6	0.24	0.864
Final length[1]	17.0 ± 1.1[a]	16.4 ± 1.0[b]	16.8 ± 1.1[a]	15.4 ± 1.1[c]	32.5	<0.001
SGR[3]	1.3 ± 0.03[a]	1.1 ± 0.05[a]	1.2 ± 0.1[a]	0.9 ± 0.1[b]	14.0	0.002
CF[4]	1.01 ± 0.1	0.98 ± 0.1	0.97 ± 0.1	0.94 ± 0.8	3.11	0.089
HSI[5]	6.8 ± 1.5	6.9 ± 1.4	7.6 ± 1.5	6.0 ± 1.8	3.75	0.060
AFI[6]	33.0 ± 1.8[a]	28.2 ± 1.4[b]	29.3 ± 1.0[b]	22.2 ± 1.4[c]	30.1	<0.001
FCR[7]	0.90 ± 0.02[a]	0.97 ± 0.03[a]	0.91 ± 0.1[a]	1.1 ± 0.1[b]	12.3	0.002

[1]Measurement calculated by individual fish, n is variable depending on number of fish remaining in tank for final measurements. Initial measurements, n = 9. Final measurements: FO= 99, 100CO= 104, 100COSEFM= 105, 100CO15CM= 98

[2]Weight gain (g $fish^{-1}$) = Final weight – Initial Weight. Measurement calculated from tank means, n=3

[3]Specific growth rate (% day^{-1}) = 100 x [ln (final body weight) – ln(initial body weight)]/days. Measurement calculated from tank means, n=3.

[4]Condition factor = Body mass/ $length^3$. Measurement calculated by individual fish.

[5]Hepatosomatic index = 100 x (liver mass/ body mass). Measurement calculated by individual fish.

[6]Apparent feed intake (g $fish^{-1}$) = Feed consumed (g) / number of fish per tank. Measurement calculated by tank means, n=3.

[7]Feed conversion ratio = Feed intake / Weight gain. Measurement calculated by tank means, n=3.

Table 3.4. Lipid and fatty acid composition of cod muscle tissue[1]

Lipid composition	Initial	FO	100CO	100COSEFM	100CO15CM	F-stat	p-value
(% ww^{-1})							
Total lipid	0.6 ± 0.2	0.7 ± 0.2	1.0 ± 0.4	0.7 ± 0.2	0.8 ± 0.3	1.58	0.27
Neutral lipid	0.1 ± 0.04	0.1 ± 0.04^{a}	0.2 ± 0.04^{b}	0.05 ± 0.02^{a}	0.1 ± 0.04^{a}	13.0	0.002
Polar lipid	0.5 ± 0.2	0.6 ± 0.2	0.8 ± 0.4	0.7 ± 0.2	0.6 ± 0.3	1.13	0.39
(% Total lipid)							
Triacylglycerol	0.5 ± 0.0	0.0 ± 0.0^{a}	0.3 ± 0.1^{a}	1.3 ± 0.3^{b}	0.8 ± 0.2^{b}	11.8	0.001
Free fatty acid	0.0 ± 0.0	0.0 ± 0.0^{a}	2.5 ± 0.8^{b}	0.0 ± 0.0^{a}	2.0 ± 0.3^{b}	7.7	0.01
Sterol	12.5 ± 3.7	11.4 ± 3.7^{a}	7.7 ± 1.3^{b}	6.4 ± 1.1^{b}	7.4 ± 1.8^{b}	8.49	0.005
AMPL[2]	2.2 ± 0.8	2.1 ± 0.8^{a}	2.4 ± 0.6^{a}	4.1 ± 1.1^{b}	1.2 ± 0.3^{c}	9.41	0.005
Phospholipid	83.7 ± 5.3	84.4 ± 5.3	79.4 ± 9.2	88.9 ± 3.2	85.7 ± 4.4	3.2	0.08
Fatty acids[3]							
14:0	1.4 ± 0.2	1.3 ± 0.2^{a}	0.7 ± 0.1^{b}	0.6 ± 0.2^{b}	0.5 ± 0.1^{b}	64.6	<0.001
16:0	17.7 ± 0.9	17.9 ± 3.3^{a}	14.7 ± 0.7^{b}	13.8 ± 0.9^{b}	14.9 ± 1.6^{b}	7.39	0.01
16:1ω9	2.8 ± 0.3	2.5 ± 0.4^{a}	1.3 ± 0.2^{b}	0.9 ± 0.1^{c}	1.0 ± 0.2^{bc}	167	<0.001
18:0	4.0 ± 0.4	4.2 ± 0.9	4.3 ± 0.3	4.0 ± 0.2	4.2 ± 0.6	0.29	0.83
18:1ω9	8.9 ± 0.6	9.3 ± 1.3^{a}	11.9 ± 0.7^{bc}	12.6 ± 0.7^{b}	11.1 ± 1.1^{c}	16.8	0.001
18:1ω7	2.8 ± 0.1	3.1 ± 0.4^{a}	2.4 ± 0.1^{b}	2.1 ± 0.1^{b}	2.3 ± 0.2^{b}	25.4	<0.001
18:2ω6 (LNA)	4.5 ± 0.3	5.2 ± 0.6^{a}	11.7 ± 1.1^{b}	13.8 ± 0.7^{c}	10.6 ± 1.4^{b}	169	<0.001
18:3ω6	0.1 ± 0.03	0.1 ± 0.01^{a}	0.06 ± 0.01^{b}	0.04 ± 0.02^{b}	0.06 ± 0.01^{b}	36.7	<0.001
18:3ω3 (ALA)	0.6 ± 0.1	0.6 ± 0.1^{a}	7.8 ± 0.1^{b}	11.3 ± 1.1^{c}	7.3 ± 1.1^{b}	148	<0.001
18:4ω3	0.6 ± 0.1	0.6 ± 0.1^{a}	0.3 ± 0.1^{b}	0.2 ± 0.04^{b}	0.3 ± 0.04^{b}	61.1	<0.001
20:1ω9	2.2 ± 0.3	1.5 ± 0.2^{a}	2.3 ± 0.2^{b}	2.2 ± 0.7^{b}	2.2 ± 0.1^{b}	9.42	0.005
20:4ω6	1.6 ± 0.1	1.7 ± 0.1^{a}	1.1 ± 0.1^{bc}	0.9 ± 0.1^{b}	1.2 ± 0.2^{c}	66.6	<0.001
20:3ω3	0.0 ± 0.0	0.0 ± 0.0^{a}	0.3 ± 0.03^{b}	0.4 ± 0.1^{b}	0.5 ± 0.1^{b}	6.41	0.02
20:4ω3	0.2 ± 0.0	0.5 ± 0.1^{a}	0.3 ± 0.1^{b}	0.2 ± 0.03^{b}	0.3 ± 0.04^{b}	34.8	<0.001
20:5ω3 (EPA)	17.7 ± 0.7	18.0 ± 2.5^{a}	12.9 ± 0.5^{bc}	11.1 ± 0.4^{b}	13.4 ± 1.1^{c}	57.9	<0.001

22:1ω9	0.7 ± 0.1	0.5 ± 0.1[a]	0.4 ± 0.1[a]	0.2 ± 0.1[b]	0.4 ± 0.1[a]	25.5	<0.001
22:5ω6	0.5 ± 0.04	0.5 ± 0.04[a]	0.4 ± 0.04[b]	0.3 ± 0.03[c]	0.4 ± 0.04[b]	19.9	<0.001
22:5ω3	2.4 ± 0.2	2.4 ± 0.2[a]	1.9 ± 0.1[b]	1.6 ± 0.1[c]	2.0 ± 0.2[b]	40.1	<0.001
22:6ω3 (DHA)	27.2 ± 1.6	25.7 ± 3.8[a]	22.1 ± 2.2[ab]	20.3 ± 2.2[b]	24.2 ± 1.1[ab]	4.27	0.04
∑SFA[4]	23.9 ± 1.1	22.5 ± 1.0[a]	20.0 ± 0.9[b]	18.8 ± 1.2[b]	20.0 ± 2.2[b]	5.61	0.02
∑MUFA[5]	18.7 ± 1.5	18.1 ± 1.8[a]	20.1 ± 1.1[b]	19.8 ± 1.1[ab]	18.8 ± 1.5[ab]	4.49	0.04
∑PUFA[6]	57.0 ± 1.6	59.2 ± 1.3	59.8 ± 1.1	61.3 ± 1.4	61.2 ± 3.1	1.31	0.34
∑ω3	49.2 ± 1.0	50.2 ± 1.9[a]	45.8 ± 1.9[b]	45.5 ± 1.8[b]	48.1 ± 1.8[b]	4.55	0.04
∑ω6	6.8 ± 0.9	7.7 ± 0.6[a]	13.4 ± 1.0[b]	15.2 ± 0.7[c]	12.4 ± 1.2[b]	171	<0.001
ω3/ω6	7.5 ± 1.1	6.3 ± 0.9[a]	3.4 ± 0.4[bc]	3.0 ± 0.2[b]	3.9 ± 0.6[c]	47.4	<0.001
DHA/EPA	1.5 ± 0.1	1.4 ± 0.2[a]	1.7 ± 0.1[b]	1.8 ± 0.2[b]	1.8 ± 0.2[b]	10.4	0.004
∑Terrestrial[7]	4.9 ± 0.9	5.8 ± 0.7[a]	19.6 ± 2.1[b]	25.2 ± 1.8[c]	18.0 ± 2.6[b]	165	<0.001

[1]Values are mean (n=9) ± SD. Means with different superscripts indicate significant differences at the end of the experiment.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester)
[4]Saturated fatty acid
[5]Monounsaturated fatty acid
[6]Polyunsaturated fatty acid
[7]Terrestrial = 18:2ω6 + 18:3ω3

Table 3.5. Lipid and fatty acid composition of cod liver tissue[1]

Lipid composition	Initial	FO	100CO	100COSEFM	100CO15CM	F-stat	p-value
(% ww^{-1})							
Total lipid	21.0 ± 3.1	22.2 ± 6.0^{a}	21.9 ± 6.6^{a}	34.5 ± 3.6^{b}	36.4 ± 3.0^{b}	8.77	0.004
Neutral lipid	18.9 ± 1.8	20.2 ± 5.5^{a}	20.2 ± 6.4^{a}	31.7 ± 3.6^{b}	33.2 ± 3.0^{b}	7.43	0.008
Polar lipid	2.1 ± 0.3	1.9 ± 0.8	1.7 ± 0.9	2.6 ± 0.5	3.0 ± 0.4	2.55	0.129
(% Total lipid)							
Triacylglycerol	70.1 ± 6.8	68.8 ± 8.3	73.7 ± 11	77.2 ± 8.6	74.2 ± 11	0.38	0.772
Free fatty acid	0.8 ± 0.1	1.0 ± 0.5	1.0 ± 0.2	0.5 ± 0.1	0.7 ± 0.1	1.14	0.385
Sterol	14.1 ± 1.9	15.2 ± 3.2	16.4 ± 2.7	14.5 ± 1.8	16.1 ± 3.2	0.04	0.987
AMPL[2]	3.5 ± 0.5	3.9 ± 0.4	4.7 ± 0.9	2.4 ± 0.4	3.4 ± 0.9	2.54	0.129
Phospholipid	6.2 ± 1.0	5.0 ± 1.0^{a}	2.5 ± 0.7^{b}	4.1± 0.7^{ab}	5.4 ± 0.7^{a}	8.08	0.006
Fatty acids[3]							
14:0	3.3 ± 0.2	3.1 ± 0.3^{a}	2.0 ± 0.2^{b}	1.4 ± 0.1^{c}	2.0 ± 0.2^{b}	65.4	<0.001
16:0	13.6 ± 0.4	13.2 ± 0.8^{a}	11.8 ± 1.4^{b}	9.7 ± 0.5^{c}	10.7 ± 0.7^{bc}	27.1	<0.001
16:1ω7	7.0 ± 0.3	7.3 ± 0.5^{a}	4.5 ± 0.5^{b}	3.2 ± 0.3^{c}	4.4 ± 0.6^{b}	92.7	<0.001
18:0	4.2 ± 0.5	4.8 ± 0.7^{a}	4.4 ± 0.4^{a}	3.8 ± 0.3^{b}	3.5 ± 0.4^{b}	5.81	0.020
18:1ω9	18.2 ± 0.8	22.9 ± 1.4^{a}	28.2 ± 1.9^{b}	28.5 ± 1.0^{b}	24.0 ± 2.5^{a}	30.6	<0.001
18:2ω6 (LNA)	6.2 ± 0.2	7.0 ± 0.3^{a}	13.2 ± 1.7^{b}	15.1 ± 0.8^{c}	13.6 ± 1.6^{b}	36.1	<0.001
18:3ω3 (ALA)	1.0 ± 0.04	1.0 ± 0.1^{a}	10.6 ± 1.5^{b}	15.0 ± 1.3^{c}	12.2 ± 2.0^{b}	89.8	<0.001
18:4ω3	1.6 ± 0.1	1.5 ± 0.1^{a}	0.7 ± 0.1^{b}	0.5 ± 0.1^{c}	0.7 ± 0.1^{b}	174	<0.001
20:1ω9	6.7 ± 0.4	5.7 ± 0.3^{a}	8.9 ± 1.5^{b}	8.0 ± 0.5^{b}	8.2 ± 0.6^{b}	37.3	<0.001
20:4ω6	0.6 ± 0.04	0.7 ± 0.1^{a}	0.3 ± 0.1^{b}	0.2 ± 0.03^{b}	0.3 ± 0.1^{b}	69.1	<0.001
20:3ω3	0.0 ± 0.0	0.0 ± 0.0^{a}	0.6 ± 0.01^{b}	0.8 ± 0.02^{c}	0.6 ± 0.03^{b}	124	<0.001
20:4ω3	0.2 ± 0.0	0.2 ± 0.01	0.3 ± 0.01	0.2 ± 0.01	0.3 ± 0.01	1.94	1.100
20:5ω3 (EPA)	10.8 ± 0.4	11.6 ± 0.9^{a}	4.8 ± 0.8^{b}	3.7 ± 0.5^{c}	4.7 ± 0.9^{bc}	122	<0.001
22:1ω9	4.9 ± 0.5	0.3 ± 0.04^{a}	0.6 ± 0.2^{b}	0.7 ± 0.1^{b}	0.6 ± 0.2^{b}	6.21	0.020
22:5ω3	1.5 ± 0.1	1.6 ± 0.2^{a}	0.7 ± 0.1^{bc}	0.5 ± 0.1^{b}	0.7 ± 0.1^{c}	67.1	<0.001
22:6ω3 (DHA)	7.7 ± 0.4	8.8 ± 0.4^{a}	3.9 ± 0.9^{b}	3.4 ± 0.5^{b}	3.5 ± 1.0^{b}	87.3	<0.001

∑SFA[4]	22.2 ± 0.5	21.8 ± 1.3[a]	18.3 ± 0.7[b]	15.3 ± 0.6[c]	16.7 ± 1.1[d]	47.2	<0.001
∑MUFA[5]	42.9 ± 1.0	40.2 ± 0.9	45.8 ± 1.2	43.2 ± 0.7	44.0 ± 1.0	106	<0.001
∑PUFA[6]	33.8 ± 1.0	37.3 ± 1.9[ab]	35.5 ± 1.9[a]	41.1 ± 0.9[c]	38.8 ± 1.8[b]	19.7	<0.001
∑ω3	23.4 ± 0.8	25.8 ± 1.5[a]	20.8 ± 1.4[b]	24.4 ± 0.4[ac]	22.9 ± 1.4[c]	37.2	<0.001
∑ω6	8.0 ± 0.8	8.3 ± 0.4[a]	13.7 ± 1.8[b]	15.5 ± 0.7[c]	14.4 ± 1.4[bc]	36.0	<0.001
ω3/ ω6	2.9 ± 0.2	3.1 ± 0.1[a]	1.6 ± 0.1[b]	1.6 ± 0.1[b]	1.6 ± 0.2[b]	229	<0.001
DHA/EPA	0.7 ± 0.02	0.8 ± 0.04[a]	0.8 ± 0.1[a]	0.9 ± 0.1[b]	0.7 ± 0.1[a]	13.5	0.002
∑Terrestrial[7]	7.2 ± 0.2	8.0 ± 0.4[a]	22.7 ± 0.9[b]	30.2 ± 2.0[c]	24.8 ± 1.9[b]	149	<0.001

[1]Values are mean (n=9) ± SD. Means with different superscripts indicate significant differences at the end of the experiment.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester)
[4]Saturated fatty acid
[5]Monounsaturated fatty acid
[6]Polyunsaturated fatty acid
[7]Terrestrial = 18:2ω6 + 18:3ω3

Table 3.6. Major fatty acids in selected cod tissues[1]

Tissue/Fatty acid[2]	FO	100CO	100COSEFM	100CO15CM	F-stat	p-value
Skin						
18:2ω6 (LNA)	3.9 ± 0.2[a]	9.5 ± 0.9[b]	11.8 ± 0.7[c]	9.4 ± 1.1[b]	86.4	<0.001
18:3ω3 (ALA)	0.4 ± 0.1[a]	4.5 ± 1.2[b]	6.7 ± 0.5[c]	4.5 ± 1.5[b]	53.2	<0.001
20:3ω3	0.0 ± 0.0[a]	0.5 ± 0.1[b]	0.7 ± 0.1[c]	0.5 ± 0.1[b]	112	<0.001
20:5ω3 (EPA)	13.5 ± 1.3[a]	9.0 ± 1.0[b]	7.9 ± 0.7[c]	8.7 ± 0.9[b]	45.9	<0.001
22:6ω3 (DHA)	20.2 ± 2.5[a]	16.6 ± 3.2[b]	16.5 ± 2.9[b]	16.8 ± 2.7[b]	4.43	<0.041
∑PUFA[3]	43.6 ± 3.6	45.8 ± 6.5	49.1 ± 4.0	46.1 ± 6.8	0.91	0.478
∑ω3	37.1 ± 3.7	32.6 ± 5.9	33.6 ± 4.2	32.2 ± 6.3	2.56	1.280
∑ω6	7.8 ± 0.2[a]	12.7 ± 0.8[b]	14.9 ± 0.7[c]	12.9 ± 0.7[b]	82.1	<0.001
Brain						
18:2ω6 (LNA)	0.8 ± 0.1[a]	1.4 ± 0.2[b]	1.8 ± 0.3[c]	1.5 ± 0.3[bc]	26.4	<0.001
18:3ω3 (ALA)	0.1 ± 0.01[a]	0.8 ± 0.1[b]	1.1 ± 0.2[c]	0.7 ± 0.1[b]	20.1	<0.001
20:3ω3	0.0 ± 0.0[a]	0.2 ± 0.01[b]	0.6 ± 0.1[c]	0.4 ± 0.1[bc]	8.61	0.007
20:5ω3 (EPA)	6.7 ± 1.2	6.1 ± 0.6	5.0 ± 1.4	5.8 ± 0.4	3.48	0.070
22:6ω3 (DHA)	23.2 ± 1.8	21.6 ± 1.3	21.2 ± 1.4	20.4 ± 1.6	2.35	0.157
∑PUFA	39.9 ± 2.5	39.0 ± 3.5	35.2 ± 2.7	35.3 ± 2.9	2.63	0.120
∑ω3	36.3 ± 2.6[a]	34.6 ± 4.2[ab]	30.1 ± 1.7[bc]	29.8 ± 2.8[c]	4.53	0.037
∑ω6	2.2 ± 0.5[a]	3.1 ± 0.6[ab]	3.6 ± 1.0[b]	3.3 ± 0.8[ab]	4.94	0.031
Gut						
18:2ω6 (LNA)	4.0 ± 0.6[a]	10.5 ± 0.4[b]	12.9 ± 0.3[c]	10.3 ± 0.9[b]	204	<0.001
18:3ω3 (ALA)	0.4 ± 0.1[a]	6.0 ± 0.6[b]	8.7 ± 0.9[c]	5.9 ± 0.9[b]	163	<0.001
20:3ω3	0.0 ± 0.0[a]	0.6 ± 0.04[b]	0.9 ± 0.1[c]	0.7 ± 0.1[b]	136	<0.001
20:5ω3 (EPA)	13.4 ± 0.5[a]	8.3 ± 0.6[b]	7.3 ± 0.5[c]	8.4 ± 0.4[b]	255	<0.001
22:6ω3 (DHA)	24.3 ± 2.0[a]	16.5 ± 1.5[b]	15.5 ± 1.5[b]	17.5 ± 0.5[b]	57.0	<0.001

∑PUFA	50.9 ± 1.8[a]	47.5 ± 2.0[b]	51.2 ± 1.6[a]	49.8 ± 1.7[a]	8.10	0.001
∑ω3	41.8 ± 2.1[a]	32.7 ± 2.3[b]	34.4 ± 1.4[bc]	35.4 ± 1.3[c]	47.9	<0.001
∑ω6	4.0 ± 0.3[a]	3.1 ± 0.1[b]	3.2 ± 0.3[bc]	3.5 ± 0.2[c]	26.3	<0.001
Spleen						
18:2ω6 (LNA)	4.1 ± 0.3[a]	8.6 ± 0.7[b]	9.3 ± 1.7[b]	9.0 ± 0.9[b]	22.0	<0.001
18:3ω3 (ALA)	0.4 ± 0.1[a]	4.0 ± 1.6[b]	5.2 ± 1.8[b]	5.1 ± 1.2[b]	12.4	<0.001
20:3ω3	0.0 ± 0.0[a]	0.5 ± 0.1[b]	0.7 ± 0.1[c]	0.6 ± 0.1[bc]	72.1	<0.001
20:5ω3 (EPA)	9.6 ± 1.7[a]	6.9 ± 1.4[ab]	4.9 ± 1.9[b]	7.0 ± 1.6[ab]	13.9	0.002
22:6ω3 (DHA)	12.3 ± 2.8[a]	11.6 ± 1.9[b]	8.4 ± 1.9[c]	11.8 ± 1.0[b]	4.50	0.042
∑PUFA	34.0 ± 1.9	37.8 ± 3.0	33.4 ± 3.4	39.2 ± 2.0	1.21	0.366
∑ω3	25.1 ± 1.6	24.8 ± 3.1	20.5 ± 2.8	26.4 ± 1.6	1.53	0.279
∑ω6	8.1 ± 0.9[a]	11.9 ± 1.5[b]	11.9 ± 2.0[b]	11.9 ± 0.9[b]	9.5	0.005
Carcass						
18:2ω6 (LNA)	3.9 ± 0.2[a]	9.3 ± 0.6[b]	11.2 ± 0.8[c]	8.9 ± 1.3[b]	132	<0.001
18:3ω3 (ALA)	0.4 ± 0.03[a]	5.4 ± 0.5[b]	7.8 ± 0.9[c]	5.5 ± 1.2[b]	89.3	<0.001
20:3ω3	0.07 ± 0.01[a]	0.6 ± 0.05[b]	0.8 ± 0.1[c]	0.5 ± 0.02[b]	41.5	<0.001
20:5ω3 (EPA)	15.1 ± 0.6[a]	10.3 ± 0.4[b]	9.2 ± 0.3[c]	10.3 ± 0.5[b]	173	<0.001
22:6ω3 (DHA)	23.6 ± 0.9[a]	20.2 ± 0.8[bc]	19.1 ± 1.0[b]	20.8 ± 1.8[c]	32.8	<0.001
∑PUFA	51.8 ± 1.8	52.1 ± 1.4	55.3 ± 2.7	53.2 ± 2.2	3.49	0.079
∑ω3	42.2 ± 0.8[a]	38.8 ± 0.6[b]	39.0 ± 0.9[b]	39.2 ± 1.4[b]	20.3	0.001
∑ω6	7.9 ± 0.4[a]	12.0 ± 0.6[b]	14.0 ± 0.6[c]	12.1 ± 1.2[b]	119	<0.001

[1]Values are mean (n=9) ± SD. Means with different superscripts indicate significant differences at the end of the experiment.
[2]Data expressed as area percentage of FAME (fatty acid methyl ester)
[3]Polyunsaturated fatty acid

Table 3.7. ANOSIM results between tissues within each dietary group

Diet	Least Similar		R	Most Similar		R
	Tissue A	Tissue B		Tissue A	Tissue B	
FO	Liver	Muscle Skin Spleen Gut Carcass	1	Brain	Gut	0.498
100CO	Liver	Muscle Skin Brain Carcass	1	Skin	Spleen	0.248
100COSEFM	Liver	Muscle Skin Spleen Gut Brain Carcass	1	Skin	Spleen	0.515
100CO15CM	Liver	Muscle Spleen Gut Carcass	1	Skin	Spleen	0.261

[1]Comparison between tissue and diet results were significant at p=0.01

Table 3.8. SIMPER dissimilarities between diet and tissue fatty acid profiles

Diet	Tissue	Average Dissimilarity (%)	Major Fatty Acid Contributor [1]	Contribution (%)
FO	Liver	24.7	18:1ω9	28.8
	Muscle	27.3	22:6ω3	33.7
	Brain	41.8	22:6ω3	16.5
100CO	Liver	22.5	18:1ω9	22.9
	Muscle	40.6	22:6ω3	23.5
	Brain	50.5	22:6ω3	17.5
100COSEFM	Liver	17.0	18:1ω9	22.8
	Muscle	38.6	22:6ω3	24.0
	Brain	52.2	18:3ω3	18.4
100CO15CM	Liver	22.6	18:1ω9	22.8
	Muscle	46.2	22:6ω3	23.8
	Brain	53.0	22:6ω3	17.0

[1]Major contributing fatty acid abbreviations: 22:6ω3 (DHA) and 18:3ω3 (ALA)

Table 3.9. SIMPER dissimilarities between tissue fatty acid profiles compared across dietary groups, where dissimilarities are quantified according to diet among a specific tissue

Diet A	Diet B	Tissue	Average Dissimilarity (%)	Major Fatty acid Contributor[1]	(%)
FO	100COSEFM	Liver	31.2	18:3ω3	22.6
		Muscle	24.9	18:3ω3	21.8
		Brain	27.4	18:1ω9	21.5
\		Skin	21.4	18:2ω6	18.6
		Carcass	23.4	18:3ω3	16.2
		Gut	29.2	18:2ω6	15.4
		Spleen	25.2	18:1ω9	11.8
		Overall Mean	26.1	-	-
FO	100CO	Liver	25.6	18:3ω3	19.1
		Muscle	18.8	18:3ω3	19.4
		Brain	22.2	18:1ω9	20.4
		Skin	17.2	18:2ω6	16.6
		Carcass	17.5	18:2ω6	15.4
		Gut	24.8	22:6ω3	19.6
		Spleen	20.4	22:6ω3	13.1
		Overall Mean	20.9	-	-
FO	100CO15CM	Liver	26.0	18:3ω3	21.9
		Muscle	17.4	18:3ω3	19.6
		Brain	22.7	18:1ω9	21.7
		Skin	17.5	18:2ω6	15.8
		Carcass	17.9	18:3ω3	17.9
		Gut	21.9	22:6ω3	14.6
		Spleen	20.3	18:2ω6	12.3
		Overall Mean	20.5	-	-
100CO	100COSEFM	Liver	11.2	18:3ω3	20.5
		Muscle	8.00	18:3ω3	22.2
		Brain	14.1	22:6ω3	20.9
		Skin	9.47	22:6ω3	17.9
		Carcass	7.76	22:6ω3	15.5
		Gut	10.6	18:3ω3	16.3
		Spleen	15.6	22:6ω3	20.8
		Overall Mean	11.0	-	-
100COSEFM	100CO15CM	Liver	11.0	18:1ω9	21.1
		Muscle	10.6	22:6ω3	21.9
		Brain	12.7	22:6ω3	21.5

		Skin	9.61	22:6ω3	15.9
		Carcass	8.36	18:0	15.1
		Gut	8.69	18:3ω3	16.7
		Spleen	14.5	22:6ω3	19.1
		Overall Mean	10.8	-	-
100CO	100CO15CM	Liver	10.7	18:1ω9	28.0
		Muscle	6.54	22:6ω3	28.7
		Brain	6.25	22:6ω3	17.6
		Skin	9.01	22:6ω3	19.6
		Carcass	5.37	22:6ω3	15.3
		Gut	7.83	22:6ω3	23.5
		Spleen	12.2	22:6ω3	21.6
		Overall Mean	8.27	-	-

[1]Major contributing fatty acid abbreviations: 18:2ω6 (LNA), 18:3 ω3 (ALA), 22:6ω3 (DHA)

Table 3.10. Partitioning of ω3 PUFA by the whole-body fatty acid balance method; data are relative to the ω3 balance after 13 weeks of feeding

	FO	100CO	100COSEFM	100CO15CM	*F*-stat	*p*-value
Total fatty acid (mg fish^{-1})						
18:3ω3(ALA)						
Intake[1]	26.9 ± 1.2^{a}	330.5 ± 14^{b}	525.9 ± 15^{c}	245.9 ± 13^{d}	2499	<0.001
Excretion[2]	1.0 ± 0.05^{a}	12.9 ± 0.5^{b}	20.5 ± 0.5^{c}	9.6 ± 0.5^{b}	2499	<0.001
Initial body content	6.8 ± 1.0	6.8 ± 1.0	6.8 ± 1.0	6.8 ± 1.0	-	-
Final body content	6.8 ± 1.0^{a}	79.6 ± 11^{b}	221.4 ± 20^{c}	89.5 ± 9.1^{b}	14.3	<0.001
Accumulation	0.0 ± 0.0^{a}	72.8 ± 11^{b}	214.6 ± 21^{c}	82.7 ± 9.0^{b}	14.9	<0.001
Net Intake	25.8 ± 1.2^{a}	317.5 ± 4.5^{b}	505.5 ± 4.9^{c}	236.4 ± 4.2^{d}	2499	<0.001
Appearance/Disappearance	-25.9 ± 1.0^{a}	-244.8 ± 14^{bc}	-290.9 ± 20^{b}	-153.7 ± 10^{c}	22.9	<0.001
18:4ω3						
Intake	41.5 ± 1.5^{a}	8.5 ± 0.4^{b}	5.6 ± 0.2^{c}	3.9 ± 0.2^{d}	2885	<0.001
Excretion	1.6 ± 0.1	0.3 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	2885	<0.001
Initial body content	10.5 ± 1.0	10.5 ± 1.0	10.5 ± 1.0	10.5 ± 1.0	-	-
Final body content	10.5 ± 1.0^{a}	4.6 ± 2.1^{b}	6.8 ± 3.8^{ab}	4.5 ± 2.7^{b}	4.8	0.007
Accumulation	0.0 ± 0.0^{a}	-5.5 ± 2.1^{b}	-3.4 ± 1.2^{ab}	-5.6 ± 2.7^{b}	4.8	0.007
Net Intake	39.9 ± 1.8^{a}	8.2 ± 0.4^{b}	5.4 ± 0.2^{c}	3.8 ± 0.2^{d}	2885	<0.001
Appearance/Disappearance	-39.9 ± 5.1^{a}	-13.7 ± 2.4^{b}	-8.8 ± 3.9^{c}	-9.3 ± 2.6^{bc}	145	<0.001
20:3ω3						
Intake	ND	ND	ND	ND		
Excretion	ND	ND	ND	ND		
Initial body content	ND	ND	ND	ND		
Final body content	0.05 ± 0.01^{a}	4.6 ± 0.6^{b}	11.6 ± 1.9^{c}	4.9 ± 0.9^{b}	17.6	<0.001
Accumulation	0.05 ± 0.01^{a}	4.6 ± 0.6^{b}	11.6 ± 1.9^{c}	4.9 ± 0.9^{b}	17.6	<0.001
Net Intake	-	-	-	-	-	-
Appearance/Disappearance	0.05 ± 0.01^{a}	4.6 ± 0.6^{b}	11.6 ± 1.9^{c}	4.9 ± 0.9^{b}	17.6	<0.001

20:4ω3						
Intake	10.7 ± 0.5[a]	2.2 ± 0.1[b]	1.6 ± 0.04[c]	1.0 ± 0.1[c]	2821	<0.001
Excretion	0.4 ± 0.01[a]	0.09 ± 0.01[b]	0.06 ± 0.001[c]	0.04 ± 0.002[d]	2821	<0.001
Initial body content	4.7 ± 1.9	4.7 ± 1.9	4.7 ± 1.9	4.7 ± 1.9	-	-
Final body content	4.7 ± 1.9[a]	2.2 ± 0.8[b]	2.9 ± 0.5[ab]	2.1 ± 0.9[b]	6.48	0.001
Accumulation	0.0 ± 0.0[a]	-2.5 ± 0.8[b]	-1.7 ± 0.5[ab]	-2.6 ± 1.9[b]	6.48	0.001
Net Intake	10.3 ± 0.5[a]	2.2 ± 0.1[b]	1.6 ± 0.04[c]	1.0 ± 0.05[d]	2821	<0.001
Appearance/Disappearance	-10.3 ± 0.5[a]	-4.6 ± 0.9[b]	-3.3 ± 0.5[b]	-3.5 ± 0.9[b]	49.5	<0.001
20:5ω3 (EPA)						
Intake	296.4 ± 13[a]	71.6 ± 3.0[b]	54.7 ± 1.6[c]	34.6 ± 1.8[d]	2592	<0.001
Excretion	12.1 ± 0.6[a]	2.9 ± 0.1[b]	2.2 ± 0.1[c]	1.4 ± 0.1[d]	2592	<0.001
Initial body content	107.9 ± 11	107.9 ± 11	107.9 ± 11	107.9 ± 11	-	-
Final body content	107.9 ± 11[a]	58.1 ± 6.1[b]	73.1 ± 9.3[b]	49.0 ± 6.0[b]	10.2	<0.001
Accumulation	0.0 ± 0.0[a]	-50.0 ± 6.1[b]	-34.9 ± 9.3[b]	-59.0 ± 6.0[b]	10.2	<0.001
Net Intake	284.3 ± 13[a]	68.9 ± 2.9[b]	52.5 ± 1.5[c]	33.2 ± 1.8[d]	2592	<0.001
Appearance/Disappearance	-284.3 ± 10[a]	-118.6 ± 6.6[b]	-87.3 ± 9.4[b]	-92.2 ± 5.9[b]	129	<0.001
22:5ω3						
Intake	33.5 ± 1.5[a]	7.7 ± 0.3[b]	5.5 ± 0.2[c]	3.6 ± 1.2[d]	2707	<0.001
Excretion	1.3 ± 0.06	0.3 ± 0.01	0.2 ± 0.01	0.1 ± 0.01	2707	<0.001
Initial body content	15.5 ± 1.4	15.5 ± 1.9	15.5 ± 1.9	15.5 ± 1.9	-	-
Final body content	15.5 ± 1.4[a]	9.3 ± 1.8[b]	10.2 ± 1.5[b]	7.0 ± 2.6[c]	7.78	<0.001
Accumulation	0.0 ± 0.0[a]	-6.2 ± 1.2[b]	-5.4 ± 1.5[b]	-8.5 ± 2.6[c]	7.78	<0.001
Net Intake	32.2 ± 1.5[a]	7.4 ± 0.3[b]	5.3 ± 0.2[c]	3.4 ± 1.2[d]	2707	<0.001
Appearance/Disappearance	-32.2 ± 1.5[a]	-13.6 ± 3.7[b]	-10.7 ± 1.7[b]	-12.0 ± 0.9[b]	60.3	<0.001
22:6ω3 (DHA)						
Intake	256.5 ± 12[a]	90.3 ± 3.8[b]	74.2 ± 2.2[c]	45.2 ± 2.4[d]	1921	<0.001
Excretion	19.2 ± 0.9[a]	6.8 ± 0.2[b]	5.6 ± 0.1[c]	3.4 ± 0.2[c]	1921	<0.001
Initial body content	117.6 ± 10	117.6 ± 10	117.6 ± 10	117.6 ± 10	-	-
Final body content	117.6 ± 10[a]	79.3 ± 7.2[bc]	96.2 ± 12[ab]	61.4 ± 5.5[c]	10.5	<0.001
Accumulation	0.0 ± 0.0[a]	-38.3 ± 7.3[bc]	-21.4 ± 5.0[b]	-56.2 ± 5.8[c]	10.5	<0.001
Net Intake	237.3 ± 11[a]	83.6 ± 3.5[a]	68.6 ± 2.0[c]	41.8 ± 2.3[d]	1921	<0.001

Appearance/Disappearance	-237.3 ± 6.7[a]	-122.0 ± 7.9[b]	-90.1 ± 9.2[c]	-98.0 ± 5.8[bc]	83.1	<0.001

[1]Values are mean ± SD. Means with different superscripts indicate significant differences at the end of the experiment
[2]Intake = (g feed intake * mg of fatty acid per g of feed)
[3]Excretion = (mg of fatty acid intake * fatty acid digestibility)

Table 3.11. Calculation of 18:3ω3 (ALA) balance by the whole-body fatty acid balance method; data is relative to the ω3 balance after 13 weeks of feeding[1]

	FO	100CO	100COSEFM	100CO15CM	F-stat	*p*-value
Net intake 18:3ω3	92.7 ± 1.5^{a}	1140.3 ± 48^{b}	1815.5 ± 53^{c}	849.0 ± 46^{d}	2499	<0.001
Disappearance 18:3ω3	92.9 ± 11^{a}	244.8 ± 44^{bc}	290.9 ± 42^{c}	153.7 ± 20^{ab}	13.2	<0.001
Elongation	1.7 ± 0.8^{a}	15.0 ± 1.2^{ab}	37.9 ± 6.1^{c}	16.1 ± 3.1^{b}	17.6	<0.001
Oxidized	91.2 ± 11^{a}	229.8 ± 49^{bc}	253 ± 48^{c}	137.6 ± 23^{ab}	7.43	0.001
Oxidized (%)	98.4 ± 0.9^{a}	93.9 ± 4.5^{a}	86.9 ± 20^{b}	89.5 ± 14^{a}	5.85	0.003
Elongated (%)	1.6 ± 0.3^{a}	6.1 ± 1.5^{b}	13.1 ± 2.1^{c}	10.5 ± 1.2^{d}	112	<0.001

[1]Values are mean (n=9) ± SD (mmol fish^{-1}). Means with different superscripts indicate significant differences

Table 3.12. Energy budget of Atlantic cod fed experimental diets

	FO	100CO	100COSEFM	100CO15CM	F-stat	*p*-value
Lipid content of feed (%)	8.8 ± 0.2	9.1 ± 1.3	9.9 ± 1.4	9.3 ± 1.2		
Feed intake (g fish)	33.0 ± 1.8^{a}	28.2 ± 1.4^{b}	29.3 ± 1.0^{b}	22.2 ± 1.4^{c}	30.1	<0.001
Lipid intake (mg fish^{-1})	2906 ± 157^{a}	2567 ± 127^{b}	2898 ± 98^{a}	2064 ± 129^{c}	28.1	<0.001
Muscle lipid[1]	19.2 ± 2.8^{ab}	24.7 ± 1.1^{a}	8.5 ± 1.4^{c}	11.2 ± 1.8^{b}	12.9	0.002
Liver[1]	658 ± 129^{a}	715 ± 109^{ab}	1422 ± 189^{b}	756 ± 166^{ab}	6.02	0.019
Skin[1]	3.3 ± 1.2	3.4 ± 1.0	3.1 ± 1.1	2.7 ± 0.9	0.38	0.769
Brain[1]	6.0 ± 1.2	7.2 ± 1.9	4.8 ± 0.6	5.0 ± 1.7	3.86	0.056
Spleen[1]	1.3 ± 0.5	1.6 ± 1.5	1.3 ± 0.3	0.8 ± 0.3	1.96	0.198
Gut[1]	17.5 ± 1.4^{ab}	17.8 ± 2.0^{ab}	24.9 ± 3.1^{a}	12.4 ± 1.2^{b}	5.63	0.023
Carcass[1]	73.7 ± 14^{a}	50.0 ± 11^{b}	77.6 ± 6.4^{a}	29.7 ± 8.9^{c}	34.1	<0.001
Total lipid deposition (mg fish^{-1})[2]	779 ± 128^{a}	820 ± 112^{a}	1542 ± 293^{b}	818 ± 164^{a}	6.18	0.018
% of Total lipid in stored in liver	80.1 ± 11	85.9 ± 4.3	89.9 ± 6.2	86.0 ± 6.1	1.31	0.336
Oxidized lipid (mg)[3]	2127 ± 383^{a}	1747 ± 336^{ab}	1590 ± 569^{b}	1246 ± 224^{b}	5.93	<0.001
Oxidized lipid (%)[4]	73.2 ± 4.3^{a}	68.1 ± 4.3^{a}	54.9 ± 6.9^{b}	60.4 ± 8.0^{b}	16.7	<0.001
Stored lipid (%)[5]	26.8 ± 4.4^{a}	31.9 ± 4.4^{a}	53.2 ± 10^{b}	39.7 ± 8.0^{c}	33.5	<0.001
Energy intake from lipid (kJ fish^{-1})	113.3 ± 6.1^{a}	100.1 ± 4.9^{b}	113.0 ± 3.8^{a}	80.5 ± 5.0^{c}	28.1	<0.001
Deposited energy (kJ fish^{-1})[2]	30.4 ± 4.9^{a}	31.9 ± 6.4^{a}	60.1 ± 11^{b}	31.9 ± 4.4^{a}	3.14	0.019
Total Energy Expenditure (kJ)[3]	82.9 ± 4.9^{a}	68.1 ± 4.4^{ab}	52.9 ± 11^{b}	48.6 ± 6.4^{b}	4.54	0.009

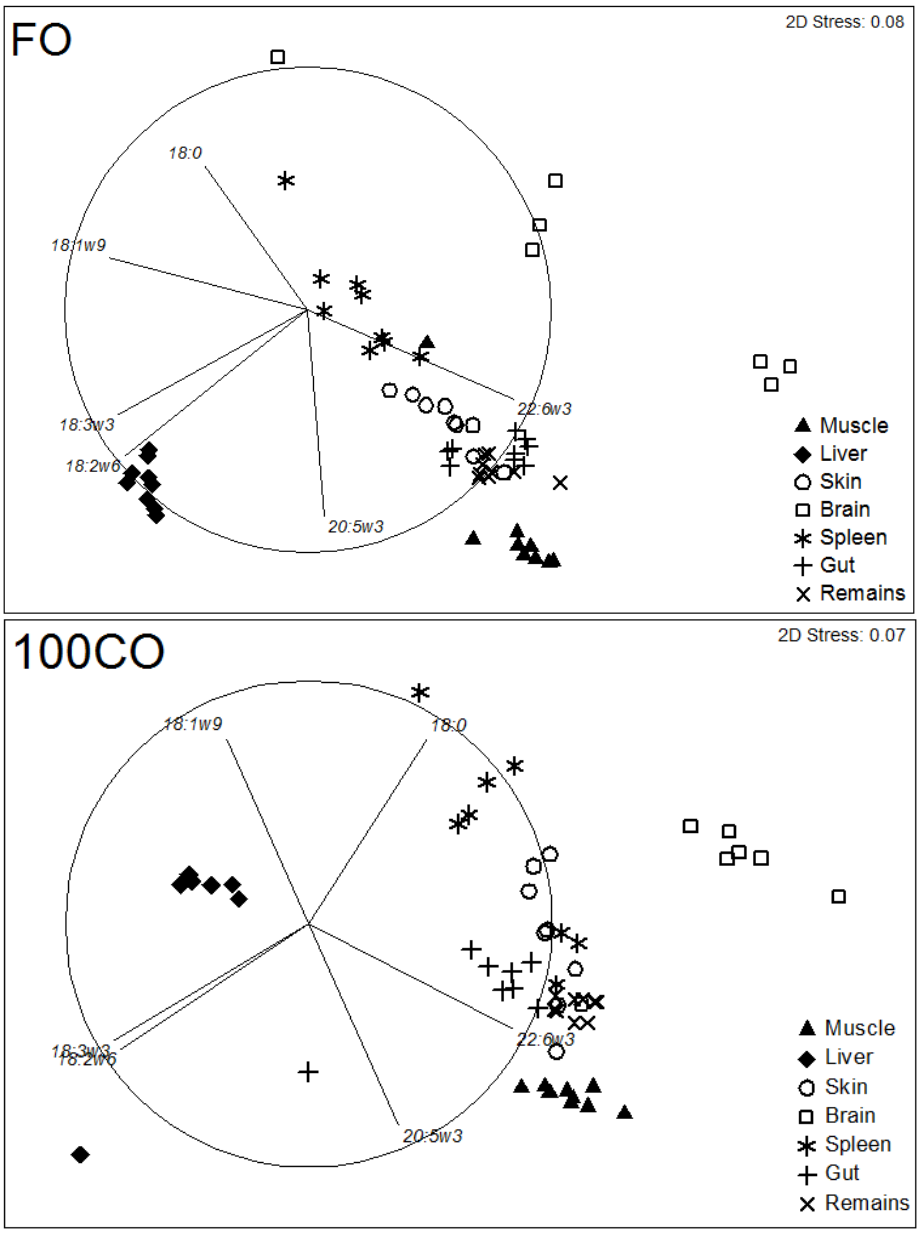
[1]Neutral lipid (mg fish^{-1}); values are mean (n=9) ± SD. Means with different superscripts indicate significant differences at the end of the experiment.

[2]Total lipid = $\sum$ of organ lipids (mg or kJ)

[3]Oxidized lipid (or Energy Expenditure) = Lipid intake (mg or kJ per fish) – Total lipid (mg or kJ per fish)

[4]Oxidized (%) = (Oxidized lipid (mg) / Lipid intake (mg per fish)) *100

[5]Stored (%) = (Total lipid (mg per fish) / Lipid intake (mg per fish)) *100


FO
2D Stress: 0.08
18:0
18:1w9
18:3w3
18:2w6
20:5w3
22:6w3
Muscle
Liver
Skin
Brain
Spleen
Gut
Remains
100CO
2D Stress: 0.07
18:1w9
18:0
18:3w3
18:2w6
20:5w3
22:6w3
Muscle
Liver
Skin
Brain
Spleen
Gut
Remains

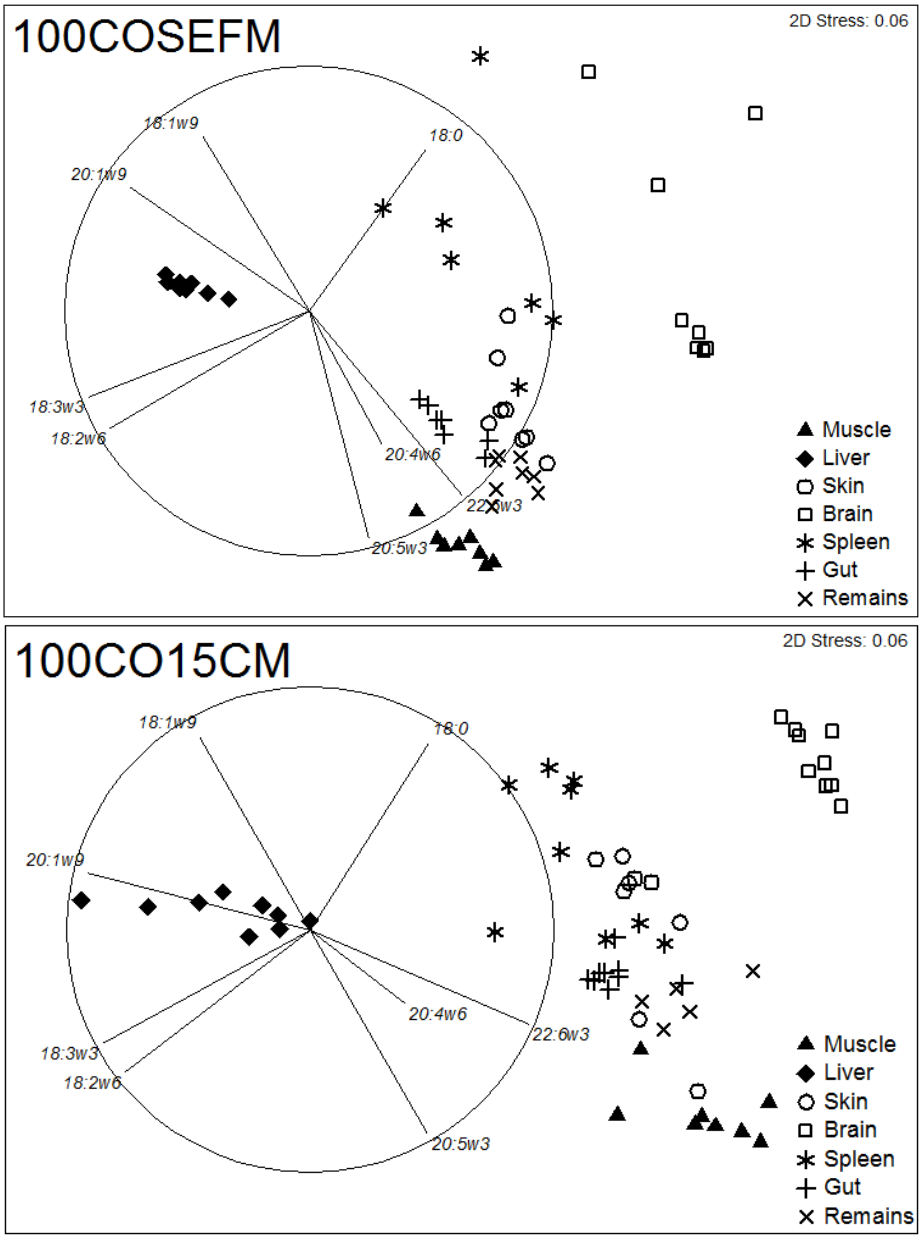


Figure 3.1. Comparison of tissues within each dietary group using multi-dimensional scaling. Vector fatty acid abbreviations: 18:2ω6 (LNA), 18:3ω3 (ALA), 20:5ω3 (EPA), 22:6ω3 (DHA)

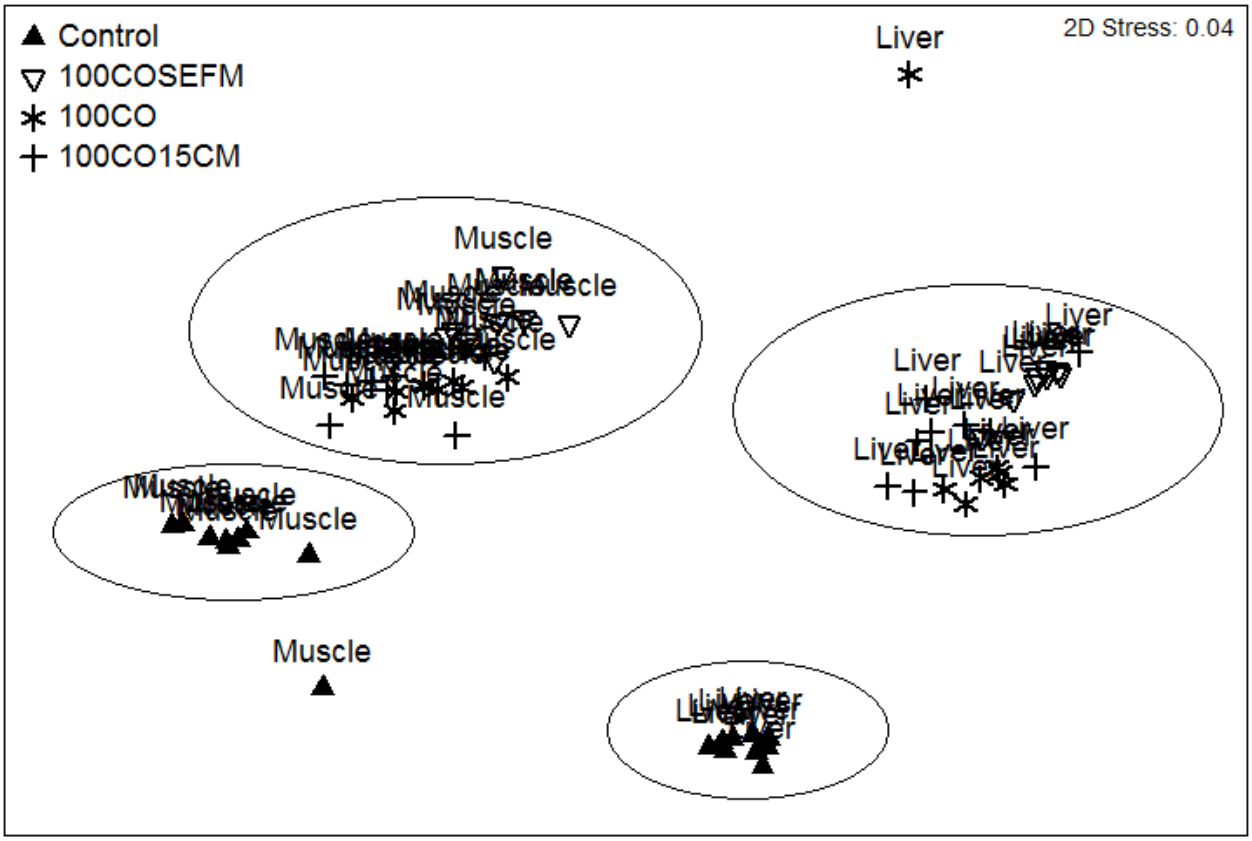


Figure 3.2. Comparison of liver and muscle tissue across all experimental diets after 13 weeks using multi-dimensional scaling. Cluster groupings were determined according to Bray Curtis similarity cluster analysis.

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Chapter 4. Partial replacement of fish oil with camelina oil in diets for juvenile Atlantic cod (*Gadus morhua*): effect on growth and lipid composition of muscle and liver, with incidence of a microsporan parasite, *Loma morhua*

4.1 Abstract

Atlantic cod were fed diets that replaced 40% or 80% of fish oil (FO) with camelina oil (CO) for 12 weeks. During the experiment, cod were unintentionally exposed to the microsporan parasite, *Loma morhua*, which confounded the experiment by introducing health as a factor affecting growth performance. The specific growth rate for each treatment after 12 weeks (0.3-0.4% day^{-1}) was lower than typical values found for farmed cod at approximately this weight. Growth data was analyzed according to health status of two groups: cod with <10 xenomas on each of the heart, spleen and gills were considered healthy and cod with >10 xenomas on the heart, spleen or gills were considered heavily infested. There was a significant difference in growth between healthy fish and fish heavily infested with *Loma*, regardless of diet. Considering the population as a whole, including both healthy and *Loma* infected fish; there was no significant difference in the growth rate depending on dietary treatment. However when both health categories were considered separately, unhealthy fish fed the FO diet had a significantly higher growth rate (0.43% day^{-1}) than unhealthy fish fed 80% CO (0.19% day^{-1}). Essential fatty acids, particularly 20:5ω3 and 22:6ω3, were not altered in cod muscle after being fed CO diets, however they were reduced significantly in the liver compared to cod fed the FO diet.

4.2 Introduction

Fish is the only major source of long chain (LC) polyunsaturated ω3 fatty acids (PUFAs) for humans, particularly docosahexaenoic acid (DHA, 22:6ω3) and eicosapentaenoic acid (EPA, 20:5ω3) which are essential to heart and brain health (Santerre, 2010). With the increase in fish consumption due to both a recent interest in the health benefits of seafood and the natural world population increase, wild fisheries have dramatically declined and aquaculture has become a significant seafood provider. As a result, it is the fastest growing animal production industry in the world (FAO, 2009). However, marine fish aquaculture intensely depends on fish oil (FO) in feeds which is costly, unsustainable, and contributes to the exploitation of wild fish stocks (Farrell et al., 2010). If aquaculture continues to rely on FO as a main source of lipid, there will be repercussions on both aquaculture and wild fisheries. Sustainable alternatives of FO for use in aquaculture must be investigated, particularly terrestrial plant oils. They are readily attainable, sustainable and rich in unsaturated fatty acids. Studies have replaced FO with plant oils in fish feeds for several different fish species, including rapeseed oil (Fountoulaki et al., 2009), palm oil (Bell et al., 2002), linseed oil (Menoyo et al., 2005), sunflower oil (Brandsen et al., 2003) and plant oil blends (Jordal et al., 2007) without affecting growth and without significant mortality. However, use of many terrestrial plant oils is limited due to fatty acid profiles that lack key components, namely sufficient levels of long chain polyunsaturated fatty acids (PUFA) from the ω3 series.

The false flax, camelina (*Camelina sativa*) holds significant potential for aquaculture. Camelina is a member of the Brassicaceae family which includes mustards,

rapes, and canola (Budin et al., 1995). The high lipid content in camelina (40%) (Zubr, 1997) makes it an attractive candidate for aquaculture feed. Its most unique feature is the high content of polyunsaturated fatty acids (PUFA), particularly α-linolenic acid (ALA,18:3ω3), an essential ω3 fatty acid that constitutes up to 40% of the fatty acids (Ni Eidhin et al., 2003). The ω3 PUFAs are essential for growth and development in fish and must be included in diets. In comparison, the ALA content in soybean, rapeseed, sunflower, peanut, and corn oil is less than 12% (Ni Eidhin et al., 2003). Therefore, camelina is a superior source of ω3 PUFA than other plant oils previously used in aquaculture studies. However, to date, camelina as a lipid source for fish has not been evaluated.

It is important to consider how new dietary ingredients will affect health and growth of farmed fish and how this may impact the aquaculture industry. It is well known that Canadian aquaculture is affected by several different pathogens annually that can devastate a farm (Khan, 2005). Good nutrition may lessen the impact caused by such pathogens. The microsporan parasite, *Loma morhua* is widely distributed in Atlantic cod of coastal Newfoundland (Lom and Laird, 1976; Morrison and Sprague, 1981; Khan and Tuck, 1995). Cod aquaculture is regularly exposed to *Loma morhua* (Khan, 2005). Fish show signs of emaciation, lethargy and exhibit pale gills. It is known to significantly negatively affect weight gain, condition factor and feeding rates and ultimately leads to mortality (Khan, 2005). Experimentation of new diets under conditions typical in Canadian aquaculture is a key aspect of this study. Therefore, it is of interest to determine if cod fed camelina-containing diets perform as well as cod fed a commercial type diet when exposed to a natural pathogen that is common in cod aquaculture. This study also

provides insight regarding how to statistically analyze data that were confounded by an uncontrollable external factor such as *Loma* and how to isolate its effect on the experiment.

This study focused on utilizing camelina oil (CO) as an alternative lipid for FO. The overall objectives of this study are to: 1) conduct a feeding trial to determine growth and lipid and fatty acid profiles of cod fed diets containing CO; 2) determine if *Loma morhua* affects growth of cod while fed CO containing diets compared to cod fed a FO diet and 3) determine if feeding a CO diet can help minimize negative effects on cod during an infestation of *Loma morhua*.

4.3 Methods

Camelina oil

Camelina (Calena cultivar) was grown and harvested by the Department of Plant and Animal Sciences, Dalhousie University at an off-campus location (Canning, Nova Scotia, Canada). The seeds were single pressed using a KEK 0500 press at Atlantic Oilseed Processing, Ltd. (Summerside, Prince Edward Island, Canada) to extract the oil, and ethoxyquin was added to the final product as an antioxidant.

Experimental diets

Three practical diets were produced at the Faculty of Agriculture Campus, Dalhousie University (Table 4.1). Diets were formulated to meet the nutritional requirements of gadoids based on previous formulations (Tibbetts et al. 2004; 2006), and were isonitrogenous and isolipidous. CO was used as the test ingredient in each of the

experimental diets, and herring oil was used in the control diet (FO). CO replaced 40% (CO40) or 80% (CO80) of the total FO in two experimental diets. The maximum replacement level of 80% was chosen because marine fish have essential fatty acid requirements (NRC, 2011), which may not have been met if 100% of FO was replaced, therefore health and welfare of cod was considered when formulating the diets. Experimental diets were steam pelleted using a laboratory pelleting mill (California Pellet Mill, San Francisco, USA). The initial size of pellet was 1.5 mm and was increased to 2.5 mm as the fish grew larger throughout the trial. Diets were stored at -20°C until needed.

Experimental fish

An experiment was conducted with juvenile cod (131.4 ± 19.4 g fish $^{-1}$ mean initial weight ± SD; 22.1± 1.2 cm fish $^{-1}$ mean initial length) at the Ocean Sciences Centre (St. John's, Newfoundland and Labrador, Canada) where fish were cultured from hatch, passive integrated transponder (PIT) tagged, and reared to initial experimental size. Fish were randomly distributed (660 total) into 6 experimental tanks (3000 L), each tank with 110 fish. This study was approved by the Institutional Animal Care Committee of Memorial University of Newfoundland, protocol number 10-50-CP. Fish were measured for length and weight prior to tank distribution. The fish were acclimated for one week on the control diet prior to the start of the feeding trial. The fish were also acclimated on the experimental diets for one week after initial sampling. Dietary treatments were fed to fish in duplicate tanks for the 12 week trial. Throughout the duration of the trial, a flow-through system of filtered (40 µm) seawater was supplied to each tank at a rate of 8 L min^{-1} and a photoperiod of 12 hours. The dissolved oxygen (10 mg L^{-1}) and water

temperature (10°C) were monitored daily. Fish were fed 1% of body weight of feed per tank per day in order to ensure equal consumption of feed between treatments. Mortalities were weighed and recorded throughout the trial. After initial sampling, a number of cysts were noticed on the spleens of several fish. As the experiment progressed, the number of fish with cysts on the spleen, gills and heart were documented. It was determined that fish were infected with the microsporian parasite, *Loma morhua*, and this became a focus of the experiment.

Sampling

Sampling occurred at week 0 (the day before experimental diets were fed), week 1, 2, 4, 6 and 12. Six fish per tank were randomly sampled and scanned for PIT tag identification and measured for length and weight. The whole liver was removed, weighed and sampled for dry matter and lipid analysis. The skin was removed on the left side and muscle tissue was subsampled for dry matter and lipid analysis. Lipid samples were stored on ice during sampling of each tank and processed hourly to weigh, cover with chloroform, flush with nitrogen and wrap in Teflon. Lipid samples were stored at -20°C until analysis. Sampling procedures for weeks 4 and 6 differed since fish were sampled for tissues selectively based on severity of *Loma* in individual fish. All fish were randomly selected from the tank, scanned for identification and measured for length and weight. Fish were then dissected to observe tissues that are known to be affected by *Loma* including the heart, gill and spleen (Khan, 2005). Tissues were sampled for lipid and dry matter analysis only if the fish exhibited less than 10 xenomas in each of the three *Loma* targeted tissues (i.e., fish had to have less than 10 xenomas on each tissue, the spleen,

heart and gills for the fish to be sampled for analysis). Fish with more than 10 xenomas in any one of the three specified tissues were discarded (i.e., if a fish had 10 xenomas on the spleen, but had 9 on the heart and 9 in the gills, the fish was not sampled for tissues because of the number of xenomas on the spleen). Tissues were inspected by the naked eye for xenomas. The heart and spleen were removed from the body and inspected individually. Each gill raker was inspected individually, however the count of 10 xenomas applied to the gills as a whole, not per gill raker. One person inspected the gills and heart for xenomas, and another person inspected the spleen. These two individuals completed inspection on their assigned organs throughout the sampling process; therefore inspection was consistent between fish. Growth data from fish with signs of high infection was compared to that of fish with low infection to determine if *Loma* had an effect on growth in this experiment. Thus, the *Loma* infection was categorized as "healthy" for those fish with less than 10 xenomas on each organ, or "unhealthy" with more than 10 xenomas on one of the three organs.

Lipid extracts

Lipid samples were extracted according to Parrish (1999). Samples were homogenized in a 2:1 mixture of ice-cold chloroform: methanol. Samples were homogenized with a Polytron PCU-2-110 homogenizer (Brinkmann Instruments, Rexdale, Ontario, Canada). Chloroform extracted water was added to bring the ratio of cholorform:methanol:water to 8:4:3. The sample was sonicated for six min in an ice bath and centrifuged at 4000 rpm for two min at room temperature. The bottom organic layer was removed using a double pipetting technique, placing a 2 ml lipid-cleaned Pasteur

pipette inside a 1 ml pipette, to remove the organic layer without disturbing the top aqueous layer. Chloroform was then added back to the extraction test tube and the entire procedure was repeated 3 times for muscle samples and 5 times for liver samples. All organic layers were pooled into a lipid-cleaned vial. The samples were concentrated using a flash-evaporator (Buchler Instruments, Fort Lee, New Jersey, USA).

Lipid class separation

Lipid class composition was determined using an Iatroscan Mark VI TLC-FID (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan), silica coated Chromarods and a three-step development method (Parrish, 1987). The lipid extracts were applied to the Chromarods and focused to a narrow band using 100% acetone. The first development system was hexane: diethyl ether: formic acid (99.95:1.0:0.05). The rods were developed for 25 min, removed from the system for 5 min and replaced for 20 min. The second development was for 40 min in hexane: diethyl ether: formic acid (79:20:1). The final development system had two steps, the first was 100% acetone for two 15 min time periods, followed by two 10 min periods in chloroform: methanol: chloroform-extracted water (5:4:1). Before using each solvent system the rods were dried in a constant humidity chamber. After each development system, the rods were partially scanned in the Iatroscan and the data were collected using Peak Simple software (version 3.67, SRI Inc). The Chromarods were calibrated using standards from Sigma Chemicals (Sigma Chemicals, St. Louis, Missouri, USA).

Fatty acid methyl ester (FAME) derivatization

For all muscle samples, lipid extracts were transesterified using 14% BF_3 in MeOH for 1.5 hours at 85°C. For all liver samples, lipid extracts were transesterified using the Hilditch reagent (1.5 H_2SO_4: 98.5 anhydrous MeOH) for 1 hour at 100°C. Reagents were added in the proportion of 1.5 ml reagent per 4-16 mg of lipid (Morrison and Smith, 1964). Samples were vortexed halfway through each derivatization reaction. To analyze the derivatization efficiency of both procedures, 18 samples of liver and muscle were transesterified using both methods, and then the lipid class composition of the methyl ester solution was determined by TLC-FID. The derivatization efficiency was calculated from the amount of underivatized acyl lipids. This value was divided by the amount of acyl lipids in the extract before transmethylation, expressed as a percentage, and subtracted from 100%.

All FAMEs were analyzed on a HP 6890 GC FID equipped with a 7683 autosampler. The GC column was a ZB wax+ (Phenomenex, Torrance, California, USA). The column length was 30 m with an internal diameter of 0.32 mm. The column temperature began at 65°C where it was held for 0.5 min. The temperature ramped to 195°C at a rate of 40°C min^{-1}, held for 15 min then ramped to a final temperature of 220°C at a rate of 2°C min^{-1}. This final temperature was held for 45 sec. The carrier gas was hydrogen flowing at 2 ml min^{-1}. The injector temperature started at 150°C and ramped to a final temperature of 250°C at 120°C min^{-1}. The detector temperature stayed at 260°C. Peaks were identified using retention times from standards purchased from Supelco (Bellefonte, Pennsylvania, USA): 37 component FAME mix (Product number 47885-U), PUFA 3 (product number 47085-U) and PUFA 1 (product number 47033-U).

Chromatograms were integrated using the Varian Galaxie Chromatography Data System, version 1.9.3.2 (Agilent Technologies, Colorado, USA).

Statistical methods

Statistical analysis followed methods outlined by Sokal and Rolf (1994). For analysis of growth data, lipid class data, and fatty acid data, where individual fish were weighed, measured and sampled, a three-way nested ANOVA was performed using the General Linear Model (Minitab 16 Statistical Software, State College, Pennsylvania, USA). The model was designed to test the effect of diet on the response variable and nested fish individuals within tanks to negate variability among tanks and individuals, while also testing for tank effects. Significant differences among treatment means were differentiated using the Tukey HSD multiple comparison test and all residuals were evaluated for homogeneity and normality. To analyze differences between initial and final fatty acids in muscle and liver, a two-sample t-test was performed to test between the initial and final CO80 fatty acids.

Four analyses were conducted in order to answer two major research questions with two models. In the first model, it was important to determine whether there were tank effects based on health status (healthy or unhealthy determined by the number of xenomas on the organs) that may have an effect on growth. In other words, did each tank have a consistent or insignificant proportion of *Loma* infected and healthy fish, or were some tanks more heavily infected which would create a tank bias. In order to answer this question, two analyses were conducted: 1) to determine if there was a growth differential

based on health and time and each tank, and 2) if there was a tank effect in either the healthy fish or heavily infected fish.

In the second model, the purpose was to isolate whether diet and health affected growth. In order to answer this question, two more analyses were conducted: 3) to determine whether substituting CO for FO had an effect on growth and did the presence of *Loma* confound this effect, and 4) to determine if there was a tank effect based on individual PIT tagged fish sampled for each dietary group.

All four analyses were conducted using the general linear model in Minitab. A multi-way ANCOVA was used for all analyses, using time as a regression covariate. Two *a priori* tests were hypothesized: (1) growth rate in cod fed the control diet is higher or lower than cod fed the lowest level of CO; (2) growth rate in cod fed the lowest level of CO is higher than cod fed the highest level of CO.

4.4 Results

Camelina oil fatty acid composition

The majority of fatty acids (%) were 18-carbon chains including 18:3ω3 (35.6 ± 0.6), 18:2ω6 (18.4 ± 0.3) and 18:1ω9 (11.5 ± 0.2). The next most abundant fatty acids were those with 20 carbons or greater in length: 20:1ω9 (12.9 ± 0.5), 22:1ω9 (2.6 ± 0.4) and 20:2ω6 (2.0 ± 0.1).

Experimental diet composition

Diets were isonitrogenous (45.7-47.6%) and isolipidous (12.9-13.2%) (Table 4.1). The FO diet contained slightly more moisture than the CO40 diet, with a difference of

3.5%. The diets were mostly composed of neutral lipid (7.7-8.7% ww^{-1}) (Table 4.2). Triacylglycerol (TAG) was the most predominant lipid class (45.9-49.4%), followed by phospholipid (23.7-30.3%). Free fatty acid (FFA) and sterol (ST) were both significantly higher in CO80 than FO diets, but CO40 did not differ from either diet. Acetone-mobile polar lipid (AMPL) was significantly higher in CO40 than FO and CO80. Most fatty acids were significantly different among the three experimental diets (Table 4.2). Saturated fatty acids (SFA) decreased with inclusion of CO; monounsaturated fatty acids (MUFA) and PUFA increased with CO inclusion. Total ω3 fatty acids were lower in CO80 than FO and CO40. The sum of ω6 fatty acids increased with inclusion of CO, which decreased ω3/ω6 ratio in CO40 and CO80 diets compared to the FO diet. Terrestrial plant fatty acids also increased with inclusion of CO. Generally, PUFA that were not present in CO (e.g., DHA, 22:5ω3, EPA) decreased in the diets. Similar results were observed with SFAs. Fatty acids present in CO (e.g., LNA, ALA, 20:1ω9) increased when FO was removed from the diet.

Health assessment and growth

Four analyses were conducted based on two research questions in order to isolate specific factors that may have affected growth of cod in this study. All analyses tested a regression model (growth rate, weight gain over time) and included several factors (health status, tank effect) to determine if the main factor of interest (diet treatment) could actually explain the difference in the response. Several analyses were undertaken based on the growth data (Table 4.3) to explain these differences and isolate the effect of diet treatment.

1) *Is there a difference in growth depending on health status and time?*

The first analysis was conducted to determine whether growth was dependent on health status and time and if health and time interact to affect growth (Table 4.4). The response variable used was a growth parameter, the specific growth rate (100% x [ln(Final body weight)-ln(Initial body weight)] measured as a percentage over the explanatory variable time, a regression variable to give a slope with units % day^{-1}. In this analysis, there were two explanatory variables: health (categorical variable, 2 levels-healthy or not based on number of xenomas outlined in methods section) and time (regression covariate). The interaction between health and time were tested and was not significant ($F = 2.02$; $p = 0.155$). Both main effects, however, were significant factors. Health status was found to significantly affect growth ($F = 8.84$; $p = 0.003$) as well as time ($F = 129$; $p < 0.0001$). The interaction term was not significant; therefore the slope did not significantly differ between healthy and unhealthy fish; however health and time were both significant factors separately.

2) *Is there a difference in growth within each tank for healthy and unhealthy groups?*

The second analysis was conducted to evaluate tank effects based on health status to determine if there were differences in growth within each tank for each health group (Table 4.5). The presence of *Loma* had a significant effect on growth in the fish in this experiment based on the results of the first analysis; this analysis will determine whether *Loma* consistently affected each tank, so that any tank effects can be identified. Both groups of fish (healthy and unhealthy) were tested for tank effects to ensure certain tanks were not more heavily infested than others which would affect dietary treatments. The response variable used was the specific growth rate measured as a percentage over the

explanatory variable time, a regression variable to give a slope with units % day^{-1}. In this analysis, there were two explanatory variables: tank (categorical variable, 6 levels- 2 tanks per dietary treatment = 6 tanks total) and time (regression covariate). The interaction between time and tank was not significant ($F = 1.61$; $p = 0.160$). Tank did not significantly affect growth ($F = 0.26$; $p = 0.936$). Time was a significant factor ($F = 48.77$; $p < 0.0001$). The interaction between time and tank was not significant ($F = 1.07$; $p = 0.380$). Tank did not significantly affect growth ($F = 1.00$; $p = 0.418$). Time was a significant factor ($F = 77.36$; $p < 0.0001$). The interaction between tank and time was not significant for healthy or unhealthy fish groups. There was no effect of tank for either health groups, however time significantly affected growth rate.

3) *Does substituting CO for FO have an effect on growth and did the presence of Loma confound this?*

The main purpose of this study was to determine whether cod can grow effectively while being fed a diet containing CO instead of FO. However, during the study the experimental cod were naturally exposed to the parasite, *Loma morhua* which may have confounded the effect of diet. This analysis was conducted to determine whether cod fed camelina diets grew significantly more or less than cod fed a FO diet and whether diet and health were interacting factors in this experiment. In this analysis, the focus was on the effect of CO on growth and whether health confounded this effect. Time was the regression covariate, and the previous analysis showed there is no interaction between health and time. Time obviously influences weight gain in fish, so the interaction term between diet and time may influence the effect of diet alone. A new question arises, was the difference in growth rate between diets significant after time is controlled? This

statistical control in the ANCOVA model will allow the effect of the regression variable (time) to be removed in order to arrive at a better analysis of the effect of diet. This will improve the analysis by achieving a lower error MS and a better analysis of the factors of interest in this study. First, the full model was analyzed to establish that there was no interaction term between diet and time, and then a second analysis was run with this interaction term removed to test whether diet had an effect on growth and if diet and health interacted to affect growth. The response variable used was a growth parameter, the specific growth rate measured as a percentage over the explanatory variable time, a regression variable to give a slope with units % day^{-1}. In this analysis, there were three explanatory variables: diet (categorical variable, 3 levels- FO diet, CO40 and CO80); health (categorical variable, 2 levels- healthy or unhealthy based on number of xenomas outlined in methods section) and time (regression covariate). The previous analysis showed that there was no interaction between health and time and it is assumed that time significantly affects weight gain across all diets, therefore the three way interaction between health, diet and time did not need to be tested. The interaction between diet and time was insignificant ($F = 2.37$; $p = 0.094$), therefore the model was rerun without this interaction term to arrive at a better analysis of diet and health.

The new model will detect if there was a significant difference in growth between diets after time is controlled. The effects of diet, health, time and the interaction of diet and health were tested (Table 4.6). The interaction term between diet and health was not significant ($F = 1.80$; $p = 0.167$). Diet and health can be examined as main effects. As observed in previous models, both health ($F= 13.04$; $p < 0.0001$) and time ($F = 125.21$; $p < 0.0001$) were significant factors. Diet, however, did not significantly affect growth ($F =$

0.09; p = 0.915). In the first model without controlling for time, there was a significant interaction between diet and health, which prevents us from looking at diet as a main effect. In the improved model controlling for time, this interaction was not significant which allows examination of the factor of interest. However, the effect of diet was not significant.

4) *Is there a tank effect based on the individual fish sampled for each diet group?*

All fish were PIT tagged prior to the study, therefore each fish was tracked individually and an exact measurement of weight gain was obtained for each fish. It is important to ensure that there is no bias of weight gain for individual fish in each tank in order to effectively draw conclusions regarding the factors of interest. Was there an effect of fish within tanks? This analysis tested the effect of time, tank and individual fish within tanks (nested ANCOVA). The response variable used was a growth parameter, the specific growth rate measured as a percentage over the explanatory variable time, a regression variable to give a slope with units % day^{-1}. In this analysis, there were three explanatory variables: tank (categorical variable, 6 levels- 2 tanks per dietary treatment = 6 tanks total), time (regression variable) and fish within tank (Table 4.7). Fish and tank were random factors, therefore there is no interaction. The interaction between tank and time was already tested; therefore this interaction was not of interest here and did not need to be tested since tank was a random factor. The focus of this analysis was on individual fish causing unwanted variation in the analysis. The nested term (fish within tanks) was not significant (F = 1.19; p = 0.095). The tank term was also not significant (F = 1.75; p = 1.22). Again, time was a significant factor (F = 43.44; p < 0.0001).

Remaining growth parameters

Remaining growth parameters were based on measurements taken from healthy fish at both 6 weeks and 12 weeks (Table 4.3). Cod were initially 128-132 g $fish^{-1}$and gained 54.5-61.1 g $fish^{-1}$ after 6 weeks of growth, to reach a final weight of 183-194 g $fish^{-1}$; however, final mass and weight gain were not different among dietary treatments. Cod were initially 22.3-22.8 cm in length and grew to 24.0-24.3 cm after 6 weeks. At the week 6 sampling, the growth rate was 0.83-0.87 % day^{-1}. There was no difference in CF, SGR, HSI or FCR among dietary treatments at week 6, and there was no difference between replicate tanks within treatments. After 12 weeks of the feeding trial, cod fed CO40 and CO80 lost weight compared to final weight measurements at week 6. The CF was lower at week 12 than week 6. The growth rate was about half the rate measured at week 6. HSI was also lower at week 12 than week 6. There was no significant difference among treatments for any of the measured growth parameters at week 12, nor was there a difference between tanks within treatments.

Lipid analysis

Due to the decline in health and decreased growth by week 12, results from tissue analysis were presented from week 6 instead of week 12 for a more accurate representation of how CO affects tissue fatty acids.

Muscle

Initial muscle samples were 0.7% lipid (w/w) (Table 4.8). The lipid proportion in the muscle did not change after 6 weeks, and did not differ among treatments (0.7%). Phospholipid was the predominant lipid class initially (89%). This proportion did not

change after 6 weeks, and did not differ among treatments (87-89%). TAG was 1.5% initially and increased in all treatments after 6 weeks, but the difference among treatments was not significant (3.3-4.0%). FFA were minimal throughout the experiment (0.3%) and did not differ among treatments after 6 weeks. ST were 3.8% initially and increased slightly after 6 weeks of feeding; however, the proportion among treatments did not differ (4.3-4.6%). AMPL was initially 3.0% and was 1.7% (FO), 1.4% (CO40) and 2.7 (CO80) after 6 weeks. The sum of neutral lipids slightly decreased from initial (0.1%) to final sampling (0.06-0.07%); the sum of polar lipids remained the same throughout the trial and the same among treatments (0.6%).

Fatty acids present at high levels in CO tended to increase significantly in the muscle tissue (Table 4.8). Proportions of 18:0, LNA, ALA, total ω6 and the sum of 18:2ω6 + 18:3ω3 ("terrestrial") were significantly higher in cod fed CO80 than FO fed cod. Although the level of ω3 was not different among treatments, the increase in total ω6 fatty acids caused the ω3/ω6 ratio to decrease progressively as CO was added to the diet. In contrast, 14:0, 16:0 and 16:1ω7 in cod fed CO80 significantly decreased in the flesh compared to the FO group. There was no difference among groups for the remaining fatty acid profile in the muscle tissue.

Liver

Initial liver samples were 30.7% ww^{-1}, most of which was neutral lipid (29.0% ww^{-1}) (Table 4.9). Total lipid was slightly lower after 6 weeks (23.7-25.6% ww^{-1}); however, both total lipid and neutral lipid did not differ among treatments. TAG was the predominant lipid class initially (75.7%), and was similar after 6 weeks of feeding (74.8-

77.1%), which did not differ among treatments. FFA, ST and AMPL in initial livers were similar to FO livers after 6 weeks, and did not differ among treatments at final sampling. Phospholipid in initial livers (3.6%) was similar to FO after 6 weeks (3.8%). Cod fed CO80 had significantly lower levels of phospholipid than FO after 6 weeks of feeding; however, CO40 did not differ from either treatment.

As observed in the muscle tissue, fatty acids present in high levels in CO tended to increase significantly in the liver tissue (Table 4.9). Levels of LNA, ALA, the sum of ω6 and the sum of 18:2ω6 + 18:3ω3 ("terrestrial") were significantly higher in cod fed CO40 and CO80 than FO fed cod. LC ω3 PUFA such as EPA, 22:5ω3 and DHA were significantly reduced in CO40 and C080 compared to FO. SFA levels decreased significantly with addition of CO in the diet, whereas MUFA increased with CO inclusion. PUFA remained the same despite dietary treatment. Total ω3 levels were lower in CO80 than FO, but CO40 did not differ between either treatments. Total ω6 levels were higher in CO40 and CO80 than FO. As a result, the ω3/ ω6 ratio was lower in camelina groups compared to FO.

4.5 Discussion

This study evaluated the use of CO to partially replace FO in diets for juvenile Atlantic cod for 12 weeks. CO is known to be high in ALA, the precursor fatty acid in the ω3 pathway (Zubr, 2009). The oil is also abundant in LNA, 18:1ω9 and 20:1ω9. After replacing 40% and 80% of FO with CO, the lipid and fatty acid profiles of the experimental diets were significantly different. In general, the substitution decreased SFA, and increased MUFA and PUFA, although LC ω3 PUFA decreased with the

removal of FO. Fatty acids abundant in CO increased significantly in CO40 and CO80 diets, mainly LNA, ALA, 20:1ω9 and total ω6 fatty acids, except 20:4ω6 which is present in FO. In order to determine if the change in dietary oil had an effect on growth and tissue lipids, growth was measured over 12 weeks and tissue samples were obtained throughout the experiment.

After initial sampling, a number of cysts were noticed on the spleens of several fish. As the experiment progressed, the number of fish with cysts on the spleen, gills and heart were documented. It was determined that fish were infected with the microsporian parasite, *Loma morhua*. Fish infected with *Loma* typically show signs of emaciation, lethargy and exhibit pale gills (Khan, 2005). Cysts or xenomas are most prevalent in the gills, spleen and heart (Morrison, 1983), with the gills being the preferred site of infection because of the high oxygen supply and good circulation which provide the ideal environment for maturation of spores (Morrison, 1983). It has been known to significantly affect weight gain, condition factor and feeding rates, and ultimately leads to mortality (Khan, 2005). Presence of the infection in hatchery-reared cod suggests that it might originate from infected wild fish living in the vicinity of the intake pipe and gaining entry via the incoming unfiltered seawater (Ramsay et al., 2002). This is likely the case in the present study, since the cod used in this experiment were hatched and reared in a land-based facility. Prevention of *Loma* in a hatchery system is difficult, unless the filtration system is 4-7 µm to remove spores (Khan, 2005). Despite signs of *Loma*, the experiment continued in order to determine if the camelina treated fish were less susceptible to illness caused by *Loma*. Therefore, it was important to statistically

isolate the main factor of interest in this study, dietary treatment, from other sources of variation that may have confounded the actual results of the experiment. Obviously, the health of fish became a factor that significantly affected the growth of the experimental fish. As a result, variation caused by *Loma* infection and tank effects needed to be accounted for prior to the analysis of differences in growth caused by diet alone in order to truly test the effect of diet. In general these analyses seemed effective in isolating the main diet factor, with the data generally fitting each model without major violation of assumptions. However, there were statistical issues with setting up the original models, likely with using time as a covariate when the slope cannot be estimated within levels of other factors. Therefore, the original two models (to test health and tank effects; and to test diet and health effects) were broken down into four analyses to answer the same questions.

The first model was used to determine if there were tank effects based on health status. The first analysis confirmed that there was a difference in growth based on health for each treatment, therefore growth was dependent on health and time, but the two factors did not interact. The second analysis determined that there was no effect of tank in either healthy fish or infected fish; therefore certain tanks were not more heavily infested than others which would affect the analysis of dietary treatments. The second model was used to determine if diet and health affect growth. The first analysis confirmed that health status significantly affected growth; however dietary treatment did not influence growth. The second analysis confirmed that there was no tank effect based on PIT tagged individuals for each treatment. There were no interactions detected between diet and other factors and the *a priori* comparisons were not undertaken since there was no significant

difference detected. Other post-hoc tests were not conducted because the only significant factor detected was health which contained only two levels, so post-hoc comparisons were not necessary. Therefore, after isolating health and tank effects from confounding the original hypothesis of the experiment, we were able to determine that there was no difference in growth based on dietary treatment. However, throughout most of the analyses, the error term was high. This limited the ability to detect any significant differences in diet since variation between diets was quite low. However, there may be other reasons due to experimental design that would explain why the effect of diet did not influence growth rate in cod.

The SGR for each treatment after 12 weeks (0.3-0.4% day^{-1}) was lower than typical values found for farmed cod at approximately this weight (0.8-0.9% day^{-1}) (Bjornsson and Steinarsson, 2002; Couture et al., 1998; Jobling et al., 1991). There was a significant difference in growth between fish that were considered healthy (less than 10 xenomas on each organ) and fish that were heavily infected with *Loma*; however, the criteria that categorized fish as healthy or not was determined by the number of xenomas observed in organs upon sampling. Fish that were considered "healthy" may have exhibited some signs of *Loma* infection; necropsy reveals massive infection with xenomas on spleen, heart, gills and inner body wall (Khan, 2005). However, only those fish with more than 10 xenomas in any of the 3 tissues examined were categorized as unhealthy. Most fish were infected with *Loma* by final sampling which explains such low SGR values compared to a normal growth rate for this size cod.

Growth data from week 6 of the experiment was compared to week 12 to observe the decline in fish health over 6 weeks. Cod fed CO40 and CO80 lost 3.9 and 10 g $fish^{-1}$

between week 6 and 12. Cod fed the FO diet gained 5.4 g $fish^{-1}$ between week 6 and 12. The SGR in week 12 was half compared to the SGR in week 6. The condition factor and HSI were both lower after 6 weeks. Khan (2005) found that the condition factor and hepatosomatic index, as well as other measures of health such as hematocrit, hemoglobin, plasma protein and lymphocytes were significantly lower in infected groups than a control group. The comparison of these two intervals demonstrates how quickly the parasite can deteriorate fish health. From the lowered growth rate between week 6 and week 12 as well as confirmation of multiple statistical tests that health was a factor in the growth of fish, it can be concluded that the microsporan parasite, *Loma morhua* had a negative effect on growth of cod. However, it was difficult to determine if any dietary treatments helped minimize negative effects caused by the infestation. For example, considering the population as a whole for each treatment, including both healthy and *Loma* infected fish; there was no significant difference in the growth rate. However if both health categories are considered separately, unhealthy fish fed the FO diet had a significantly higher growth rate than unhealthy fish fed CO80. The lower SGR in unhealthy cod fed CO80 was not due to any tank effects for this particular diet, since this was tested in one of the models. It is possible that diet acceptability was affected after fish health deteriorated, resulting in lower feed consumption, which has been documented in cod infected with *Loma* (Khan, 2005). It is also possible that the lower ω3 levels in CO80 diet was not significant enough to support health and growth compared to the FO group. A recent experiment found that when barramundi (*Lates calcarifer*) were fed a diet with rapeseed oil or FO for 6 weeks and then subjected to a pathogen (*Streptococcus iniae),*

fish fed on FO had a longer lasting and enduring response in their fatty acid and eicosanoid concentrations, compared with those fed on rapeseed oil (Alhazzaa et al., 2013). Therefore, the immune response under biologically stressful conditions caused by disease is highly influenced by dietary history. Further experimentation is needed to conclude whether fish fed CO express an appropriate immune response after a disease challenge compared to FO and other vegetable oils.

Although effort and caution were taken to effectively test the effect of diet without confounding variables, a significant difference in growth rate was not detected in cod fed a FO diet compared to CO diets. One reason for this could be due to the diet formulation itself. Both CO diets contained a portion of FO. Diets were formulated to meet the requirements for Atlantic cod, which contain high protein (45%) and low lipid (12%). Herring meal supplied almost half of the diet in all three experimental diets. Fish meal typically contains 7-10% lipid, so even in the highest camelina replacement diet (CO80), 20% of lipid is supplied from FO and an additional 7-10% lipid supplied from fish meal. Therefore lipid and fatty acid differences between these diets may be slight compared to the same replacement levels formulated for a high-lipid diet for other species, such as Atlantic salmon.

However, even subtle differences in the fatty acid profile of the diet were enough to significantly change the fatty acid profile of the muscle and liver tissue. Although, muscle tissue fatty acids were not altered as much as in the liver because cod muscle tissue is less susceptible to dietary changes than liver tissue, since tissues high in neutral lipid respond more readily to changes in dietary lipids than polar lipid (Sargent et al., 1989; Higgs and Dong, 2000). After 6 weeks of feeding, profiles from cod fed CO80

began to shift toward a slightly more "terrestrial" profile. Total ω6 fatty acids and the sum of LNA and ALA (terrestrial) increased significantly in the muscle and liver with inclusion of CO. Increases ω6 PUFA series is a typical result for fish fed a vegetable oil diet (Bell et al., 2003; Turchini et al., 2009; Turchini & Mailer, 2010). Although total ω3 fatty acids were not different among diets, the increase in ω6 fatty acids lowered the ω3/ω6 ratio. Low ω3/ω6 ratios typical of the Western diet induces pro-inflammation in humans (Egger and Dixon, 2010). Consuming fish is one of the best ways to increase this ratio (Egger and Dixon, 2010), however when fillets are high in ω6 PUFA it poses a problem for consumers purchasing fish for ω3 intake. The lowered ω3/ω6 ratio is also problematic for fish health. In the present study, heavily infected fish fed the FO diet had a significantly higher growth rate than heavily infected fish fed CO80. This may be directly related to the immune response, which was likely influenced by dietary history, particularly the ω3/ω6 ratio. The ω3/ω6 ratio in the diet strongly affects eicosanoid production; increased levels of ω3 LC-PUFA in the diet reduces the synthesis of ω6 LC-PUFA-derived pro-inflammatory eicosanoids and increases the production of anti-inflammatory eicosanoids from ω3 PUFA (Schmitz, et al., 2008; Wada et al., 2007). In fish, the use of dietary vegetable oils with lowered ω3/ω6 ratio (compared to dietary FO) induced the expression of pro-inflammatory markers such as COX-2, pro-inflammatory cytokines, such as TNF-a and IL-1b and other systemic inflammation markers after a pathogen challenge (Alhazzaa et al., 2013; Martens et al., 2010; Montero et al., 2010; Oxley et al., 2010). Although CO provides a significant level of ω3 PUFA, the high level

of ω6 PUFA likely causes competition between pro-inflammatory (ω6-derived) and anti-inflammatory (ω3-derived) eicosanoid families.

Essential fatty acids, particularly EPA and DHA, were not altered in cod muscle after fed CO diets, which suggest that fillets are generally equally as healthful as in FO fed cod. However, significant decreases in EPA and DHA in the liver highlight the effect of lipid storage and how certain tissues respond to dietary change. This evidence suggests that these essential fatty acids were either supplied in enough quantity to maintain these levels in the tissues, or they were sequestered in the muscle tissue so that levels can be maintained or PUFA synthesis has occurred to maintain these levels, which has been documented in other studies (Alhazzaa et al., 2013; Wijekoon, 2012). The lack of difference in essential fatty acids between FO and CO80 muscle also suggests that certain tissues may be able to compensate for alterations in conditions such as infection and changes in diet.

After careful statistical analysis of the growth rate of cod fed camelina diets and also infected with *Loma morhua*, there was no difference in growth between a commercial-type diet and diets containing CO. However, health was a significant factor that affected growth in this experiment. Cod fed CO80 that were also heavily infected with *Loma* grew significantly less than healthy cod fed the same diet. Therefore, the effects of *Loma* were not minimized by feeding a diet containing CO. Lipid and fatty acid profiles suggest that after 6 weeks of feeding, the effect of CO on muscle and liver was significant, but minimal in the muscle.

Table 4.1. Formulation and proximate composition of juvenile cod experimental diets

Diet	FO	CO40	CO80
Ingredient (% of diet)			
Herring meal	48.0	48.0	48.0
Wheat Gluten Meal	5.0	5.0	5.0
CPSP-G 5.0[1]	5.0	5.0	5.0
Wheat Middlings	16.8	16.8	16.8
Whey Powder	7.0	7.0	7.0
Krill Hydrolysate	2.0	2.0	2.0
Corn Starch (pre-gel)	5.6	5.6	5.6
Vitamin Mixture[2]	1.95	1.95	1.95
Mineral Mixture[3]	1.95	1.95	1.95
Choline Chloride	0.3	0.3	0.3
Herring Oil	6.4	3.84	1.28
Camelina Oil	---	2.56	5.12
Proximate composition analyzed (as fed)			
Moisture (%)	9.6	6.1	8.1
Ash (%)	2.5	2.0	2.5
Crude protein (%)	45.7	47.6	46.8
Lipid (%)	13.2	12.9	13.0

[1]Concentre proteique soluble de poisson (soluble fish protein concentrate)
[2]Northeast Nutrition, Truro, Nova Scotia, Canada. Vitamin Premix contains per kg: Vitamin A 0.9 g, Vitamin D3 8.0 g, Vitamin E 50.0 g, Vitamin K 3.0 g, Thiamin 2.8 g, Riboflavin 4.0 g, Pantothenic acid 24.0 g, Biotin 0.1 g, Folic acid 26.7 g, Vitamin B12 0.03 g, Niacin 15.1 g, Pyridixine 3.3 g, Ascorbic acid 10.8 g, Wheat middlings (carrier) 851.3 g
[3]Northeast Nutrition, Truro, Nova Scotia, Canada. Mineral Premix contains per kg: Mangonese oxide 12.3 g, Zinc oxide 20.6 g, Copper sulphate 6.1 g, Iodine 15.8 g, Wheat middlings (carrier) 954.2 g.

Table 4.2. Lipid and fatty acid composition of cod FO and CO diets[1]

Lipid composition	FO	CO40	CO80	*F*-stat	*p*-value
(% *ww*$^{-1}$)					
Neutral lipid	8.7 ± 0.4	8.4 ± 4.5	7.7 ± 1.1	0.08	0.92
Polar lipid	4.5 ± 0.6	4.5 ± 0.9	4.3 ± 0.2	0.09	0.91
(*% total lipid)*					
Triacylglycerol	45.9 ± 4.9	49.0 ± 3.9	49.4 ± 4.5	0.10	0.99
Free fatty acid	3.2 ± 0.3^{a}	4.8 ± 1.0^{ab}	8.0 ± 1.6^{b}	13.9	0.01
Sterol	2.5 ± 1.0^{a}	4.5 ± 1.8^{ab}	6.9 ± 0.8^{b}	7.78	0.02
AMPL[2]	3.9 ± 0.6^{a}	7.8 ± 1.4^{b}	3.5 ± 0.9^{a}	20.7	0.002
Phospholipid	30.3 ± 3.1	29.6 ± 2.5	23.7 ± 1.3	1.69	0.23
Fatty acids[3]					
14:0	5.5 ± 0.2^{a}	4.2 ± 0.3^{b}	2.9 ± 0.03^{c}	126	<0.001
16:0	18.7 ± 0.3^{a}	16.9 ± 1.3^{ab}	16.1 ± 0.4^{b}	8.29	0.020
16:1ω9	6.6 ± 0.1^{a}	5.2 ± 0.3^{b}	3.9 ± 0.02^{c}	190	<0.001
18:0	2.7 ± 0.03^{a}	2.5 ± 0.1^{b}	2.4 ± 0.05^{b}	20.0	0.002
18:1ω9	10.9 ± 1.7	13.1 ± 1.6	14.1 ± 0.06	4.7	0.070
18:1ω7	3.0 ± 0.01	2.8 ± 0.01	2.5 ± 0.1	56.4	0.001
18:2ω6 (LNA)	7.9 ± 0.2^{a}	10.9 ± 0.7^{b}	13.5 ± 0.2^{c}	139	0.001
18:3ω3 (ALA)	1.3 ± 0.04^{a}	5.0 ± 0.9^{b}	8.1 ± 0.3^{c}	118	<0.001
20:1ω9	4.8 ± 0.09^{a}	6.4 ± 0.4^{b}	7.4 ± 0.2^{c}	82.1	<0.001
20:4ω6	0.6 ± 0.01^{a}	0.5 ± 0.02^{b}	0.4 ± 0.02^{c}	126	<0.001
20:5ω3 (EPA)	11.1 ± 0.3^{a}	8.7 ± 0.3^{b}	6.0 ± 0.1^{c}	373	<0.001
22:1ω9	5.5 ± 0.1	5.3 ± 0.3	4.9 ± 0.3	4.6	<0.060
22:1ω7	0.7 ± 0.006^{a}	1.1 ± 0.1^{b}	1.3 ± 0.06^{c}	83.7	<0.001
22:5ω3	1.2 ± 0.2^{a}	0.9 ± 0.1^{a}	0.5 ± 0.3^{b}	13.5	0.006
22:6ω3 (DHA)	10.3 ± 0.2^{a}	9.4 ± 0.04^{ab}	8.5 ± 0.3^{b}	6.7	0.003
∑SFA[4]	27.6 ± 0.4^{a}	24.3 ± 1.0^{b}	22.6 ± 0.6^{b}	37.8	<0.001
∑MUFA[5]	33.7 ± 0.1^{a}	35.6 ± 1.0^{b}	36.3 ± 0.6^{b}	12.1	0.010
∑PUFA[3]	38.7 ± 0.2^{a}	39.8 ± 0.3^{b}	40.6 ± 0.1^{c}	38.3	<0.001
∑ω3	25.7 ± 0.4^{a}	25.7 ± 0.5^{a}	24.3 ± 0.2^{b}	11.5	0.010
∑ω6	8.9 ± 0.2^{a}	11.3 ± 0.6^{b}	14.5 ± 0.2^{c}	164	0.001
ω3/ω6	2.9 ± 0.1^{a}	2.3 ± 0.2^{b}	1.7 ± 0.03^{c}	91.1	<0.001
Terrestrial[6]	9.2 ± 0.1^{a}	15.2 ± 0.2^{b}	21.6 ± 0.1^{c}	3743	<0.001

[1]Values are mean ± SD (n=3). Different superscripts indicate significant differences

[2]Acetone mobile polar lipid

[3] Data expressed as area percentage of FAME (fatty acid methyl ester), values are mean (n=3) ± SD.

[4] Saturated fatty acid

[5] Monounsaturated fatty acid

[6] Polyunsaturated fatty acid

[7] 18:2ω6 + 18:3ω3

Table 4.3. Growth performance of juvenile Atlantic cod fed experimental diets after 6 and 12 weeks of growth[1]

	FO	CO40	CO80	F-stat (*Diet)*	*p*-value	*F*-stat *(Tank)*	*p*-value
6 weeks							
Initial body mass (g)	129 ± 20	128 ± 18	132 ± 14	-	-	-	-
Final body mass (g)	183 ± 36	183 ± 30	194 ± 38	0.30	0.76	1.36	0.27
Weight gain (g fish^{-1})	54.5 ± 19	54.6 ± 16	61.1 ± 33	0.67	0.57	0.40	0.76
Initial length (cm)	22.8 ± 1.8	22.3 ± 0.7	22.3 ± 1.8	-	-	-	-
Final length (cm)	24.3 ± 1.3	24.0 ± 1.0	24.3 ± 0.9	0.34	0.74	1.03	0.39
CF[2]	1.26 ± 0.1	1.31 ± 0.1	1.33 ± 0.2	1.71	0.32	0.40	0.76
SGR[3] (% day^{-1})	0.83 ± 0.2	0.84 ± 0.3	0.87 ± 0.4	0.96	0.48	0.06	0.98
HSI[4]	6.38 ± 1.5	6.51 ± 5.1	6.42 ± 2.0	0.08	0.93	0.27	0.85
FCR[5]	0.34 ± 0.1	0.29 ± 0.02	0.27 ± 0.05	0.32	0.75		
12 weeks							
Final body mass (g)	194 ± 39	180 ± 48	183 ± 39	0.23	0.84	1.77	0.18
Weight gain (g fish^{-1})	59.9 ± 40	50.7 ± 39	50.1 ± 36	0.12	0.89	2.32	0.09
Final length (cm)	25.3 ± 1.8	24.8 ± 1.4	25.4 ± 1.2	0.45	0.68	1.06	0.38
CF	1.19 ± 0.2	1.15 ± 0.2	1.1 ± 0.2	0.93	0.49	0.93	0.44
SGR (% day^{-1})	0.49 ± 0.2	0.32 ± 0.2	0.39 ± 0.2	0.13	0.88	2.18	0.11
HSI	5.74 ± 1.2	4.98 ± 1.7	5.75 ± 1.8	0.58	0.61	1.63	0.20

[1]Values are mean (n=12) ± SD. Means with different superscripts indicate significance.
[2]CF, Condition factor = body mass/ length^3
[3]SGR, Specific growth rate = 100 x [ln (final body weight) – ln(initial body weight)]/days
[4]HSI, Hepatosomatic index = 100 x (liver mass/ body mass
[5]FCR, Feed conversion ratio = Feed consumed (g fish^{-1}) / weight gain (g fish^{-1})

Table 4.4. Regression equations (slope is SGR, % day^{-1}) for each dietary treatment for both health groups, healthy and unhealthy fish

Diet	Healthy SGR	Unhealthy SGR
FO	y = 0.4993x + 5.610	y = 0.4303x – 12.58
CO40	y = 0.3282x + 8.195	y = 0.3265x + 1.664
CO80	y = 0.3917x – 0.523	y = 0.1893x + 12.26

Table 4.5. Regression equations (slope is SGR, % day^{-1}) for each tank in both healthy and unhealthy fish

Tank	Healthy SGR	Unhealthy SGR
1	y = 0.5859x - 16.61	y = 0.5354x + 5.205
2	y = 0.4299x - 1.234	y = 0.1068x + 11.82
7	y = 0.4262x - 5.129	y = 0.3725x + 8.905
8	y = 0.3419x + 0.763	y = 0.2821x + 12.49
10	y = 0.3671x – 7.563	y = 0.3253x + 6.015
12	y = 0.2804x + 7.484	y = 0.2380x + 6.831

Table 4.6. Regression equations (slope is SGR, % day^{-1}) for each dietary treatment including both healthy and unhealthy fish

Diet	Regression Equation (Slope SGR, % day^{-1})
FO	y = 0.3929x + 1.361
CO40	y = 0.2872x + 6.883
CO80	y = 0.2864x + 7.222

Table 4.7. Regression equations (slope is SGR, % day^{-1}) for each tank

Tank	Diet	SGR (% day^{-1})
1	FO	y = 0.4506x + 0.981
10	FO	y = 0.3053x + 2.430
7	CO40	y = 0.3160x + 7.549
12	CO40	y = 0.2456x + 5.703
2	CO80	y = 0.3046x + 5.703
8	CO80	y = 0.2681x + 8.69

Table 4.8. Lipid class and fatty acid composition of cod muscle after six weeks of growth[1]

Lipid composition	Initial	FO	CO40	CO80	*F*-stat	*p*-value
(% ww^{-1})						
Total lipid	0.7 ± 0.2	0.7 ± 0.03	0.7 ± 0.1	0.7 ± 0.1	1.83	0.30
Neutral lipid	0.1 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.92	0.49
Polar lipid	0.6 ± 0.02	0.7 ± 0.02	0.6 ± 0.1	0.6 ± 0.04	0.91	0.49
(*% total lipid)*						
Triacylglycerol	1.5 ± 0.7	3.3 ± 1.0	3.5 ± 1.2	4.0 ± 1.3	0.07	0.93
Free fatty acid	0.3 ± 0.1	0.3 ± 0.03	0.4 ± 0.01	0.3 ± 0.03	0.66	0.58
Sterols	3.8 ± 0.4	4.5 ± 1.0	4.6 ± 0.6	4.3 ± 0.3	0.07	0.94
AMPL[2]	3.0 ± 0.8	1.7 ± 0.3	1.4 ± 0.5	2.7 ± 1.0	0.44	0.68
Phospholipid	89 ± 2.7	89 ± 2.5	89 ± 3.9	87 ± 3.7	0.37	0.72
Fatty acids[3]						
14:0	0.9 ± 0.1	1.0 ± 0.03[a]	0.7 ± 0.02[ab]	0.4 ± 0.004[b]	6.73	0.01
16:0	20.8 ± 1.9	19.5 ± 0.6[a]	18.4 ± 0.8[b]	18.5 ± 0.5[b]	4.50	0.03
16:1ω7	1.4 ± 0.7	1.8 ± 0.04	1.4 ± 0.04	1.2 ± 0.02	4.20	0.07
18:0	4.2 ± 0.5	3.8 ± 0.03[a]	4.1 ± 0.4[ab]	4.5 ± 0.4[b]	6.09	0.01
18:1ω9	7.2 ± 0.5	7.2 ± 0.9	8.9 ± 1.0	8.6 ± 1.0	2.47	0.12
18:1ω7	2.4 ± 0.1	2.4 ± 0.02	1.5 ± 0.004	1.9 ± 0.9	1.58	0.24
18:2ω6 (LNA)	2.3 ± 0.2	3.0 ± 0.05[a]	3.6 ± 0.8[a]	4.9 ± 0.8[b]	10.9	0.001
18:3ω3 (ALA)	0.3 ± 0.1	0.4 ± 0.001[a]	1.3 ± 0.01[a]	2.4 ± 0.08[b]	14.9	<0.001
20:1ω9	1.0 ± 0.1	1.2 ± 0.03	1.6 ± 0.04	1.2 ± 0.9	0.62	0.55
20:4ω6	2.3 ± 0.1	2.3 ± 0.3	2.4 ± 0.2	2.3 ± 0.4	0.34	0.72
20:5ω3 (EPA)	17.2 ± 1.6	19.0 ± 0.6	18.0 ± 0.7	17.0 ± 0.2	2.08	0.162
22:1ω9	-	0.1 ± 0.003	0.1 ± 0.001	0.2 ± 0.001	2.23	0.19
22:5ω3	2.5 ± 0.2	2.5 ± 0.2	2.3 ± 0.07	2.1 ± 0.6	2.37	0.17
22:6ω3 (DHA)	31.5 ± 2.7	30.5 ± 2.2	30.2 ± 1.2	30.6 ± 2.0	0.07	0.94
∑SFA[4]	26.4 ± 2.3	24.4 ± 0.5	23.9 ± 0.8	23.4 ± 0.5	0.81	0.47
∑MUFA[5]	13.9 ± 1.0	13.9 ± 1.7	14.7 ± 1.7	14.6 ± 2.0	0.57	0.58

∑PUFA[6]	59.2 ± 2.8	61.0 ± 1.3	61.0 ± 1.3	61.8 ± 2.3	0.54	0.60
∑ω3	52.6 ± 2.8	53.5 ± 2.0	52.8 ± 1.8	52.9 ± 1.6	0.90	0.43
∑ω6	5.4 ± 0.2	6.2 ± 0.4[a]	7.0 ± 0.6[b]	8.1 ± 0.6[c]	14.4	0.01
ω3/ω6	9.7 ± 0.5	8.6 ± 0.8[a]	7.6 ± 0.9[b]	6.6 ± 0.7[c]	8.08	0.02
Terrestrial[7]	2.6 ± 0.3	3.4 ± 0.6[a]	4.9 ± 1.5[b]	7.2 ± 1.6[c]	12.4	0.01

[1]Values are mean (n=6) ± SD. Different superscripts indicate significant differences.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester), values are mean (n=6) ± SD.
[4]Saturated fatty acid
[5]Monounsaturated fatty acid
[6]Polyunsaturated fatty acid
[7]18:2ω6 + 18:3ω3

Table 4.9. Lipid class and fatty acid composition of cod liver after six weeks of growth[1]

Lipid composition	Initial	FO	CO40	CO80	*F*-stat	*p*-value
(% *ww*$^{-1}$)						
Total lipid	30.7 ± 2.3	24.0 ± 0.5	23.7 ± 2.2	25.6 ± 3.1	1.33	0.294
Neutral lipid	29.0 ± 1.6	20.3 ± 2.3	19.9 ± 1.0	21.5 ± 2.9	0.85	0.447
Polar lipid	1.8 ± 0.1	3.8 ± 0.3	3.8 ± 1.5	4.1 ± 1.0	0.16	0.852
(*% total lipid)*						
Triacylglycerol	75.7 ± 6.8	75.8 ± 8.0	74.8 ± 6.5	77.1 ± 5.9	0.17	0.846
Free fatty acid	6.6 ± 1.2	6.6 ± 1.5	5.4 ± 1.0	4.9 ± 0.9	3.38	0.061
Sterols	3.0 ± 1.1	3.2 ± 0.5	3.7 ± 1.0	3.8 ± 1.1	0.76	0.487
AMPL[2]	12.1 ± 1.0	12.1 ± 2.1	13.4 ± 1.2	11.8 ± 1.2	1.78	0.201
Phospholipid	3.6 ± 0.9	3.8 ± 1.0^{a}	2.9 ± 0.8^{ab}	2.4 ± 0.6^{b}	4.53	0.029
Fatty acids[3]						
14:0	3.9 ± 0.3	3.8 ± 0.3^{a}	3.3 ± 0.1^{ab}	3.1 ± 0.1^{b}	8.53	0.007
16:0	13.9 ± 0.4	13.6 ± 0.3^{a}	12.6 ± 0.4^{b}	12.7 ± 0.1^{b}	13.5	0.001
16:1ω7	6.8 ± 1.7	7.1 ± 0.5^{a}	6.2 ± 0.3^{b}	5.6 ± 0.5^{b}	11.9	0.002
18:0	4.3 ± 0.4	4.3 ± 0.4	4.3 ± 0.2	4.3 ± 0.5	0.00	0.997
18:1ω9	17.9 ± 1.0	17.4 ± 0.5^{a}	18.7 ± 0.2^{ab}	19.3 ± 1.5^{b}	6.07	0.019
18:1ω7	4.5 ± 0.4	4.7 ± 0.2^{a}	4.4 ± 0.3^{ab}	4.3 ± 0.1^{b}	5.95	0.020
18:2ω6 (LNA)	4.5 ± 0.3	5.0 ± 0.3^{a}	7.0 ± 0.6^{b}	7.7 ± 0.7^{b}	38.9	0.006
18:3ω3 (ALA)	1.6 ± 0.1	1.0 ± 0.04^{a}	3.8 ± 0.9^{b}	4.9 ± 1.0^{b}	41.0	<0.001
20:1ω9	4.7 ± 0.8	5.1 ± 0.2^{a}	6.1 ± 0.4^{b}	6.4 ± 0.3^{b}	24.4	<0.001
20:4ω6	0.8 ± 0.1	0.7 ± 0.1^{a}	0.5 ± 0.004^{b}	0.6 ± 0.1^{b}	14.0	0.002
20:3ω3	0.0 ± 0.0	0.0 ± 0.0^{a}	0.2 ± 0.04^{b}	0.2 ± 0.02^{b}	7.95	0.004
20:5ω3 (EPA)	13.2 ± 0.5	13.5 ± 0.5^{a}	10.5 ± 1.2^{b}	9.7 ± 1.3^{b}	19.4	<0.001
22:1ω9	3.5 ± 0.3	3.4 ± 0.2	3.8 ± 0.2	3.6 ± 0.1	3.14	0.087
22:5ω3	2.0 ± 0.2	2.1 ± 0.1^{a}	1.8 ± 0.04^{b}	1.6 ± 0.1^{b}	25.7	<0.001
22:6ω3 (DHA)	8.9 ± 0.5	9.0 ± 0.4^{a}	7.8 ± 0.2^{b}	7.4 ± 0.9^{b}	10.9	0.003
∑SFA[4]	23.5 ± 0.8	22.6 ± 0.6^{a}	20.9 ± 0.7^{b}	20.9 ± 0.8^{b}	9.45	0.005

∑MUFA[5]	40.4 ± 0.8	40.0 ± 0.8[a]	42.1 ± 0.3[b]	42.1 ± 1.3[b]	7.08	0.012
∑PUFA[6]	36.2 ± 1.0	37.4 ± 0.5	37.0 ± 0.4	37.0 ± 0.8	0.41	0.672
∑ω3	27.8 ± 0.7	28.0 ± 0.3[a]	26.6 ± 0.2[ab]	25.8 ± 1.8[b]	6.39	0.016
∑ω6	6.3 ± 0.3	6.7 ± 0.6[a]	8.5 ± 0.6[b]	9.5 ± 0.7[b]	25.9	<0.001
ω3/ω6	4.4 ± 0.2	4.2 ± 0.4[a]	3.2 ± 0.3[b]	2.7 ± 0.4[b]	22.8	<0.001
Terrestrial[7]	6.1 ± 0.3	6.5 ± 0.3[a]	8.3 ± 0.4[b]	9.0 ± 0.6[b]	43.3	<0.001

[1]Values are mean (n=6) ± SD. Different superscripts indicate significant differences.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester), values are mean (n=6) ± SD.
[4]Saturated fatty acid
[5]Monounsaturated fatty acid
[6]Polyunsaturated fatty acid
[7]18:2ω6 + 18:3ω3

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Chapter 5. Changes in tissue lipid and fatty acid composition of farmed rainbow trout in response to dietary camelina oil as a replacement of fish oil

5.1 Abstract

Camelina oil (CO) replaced 50% and 100% of fish oil (FO) in diets for farmed rainbow trout (initial weight 44 ± 3 g fish^{-1}). The oilseed is particularly unique due to its high lipid content (40%) and high amount of α-linolenic acid (ALA, 18:3ω3) (30%). Replacing 100% of FO with CO did not negatively affect growth of rainbow trout after a 12 week feeding trial (FO= 168 ± 32 g fish^{-1}; CO= 184 ± 35 g fish^{-1}). Lipid and fatty acid profiles of muscle, viscera and skin were significantly affected by the addition of CO after 12 weeks of feeding. However, the final amount of docosahexaenoic acid (DHA, 22:6ω3) and eicosapentaenoic acid (EPA, 20:5ω3) was 563 mg in a 75 g fillet (1 serving) were enough to satisfy daily DHA and EPA requirements (250 mg) set by World Health Organization. Other health benefits include lower SFA and higher MUFA in filets fed CO *vs* FO. Compound-specific stable isotope analysis (CSIA) confirmed that the δ^{13}C isotopic signature of DHA in CO fed trout shifted significantly compared to DHA in FO fed trout. The shift in DHA δ^{13}C indicates mixing of a terrestrial isotopic signature compared to the isotopic signature of DHA in fish oil-fed tissue. These results suggest that ~27% of DHA was synthesized from the terrestrial and isotopically lighter ALA in the CO diet rather than incorporation of DHA from fish meal in the CO diet. This was the first study to use CSIA in a feeding experiment to demonstrate synthesis of DHA in fish.

5.2 Introduction

Long chain (LC) ω3 polyunsaturated fatty acids (PUFA) have been recognized for their health benefits for decades. This group of fatty acids is essential as they cannot be synthesized and therefore must be consumed in adequate amounts from the diet. Docosahexaenoic acid (DHA 22:6ω3) and eicosapentaenoic acid (EPA, 20:5ω3) are most associated with benefits in heart, brain and eye health (Swanson et al., 2012). They are synthesized by marine microorganisms and travel through the ocean food chain. Therefore, the most efficient source of EPA and DHA is through consumption of lipid-rich seafood, particularly from cold-water (Kris-Etherton et al., 2002). Nearly half of the world's seafood today is produced from aquaculture (FAO, 2012). Salmonids like rainbow trout (*Oncorhynchus mykiss*) are an excellent source of DHA and EPA, and are also a popular farmed fish species. Like humans, rainbow trout also have a requirement for essential fatty acids, therefore DHA and EPA must be provided in the diet through other fish oils, such as herring (NRC, 2011). Reducing the amount of fish oil (FO) in aquaculture feeds is critical for sustainability and economic reasons (Turchini et al., 2009); therefore research into alternative dietary oils is necessary.

Research in this area has focused on testing various terrestrial plant and animal lipids in aquaculture feeds and its effect on growth and lipid biochemistry. Most terrestrial plant oils, such as palm, sunflower, soybean, and linseed, have a higher proportion of ω6 and ω9 fatty acids and a lower proportion of ω3 fatty acids in comparison to fish oil. Although replacing FO with these oils generally does not affect overall health and growth of the fish, most studies have shown reduced levels of ω3 PUFA in the fillet, particularly DHA and EPA (Bell et al., 2002; Bell et al., 2003;

Brandsen et al., 2003; Menoyo et al., 2005), which lowers health benefits normally provided to humans that consume fish. Therefore, research in terrestrial plant oils with significant amounts of ω3 is of particular interest.

The oilseed, *Camelina sativa* (commonly known as false flax or gold of pleasure) is a member of the Brassicaceae family which includes mustards, rapes, and canola (Budin et al., 1995). The crop originated in southeastern Europe and southwestern Asia, however it has recently been reintroduced to Canadian agriculture on account of its agriculturally robust nature (Warwick, 2011). The oilseed presents many beneficial attributes useful to the agricultural sector, including ability to grow in low-fertility soils, tolerance to insects and weeds, and it can survive frost and freeze-thaw cycles (Budin et al., 1995). The biochemical composition of camelina makes it an attractive nutritional source for the aquaculture feed industry. The oilseed is particularly unique due to its high lipid content (40%) and high amount of α-linolenic acid (ALA, 18:3ω3) (30%), an essential medium chain ω3 PUFA which is a precursor to longer chain ω3 PUFA (Zubr, 1997, Ni Eidhin et al., 2003). Camelina oil (CO) is also naturally protected against lipid oxidation, despite increased PUFA content because it contains a high amount of γ-tocopherol, the most potent antioxidant tocopherol isomer (Ni Eidhin et al., 2003). Generally, CO has more PUFA and monounsaturated fatty acids (MUFA) and lower saturated fatty acids (SFA) than other terrestrial plant oils, a fatty acid profile that benefits both fish and humans. These characteristics potentially give CO a unique nutritional advantage over other plant oils that have been previously used in aquaculture. The use of camelina oil has been shown to be suitable in feeds for Atlantic cod (Morais et al., 2012; Hixson et al., 2013, Chapter 2), and vegetable oil blends containing 20% CO have been

tested in Atlantic salmon (Bell et al., 2010); however it has not been tested in rainbow trout.

Although terrestrial plants do not naturally produce EPA and DHA, it is possible that the amount of ALA provided in the feeds may encourage synthesis of EPA and DHA in fish when these fatty acids are low or absent in the diet. Humans can synthesize DHA and EPA from ALA, however this process is inefficient with a conversion rate of <1% (Brenna et al., 2009). Anadromous fish, those that migrate from sea water to fresh, are known to more efficiently convert ALA to DHA and EPA. For example, Atlantic salmon (*Salmo salar*) express the Δ5 and 6 desaturase genes that are responsible for desaturation of ALA (Hastings et al., 2004; Zheng et al., 2005) and those fed a rapeseed oil diet showed even greater expression of these genes compared to the fish oil fed group (Jordal et al., 2005). In fact, this metabolic pathway is not just simply present and expressed, but is actually functional in the majority of the freshwater fish and salmonid species studied (Tocher, 2003). Consequently, studies have shown the LC ω3 PUFA input/ LC ω3 PUFA output ratio in fish fed vegetable oil diets is <1, indicating that salmonid aquaculture is a net producer of FO (Crampton et al., 2010; Turchini et al., 2011). Based on this premise, we hypothesize that rainbow trout will synthesize DHA and EPA to some degree from the high amount of ALA provided in the diet from CO. As a tool to confirm this hypothesis, compound specific stable isotope analysis (CSIA) was used. Fatty acid isotopic signatures are frequently used in food web studies to determine the transfer of fatty acids from prey to predator based on their $^{13}C/^{12}C$ ratio (Budge et al., 2008). In chemical and physical reactions, compounds containing the lighter ^{12}C isotope preferentially react, resulting in a fractionation of ^{12}C and ^{13}C isotopes. The ratio of $^{13}C/^{12}C$ is expressed as $\delta^{13}C$. The $\delta^{13}C$

of animal tissues is thought to reflect a mixture of $\delta^{13}C$ from all diet items (Voight et al., 2008). The ratio of carbon isotopes changes little as carbon moves through a food chain, therefore when dietary sources have distinctly different isotopic signatures, the ultimate sources of carbon and the relative contribution of different dietary items to a consumer can be determined (Post 2002; Budge et al., 2008). As a terrestrial plant, camelina fatty acids such as ALA and LNA have distinctly different isotopic signatures than the same fatty acids in marine sources like FO due to differences in the source of carbon (terrestrial carbon in the form of CO_2 gas vs. marine carbon as carbonate), which results in differences in $\delta^{13}C$ values due to how the stable isotopes are fractionated as CO_2 dissolves (O'Leary, 1988). Using CSIA, we can determine the dietary contribution of DHA and EPA in rainbow trout tissue fed a camelina oil diet and conclude whether these fatty acids were synthesized products from ALA in camelina oil or whether they have been incorporated in the tissue directly from the limited amount of DHA provided in the residual lipid in fish meal, depending on the $\delta^{13}C$ values.

The purpose of this study was to evaluate camelina oil as a suitable lipid source for farmed rainbow trout. A nutritional feeding trial was conducted with diets containing camelina oil in order to determine lipid and tissue fatty acid composition in tissues in response to changes in diet composition. Using CSIA, this study determined the dietary contribution that camelina oil may have on the synthesis of DHA and EPA in rainbow trout.

5.3 Methods

Experimental diets

Camelina (Calena cultivar) was grown and harvested by the Department of Plant and Animal Sciences, Dalhousie University at an off-campus location (Canning, Nova Scotia, Canada). The seeds were single pressed using a KEK 0500 press at Atlantic Oilseed Processing, Ltd. (Summerside, Prince Edward Island, Canada) to extract the oil and ethoxyquin was added to the final product.

All diets were formulated as isonitrogenous, isolipidous, and isocaloric practical diets and were produced at the Faculty of Agriculture Campus, Dalhousie University (Truro, Nova Scotia, Canada). The experimental treatments were as follows: a control diet with fish oil (FO); 50% fish oil replacement with camelina oil (CO50); and 100% fish oil replacement (CO100). Diets were formulated to meet nutritional requirements of rainbow trout (NRC, 2011). All diets were steam pelleted using a laboratory pelleting mill (California Pellet Mill, San Francisco, USA). The initial size of pellet was 1.5 mm and increased to 2.5 mm as the fish grew larger throughout each trial. Diets were stored at -20°C until needed.

Experimental fish

An experiment was conducted with juvenile rainbow trout (44.9 ± 10 g $fish^{-1}$ mean initial weight ± SD; 15.7 ± 1.2 cm mean initial length) at the Faculty of Agriculture Campus, Dalhousie University (CCAC, 2011-016). Fish were received from Fraser Mill's hatchery (Antigonish, Nova Scotia, Canada). Fish were randomly distributed (837 total) into 9 experimental tanks (200 L capacity), each tank with 93 fish. Dietary treatments were administered to triplicate tanks for the 12 week trial. The fish were acclimated on the control diet for one week prior to initial sampling. A flow through system of

freshwater was supplied to each tank at a rate of 10 L min^{-1} and a photoperiod of 12 hours. The dissolved oxygen (10 mg L^{-1}) and water temperature (14°C) was monitored daily. Fish were fed to apparent satiation twice daily (0900 hr and 1600 hr) and feed consumption was recorded weekly. Mortalities were weighed and recorded throughout the trial.

Sampling methods

Sampling occurred at Dalhousie University Agriculture Campus (Truro, Nova Scotia, Canada) at week 0 (the day before experimental diets were fed) and 12. Six fish per tank were randomly sampled on each sampling date and measured for length and weight. The skin was removed on the left side and muscle tissue was subsampled for dry matter and lipid analysis. The whole viscera and surrounding fat were weighed prior to sampling to measure the visceral somatic index. Lipid samples were stored on ice during sampling of each tank and were processed within an hour. Samples were collected in 50 ml test tubes that had been rinsed three times with methanol followed by three rinses with chloroform. The tubes were allowed to dry completely before they were weighed. The tubes were weighed again following the addition of the sample. After wet weights of samples were recorded, samples were covered with 8 ml of chloroform, the headspace in the tube was filled with nitrogen, the Teflon-lined caps sealed with Teflon tape and the samples were stored at -20°C until shipment to the Ocean Sciences Centre, Memorial University (St. John's, Newfoundland, Canada) for analysis (CCAC 12-09-MR, approved protocol for use of fish tissues from Dalhousie University).

Lipid extracts

Lipid samples were extracted according to Parrish (1999). Samples were homogenized in a 2:1 mixture of ice-cold chloroform: methanol. Samples were homogenized with a Polytron PCU-2-110 homogenizer (Brinkmann Instruments, Rexdale, Ontario, Canada). Chloroform extracted water was added to bring the ratio of cholorform:methanol:water to 8:4:3. The sample was sonicated for 6 min in an ice bath and centrifuged at 4000 rpm for two min. The bottom organic layer was removed using a double pipetting technique, placing a 2 ml lipid cleaned Pasteur pipette inside a 1 ml pipette, to remove the organic layer without disturbing the top aqueous layer. Chloroform was then added back to the extraction test tube and the entire procedure was repeated 3 times. All organic layers were pooled into a lipid-cleaned vial. The samples were concentrated using a flash-evaporator (Buchler Instruments, Fort Lee, New Jersey, USA).

Lipid class separation

Lipid class composition was determined using an Iatroscan Mark VI TLC-FID, silica coated Chromarods and a three-step development method (Parrish, 1987). The lipid extracts were applied to the Chromarods and focused to a narrow band using 100% acetone. The first development system was hexane: diethyl ether: formic acid (99.95:1.0:0.05). The rods were developed for 25 min, removed from the system for 5 min and replaced for 20 min. The second development was for 40 min in hexane: diethyl ether: formic acid (79:20:1). The final development system had two steps, the first was 100% acetone for two 15 min time periods, followed by two 10 min periods in chloroform: methanol: chloroform-extracted water (5:4:1). Before using each solvent system the rods were dried in a constant humidity chamber. After each development

system, the rods were scanned in the Iatroscan and the data were collected using Peak Simple software (ver 3.67, SRI Inc). The Chromarods were calibrated using standards from Sigma Chemicals (Sigma Chemicals, St. Louis, Missouri, USA).

Fatty acid methyl ester (FAME) derivatization

Lipid extracts were transesterified using the Hilditch reagent (1.5 H_2SO_4: 98.5 anhydrous MeOH) for 1 hour at 100°C. Reagents were added in the proportion of 1.5 ml reagent per 4-16 mg of lipid (Morrison & Smith, 1964). Samples were vortexed half way through each derivatization reaction. To check the derivatization efficiency, samples were transesterified and then the lipid class composition of the methyl ester solution was determined by TLC-FID. The derivatization efficiency is calculated from the amount of underivatized acyl lipids compared to the amount of methyl esters in a sample.

All FAMEs were analyzed on a HP 6890 GC FID equipped with a 7683 autosampler. The GC column was a ZB wax+ (Phenomenex, Torrance, California, USA). The column length was 30 m with an internal diameter of 0.32 mm. The column temperature began at 65°C where it was held for 0.5 min. The temperature ramped to 195°C at a rate of 40°C min^{-1}, held for 15 min then ramped to a final temperature of 220°C at a rate of 2°C min^{-1}. This final temperature was held for 45 sec. The carrier gas was hydrogen flowing at 2 ml min^{-1}. The injector temperature started at 150°C and ramped to a final temperature of 250°C at 120°C min^{-1}. The detector temperature stayed at 260°C. Peaks were identified using retention times from standards purchased from Supelco: 37 component FAME mix (Product number 47885-U), PUFA 3 (product number 47085-U) and PUFA 1 (product number 47033-U). Chromatograms were

integrated using the Varian Galaxie Chromatography Data System, version 1.9.3.2 (Agilent Technologies, Colorado, USA).

Compound-specific stable carbon isotope analysis

The δ^{13} values of 18:0, 18:1ω9, 18:2ω6, 18:3ω3, 20:5ω3, and 22:6ω3 were analyzed by GC-combustion-isotope mass spectrometry (GC-C-IRMS) at the Core Research Equipment and Instrument Training Network (CREAIT Network) at Memorial University of Newfoundland. FAMES from dietary camelina oil, fish oil, and rainbow trout muscle tissue from FO and CO100 groups were analyzed. All δ^{13}C values were reported relative to the Vienna Pee Dee Belemnite (VPDB) standard using standard Δ notation:

$$\delta^{13}C = \left[\frac{\frac{13c}{12c}(sample)}{\frac{13c}{12c}(standard)} - 1 \right] \times 1000$$

An aliquot of the methanol used during methylation of fatty acids was analyzed for the δ^{13}C composition. The average δ^{13}C of methanol was used to correct for the additional methyl group added to fatty acids during transesterification, by subtracting the proportional contribution of methanol to the δ^{13}C of FAME using the following equation:

$\delta^{13}C = (n + 1)[\delta^{13}C_{FAME}] - n[\delta^{13}C_{FFA}]$,

where n is the number of C atoms in the fatty acid (Budge et al., 2011). All FAME data were corrected for the contribution of this methyl group from this equation.

A two-end member mixing model (Budge et al., 2008) was used to determine the relative contribution of camelina oil and fish oil to DHA and EPA in muscle tissue:

$\delta^{13}C_{Tissue,\,k} = x_k\,\delta^{13}C_{CO} + (1- x_k)\,\delta^{13}C_{FO}$,

where x_k is the proportion of camelina oil C contribution to *k*, the fatty acid of interest, either DHA or EPA. $\delta^{13}C_{CO}$ is the isotope ratio of ALA in camelina oil, and $\delta^{13}C_{FO}$ is the isotope ratio of DHA in fish oil.

Statistical methods

Statistical analysis followed methods outlined by Sokal & Rolf (1994). To avoid committing a type II error (accepting a false null hypothesis), this study rigorously tested growth data in different ways to ensure conclusions made about camelina oil truly reflected the results. For analysis of growth data, lipid class data, and fatty acid data, where individual fish were weighed, measured and sampled, a three-way nested ANOVA was performed using the General Linear Model (Minitab 16 Statistical Software). The model was designed to test the effect of diet on the response variable and nested fish individuals within tanks to negate variability among tanks and individuals, while also testing for tank effects. For analysis of growth data that depend on comparison to an initial measurement and thus must be pooled per tank (i.e., mean weight gain and specific growth rate), a two-way ANOVA was performed to test the effect of diet and tank variability. In both cases, where significant differences occurred, treatment means were differentiated using the Tukey HSD multiple comparison test and all residuals were evaluated for homogeneity and normality. For analysis of growth data, *t*-tests for each growth parameter were additionally performed between FO and CO100 treatment groups (highest camelina replacement) to verify results from the ANOVA. For analysis of stable isotope data, two-sample t-tests were performed to detect the differences between $\delta^{13}C$

values of specific fatty acids (fish oil *vs* camelina oil; rainbow trout fed FO *vs* rainbow trout fed CO100). For each model tested, the residuals were examined to evaluate the appropriateness of the model, therefore normality, homogeneity and independence of residuals were considered. If a p-value was close to 0.05 and residuals were not normal, a p-randomization was conducted >1,000 times to test the data empirically.

5.4 Results

Experimental diet composition

The dry matter (89.1-90.3%), ash (2.7-3.2%), protein (44.4-46.9%) and lipid (14.2-15.7%) were similar across all diets and accurately reflected the formulation (Table 5.1). Camelina oil is composed mainly PUFA (60%) and MUFA (32%) and is low in SFA (11%) (Table 5.2). The unsaturated ALA (30%), LNA (24%), 18:1ω9 (15%) and 20:1ω9 (11%) were the most abundant fatty acids in the oil. The diets were mainly composed of neutral lipid (13-15%), but CO diets were significantly lower in polar lipid (0.3%) than the fish oil diet (1%) (Table 5.3). Triacylglycerol (TAG) and sterol (ST) significantly increased with the addition of CO from 79% (FO) to 83% (CO50 and CO100) and 0.2% (FO) to 4% (CO50 and CO100). Phospholipid (PL) was significantly higher in FO (5%) than CO diets (1%). Saturated fatty acids (SFA) significantly decreased when fish oil was replaced with camelina (FO>CO50>CO100), but monounsaturated fatty acids (MUFA), PUFA and total ω3 increased significantly with the addition of camelina oil (FO<CO50≤CO100). Fatty acids that were typically high in CO increased significantly in the CO50 and CO100 diets compared to FO, particularly 18:1ω9, 18:2ω6 and 18:3ω3.

Growth performance

The initial weights of the fish were not significantly different among treatments at the start of the experiment (43-48 g fish^{-1}; Table 5.4) and did not vary among tanks within treatments (F= 0.93; p= 0.49). The initial lengths (standard) of the fish did not vary significantly at the start of the experiment (15-16 cm) and did differ significantly among tanks (F= 0.65; p= 0.69). The final weight of the fish ranged from 168 g fish^{-1} (FO) to 184 g fish^{-1} (CO100), and did not vary among treatments or tanks within a treatment (F= 0.99; p= 0.45). Since the maximum difference in final weight was between FO and CO100 fish, the minimum detectable difference was calculated, which is the difference between means that could have been significantly different. The purpose of this calculation was two-fold: to ensure that the difference in means between FO and CO100 fish was in fact different when compared directly without CO50 included, and to ensure that the statistical design was sufficient to detect differences between these treatments. Given the same variance and sample size as the original data, two-sample *t*-tests were performed with two different means until a significant difference was observed. Each time a *t*-test was performed the difference between the two means was increased. The difference between FO fish final mass (168 g) and CO100 fish final mass (184 g) was 16 g. If the final weight of FO fish was 164 ± 32 and CO100 fish 188 ± 35 (n= 18), the difference in weight would be 24 g and is significantly different (p= 0.039). A difference of 22 g between means was not significant (p= 0.057). The difference between FO and CO100 final weights would have to increase by 1.5 times before a difference could be detected. Alternatively, by increasing the sample size from 6 fish per tank (n=18) to 12 fish per tank (n=36), a significant difference between means could be observed (p= 0.047). Therefore, the experimental design was accurate in accepting the null hypothesis, since the design would

need to alter dramatically and a significant biological difference would need to occur in order for a significant difference between FO fish and CO100 fish to be detected. At the end of the experiment, fish gained between 125 g $fish^{-1}$ (FO) and 136 g $fish^{-1}$ (CO100), again with no significant difference among treatments or tanks. The final length was the same across all treatments (23 cm) and no difference among tanks in a treatment ($F= 0.98$; $p= 0.452$). The condition factor did not vary among treatments or tanks ($F= 0.66$; $p= 0.684$). There was no difference in SGR among treatments (1.59-1.62% day^{-1}) and no difference among tanks in treatments. The visceral somatic index did not differ among treatments (12.2-12.5) or tanks ($F= 2.18$; $p= 0.06$). The FCR did not differ among treatments or tanks; however, a two sample *t*-test was performed between FO and CO100 fish which found that the FCR was significantly lower in FO than CO100 fish.

Muscle tissue lipid and fatty acid composition

Initially, rainbow trout muscle contained 1.2% total lipid (ww^{-1}), composed of neutral lipid (0.8%) and polar lipid (0.4%) (Table 5.5). TAG was the dominant lipid class with 71%, followed by phospholipid (PL) (21%), AMPL (6%) and sterol (ST) (2%). After the 12 week experiment, there was no significant difference in total lipid (2-4%ww^{-1}), neutral lipid (2-3%ww^{-1}) and polar lipid (0.4%ww^{-1}) among all treatments. TAG remained the same among treatments (75-86%), however since the original p-value was close to 0.05 and residuals were not homogenous or normal, a p-value randomization was conducted 5000 times. The randomized p-value was 0.08, confirming TAG was not significantly different among treatments. PL was significantly lower in CO50 (9%) than

FO (17%).There were no significant differences among tanks within each treatment for muscle lipid classes.

Initial rainbow trout muscle tissue composition was composed mainly of PUFAs (40%) and MUFAs (35%), with some SFAs (22%) (Table 5.5). The ω3 fatty acids accounted for 26% of the total fatty acid composition and the ω6's accounted for 13%. Of the 18-carbon fatty acids, 18:1ω9 was highest (20%) followed by LNA (10%). After 12 weeks, replacing FO with CO significantly decreased the SFA content in muscle tissue from 25% (FO) to 15% (CO100) and increased the MUFA content from 31% (FO) to 39% (CO100). The PUFA content in the muscle was the same whether FO or CO diets were consumed. The total ω3 content of the muscle tissue decreased significantly and the ω6 content increased significantly comparing FO-fed fish and CO100-fed fish. EPA in the muscle decreased significantly with the addition of CO, from 10% (FO) to 3% (CO100). DHA levels in the muscle decreased by about half with the addition of CO; from 15% (FO) to 8% (CO100). 20:3ω3 in the muscle was 10 times higher in CO100 (1%) than FO (0.09%). In general, the muscle tissue of fish fed-CO50 was significantly different from both FO fish and CO100 fish, with values in between those of FO fish and CO100 fish. There were no significant differences among tanks within each treatment for any of the muscle tissue fatty acids.

Skin tissue lipid class and fatty acid composition

Rainbow trout skin increased in total lipid from 2.2% (ww^{-1}) (FO) to 3.7% (ww^{-1}) (CO100) (Table 5.6). Neutral lipid in the skin was also significantly higher in CO100 (3.2 %) than FO (1.8%). Polar lipid was significantly higher in CO50 (0.8%) than FO (0.4%)

and CO100 (0.5%). The skin of fish fed CO50 was significantly lower in TAG (79%) than FO fish (83%) and CO100 fish (85%). There were no significant differences among tanks within each treatment for skin lipid classes.

In the skin, including subcutaneous fat, SFA decreased significantly with the addition of CO, while MUFA and PUFA and terrestrial fatty acids increased significantly with CO inclusion (Table 5.6). Total ω3 fatty acids decreased significantly in the skin of trout fed CO100 (25%) compared to trout fed FO (28%). Total ω6 fatty acids in the skin increased significantly in trout fed CO100 (18%) compared to trout fed FO (8%). DHA decreased significantly in the skin with the addition of CO from 11% (FO) to 5% (CO100), as did DHA, from 10% (FO) to 2% (CO100). There were no significant differences among tanks within each treatment for any of the skin fatty acids.

Visceral fat lipid class and fatty acid composition

Fat surrounding the viscera in CO100-fed fish had significantly more total lipid (56% ww^{-1}) than CO50-fed fish (32% ww^{-1}) and FO-fed fish (35% ww^{-1}) (Table 5.7). Neutral lipid was also significantly higher in fish fed CO100 (52% ww^{-1}) than CO50 (26% ww^{-1}) and FO (30% ww^{-1}). TAG was significantly higher in fish fed CO100 (83%) than CO50 (72%) and FO (74%). AMPL was significantly higher in FO-fed fish (12%) and CO50-fed fish (15%) than CO100-fed fish (3%). There were no significant differences among tanks within each treatment for viscera fat lipid classes.

As observed in both muscle tissue and skin, SFA in visceral fat decreased significantly in CO100 (15%) compared to FO (24%) (Table 5.7). MUFA, PUFA and terrestrial fatty acids increased significantly with CO inclusion. Total ω3 fatty acids in the

viscera fat were significantly lower in CO100 fish (23%) and CO50 fish (24%) compared to FO fish (25%). DHA in the viscera fat was significantly higher in FO fish (9%) compared to both CO50 (6%) and CO100 fish (4%). EPA in the viscera fat was significantly higher in FO (10%) > CO50 (5%) > CO100 (2%). There were no significant differences among tanks within each treatment for any of the fatty acids in fat surrounding the viscera.

Compound-specific stable carbon isotope analysis

The $\delta^{13}C$ values for 18:0, 18:1ω9, LNA and ALA were significantly different between dietary camelina oil and herring oil used in CO100 and FO diets (Table 5.8). The $\delta^{13}C$ values in muscle tissue were significantly different for 18:0, 18:1ω9, LNA, ALA, DHA between CO-fed and FO-fed rainbow trout. The $\delta^{13}C$ values for muscle tissue fed either FO or CO100 differed significantly for all selected fatty acids, except for 20:5ω3. The tissue $\delta^{13}C$ for 18:1ω9 was most different between FO (-24.2‰) and CO100 (-29.5‰) than any other fatty acid. The $\delta^{13}C$ value for DHA in muscle tissue was significantly higher in CO100 fed fish than FO fed fish; which is a 1.3‰ difference from 22:6ω3 in muscle fed FO and a 2.4‰ difference from dietary FO.

5.5 Discussion

Replacing 100% of FO with CO did not negatively affect growth of rainbow trout after a 12 week feeding trial. Several different vegetable oils have been fed to farmed rainbow trout partially or totally substituting FO without compromising growth (Greene & Selivonchick, 1990; Caballero et al., 2002; Nadege et al., 2006), including camelina oil

in Atlantic cod (Morais et al., 2012; Hixson et al., 2013, Chapter 2). This was the first study to use CO to replace FO in diets for rainbow trout.

The higher FCR values observed in trout fed diets CO50 and CO100 could be due to slightly higher feed consumption by these fish, since growth rate and final weight were not significantly different among treatments. However, FCR values for rainbow trout that are less than 1 indicate successful feed conversion (Cowx, 2005; Bailey & Alanara, 2006; Thanuthong et al., 2011a); therefore all treatments are comparable to this standard for this species, fed at 15°C. Weight gain, condition factor, growth rate and visceral somatic index were not different among treatments, and typical for farmed rainbow trout at this size and grown at this temperature. In terms of growth performance, camelina oil can fully replace fish oil in farmed rainbow trout without negative effects.

Viscera was the preferential lipid deposition site, in agreement with other studies (Figueiredo-Silva et al., 2005; Corraze & Kaushik, 1999), but the total lipid content with CO100 feeding was much higher (56% ww^{-1}) than in FO (35% ww^{-1}). Similarly in the skin, total lipid was higher in camelina-fed groups than the control. Salmon fed a vegetable oil blend containing 20% CO had significantly higher lipid deposition and increased TAG content (Leaver et al., 2011), and since viscera was the primary lipid deposition site, it is not surprising that increased lipid was stored in this tissue. The muscle lipid composition was unaffected by dietary oil type, a result also observed by other studies that fed different vegetable oils to rainbow trout and salmon (Menoyo et al., 2005; Figueiredo-Silva et al., 2005). The lipid composition of the viscera most reflected that of the diet, because it was accumulated throughout the experiment from dietary fat and also accounted for most of the stored lipid. However, it should also be mentioned that

several studies noted that fat content in salmonid tissues responded differently to different lipid sources. For example, parallel studies with salmon of the same size revealed that differences in flesh adiposity depended on whether fish were fed palm, rapeseed or linseed oils (Menoyo et al., 2005). Therefore, it is difficult to compare changes in lipid composition in this study to other studies that tested different types of vegetable oils, since the response may be different, even when comparing fish of the same species and size.

In general, fatty acid profiles of all three tissues were significantly affected by the addition of CO, and the differences between FO and CO fish showed similar proportions and trends across all three tissues. In the muscle, SFA were significantly lower, MUFA significantly higher and PUFA the same across all diets. Although, the proportion of ω3 fatty acids in trout muscle was significantly lower in CO fish compared to FO, these differences were very small. There was a much greater difference in total ω6 fatty acids, which were about twice as high in CO100 muscle as FO muscle, which inevitably reduced the ω3/ω6 ratio by about one third, from 4.4 to 1.7. Despite the lower ω3/ω6 ratio, there is still almost twice as much ω3 than ω6 which is enough to help increase the low ω3/ω6 ratio in the typical Western diet, which is as low as 0.06 and the cause of many diseases, particularly cardiovascular (Simopoulus, 2002). Terrestrial fatty acids in the tissues were about four times higher in CO100-fed fish compared to FO-fed fish. Increases in these fatty acids is a common result when feeding vegetable oils to farmed salmonids (Rosenlund et al., 2001; Bell et al., 2003; Drew et al., 2007; Thanuthong et al., 2011b). High levels of ALA in fish fillets are still beneficial for human health, as it is an essential fatty acid in human nutrition (Simopoulus, 2002, Brenna et al., 2009). But,

proportions of DHA in the muscle tissue were about half the amount in CO100-fed trout compared to FO-fed trout; and EPA in the muscle tissue was about one fifth the level in CO100-fed trout compared to FO-fed trout. Most studies have shown that the proportions of EPA and DHA are decreased in fish fed diets containing vegetable oils; however the quantitative amount of DHA and EPA in fatty fish filets like rainbow trout likely still meets human nutritional requirements for these EFA. According to the World Health Organization (WHO), the daily requirement for DHA + EPA is 250 mg (WHO, 2008). Based on Canada's Food Guide, one serving of cooked fish is 75 g (Health Canada, 2011). Therefore, if one serving (75 g) of rainbow trout fed 100% camelina oil was consumed, the amount of DHA+EPA (563 mg) would be more than sufficient to meet the daily requirement recommended by the WHO (250 mg).

Including high levels of ALA in the diet did not prevent reductions in DHA and EPA in any tissue when fish oil was removed. Previous studies in rainbow trout and salmon have reported increases in DHA when fed a vegetable oil diet and have implied some conversion of ALA to DHA (Caballero et al., 2002; Menoyo et al., 2005) but in the present study, loss of DHA was half compared to the fish oil diet, for all tissues. Studies have suggested a possible metabolic competition between 18:2ω6 (linoleic acid, LNA) and ALA, since both fatty acids are substrates for the same Δ6 desaturase enzymes (Caballero et al., 2005, Skonberg et al. 1994).Therefore high content of dietary LNA in camelina oil may inhibit metabolism of ALA into EPA and DHA or high content of ALA may inhibit metabolism of LNA into ARA. Either situation may have been the case in the present study, since neither ω3 or ω6 fatty acids showed extensive desaturation or elongation products in fish fed the CO100 diet. Limited substrate availability also inhibits

the efficiency of Δ6 desaturase (Thanuthong et al., 2011a); however, 40% of fatty acids (LNA+ALA) in the present study were available substrates, so this may not be the issue. But, excessive C_{18} PUFA substrates could limit the availability of Δ6 desaturase to act on C_{24} fatty acids, and thus potentially limit final DHA production (Thanuthong et al., 2011b). However, a few fatty acids were observed that suggest elongation and desaturation had occurred, despite decreased levels of EPA and DHA. In muscle, viscera and skin, 18:4ω3, 18:5ω3, 20:3ω3 and 20:3ω6 increased significantly with the addition of camelina oil, although some of these fatty acids increased in very small proportions, therefore their biological significance is questionable. Also, some of these fatty acids are not typically known as intermediates in the ω3 pathway. The conventional ω3 pathway begins with Δ6 desaturation of ALA to 18:4ω3, elongation to 20:4ω3 and then Δ5 desaturation to EPA and two elongations followed by a second Δ6 desaturation and β-oxidation to DHA (Sprecher, 2000). Wei-Chun et al. (2012) postulated a bypass in the ω3 pathway whereby ALA is chain elongated to 20:3ω3. The study found that the barramundi elongase has the capacity for conversion of ALA and LA to their C_{20} products, suggesting an alternative pathway for PUFA synthesis bypassing the first Δ6 desaturation step, which is rate limiting and may slow the pathway (Park et al., 2009). Desaturation of 20:3ω3 continues in the usual pathway with Δ5 desaturase. Therefore PUFA synthesis may be more efficient with the option of alternative pathway when there is ALA and LNA competition at this rate limiting step of Δ6 desaturation, which may explain the increase in 20:3ω3 when camelina oil is supplied in the diet. However, this alternative pathway is yet to be confirmed with rainbow trout.

Although the level of DHA in the muscle was half that compared to the initial DHA level, it was still twice the level provided in the diet. It is possible that DHA was selectively incorporated in the muscle through the duration of the trial because levels in the diet were low, which has been suggested in other studies (Leaver et al., 2011; Wijekoon, 2012). Catabolism of DHA may have also occurred in order to meet metabolic needs of the fish, which explains lowered levels of DHA in the tissue compared to the initial samples. However, higher proportions of DHA were observed in the tissue than was provided in the diet, therefore it is also possible that some DHA was synthesized *de novo*. In order to confirm this, CSIA was used. Camelina oil and fish oil were isotopically distinct with differences in 18:0, 18:1ω9, LNA and ALA. In terrestrial ecosystems, $\delta^{13}C$ is based on plants with different photosynthetic pathways (e.g., c3, c4 and CAM) and in pelagic marine ecosystems $\delta^{13}C$ is based on phytoplankton (Post, 2002). These fatty acids (18:0, 18:1ω9, LNA and ALA) were slightly more isotopically enriched in fish oil than camelina oil, as camelina is a c3 plant and has $\delta^{13}C$ values between -25‰ to -35‰ (O'Leary 1988) and marine origins are isotopically heavier or enriched with ^{13}C (Phillips et al., 2005).

Different $\delta^{13}C$ values for DHA in tissues fed CO100 indicate that substantial fractionation occurred that resulted in isotopically lighter DHA in trout fed CO100 (29.7‰) compared to FO (28.4‰), a difference of 1.3‰. 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 shift in DHA $\delta^{13}C$ indicates mixing of a terrestrial and marine isotopic signature and significant fractionation. The mixing model estimates camelina oil contributed 27% to DHA in muscle tissue of trout fed CO100 and 14% to EPA. One

assumption however, is that the fractionation which occurred during elongation and desaturation was minimal. Kinetic fractionation of carbon is associated with the formation or breaking of a carbon bond. Elongation and desaturation of precursors results in fractionation, because enzymes preferentially utilize the lighter precursor resulting in depleted ^{13}C products (Monson & Hayes, 1982; Budge et al., 2011). This fractionation likely contributed very little to the overall shift toward the terrestrial isotopic signature, as it has been suggested that desaturation of fatty acids does not have any significant carbon isotopic fractionation effects (Schouten et al., 1998). The kinetic isotope effect expected for chain elongation from ALA to DHA should result in lighter DHA (Abrajano et al., 1994), which was observed in this experiment. The degree to which the shift in $\delta^{13}C$ in DHA was due to fractionation caused by biosynthesis and/or conservation of the $\delta^{13}C$ of ALA is not distinguishable, but fatty acids synthesized *de novo* are expected to reflect the $\delta^{13}C$ values of the carbon utilized from the diet (Ruess et al., 2005), in this case from ALA. Lipid-transport processes supplying TAG to tissues involve hydrolysis and re-esterification could explain the fractionation that occurred. There are many potential sources of fractionation of fatty acids, but most work to date in real systems has found little evidence to support it.

Therefore, the isotopically lighter DHA in CO100 fed trout compared to the significantly heavier DHA in FO fed trout suggests that the origins of DHA may have been terrestrial, i.e., DHA was likely synthesized from the terrestrial and isotopically lighter ALA in the CO100 diet. Although some residual DHA from fish meal in the CO100 diet may have been selectively incorporated in tissues, the $\delta^{13}C$ is assumed to be equivalent to that of fish oil. Other dietary components provide 1% lipid to the diet,

therefore their $\delta^{13}C$ values contributes very minimally to the $\delta^{13}C$ values in the muscle tissue. Therefore the isotopic shift observed in CO100 tissues indicates that some DHA was synthesized *de novo* (~27%); otherwise the $\delta^{13}C$ of DHA in CO100 muscle tissue would have matched the $\delta^{13}C$ in FO muscle tissue. Also, it is highly unlikely that DHA in the fish fed CO100 was simply DHA remaining from the originally fed diets because of its $\delta^{13}C$. If DHA was simply being catabolized and removed from the tissues, the isotopically lighter DHA would be removed preferentially, consequently leaving the more enriched DHA in the tissue; however DHA remaining in CO100 muscle tissue was in fact isotopically lighter. Biosynthesis in fish has been demonstrated using CSIA in a previous study. A controlled feeding experiment with mummichogs (*Fundulus heteroclitus*) found that shifts in nonessential amino acid $\delta^{13}C$ values in muscle tissue indicate a high degree of *de novo* biosynthesis, particularly in a diet that was low in protein content, and it was suggested that such a diet required biosynthesis to meet muscle composition demand (McMahon et al., 2010). Similarly, in the present study it is reasonable to suggest the biosynthesis of DHA by rainbow trout, as this fatty acid was depleted in the CO100 diet. This is the first study to use CSIA in a controlled feeding experiment to demonstrate synthesis of DHA in fish.

CO used to replace 100% of FO in diets for rainbow trout shows promise for the aquaculture industry. Growth performance of fish fed CO diets was not affected compared to a typical commercial diet. Although fatty acid proportions were significantly altered after 12 weeks of feeding, final DHA and EPA amounts in a 75 g filet were enough to satisfy daily DHA and EPA requirements set by the WHO. Other human health benefits include lower SFA and higher MUFA in filets of fish fed CO *vs* FO. Biosynthesis

of LC PUFA was demonstrated by evidence of elongated products and by the unique isotopic signature of DHA in CO100 tissue, confirmed by CSIA. Feeding CO diets for a full production cycle, including first feeding, would be beneficial to monitor fatty acid profiles to determine if DHA and EPA levels remain the same as the levels observed after 12 weeks, particularly since there is a difference between life stages and the ability of desaturation and elongation in salmonids.

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Table 5.1. Formulation and proximate composition of control and experimental diets

Diet	FO	CO50	CO100
Ingredient (% of diet)			
Herring meal	33.5	33.5	33.5
Soybean meal	10.0	10.0	10.0
Empyreal ®	8.0	8.0	8.0
Wheat middlings	16.75	16.75	16.75
Feather meal	5.0	5.0	5.0
Poultry by-product meal	5.0	5.0	5.0
Lignisol	2.5	2.5	2.5
Salt (Iodized)	0.3	0.3	0.3
D/L Methionine	0.2	0.2	0.2
Vitamin/Mineral premix[1]	0.2	0.2	0.2
Special premix[1]	0.25	0.25	0.25
Choline chloride	0.5	0.5	0.5
Lysine HCL	0.3	0.3	0.3
Herring oil	17.5	8.75	-
Camelina oil	-	8.75	17.5
Proximate composition analyzed, as-fed basis			
Moisture	89.1	90.1	90.3
Ash	3.2	2.7	2.9
Crude protein	44.4	46.2	46.9
Lipid	14.2	15.7	15.6

[1] Northeast Nutrition, Truro, Nova Scotia, Canada. Vitamin/Mineral Premix contains per kg: Zinc 77.5 mg, Manganese 125 mg, Iron 84 mg, Copper 2.5 mg, Iodine 7.5 mg, Vitamin A 5000IU, Vitamin D 4000IU, 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.
[2] Northeast Nutrition, Truro, Nova Scotia, Canada Special Premix contains per kg: Selenium 0.220 mg, Vitamin E 250 IU, Vitamin C 200 mg, Astaxanthin 60 mg, Wheat shorts 1988 mg.

Table 5.2. Lipid class and fatty acid composition of camelina oil[1]

Lipid composition	Camelina oil
(*% total lipid*)	
Triacylglycerol	49.9 ± 6.2
Free fatty acid	19.6 ± 3.2
Sterol	9.0 ± 0.6
AMPL[2]	10.2 ± 3.5
Phospholipid	11.0 ± 0.7
Fatty acid[3]	
18:0	2.2 ± 0.2
18:1ω9	15.1 ± 0.8
18:2ω6	23.8 ± 0.2
18:3ω3	29.3 ± 0.3
20:0	1.6 ± 0.1
20:1ω9	11.9 ± 0.1
20:1ω7	1.1 ± 0.01
20:2ω6	1.9 ± 0.02
22:1ω9	3.3 ± 0.02
∑SFA[4]	10.6 ± 1.2
∑MUFA[5]	32.2 ± 0.3
∑PUFA[6]	59.6 ± 1.2
∑ω3	30.4 ± 0.2
∑ω6	25.7 ± 0.2
ω3/ω6	1.2 ± 0.001

[1]Values are mean (n=3) ± SD.
[2]Acetone-mobile polar lipid
[3]Data expressed as area percentage of FAME (Fatty Acid Methyl Ester)
[4] Saturated fatty acid
[5] Monounsaturated fatty acid
[6] Polyunsaturated fatty acid

Table 5.3. Lipid class and fatty acid composition of control and experimental diets[1]

Lipid composition	FO	CO50	CO100	F-stat	p-value
(*% ww^{-1}*)					
Neutral lipid	13.2 ± 2.3	15.4 ± 2.2	15.3 ± 1.3	1.18	0.369
Polar lipid	1.0 ± 0.5^{a}	0.3 ± 0.1^{b}	0.3 ± 0.1^{b}	6.37	0.033
(*% total lipid*)					
Triacylglycerol	79.0 ± 1.8^{a}	82.6 ± 1.7^{b}	82.8 ± 1.4^{b}	5.35	0.046
Free fatty acid	14.1 ± 2.0	11.7 ± 2.0	11.4 ± 2.0	1.66	0.267
Sterol	0.2 ± 0.02^{a}	3.8 ± 0.3^{b}	3.8 ± 0.7^{b}	5.86	0.039
AMPL[2]	1.6 ± 0.8	1.1 ± 0.5	0.8 ± 0.3	1.81	0.242
Phospholipid	5.3 ± 3.2^{a}	0.8 ± 0.07^{b}	1.3 ± 0.3^{b}	5.24	0.048
Fatty acid[3]					
14:0	7.5 ± 0.3^{a}	3.9 ± 0.1^{b}	1.1 ± 0.02^{c}	1095	<0.001
16:0	21.2 ± 0.1^{a}	13.0 ± 0.1^{b}	11.7 ± 0.3^{c}	1401	<0.001
16:1ω7	8.8 ± 0.3^{a}	4.6 ± 0.05^{b}	1.1 ± 0.1^{c}	1054	<0.001
18:0	3.4 ± 0.1^{a}	3.0 ± 0.02^{b}	2.4 ± 0.1^{c}	75.9	0.01
18:1ω9	10.8 ± 0.2^{a}	18.2 ± 0.1^{b}	19.0 ± 0.4^{b}	429	<0.001
18:1ω7	3.5 ± 0.2^{a}	3.0 ± 0.1^{ab}	2.6 ± 0.2^{b}	21.7	0.02
18:2ω6 (LNA)	7.9 ± 0.3^{a}	11.7 ± 0.1^{b}	20.3 ± 0.3^{c}	1330	<0.001
18:3ω3 (ALA)	1.1 ± 0.06^{a}	12.0 ± 0.1^{b}	19.9 ± 0.7^{c}	822	<0.001
20:0	0.3 ± 0.04^{a}	0.7 ± 0.01^{ab}	0.9 ± 0.2^{b}	12.1	0.02
20:1ω9	2.0 ± 0.1^{a}	7.4 ± 0.7^{b}	8.3 ± 0.4^{b}	127	<0.001
20:2ω6	0.3 ± 0.2^{a}	0.6 ± 0.02^{ab}	1.0 ± 0.2^{b}	15.3	0.01
20:4ω6	0.7 ± 0.06^{a}	0.4 ± 0.01^{b}	0.2 ± 0.07^{b}	36.5	0.003
20:5ω3 (EPA)	12.8 ± 0.1^{a}	8.5 ± 0.1^{b}	2.4 ± 0.06^{c}	6015	<0.001
22:1ω9	1.4 ± 0.04	1.3 ± 0.01	1.3 ± 0.4	0.8	0.1
22:6ω3 (DHA)	7.0 ± 0.4^{a}	4.8 ± 0.3^{b}	3.9 ± 0.4^{c}	64.1	0.001
∑SFA[4]	33.3 ± 0.3^{a}	21.3 ± 0.1^{b}	16.4 ± 0.3^{c}	5361	<0.001
∑MUFA[5]	27.7 ± 0.3^{a}	35.9 ± 0.9^{b}	34.3 ± 0.5^{b}	192	<0.001
∑PUFA[6]	37.1 ± 2.7^{a}	43.0 ± 0.6^{b}	49.1 ± 0.6^{c}	41.0	0.002
∑ω3	23.1 ± 0.6^{a}	27.4 ± 0.7^{b}	27.0 ± 0.6^{b}	37.7	0.003
∑ω6	9.1 ± 0.5^{a}	12.8 ± 0.1^{b}	21.6 ± 0.1^{c}	1468	<0.001
ω3/ω6	2.6 ± 0.1^{a}	2.1 ± 0.01^{b}	1.25 ± 0.02^{c}	407	<0.001

[1]Values are mean (n=3) ± SD. Means with different superscripts indicate significance.
[2]Acetone-mobile polar lipid
[3]Data expressed as area percentage of FAME (Fatty Acid Methyl Ester)
[4] Saturated fatty acid
[5] Monounsaturated fatty acid
[6] Polyunsaturated fatty acid

Table 5.4. Growth performance of rainbow trout fed experimental diets for 12 weeks[1]

	FO	CO50	CO100	Max. Diff (%)	F-stat	p-value	*t*-stat	p-value
Initial body mass (g)	43 ± 12	42 ± 9	48 ± 12	-	1.52	0.29	-	-
Initial length (cm)	15.4 ± 1.2	15.5 ± 1.1	16.0 ± 1.2	-	2.07	0.21	-	-
Final body mass (g)	168 ± 32	166 ± 26	184 ± 35	9.5	1.77	0.25	-1.4	0.2
Weight gain (g $fish^{-1}$)	125 ± 4.0	123 ± 16	136 ± 19	8.8	0.66	0.55	-1.0	0.4
Final length (cm)	23.3 ± 1.4	23.1 ± 1.4	23.7 ± 1.5	1.7	0.99	0.43	-0.8	0.4
CF[2]	1.32 ± 0.08	1.34 ± 0.08	1.36 ± 0.08	3.0	1.78	0.25	-1.5	0.1
SGR[3] (% day^{-1})	1.62 ± 0.08	1.61 ± 0.2	1.59 ± 0.2	1.9	0.02	0.98	0.6	0.6
VSI[4]	12.4 ± 1.2	12.2 ± 1.4	12.5 ± 1.4	0.8	0.11	0.89	-0.2	0.8
FCR[5]	0.86 ± 0.05	0.93 ± 0.05	0.93 ± 0.02	8.1	2.10	0.20	**-5.5**	**<0.01**

[1] Values are mean (n=18) ± SD for measurements on individual fish. Values are mean (n=3) for measurements on tank means (weight gain, SGR, FCR).
[2] CF, Condition factor = body mass/ $length^3$
[3] SGR, Specific growth rate = 100 x [ln (final body weight) – ln(initial body weight)]/days
[4] VSI, Visceral somatic index = 100 x (viscera mass/ body mass)
[5] FCR, Feed conversion ratio = Feed consumption/ weight gain

Table 5.5. Lipid class and fatty acid composition of rainbow trout muscle tissue[1]

Lipid composition	Initial	FO	CO50	CO100	F-stat	p-value
(*% ww^{-1}*)						
Total lipid	1.2 ± 0.4	2.0 ± 1.1	3.7 ± 1.6	2.5 ± 1.2	3.3	0.10
Neutral lipid	0.8 ± 0.1	1.5 ± 0.8	3.3 ± 1.6	2.1 ± 1.0	3.9	0.09
Polar lipid	0.4 ± 0.02	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3	0.79
(*% total lipid*)						
Triacylglycerol	71.1 ± 7.1	74.9 ± 6.3	86.3 ± 5.5	79.4 ± 7.4	-	0.08
Sterol	1.5 ± 0.2	1.6 ± 0.4	2.1 ± 1.0	3.0 ± 1.0	2.9	0.14
AMPL[2]	6.4 ± 1.9	5.9 ± 2.9	2.5 ± 1.2	4.3 ± 2.6	3.8	0.09
Phospholipid	20.8 ± 6.5	17.2 ± 6.6^{a}	9.0 ± 3.3^{b}	13.0 ± 5.0^{ab}	4.6	0.02
Fatty acid[3]						
14:0	2.7 ± 0.3	4.7 ± 0.3^{a}	3.1 ± 0.1^{b}	1.4 ± 0.1^{c}	295	< 0.001
16:0	15.2 ± 1.1	15.5 ± 0.4^{a}	13.2 ± 0.5^{b}	10.3 ± 0.3^{c}	338	< 0.001
16:1ω7	5.8 ± 0.5	7.1 ± 0.3^{a}	4.7 ± 0.1^{b}	2.5 ± 0.2^{c}	617	< 0.001
18:0	3.8 ± 0.3	3.2 ± 0.1^{a}	3.0 ± 0.1^{b}	2.8 ± 0.1^{c}	104	< 0.001
18:1ω9	19.9 ± 1.8	13.1 ± 0.2^{a}	17.1 ± 0.4^{b}	20.8 ± 0.4^{c}	474	< 0.001
18:2ω6 (LNA)	10.3 ± 0.9	5.8 ± 0.3^{a}	10.3 ± 0.4^{b}	14.5 ± 0.3^{c}	1141	< 0.001
18:3ω3 (ALA)	1.1 ± 0.04	1.2 ± 0.1^{a}	7.9 ± 0.4^{b}	12.9 ± 0.4^{c}	2421	< 0.001
18:4ω3	0.7 ± 0.06	1.2 ± 0.1^{a}	1.1 ± 0.1^{b}	1.6 ± 0.2^{c}	17.5	0.003
18:5ω3	0.1 ± 0.04	0.2 ± 0.02^{a}	0.5 ± 0.02^{b}	0.7 ± 0.03^{c}	378	< 0.001
20:1ω9	2.8 ± 0.5	3.0 ± 0.2^{a}	6.0 ± 0.3^{b}	8.5 ± 0.4^{c}	377	< 0.001
20:2ω6	0.4 ± 0.06	0.3 ± 0.03^{a}	0.9 ± 0.04^{b}	1.4 ± 0.1^{c}	749	< 0.001
20:3ω6	0.4 ± 0.05	0.2 ± 0.02^{a}	0.2 ± 0.02^{a}	0.4 ± 0.01^{b}	231	< 0.001
20:4ω6	0.9 ± 0.1	0.8 ± 0.05^{a}	0.6 ± 0.05^{b}	0.3 ± 0.06^{c}	108	< 0.001
20:3ω3	-	0.09 ± 0.01^{a}	0.6 ± 0.04^{b}	1.01 ± 0.07^{c}	481	< 0.001
20:4ω3	0.5 ± 0.08	0.8 ± 0.07	0.8 ± 0.08	0.9 ± 0.2	4.69	0.06
20:5ω3 (EPA)	5.5 ± 0.5	10.1 ± 0.4^{a}	5.7 ± 0.2^{b}	2.5 ± 0.3^{c}	897	< 0.001
22:1ω9	2.9 ± 0.5	2.8 ± 0.2^{a}	2.6 ± 0.2^{a}	2.4 ± 0.1^{b}	8.3	0.02
22:5ω3	1.5 ± 0.2	2.9 ± 0.1^{a}	1.7 ± 0.1^{b}	0.7 ± 0.05^{c}	1987	< 0.001
22:6ω3 (DHA)	15.8 ± 2.2	15.4 ± 1.0^{a}	11.2 ± 1.1^{b}	7.5 ± 1.3^{c}	43.0	< 0.001
∑SFA[4]	22.2 ± 1.5	24.7 ± 0.6^{a}	20.2 ± 0.7^{b}	14.9 ± 0.3^{c}	1083	< 0.001
∑MUFA[5]	35.4 ± 1.1	30.6 ± 0.7^{a}	35.0 ± 0.8^{b}	39.0 ± 0.9^{c}	117	< 0.001
∑PUFA[6]	40.2 ± 4.0	44.2 ± 1.1	44.5 ± 1.2	45.7 ± 0.8	3.66	0.091
∑ω3	25.5 ± 3.0	32.7 ± 1.2^{a}	29.6 ± 1.1^{b}	27.8 ± 1.1^{c}	22.0	0.002
∑ω6	12.6 ± 1.1	7.5 ± 0.4^{a}	12.1 ± 0.5^{b}	16.8 ± 0.3^{c}	1536	< 0.001
Terrestrial[7]	11.4 ± 1.1	7.0 ± 0.4^{a}	18.1 ± 0.8^{b}	27.4 ± 0.7^{c}	1887	< 0.001
ω3/ω6	2.0 ± 0.2	4.4 ± 0.3^{a}	2.5 ± 0.1^{b}	1.7 ± 0.1^{c}	823	< 0.001

[1]Values are mean (n=9) ± SD. Means with different superscripts indicate significant differences at the end of the experiment.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester)
[4]Saturated fatty acid
[5] Monounsaturated fatty acid
[6] Polyunsaturated fatty acid
[7] Terrestrial = 18:2ω6 + 18:3ω3

Table 5.6. Lipid class and fatty acid composition of rainbow trout skin after 12 weeks[1]

Lipid composition	FO	CO50	CO100	F-stat	p-value
(*% ww^{-1}*)					
Total lipid	2.2 ± 0.6^{a}	3.6 ± 0.8^{b}	3.7 ± 1.2^{b}	4.2	0.04
Neutral lipid	1.8 ± 0.5^{a}	2.8 ± 0.8^{ab}	3.2 ± 1.1^{b}	3.8	0.04
Polar lipid	0.4 ± 0.1^{a}	0.8 ± 0.1^{b}	0.5 ± 0.1^{a}	11.2	0.001
(*% total lipid*)					
Triacylglycerol	83.3 ± 3.2^{a}	78.6 ± 4.9^{b}	84.6 ± 1.0^{a}	10.1	0.01
AMPL[2]	11.6 ± 1.6	13.4 ± 3.2	7.2 ± 1.7	2.8	0.33
Phospholipid	5.0 ± 3.0	8.8 ± 5.2	6.5 ± 2.2	1.4	0.31
Fatty acid[3]					
14:0	5.2 ± 0.1^{a}	3.3 ± 0.1^{b}	1.5 ± 0.1^{c}	1620	< 0.001
16:0	15.1 ± 0.2^{a}	12.7 ± 0.3^{b}	9.8 ± 0.2^{c}	99.3	< 0.001
16:1ω9	5.2 ± 0.2^{a}	3.2 ± 0.2^{b}	1.0 ± 0.01^{c}	813	< 0.001
18:0	3.3 ± 0.1^{a}	3.1 ± 1.0^{b}	2.8 ± 0.1^{c}	35.3	< 0.001
18:1ω9	14.6 ± 0.4^{a}	18.7 ± 0.2^{b}	22.2 ± 0.4^{c}	705	< 0.001
18:2ω6 (LNA)	6.2 ± 0.3^{a}	10.8 ± 0.2^{b}	14.7 ± 0.4^{c}	1311	< 0.001
18:3ω3 (ALA)	1.3 ± 0.05^{a}	8.0 ± 0.3^{b}	12.9 ± 0.5^{c}	1818	< 0.001
18:4ω3	1.2 ± 0.05^{a}	1.1 ± 0.1^{a}	1.6 ± 0.1^{b}	45.8	< 0.001
18:5ω3	0.2 ± 0.01^{a}	0.5 ± 0.01^{b}	0.7 ± 0.03^{c}	1014	< 0.001
20:1ω9	3.6 ± 0.2^{a}	6.2 ± 0.2^{b}	9.2 ± 0.2^{c}	1746	< 0.001
20:2ω6	0.4 ± 0.03^{a}	0.9 ± 0.03^{b}	1.6 ± 0.05^{c}	1286	< 0.001
20:3ω6	0.2 ± 0.02^{a}	0.2 ± 0.01^{a}	0.4 ± 0.03^{b}	211	< 0.001
20:4ω6	0.9 ± 0.07^{a}	0.6 ± 0.05^{b}	0.4 ± 0.06^{c}	143	< 0.001
20:3ω3	0.1 ± 0.01^{a}	0.6 ± 0.02^{b}	1.1 ± 0.06^{c}	383	< 0.001
20:4ω3	0.9 ± 0.1^{a}	0.7 ± 0.1^{b}	0.9 ± 0.1^{ab}	4.64	0.03
20:5ω3 (EPA)	9.8 ± 0.3^{a}	5.4 ± 0.2^{b}	2.1 ± 0.3^{c}	1207	< 0.001
22:1ω9	3.1 ± 0.2^{a}	2.8 ± 0.3^{b}	2.6 ± 0.1^{b}	11.6	0.01
22:5ω6	0.2 ± 0.01	0.1 ± 0.02	0.2 ± 0.01	0.26	
22:5ω3	2.7 ± 0.01^{a}	1.5 ± 0.1^{b}	0.6 ± 0.1^{c}	1414	< 0.001
22:6ω3 (DHA)	11.0 ± 0.6^{a}	8.1 ± 0.5^{b}	5.0 ± 0.4^{c}	213	< 0.001
∑SFA[4]	24.1 ± 0.3^{a}	19.5 ± 0.3^{b}	14.3 ± 0.3^{c}	1552	< 0.001
∑MUFA[5]	34.4 ± 0.5^{a}	38.0 ± 0.6^{b}	42.1 ± 0.3^{c}	332	< 0.001
∑PUFA[6]	40.9 ± 0.6^{a}	41.8 ± 0.6^{b}	43.4 ± 0.3^{c}	32.3	< 0.001
∑ω3	28.0 ± 0.8^{a}	26.3 ± 0.7^{b}	24.8 ± 0.3^{c}	37.4	< 0.001
∑ω6	8.3 ± 0.3^{a}	13.1 ± 0.2^{b}	17.5 ± 0.8^{c}	465	< 0.001
Terrestrial[7]	7.5 ± 0.3^{a}	18.8 ± 0.5^{b}	27.6 ± 0.7^{c}	2047	< 0.001
ω3/ω6	3.2 ± 0.1^{a}	1.9 ± 0.1^{b}	1.3 ± 0.1^{c}	375	< 0.001

[1]Values are mean (n=9) ± SD. Means with different superscripts indicate significant differences at the end of the experiment.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester)
[4]Saturated fatty acid
[5] Monounsaturated fatty acid
[6] Polyunsaturated fatty acid
[7] Terrestrial = 18:2ω6 + 18:3ω3

Table 5.7. Lipid class and fatty acid composition of rainbow trout viscera fat after 12 weeks[1]

Lipid composition	FO	CO50	CO100	F-stat	p-value
(*% ww^{-1}*)					
Total lipid	34.8 ± 10[a]	32.0 ± 6.1[a]	56.1 ± 19[b]	6.2	0.01
Neutral lipid	30.2 ± 9.0[a]	26.3 ± 6.2[a]	51.5 ± 19[b]	6.8	0.01
Polar lipid	4.6 ± 1.7	5.7 ± 1.0	4.6 ± 2.5	0.7	0.52
(*% total lipid*)					
Triacylglycerol	74.4 ± 11.0[a]	71.8 ± 11.1[a]	83.1 ± 9.5[b]	4.1	0.04
Sterol	11.6 ± 3.8	9.6 ± 2.2	8.6 ± 2.2	0.3	0.78
AMPL[2]	11.7 ± 4.5[a]	15.2 ± 3.0[a]	2.7 ± 0.8[b]	10	0.01
Phospholipid	1.9 ± 0.5	2.8 ± 0.9	4.9 ± 1.2	2.0	0.21
Fatty acid[3]					
14:0	5.2 ± 0.7[a]	3.6 ± 0.04[b]	1.8 ± 0.1[c]	138	< 0.001
16:0	14.2 ± 0.3[a]	12.1 ± 0.4[b]	9.6 ± 0.3[c]	445	< 0.001
16:1ω7	8.0 ± 0.6[a]	5.5 ± 0.1[b]	3.4 ± 0.2[c]	285	< 0.001
18:0	3.2 ± 0.7	2.9 ± 0.2	2.6 ± 0.1	2.99	0.123
18:1ω9	15.9 ± 0.9[a]	19.6 ± 0.4[b]	22.8 ± 0.4[c]	268	< 0.001
18:2ω6 (LNA)	6.7 ± 0.6[a]	11.4 ± 0.5[b]	14.9 ± 0.4[c]	369	< 0.001
18:3ω3 (ALA)	1.3 ± 0.2[a]	8.4 ± 0.4[b]	12.5 ± 0.7[c]	751	< 0.001
18:4ω3	1.3 ± 0.2[a]	1.1 ± 0.1[b]	1.6 ± 0.3[c]	17.1	< 0.001
18:5ω3	0.2 ± 0.02[a]	0.5 ± 0.02[b]	0.7 ± 0.03[c]	662	< 0.001
20:1ω9	3.6 ± 0.5[a]	6.7 ± 0.2[b]	9.1 ± 0.2[c]	62.1	< 0.001
20:2ω6	0.4 ± 0.03[a]	1.0 ± 0.04[b]	1.4 ± 0.1[c]	743	< 0.001
20:3ω6	0.2 ± 0.01[a]	0.2 ± 0.02[a]	0.4 ± 0.02[b]	92.6	< 0.001
20:4ω6	0.7 ± 0.02[a]	0.4 ± 0.02[b]	0.3 ± 0.04[c]	578	< 0.001
20:3ω3	0.1 ± 0.02[a]	0.7 ± 0.05[b]	1.0 ± 0.1[c]	287	< 0.001
20:5ω3 (EPA)	9.6 ± 0.8[a]	4.9 ± 0.3[b]	2.1 ± 0.3[c]	447	< 0.001
22:1ω9	3.5 ± 0.5	3.1 ± 0.1	2.9 ± 0.3	5.92	0.036
22:1ω7	0.4 ± 0.04	1.1 ± 0.05	1.7 ± 0.05	659	< 0.001
22:5ω3	2.6 ± 0.4[a]	1.5 ± 0.1[b]	0.7 ± 0.1[c]	270	< 0.001
22:6ω3 (DHA)	9.2 ± 0.8[a]	6.3 ± 0.3[b]	3.8 ± 0.4[c]	206	< 0.001
∑SFA[4]	24.2 ± 0.4[a]	19.3 ± 0.5[b]	14.5 ± 0.4[c]	1052	< 0.001
∑MUFA[5]	28.5 ± 2.5[a]	34.8 ± 0.4[b]	40.0 ± 0.5[c]	63.4	< 0.001
∑PUFA[6]	38.4 ± 1.9[a]	40.2 ± 0.7[b]	42.1 ± 0.9[c]	14.3	0.005
∑ω3	25.3 ± 0.7[a]	24.1 ± 0.4[b]	23.3 ± 0.7[c]	27.3	0.001
∑ω6	8.6 ± 0.7[a]	13.5 ± 0.5[b]	17.6 ± 0.6[c]	101	< 0.001
Terrestrial[7]	8.1 ± 0.8[a]	19.8 ± 0.7[b]	27.5 ± 1.0[c]	167	< 0.001
ω3/ω6	3.0 ± 0.2[a]	1.8 ± 0.07[b]	1.3 ± 0.041[c]	106	< 0.001

[1]Values are mean (n=9) ± SD. Means with different superscripts indicate significant differences at the end of the experiment.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester)
[4]Saturated fatty acid
[5] Monounsaturated fatty acid
[6] Polyunsaturated fatty acid
[7] Terrestrial plant = 18:2ω6 + 18:3ω3

Table 5.8. $\delta^{13}C$ values (‰) of selected fatty acids for fish oil[1], camelina oil[1], and rainbow trout muscle tissue[2] fed FO and CO100 and the relative percent contribution (RC) of camelina oil to tissue fatty acids.

Fatty acid (‰)	Fish Oil	Camelina Oil	T-stat	p-value	Muscle FO	Muscle CO	T-stat	p-value	RC (%)
18:0	-24.5 ± 0.6	-32.8 ± 0.4	-17.5	0.036	-23.5 ± 0.7	-27.1 ± 0.6	-11.8	<0.001	
18:1ω9	-25.3 ± 0.3	-32.4 ± 0.5	-10.0	0.044	-24.2 ± 0.9	-29.5 ± 0.9	-12.1	<0.001	
18:2ω6 (LNA)	-32.1 ± 0.1	-33.7 ± 0.2	-12.4	0.006	-28.7 ± 0.4	-31.6 ± 1.1	-7.2	<0.001	
18:3ω3 (ALA)	-35.7 ± 0.1	-36.1 ± 0.1	-4.9	0.008	-33.0 ± 0.8	-34.3 ± 1.3	-2.7	0.020	
20:5ω3 (EPA)	-28.4 ± 1.2	NP	---	---	-28.7 ± 1.0	-29.5 ± 0.7	-1.9	0.084	14.3
22:6ω3 (DHA)	-27.3 ± 1.0	NP	---	---	-28.4 ± 0.9	-29.7 ± 1.2	-2.5	0.026	27.3

NP= Not present
[1]Values are mean (n=3) ± SD
[2]Values are mean (n=9) ± SD

5.6 References

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Chapter 6. Full substitution of fish oil with camelina oil, with partial substitution of fish meal with camelina meal, in diets for farmed Atlantic salmon (*Salmo salar*) and its effect on tissue lipids and sensory quality

6.1 Abstract

Camelina oil (CO) and meal (CM) are potential replacements of fish meal (FM) and oil (FO) in aquaculture feeds. CO is high in α-linolenic acid (ALA; 18:3ω3) (30%), with a ω3/ω6 ratio >1. This study tested diets with 100% CO, solvent extracted FM (SEFM) and partially substituted FM with 10% CM, in a 16 week feeding trial with Atlantic salmon (mean initial weight 240 g fish^{-1}). Growth varied among dietary groups (529-691 g fish^{-1}), but was not affected by feeding 100% CO; however it was lower in groups fed SEFM and 10% CM diets (p=0.001). Total lipid in salmon flesh fed a diet with CO, SEFM and CM (22% ww^{-1}) was significantly higher than FO flesh (14% ww^{-1}) (p=0.004). There was no difference in the sensory quality of salmon fillets that were fed either FO or 100% CO diets. This was the first study to use CO as a complete FO replacement in diets for farmed Atlantic salmon.

6.2 Introduction

Seafood is a major source of long chain (LC) ω3 polyunsaturated fatty acids (PUFA) in the human diet. Aquaculture supplies nearly half of the world's seafood supply, which requires a steady and sustainable supply of feed ingredients (FAO, 2012). Fish oil (FO) is a critical lipid source in feeds for aquaculture, which is highly dependent on wild fisheries. Availability, sustainability and cost are concerns for future use of FO in aquaculture. The FO supply is under severe pressure from a number of industries including pharmaceutical, agricultural, functional foods and aquaculture (Turchini et al., 2009); therefore its availability and cost are inconsistent.

Several different vegetable oils are commercially used in fish feeds (Turchini et al., 2009), such as canola, soybean and sunflower oil, however only a small proportion of FO can be replaced by these alternative oils due to their lack of LC ω3 PUFA. The ideal FO replacement should have a fatty acid composition that is highly digestible and which provides high retention of ω3 PUFAs. It should also provide high levels of precursor ω3 fatty acids for biosynthesis of LC ω3 PUFA and low levels of ω6 PUFA to maintain a high ω3/ω6 ratio which is beneficial for fish and human health (Seierstad et al., 2005; Torstensen et al., 2005).

The oilseed camelina (*Camelina sativa*), possesses many of these qualities. It yields 40% total lipid and is high in α-linolenic acid (ALA, 18:3ω3) at a proportion of 30% and with lower levels of ω6, to produce a ω3/ω6 ratio >1. ALA is a LC ω3 precursor, and some fish species like Atlantic salmon (*Salmo salar*) have the metabolic enzymes necessary for converting ALA to eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) (Hastings et al., 2004; Zheng et al., 2005; Jordal et al., 2005). Higher levels of ALA in the diet will increase substrate availability for desaturase and elongase enzymes to convert ALA to EPA and DHA in salmon tissues (Thanuthong et al., 2011). Therefore, it is hypothesized that Atlantic salmon will synthesize DHA and EPA to some degree from the high amount of ALA provided in the diet from camelina oil (CO), in order to maintain adequate levels of these essential fatty acids.

Replacing FO with vegetable oils (VO) has been studied extensively in Atlantic salmon. These alternative oils, such as rapeseed oil, palm oil, linseed oil or soy oil, can usually replace 100% of FO without compromising growth (Turchini et al., 2009). However levels of DHA and EPA in the fillet normally reduce to a fraction of that compared to salmon that were fed FO (Bell et al., 2002; Bell et al., 2003; Brandsen et al., 2003; Menoyo et al., 2005). This affects the flesh

nutritional quality for human consumption with implications for human health (Seierstad et al., 2005; Midtbø et al., 2013). Other quality aspects of Atlantic salmon fillet such as sensory quality, flesh texture and colour have been affected by dietary VO (Regost et al., 2004; Torstensen et al., 2005). Consideration of the final product is very important when investigating alternative diet sources in order to adhere to a general standard for consumer health and benefit.

Commercial feed companies replace portions of both fish meal (FM) and FO with vegetable sources (Crampton and Carr, 2012). Therefore, in order to test the effect of CO in a practical diet with the intention of its use in the aquaculture industry, replacing FO with CO along with a partial replacement of FM with plant meal should be considered. Camelina meal (CM) is also considered as a fish meal replacement, on account of its crude protein level (38%), inclusion of some essential amino acids and its availability after oil extraction. CO has replaced FO in diets for Atlantic cod (Morais et al., 2012; Hixson et al., 2013); however a full replacement has yet to be tested with Atlantic salmon. The purpose of this study was to evaluate CO as a suitable lipid source for farmed Atlantic salmon. A nutritional feeding trial was conducted with diets containing CO and CM in order to determine lipid and tissue fatty acid composition in tissues and to assess the final fillet for flesh composition and quality for human consumption.

6.3 Methods

Experimental diets

Camelina (Calena cultivar) was grown and harvested by the Department of Plant and Animal Sciences, Faculty of Agriculture, Dalhousie University at an off-campus location (Canning, Nova Scotia, Canada). The seeds were single pressed using a KEK 0500 press at

Atlantic Oilseed Processing, Ltd. (Summerside, Prince Edward Island, Canada) to extract the oil and ethoxyquin was added to the final product as an antioxidant. The meal was pressed with a hammer mill (screen size 8 mm) into a pre-pressed meal cake at Atlantic Oilseed Processing, Ltd., then solvent extracted with petroleum ether at a concentration of 3 ml g^{-1} at the Faculty of Agriculture, Dalhousie University (Truro, Nova Scotia, Canada).

All diets were formulated as isonitrogenous, iso-energetic diets and were produced at the Faculty of Agriculture, Dalhousie University (Truro, Nova Scotia, Canada) (Table 6.1). The experimental treatments were as follows: a control diet with fish oil (FO); 100% FO replacement with camelina oil (100CO); 100% FO replacement with CO with solvent extracted FM (100COSEFM); 100% FO replacement with CO and 10% inclusion of CM (100CO10CM); and 100% FO replacement with CO with solvent extracted fish meal (SEFM) and 10% inclusion of camelina meal (100COSEFM10CM). SEFM was used in two experimental diets in order to remove all marine lipids from the diet to test the full effect of CO. The FM was solvent extracted with petroleum ether at a concentration of 3 ml g^{-1}. CM was added in two experimental diets as a partial substitution of FM, with CO as a full replacement of FO. Diets were formulated to meet nutritional requirements of Atlantic salmon (NRC, 2011). All diets were steam pelleted using a laboratory pelleting mill (California Pellet Mill, San Francisco, USA). The initial size of the pellet was 4.0 mm; it increased to 6.0 mm as the fish grew larger throughout the trial. Diets were stored at -20°C until needed.

Experimental fish

An experiment was conducted with salmon smolts in seawater (242 ± 46 g fish $^{-1}$ mean initial weight ± SD; 27 ± 1.8 cm mean initial length) at the Ocean Sciences Centre (Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, Canada). Fish were

received from Cooke Aquaculture (St. Alban's, Newfoundland and Labrador, Canada). The salmon (Saint John River stock) were transferred from the hatchery (freshwater) to the Ocean Sciences Centre, Dr. Joe Brown Aquatic Research Building (JBARB) (seawater) to undergo smoltification (Memorial University Institutional Animal Care Protocol Approved 12-50-MR). The smolts (n=750) were randomly distributed into 15 experimental tanks (500 L), each tank with 50 fish. The fish were acclimated on the control diet for one week prior to initial sampling. Throughout the duration of the trial, a flow-through system of filtered seawater was supplied to each tank at a rate of 12 L min^{-1} and a photoperiod of 12 hours. The dissolved oxygen (10 mg L^{-1}) and water temperature (14°C) was monitored daily. Fish were fed to apparent satiation. Mortalities were weighed and recorded throughout the trial.

Tissue sampling

Sampling occurred at week 0 (the day before experimental diets were fed), week 1 (3 days of acclimation on the test diets plus 7 days on full test diet), 8, and 16. Three fish per tank were randomly sampled and measured for length and weight. The whole viscera was removed and weighed and sampled for lipid analysis. The skin was removed on the left side and white muscle tissue was subsampled for dry matter and lipid analyses. At the final sampling, additional tissues were sampled for lipid analysis, including white muscle, dark muscle, skin and belly flap (the lower region of muscle tissue that surrounds the abdominal cavity). The skin was separated without carrying away any adherent dark muscle. The subdermal fat layer external to the dark muscle was then scraped off as completely as possible. A wedge-shaped cut was made along the entire length above lateral line and directly below the dorsal fin and the dark muscle was carefully separated from the white muscle. The locations of these samples and sampling methods for these tissues were based on Zhou et al. (1995). Lipid samples were stored on ice during

sampling of each tank and were processed within an hour. Samples were collected in 50 ml test tubes that had been rinsed three times with methanol followed by three rinses with chloroform. The tubes were allowed to dry completely before they were weighed. The tubes were weighed again following the addition of the sample. After wet weights were recorded, samples were covered with 8 ml of chloroform (HPLC-grade), the headspace in the tube was filled with nitrogen, the Teflon-lined caps were sealed with Teflon tape, and the samples were stored at -20°C.

Lipid extracts

Lipid samples were extracted according to Parrish (1999). Samples were homogenized in a 2:1 mixture of ice-cold chloroform: methanol. Samples were homogenized with a Polytron PCU-2-110 homogenizer (Brinkmann Instruments, Rexdale, Ontario, Canada). Chloroform extracted water was added to bring the ratio of chloroform: methanol: water to 8:4:3. The sample was sonicated for 6 min in an ice bath and centrifuged at 4000 rpm for 2 min at room temperature. The bottom, organic layer was removed using a double pipetting technique, placing a 2 ml lipid-cleaned Pasteur pipette inside a 1 ml pipette, to remove the organic layer without disturbing the top aqueous layer. Chloroform was then added back to the extraction test tube and the entire procedure was repeated 3 times for muscle samples and 5 times for liver samples. All organic layers were pooled into a lipid-cleaned vial. The samples were concentrated using a flash-evaporator (Buchler Instruments, Fort Lee, New Jersey, USA).

Lipid class separation

Lipid class composition was determined using an Iatroscan Mark VI TLC-FID (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan), silica coated Chromarods and a three-step

development method (Parrish, 1987). The lipid extracts were applied to the Chromarods and focused to a narrow band using 100% acetone. The first development system was hexane: diethyl ether: formic acid (99.95:1.0:0.05). The rods were developed for 25 min, removed from the system for 5 min to dry and replaced for 20 min. The second development was for 40 min in hexane: diethyl ether: formic acid (79:20:1). The final development system had two steps, the first was 100% acetone for two 15 min time periods, followed by two 10 min periods in chloroform: methanol: chloroform-extracted water (5:4:1). Before using each solvent system the rods were dried in a constant humidity chamber. After each development system, the rods were partially scanned in the Iatroscan and the data were collected using Peak Simple software (version 3.67, SRI Inc). The Chromarods were calibrated using standards from Sigma Chemicals (Sigma Chemicals, St. Louis, Missouri, USA).

Fatty acid methyl ester (FAME) derivatization

Lipid extracts were transesterified using the Hilditch reagent (1.5 H_2SO_4: 98.5 anhydrous MeOH) for 1 hour at 100°C. Reagents were added in the proportion of 1.5 ml reagent per 4-16 mg of lipid. Samples were vortexed half way through each derivatization reaction. To check the derivatization efficiency, samples were transesterified and then the lipid class composition of the methyl ester solution was determined by TLC-FID. The derivatization efficiency is calculated from the amount of underivatized acyl lipids compared to the amount of methyl esters in a sample.

All FAMEs were analyzed on a HP 6890 GC FID equipped with a 7683 autosampler. The GC column was a ZB wax+ (Phenomenex, Torrance, California, USA). The column length was 30 m with an internal diameter of 0.32 mm. The column temperature began at 65°C where it was held for 0.5 min. The temperature ramped to 195°C at a rate of 40°C min^{-1}, held there for 15

min and then ramped to a final temperature of 220°C at a rate of 2°C min^{-1}. This final temperature was held for 45 sec. The carrier gas was hydrogen flowing at 2 ml min^{-1}. The injector temperature started at 150°C and ramped to a final temperature of 250°C at 120°C min^{-1}. The detector temperature stayed at 260°C. Peaks were identified using retention times from standards purchased from Supelco (Bellefonte, Pennsylvania, USA): 37 component FAME mix (Product number 47885-U), PUFA 3 (product number 47085-U) and PUFA 1 (product number 47033-U). Chromatograms were integrated using the Varian Galaxie Chromatography Data System, version 1.9.3.2 (Agilent Technologies, Colorado, USA).

Sensory analysis

Fish were sampled for sensory analysis two weeks after final sampling for lipid analyses (week 18) from the FO and CO100 treatments. Three fish from each dietary group were killed by a blow to the head, gutted and chilled on ice. Fish were then filleted and portioned into 3 cm x 3 cm squares and placed into sampling cups and covered with lids. Raw samples were carried from the JBARB to an isolated, air-freshened room with standardized light at the Ocean Sciences Centre. The room was prepared according to standards outlined by Carpenter et al. (2000). An untrained panel (n=24) composed of volunteers performed a triangle test, a hedonic test, and a quantitative descriptive analysis (QDA) to profile salmon treatments in terms of smell, appearance and texture. The tests in this sensory evaluation were designed based on methods outlined by Cardello et al. (1982), the Food and Agricultural Organization of the United Nations (FAO, 1999) and Carpenter et al. (2000). These types of tests have been used by the Canadian Food Inspection Agency for sensory evaluations, and have been used in a number of other studies in the aquaculture field (Monterro et al., 2005; Torstenssen et al., 2005; Veiseth-Kent et al., 2010). Panelists were given specific instructions about how to evaluate the samples for

appearance, odour and texture (Carpenter et al., 2000). The triangle test required panelists to determine which one of three samples seemed different in terms of appearance, texture and odor (two FO samples and one CO100 sample). The hedonic test required panelists to score on a two anchored (e.g. firm and soft) linear scale (1-7) for each of appearance, odour and texture (FAO, 1999). The QDA asked panelists to rate the intensity on a scale of 1-7 (1= no intensity; 7= distinct intensity) for a number of descriptions (e.g. brightness, orange intensity, surface moistness). Both QDA tests were objective and had nothing to do with the likes or dislikes of each panelist.

Texture analysis

Texture analysis was completed at the Marine Institute of Memorial University of Newfoundland (St. John's, Newfoundland and Labrador, Canada). For logistical reasons, texture analysis was performed on frozen fillets. Salmon from the FO and 100CO treatments (n=3) were killed by an overdose of anaesthetic (MS-222) and carcasses stored in plastic bags in a freezer at -20°C until analysis (6 months). Whole salmon in bags were thawed at ambient temperature and were monitored regularly to evaluate if thawing was completed, and then transferred to a refrigerator (4°C) prior to sampling (FAO, 1999). The salmon were eviscerated and filleted, and fillets were washed, bagged and stored on ice prior to analysis. The texture profile analyses were performed at three adjacent locations on the fillet mid-loin above the lateral line, and the average of these three measurements was used in the data analysis. Fillet texture was evaluated instrumentally using a Texture Analyzer (TA.XT Plus, Texture Technologies Corporation, Scarsdale, New York, USA) equipped with a 9.5 mm cylindrical probe, and data were recorded using the Texture Exponent software (Stable Micro Systems version 6.0, London, UK). The

probe was pressed into the fillet at a pre-test speed of 5.0 mm s^{-1}, tested at a constant speed of 1.0 mm s^{-1} and a post-test speed at 5.0 mm s^{-1}. The penetration depth was 50% of the fillet thickness. The force necessary to maintain a constant speed of the probe to reach the specified fillet thickness was recorded during compression, which resulted in a texture profile curve (force vs. time). The force required to penetrate the surface (breakpoint force) and the maximum force (max force) reached during compression were recorded, as well as the fillet thickness (mm). The temperature of the fillets was 2°C immediately prior to analysis.

Colour analysis

The instrumental colour analysis was done at the Marine Institute of Memorial University of Newfoundland (St. John's, Newfoundland and Labrador, Canada) by ColorTec-PCM (Accuracy Microsensors, Inc, Pittsford, New York), on the same fillets used in the texture analysis. The Hunter colour scale was used, with light source D, where L (lightness), a (redness), and b (yellowness) were recorded and chromaticity [$(a^2 + b^2)^{1/2}$] and hue [$\tan^{-1}(b/a)$] were calculated. Colour measurements were taken on the same mid-loin portion of the fillet as outlined in the texture analysis section.

Statistical methods

Statistical analysis followed methods outlined by Sokal and Rolf (1994). For analysis of growth data, lipid class data, and fatty acid data, where individual fish were weighed, measured and sampled, a three-way nested ANOVA was performed using the General Linear Model (Minitab 16 Statistical Software, State College, Pennsylvania, USA). The model was designed to test the effect of diet on the response variable and nested fish individuals within tanks to negate variability among tanks and individuals, while also testing for tank effects. For analysis of

growth data that depend on comparison to an initial measurement and thus must be pooled per tank (i.e., mean weight gain and specific growth rate), a two-way ANOVA was performed to test the effect of diet and tank variability. In both cases, where significant differences occurred, treatment means were differentiated using the Tukey HSD multiple comparison. For each model tested, the residuals were examined to evaluate the appropriateness of the model; therefore normality, homogeneity and independence of residuals were considered. If a p-value was close to 0.05 and residuals were not normal, a p-randomization was conducted >5,000 times to test the data empirically. In addition, PRIMER (Plymouth Routines in Multivariate Ecological Research; PRIMER-E Ltd, Version 6.1.15, Ivybridge, UK) was used to analyze selected fatty acid data using SIMPER (similarity of percentages analysis) and ANOSIM (analysis of similarities) to define similarities and differences among tissue and dietary fatty acid data. Fatty acids that accounted for > 0.05% of total fatty acids were included in the analyses. SIMPER and ANOSIM are multivariate analyses that use a resemblance matrix and the latter carries out an approximate analogue of ANOVA. In both cases non-parametric Bray-Curtis similarity was chosen.

The sensory, texture and colour measurements of salmon fillets were analyzed by t-tests and principal component analysis (PCA). A two-sample t-test between salmon fillets that were fed either FO or CO100 was performed for each sensory attribute for both the hedonic test and QDA. The purpose of the PCA was to explore the main trends in the data, and the score plot reveals samples with similar sensory attributes located in the same area in the score plot.

6.4 Results

Experimental diets

100% FO was replaced by CO in all experimental diets except the FO diet (Table 6.1), with addition of SEFM, CM or both. Total lipid levels in the diets varied slightly (18-22%). Lipid classes in the diets were composed mainly of triacylglycerol (TAG) and phospholipid (Table 6.2). TAG was significantly higher in diets with SEFM and/or CM than FO. Acetone mobile polar lipid (AMPL) was significantly lower in diets with SEFM and/or CM than FO. Phospholipid levels in diets with CM were significantly lower than remaining diets. Generally, fatty acid profiles of all CO experimental diets were significantly different than the FO diet (Table 6.2). Differences in fatty acid profiles between CO diets were minimal, but often significant. CO diets were significantly lower in SFA, and higher in MUFA and PUFA than the FO diet. The ω3 PUFA were significantly lower in CO diets compared to FO, and CO diets were about three times, and significantly, higher in the ω6 series compared to the FO diet. ALA and LNA were predominant in CO diets (>40% total), but minimal in the FO diet (6%). EPA and DHA dropped significantly in CO diets (~2% sum of EPA+DHA) compared to FO (16% EPA, 8% DHA).

Growth performance

Initially, salmon were 230-255 g $fish^{-1}$ and grew to 529-691 g $fish^{-1}$ after 16 weeks (Table 6.3). Salmon fed the FO diet gained significantly more weight (471 g $fish^{-1}$) than salmon fed 100COSEFM, 100CO10CM and 100COSEFM10CM, but did not differ from salmon fed 100CO. Salmon fed 100COSEFM10CM gained the least amount of weight at the end of the experiment (281 g $fish^{-1}$). There were no tank differences in final weight ($F=1.2$; $p=0.29$) or weight gain ($F=4.3$; $p=0.06$). The growth rate (SGR) for FO (0.99% day^{-1}) and 100CO (0.86% day^{-1}) was not different, although remaining groups grew at a significantly slower rate than

salmon fed FO. All salmon were similar in condition factor (~1.5). Salmon fed 100COSEFM10CM stored significantly more fat around the viscera (12%) than salmon fed FO (10%). CO-fed salmon consumed significantly less feed (381-436 g) than salmon fed FO (515 g) over 16 weeks; however this did not significantly affect the FCR (1.0-1.25).

White muscle tissue lipid and fatty acid composition

Salmon flesh was 3% lipid at the start of the experiment, composed mostly of neutral lipid (Table 6.4). TAG was the predominant lipid class (78%), followed by phospholipid (15%). After 16 weeks of growth, total lipid increased in the FO-fed salmon (14% ww^{-1}) and CO groups increased in total lipid with either inclusion of CM or SEFM, particularly the 100COSEFM10CM-fed salmon (22% ww^{-1}). Total lipid in white muscle of salmon fed 100CO was not different than the control (10% ww^{-1}). The increase in total lipid inevitably increased the neutral lipid proportion, which was significantly higher in 100COSEFM, 100CO10CM and 100COSEFM10CM-fed salmon. Lipid classes were not significantly different among treatments; however TAG increased in proportion (~90%) compared to the initial profile (80%) and phospholipid decreased (2-5%) compared to the initial profile (15%).

Fatty acid profiles in white muscle tissue were significantly altered when FO was replaced by CO (Table 6.4). SFA were reduced by about half in CO groups (15%) compared to FO (25%), with SFA in 100COSEFM10CM-fed salmon significantly lower than both FO and CO100-fed salmon. MUFA was significantly higher in CO groups, and PUFA was either the same or higher in CO groups compared to FO. PUFA of the ω6 series more than doubled in CO groups compared to FO, which was significant. The ω3/ω6 ratio was significantly lower in CO groups compared to FO, due to the significant increase in the ω6 series. LNA was over twice as

high and significantly different in CO groups than in FO and ALA was significantly higher in CO groups than FO, particularly 100COSEFM10CM, which was significantly higher than all CO groups. DHA and EPA reduced significantly after 16 weeks in groups containing CO. However, because salmon fed CO generally had more lipid stored in the white muscle, the quantitative amounts of DHA and EPA (μg g^{-1}) were considered, which accounts for the actual amount of the fatty acid in the tissue, rather than the level of DHA as a percentage of the total fatty acids that were measured. After calculating the quantitative amounts of DHA and EPA, there was no significant difference in the amount of DHA in the muscle between 100COSEFM, 100CO10CM and 100COSEFM10CM compared to FO, despite lower levels (%) of DHA in these groups (Figure 6.1). This was due to the increase in flesh lipid in salmon fed these CO diets. Several fatty acids were noted in groups fed CO that suggest LC PUFA synthesis occurred, e.g., 18:4ω3, 20:3ω3, 20:4ω3, 22:4ω3, and were significantly higher in these groups than in FO. Generally, fatty acids in all CO groups were similar to each other, but different compared to FO; however, removing residual lipid from fish meal and adding 10% CM (100COSEFM10CM) tended to change the white muscle fatty acid profile compared to the other CO-fed salmon. ANOSIM revealed white muscle tissue following FO and CO feeding as completely dissimilar (R=1, p=0.001); 100COSEFM10CM and 100CO were the least similar among CO diets (R=0.787, p=0.001) and 100COSEFM and 100CO10CM were the most similar (R=0.093, p=0.08). Analysis by SIMPER revealed that FO white muscle was between 25.5-28.6% dissimilar from all white muscle after feeding CO diets, with 100COSEFM10CM white muscle having the most dissimilar fatty acid profile from FO white muscle. All CO diets were <4.78% dissimilar from one another. When tissue and diet fatty acid profiles for each dietary group were compared with

each other, SIMPER revealed that FO white muscle tissue and the FO diet was 8.7% dissimilar; but CO white muscle tissue and respective diets were >20% dissimilar (Table 6.9).

Dark muscle and belly flap lipid and fatty acid composition

Total lipid and neutral lipid in dark muscle were significantly higher in salmon fed 100CO and 100COSEFM than salmon fed FO and 100COSEFM10CM, and dark muscle was higher in total lipid than white muscle (Table 6.5). Proportions of TAG were higher in dark muscle of salmon fed CO than FO, but all dark muscle, regardless of diet fed was >94% TAG. Phospholipid, AMPL and free fatty acid (FFA) were not different among groups. Dark muscle tissue showed similar trends in fatty acid profiles as in white muscle tissue. Increases in ALA (10 times) and LNA (2 times) in dark muscle tissue from CO groups compared to FO salmon were notable and significantly different. EPA was about one third lower in CO groups than FO; and the proportion of DHA was about half in CO groups compared to FO. SFA, MUFA and PUFA in FO were similar in proportion compared to that in white muscle and also showed the same trends when CO was added in the diets.

Total lipid, neutral lipid and lipid classes were not different among groups in the belly flap (Table 6.6). The belly flap lipid composition was similar to dark muscle, mostly composed of TAG (91-97%). ALA and LNA were predominant fatty acids in CO fed groups, and their sum was over triple the proportion in CO groups compared to FO. EPA in the belly flap was significantly lower in salmon fed 100COSEFM10CM than 100CO and FO, with all groups lower than FO. DHA in the belly flap was more than twice as high in salmon fed FO than CO, but salmon fed CO did not differ amongst each other. SFA, MUFA and PUFA in the belly flap of salmon fed FO were similar in proportion compared to that in white and dark muscle and also showed the same trends in the belly flap of salmon fed any CO diet.

Skin and viscera fat lipid and fatty acid composition

Skin total lipid was higher in salmon fed 100COSEFM and 100COSEFM10CM compared to all other groups, and contained more lipid than white muscle, dark muscle and belly flap tissue (Table 6.7). Neutral lipid and polar lipid were not significantly different among groups. Lipid classes in the skin did not differ among groups, with TAG the predominant lipid class in the skin (86-92%). ST was found in the skin tissue of salmon fed all diets, and did not differ significantly (1-4%). The sum of ALA and LNA in the skin of salmon fed any CO diet was over triple that in the skin of salmon fed FO, with the highest proportion of these fatty acids in the 100COSEFM10CM group. The 100COSEFM10CM group had significantly less DHA in the skin than salmon fed 100CO and FO. SFA, MUFA and PUFA in the skin of salmon fed FO were similar in proportion compared to that in white muscle, dark muscle and belly flap, and also showed the same trends in salmon fed any CO diet.

Viscera fat contained the most lipid compared to all tissues analyzed (>70% ww^{-1}). The FO group was higher in total lipid than all CO groups (Table 6.8). Neutral lipid was significantly different among groups and also showed the same trends among groups as total lipid. Viscera fat was mostly composed of TAG for all groups (90-93%). ST was significantly higher in 100COSEFM than other diets. The sum of ALA and LNA in the viscera fat of CO groups was over triple that of FO skin, with the highest proportion of these fatty acids in the 100COSEFM10CM group. DHA proportions in the viscera fat were reduced by half in salmon fed 100CO, 100COSEFM and 100CO10CM and less than half in salmon fed 100COSEFM10CM compared to salmon fed FO. EPA proportions in the viscera fat were also reduced by half with addition of CO, with the lowest proportions in salmon fed 100COSEFM10CM. SFA, MUFA and PUFA in the viscera fat of salmon fed FO were similar in

proportion compared to that in white muscle, dark muscle and belly flap, and also showed the same trends when CO was added in the diets.

Sensory evaluation

The three sensory evaluations (triangle test, hedonic test and QDA) showed no significant differences in appearance, odour and texture between salmon fillets that were fed either FO or 100CO (Table 6.10). The triangle test revealed that participants could not distinguish an overall difference between FO and CO100 fillets when presented with three samples: two FO fillets and one 100CO fillet. FO and 100CO fillets scored similarly for attributes in the QDA, and the radial plot revealed no difference in pattern between the two groups (Figure 6.2). Participants scored raw salmon fillets that were fed either FO and 100CO evenly in the hedonic test, and both fillets appeared natural, fresh smelling, and firm-elastic in texture (Figure 6.3). The similarity between groups was demonstrated by principal components and analysis in which no distinct groups were formed (data not shown).

Texture and colour analyses

The texture analysis revealed no significant differences in the breakpoint force, maximum force and fillet thickness between FO and 100CO fillets (Table 6.10). The colour analysis revealed no significant differences in l, a, b, chromaticity or hue; however, the instrument defined FO fillets as “dark yellow” and 100CO fillets as “dark orange” (Table 6.10).

6.5 Discussion

Replacing 100% of FO with CO did not significantly affect weight gain in Atlantic salmon after 16 weeks of growth. This result is in agreement with previous studies that found replacing 100% FO with VO in diets for salmon did not affect growth or health (Bell et al., 2001;

Bell et al., 2003; Menoyo et al., 2005); however, as long as low levels of EPA and DHA were provided by the lipid in FM. Consequently, removing lipid from FM and/or including 10% CM reduced growth of salmon in this experiment. Growth rate, feed conversion and visceral somatic index were not affected by using 100% CO, but including SEFM and CM affected these growth parameters. Although using 100% CO to replace FO did not affect fish performance, elimination of EFAs that were supplied in marine lipid, either in the form of FM or FO, had a significant detrimental effect on growth. These results indicate that a minimum level of marine lipid must be supplied in diets for Atlantic salmon to support growth, at least through FM if 100% of FO is replaced, or through other marine lipid sources such as algae.

Changing the lipid and fatty acid composition of the diet can affect the composition and quantity of flesh lipid stores (Bell et al., 2004). As observed in previous studies using VO, including CO, the dietary lipid and fatty acid composition of the oil directly influenced tissue compositions (Hixson et al., 2013, Chapter 2). After 16 weeks, lipid in the flesh increased from 3% ww^{-1} to 14% ww^{-1} in the FO group, which is expected as salmon accumulate lipid in the flesh as market size is reached (10-20% ww^{-1}) (Bell et al., 1998; Aursand et al., 2000). Also, smolt transformation significantly depletes total lipid content of white muscle and reduces TAG concentrations (Sheridan et al., 1983); therefore lower lipid values at the start of the experiment were expected since fish experienced this life stage transformation immediately prior to the experiment. Final total lipid levels in salmon fed CO with SEFM or CM had higher total lipid in the flesh compared to FO or 100CO, which may be due to the small increase in total lipid in the diets. However, increases in flesh lipid in response to VO in the diet have been documented in other studies, particularly when substituting both FO and FM. A study that replaced both FM and FO with VO and plant protein found that salmon developed increased adiposity in the fillet

through interactions between high levels of VO and plant proteins compared to salmon fed FO (Bendiksen et al., 2011; Torstensen et al., 2011). FO and 100CO diets were identical in total lipid, and there was no resulting significant difference in total lipid in the flesh, although some studies have also reported decreases in salmon flesh lipid after feeding a VO diet (Bell et al., 2001; Bell et al., 2002). Other parts of the muscle (dark muscle, belly flap) naturally have a higher proportion of lipid than white muscle (Zhou et al., 1995; Nanton et al., 2007), which explains higher total lipid amounts across these muscle sections. Considering the anatomical and physiological differences between these muscle types, the observed difference in lipid storage was expected. White muscle is responsible for a burst of activity such as fast swimming, while the dark muscle is for slow, steady swimming and for maintaining the horizontal balance of the body (Hudson, 1973; Walker and Pull, 1973). Fish muscle lipids exist mainly in three forms: neutral lipids (mainly TG) in adipocytes for storage of energy, polar lipids serving as major components of the cell membrane, and intracellular lipid droplets distributed in cell cytoplasm as local energy stores. Histology has shown the presence of lipid droplets inside dark muscle fibres between the myofibrils (Ackman and Zhou, 1994). The excess stores of lipid in dark muscle likely supply a consistent source of energy necessary to maintain constant movement and balance.

Comparatively more lipid was stored surrounding the viscera in salmon fed a CO diet with SEFM and CM; the interaction between plant oil and protein has also been found to increase visceral adipose tissue in salmon (Torstensen et al., 2011). Atlantic salmon adipocytes decrease their lipid accumulation when stimulated with marine ω3 fatty acids (Torstensen et al., 2011; Todorcevic et al., 2008). In addition, LC ω3 PUFAs were found to prevent the growth of adipocyte size and adipose tissue mass in rats (Parrish et al., 1990) by down-regulating the late

phase of adipocyte differentiation (Okuno et al. 1997). Although more fat was stored here, the composition was significantly lower in total lipid (75% ww^{-1}) than FO (79% ww^{-1}), suggesting the fat may have had a network of connective tissue and blood vessels to support the adipose tissue surrounding the viscera. All salmon tissues contained considerable amounts of TAG (>90%) and neutral lipid, as TAG is the only major component in stored fat and is typical of farmed salmon (Zhou et al., 1995), therefore differences in more polar lipid classes were subtle and reflected the different dietary treatments.

It is well known that tissue fatty acid composition in fish is directly influenced by dietary fatty acid composition and that feeding high levels of VO will strongly influence the fatty acid profile of various tissues (Torstensen et al., 2000; Bell et al., 2003; Bell et al., 2004; Hixson et al., 2013, Chapter 2). CO significantly altered fatty acid profiles across all tissues examined in this study. Generally, removing lipid from FM had more of an effect on fatty acid composition of the tissues than the addition of CM, and the combination of SEFM and CM had a greater effect on tissue fatty acid composition than any CO diet. Replacing FO with CO decreased SFA, and increased MUFA and did not change PUFA levels in the tissues examined. Based on these characteristics, consuming salmon fillets fed 100% CO diets would be more beneficial to human health than consuming salmon fed FO. However, the proportion of ω3 fatty acids dropped significantly in CO groups compared to FO, and the proportion of ω6 fatty acids increased two-fold. This inevitably changed the ω3/ω6 ratio, although the ratio was still >1.5 in all tissues. From a fish health standpoint, the change in the ω3/ω6 ratio can be detrimental to eicosanoid production. Increased consumption of LC ω3 PUFA reduces the synthesis of LC ω6 PUFA-derived pro-inflammatory eicosanoids and elevates the production of anti-inflammatory eicosanoids from ω3 PUFA; therefore decreases in this ratio over 100-200% reflect directly on

anti-inflammatory eicosanoid production (Alhazzaa et al., 2013). From a human health standpoint, increases in ω6 fatty acids in salmon fillets are a concern. When mice were fed farmed salmon raised on diets with 100% soybean oil, the excessive dietary LNA elevated endocannabinoids in the liver, increased weight gain and counteracted the anti-inflammatory properties of EPA and DHA (Alvheim et al., 2013). Although soybean oil characteristically has higher levels of LNA than CO, the four-fold increase in total ω6 observed in the tissues of CO-fed groups may be problematic for both fish and human health.

Multivariate statistics provided quantitative insight into the similarities among tissue fatty acids in white muscle tissue compared amongst different dietary treatments. It was clear that fatty acid profiles of FO muscle tissue were quite different compared to profiles of CO muscle tissue, but fatty acid profiles of muscle tissue among CO diets were 95% similar. Of the CO groups, flesh from 100CO and 100COSEFM10CM were the least similar and 100COSEFM and 100CO10CM were the most similar. Comparison of muscle tissue fatty acid profiles to fatty acid profiles of the diet fed, showed that FO tissue and FO diet profiles were 8.7% dissimilar, while CO tissues and CO diet profiles were >20% dissimilar. Generally it is thought that tissue fatty acid profiles should essentially match fatty acid profiles of the diet that was fed; however these results suggest that some biochemical conversion of fatty acids occurs which alters body composition compared to the diet, therefore the equilibrium between diet and flesh fatty acid composition may not be equal proportions between what is fed and what is deposited into tissues, a result which has been previously reported in salmon (Budge et al., 2011). In the CO groups, ALA was the major contributor to this inequality or dissimilarity between tissue and diet fatty acid profiles. This suggests that the level of ALA provided in the diet was in excess, since it was the major contributor to the ~20% dissimilarity between tissue and diet. There could be three

possible explanations for the disappearance of ALA: excretion through feces, β-oxidation, or elongation/desaturation into longer, more unsaturated fatty acid chains. Digestibility of ALA is over 96% in salmon (Menoyo et al., 2003), so it is unlikely that the dissimilarity was due to loss through feces. Salmonids tend to preferentially metabolize C_{18} PUFA by β-oxidation; therefore ALA is often selected against in terms of flesh deposition, so some of the discrepancy between diet ALA and tissue ALA could be due to β-oxidation for energy production. Also, fatty acids present in relatively larger proportions in the diet are more readily catabolized than those present in smaller proportions (Budge et al., 2011). However, ALA is a substrate for Δ6 desaturase. In salmon flesh after feeding CO diets, levels of DHA were slightly higher than what was provided in the diet, which was also confirmed by 9% dissimilarity recognized by SIMPER. This is an indication that some ALA was converted to DHA *de novo*.

It has been shown that salmon can biosynthesize some DHA and EPA from ALA (Hastings et al., 2004; Jordal et al., 2005; Zheng et al., 2005). Evidence of LC ω3 PUFA synthesis was shown through specific ω3 fatty acids that were not present in the diet or FO tissues; for example significant increases in 18:4ω3, 20:3ω3, 20:4ω3 and 22:4ω3 were observed in CO groups, particularly in salmon fed diets with SEFM. However, this only shows that these fatty acids in the ω3 pathway were synthesized, rather than completion of the pathway, since EPA and DHA were reduced significantly. But, it is likely that some DHA and EPA were synthesized, particularly by salmon fed 100COSEFM10CM, because these fatty acids were supplied at <1% of the diet, yet were found at levels of 4% (DHA) and 2% (EPA) in white muscle tissue. This may also be a function of selective incorporation of DHA and EPA in the flesh due to preferential deposition and retention, which has been observed in previous studies that fed salmon VO diets (Bell et al., 2004; 2003; 2002). Despite significant decreases in DHA

and EPA levels, increases in flesh lipid that were found in SEFM and CM diets caused quantitative amounts of DHA to be relatively equal to the amount of DHA in FO fed white muscle, despite that the proportion of DHA in FO is double that of the CO groups. The quantitative amount of DHA and EPA in fatty fish likely still meets human nutritional requirements for these EFA. According to the World Health Organization (WHO), the daily requirement for DHA + EPA is 250 mg (WHO, 2008). Based on Canada's Food Guide, one serving of cooked fish is 75 g (Health Canada, 2011). The sum of DHA and EPA in salmon white muscle fed 100% CO is 4086 µg g^{-1} (wet weight), which is 306 mg for a 75 g serving. In salmon white muscle fed a double substitution with 100% CO and 10% CM, the sum of DHA and EPA is 9347 µg g^{-1} (wet weight), which is 701 mg per serving. Therefore, consuming one serving of salmon fillet that was fed either 100% CO or a double replacement diet, the amount of DHA and EPA would be more than sufficient to meet the daily requirement recommended by the WHO.

There were no observed differences in the sensory quality of salmon fed either FO or 100% CO diets. The QDA and hedonic test performed by untrained panelists revealed no significant differences in appearance, odour or texture between camelina fed and FO fed salmon fillets. Salmon fillets fed camelina had a natural appearance, smelled fresh, and had a firm-elastic texture; results that were nearly identical to salmon fillets fed CO. Panelists also could not distinguish the CO-fed fillet among two other FO-fed fillets. Radial plots reveal similar patterns for all sensory parameters and PCA score plots do not show distinct components, confirmed by t-tests between the two groups. FO-fed fillets scored slightly higher in marine odour than CO, a result that has been documented in other studies (Turchini et al., 2009). Previous experiments that replaced FO with different VO, even at lower replacement levels, reported differences in

taste, odour and fish aroma (Thomassen and Rosjo 1989; Skonberg et al., 1993), marine and rancid odour (Torstensen et al., 2005); but panelists ranked salmon fillets that were fed 100% VO diets more preferable than commercially fed salmon (Torstensen et al., 2005). Although many studies have reported that alternative lipid sources can affect some of the principal characteristics of the final sensory qualities of farmed fish, results in the literature are divided and contradictory (Turchini et al., 2009). It is possible that differences in sensory qualities between FO and CO fed fish remained unnoticed due to the use of untrained panelists, but ultimately it is the untrained consumer that would purchase the salmon.

The texture of fish muscle is also an important attribute in regards to both consumer satisfaction and mechanical processing of the fillets (Veiseth-Kent et al., 2010). There was no difference in texture between FO and CO fed fillets, as found in previous studies that fed fish VO diets (Montero et al., 2005; Regost et al., 2004; Hardy et al., 1987). For logistical reasons, texture analysis was performed on frozen fillets. Texture maximal force of raw fillets tends to decrease with frozen storage (Regost et al., 2004), which explains lower maximum force and break force values found in this study compared to previous studies (Regost et al., 2004; Veiseth-Kent et al., 2010). Regardless of frozen storage, dietary oil did not appear to affect the texture of FO and CO fed fillets that were stored in the same conditions. Further, the results found by instrumental texture analysis agree with the texture results evaluated by sensory analysis, in that there was no detectable difference between treatments. Pigmentation is also an important attribute in consumer perception of raw salmon fillets. There was no difference in lightness, redness, yellowness. Chromaticity and hue were within range of previous studies on salmon colour (Veiseth-Kent et al., 2010). The colour instrument detected differences in colour descriptions: FO fillets were reported as dark yellow and CO fillets were reported as dark orange. The

description was contradictory to both the L, a, b measurements and the sensory analysis, which did not find differences in colour between fillets fed FO or CO, suggesting that the difference in colour are not detectable by the human eye. Freezing tends to decrease colour scores, however differences that have been found between FO and VO diets remained after frozen storage in a previous experiment (Regost et al., 2004). Therefore if there were differences in colour between diets before frozen storage in the present study, it is likely that storage would not have affected the difference in colour caused by differences in diet. Most studies did not find differences in flesh astaxanthin levels when using VO diets (Bell et al. 2002; Torstensen et al. 2005). Instrumental analysis of colour and texture confirmed results found in the sensory analysis: there is no apparent difference in appearance, odour and texture of raw salmon fillets fed FO or CO.

The present study replaced 100% of FO with CO, removed lipids from FM and partially substituted FM (10%) with CM. This was the first study to use CO as a full FO replacement for Atlantic salmon. Weight gain and growth rate were not affected by using 100% CO, however removing lipid from the FM and adding 10% CM significantly reduced weight gain and growth rate. These results show that there must be a minimum level of marine lipids provided in the diet, at least through FM in order to maintain normal growth of salmon. Double substituting both FO with CO and some FM with CM did not support growth to the expected level. It is questionable whether the combination of CO and CM, or specifically CM at the level of inclusion used in this study, cannot support growth. It is possible that other plant protein sources may be more suitable to include with CO in diets for salmon. These data lend itself to a growing list of possible VO sources that may be used to replace FO. However, considering decreases in EFAs like DHA and EPA and increases in PUFAs from the ω6 series in salmon muscle tissue after a 16 week period, replacing FO with CO for an entire production cycle may not be ideal, however using CO during

the juvenile and grow-out phase is recommended. In the future it may become necessary to use VO sources like CO at non-critical points in the production cycle, while feeding FO at critical life stages and finishing periods.

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Table 6.1. Formulation and proximate composition of control and experimental diets[1]

Diet	1	2	3	4	5
Ingredient (% of diet)					
Fish oil	14.0	-	-	-	-
Fish meal	34.9	34.9	-	31.8	-
SEFM[2]	-	-	32.9	-	30.0
Camelina oil	-	14.0	17.8	16.1	19.5
Camelina meal	-	-	-	10.0	10.0
Empyreal 75	5.0	5.0	5.0	5.0	5.0
Wheat gluten meal	15.0	15.0	15.0	15.0	15.0
Whey	5.0	5.0	5.0	5.0	5.0
Wheat	22.4	22.4	20.7	13.5	11.9
Pregelatinized starch	2.5	2.5	2.5	2.5	2.5
Vitamin/Mineral premix[3]	0.2	0.2	0.2	0.2	0.2
Special premix[4]	0.25	0.25	0.25	0.25	0.25
D/L Methionine	0.17	0.17	0.17	0.17	0.17
Choline chloride	0.5	0.5	0.5	0.5	0.5
Proximate composition, as fed basis (n=3)					
Moisture	8.6 ± 0.2	9.3 ± 0.2	10.9 ± 0.1	8.0 ± 0.04	9.7 ± 0.5
Ash	2.9 ± 0.1	3.2 ± 0.2	2.0 ± 0.1	2.7 ± 0.4	2.9 ± 0.2
Protein	41.3 ± 0.2	42.2 ± 0.5	40.7 ± 0.3	42.2 ± 0.8	41.0 ± 0.7
Lipid	17.6 ± 1.2	18.5 ± 0.2	20.0 ± 0.8	19.3 ± 0.6	22.2 ± 1.2

[1] FO (1), 100CO (2), 100COSEFM (3), 100CO10CM (4), 100COSEFM10CM (5)
[2] Solvent-extracted fish meal
[3] Northeast Nutrition, Truro, Nova Scotia, Canada. Vitamin/Mineral Premix contains per kg: Zinc 77.5 mg, Manganese 125 mg, Iron 84 mg, Copper 2.5 mg, Iodine 7.5 mg, Vitamin A 5000IU, Vitamin D 4000IU, 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.
[4] Northeast Nutrition, Truro, Nova Scotia, Canada Special Premix contains per kg: Selenium 0.220 mg, Vitamin E 250 IU, Vitamin C 200 mg, Astaxanthin 60 mg, Wheat shorts 1988 mg.

Table 6.2. Lipid class and fatty acid profile of control and experimental diets[1]

Lipid composition	1	2	3	4	5	F-stat	*p*-value
(% ww^{-1})							
Neutral lipid	15.5 ± 1.4^{a}	16.8 ± 0.2^{ab}	18.3 ± 0.9^{b}	18.6 ± 0.6^{bc}	21.1 ± 1.3^{c}	13.7	<0.001
Polar lipid	2.1 ± 0.2^{a}	1.7 ± 0.1^{b}	1.8 ± 0.1^{b}	0.8 ± 0.03^{c}	1.1 ± 0.1^{c}	58.4	<0.001
(% total lipid)							
Triacylglycerol	82.1 ± 2.7^{a}	86.6 ± 2.2^{ab}	88.0 ± 1.3^{b}	91.6 ± 2.2^{b}	91.6 ± 1.7^{b}	10.5	0.001
Free fatty acid	3.1 ± 0.7	1.1 ± 0.1	0.9 ± 0.3	2.0 ± 0.5	1.1 ± 0.1	2.14	0.15
Sterol	2.7 ± 0.7	2.9 ± 1.0	2.2 ± 0.8	2.4 ± 0.2	2.4 ± 0.8	0.17	0.95
AMPL[2]	3.4 ± 0.5^{a}	2.6 ± 0.4^{ab}	1.8 ± 0.5^{bc}	0.6 ± 0.1^{c}	1.2 ± 0.3^{bc}	12.6	0.001
Phospholipid	8.6 ± 1.1^{a}	6.7 ± 0.3^{a}	7.1 ± 0.7^{a}	3.4 ± 0.4^{b}	3.7 ± 0.9^{b}	27.0	<0.001
Fatty acids[3]							
14:0	6.5 ± 0.04^{a}	0.8 ± 0.04^{b}	0.6 ± 0.08^{c}	0.7 ± 0.01^{c}	0.4 ± 0.02^{d}	8353.4	<0.001
16:0	15.8 ± 0.08^{a}	7.2 ± 0.06^{b}	7.1 ± 0.1^{b}	7.0 ± 0.06^{b}	6.6 ± 0.2^{c}	3522.8	<0.001
16:1ω7	8.3 ± 0.05^{a}	1.7 ± 0.06^{b}	1.0 ± 0.1^{c}	1.3 ± 0.03^{d}	0.7 ± 0.02^{e}	8879.2	<0.001
18:0	2.8 ± 0.01^{a}	2.2 ± 0.2^{b}	2.4 ± 0.3^{ab}	2.2 ± 0.1^{b}	2.2 ± 0.01^{b}	7.98	0.004
18:1ω9	8.5 ± 0.04^{a}	19.5 ± 0.2^{b}	20.7 ± 0.3^{c}	20.0 ± 0.1^{d}	21.2 ± 0.1^{e}	2188.2	<0.001
18:2ω6 (LNA)	5.6 ± 0.1^{a}	18.3 ± 0.2^{b}	19.1 ± 0.1^{c}	18.6 ± 0.4^{bc}	19.0 ± 0.2^{c}	2217.7	<0.001
18:3ω3 (ALA)	0.8 ± 0.07^{a}	20.7 ± 0.4^{b}	22.5 ± 0.8^{c}	22.3 ± 0.2^{c}	24.2 ± 0.1^{d}	1481.5	<0.001
20:1ω9	3.4 ± 0.05^{a}	11.3 ± 0.2^{b}	11.0 ± 0.3^{b}	11.4 ± 0.1^{b}	11.4 ± 0.1^{b}	1079.7	<0.001
20:4ω6	0.7 ± 0.1^{a}	0.1 ± 0.01^{b}	0.0 ± 0.0^{b}	0.04 ± 0.01^{b}	0.0 ± 0.0^{b}	110.9	<0.001
20:5ω3 (EPA)	15.5 ± 0.08^{a}	1.5 ± 0.05^{b}	0.9 ± 0.1^{c}	1.1 ± 0.06^{c}	0.6 ± 0.03^{d}	19921	<0.001
22:1ω9	0.6 ± 0.02^{a}	2.8 ± 0.06^{b}	2.8 ± 0.1^{b}	2.8 ± 0.05^{b}	2.9 ± 0.04^{b}	932.8	<0.001
22:5ω3	1.8 ± 0.01^{a}	0.2 ± 0.01^{b}	0.0 ± 0.0^{b}	0.1 ± 0.01^{b}	0.0 ± 0.0^{b}	928.3	<0.001
22:6ω3 (DHA)	7.9 ± 0.07^{a}	1.5 ± 0.05^{b}	1.2 ± 0.1^{bc}	1.3 ± 0.03^{bc}	0.9 ± 0.2^{b}	1294.9	<0.001
∑SFA[4]	27.7 ± 0.7^{a}	12.2 ± 0.4^{b}	12.2 ± 0.7^{b}	11.9 ± 0.2^{b}	11.6 ± 0.3^{b}	621.0	<0.001
∑MUFA[5]	32.5 ± 0.1^{a}	42.8 ± 0.7^{b}	40.9 ± 0.6^{cd}	41.9 ± 0.1^{bc}	40.7 ± 0.2^{d}	800.7	<0.001
∑PUFA[6]	39.0 ± 0.8^{a}	44.8 ± 0.02^{b}	46.6 ± 0.2^{c}	46.1 ± 0.08^{b}	47.6 ± 0.2^{c}	224.1	<0.001
∑ω3	29.2 ± 0.6^{a}	24.1 ± 0.2^{b}	24.9 ± 0.2^{b}	24.9 ± 0.3^{b}	25.8 ± 0.1^{c}	86.2	<0.001
∑ω6	7.2 ± 0.2^{a}	20.5 ± 0.3^{b}	21.7 ± 0.1^{c}	21.0 ± 0.3^{b}	21.7 ± 0.1^{c}	2272.2	<0.001
ω3/ω6	4.1 ± 0.04^{a}	1.2 ± 0.02^{b}	1.1 ± 0.03^{b}	1.2 ± 0.03^{b}	1.2 ± 0.003^{b}	6772.2	<0.001

Terrestrial[7]	7.6 ± 0.1[a]	18.4 ± 0.2[b]	19.2 ± 0.1[c]	18.7 ± 0.4[bc]	19.0 ± 0.1[c]	1646.7	<0.001

[1] FO (1), 100CO (2), 100COSEFM (3), 100CO10CM (4), 100COSEFM10CM (5).Values are mean (n=9) ± SD. Means with different superscripts indicate significant differences at the end of the experiment.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester)
[4]Saturated fatty acid
[5]Monounsaturated fatty acid
[6]Polyunsaturated fatty acid
[7]Terrestrial = 18:2ω6 + 18:3ω3

Table 6.3. Growth performance after 16 week feeding trial[1, 2]

	1	2	3	4	5	F-stat	*p-value*
Initial Weight (g)	230 ± 41	236 ± 56	231 ± 46	255 ± 45	247 ± 11	0.65	0.64
Final Weight (g)	691 ± 153^{a}	613 ± 117^{b}	537 ± 113^{c}	573 ± 136^{bc}	529 ± 121^{c}	11.4	0.001
Weight Gain (g) [3]	471 ± 39^{a}	378 ± 31^{ab}	306 ± 44^{b}	320 ± 57^{b}	281 ± 33^{b}	9.94	0.002
Initial Length (cm)	26.2 ± 2.4	26.8 ± 2.1	26.3 ± 2.3	27.3 ± 1.4	27.6 ± 1.2	1.56	0.26
Final Length (cm)	35.0 ± 4.1^{a}	34.3 ± 2.1^{ab}	33.1 ± 2.4^{bc}	33.3 ± 2.8^{bc}	32.6 ± 2.6^{c}	5.65	0.01
SGR (% day^{-1}) [4]	0.99 ± 0.1^{a}	0.86 ± 0.1^{ab}	0.76 ± 0.1^{b}	0.73 ± 0.1^{b}	0.68 ± 0.1^{b}	8.38	0.006
CF [5]	1.53 ± 0.1	1.50 ± 0.1	1.46 ± 0.1	1.53 ± 0.1	1.51 ± 0.1	2.30	0.13
VSI (%) [6]	9.8 ± 1.1^{a}	10.1 ± 3.6^{a}	11.1 ± 1.2^{ab}	11.1 ± 1.5^{ab}	12.0 ± 1.6^{b}	4.91	0.001
AFI[2]	515 ± 7.6^{a}	436 ± 11^{b}	400 ± 29^{b}	391 ± 15^{b}	381 ± 46^{b}	22.6	0.001
FCR[2]	1.01 ± 0.1	1.06 ± 0.1	1.21 ± 0.1	1.14 ± 0.2	1.25 ± 0.1	2.15	0.17

[1]FO (1), 100CO (2), 100COSEFM (3), 100CO10CM (4), 100COSEFM10CM (5)
[2]Weight, length, condition factor, apparent feed intake, feed conversion ratio measurements calculated from individual fish. Initial measurements; n=9. Final measurements; n= 48 (FO); 48 (100CO); 67 (100COSEFM); 66 (100CO10CM); 66 (100COSEFM10CM)
[3]Weight gain = Final weight – Initial Weight. Measurement calculated from tank means, n=3
[4]Specific growth rate = 100 x [ln (final body weight) – ln(initial body weight)]/days. Measurement calculated from tank means, n=3.
[5]Condition factor = Body mass/ length3. Measurement calculated by individual fish.
[6]Viscera somatic index = 100 x (viscera mass/ body mass). Measurement calculated by individual fish, n=27.
[7]Apparent feed intake = Feed consumed (g) / number of fish per tank. Measurement calculated by tank means, n=3.
[8]Feed conversion ratio = Feed intake / Weight gain. Measurement calculated by tank means, n=3.

Table 6.4. Lipid class and fatty acid profiles in initial and final white muscle tissues[1]

Lipid composition	Initial	1	2	3	4	5	F-stat	*p*-value
(% ww^{-1})								
Total lipid	3.3 ± 1.3	14.0 ± 2.4^{a}	9.8 ± 1.6^{a}	16.8 ± 3.5^{b}	15.2 ± 4.0^{ab}	22.0 ± 2.8^{c}	7.3	0.004
Neutral lipid	2.7 ± 1.2	8.6 ± 2.4^{a}	5.3 ± 1.6^{a}	13.3 ± 2.2^{b}	13.4 ± 3.8^{b}	19.3 ± 3.2^{c}	9.0	0.002
Polar lipid	0.6 ± 0.01	0.5 ± 0.1	0.3 ± 0.03	0.6 ± 0.1	0.3 ± 0.1	0.6 ± 0.3	1.9	0.78
(% total lipid)								
Triacylglycerol	78.3 ± 8.1	88.7 ± 12	93.5 ± 2.5	92.4 ± 7.2	94.3 ± 4.6	90.3 ± 17	0.6	0.61
Free fatty acid	0.5 ± 0.1	2.0 ± 1.0	1.1 ± 0.8	0.5 ± 0.1	1.5 ± 0.5	2.2 ± 1.0	0.7	0.62
Sterol	3.4 ± 1.0	0.5 ± 0.2	-	0.7 ± 0.3	-	1.7 ± 0.3	1.1	0.4
AMPL[2]	2.8 ± 0.5	3.9 ± 2.1	2.6 ± 0.6	1.6 ± 0.6	2.0 ± 0.9	2.1 ± 1.1	0.4	0.8
Phospholipid	14.9 ± 1.6	4.9 ± 1.0	2.6 ± 0.7	5.2 ± 1.5	2.2 ± 0.5	1.8 ± 0.5	1.7	0.23
Fatty acids[3]								
14:0	4.5 ± 0.6	5.2 ± 0.3^{a}	1.8 ± 0.1^{b}	1.6 ± 0.2^{b}	1.7 ± 0.3^{b}	1.5 ± 0.2^{b}	200	<0.001
16:0	15.8 ± 1.0	15.7 ± 0.5^{a}	10.0 ± 0.5^{b}	9.5 ± 0.5^{b}	9.5 ± 0.5^{b}	8.8 ± 0.5^{c}	160	<0.001
16:1ω7	6.4 ± 1.6	7.5 ± 0.5^{a}	3.1 ± 0.2^{b}	2.8 ± 0.5^{bc}	2.8 ± 0.6^{bc}	2.1 ± 0.8^{c}	82.3	<0.001
18:0	4.1 ± 0.3	3.4 ± 0.2^{a}	2.9 ± 0.1^{b}	2.8 ± 0.2^{b}	2.8 ± 0.1^{b}	2.7 ± 0.1^{b}	28.8	<0.001
18:1ω9	18.8 ± 2.3	14.2 ± 1.2^{a}	22.6 ± 0.6^{b}	23.3 ± 0.7^{b}	23.1 ± 0.6^{b}	23.6 ± 0.6^{b}	186	<0.001
18:2ω6 (LNA)	5.8 ± 0.8	5.6 ± 0.4^{a}	12.9 ± 0.5^{b}	13.3 ± 0.4^{b}	13.1 ± 0.7^{b}	14.0 ± 0.6^{c}	304	<0.001
18:3ω3 (ALA)	1.7 ± 0.2	0.9 ± 0.1^{a}	10.6 ± 0.7^{b}	11.1 ± 0.4^{b}	11.3 ± 1.0^{b}	12.6 ± 1.0^{c}	244	<0.001
18:4ω3	1.0 ± 0.1	1.3 ± 0.1^{a}	3.5 ± 0.4^{b}	4.1 ± 0.5^{c}	4.1 ± 0.3^{c}	4.4 ± 0.4^{c}	155	<0.001
20:1ω9	1.7 ± 0.2	2.8 ± 0.3^{a}	8.6 ± 0.4^{b}	8.1 ± 0.4^{b}	8.4 ± 0.6^{b}	8.7 ± 0.3^{b}	194	<0.001
20:4ω6	1.1 ± 0.1	0.8 ± 0.1^{a}	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	49.4	<0.001
20:3ω3	0.0 ± 0.0	0.0 ± 0.0^{a}	0.7 ± 0.1^{b}	0.8 ± 0.03^{bc}	0.8 ± 0.1^{bc}	0.9 ± 0.04^{c}	96.1	<0.001
20:4ω3	0.0 ± 0.0	0.0 ± 0.0^{a}	0.7 ± 0.1^{b}	0.9 ± 0.1^{bc}	0.8 ± 0.1^{c}	0.9 ± 0.1^{c}	131	<0.001
20:5ω3 (EPA)	6.4 ± 0.1	9.0 ± 0.7^{a}	2.5 ± 0.2^{b}	2.3 ± 0.3^{b}	2.3 ± 0.4^{b}	2.1 ± 0.2^{c}	396	<0.001
22:1ω9	1.2 ± 0.3	0.3 ± 0.05^{a}	1.5 ± 0.1^{b}	1.4 ± 0.1^{b}	1.5 ± 0.1^{b}	1.6 ± 0.1^{c}	202	<0.001
22:4ω3	0.0 ± 0.0	0.0 ± 0.0^{a}	0.1 ± 0.01^{b}	0.1 ± 0.02^{bc}	0.1 ± 0.02^{bc}	0.2 ± 0.02^{c}	19.3	<0.001
22:5ω3	2.7 ± 0.2	3.8 ± 0.1^{a}	1.1 ± 0.1^{b}	1.0 ± 0.1^{b}	1.0 ± 0.2^{b}	1.0 ± 0.1^{b}	489	<0.001
22:6ω3 (DHA)	16.3 ± 1.6	13.3 ± 2.9^{a}	5.3 ± 0.5^{b}	5.6 ± 1.2^{b}	5.1 ± 0.7^{b}	3.9 ± 0.5^{c}	58.7	<0.001
∑SFA[4]	25.3 ± 1.2	25.2 ± 0.6^{a}	15.9 ± 0.6^{b}	15.2 ± 0.6^{bc}	15.2 ± 0.8^{bc}	14.3 ± 0.8^{c}	253	<0.001
∑MUFA[5]	33.5 ± 3.6	32.8 ± 2.5^{a}	41.9 ± 0.3^{b}	38.6 ± 3.6^{c}	41.2 ± 0.8^{bc}	41.0 ± 0.8^{bc}	19.9	<0.001

∑PUFA[6]	40.1 ± 3.8	41.2 ± 2.4[a]	42.0 ± 0.6[ab]	45.9 ± 3.8[c]	43.2 ± 1.2[abc]	44.4 ± 1.3[bc]	4.4	0.030
∑ω3	29.0 ± 4.5	29.2 ± 3.1[a]	24.6 ± 0.3[b]	27.9 ± 3.7[ab]	25.6 ± 0.9[bc]	25.9 ± 1.3[bc]	4.6	0.020
∑ω6	8.1 ± 0.6	7.6 ± 0.6[a]	16.3 ± 0.4[b]	17.0 ± 0.3[bc]	16.7 ± 0.7[b]	17.8 ± 0.5[c]	338	<0.001
ω3/ω6	3.6 ± 1.0	3.9 ± 0.8[a]	1.5 ± 0.03[b]	1.6 ± 0.2[b]	1.5 ± 0.04[b]	1.5 ± 0.1[b]	64.2	<0.001
DHA/EPA	2.5 ± 0.5	1.5 ± 0.2[a]	2.1 ± 0.3[b]	2.4 ± 0.3[b]	2.2 ± 0.4[b]	1.9 ± 0.6[ab]	6.4	0.008
Terrestrial[7]	7.4 ± 0.9	6.5 ± 0.5[a]	23.5 ± 1.1[b]	24.4 ± 0.7[b]	24.4 ± 1.9[b]	26.6 ± 1.6[c]	284	<0.001

[1]FO (1), 100CO (2), 100COSEFM (3), 100CO10CM (4), 100COSEFM10CM (5). Values are mean (n=9) ± SD. Means with different superscripts indicate significant differences at the end of the experiment.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester)
[4]Saturated fatty acid
[5]Monounsaturated fatty acid
[6]Polyunsaturated fatty acid
[7]Terrestrial = 18:2ω6 + 18:3ω3

Table 6.5. Final lipid class and fatty acid profiles in dark muscle tissue[1]

Lipid composition	1	2	3	4	5	F-stat	*p*-value
(% ww^{-1})							
Total lipid	24.9 ± 4.5^{a}	32.8 ± 3.4^{b}	29.9 ± 3.9^{b}	29.8 ± 4.8^{b}	23.8 ± 3.0^{a}	6.4	0.007
Neutral lipid	24.3 ± 4.5^{a}	32.3 ± 3.8^{b}	28.8 ± 3.9^{b}	29.2 ± 4.8^{b}	23.2 ± 3.0^{a}	6.3	0.007
Polar lipid	0.6 ± 0.1^{a}	0.6 ± 0.1^{a}	1.1 ± 0.4^{b}	0.6 ± 0.1^{a}	0.6 ± 0.1^{a}	6.4	0.007
(% total lipid)							
Triacylglycerol	94.6 ± 3.7^{a}	97.9 ± 1.4^{b}	96.2 ± 1.7^{bc}	97.8 ± 1.3^{b}	95.5 ± 3.9^{c}	5.6	0.01
Free fatty acid	0.9 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	-	1.2 ± 0.1	0.4	0.79
AMPL[2]	2.4 ± 0.2	0.6 ± 0.1	2.1 ± 0.4	1.1 ± 0.4	1.9 ± 0.8	2.1	0.16
Phospholipid	2.1 ± 0.6	0.8 ± 0.2	1.3 ± 0.6	1.1 ± 0.5	1.3 ± 0.1	2.2	0.15
Fatty acids[3]							
14:0	5.1 ± 0.1^{a}	2.3 ± 0.2^{b}	2.3 ± 0.2^{b}	2.2 ± 0.1^{bc}	1.9 ± 0.3^{c}	189	<0.001
16:0	14.6 ± 0.5^{a}	9.8 ± 0.4^{b}	9.4 ± 0.5^{bc}	9.5 ± 0.3^{b}	8.7 ± 0.5^{c}	133	<0.001
16:1ω7	7.8 ± 0.2^{a}	4.1 ± 0.3^{b}	3.9 ± 0.4^{b}	3.7 ± 0.2^{bc}	3.2 ± 0.6^{c}	105	<0.001
18:0	3.0 ± 0.8	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	1.15	0.387
18:1ω9	15.8 ± 1.2^{a}	22.5 ± 0.5^{b}	23.4 ± 0.4^{b}	23.0 ± 0.4^{b}	23.0 ± 0.4^{b}	309	<0.001
18:2ω6 (LNA)	6.0 ± 0.3^{a}	12.1 ± 0.5^{b}	12.6 ± 0.5^{b}	12.4 ± 0.4^{b}	13.5 ± 0.9^{c}	152	<0.001
18:3ω3 (ALA)	1.1 ± 0.1^{a}	9.4 ± 0.7^{b}	10.0 ± 0.7^{b}	9.9 ± 0.4^{b}	11.6 ± 1.2^{c}	216	<0.001
18:4ω3	1.4 ± 0.04^{a}	3.1 ± 0.3^{b}	3.6 ± 0.4^{c}	3.8 ± 0.5^{c}	3.8 ± 0.4^{c}	53.2	<0.001
20:1ω9	2.9 ± 0.2^{a}	7.8 ± 0.4^{b}	7.6 ± 0.4^{b}	7.7 ± 0.3^{b}	8.0 ± 0.5^{b}	239	<0.001
20:4ω6	0.8 ± 0.02^{a}	0.4 ± 0.04^{b}	0.4 ± 0.02^{b}	0.4 ± 0.05^{b}	0.4 ± 0.04^{b}	134	<0.001
20:3ω3	0.0 ± 0.0^{a}	0.6 ± 0.04^{b}	0.7 ± 0.02^{c}	0.6 ± 0.1^{b}	0.7 ± 0.1^{c}	463	<0.001
20:4ω3	0.1 ± 0.01^{a}	0.7 ± 0.1^{b}	0.8 ± 0.1^{b}	0.8 ± 0.1^{b}	0.8 ± 0.1^{b}	3.48	0.040
20:5ω3 (EPA)	8.3 ± 0.6^{a}	3.0 ± 0.3^{b}	2.7 ± 0.2^{b}	2.7 ± 0.3^{b}	2.5 ± 0.2^{b}	507	<0.001
22:1ω9	0.4 ± 0.02^{a}	1.4 ± 0.1^{b}	1.4 ± 0.1^{b}	1.4 ± 0.1^{b}	1.5 ± 0.1^{c}	125	<0.001
22:4ω3	0.2 ± 0.02^{a}	0.1 ± 0.01^{b}	0.1 ± 0.01^{b}	0.1 ± 0.01^{b}	0.1 ± 0.01^{b}	155	<0.001
22:5ω3	3.6 ± 0.2^{a}	1.4 ± 0.1^{b}	1.3 ± 0.1^{b}	1.3 ± 0.1^{b}	1.2 ± 0.1^{c}	782	<0.001
22:6ω3 (DHA)	11.7 ± 0.7^{a}	5.9 ± 0.4^{b}	5.7 ± 0.4^{bc}	5.6 ± 0.5^{bc}	5.2 ± 0.5^{c}	260	<0.001
∑SFA[4]	23.6 ± 1.0^{a}	16.2 ± 0.6^{b}	15.8 ± 0.7^{bc}	15.8 ± 0.5^{bc}	14.8 ± 0.8^{c}	170	<0.001
∑MUFA[5]	32.2 ± 1.2^{a}	42.3 ± 0.4^{b}	41.6 ± 0.6^{bc}	41.7 ± 0.6^{b}	40.7 ± 0.8^{c}	156	<0.001

∑PUFA[6]	40.4 ± 0.6[a]	41.2 ± 0.6[ab]	42.3 ± 0.8[c]	42.2 ± 0.6[bc]	44.1 ± 1.3[d]	13.3	0.001
∑ω3	27.6 ± 0.8[a]	24.2 ± 0.4[b]	24.8 ± 0.4[b]	24.9 ± 0.4[b]	26.0 ± 0.8[c]	22.2	<0.001
∑ω6	8.6 ± 0.4[a]	15.4 ± 0.5[b]	16.1 ± 0.6[b]	15.9 ± 0.4[b]	16.9 ± 0.8[c]	162	<0.001
ω3/ω6	3.2 ± 0.2[a]	1.6 ± 0.04[b]	1.5 ± 0.04[b]	1.6 ± 0.03[b]	1.5 ± 0.04[b]	181	<0.001
DHA/EPA	1.4 ± 0.1[a]	2.0 ± 0.1[b]	2.1 ± 0.2[b]	2.1 ± 0.2[b]	2.1 ± 0.1[b]	40.0	<0.001
Terrestrial[7]	7.1 ± 0.5[a]	21.5 ± 0.2[b]	22.6 ± 1.1[b]	22.3 ± 0.7[b]	25.2 ± 2.1[c]	193	<0.001

[1]FO (1), 100CO (2), 100COSEFM (3), 100CO10CM (4), 100COSEFM10CM (5). Values are mean (n=9) ± SD. Means with different superscripts indicate significant differences at the end of the experiment.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester)
[4]Saturated fatty acid
[5]Monounsaturated fatty acid
[6]Polyunsaturated fatty acid
[7]Terrestrial = 18:2ω6 + 18:3ω3

Table 6.6. Lipid class and fatty acid profile final belly flap tissue[1]

Lipid composition	1	2	3	4	5	F-stat	*p*-value
(% ww^{-1})							
Total lipid	20.5 ± 2.7	21.7 ± 3.3	26.0 ± 2.2	25.9 ± 2.7	22.5 ± 3.2	0.6	0.67
Neutral lipid	19.9 ± 2.7	20.8 ± 3.0	25.3 ± 2.3	24.8 ± 2.5	22.1 ± 2.4	0.6	0.67
Polar lipid	0.6 ± 0.1	0.9 ± 0.3	0.8 ± 0.2	1.1 ± 0.3	0.4 ± 0.1	0.5	0.71
(% total lipid)							
Triacylglycerol	91.1 ± 3.1	92.7 ± 4.7	93.1 ± 6.2	93.3 ± 3.7	97.2 ± 2.7	2.2	0.14
Free fatty acid	4.6 ± 1.4	3.9 ± 1.6	3.4 ± 0.8	2.8 ± 0.7	0.9 ± 0.3	2.2	0.15
AMPL[2]	2.0 ± 0.6	1.8 ± 0.8	2.4 ± 0.5	1.3 ± 0.6	1.6 ± 0.5	0.2	0.94
Phospholipid	1.4 ± 0.3	1.6 ± 0.4	1.1 ± 0.4	2.7 ± 1.0	1.0 ± 0.4	1.2	0.35
Fatty acids[3]							
14:0	5.2 ± 0.2^{a}	2.0 ± 0.1^{b}	1.8 ± 0.2^{bc}	1.9 ± 0.2^{b}	1.6 ± 0.2^{c}	685	<0.001
16:0	15.1 ± 0.6^{a}	10.1 ± 0.3^{b}	9.4 ± 0.3^{c}	9.6 ± 0.3^{bc}	9.0 ± 0.5^{c}	288	<0.001
16:1ω7	7.9 ± 0.1^{a}	3.4 ± 0.3^{b}	3.0 ± 0.3^{b}	3.2 ± 0.2^{b}	2.5 ± 0.3^{c}	425	<0.001
18:0	3.4 ± 0.1^{a}	2.9 ± 0.1^{b}	2.8 ± 0.1^{b}	2.8 ± 0.2^{b}	2.8 ± 0.1^{b}	27.3	<0.001
18:1ω9	15.9 ± 1.2^{a}	23.4 ± 0.6^{b}	24.6 ± 0.5^{bc}	23.9 ± 0.5^{bc}	24.1 ± 0.4^{c}	202	<0.001
18:2ω6 (LNA)	5.9 ± 0.1^{a}	12.4 ± 0.3^{b}	13.1 ± 0.4^{bc}	12.5 ± 0.5^{cd}	13.7 ± 0.9^{d}	343	<0.001
18:3ω3 (ALA)	1.0 ± 0.1^{a}	9.7 ± 0.6^{b}	10.5 ± 0.5^{b}	10.1 ± 0.6^{b}	11.8 ± 1.1^{c}	316	<0.001
18:4ω3	1.4 ± 0.1^{a}	3.4 ± 0.4^{b}	4.1 ± 0.5^{c}	4.3 ± 0.6^{c}	4.4 ± 0.3^{c}	130	<0.001
20:1ω9	3.0 ± 0.1^{a}	8.6 ± 0.4^{b}	8.4 ± 0.4^{b}	8.4 ± 0.4^{b}	8.7 ± 0.4^{b}	405	<0.001
20:4ω6	0.8 ± 0.02^{a}	0.4 ± 0.03^{b}	0.3 ± 0.02^{b}	0.4 ± 0.05^{b}	0.3 ± 0.04^{b}	184	<0.001
20:3ω3	0.0 ± 0.0^{a}	0.7 ± 0.1^{b}	0.8 ± 0.03^{c}	0.7 ± 0.1^{b}	0.8 ± 0.04^{c}	188	<0.001
20:5ω3 (EPA)	8.9 ± 0.6^{a}	2.7 ± 0.2^{b}	2.2 ± 0.2^{bc}	2.4 ± 0.3^{bc}	2.2 ± 0.2^{c}	860	<0.001
22:1ω9	0.3 ± 0.05^{a}	1.4 ± 0.1^{b}	1.4 ± 0.1^{b}	1.5 ± 0.1^{b}	1.6 ± 0.1^{c}	233	<0.001
22:5ω3	3.8 ± 0.1^{a}	1.1 ± 0.1^{b}	1.0 ± 0.1^{bc}	1.1 ± 0.1^{b}	0.9 ± 0.1^{c}	800	<0.001
22:6ω3 (DHA)	11.4 ± 0.9^{a}	4.8 ± 0.3^{b}	4.6 ± 0.4^{b}	4.7 ± 0.6^{b}	4.0 ± 0.7^{b}	202	<0.001
∑SFA[4]	24.5 ± 0.9^{a}	16.3 ± 0.4^{b}	15.2 ± 0.6^{cd}	15.6 ± 0.6^{bc}	14.7 ± 0.8^{d}	340	<0.001
∑MUFA[5]	35.5 ± 1.0^{a}	43.3 ± 0.7^{b}	42.6 ± 0.4^{bc}	42.8 ± 0.6^{bc}	41.9 ± 0.5^{c}	151	<0.001
∑PUFA[6]	39.3 ± 0.9^{a}	40.1 ± 0.7^{a}	41.9 ± 0.7^{b}	41.3 ± 0.9^{b}	43.2 ± 1.0^{c}	16.4	<0.001
∑ω3	27.6 ± 0.8^{a}	23.4 ± 0.5^{b}	24.3 ± 0.3^{c}	24.3 ± 0.5^{c}	25.2 ± 0.5^{d}	43.9	<0.001

∑ω6	8.5 ± 0.2[a]	15.9 ± 0.3[b]	16.8 ± 0.5[c]	16.2 ± 0.5[b]	17.3 ± 0.6[c]	374	<0.001
ω3/ω6	3.2 ± 0.1[a]	1.5 ± 0.03[b]	1.4 ± 0.03[b]	1.5 ± 0.04[b]	1.5 ± 0.04[b]	1468	<0.001
DHA/EPA	1.3 ± 0.2[a]	1.8 ± 0.2[b]	2.0 ± 0.1[b]	2.0 ± 0.3[b]	1.8 ± 0.2[b]	24.5	<0.001
Terrestrial[7]	6.9 ± 0.2	22.1 ± 0.9	23.6 ± 0.8	22.5 ± 1.0	25.5 ± 2.0	332	<0.001

[1]FO (1), 100CO (2), 100COSEFM (3), 100CO10CM (4), 100COSEFM10CM (5). Values are mean (n=9) ± SD. Means with different superscripts indicate significant differences at the end of the experiment.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester)
[4]Saturated fatty acid
[5]Monounsaturated fatty acid
[6]Polyunsaturated fatty acid
[7]Terrestrial = 18:2ω6 + 18:3ω3

Table 6.7. Lipid class and fatty acid profile final skin tissue[1]

Lipid composition	1	2	3	4	5	F-stat	*p*-value
(% ww^{-1})							
Total lipid	31.1 ± 3.9^{a}	32.0 ± 2.9^{a}	44.9 ± 4.9^{b}	35.3 ± 3.5^{ab}	39.7 ± 3.5^{b}	3.9	0.04
Neutral lipid	30.6 ± 3.9	31.3 ± 2.9	42.7 ± 4.4	32.9 ± 3.6	37.5 ± 3.3	3.0	0.07
Polar lipid	0.5 ± 0.1	0.7 ± 0.3	2.2 ± 0.8	2.4 ± 0.6	2.1 ± 0.4	2.2	0.15
(% total lipid)							
Triacylglycerol	91.9 ± 3.3	90.3 ± 5.2	92.3 ± 5.8	85.9 ± 8.1	90.2 ± 2.5	1.1	0.38
Free fatty acid	2.6 ± 1.2	2.9 ± 0.6	1.9 ± 0.7	1.9 ± 0.5	1.9 ± 0.8	1.2	0.36
Sterol	3.6 ± 0.6	4.1 ± 0.2	1.1 ± 0.6	4.1 ± 1.0	2.1 ± 0.5	1.2	0.38
AMPL[2]	1.0 ± 0.4	1.8 ± 0.4	1.1 ± 0.3	2.9 ± 0.6	4.1 ± 0.8	2.7	0.09
Phospholipid	0.9 ± 0.5	0.7 ± 0.03	2.6 ± 1.0	1.8 ± 0.8	1.4 ± 0.1	0.4	0.81
Fatty acids[3]							
14:0	5.0 ± 0.3^{a}	2.0 ± 0.2^{b}	1.8 ± 0.2^{b}	1.7 ± 0.4^{b}	1.7 ± 0.3^{b}	331	<0.001
16:0	15.1 ± 1.3^{a}	10.0 ± 0.4^{b}	9.3 ± 0.4^{bc}	9.4 ± 0.5^{bc}	9.0 ± 0.6^{c}	114	<0.001
16:1ω7	7.6 ± 0.2^{a}	3.5 ± 0.3^{b}	3.3 ± 0.4^{b}	3.0 ± 0.5^{bc}	2.6 ± 0.4^{c}	294	<0.001
18:0	3.4 ± 0.4^{a}	2.9 ± 0.1^{b}	2.9 ± 0.1^{b}	2.8 ± 0.2^{b}	2.8 ± 0.1^{b}	10.6	0.001
18:1ω9	16.0 ± 1.2^{a}	23.6 ± 0.5^{b}	24.4 ± 0.7^{b}	24.1 ± 0.5^{b}	24.3 ± 0.3^{b}	286	<0.001
18:2ω6 (LNA)	5.7 ± 0.2^{a}	12.4 ± 0.5^{b}	13.3 ± 0.5^{cd}	12.8 ± 0.6^{bc}	13.6 ± 0.8^{d}	563	<0.001
18:3ω3 (ALA)	1.0 ± 0.1^{a}	9.7 ± 0.7^{b}	10.8 ± 0.4^{cd}	10.3 ± 0.9^{bc}	11.7 ± 1.2^{d}	505	<0.001
18:4ω3	1.3 ± 0.1^{a}	3.4 ± 0.3^{b}	4.0 ± 0.4^{c}	4.3 ± 0.6^{c}	4.3 ± 0.3^{c}	106	<0.001
20:1ω9	2.9 ± 0.1^{a}	8.5 ± 0.5^{b}	8.4 ± 0.4^{b}	8.6 ± 0.8^{b}	8.8 ± 0.4^{b}	521	<0.001
20:4ω6	0.8 ± 0.1^{a}	0.4 ± 0.1^{b}	0.4 ± 0.04^{b}	0.4 ± 0.1^{b}	0.3 ± 0.04^{b}	46.5	<0.001
20:3ω3	0.0 ± 0.0^{a}	0.7 ± 0.04^{b}	0.8 ± 0.05^{b}	0.8 ± 0.1^{b}	0.9 ± 0.05^{b}	82.3	<0.001
20:5ω3 (EPA)	8.9 ± 0.6^{a}	2.7 ± 0.3^{b}	2.4 ± 0.3^{b}	2.3 ± 0.4^{b}	2.2 ± 0.2^{b}	745	<0.001
22:1ω9	0.4 ± 0.03^{a}	1.4 ± 0.1^{b}	1.4 ± 0.1^{b}	1.5 ± 0.1^{bc}	1.6 ± 0.1^{c}	230	<0.001
22:5ω3	3.8 ± 0.2^{a}	1.0 ± 0.4^{b}	1.0 ± 0.1^{b}	1.0 ± 0.1^{b}	0.9 ± 0.1^{b}	249	<0.001
22:6ω3 (DHA)	11.6 ± 0.9^{a}	4.7 ± 0.4^{b}	4.5 ± 0.3^{bc}	4.5 ± 0.5^{bc}	3.9 ± 0.7^{c}	292	<0.001
∑SFA[4]	24.3 ± 1.7^{a}	16.1 ± 0.4^{b}	14.9 ± 0.8^{b}	15.2 ± 0.9^{b}	14.7 ± 1.3^{b}	118	<0.001
∑MUFA[5]	35.2 ± 1.6^{a}	43.4 ± 0.6^{b}	42.4 ± 0.7^{bc}	42.9 ± 0.6^{bc}	42.1 ± 0.5^{c}	135	<0.001
∑PUFA[6]	39.8 ± 1.0^{a}	40.1 ± 0.5^{a}	42.5 ± 0.7^{bc}	41.7 ± 0.8^{b}	43.1 ± 1.1^{c}	22.0	<0.001

∑ω3	27.5 ± 0.8[a]	23.1 ± 0.3[b]	24.4 ± 0.3[cd]	24.1 ± 0.4[c]	24.9 ± 0.4[d]	81.8	<0.001
∑ω6	7.6 ± 0.4[a]	15.5 ± 0.5[b]	16.6 ± 0.5[c]	16.2 ± 0.8[bc]	16.9 ± 0.7[c]	417	<0.001
ω3/ω6	3.6 ± 0.2[a]	1.5 ± 0.1[b]	1.5 ± 0.03[b]	1.5 ± 0.1[b]	1.5 ± 0.04[b]	281	<0.001
DHA/EPA	1.3 ± 0.2[a]	1.8 ± 0.2[b]	1.9 ± 0.2[b]	2.0 ± 0.3[b]	1.7 ± 0.2[b]	10.8	0.001
Terrestrial[7]	6.7 ± 0.3[a]	22.1 ± 1.1[b]	24.1 ± 0.7[cd]	23.1 ± 1.5[bc]	25.1 ± 2.0[d]	449	<0.001

[1]FO (1), 100CO (2), 100COSEFM (3), 100CO10CM (4), 100COSEFM10CM (5). Values are mean (n=9) ± SD. Means with different superscripts indicate significant differences at the end of the experiment.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester)
[4]Saturated fatty acid
[5]Monounsaturated fatty acid
[6]Polyunsaturated fatty acid
[7]Terrestrial = 18:2ω6 + 18:3ω3

Table 6.8. Lipid class and fatty acid profile final viscera fat[1]

Lipid composition	1	2	3	4	5	F-stat	*p*-value
(% ww^{-1})							
Total lipid	79.3 ± 8.2^{a}	70.3 ± 4.6^{b}	69.3 ± 10^{b}	71.0 ± 11^{b}	65.3 ± 13^{c}	6.5	0.01
Neutral lipid	74.9 ± 7.1^{a}	68.5 ± 4.2^{b}	66.1 ± 11^{b}	66.6 ± 11^{b}	71.6 ± 12^{c}	7.5	0.003
Polar lipid	4.1 ± 0.8	1.8 ± 0.5	3.1 ± 1.5	3.8 ± 1.1	1.6 ± 0.6	0.6	0.71
(% total lipid)							
Triacylglycerol	90.5 ± 4.0	93.3 ± 3.0	89.8 ± 5.0	90.5 ± 7.3	92.7 ± 4.8	0.8	0.54
Free fatty acid	3.4 ± 1.0	3.9 ± 0.7	3.4 ± 0.6	4.1 ± 1.0	3.9 ± 1.1	0.4	0.88
Sterol	1.8 ± 0.7^{a}	2.2 ± 0.5^{a}	4.4 ± 0.5^{b}	1.9 ± 0.8^{a}	1.7 ± 0.7^{a}	8.9	0.002
AMPL[2]	3.2 ± 0.5	1.7 ± 0.4	2.0 ± 0.4	2.4 ± 1.0	1.8 ± 0.5	1.0	0.45
Phospholipid	1.1 ± 0.4	1.0 ± 0.3	1.9 ± 0.5	1.9 ± 0.4	0.9 ± 0.3	0.5	0.72
Fatty acids[3]							
14:0	5.2 ± 0.3^{a}	2.0 ± 0.2^{b}	1.8 ± 0.3^{b}	2.0 ± 0.2^{b}	1.7 ± 0.4^{b}	203	<0.001
16:0	14.1 ± 0.5^{a}	9.4 ± 0.4^{b}	8.9 ± 0.4^{bc}	9.0 ± 0.3^{b}	8.4 ± 0.6^{c}	232	<0.001
16:1ω7	8.1 ± 0.3^{a}	3.6 ± 0.4^{b}	3.1 ± 0.5^{bc}	3.3 ± 0.3^{b}	2.7 ± 0.6^{c}	154	<0.001
18:0	3.1 ± 0.2^{a}	2.9 ± 0.2^{ab}	2.7 ± 0.1^{ab}	2.7 ± 0.2^{ab}	2.3 ± 0.9^{b}	5.11	0.017
18:1ω9	16.0 ± 1.1^{a}	23.6 ± 0.6^{b}	24.5 ± 0.5^{b}	24.1 ± 0.6^{b}	24.2 ± 0.4^{b}	300	<0.001
18:2ω6 (LNA)	6.1 ± 0.3^{a}	12.5 ± 0.4^{b}	13.3 ± 0.5^{cd}	12.7 ± 0.3^{bc}	13.8 ± 0.9^{d}	327	<0.001
18:3ω3 (ALA)	1.1 ± 0.1^{a}	9.8 ± 0.6^{b}	10.7 ± 0.7^{b}	10.1 ± 0.3^{b}	11.9 ± 1.1^{c}	306	<0.001
18:4ω3	1.4 ± 0.1^{a}	3.4 ± 0.3^{b}	4.2 ± 0.6^{c}	4.3 ± 0.7^{c}	4.4 ± 0.4^{c}	76.4	<0.001
20:1ω9	3.0 ± 0.2^{a}	8.6 ± 0.5^{b}	8.3 ± 0.6^{b}	8.3 ± 0.3^{b}	8.6 ± 0.7^{b}	140	<0.001
20:4ω6	0.7 ± 0.01^{a}	0.3 ± 0.04^{b}	0.3 ± 0.1^{b}	0.4 ± 0.03^{b}	0.3 ± 0.1^{b}	115	<0.001
20:3ω3	0.1 ± 0.01^{a}	0.7 ± 0.1^{b}	0.8 ± 0.04^{c}	0.7 ± 0.1^{b}	0.8 ± 0.01^{c}	193	<0.001
20:5ω3 (EPA)	8.8 ± 0.9^{a}	2.8 ± 0.3^{b}	2.3 ± 0.2^{b}	2.4 ± 0.2^{b}	2.2 ± 0.3^{b}	656	<0.001
22:1ω9	0.4 ± 0.03^{a}	1.5 ± 0.1^{b}	1.4 ± 0.2^{b}	1.4 ± 0.1^{b}	1.6 ± 0.2^{b}	86.1	<0.001
22:5ω3	3.8 ± 0.2^{a}	1.2 ± 0.2^{b}	1.1 ± 0.1^{bc}	1.1 ± 0.1^{bc}	1.0 ± 0.2^{c}	737	<0.001
22:6ω3 (DHA)	10.9 ± 0.5^{a}	4.5 ± 0.4^{b}	4.4 ± 0.7^{b}	4.6 ± 0.6^{b}	3.9 ± 0.9^{b}	155	<0.001
∑SFA[4]	23.3 ± 0.6^{a}	15.6 ± 0.6^{b}	14.8 ± 0.8^{bc}	14.9 ± 0.7^{bc}	13.9 ± 1.3^{c}	254	<0.001
∑MUFA[5]	35.8 ± 1.0^{a}	43.7 ± 0.6^{b}	42.5 ± 0.5^{cd}	43.0 ± 0.6^{bc}	42.1 ± 0.4^{d}	244	<0.001
∑PUFA[6]	40.3 ± 0.8^{a}	40.4 ± 0.7^{a}	42.4 ± 0.8^{b}	41.8 ± 1.1^{b}	43.9 ± 1.0^{c}	27.6	<0.001

∑ω3	27.0 ± 0.6[a]	23.2 ± 0.3[b]	24.4 ± 0.4[c]	24.3 ± 0.7[c]	25.2 ± 0.2[d]	64.9	<0.001
∑ω6	8.8 ± 0.3[a]	15.8 ± 0.4[b]	17.0 ± 0.6[cd]	16.3 ± 0.4[bc]	17.3 ± 0.8[d]	380	<0.001
ω3/ω6	3.1 ± 0.1[a]	1.5 ± 0.04[b]	1.4 ± 0.04[b]	1.5 ± 0.03[b]	1.5 ± 0.1[b]	1896	<0.001
DHA/EPA	1.3 ± 0.1[a]	1.6 ± 0.2[b]	1.9 ± 0.2[b]	1.9 ± 0.3[b]	1.8 ± 0.2[b]	11.9	0.001
Terrestrial[7]	7.2 ± 0.4[a]	22.2 ± 1.0[b]	24.0 ± 1.1[bc]	22.8 ± 0.6[b]	25.6 ± 2.4[c]	327	<0.001

[1]FO (1), 100CO (2), 100COSEFM (3), 100CO10CM (4), 100COSEFM10CM (5). Values are mean (n=9) ± SD. Means with different superscripts indicate significant differences at the end of the experiment.
[2]Acetone mobile polar lipid
[3]Data expressed as area percentage of FAME (fatty acid methyl ester)
[4]Saturated fatty acid
[5]Monounsaturated fatty acid
[6]Polyunsaturated fatty acid
[7]Terrestrial = 18:2ω6 + 18:3ω3

Table 6.9. The dissimilarity (%) between white muscle tissue fatty acids and dietary fatty acids for each dietary group, as interpreted by SIMPER[1]

	FO	100CO	100COSEFM	100CO10CM	100COSEFM10CM
Mean Dissimilarity	8.73	21.3	22.0	22.3	19.6
Major Contributors	15.3 (20:5ω3)	24.4 (18:3ω3)	27.8 (18:3ω3)	25.5 (18:3ω3)	31.4 (18:3ω3)
	13.4 (18:1ω9)	12.8 (18:2ω6)	14.2 (18:2ω6)	12.7 (18:2ω6)	13.4 (18:2ω6)
	12.8 (22:6ω3)	9.22 (22:6ω3)	11.7 (20:1ω9)	9.34 (22:6ω3)	8.39 (22:6ω3)

[1]Values are based on n= 9 for tissue samples and n=3 for diet sample

Table 6.10. Sensory, texture and colour evaluations performed on salmon fillets that were fed either FO or CO100 diets

	FO	CO100	*t*-stat	p-value
Sensory Evaluation[1]				
Brightness	4.4 ± 1.4	4.1 ± 1.7	-0.65	0.52
Orange intensity	4.1 ± 1.5	4.0 ± 1.7	-0.36	0.72
Surface moistness	4.1 ± 1.3	4.5 ± 1.5	-0.90	0.38
Firmness	4.8 ± 1.5	4.6 ± 1.4	-0.48	0.63
Marine odour	4.0 ± 1.9	3.5 ± 1.9	-0.84	0.41
Vegetable odour	2.0 ± 1.3	2.0 ± 1.5	-0.10	0.92
Rancid odour	1.2 ± 0.5	1.3 ± 1.0	0.69	0.48
Appearance[2]	3.3 ± 1.3	3.2 ± 1.3	-0.23	0.82
Odour[3]	2.1 ± 0.8	2.5 ± 0.8	1.83	0.07
Texture[4]	2.2 ± 1.3	2.4 ± 1.4	0.53	0.60
Texture				
Breakpoint force (N)	2.65 ± 0.03	2.32 ± 0.2	-1.63	0.35
Maximum force (N)	2.88 ± 0.1	2.74 ± 0.8	-0.23	0.85
Fillet thickness (mm)	15.3 ± 2.3	15.4 ± 2.0	0.04	0.97
Colour				
Lightness (L)	4561 ± 235	4575 ± 237	0.07	0.95
Redness (a)	1074 ± 154	1109 ± 68	0.36	0.76
Yellowness (b)	2299 ± 88	2711 ± 437	1.60	0.25
Chromaticity (C)	2541 ± 64	2931 ± 421	1.58	0.25
Hue (H)	64.9 ± 3.7	67.5 ± 2.6	0.98	0.41
Description	Dark yellow	Dark orange		

[1]Scores from both tests were on a scale from 1-7
[2]Scale ranged from 1 (translucent) - 7 (discoloured)
[3]Scale ranged from 1 (marine) – 7 (putried)
[4]Scale ranged from 1 (firm) – 7 (plastic)

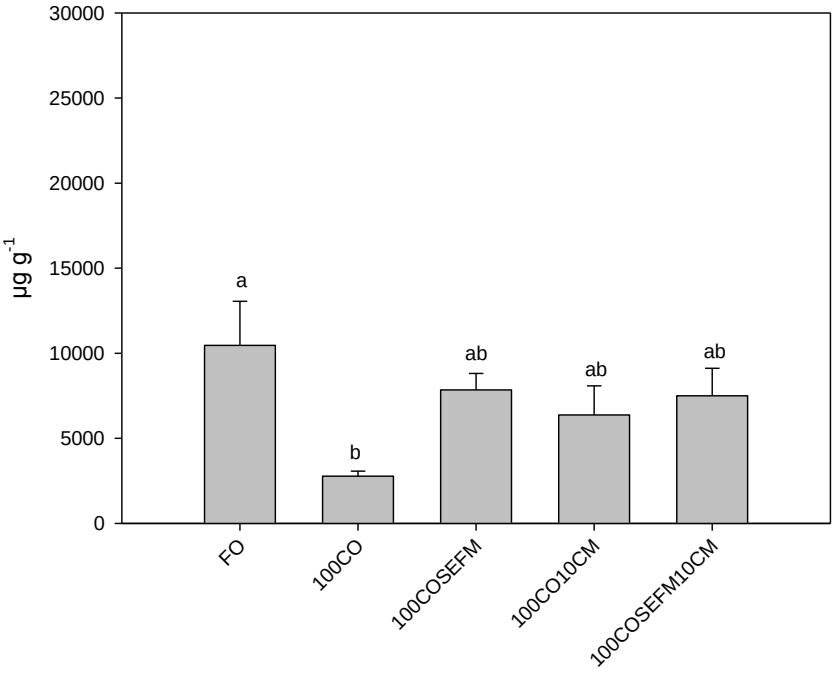

Figure 6.1. Quantitative amount of DHA (µg g^{-1}) in white muscle tissue after 16 weeks

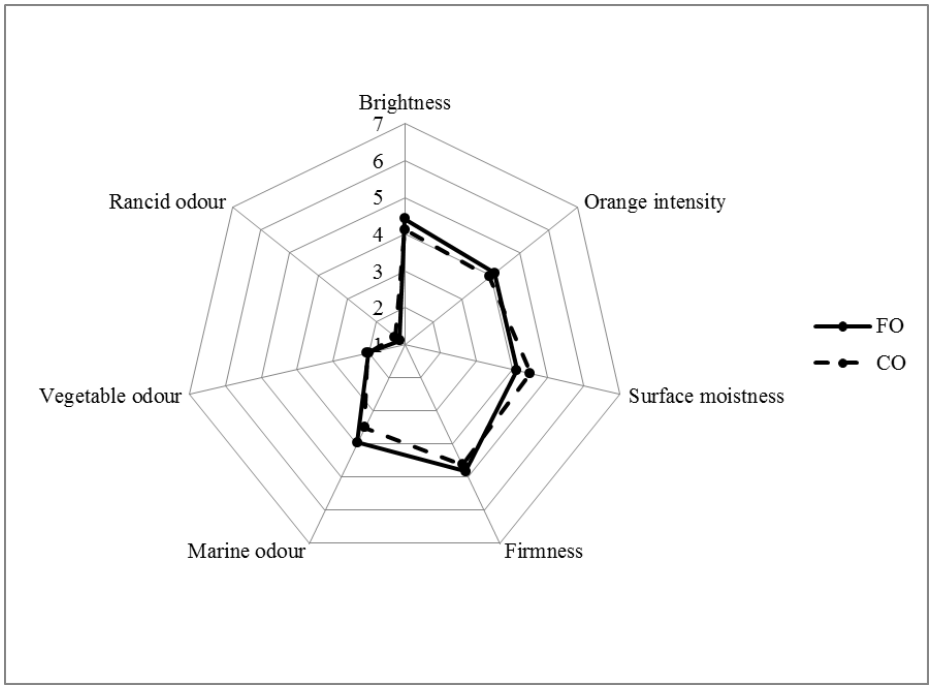

Figure 6.2. Sensory attributes of salmon fillets after 16 weeks of feeding either FO or 100CO diets

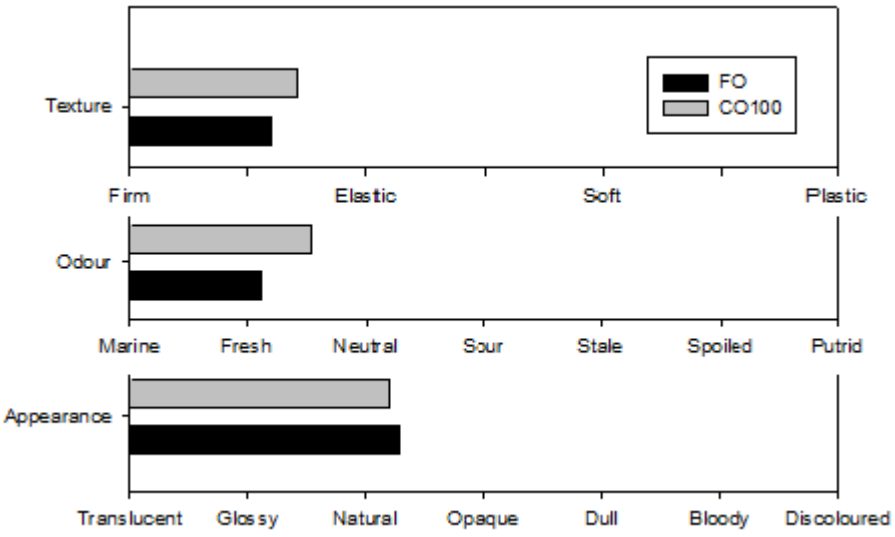

Figure 6.3. Sensory attributes of salmon fillets after 16 weeks of feeding either FO or 100CO diets

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Chapter 7. Conclusion

This study examined the effects of using CO as a FO replacement in diets for farmed Atlantic cod, rainbow trout and Atlantic salmon. The main focus of each study was the effect on growth, lipid class and fatty acid composition of tissues, lipid metabolism and fatty acid biosynthesis. Several quantitative analyses were used in order to address these research questions, specifically lipid instrumental analyses, compound specific stable isotope analysis, the fatty acid mass balance method, energy budgeting, multivariate statistics, sensory evaluation and sensory instrumental analyses. Five experimental feeding trials were conducted with Atlantic cod, rainbow trout and Atlantic salmon in order to determine the effectiveness of CO in diets.

Growth of Atlantic cod was not affected when 80% of fish oil was replaced with camelina oil; however, replacing 100% fish oil depressed weight gain over a 13 week feeding period, but did not affect growth rate. Tissue fatty acid profiles showed reductions in DHA and EPA, while levels of ALA, LNA and 18:1ω9 increased. Tissue fatty acid profiles also indicated selective catabolism and retention of certain fatty acids based on the comparison of tissue to diet fatty acid profiles using multivariate statistics. The fatty acid mass balance method estimated that cod fed CO were able to elongate some ALA, while the remaining ALA was oxidized. Energy budget estimates indicated that excess CO lipids were deposited in the liver and not utilized for energy, which impacted growth. The use of SEFM in CO diets showed that diets for cod must include a minimum level of DHA and EPA, even if it is supplied in the form of FM, in order to prevent reductions in growth and severe changes in tissue fatty acid profiles. Substituting

both FO and some FM with CO and CM appeared to be problematic for growth and lipid metabolism, likely as a result of reduced feed intake due to poor diet palatability caused by antinutritional factors. CO did not prevent or ameliorate symptoms of disease and reductions in growth after infection with the parasite, *Loma morhua*.

Rainbow trout were not affected by replacing 100% FO with CO in terms of growth performance. Although levels of DHA and EPA were reduced compared to trout fed fish oil, the amount of DHA and EPA in one serving of rainbow trout fillet was enough to satisfy human health requirements according to the World Health Organization. Compound specific stable isotope analysis confirmed that the isotopic signature of DHA in CO fed trout shifted significantly compared to DHA in fish oil fed trout. These results suggest that some of the DHA in trout muscle was synthesized from the terrestrial and isotopically lighter ALA in the CO diet rather than incorporation of DHA from FM in the CO diet. Therefore, feeding CO encouraged some synthesis of ALA into more beneficial fatty acids.

Atlantic salmon were also not affected by replacing 100% FO with CO in terms of growth performance. However, when FM was solvent extracted to remove all lipids, a significant reduction in growth was observed. Additionally, feeding a diet with essentially no marine lipid and inclusion of CM yielded the poorest growth in the experiment. These results indicate that a minimum amount of DHA and EPA must be included in the diet, at least in the form of full fat FM; and also that inclusion of CM with camelina oil causes problems in growth and lipid metabolism, perhaps due to the presence of antinutritional factors in the meal. Excess lipid storage may indicate that the use of camelina fatty acids is limited; once energy requirements are fulfilled, the remainder is stored without much

purpose for other functions, in contrast to FO which is plentiful in other fatty acids such as DHA and EPA with a multitude of physiological functions. A sensory evaluation with a triangle test, hedonic test and quantitative description analysis, as well as instrumental analysis of texture and colour revealed no differences in appearance, odour, texture and colour of salmon fillets that were fed either CO or FO diets.

Generally, the use of CO is acceptable for commercial use in aquaculture, and appears to be a superior lipid source compared to already commercially used plant oils, such as soybean and canola oil, due to the relatively high ω3 content. There appeared to be slight species differences, for example cod growth was affected by the 100% CO diet, whereas the salmonids were not affected. Although lipid storage in cod and salmonids is markedly different, there were similarities in how camelina oil was metabolized. Excess lipid was stored in either the muscle for salmonids or the liver for cod, but it appeared that high levels of ALA and LNA were not well utilized, since much of it was stored and oxidized for energy and likely was not used for other physiological functions. Cod and rainbow trout showed varying abilities to biosynthesize ALA in camelina oil to longer, more unsaturated PUFAs. Cod appeared to only have an ability to elongate to an apparent dead-end product, while trout were able to synthesize some of their own DHA from ALA in camelina oil. The excess ALA substrate and lack of DHA and EPA in the diet likely encouraged biosynthesis for both cod and rainbow trout, but due to evolution, life history and natural diets, these two species clearly show varying abilities in fatty acid biosynthesis. Therefore, inclusion of CO in commercial diets must consider lipid

requirements and lipid storage in each species; salmonids can utilize CO as a full replacement of FO, whereas cod can tolerate upwards of 80% replacement of FO.

Future research in the area of FO replacements and CO will likely use biotechnology tools, such as transgenic technology, to improve camelina for aquaculture feed purposes. Improved lines of camelina that are specifically bred for certain qualities that are attractive in aquaculture, for example increased ALA content. Currently, camelina is considered as a model oilseed for genetic modification, with inclusion of fungal or yeast transgenes that enable the plant to produce endogenous DHA and EPA. Transgenic camelina would be ideal for use in aquaculture; however future research must be conducted using cultured fish and animal trials to ensure food safety and efficient fish production. Future research may also focus on developing broodstock strains that can efficiently biosynthesize ALA from CO(or other plant oils) into DHA and EPA. In this study, rainbow trout synthesized some of the DHA from ALA in camelina oil. This may be improved by choosing certain fish that have a greater capacity to do this, and develop a broodstock program with fish that have an enhanced ability to utilize terrestrial plant oils.

Appendix I. Energy budget calculation

The energy budget calculations were calculated based on total lipid intake and neutral lipid remaining in all tissues. Alternatively, this method was also calculated based on total lipid intake and neutral acyl lipid in tissues, which eliminates energy accounted for in the glycerol backbone, hydrocarbons, ketones, alcohols and sterols. The acyl lipid method was calculated for the FO and 100COSEFM treatment groups, since these groups showed the greatest difference in energy budget results. The results of this comparison showed that there was minimal difference between the two methods. For simplicity, the neutral lipid method was presented in the thesis; however, the comparison between methods is here:

	Acyl Neutral Lipid		Neutral Lipid	
	FO	100COSEFM	FO	100COSEFM
Lipid intake	2755	2707	2906	2898
Total lipid deposition	694.0	1343	779.0	1422
Oxidized lipid (%)	74.81	50.39	73.24	54.89
Stored lipid (%)	25.19	49.61	26.82	53.24
Energy intake from lipid (kJ per fish)	109.9	108.0	113.3	113.0
Deposited energy (kJ per fish)	27.69	53.59	30.43	60.14
Total energy expenditure (kJ per fish)	82.23	54.42	82.86	52.93