LONG CHAIN-POLYUNSATURATED FATTY ACID SYNTHESIS IN THE MUSCLE AND LIVER OF LANDLOCKED AND SAINT JOHN RIVER ATLANTIC SALMON FED A FISH OIL-FREE DIET

by

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ABSTRACT

The first trial compared tissue omega-3 long chain polyunsaturated fatty acid (n-3 LC-PUFA) content between the landlocked Grand Lake strain (GL) and the farmed Saint John River (SJR) strain. After 16 weeks, GL and SJR salmon fed the fish oil-free diet had a similar n-3 LC-PUFA level in the muscle and liver. The significantly highest muscle DHA level was observed in the GL/control diet, showing that GL strain conserved a higher muscle DHA content than SJR strain when fed the control diet. GL strain had a genetic potential of increased muscle n-3 LC-PUFA storage.

In the second trial, fifty Atlantic salmon families of SJR strain were offered a control or a fish oil-free diet. After 16 weeks, the n-3 LC-PUFA level clearly upregulated in tissue when fed the fish oil-free diet. Certain families exhibited the affinity to n-3 fatty acid storage when fed the fish oil-free diet.

LIST OF ABBREVIATIONS

ALA: α linolenic acid

ARA: arachidonic acid

ANOVA: analysis of variance

BW: body weight

CF: condition factor

DHA: docosahexaenoic acid

EFA: essential fatty acid

EPA: eicosapentaenoic acid

FAME: fatty acid methyl ester

FCR: feed conversion ratio

FO: fish oil

GC: gas chromatography

GL: Grand Lake

LNA: linoleic acid

LC-PUFA: long chain polyunsaturated fatty acid

MUFA: monounsaturated fatty acid

N-3: omega-3

N-6: omega-6

PL: phospholipids

PIT: passive integrated transponders

SFA: saturated fatty acids

SGR: specific growth rate

SJR: Saint John River

TAG: triacylglycerol

VSI: visceral somatic index

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CHAPTER 1. INTRODUCTION

1.1 RATIONALE

Post-smolt Atlantic salmon require 1 to 1.5% (10 to 15 g/kg) of omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) in the diet for optimal growth (Glencross et al., 2014; Bou et al., 2017a; Huyben et al., 2021a). Fish oil supplies the major source of n-3 LC-PUFA in aquaculture (FAO, 2022). This is a challenge in aquaculture to continuously supply dietary n-3 LC-PUFA from finite wild fisheries. Terrestrial plant oils such as canola oil and camelina oil, have been used as alternative oils in aquaculture feeds. However, they do not naturally provide essential n-3 LC-PUFA, DHA and EPA. The deficiency of n-3 LC-PUFA negatively affects fish growth and health. Salmonids can synthesize a part of n-3 LC-PUFA from the n-3 precursor (Hixson et al., 2014a; Tocher, 2015). Moreover, freshwater landlocked Atlantic salmon (*Salmo salar*) have a higher capacity for n-3 LC-PUFA biosynthesis compared with the farmed strain (Betancor et al., 2016). The trait of LC-PUFA synthesis is strongly related to both the genetic effect and dietary effect (Leaver et al., 2011; Horn et al., 2018).

The objective of the current study was to compare enhanced n-3 LC-PUFA content and storages between freshwater landlocked and commercial Atlantic salmon in North America. If the landlocked strain exhibited significantly better n-3 LC-PUFA content and storage when fed a diet without fish oil or n-3 LC-PUFA, it would be potentially a valuable genetic resource for the commercial Atlantic salmon broodstock and reduce the demand for fish oil as a source of n-3 LC-PUFA in the diet. Similarly, the ability of n-3 LC-PUFA storages in the fish muscle and liver were compared among fifty families within a commercial strain of Atlantic salmon in the second experiment. Families with the best or lowest growth performance were analyzed for fatty acid composition. The analyses determined whether there were significant differences in the n-3 LC-PUFA content in the fish muscle and liver among selected families. SJR families with enhanced n-3 LC-PUFA content have the genetic potential for future commercial broodstock programs.

1.2 THE PRODUCTION AND NUTRITIONAL VALUE OF ATLANTIC SALMON

From 2000 to 2020, the global production of Atlantic salmon tripled from 0.9 million tonnes to 2.7 million tonnes (FAO, 2022, p. 43). During the same period, Grass carp (*Ctenopharyngodon idellus*) production increased from 3 million tonnes to 5.8 million tonnes,

representing the highest finfish production in the world (FAO, 2022, p. 43). It was followed by Silver carp (*Hypophthalmichthys molitrix*), Nile tilapia (*Oreochromis niloticus*), Common carp (*Cyprinus carpio*), Bighead carp (*Hypophthalmichthys nobilis*), and Atlantic salmon. In terms of the nutritional value, a 100 g farmed Atlantic salmon fillet contains about 18% protein, 12 % lipid and provides approximately 1 g of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) (Jensen et al., 2012). The muscle absolute content of EPA and DHA (g/100g) is approximately 0.06 in Grass carp, 0.5 in Bighead carp (Pyz-Łukasik and Kowalczyk-Pecka, 2017), 0.08 in Nile tilapia, and 0.2 in Common carp (Usydus et al., 2011). The dietary recommendation of EPA and DHA consumption is between 0.2 - 0.5 g/day for an adult, in order to lower the risk of cardiovascular and inflammatory disease (EFSA, 2012). By comparison to cyprinids, farmed Atlantic salmon provides a superior content of EPA and DHA for human consumption. Atlantic salmon production accounted for 33% of marine and coastal finfish aquaculture worldwide in 2020 (FAO, 2022. p.43). The growing demand for farmed fish, including Atlantic salmon, stimulates the expansion of aquaculture production and increases the demand for aquaculture feed. The pricey fish meal and fish oils for aquafeeds are gradually substituted with cheaper vegetable ingredients in the aquaculture production, which inevitably changes the tissue fatty acid composition, particularly EPA and DHA in the final fish product (Hixson et al., 2014a; Sprague et al., 2016; 2020).

1.3 ATLANTIC SALMON LIFE HISTORY

Atlantic salmon is native to temperate regions in the northern hemisphere of eastern North America to northwestern Europe and Russia. Atlantic salmon mostly exhibit an anadromous life cycle. They start their lives in freshwater streams where eggs hatch, and develop into alevin, fry and parr - three forms of salmon early life stages. Young salmon in fresh water go through a physiological process called smoltification before migration to the marine environment where they grow rapidly. Mature Atlantic salmon in the ocean return to spawn at their natal freshwater environment, which usually occurs in late autumn until early winter (Thorstad et al., 2011, p. 14). Depending on the geographical variations, food availability and flexible migratory patterns, some salmon populations have a different life history and experience a short migration distance or even live in the freshwater environment their whole life. They are termed landlocked Atlantic salmon (refer to Chapter 1.4).

Figure 1.1. The comparison of morphological characteristics between Atlantic salmon parr and smolt (McCormick, 2012, p. 201).

Commercial salmon producers grow young salmon in land-based freshwater hatchery until smoltification (Bergheim et al., 2009). This transformation from salmon parr to smolt is a process that fish undergo in a series of changes in morphology, physiology and behavior to better survive and thrive in a marine environment (McCormick, 2012, p. 199). In the northern hemisphere, smolt development usually takes place in spring and is triggered by temperature rise and a longer photoperiod (Björnsson, 1997; McCormick 2012, p. 202). The morphological differences between parr and smolt are apparent. Salmon parr have dark stripes/ parr marks with some red spots in-between along its small body. Salmon smolts gradually lose their parr marks and red spots, showing dark fin margins, gaining silver scales, and developing a slender body shape (Fig 1.1). Smolts also display active swimming and schooling behaviors during this stage. These changes could be partly explained by an elevated metabolic rate and a decreased lipid content (McCormick and Saunders, 1987).

Morphological and physiological development are different between landlocked and anadromous Atlantic salmon during the period of smoltification. The high incidence of early sexual maturity in landlocked Atlantic salmon is associated with their smaller body size and negatively affects their hypo-osmoregulatory ability in saltwater (Birt and Green, 1993). Compared with the anadromous salmon, landlocked salmon show slower changes of losing parr marks and silvering (Fig 1.1), and less salinity tolerance, survival rate and initial growth in seawater (Birt et al., 1991; Nilsen et al., 2003; McCormick et al., 2019). Genetic and

physiological differences during the spring smoltification are evident between anadromous and landlocked Atlantic salmon (Hauge et al., 2016).

1.4 LANDLOCKED ATLANTIC SALMON

Landlocked or freshwater Atlantic salmon (*Salmo salar*) have a non-anadromous life cycle, characterized by good adaptation to the freshwater environment its entire life. Their morphology is similar in appearance to anadromous Atlantic salmon. Native landlocked salmon inhabit freshwater rivers or lakes in eastern North America, northern Europe and Russia (Hutchings et al., 2019). Instead of swimming downstream to the ocean, juvenile landlocked salmon migrate to freshwater lakes until maturation and then return to spawn at their natal rivers. The emergence of landlocked salmon is probably due to the geographical isolation between inland waters and the ocean. About ten thousand years ago, the retreat of glaciers driven by warming climate shaped the landscape and created ice-melting water lakes. These Atlantic salmon populations have been gradually developing the adaptation to inland freshwater ecosystem (Berg, 1985; Lumme et al., 2016). Some landlocked salmon populations in Newfoundland have no geographical barriers to the sea (Hutching et al., 2019). Certain freshwater salmon populations in Magaguadavic river, New Brunswick are not strictly "landlocked" and can move to the costal environment for a period of time (Carr et al., 2005). This shows that the phylogeny of freshwater landlocked salmon populations is diverse and geographically specific.

In Canada, landlocked Atlantic salmon are mostly reported in Quebec (Riley and Power, 1987; Leclerc et al., 1996) and Newfoundland (Hutchings et al., 2019). There are also landlocked Atlantic salmon in Nova Scotia (Alexander, 1975; MacCrimmon and Gots, 1979) and New Brunswick rivers (Carr et al., 2005). In Maine, USA, four populations of landlocked Atlantic salmon were documented before 1868: Sebago Lake, West Grand Lake, Green Lake, and Sebec Lake (Havey and Warner, 1985. p, 2; Pellerin and Pierce, 2015). According to an early restoring management program in 1868, landlocked Atlantic salmon eggs were collected at the West Grand Lake and then a year later, relocated to Lake Cathance. The Grand Lake stream hatchery has been managing fish stocking right up to today in Maine (MDIFW, 2021a). Wild landlocked salmon from the West Grand Lake are regularly captured and cultured in this hatchery, and then released to rivers in Maine to support salmon populations. Currently, this facility produces about 80,000 fish every year to sustain sport angling in Maine, including landlocked salmon and brook

trout (*Salvelinus fontinalis*) (MDIFW, 2021b). The restoration and re-introduction of hatchery-reared Atlantic salmon are also reported in Europe, such as Lake Vänern in Sweden, and Lake Saimaa and Pielinen in Finland (Piccolo et al., 2012; Vehanen, 2006).

Lake surface area and food availability dictate the landlocked fish body size. Some landlocked Atlantic salmon populations have a smaller body size of 10 to 30 cm when they solely live in rivers and prey on aquatic insects (Hutchings et al., 2019). There is a significant positive relationship between the maximum fish length and lake surface area according to a study comparing 64 populations of landlocked salmon in North America and Europe (Hutchings et al., 2019). Landlocked salmon can reach a fork length of 50 to 85 cm when prey is abundant. The primary prey for wild salmonid is rainbow smelt (*Osmerus mordax*) in North America, vendace (*Coregonus albula)* and European smelt (*Osmerus eperlanus*) in Europe. Fish fork length at maturity is less than 50 cm in wild populations of landlocked Atlantic salmon, while it is larger than 50 cm in wild anadromous salmon populations (Hutchings et al., 2019). There is a wide variation in maximum fork length among different populations of landlocked Atlantic salmon. For example, the landlocked Atlantic salmon population from West Grand Lake, Maine reaches sexual maturity at 42 cm and has a maximum fork length of 54 cm (Havey and Warner, 1970; Hutchings et al., 2019). The fork length at maturity and the maximum length of Gullspångsälven population in Vänern, Sweden are double the West Grand Lake population (Ros, 1981). Moreover, recreational angling has caused the depletion of the larger salmon in Maine (MDIFW, 2021a), which indicates that some wild landlocked populations may no longer achieve their historical maximum body size (Hutchings et al., 2019).

The ability of LC-PUFA biosynthesis in landlocked salmon can be stimulated when there is poor dietary LC-PUFA in a freshwater environment. The potential mechanism is that salmonids are stimulated to upregulate the ability of LC-PUFA biosynthesis to meet their nutritional requirement due to the deficiency of LC-PUFA in the diet (Hixson et al., 2014c; Betancor et al., 2016). Aquatic microalgae, as the primary producer of LC-PUFA, are consumed and accumulated to higher trophic levels through the food chain (Sprague et al., 2016). Higher forms of terrestrial plants do not *de novo* synthesize LC-PUFA, such as EPA and DHA (Sayanova and Napier, 2004). Compared with the freshwater environment, the large amounts and variety of microalgae and plankton such as diatoms and dinophytes, contain greater amounts of EPA and DHA in the marine environment (Gladyshev et al., 2013). Sea-run Atlantic salmon

consume LC-PUFA rich food and store high EPA and DHA contents in their fish muscle. By comparison, landlocked salmon have relatively high LNA (linoleic acid, 18:2n-6) and ARA (arachidonic acid, 20:4n-6), indicating that they rely on freshwater diets from the nearby terrestrial ecosystem (Parzanini et al., 2020). The trait of upregulated LC-PUFA biosynthesis in landlocked Atlantic salmon, especially EPA and DHA, is highly valuable for producing future generations of farmed Atlantic salmon.

The diverse life-histories and restoring management of landlocked Atlantic salmon complicate the genetic lineages between landlocked Atlantic salmon and anadromous salmon. In the present study, landlocked strain Atlantic salmon from Grand Lake Stream Hatchery, were descendants of the West Grand Lake population in Maine (refer to Chapter 2.3.2). Previous studies analyzed fin and scale samples from different salmon populations and reported that there is a genetic difference between Maine landlocked strain Atlantic salmon and Canadian SJR strain Atlantic salmon (King et al., 2000; 2001; Spidle et al., 2003). More specifically, the genetic variation was presented by gene diversity, the total number of alleles across loci, and the number of unique alleles. Genetic differences were investigated between West Grand Lake strain Atlantic salmon and anadromous Atlantic salmon populations in Canada and Maine (Spidle et al., 2003; King et al. 2001). Microsatellite DNA loci on the Atlantic salmon indicated that West Grand Lake strain in Maine had significantly less genetic diversity and could be differentiated from 27 other anadromous Atlantic salmon populations in North America, including the Saint John River strain, NB (King et al., 2001). The mitochondrial DNA haplotype diversity in the landlocked strain of West Grand Lake in Maine was estimated at 0.506, while it was 0.351 in the SJR strain Atlantic salmon in NB, which had a significant difference in the heterogeneity of haplotype frequency (King et al., 2000). In addition, Maine landlocked strains and North American anadromous Atlantic salmon had fewer alleles and fewer unique alleles. They were less historically developed compared with 13 other European anadromous Atlantic salmon populations (King et al., 2001).

1.5 SAINT JOHN RIVER (SJR) ATLANTIC SALMON

In Atlantic Canada, SJR strain is the only permitted commercial Atlantic salmon strain for production in sea-cages (Glebe, 1998; Quinton et al., 2005). During the 1970 - 1980s, the salmon breeding programs in New Brunswick brought wild Atlantic salmon stocks from different rivers,

including Saint John, Miramichi, Big Salmon, Dennis Streams, Digdeguash and Magaguadavic. Both the wild SJR salmon stocks with superior growth performance and the salmon collected from the Mactaquac Hatchery (Mactaquac Biodiversity Facility), NB, largely support the production of SJR Atlantic salmon. Four separate year classes of Atlantic salmon were collected by the Mactaquac Hatchery. This early breeding program lays the foundation of the genetic improvement within the SJR strain. In the late 1980s, the Atlantic Salmon Broodstock Development Program, which is based in St. Andrews, New Brunswick, gathered researchers and salmon producers to further explore the genetic improvements of Atlantic salmon growth traits (Quinton et al., 2005). Later, Cooke Aquaculture Inc. maintains and developed their own SJR strain Atlantic salmon breeding program. The recent generations derived from the Mactaquac Biodiversity Facility were produced in a paternal half-sibling design where fifty male and one hundred female broodstock with the highest saltwater growth were cross-bred (Liu et al., 2017).

Multiple integral traits are investigated in producing Canadian SJR strain Atlantic salmon besides optimal fish growth (Friars et al., 1995; O'Flynn et al., 1999) and low early sexual maturity rate (Boulding et al., 2019). Selection efforts also focus on harvest weight and meat quality (Quinton et al., 2005), and disease resistance in parr (Holborn et al., 2018; 2020). Meat quality includes flesh carotenoid pigments, flesh color, and fat content. In the present study, the trait of LC-PUFA biosynthesis especially EPA and DHA, in fish muscle and liver are evaluated in the SJR strain Atlantic salmon (refer to Chapter 3).

1.6 FISH NUTRITION IN THE FORMULATED DIET

The nutrition requirements of fish are broadly dependent on species, feeding habits, and life stages (NRC, 2011). In the wild, freshwater herbivorous fish mainly feed on terrestrial plants, which supply carbohydrates including starch, sugar, and fibre. Marine carnivorous fish, such as salmonids have limited capacity to use carbohydrates and rather use lipids as the primary storage of energy. Different food sources result in physiological differences in the digestive systems between carnivorous and herbivorous or omnivorous fish, including the length and structure of the intestine, enzyme activities, and microbial reactions in food digestion (Colombo, 2020, p. 54).

Fish cultured in aquaculture facilities require a variety of essential nutrients for growth and reproduction: macronutrients including protein, lipid and carbohydrate; and micronutrients containing amino acids, fatty acids, vitamins and minerals. Recommended protein level in the diet for farmed Atlantic salmon ranges from 34 to 48% where salmon in the early development requires a relatively higher protein than post-smolts Atlantic salmon (NRC, 2011, p. 70). The level of soluble carbohydrates in the diets is less than 20% for carnivorous fish and ranges from 25 to 40% for omnivorous fish (Lall and Tibbetts, 2009). Commercial fish feeds contain 28 to 40% lipid for oily fish, such as Atlantic salmon. The lipid requirement for fish is affected by the levels of protein and carbohydrate, which can be utilized as sources of energy. Dietary lipids can serve as energy supply and spare protein consumption for energy. The lipid requirement for Atlantic salmon is often determined by optimal growth and the requirement of essential fatty acids, including α-linolenic acid (ALA, 18:3n-3), linoleic acid (LNA, 18:2n-6), EPA and DHA. These essential fatty acids play an important role in lipid metabolism (refer to Chapter 1.8) and can be stored in the salmon flesh for human consumption.

Freshwater landlocked salmon have relatively high levels of LNA and ARA (arachidonic acid, 20:4n-6) in the muscle and liver, reflecting that they rely on freshwater diets in their terrestrial ecosystem (Parzanini et al., 2020). The presence of EPA and DHA in the muscle and liver demonstrates that landlocked salmon produce n-3 LC-PUFA for physiological needs when they are conditioned by the lower levels of EPA and DHA in the freshwater diets (refer to Chapter 1.9). Marine salmon have access to abundant n-3 fatty acids and acquire dietary ALA, LNA, EPA and DHA through marine food chain (primary producers, invertebrates and small pelagic fish). Thus, marine salmon are not under the pressure of EPA and DHA deficiency, which may weaken the capability to synthesize LC-PUFA (Sargent et al., 2002). In the present study, the LC-PUFA content in anadromous Atlantic salmon was compared with landlocked salmon when they were fed a fish oil-free diet (refer to Chapter 2).

Vitamins are a group of organic compounds that are important cofactors for cellular membrane integrity, endocrine regulation, and immune system function (Mai et al., 2022). Minerals are a group of inorganic substances that fish require for sustaining tissues (mainly skeletal structures), cellular ionic balance, and endocrine regulation (Lall, 2022).

Based on the understanding of fish nutrition requirements, the aquaculture feed industry manufactures formulated fish feeds for optimal fish growth performance while considering the

cost and demand of the animal. Sourcing sustainable and economical feed ingredients without compromising the fish growth and health requirements has been a key consideration in aquaculture nutrition.

1.7 LIPID AND FATTY ACID CLASS IN FISH

1.7.1 Lipid Classes

Lipids are a group of fat-soluble organic compounds in plant and animal tissues, in the form of triacylglycerol, phospholipids, sphingomyelins, waxes, and sterols. Dietary lipids play an important role as the primary energy source for salmonids, and provide the structural components of cell membranes, and the functional role of intracellular signaling (Turchini et al., 2022).

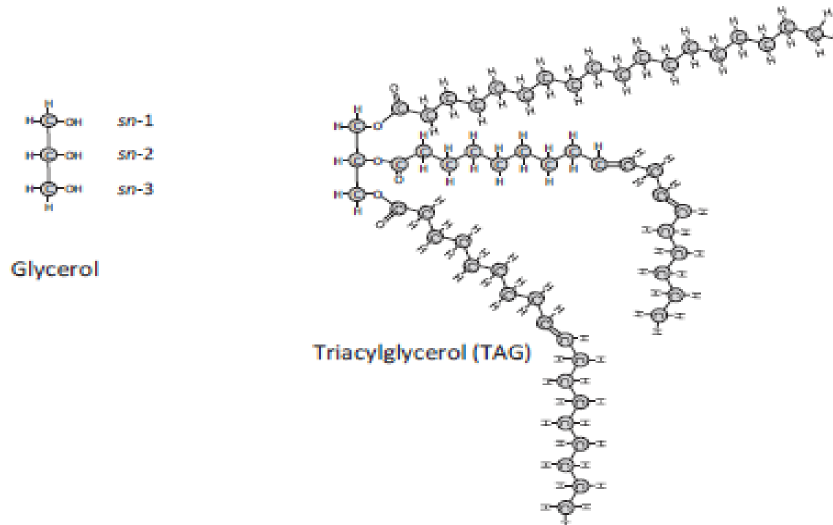

Figure 1.2. The structure of glycerol and an example of triacylglycerols (TAG) that have three fatty acids, 18:0, 18:2n-6 and 18:1n-9. Three fatty acids are esterized to three *sn* positions when enzymatic esterification occurs (Turchini et al., 2022, p. 310).

Triacylglycerols (TAG) are neutral lipids due to the absence of charged groups and acidic or basic groups. They provide the primary energy storage and also the predominant lipid class in fish tissues, dietary oils and fats (Turchini et al., 2022). In Atlantic salmon fillets, TAG accounted for over 90% of the lipid, while phosphatidylcholine (a major phospholipid class) was only 3.6% (Ruiz-Lopez et al., 2015). Triacylglycerols are composed of three fatty acids esterified to three hydroxyl groups of glycerol in the TAG (Fig 1.2). Saturated and monosaturated fatty acids are located at the *sn*-1 and *sn*-3 position, while polyunsaturated fatty

acids and long-chain PUFA tend to locate at the *sn*-2 position (Tocher, 2003; Bell and Koppe, 2010).

Phospholipids (PL) are the major group of polar lipids or complex lipids. They are comprised of a phosphatidic acid and a phosphate group. Phosphatidic acid contains two fatty acids esterified to the glycerol. PL and sterols play an important role as the structural components of cellular membranes and function as signaling molecules (Turchini et al., 2022, p. 305). All lipid classes except cholesterol contain fatty acids in esterified form. LC-PUFA are present in both PL and TAG. Farmed Atlantic salmon muscle lipids are 70 to 90% TAG (Bell et al., 1998). Therefore, the present study focused on the fatty acid compositions in TAG in the fish muscle and liver.

1.7.2 Fatty Acid Classes

Fatty acids contain a carboxylic acid (-COOH) with an aliphatic chain ($-CH_2-$) and a methyl end ($-CH_3$). The carbon backbone of fatty acids is either saturated by hydrogen or unsaturated when a double bond is formed. The number of double bonds (ethylenic bonds) ranges from zero to six, indicating different degrees of unsaturation. Fatty acids which have only one double bond are called monounsaturated fatty acids (MUFA). Unsaturated fatty acids containing two or more double bonds are termed polyunsaturated fatty acids (PUFA). The chain length of a fatty acid is defined by the number of carbon atoms. Polyunsaturated fatty acids contain 20 or more carbons and those with ≥2 double bonds are termed long chain polyunsaturated fatty acids (LC-PUFA). In some literature, HUFA refers to highly unsaturated fatty acids, which are comprised of more than 18 carbons and more than 2 double bonds.

1.8 ROLES OF LC-PUFA IN TELEOSTS

Dietary LC-PUFA provides the functions of preserving energy, supporting cellular membranes, and metabolic regulation in teleosts. These functions also reflect the important roles of lipids in fish physiology.

1.8.1 LC-PUFA Oxidation

During the process of lipid metabolism, the oxidation of LC-PUFA can provide metabolic energy; in reverse, the LC-PUFA are retained in TAG in fish lipids when enzymatic esterification occurs (Tocher, 2003; Turchini et al., 2022, p. 311). The oxidation of LC-PUFA as C_{20} and C_{22} fatty acids, is at a significantly lower rate of energy release than the oxidation of

PUFA (18:2n-6 and 18:3n-3) in rainbow trout (*Oncorhynchus mykiss*) (Henderson and Sargent, 1985). The lipid level and total energy content in salmon feeds play a more significant role than the oxidation of LC-PUFA on the growth of Atlantic salmon (Huyben et al., 2021a; 2021b)

1.8.2 LC-PUFA in Cellular Membranes

Fatty acids, including LC-PUFA, are a constituent of phospholipids (PL). Phospholipids coupled with protein form the basic components of the cellular membrane, as lipid bilayers. Marine fish have abundant DHA in neural membranes in brains and retinas. Lipid accounts for up to 40% in neural tissue in marine fish; up to 40% of the total fatty acid is DHA in the glycerophospholipid called cephalin (Glencross, 2009). In rainbow trout retinal cells, DHA is at least 40% of the total fatty acids, while it is at least 70% in Atlantic cod (*Gadus morhua*) (Bell and Dick, 1991). Juvenile herring (*Clupea harengus L.*) fed on a diet containing DHA show a better visual performance and are active in predation at the low light intensity, compared to fish fed a DHA-free diet (Bell et al., 1995).

The changes of the LC-PUFA content can affect cell membrane fluidity and regulate the membrane proteins and enzymes (Tocher, 1995). There are multiple double bonds and more complex chemical structures in LC-PUFA than saturated fatty acids. A higher portion of LC-PUFA in PL bilayers extends the space between the molecules of PL and then, increases the membrane fluidity. The enhanced membrane fluidity contributes to salmonid smoltification by the increased water absorption in the fish gut when fish acclimate to the reduced water temperature and increased salinity of the marine environment (Sundell and Sundh, 2012). The alteration of fatty acid composition, particularly the higher percentage of n-3 PUFA in intestinal membranes is observed in both Masu salmon (*Oncorhynchus masou*) and rainbow trout in seawater (Li and Yamada, 1992; Ge et al., 2021).

1.8.3 LC-PUFA in Metabolic Regulation

Both n-3 and n-6 LC-PUFA are active in metabolic regulation. Within the cell, LC-PUFA are ligands for transcription factors, which modulate the gene expression of fatty acid desaturase and elongase (Leaver et al., 2018; Tocher, 2010). The n-3 and n-6 LC-PUFA often have opposite effects on fish physiology but compete for the same fatty acid desaturase and elongase (Fig 1.4). Outside the cell, the regulatory functions of LC-PUFA include both homeostasis and the stress response in the endocrine system (Tocher, 2010; Turchini et al., 2022, p. 347). These regulations are achieved by some LC-PUFA derivatives, such as eicosanoids, which are derived from

oxidized products of ARA (arachidonic acid, 20:4n-6), EPA, and DHA (Fig 1.3). Eicosanoids are hormone-related mediators which regulate the fish immune and inflammatory response. In general, eicosanoids derived from n-6 LC-PUFA have a proinflammatory effect and enhance vascular permeability. Eicosanoids derived from n-3 LC-PUFA improve the inflammatory response and help maintain the state of homeostasis (Serhan and Petasis, 2011). Consequently, the imbalance of n-6 and n-3 LC-PUFA and their corresponding eicosanoids could reflect the immune response in Atlantic salmon when they are under stress or in a compromised health condition (Caballero-Solares et al., 2017; Seierstad et al., 2009).

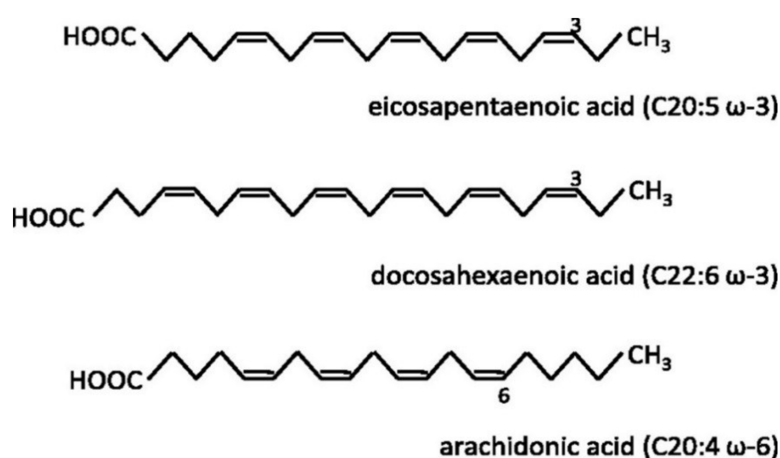

Figure 1.3. Structure and nomenclature of PUFA. Eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (ARA). The symbol of “n” or “ω” represents the methyl end of the hydrocarbon chain (Cockbain et al., 2012).

1.9 FATTY ACIDS AND LC-PUFA BIOSYNTHESIS IN TELEOSTS

Lipogenesis is the *de novo* synthesis of fatty acids in fish liver and adipose tissue by biosynthetic reactions. All vertebrates can *de novo* synthesize fatty acids to some extent. During lipogenesis, exogenous or endogenous carbon sources are oxidized and then form acetyl-CoA in mitochondria. Acetyl-CoA is catalyzed by a complex of seven enzymes called fatty acid synthetase and then produces the main product of saturated fatty acids (SFA) 16:0 (NRC, 2011, p. 105). Fatty acids synthesis of more than 16 carbons require both mitochondria and microsomes. In microsomes, the chain elongation extends from 18:0 to 24:0 (Glencross, 2009). Moreover, the desaturation of 16:0 (palmitic acid) and 18:0 (stearic acid) by microsomal Δ9 desaturases produce monounsaturated fatty acids (MUFA), 18:1n-9 (oleic acid) and 16:1n-7 (palmitoleic acid). Then, 18:1n-9 can be catalyzed by Δ12 desaturase to make 18:2n-6. The

production of 18:3n-3 from 18:2n-6 needs Δ15 desaturase. However, vertebrates lack Δ12 and Δ 15 desaturases and cannot *de novo* produce 18:2n-6 and 18:3n-3 polyunsaturated fatty acids (PUFA) (Pereira et al., 2003; Tocher 2003). Therefore, PUFAs are essential in the diets. Fatty acids including SFA, MUFA, and PUFA are esterized into different lipid classes, for example, TAG and phospholipids (PL). New lipids are stored in the fish liver, adipose tissue, and muscle (red and white). The proportion of lipid storage in the fish liver contributing to the fish whole body lipid storage varies with different fish species. For example, Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) have fatty livers where the liver stores the most lipid in the whole body (Zeng et al., 2010). In contrast, Atlantic salmon and rainbow trout primarily store lipid in the adipose tissue in the muscle, even though the liver is the major site processing lipids - TAG and PL, which involve fatty acid desaturation and chain elongation (Sargent et al., 1993; Tocher, 2003).

In this study, the biosynthesis of LC-PUFA is the focus of discussion, especially n-3 LC-PUFA, EPA and DHA. The liver is the main location of LC-PUFA biosynthesis in fish where a series of fatty acid desaturation and chain elongation takes place. Vertebrates cannot produce linoleic acid (LNA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3) from 18:0 due to the absence of Δ12 and Δ15, fatty acyl desaturases (Tocher, 2003; Glencross, 2009). The desaturases of Δ12 (or n-6) desaturases and Δ15 (or n-3) are only present in natural plants. Therefore, 18:2n-6 and 18:3n-3 are essential fatty acids to vertebrates. Fatty acid 18:2n-6 is desaturated by Δ15 desaturase and creates 18:3n-3. In the reactions of the n-3 series on the left side of Fig 1.4, dietary ALA (18:3n-3) is the precursor of EPA (20:5n-3), and DHA (22:6n-3). The pathways of n-3 LC-PUFA biosynthesis demonstrate that ALA is converted to EPA and DPA (22:5n-3) with the catalyzation of specific desaturases and elongases (Fig 1.4). Subsequently, the production of DHA from DPA needs another chain elongation, followed by Δ6 desaturation, and a step of peroxisomal chain shortening, which is also called β-oxidation (Sprecher, 2000). Atlantic salmon and rainbow trout mainly follow the "Δ6 pathway" or "Sprecher pathway" for DHA biosynthesis (Oboh et al., 2017). The alternative "Δ4 pathway" for DHA biosynthesis has been identified in rabbitfish (*Siganus canaliculatus*), Senegalese sole (*Solea senegalensis*), medaka (*Oryzias latipes*) and Nile tilapia (*Oreochromis niloticus*) (Li., et al., 2010; Morais et al., 2012; Oboh et al., 2017). In the "Δ4 pathway", DHA can be synthesized from DPA through Δ4

desaturation. All these reactions except β-oxidation occur in the endoplasmic reticulum, while the reaction of β-oxidation occurs in peroxisomes.

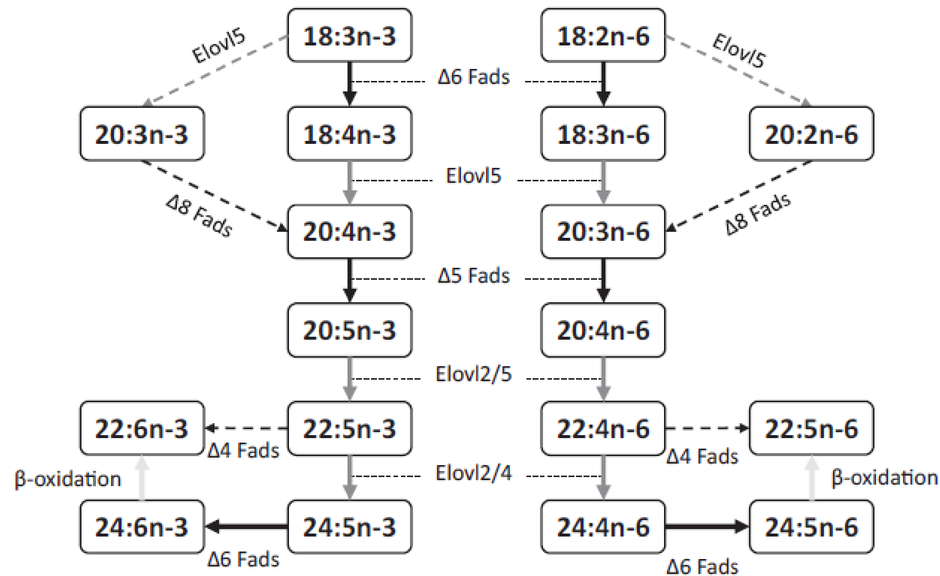

Figure 1.4. Pathways of n-3 and n-6 LC-PUFA biosynthesis in teleost fish. "Solid arrows" demonstrate the predominant pathway in most species. "Dashed arrows" show possible pathways in some species. Fatty acid desaturations are displayed in "black arrows". "Gray arrows" represent fatty acid elongation. All enzymatic activities take place in endoplasmic reticulum, while β-oxidation occurs in peroxisomes. "Fads" represents fatty acyl desaturases. "Δ6 Fads" refers to a specific fatty acyl desaturase that creates the double bond at the carbon 6th from the carboxylic group (-COOH) in the fatty acid chain. "Elovl" is fatty acid elongase or elongation of very long-chain fatty acid elongase (Turchini et al., 2022, p. 348).

The biosynthesis of both n-3 and n-6 LC-PUFA share the same enzymes, which suggests there is competition between n-3 and n-6 LC-PUFA over enzymes (Glencross, 2009; Tocher, 2003; 2010). Additionally, excess dietary LC-PUFA suppresses the Δ6 desaturation reaction and restricts PUFA synthesis (Sargent et al., 1993; Glencross, 2009; Tocher, 2015). The ratio of dietary n-6 to n-3 PUFA is an important factor in fish LC-PUFA biosynthesis.

1.10 LC-PUFA REQUIREMENTS IN SOME TELEOSTS

Accurate quantification of LC-PUFA requirements in salmonids promotes the development of appropriate formulated feeds and ensures the nutritional demand for fish growth and health. Dietary LC-PUFA including EPA, DHA and ARA (arachidonic acid, 20:4n-6) (Fig 1.3), are essential fatty acids (EFA) that fish are not able to *de novo* synthesize. Marine oils

provide a rich source of dietary LC-PUFA, particularly EPA and DHA. However, the ongoing and future trend is to replace fish oil with various terrestrial oils in formulated fish feed (Turchini et al., 2009; Colombo et al., 2020). Terrestrial plant oils do not typically contain EPA and DHA but are high in 18:3n-3 and 18:2n-6. Salmonids are capable of synthesizing LC-PUFA from dietary C_{18} PUFA in plant oils (Tocher, 2003; Glencross, 2009). Theoretically, fish having a better ability of LC-PUFA production do not need a high consumption of LC-PUFA in the diet. The upregulated LC-PUFA biosynthesis can be evaluated by the active gene expression for relevant fatty acid desaturases and elongases (Tocher, 2003; 2010).

1.10.1 Effects of EPA and DHA Deficiency in Some Teleosts

As essential fatty acids, EPA and DHA play important roles in the early development of neural tissue and fish metabolic regulation. The n-3 LC-PUFA deficiency, especially DHA results in impaired vision (Bell et al., 1995) and failed schooling behavior in marine fish larvae (Masuda et al., 1998; 2001; Ishizaki et al., 2001). Herring (*Clupea harengus L.*) larvae of 28 days post-hatch (dph) were offered experimental diets lacking DHA for 15 weeks and showed a significantly reduced DHA proportion of the total phospholipid in the eyes from 12 to 2.1% (Bell et al., 1995). Although EPA and DPA (22:5n-3) largely compensated for the lost percentage of DHA, it still decreased fish capture behavior on live prey, *Artemia nauplii*, especially at low light intensity below 1 lux (Bell et al., 1995). They implied that the impaired vision in herring larvae might not recover after early rod development. Swimming activities in response to live prey, rotifers (*Brachionus plicatilis*) were significantly reduced when gilthead seabream (*Sparus aurata*) larvae were offered a fish-oil free diet at 4 dph (Benítez-Santana et al., 2007). Atlantic bluefin tuna (*Thunnus thynnus*) larvae during 3-7 dph consumed significantly more prey when tuna larvae offered the highest rotifer DHA diet at 11 mg/g (2 vs. 5 vs. 11 mg/g). Moreover, the retinal opsin protein concentrations were 25 unit/area with the 11 mg/g DHA diet, which was significantly higher than 20 unit/area with the 5 mg/g DHA diet and 16 unit/area with the 2 mg/g DHA diet (Koven et al., 2018). Schooling behaviors describe the fish swimming synchronously in a group toward the same orientation. The larvae of yellowtail (*Seriola quinqueradiata*), Pacific threadfin (*Polydactylus sexfilis*), and striped jack (*Pseudocaranx dentex*) fed a DHA-free diet exhibited failed or impaired schooling behaviors (Masuda et al., 1998; 2001; Ishizaki et al., 2001). Importantly, the DHA-free diet was associated with decreased larvae growth and survival rate in yellowtail and Pacific threadfin (Masuda et al., 1998; 2001).

It is well established that DHA deficiency reduces the early neural development in fish brains and eyes, adversely affecting fish predation and viability (Bell et al., 1995; Benítez-Santana et al., 2007; Koven et al., 2018).

A deficiency of EPA and DHA in Atlantic salmon fry, smolt and post-smolt reduces the feed intake and the specific growth rate, and increases the fry mortality (Ruyter et al., 2000; Bou et al., 2017a). Atlantic salmon fry (initially ~ 4 g) fed 0% EPA and DHA diet in freshwater for 28 weeks displayed the lowest specific growth rate (%BW/ day) at approximately 0.17%BW/day, while the specific growth rate increased to the highest at 0.5%BW /day in the diet group of 1% EPA+ DHA (by dry weight). When the inclusion of EPA+DHA decreased from 1 to 0%, the mortality increased by 10%, while fat deposition lowered by about 4% (Ruyter et al., 2000). The ratio of EPA and DHA in the diet was not reported in the study by Ruyter et al., (2000) and the dietary lipid level only occupied 8% of total dry feed, which was approximately one-third of the dietary lipid content of current diets for salmonids. Dietary n-3 LC-PUFA is positively related to the dietary lipid level for the growth of Atlantic salmon (Huyben et al., 2021a). It indicates that the inclusion of dietary n-3 LC-PUFA needs to be increased when increasing the total lipid level in the diet for Atlantic salmon.

Atlantic salmon (initial weight ~50g) in seawater fed 0% dietary EPA+DHA for 26 weeks resulted in a reduced specific growth rate, < 1%BW/day (Bou et al., 2017a). The average mortality rate was independent of dietary EPA+DHA from 0 to 2% (Bou et al., 2017a). Additionally, a few abnormal symptoms were observed in the intestine when fish were fed a 0% DHA+ EPA diet. These symptoms included the presence of large vacuoles and swollen mucosa at the enterocytes, which suggested a mild impairment of intestinal mucosa (Bou et al., 2017a). Dietary levels of EPA and DHA each lower than 1% (10 g/kg) in feed caused lower final mean body weight when Atlantic salmon smolt (initial weight ~256 g) were fed for 16 weeks in seawater (Hixson et al., 2017). In contrast, no significant differences in fish final mean body weight were found in Atlantic salmon (initial weight ~1.2 kg) fed 0.2, 1, and 1.7% of EPA+DHA diets for 9 months. After delousing treatment at high water temperature up to 17.8 ℃ in July and August, salmon fed 1.7% of EPA+DHA (17 g/kg) had a significantly better survival rate by 47% compared with salmon fed 0.2% diet (Bou et al., 2017b). Collectively, the inclusion of dietary n-3 LC-PUFA promotes the growth rate of Atlantic salmon at both freshwater and seawater

stages; whereas some studies have observed that the excess n-3 LC-PUFA intake does not continue to improve the specific growth rate (Ruyter et al., 2000; Menoyo et al., 2003).

1.10.2 Dietary EPA and DHA Requirements in Salmonids

The essential EPA and DHA intake required by freshwater fish or salmonids is relatively low compared to C_{18} PUFA and is lower than that of marine fish. The minimum requirement level of any nutrient should satisfy normal fish growth. Optimal fish growth, fish health, and nutritional value in meat quality require a higher dietary EPA and DHA than the minimal demand. Due to the bioconversion between C20 and C22 LC-PUFA in fish tissue, EPA and DHA often appear together as n-3 LC-PUFA in most studies. Given equal levels of dietary n-3 LC-PUFA, fish fed a diet having both EPA and DHA performed better than fish fed a diet having DHA alone in terms of growth and health (Glencross et al., 2014). The goal in future salmonid production is to produce fast growth in weight with the minimal demand of fish oil.

Atlantic salmon in freshwater require at least 0.5% (5g/kg) of n-3 LC-PUFA in the diet (Ruyter et al., 2000; Qian et al., 2020). A better specific growth rate and body fat deposition in salmon fry (initial weight ~4g) was exhibited among fish which were fed 0.5% and 1% dietary n-3 PUFA (EPA: DHA =1:1) as dry weight for four months. The specific growth rate reduced from 0.5 to 0.4 %BW/day when the dietary EPA+DHA level was increased from 1 to 2% (Ruyter et al., 2000). In a six-week trial, salmon parr (initial weight ~23g) in freshwater fed a minimum 0.5% of EPA and DHA (1:1) diet showed a similar final weight as fish fed the 2% EPA and DHA (1:1) diet, which were 44g vs 42g respectively. The n-3/n-6 fatty acids ratio in the 0.5% and 2% EPA+DHA diet was 0.5 vs. 2.0, respectively (Qian et al., 2020). Some studies evaluated that maintaining optimal fish gut integrity requires more than 0.5% of n-3 LC-PUFA in the diet, particularly in seawater (Tocher, 2015; Bou et al., 2017a).

Post-smolt Atlantic salmon need 1 to 1.5% (10 to 15g/kg) of dietary n-3 LC-PUFA for optimal growth (Glencross et al., 2014; Huyben et al., 2021a). Another study recommends the n-3 LC-PUFA inclusion between 1.6 and 2.6% for Atlantic salmon in seawater (Sissener et al., 2016). In a 26-week trial, Atlantic salmon (initial weight ~50g) fed a diet including 2% EPA and DHA (1:1) at mean 10ºC seawater enhanced the mean final weight, compared to the 0.5% EPA and DHA diet, which were 366 g vs. 389 g, respectively (Bou et al., 2017b). Besides, dietary inclusion of 2% EPA and DHA also alleviated the mild intestinal damage in salmon (Bou et al., 2017b). Atlantic salmon (initial weight ~160 g) at 12 ºC seawater which were fed diets

that contained 0.4 – 1.6% (4 – 16g/kg) EPA and DHA (1:1) exhibited the highest weight gain and second highest final body weight in the 1.6% EPA and DHA diet group, compared to other diets which contained less than the 1.6% EPA and DHA (Rosenlund et al.,2016). In contrast, Atlantic salmon offered a diet of 0.4% EPA+DHA exhibited a significantly lower final weight, compared to other diets which contained more than the 0.4% EPA and DHA (Rosenlund et al.,2016). Furthermore, Atlantic salmon (initial weight ~1.4kg) fed dietary EPA+DHA from 0.4 to 2.4% (4 to 24g/kg) obtained a significantly higher mean body weight at 3.6kg in the 2.4% EPA+DHA diet, compared to 3.3kg in the 0.4% EPA+DHA diet after 21 weeks at 12 ^{o}C seawater (Rosenlund et al.,2016). The dietary DHA/EPA ratio in the 0.4% diet and the 2.4% diet groups were 1.2 and 0.8, respectively. A lower DHA/EPA ratio probably suggests some benefits of extra EPA intake in growing post-smolt Atlantic salmon (Glencross et al., 2014). Post-smolt Atlantic salmon (initial weight ~150g) reared to harvest size (~5kg) offered diets with either 1.6 or 2.6% inclusion of EPA+DHA, exhibited a relatively higher mean final body weight at 5.3kg in the 2.6% diet group, compared with 4.8kg in the 1.6% diet group, while the survival rate was similar (Sissener et al., 2016). Atlantic salmon approximately 50g, fed EPA and DHA (1:1) with progressive levels from 0 to 2% reported higher growth rates and mean final body weight in the diet groups from 0.5 to 2% n-3 LC-PUFA, compared to the 0% n-3 LC-PUFA diet group. The 2% EPA and DHA diet helped fish sustain normal intestinal mucosa (Bou et al., 2017a).

1.11 GENETIC INFLUENCES ON THE LC-PUFA LEVEL IN SALMONIDS

Fillet LC-PUFA content is a heritable trait in farmed salmon. Percentage n-3 LC-PUFA in Atlantic salmon (initial weight ~70g) fillet lipid differed between 9.5 to 15.2% after 12 weeks on the same fish oil-free diet (Leaver et al., 2011) where the amount of EPA+DHA was approximately 13g/kg in the diet. There was a significant 2 – 3% difference in fillet n-3 LC-PUFA% between salmon families having the same level of fillet lipids (Leaver et al., 2011). The heritability values of n-3 LC-PUFA percentage and absolute amount of n-3 LC-PUFA (gram per 100 g flesh) in fillet lipid were 0.77 and 0.34, respectively (Leaver et al., 2011). It indicates that approximately 77% of variation in fillet n-3 LC-PUFA percentage is caused by genetics. By comparison, another study estimated heritability for EPA, DHA percentages and absolute amount of n-3 LC-PUFA in Atlantic salmon (initial weight ~110g) flesh and found 0.09, 0.26, and 0.35, respectively (Horn et al., 2018).

The estimates of heritability for fillet EPA and DHA were relatively lower in Horn et al., (2018), compared with the estimates by Leaver et al., (2011). The reasons are probably due to different salmon strains, diets, and fish ages. Horn et al., (2018) studied a strain of Atlantic salmon from SalmoBreed AS, Norway. Fish were offered a commercial diet having a relatively high EPA+DHA of 62g/kg up to the harvest size, about 3.6kg. Salmon fillet was individually collected without considering the family factor. By comparison, Leaver et al., (2011) examined 48 families of Atlantic salmon from Scotland and they fed fish a diet having 13g/kg EPA+DHA for 12 weeks. Nevertheless, these results show that it is feasible to improve Atlantic salmon flesh n-3 LC-PUFA content, especially DHA through selectively breeding.

The salmonid liver can convert ALA to DHA since the liver is the primary organ for LC-PUFA biosynthesis and lipid metabolism (Monroig et al., 2010). Variability in expression of transcripts can be explained by liver LC-PUFA synthesis among farmed Atlantic salmon fed the same fish oil-free diet (Xue et al., 2015; Hixson et al., 2017). However, the upregulation of transcript expressions in the liver does not always correspond to the increase of EPA and DHA storage in the muscle (Xue et al., 2015; Hixson et al., 2017; Colombo et al., 2018). The relationships between liver and muscle LC-PUFA biosynthesis remain unclear, regarding gene expressions of related enzymes in LC-PUFA biosynthesis (Betancor et al., 2016; Colombo et al., 2018). Another Atlantic salmon study compared six full sibling families from SalmoBreed AS. Their parent fish were identified as being “high-producing LC-PUFA” (high desaturase expression) and “low-producing LC-PUFA” (low desaturase expression) when fed a low LC-PUFA diet (with rapeseed oil) and grown from 15 to 40g (Berge et al., 2015). Three high-producing LC-PUFA salmon families showed 1) a higher capacity for EPA and DHA synthesis in freshwater phase, 2) higher levels of EPA and DHA in the whole body, 3) lower incidence of fatty liver and 4) higher amounts of fat distributed within muscle, compared to the other three low-producing LC-PUFA salmon families, while growth was not affected. The difference in content of EPA + DHA between the high- and low- producing families was 2g/kg fillet (Berge et al., 2015).

The results exhibit that LC-PUFA synthesis is heritable and that variability among LC-PUFA synthesis capability, likely genetically dependent. The above evidence suggests that the genetic variation in salmon tissue, EPA and DHA could be selectively bred for improvement. However, it is currently unknown what this content variation might be in the North American

Atlantic salmon, such as landlocked and farmed SJR strain salmon in Canada. Salmon with an enhanced capacity to produce EPA and DHA can effectively eliminate dietary fish oil and allow salmon to maintain good health with very low levels of dietary EPA and DHA.

1.12 LIPIDS IN AQUACULTURE FEEDS

Fish oil sourced from wild fisheries is considered a natural and the most nutritious lipid source, especially for salmonid and other marine species. Fish oil is produced through the pressing of cooked fish or fish by-product. The fish remaining is dried and milled into a flour-type fishmeal, which is a highly valuable protein source in animal feeds. The common fish species harvested for making fishmeal and fish oil are marine baitfish, such as mackerel, herring, menhaden and anchovy (FAO, 2020, p. 62). The characteristics of fish oil are low in saturated fatty acids, and high in n-3 LC-PUFA, DHA and EPA. The inclusion of fish oil in the diet is a common method to enrich the content of dietary EPA and DHA for better growth in farmed fish. On the other hand, replacing fish oil with alternative lipid sources, with particular emphasis on terrestrial plant oils, has been widely studied (e.g., Turchini et al., 2009; Alhazzaa et al., 2019; Colombo et al., 2020). As a result, dietary inclusion rates of FO as dietary lipids in salmon feeds has reduced from 24 to 11% between 1990 and 2019 in Norwegian Atlantic salmon production (Ytrestøyl et al., 2015; Aas et al., 2019; MOWI, 2020). Vegetable oils gradually substitute the fish oil in fish feed.

Vegetable oils are used as an alternative lipid source for fish oil in salmonid feed due to their competitive market prices and abundance; however, the market price of both fish oil and alternatives have fluctuated. In 2020, the price of fish oil was around $2000 USD/tonne; the price of soybean oil was much cheaper, around $850 USD/tonne (Fig. 1.5). In 2022, the price of soybean oil soared up to a historical high level between $1700 to 1900 USD/tonne in 2022 (World Bank, 2022). Other terrestrial plants with a relatively lower cost and high production have the potential to be used in fish feed, including palm oil, canola oil, sunflower oil, linseed oil, and cottonseed oil (Turchini et al., 2009; World Bank, 2022). Compared with marine fish oil, vegetable oils are mostly high in n-6 and n-9 fatty acids (18:1n-9), but all are naturally devoid of EPA and DHA (Turchini et al., 2009). The nutrition requirement for n-3 LC-PUFA is essential for the growth and health of farmed salmon. In comparison with the fatty acid composition in fish oil, vegetable oils have been evaluated to partially replace FO in the fish feed. Nonetheless,

the full substitution of fish oil with vegetable oils would result in a deficiency of EPA and DHA in current farmed salmon.

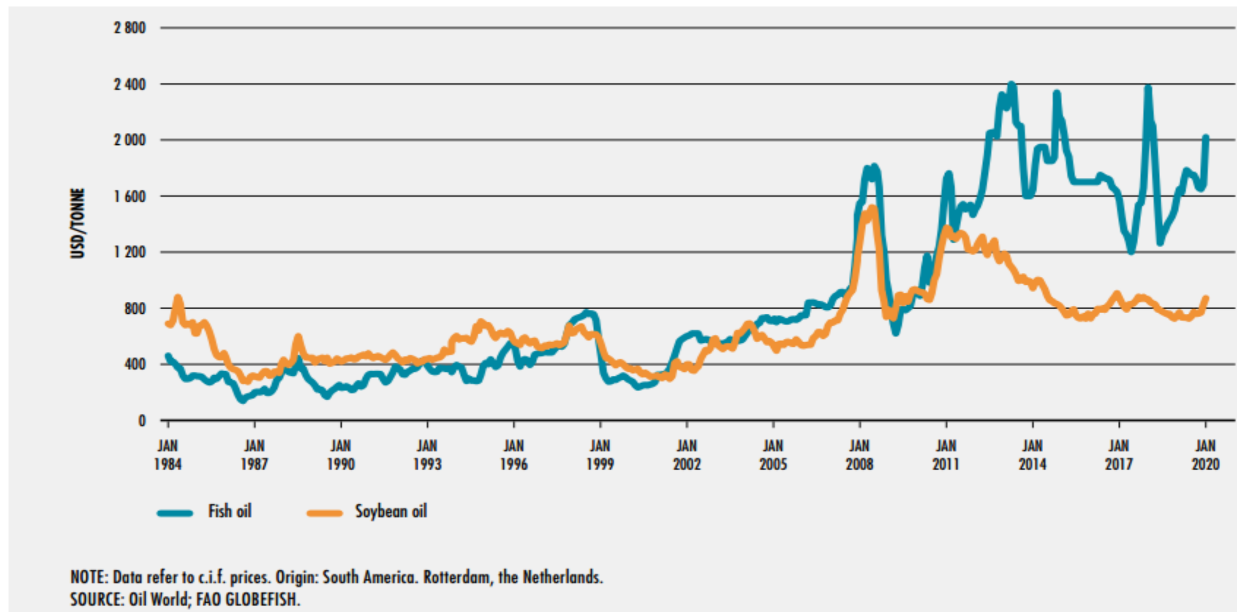

Figure 1.5. The price change of fish oil and soybean oil in the Netherlands from 1983 to 2020 (FAO, 2020, p. 89). In June 2022, soybean oil price increased to $1700 USD/tonne (USDA, 2022).

1.13 CANOLA AND CAMELINA OIL IN AQUACULTURE FEEDS

Canola oil contains plenty of 18:2n-6 (linoleic acid, LNA), while camelina oil is rich in 18:3n-3 (α-linolenic acid, ALA). Dietary ALA is the precursor of n-3 LC-PUFA - EPA and DHA. The LNA is the precursor of n-6 LC-PUFA – ARA (arachidonic acid, 20:4n-6). The ability to produce LC-PUFA in the muscle and liver by using dietary LNA and ALA is the key evaluation in the present study. In the next section, the lipid content, fatty acid profile and market value of canola and camelina oil are introduced.

1.13.1 Canola Oil

Canola (*Brassica napus*), a cross breed of rapeseed cultivar, is an important oilseed crop in global crop production. The characteristic of low-temperature tolerance favors the growth of canola in temperate regions. In 2019, global rapeseed production was 70.5 million tonnes. Canada grew the largest amount at 18.6 million tonnes, representing one-fourth of the global rapeseed production (FAOSTAT, 2019). Rapeseeds contain 22 to 60% erucic acid (22:1n-9) in the total fatty acids and 80µmol/g glucosinolates, which impairs animal growth and development. By comparison, canola has less than 2% erucic acid in lipids and less than 30µmol/g

glucosinolates in meals (Ratnayake and Daun, 2004. p. 38; Goyal et al., 2021. p. 47). The improved canola oil is widely accepted and has been proven useful in cooking, and animal feeds. The average price of canola oil was $1100 USD/tonne in Jan 2020 (USDA, 2022), while the average fish oil price was $ 2000 USD/tonne, respectively during the same period (Fig 1.5). In June 2022, the price of canola oil has since doubled and reached approximately $2000 USD/tonne (USDA, 2022) due to the strong global surge in the demand for oilseeds.

Table 1.1. Major fatty acid profile (% total fatty acids) of typical fish oils, canola oils, and camelina oils in previous studies.

Oils	SFA	MUFA	LNA	ALA	EPA	DHA	n-6 PUFA	n-3 PUFA	n-3 /n-6
Fish oil[1]									
Anchovy oil	28.8	24.9	1.2	0.8	17.0	8.8	1.3	31.2	24.0
Menhaden oil	20.0	61.7	1.3	0.2	11.0	9.1	1.5	25.1	16.7
Herring oil	20.0	56.4	1.1	0.6	8.4	4.9	1.4	17.8	12.7
Plant oils									
Canola oil[1]	4.6	62.3	20.2	12.0	/	/	20.2	12.0	0.6
Camelina oil[2]	7.9	34.7	16.9	38.1	/	/	18.0	39.0	2.2

[1]Data is organized by Turchini et al., 2009; Tanwar and Goyal, 2021.
[2]Government of Canada, 2012. "/", not detectable; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; LNA, linoleic acids (18:2n-6); ALA, α-linolenic acid (18:3n-3); EPA, eicosapentaenoic acid (20:5n-3); DHA, docosahexaenoic acid (22:6n-3).

The lipid content of canola seed is between 30 and 50% (Carré et al., 2016; Matthaus et al., 2016). The major fatty acids in canola oil are oleic acid (18:1n-9), followed by linoleic acid (18:2n-6), and α-Linolenic acid (18:3n-3), which represent approximately 60, 20, and 3 – 13% in total fatty acids, respectively (Przybylski and Eskin, 2011; Goyal et al., 2021). The ratio of n-6 and n-3 fatty acids is close to 2: 1. The level of an antinutritional factor- erucic acid has been reduced to 0.1 – 2% in dietary canola oils through genetic selection. Canola modified by transgenic technique produces DHA up to 11% of total fatty acids (Petrie et al., 2020). Like other terrestrial plant oils, canola oils do not naturally contain nutritional EPA and DHA (Table 1.1).

Fish fed a diet with its fish oil replaced by canola oil can sustain normal fish growth and survival. Red seabream (*Pagrus major*), rainbow trout and Atlantic salmon are capable of

utilizing canola oil (Glencross et al., 2003; Torstensen et al., 2005; Drew et al., 2007). In an eight-week trial, a complete replacement of fish oil (140g/kg) by canola oil in the diet significantly reduced the mean final body weight of red seabream (initial weight ~28g), which was 80g vs. 93g in the fish oil control diet (Glencross et al., 2003). However, a half or 75% substitution of fish oil with canola oil had no significant influence on red seabream mean final body weight, compared with the 140g/kg fish oil control diet (Glencross et al., 2003). Similar results are found in Bell et al., (2001), where the canola oil replaced fish oil (190g/kg) in the diet at progressive levels of 10, 25, 50, and 100%. Atlantic salmon post-smolts (initial weight ~80g) were fed those diets at 11.7℃ seawater for 17 weeks. The mean final body weight and specific growth rate were not significantly different among different diet groups, although fish fed 100% canola oil diet had a relatively lower final body weight and specific growth rate. In a similar experiment, there were no significant effects on Atlantic salmon (initial weight ~55g) mean final body weight, specific growth rate and feed conversion ratio when fish were fed 10, 25, 50, and 100% canola oil diets at 11℃ seawater for 16 weeks (Bell et al., 2003). By comparison, Atlantic salmon (initial weight ~256g) reared at 14℃ seawater and were fed a vegetable oil diet having partial substitution of fish oil with canola oil for 16 weeks (Hixson et al., 2017). Salmon fed the fish oil (240g/kg) control diet had a significantly higher mean final body weight than salmon fed the 96% canola oil diet (230g/kg canola oil +10g/kg fish oil), which were 626g vs. 549g, respectively; whereas, the weight gain, specific growth rate and feed conversion ratio were similar (Hixson et al., 2017). Overall, canola oil has been widely tested as an alternative dietary lipid in formulated fish feeds. Some studies used vegetable oils with a higher n-3 C_{18} PUFA such as linseed oils and camelina oils, to compensate for the n-3 C_{18} PUFA that natural canola oil does not contain.

Salmonids fed a diet with the fish oil replaced by canola oil show altered tissue FA composition and reduced n-3 LC-PUFA in the fillet. More than 50% replacement of fish oil by canola oil led to a significant reduction of DHA and EPA total fatty acids in the Atlantic salmon muscle (Bell et al., 2001; Bell et al., 2003; Hixson et al., 2017). Post-smolt Atlantic salmon fed 100 % canola oil diet had significantly lower DHA and EPA percentages at 7 and 3% in the fillet, compared with 15% DHA and 6% EPA in fish fed a fish oil control diet (Bell et al., 2001). Fillet DHA and EPA percentages were 8 and 3% when Atlantic salmon post-smolt were fed 100% canola oil diet, while the fillet DHA and EPA percentages were significantly higher at 16 and

6 % in fish fed fish oil control diet (Bell et al., 2003). To improve the total n-3 C_{18} PUFA level in the vegetable oil diet, canola oil and linseed oil were blended in fish feeds (~53% 18:3n-3 of total fatty acids) for rainbow trout (Drew et al., 2007). Trout (initial weight ~47g) were offered either a 200g/kg fish oil diet or the fish oil-free diet for 16 weeks. The blended vegetable diet did not negatively affect fish final body weight. When fish fed the fish oil-free diet, muscle DHA and EPA levels significantly reduced to 5 and 2%, respectively, compared with 13% DHA and 8 % EPA in the muscle when fed the fish oil-free diet (Drew et al., 2007). Muscle DHA and EPA reductions are also reported in Hixson et al., (2017) when Atlantic salmon were fed a 96% canola oil diet. Salmon muscle DHA level significantly reduced from 8 to 5 % and muscle EPA significantly decreased from 3 to 1.5% when fish were provided the fish oil control diet and the 96% canola oil diet.

1.13.2 Camelina Oil

Like canola, camelina belongs to the family *Brassicaceae* and grows as an oil crop. Camelina is a common name for the oilseed *Camelina sativa* that originally was cultivated in Europe and central Asia for food and animal feed. It thrives in cold semi-arid climate regions and tolerates low temperature and drought soil conditions (Francis and Warwick, 2009). In Canada, camelia is mainly located in the four western provinces and also found growing in Yukon and the Northwest Territories (Francis and Warwick, 2009; Government of Canada, 2011). Due to the richness of unsaturated n-3 essential fatty acids, especially ALA, many studies have evaluated camelina as a feed ingredient for livestock and fish. In 2010, Health Canada approved the use of unrefined camelina oil as a dietary ingredient. Recently, camelina oil has been used for producing biofuel in North America and Europe (El Bassam, 2010). Since the camelina crops do not have the market size as canola, the camelina prices are usually compared with canola to estimate its economic feasibility (Keske et al., 2013). In Jan 2022, the price of camelina crops was $590 CAN/tonnes, which was cheaper than $ 750 CAN/tonnes of canola seeds (SCIC, 2022). It suggests that camelina oil is cheaper than canola oil; moreover, it is much lower than fish oil price.

Camelina seeds contain around 30 to 40 % crude oil in which the unsaturated n-3 and n-6 fatty acids are largely present. The total n-3 fatty acid level accounts for approximately 39% with the majority in the form of ALA; the total n-6 FA level is approximately 18% with the majority in the form of linoleic acids (18:2n-6). The level of erucic acid (22:1n-9) is below 5%

in dietary camelina oils (Government of Canada, 2012). Compared with canola oils, camelina oils have the advantage of significantly higher n-3 C_{18} PUFA (39% vs. 12%).

It is feasible to feed salmonids the full replacement of fish oil by camelina oil in the diet. Although the 100% camelina oil diet sometimes causes slight loss of fish body weight, depending on fish species and life stage. Atlantic salmon parr (initial weight ~ 8g) were offered 100 % camelina oil diet or the fish oil (161g/kg) control diet and reared at 11.7 ℃ freshwater for 16 weeks. As a result, fish fed 100% camelina oil diet had a mean final body weight of 52g, which was similar to the mean final body weight of 49 g when salmon parr fed the fish oil control diet (Ye et al., 2016). Compared with salmon parr, Atlantic salmon smolt require a relatively higher fish oil in the diet. There was a significant decrease in fish mean final body weight when Atlantic salmon smolt (initial weight ~240g) were fed the 100% camelina oil diet at 14℃ seawater for 16 weeks. The smolt mean final weight was 613 g when fed the 100% camelina oil diet, while it was 691g when fed fish oil (140g/kg) control diet (Hixson et al., 2014c). By contrast, Atlantic salmon (initial weight ~256 g) fed 96 % camelina oil diet did not significantly affect fish mean final body weight, compared with feeding fish the fish oil (240g/kg) control diet (Hixson et al., 2017). Rainbow trout (initial weight ~2.5g) were fed the fish oil-free camelina oil diet at 14 ℃ freshwater for 16 weeks, in comparison to fish oil (140g/kg) control diet. Trout mean final body weight did not significantly differ between those two diets, which were 61g in the camelina diet group vs. 76g in the fish oil control diet group (Bullerwell et al., 2016). In addition, no significant differences in trout hindgut conditions were observed between camelina oil diet and fish oil control diet, including the villus height, width, area and intestinal wall thickness (Bullerwell et al., 2016). Feeding rainbow trout (initial weight ~45g) the diet having camelina oil as a complete replacement of fish oil did not result in a significant change in fish mean final body weight after 12 weeks, which was 184g in the 100% camelina oil diet, compared with 168 g in the fish oil (140g/kg) control diet (Hixson et al., 2014c). Therefore, it suggests that rainbow trout can better utilize the fish oil-free (or camelina oil) diet than Atlantic salmon when fish are more than 50g or after smoltification. It also suggests that freshwater rainbow trout can produce some n-3 LC-PUFA that compensate the absence of fish oil in their diet (Hixson et al., 2014b). As for other marine species, growth performance of Atlantic cod (initial weight ~ 14g) was significantly reduced in the mean final body weight, which were 44g when fish fed the 100% camelina oil diet, compared with 51g when fish fed the fish oil (54g/kg) control diet (Hixson et

al., 2014c). In an eight-week trial, red seabream had an average of 1.8g were fed camelina oil diets with progressive levels from 30 to 100% to the substitution of fish oil at 26℃ seawater (Mzengereza et al., 2021). There was not significantly difference on red seabream final mean body weight (26 to 28g) between the 100% camelina oil diet and the fish oil (60g/kg) control diet (Mzengereza et al., 2021). No severe health conditions or increased mortality due to the camelina diet were documented in the above trials.

Like canola oil, high inclusion of camelina oil in the fish feeds also changes the fatty acid composition in the fish fillet and liver compared to a fish oil diet. There was a significant linear relationship between the fish fillet DHA, EPA, ALA and LNA concentrations and those fatty acid concentrations in the diet (Hixson et al., 2014c). The Atlantic salmon fillet DHA content significantly decreased from 11 to 3% and the fillet EPA content also had significantly reduced e from 7 to 1% when salmon were fed the fish oil control diet vs. 100% camelina oil diet for 16 weeks (Hixson et al., 2014c). The results are similar to Hixson et al., (2017). Significant DHA content decrease (from 7 to 3%) and EPA content decrease (from 12 to 2%) were measured in the rainbow trout fillet when fish were fed fish oil control diet and 100% camelina oil diet for 12 weeks (Hixson et al., 2014b). Red seabream fed the fish oil diet for 8 weeks had fillet DHA and EPA level at 160 and 81 mg/g in lipids (or 16 and 8.1%), respectively; however, DHA and EPA levels significantly reduced to 120 and 53 mg/g in lipids (or 12 and 5.3%) (Mzengereza, et al., 2021). On the other hand, ALA (18:3n-3) and LNA (18:2n-6) significantly accumulated in the fillet due to the fatty acid profile of camelina oil. Overall, it shows that ALA can be synthesized to EPA and DHA at a limited level in the fish muscle, while dietary supply of EPA and DHA from fish oil can directly increase fish muscle n-3 LC-PUFA. Therefore, identifying and selectively breeding Atlantic salmon strains or families with a better capability of utilizing ALA to produce n-3 LC-PUFA is highly meaningful in less dependency on fish oil.

Irrespective of transgenic DHA and EPA improvement vegetable oils (Betancor et al., 2015), the disadvantage of using ordinary vegetable oils, including canola and camelina oil, in fish feed is the lack of essential EPA and DHA. The high level inclusion of vegetable oils in salmon feed gradually changes the fatty acid composition in the fish flesh and then weakens its nutritional value caused by the declining DHA and EPA levels (Menoyo et al., 2003; Sprague et al., 2016; Alhazzaa et al., 2019). In this context, terrestrial plant oils lacking n-3 LC-PUFA cannot perfectly replace n-3 LC-PUFA rich in Atlantic salmon feed.

1.14 RESEARCH OBJECTIVES

The principal objective is to identify the intra- and inter-strain Atlantic salmon in North America with enhanced n-3 LC-PUFA content in the fillet and liver when fish were fed a FO control diet or a FO-free diet. In the first study, the comparison of Atlantic salmon n-3 LC-PUFA content in the muscle and liver was between the landlocked Grand Lake (GL) strain and the commercial Saint John River (SRJ) strain. The objective of the second study was to assess the ability of n-3 LC-PUFA storage among fifty families within the commercial SJR strain Atlantic salmon.

CHAPTER 2: LONG CHAIN POLYUNSATURATED FATTY ACID LEVEL BY ATLANTIC SALMON: A COMPARISON OF LANDLOCKED AND ANADROMOUS STRAINS

2.1 ABSTRACT

A 2 x 2 factorial design was conducted to determine whether two Atlantic salmon strains fed two diets significantly differed in the n-3 LC-PUFA biosynthesis in the fish liver and muscle. The landlocked Grand Lake (GL) strain and the commercial Saint John River (SJR) strain (n = 480 per strain) having the similar initial mean body weight at ~57g, were fed either the control diet (10% fish oil + 5% canola oil) or the FO-free diet (15% canola oil) for 16 weeks. Each of the four treatments (strain/diet) had six replicate tanks. After 16 weeks, GL salmon fed the control diet had the significantly lowest mean final body weight among the four treatments. The mean final body weight (mean ± standard deviation) of the GL or SJR strain fed the FO-free diet did not differ significantly, 180.1 ± 3.3 vs. 202.6 ± 14.8g, respectively. Fish weight gain, percent weight increase, and TGC also exhibited the same pattern as the mean final body weight. The effect of strain on the liver fatty acid content was clearly evident. Specifically, GL strain initially had significantly higher levels ($p < 0.001$) of 18:2n-6 (LNA) and 18:3n-3 (ALA) in the liver, while SJR strain initially had significantly higher ($p < 0.05$) levels of 20:4n-6 (ARA) and 20:5n-3 (EPA) in the liver. Initial muscle fatty acid compositions did not differ between two strains.

After 16 weeks, GL and SJR strains fed the FO-free diet had a similar n-3 LC-PUFA level. Salmon in the SJR/FO-free contained 22% of n-6 PUFA in the liver fatty acids, significantly higher ($p < 0.001$) than 16% of n-6 LC-PUFA in the liver in the GL/FO-free. It indicated that the SJR strain had higher capacity for liver n-6 LC-PUFA storage. GL strain exhibited a significantly higher ($p < 0.001$) capacity of liver monounsaturated fatty acids (MUFA) storage compared with SJR strain. The GL/FO had the significantly highest ($p = 0.001$) muscle DHA level of 16.7 ± 4.8% and significantly highest ($p < 0.001$) liver DHA of 28.1 ± 3.8% among four treatments. We observed significant interaction effects of diet and strain on the muscle and liver DHA percentages. Overall, landlocked GL strain salmon had a genetic potential in terms of muscle n-3 LC-PUFA biosynthesis or storage.

2.2 INTRODUCTION

The Saint John River (SJR) Atlantic salmon is the dominant commercial salmon strain in Atlantic Canada since the late-1980s. Selective breeding of this strain has been focused primarily on optimizing growth in the ocean (O'Flynn et al., 1999; Quinton et al., 2005); low sexual maturity rate as grilse (Boulding et al., 2019); high meat quality (Quinton et al., 2005); and improved immune response among parr (Holborn et al., 2018; 2020). The trait of elevated omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) biosynthesis and storage in the muscle and liver, has yet to be considered in farmed SJR Atlantic salmon. The improvement of n-3 LC-PUFA content was observed in poultry by increasing dietary 18:3n-3 (ALA) (Kartikasari et al., 2012; Pérez et al., 2021). In a seven-week trial, there was a 4 X increase in n-3 LC-PUFA increased four-times from 5 to 20% of total lipids in chicken meat, including eicosapentaenoic acid (EPA, 20:3n-5), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) as a result of increasing dietary α-linolenic acid (ALA,18:3n-3) from 1 to 8% (Kartikasari et al., 2012). As dietary ALA was increased in the poultry diet, the n-3 PUFA related enzymes became active in the chicken hepatocytes and then produced n-3 fatty acids from C_{18} to C_{24} (Pérez et al., 2021).

Diets for farmed Atlantic salmon usually require dietary fish oils, which provide a nutritious lipid source with a high level of n-3 LC-PUFA, particularly EPA and DHA. Dietary n-3 LC-PUFA assists the functions of preserving energy, supporting cellular membranes, and regulating LC-PUFA metabolic activities in salmonids. A deficiency of n-3 LC-PUFA in Atlantic salmon can result in the reduction of feed intake and the specific growth rate and can also negatively affect the survival rate (Ruyter et al., 2000; Bou et al., 2017a). EPA and DHA requirements range from 0.5 to 1% (5 to 10 g/kg of the diet) for Atlantic salmon parr in freshwater (NRC, 2011, p. 109; Qian et al., 2020). Growing post-smolt Atlantic salmon requires a relatively higher level of EPA and DHA (1:1) from 1.6 to 2.6% (10 to 26g/kg of the diet) (Sissener et al., 2016; Bou et al., 2017a). All vertebrates including fish cannot *de novo* synthesize n-3 PUFA due to the absence of Δ15 desaturases (Tocher, 2003). Apart from the direct supply of EPA and DHA for salmonids, the increase of dietary ALA (18:3n-3) from vegetable oils is an alternative way to create EPA and DHA (Thomassen et al., 2012; Glencross et al., 2014). This ability would allow a reduction in the heavy use of fish oil in fish feeds. However, the

ability of n-3 LC-PUFA biosynthesis varies with species and depends on the genetic expression of fatty acyl desaturases and elongases (Sargent et al., 1995; Tocher, 2003).

Freshwater landlocked Atlantic salmon display the potential of enhanced n-3 LC-PUFA biosynthesis when the diet is lacking marine n-3 LC-PUFA. Marine microalgae produce a large amount of n-3 LC-PUFA, which is consumed by zooplankton, fish larvae, and organisms of higher trophic levels in the food chain (Tocher, 2010; Gladysheva et al., 2013). Atlantic salmon in the open water ecosystems can acquire n-3 LC-PUFA by preying on fish that travel from seawater to freshwater, for example, rainbow smelt (*Osmerus mordax*) (Hutchings et al., 2019). Some salmon populations in freshwater lakes have access to zero or little marine n-3 LC-PUFA depending on the habitat being isolated or far from seawater (Gladysheva et al., 2013). Therefore, landlocked Atlantic salmon have various body sizes determined by food availability and the lake area size (Hutchings et al., 2019). Freshwater lipid sources including freshwater microalgae and terrestrial plants are naturally characterized by a high level 18:2n-6 and to some extent of ALA (Sargent et al., 1995; Gladysheva et al., 2013). As a result, freshwater fish contain high levels of n-6 fatty acids in the fish flesh (Parzanini et al., 2020). The deficiency of n-3 LC-PUFA in freshwater environment stimulates landlocked salmon to produce essential EPA and DHA by using ALA as a substrate (Tocher, 2010).

The conversion rate of n-3 LC-PUFA in landlocked Atlantic salmon is higher than farmed salmon when fed the fish oil-free diet. Landlocked Atlantic salmon parr (initial weight ~5.1g; Penobscot strain, Maine, USA) exhibited significantly higher n-3 and n-6 LC-PUFA conversion rates than the farmed salmon parr (initial weight ~ 3.6 g; Ormsary strain, Scotland), when fish were fed a vegetable oil diet (100 g/kg) for 8 weeks (Rollin et al., 2003). That study also showed positive correlations and linear relationships between dietary levels of ALA and its intermediate products in the salmon body. The conversion rate of ALA to DHA in landlocked and farmed salmon (Scottish strain) was compared by the slope values in the linear equation between DHA and ALA, which were 3 in landlocked vs. 1 in farmed salmon. This shows that the DHA conversion rate in the landlocked strain is higher than in the farmed salmon when fed a fish oil-free diet (Rollin et al., 2003). However, the DHA and EPA contents were not significantly elevated in the whole body. Landlocked salmon whole body contained 3-5 % DHA and 1-2 % EPA, slightly lower than 5-6 % DHA and 2-3 % EPA in the farmed salmon (Rollin et al., 2003). The landlocked Penobscot strain and the farmed Ormsary strain of salmon were offered a marine

oil (20 % of feed) diet for 6 weeks (Peng et al., 2003). The results showed that the landlocked strain (initial weight ~0.4 g) had a significantly better daily growth coefficient than the farmed strain, 11 vs. 8. Besides, the whole body lipids contained 20 % n-3 LC-PUFA in the landlocked strain, significantly higher than 17 % in the farmed strain (initial weight ~0.8 g). Overall, landlocked salmon which were fed the fish oil-free diet exhibited a higher n-3 LC-PUFA bio-conversion rate than farmed salmon. Feeding landlocked salmon with the marine oil diet led to a better growth rate and a higher total n-3 LC-PUFA content in the whole body compared to farmed Scottish salmon (Peng et al., 2003). However, this has yet to be demonstrated in comparison to the SJR strain.

The ability of n-3 LC-PUFA biosynthesis can be determined by the metabolic activity and genetic expression of fatty acyl desaturases and elongases. Landlocked Atlantic salmon (initial weight ~25 g; Lake Gullspångsälven, Vänern, Sweden) displayed a relatively higher n-3 LC-PUFA biosynthesis than the commercial salmon (initial weight ~ 31 g; Aquagen strain, Norway) in terms of higher expression and activity of most desaturases and elongases in hepatocytes when provided the fish oil-free diet, while such improvement was significantly higher when fish were provided the fish oil (170 g/kg) control diet (Betancor et al., 2016). Landlocked salmon flesh EPA and DHA were 2 and 12 %, respectively; similar to 3 and 13 % of EPA and DHA in the farmed salmon flesh (Betancor et al., 2016). It shows that salmon flesh EPA and DHA content did not reflect the increased LC-PUFA biosynthesis in the liver, which agrees with Rolling et al., (2003). The liver is the main organ where LC-PUFA biosynthesis and lipogenesis occurs (Monroig et al., 2010); however, salmonids primarily store new lipid in the adipose tissue in the muscle (Tocher, 2003). Muscle fatty acids strongly match the fatty acids provided in the diet (Torstensen et al., 2005; Bell et al., 2010; Hixson et al., 2014a).

Collectively, landlocked salmon have the genetic potential for tissue n-3 LC-PUFA improvement in commercial Atlantic salmon. The objective of this experiment was to evaluate the ability of n-3 LC-PUFA biosynthesis in the farmed SJR and landlocked Atlantic salmon (Grand Lake strain, Maine, USA) by determining the n-3 LC-PUFA content in the muscle and liver when challenged with a fish oil-free (i.e., low n-3 LC-PUFA) diet. Thus, a two-factor experimental design was conducted for 16 weeks using two salmon strains and two diets (fish oil control diet vs. fish oil-free diet).

2.3 MATERIALS AND METHODS

2.3.1 Experimental Diets

Two isonitrogenous and isolipidic diets were produced at the Chute Animal Nutrition Lab, Faculty of Agriculture, Dalhousie University (Dal-AC), Truro, Nova Scotia, Canada. The fish oil (FO) control diet consisted of 10% fish oil and 5% canola oil, while a fish oil-free (FO-free) diet contained 15% canola oil (Table 2.1). Ingredients were first mixed by a feed homogenizer for 20 minutes (Model 2010 Marion mixer made, Rapids Machinery Company, USA). Mixtures were then prepared by blending steam and water at 96°C and 30% moisture before extrusion (type OEE 8 extruder, Amandus Kahl GmbH & Co. KG, Germany). The density of extruded wet pellets was controlled at 430-500 g/L. Pellets were then dried at 65°C for 4-5 hours when the moisture content was below 10% (JPW Design & Manufacturing, USA). The oils were each heated to ~70°C. Dried pellets were oil-coated under the pressure of -0.9 bar at ~70°C with oil in a laboratory vacuum coater (Dinnissen Process Technology, Netherlands). To ensure optimal absorption of oils, pellets were soaked, stirred and held in the vacuum coater for 5 minutes at -0.9 bar, then the pressure was increased to atmospheric pressure. Finished feed pellets were stored at -20°C to reduce the oxidization of fats. Diets were only exposed to room temperatures during periods of feeding.

The biochemical analysis of the two diets was performed by Nova Scotia Department of Agriculture Laboratory, Truro, NS, Canada. The major fatty acids in the control diet were 18:1n-9, 16:0, 18:2n-6, EPA and DHA, while the FO-free diet was abundant in 18:1n-9, followed by 18:2n-6, 16:0, and 18:3n-3 (Tale 2.2). The DHA and EPA content were both ~7% in the control diet, while DHA and EPA were both ~1% in the FO-free diet. Overall, there was 57% of total monounsaturated fatty acids (MUFA) in the FO-free diet; significantly higher ($p < 0.01$) than 38% of MUFA in the control diet. Total saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) in the FO-free diet were 13 and 30%, respectively. Total SFA and PUFA were 25 and 37% in the control diet, respectively. The n-3/n-6 ratio of the control diet was 1.3, significantly higher ($p < 0.01$) than 0.4 in the FO-free diet (Table 2.2).

2.3.2 Experimental Fish and Husbandry

Landlocked strain Atlantic salmon (Grand Lake strain, GL) were initially collected by Grand Lake Stream State Fish Hatchery from 2003 to 2005 in West Grand Lake, Maine, USA. Hatchery-reared GL salmon were used to restock the freshwater lakes in Maine according to the

Maine Department of Inland Fisheries and Wildlife (MDIFW, 2021a; b). Landlocked salmon were transferred to National Cold Water Maine Aquaculture Center (NCWMA, United States Department of Agriculture, Franklin, Maine, USA) in Feb 2019. The commercial Saint John River (SJR) strain Atlantic salmon were obtained as eyed eggs from industry stocks.

Landlocked and SJR salmon were both fed BioTrout (BioOregon, Westbrook, Maine, USA) prior to the experiment. Nine hundred and sixty salmon were randomly assigned to four treatments including GL/FO, GL/FO-free, SJR/FO, and SJR/FO-free, among twenty-four 500-liter experimental tanks. Landlocked salmon (n_1 = 480; initial weight ~59 g) were randomly distributed among twelve tanks where six random tanks were offered the FO control diet and the other six tanks were offered the FO-free diet. Similarly, SJR salmon (n_2 = 480; initial weight ~58 g) were randomly transferred to another twelve tanks with six random tanks feeding the FO control diet and the other six tanks feeding the FO-free diet. Each tank was supplied with 3-4 L/min freshwater in the flow-through systems and exposed to natural day length at the NCWMA, Franklin, Maine, USA (44°33'34.9"N, 68°15'07.6"W). The dissolved oxygen and water temperature were daily monitored and maintained at approximately 10 mg/L and 12℃. Fish were fed at a rate of 1.5% body weight per day by automatic feeders (Arvo-Tec, Innovasea Systems Inc.). The experiment was conducted for 16 weeks from April to July 2019. The study followed the guidelines of the Canadian Council of Animal Care and approved by Dalhousie Animal Care and Use Committee (protocol #2019-010) and the USDA Institutional Animal Care (protocol #2019-001).

2.3.3 Tissue Sampling

Food was withheld one day before the body size measurement and tissue sampling. At week 0, individual fish body weight and fork length were recorded when fish were anaesthetized using tricaine methane sulfonate (TMS 222; Syndel, Ferndale, USA). One fish per tank was randomly selected and euthanized with an overdose of TMS 222 for tissue sampling. A portion of skinless white dorsal muscle and a portion of liver were sampled in cryogenic vials and immediately flash frozen in liquid nitrogen. After 16 weeks, the fish in each tank were measured again. Two fish per tank were randomly euthanized for muscle and liver samples.

2.3.4 Growth Performance Analysis

After 16 weeks, fish body weight, fork length, weight gain, weight increase, thermal growth coefficient (TGC), condition factor (CF), and feed conversion ratio (FCR) were calculated as follows:

$$TGC = \frac{[(\text{Final mean body weight}^\wedge 1/3) - (\text{Initial mean body weight}^\wedge 1/3)]}{\text{Temperature in} \circ\text{C x Number of days}} \text{x}1000$$

$$FCR = \frac{\text{Feed offered (g)}}{\text{Weight gain (g)}}$$

$$CF = \frac{\text{Body weight (g)}}{\text{Fork Length (cm)}^3} \times 100$$

2.3.5 Fatty Acid Extraction and Analysis

The tissue lipid extraction was conducted at the Nutrition lab, Haley Institute, Dal-AC. Tissue samples from individual fish were weighed and dried at -50ºC for 24 hours using an Edwards Modulyo 4K freeze dryer. Each dried sample was re-weighed to determine the water content, then was ground into a fine powder using mortar and pestle under small amounts of liquid nitrogen. The powder was stored at -80ºC. Lipid extraction followed the modified Folch method (Folch et al., 1957). First, about 1 mg sample was weighed in a glass tube and was extracted three times, using a 2 ml chloroform and methanol solution (chloroform: methanol= 2:1) and then was collected into a new glass tube. Polar impurities were separated by adding 0.8 ml of 0.9% potassium chloride solution (0.9% w/v) and were centrifuged at 2000 RPM (revolutions per minute) for 5 minutes. The organic layer was transferred using a sterile glass pipette. The organic solution was evaporated to dryness by applying nitrogen gas. Two milliliters of hexane were added into the dried lipid extract as the solvent. Next, the resulting lipid extract went through a process of methylation. Two milliliters of sulfuric acid and methanol mixed solution (1% v/v; the ratio of H_2SO_4: CH_3OH= 1: 99) were added as the catalyst, Hilditch reagent (Christie and Han, 2010). The combined solution was sealed and heated at 90ºC for 90 minutes through the derivatization reaction. The fatty acid methyl esters (FAMEs) in the tube were then extracted three times by use of 4 ml hexane, and then collected in a new glass tube. The FAMEs in the organic solvent were layered by adding 1.5 ml of distilled water and centrifuged. The FAMEs extract was dried by using nitrogen gas. Finally, a 0.5 ml of hexane was added to dissolve the dried FAMEs extract and transferred in a 2 ml amber glass vial at -80℃. The FAMEs were identified and analyzed using a Bruker 436 capillary gas chromatograph (GC) with

flame ionization detection. All solvents used in the extraction and FAME derivatization procedures were of high-performance liquid chromatography (HPLC) grade. The extraction and instrument recovery efficiency were estimated by adding a known concentration of 5 α-cholestane solution (C8003, Sigma-Aldrich, St. Louis, Missouri, USA). The analysis of FAMEs by gas chromatography was conducted at the Marine Lipids Lab, Dalhousie University, Halifax, NS.

2.3.6 Statistical Analysis

Data were analyzed using Minitab 18.0 (Statistical Software, State College, PA, USA) and reported as mean values ± standard deviation (SD). The average final fish body size in tank 17 (GL/FO) and tank 9 (SJR/FO-free) were excluded as the outliers due to malfunction of automatic feeders, which were confirmed by the Grubb's test with a significance level of 0.05. Tank 16 (GL/FO-free) and tank 22 (SJR/FO) from the other two treatments were randomly removed to keep the same number of replicates per treatment (5 replicate tanks per treatment). For growth parameters including mean fish body weight, weight gain, TGC, CF, and FCR, a one-way analysis of variance (ANOVA) and a Tukey's multiple comparison test were performed to determine the effect of treatment. A two-way ANOVA and a Tukey's test were conducted to identify the diet effect, strain effect, and the interaction effect between diet and strain. Tukey's multiple comparison test used a significance level of 0.05. The comparisons of fish muscle and liver fatty acid composition among four treatment groups were conducted using a one-way ANOVA, followed by the Bonferroni test with the correction at the α-level of 0.05. The advantage of using the Bonferroni method in the fatty acid analysis is to reduce the chance of false significant results when multiple comparison tests were conducted among treatments on a single fatty acid class. Before the statistical analysis, the normality of residuals and constant variances were evaluated by Anderson-Darling test and Levene's test.

2.4 RESULTS

2.4.1 Growth Performance

The initial average body weight of GL and SJR salmon were not significantly different among the four treatment groups. Fish tripled their weight during the experiment. After 16 weeks, SJR/FO-free had the highest final mean body weight at 202.6 ± 14.8 g, which was not significantly different to the SJR/FO and GL/FO-free treatments. GL/FO had the significantly

lowest mean final body weight, 167.5 ± 2.8 g, and was similar to the GL/FO-free treatment, 180.1 ± 3.3 g (Table 2.3). Similar patterns were observed with the fish weight gain, percentage weight increase, and TGC after 16 weeks. The strain effect was significant ($p < 0.05$) on fish weight gain, percentage weight increase, and TGC (Table 2.3). The effect of diet and the interaction effect of diet and strain were not significant for any of the growth parameters.

2.4.2 Initial Tissue Fatty Acids

Initial liver fatty acid compositions differed significantly between the two strains. Among the liver fatty acids, DHA was the most abundant making up ~31% of total fatty acids in both strains. The average liver EPA content was 6.1% in the farmed SJR strain, significantly higher ($p = 0.015$) than 5.3% of EPA in the landlocked GL strain (Table 2.4). The GL strain livers had significantly higher content of 14:0, 16:1n-7, 18:1n-9, 18:2n-6, 18:3n-3, 20:1n-9, 20:1n-7, 20:2n-6, 20:4n-3, total monounsaturated fatty acids (MUFA), and the DHA/EPA ratio than the SJR strain. The commercial SJR salmon liver was significantly higher in 16:0, 20:4n-6 (arachidonic acid, ARA), 20:5n-3 (EPA), 24:1, total SFA and the n-3/n-6 ratio than in landlocked GL salmon liver. Moreover, 18:2n-6 and 18:3n-3 were both significantly higher ($p < 0.001$) in the GL strain, while intermediate LC-PUFA, 20:4n-6 and 20:5n-3 were significantly higher ($p = 0.035$ and $p = 0.015$, respectively) in the SJR strain (Table 2.4).

Initial muscle fatty acid content was independent of strain, except for 20:3n-6. The top three fatty acids in the muscle were 16:0, 18:1n-9, and DHA where 16:0 was most abundant making up ~20% of total fatty acids in the salmon muscle. Muscle DHA and EPA content was ~18% and ~5%, respectively (Table 2.5). In general, no significant effect of strain was observed in salmon muscle fatty acid profiles.

2.4.3 Final Tissue Fatty Acids

Similar to the initial liver fatty acid profiles, DHA, 16:0, and 18:1n-9 were the top three liver fatty acids in both salmon strains after 16 weeks. Liver DHA content was significantly higher ($p < 0.001$) in the GL/FO (28%) and the SJR/FO (26%), compared with the GL/FO-free (10%) and the SJR/FO-free (16%). Similarly, liver EPA content was significantly higher in the GL/FO (5%) and the SJR/FO (5%) than the GL/FO-free (1%) and the SJR/FO-free (2%) (Table 2.6). Total SFA were significantly highest in the SJR/FO, while total PUFA was significantly lowest in the GL/FO-free. Both strains fed the FO-free diet had significantly higher ($p < 0.001$) total n-6 fatty acids than fish fed the control diet. As for total n-3 fatty acids, both strains fed the

FO-free diet had significantly lower n-3 fatty acids (14 - 20%) than they fed the FO control diet (34 - 37%) (Table 2.6).

The significant interaction effect of diet and strain were observed in the liver 16:1n-9, 18:1n-9, 20:4n-6 (ARA), 22:5n-6, total PUFA, total n-6 fatty acids, and the DHA/EPA ratio (Table 2.6). Fish in GL/FO-free had significantly higher ($p = 0.007$) liver 18:1n-9 and significantly lower ($p < 0.001$) liver PUFA due to the interaction effect. Moreover, the diet x strain interaction also significantly affected 16:1n-9, 20:4n-6, 22:5n-6, total n-6 fatty acids, total PUFA and DHA/EPA ratio in the liver (Table 2.6). Liver LNA, 18:4n-3, 20:3n-6, 20:4n-3, EPA, 22:5n-3, and 24:1 depended on the diet alone, while the strain effect was less profound in the liver fatty acid where only ALA depended on the strain alone (Table 2.6).

After 16 weeks, the top three fatty acid classes in the muscle were 16:0, 18:1n:9, and DHA, regardless of treatment (Table 2.7). There were significant differences in most reported fatty acid classes among the four treatment groups. Muscle DHA levels were significantly higher ($p = 0.001$) in both strains fed the control diet compared to both strains fed the FO-free diet, which were 11 - 17% vs. 9 - 10%, respectively. Similarly, the level of 18:1n-9, EPA, 22:5n-3, total SFA, total n-3 fatty acid, the n-3/n-6 ratio followed the same pattern. Muscle LNA, ALA, ARA, total MUFA, and total n-6 fatty acid were significantly higher when both strains fed the FO-free diet (38 - 40%) than both strains fed the control diet (27 - 29%). Moreover, there was a significantly highest ($p < 0.001$) DHA/EPA ratio in the GL/FO-free among the four treatments (Table 2.7).

In terms of the two-way ANOVA results, the strain x diet interaction was also significant for muscle ARA, DPA, DHA, 14:0 and the EPA/ARA ratio (Table 2.7). Approximately 80% of muscle fatty acid classes were significantly affected by the diet, while the level of 16:1n-9, 18:0, 20:4n-3, 20:5n-6, and the DHA/EPA ratio depended on both the diet and strain. Interaction effects were observed in muscle 14:0, 22:5n-3, DHA, and EPA/ARA ratio (Table 2.7).

2.5 DISCUSSION

Different initial liver fatty acid compositions between the landlocked GL and the SJR strain Atlantic salmon reflects their different genetic background, even when both were fed the same commercial diet since first feeding. After 16 weeks, muscle DHA content in the GL/FO was the highest compared to all treatments. The EPA and DHA content did not significantly differ in the

salmon muscle between the GL/FO-free and SJR/FO-free. Among the liver fatty acids, the levels of 18:1n-9 and 18:3n-3 were significantly elevated in the GL/FO-free, while the n-6 LC-PUFA including 20:3n-6, 20:4n-6, and 20:5n-6 were significantly incorporated in the SJR/FO-free. Overall, there was a significant difference in the muscle n-3 LC-PUFA content between the two salmon strains. When both strains were fed the FO control diet, landlocked GL strain had a higher n-3 LC-PUFA content in the muscle than the farmed SJR strain (25.9 ± 6.0% vs. 19.0 ± 8.1%).

2.5.1 Growth performance

Landlocked GL strain adapted well to the FO-free diet, in terms of growth; however, those fed the FO control showed lower growth than the SJR/FO group. Salmon in the GL/FO-free had similar somatic growth with salmon in the SJR/FO-free. It was concluded that the genetic effect contributed to the significant differences in salmon growth performances, except for CF and FCR.

The growth in Atlantic salmon is under both genetic and environmental control (Quinton et al., 2005; Schaeffer et al., 2018). Environmental factors of lake surface area and food availability largely affect the wild landlocked Atlantic salmon body size. For example, wild landlocked GL salmon from West Grand Lake, Maine, USA can grow to a maximum fork length of 54 cm (Havey and Warner, 1970; Hutchings et al., 2019). The maximum body length among landlocked salmon from Lake Gullspångsälven, Vänern, Sweden, is double that of Grand Lake, Maine (Ros, 1981; Hutchings et al., 2019). Therefore, landlocked salmon exhibit various body sizes among different strains.

Landlocked Atlantic salmon parr exhibited significantly better growth rate than the anadromous salmon parr of the same age when offered either a FO diet or a FO-free diet for 8 weeks. Despite this, the mean final body weight did not differ significantly between landlocked and anadromous salmon when offered the same diet (Rollin et al., 2003; Betancor et al., 2016). The higher growth rate can be due to different metabolic pathways between landlocked and farmed strains (Bicskei et al., 2014; Betancor et al., 2018). Based on the analyses of liver transcriptome in landlocked salmon parr (Gullspångsälven strain) by Betancor et al., (2016), fish fed the FO-free diet showed upregulated gene expression of *6pfk* (+3.73 of absolute fold change), which stimulated the regulatory enzyme in glycolysis; moreover, the related gene involved in the synthesis of glucose was down-regulated (-1.69). Therefore, the increase of

glucose catabolism for energy, facilitated the lipogenic gene expression and inhibited lipolysis. As a result, landlocked salmon exhibited the higher growth rate compared to the farmed strain (Betancor et al., 2016). A further study using a high level digestible carbohydrate diet confirmed that the landlocked salmon showed a relatively faster conversion of glucose to TAG compared to the farmed strain (Betancor et al., 2018). Additionally, rainbow trout had enhanced glycolysis and decreased fatty acid oxidation when fed the FO diet; however, relevant gene expressions were not strongly elevated in the liver and muscle (Kolditz et al., 2008). Overall, landlocked salmon parr having a better growth rate is partially explained by the efficient use of carbohydrates, which reflects their natural diet in a freshwater environment. The domestication of Atlantic salmon can suppress the relevant gene expressions in glycolysis (Bicskei et al., 2014; Betancor et al., 2016).

In the current study, landlocked GL strain can efficiently utilize the FO-free diet with a poor level of n-3 LC-PUFA for normal growth. Lipolysis are stimulated in landlocked salmon fed the FO-free diet. This finding is in accordance with Rollin et al., (2003) that both anadromous and landlocked Atlantic salmon parr used FO-free vegetable oil diets without compromising the normal somatic growth. Atlantic salmon have the inherent ability to live in the freshwater environment where the availability of n-3 LC-PUFA is much lower than in the marine environment (Pickova et al., 1999; Parzanini et al., 2020). Farmed SJR strain salmon displayed a better adaptation to both diets compared to landlocked GL strain in terms of growth. At the post-smolt stage, farmed SJR strain have been selectively bred for fast growth rate and higher harvest weight since the 1970's (Glebe, 1998; Quinton et al., 2005; Schaeffer et al., 2018). Consequently, farmed SJR strain Atlantic salmon have growth advantages in comparison to the landlocked GL strain.

Regarding diet effect, landlocked GL and SJR strain had similar growth within the diet group. Dietary DHA/EPA ratio did not affect the somatic growth within the strain in this study. It aligns with Codabaccus et al., (2012) that feeding Atlantic salmon smolt (initial weight ~75 g) blended vegetable oil (200 g/kg) diets with three levels of DHA/EPA ratio: 0.5, 1.3 and 2.8, made no difference in the mean final body weight after 75 days. Moreover, the growth of salmon smolts was independent of dietary fatty acid composition (Codabaccus et al., 2012).

2.5.2 Liver and Muscle Fatty Acid Content

The initial liver fatty acid profiles differed significantly between the two strains, such as 16:0, and some C_{18}, C_{20} fatty acids, even when all salmon were offered the same commercial diet since first feeding. It suggested that differences in the liver fatty acid profile were largely the result of biological differences between the strains. The C_{18} fatty acids – 18:1n-9, LNA, and ALA, were markedly higher in the liver of the GL strain. The 16:0 and C_{20} fatty acids – EPA and ARA were markedly lower in the liver of the GL strain, compared to the liver of the SJR strain (Table 2.5). The composition of liver fatty acids shows the metabolic activities including LC-PUFA biosynthesis, lipogenesis, and fatty acid oxidation (Jordal et al., 2005). Clear evidence of a genetic effect caused the difference in the initial liver fatty acid compositions. By contrast, the initial muscle fatty acid compositions did not significantly differ between strains, except for higher muscle 20:3n-6 in the SJR strain. It concluded that the dietary fatty acid profile determined fish muscle fatty acid profile to a large extent (Torstensen et al., 2005; Bell et al., 2010; Hixson et al., 2014a).

After 16 weeks, final liver fatty acid profiles were greatly affected by the dietary treatment. The DHA and EPA content was higher among salmon fed the control diet, while both strains fed the FO-free diet resulted in significantly lower DHA and EPA levels in liver. When dietary DHA and EPA was absent in the FO-free diet, the accumulation of DHA and EPA in the liver is clear evidence of the upregulation of DHA and EPA biosynthesis in both salmon strains. Moreover, the significantly higher liver DHA/EPA ratio in the FO-free diet group illustrated that regardless of salmon strain, liver DHA tend to be synthesized and reserved in a relatively higher proportion than EPA when n-3 LC-PUFA was absent in the diet. By comparison, liver DHA, EPA percentages and the DHA/EPA ratio between strains were independent of diet. The liver DHA and EPA biosynthesis did not continue to rise and the ratio of DHA:EPA maintained at a similar level when salmon were fed excess n-3 LC-PUFA in the FO control diet. Liver DHA retention efficiency reduces when the high concentration of dietary DHA was supplied (Stubhaug et al., 2007; Glencross and Rutherford, 2014). Atlantic salmon whole-body can retain more than two-times of DHA when dietary DHA included at 1 g/kg; however, DHA retention reduces to < 50 % as the dietary DHA exceeds 10 g/kg (Glencross et al., 2022).

Regarding n-6 PUFA, SJR strain fed the FO-free diet had the highest levels of total n-6 and ARA in the liver among all treatments. The FO-free diet had a lower level of ARA but a higher level of LNA than the control diet; this showed that ARA was largely incorporated or

synthesized in the liver in the SJR strain from the abundant dietary LNA in the FO-free diet. The significantly higher ARA level in the liver in the SJR /FO-free, have led to the bioconversion of 22:5n-6, where the level of 22:5n-6 was highest among all other treatments. It was assumed that there would be a reduction of the ratio of EPA:ARA due to the large increase of ARA level in the liver in the SJR/FO-free; however, this change was not significant among the other treatments. On the other hand, the GL strain fed the FO-free diet did not significantly synthesize a higher level of ARA in the liver. Strain differences play a major effect on controlling the ARA level in the liver. Concerning the n-3 PUFA in the liver, fish in all treatments were low in ALA which was < 1% of all fatty acids. The reason could be the low incorporation of dietary ALA by the salmon liver, and lack of ALA as a substrate prevented further n-3 LC-PUFA biosynthesis toward EPA and DHA.

Compared with the SJR strain, the GL strain had a higher level of liver ALA mostly due to the strain factor ($p < 0.001$), particularly when they were fed the FO-free diet. Besides, liver 18:4n-3, 20:4n-3, EPA, 22:5n-3, and DHA in the pathway of n-3 LC-PUFA were all significantly affected by the diet factor, rather than the strain factor. 18:4n-3 could be produced by the desaturation of ALA. The level of 18:4n-3 was significantly higher in the muscle when both SJR and GL strain were fed the FO-free diet. Although the diet did supply some 18:4n-3 (0.2% total fatty acids), this does indicate some level of biosynthesis (desaturation) of ALA in salmon fed the FO-free diet. The bioconversion of n-3 PUFA from dietary ALA and n-6 PUFA from dietary LNA occurred in both strains; this was consistent with the results by Rollin et al. (2003). In addition, total MUFA, 18:1n-9, and total PUFA were higher in the liver in GL strain compared with the SJR strain when they were both fed the FO-free diet. Collectively, these results indicate that SJR strain tend to synthesize n-6 PUFA when they were fed the diet with a low n-3 LC-PUFA diet but high LNA diet. In the liver, the potential upregulation of n-3 LC-PUFA biosynthesis independent of the diet factor was minor or difficult to identify; perhaps the dietary ALA was not sufficient.

The muscle fatty acid profile in both strains largely correspond to the fatty acid profile in the diets, except for 16:1n-9 (< 1%) and the ratio of DHA:EPA. The GL strain fed the FO-free diet had a significantly higher ratio of DHA:EPA in the muscle among the four treatments. When dietary n-3 LC-PUFA was deficient, muscle DHA and EPA biosynthesis in both strains were stimulated to some extent, which was not comparable to the direct supply of excess n-3 LC-

PUFA (Tocher, 2015; Sprague et al., 2019). In particular, the landlocked GL strain can reserve a significantly higher DHA and EPA when fed the FO control diet compared with the SJR strain. Previous studies established that excess supply of dietary DHA limits the n-3 LC-PUFA biosynthesis or genetic expression involved in the LC-PUFA biosynthesis (Thomassen et al., 2012; Betancor et al., 2015). Under the interaction effect of diet and strain, the landlocked GL strain stored significantly higher muscle DHA and was less inhibited by the high DHA supply in the FO control diet compared with the SJR strain. Similar results were also found in the Swedish landlocked salmon (Betancor et al., 2016). In this sense, landlocked GL strain have the potential for higher muscle n-3 LC-PUFA storage.

Compared with the farmed Atlantic salmon, landlocked salmon fed rapeseed oil diet for 8 weeks did not enhance the n-3 LC-PUFA content in the fish muscle; however, there was an increased expression of desaturase and elongase in the metabolic pathway of n-3 LC-PUFA in the liver (Betancor et al. 2016). Since liver is the main organ for LC-PUFA biosynthesis and lipogenesis, liver transcript expression highly correlated with the muscle fatty acid profile (Xue et al., 2015; Betancor et al., 2016; Hixson et al., 2017). However, the mechanism is still unclear. More studies are needed to answer the question of how the increased capacity of LC-PUFA biosynthesis in the liver could lead to the higher storage of n-3 LC-PUFA in fish muscle.

2.6 CONCLUSIONS

There was clear evidence of genetic differences in salmon growth performance and tissue fatty acid content between the landlocked GL and the farmed SJR strain Atlantic salmon. The GL strain grew poorer when fed the FO control diet compared to the SJR strain. Both strains have the ability to synthesis n-3 LC-PUFA when dietary n-3 LC-PUFA was absent. Storage of muscle EPA and DHA levels did not significantly differ between strains within the FO-free diet group. The landlocked GL strain displayed a similar n-3 LC-PUFA level with the SJR strain in the liver. Muscle DHA level in the landlocked salmon incorporated significantly and less suppressed by the high DHA control diet. The SJR strain had a higher capacity for n-6 LC-PUFA storage. Overall, salmon muscle highly reflected dietary fatty acid composition, while liver fatty acids reflected LC-PUFA biosynthesis and fatty acid oxidation. There is a genetic potential of landlocked salmon with regard to lipid metabolism and n-3 LC-PUFA biosynthesis, which is valuable for further broodstock development.

2.7 TABLES AND FIGURES

Table 2.1. Formulation (g/kg of the feed ingredients) and proximate compositions of the fish oil (FO) control diet and the fish oil-free (FO-free) diet[1].

Ingredient (g/kg)	FO control	FO-free
Fish meal	150	150
Fish oil (Menhaden)	100	0
Ground wheat	170	170
Empyreal (corn protein)	120	120
Canola oil	50	150
Poultry by-product meal	210	210
Blood meal (porcine)	160	160
Vitamin and mineral mix[2]	2	2
Dicalcium phosphate	20	20
Pigment mix[3]	2.5	2.5
Lysine HCl	5	5
Choline chloride	10.5	10.5
Total	1000	1000
Chemical composition (g/kg)		
Dry matter	945.8	945.2
Crude protein	516.8	518.4
Crude fat	178.4	184.8
Calcium	21.7	21.1
Potassium	6.5	6.3
Magnesium	1.3	1.3
Phosphorus	16.5	16.1
Sodium	4.0	3.9
Copper	0.01	0.01
Manganese	0.02	0.02
Zinc	0.19	0.20
Ash	82.0	85.3

[1]All ingredients were donated by Northeast Nutrition (Truro, Nova Scotia, Canada)

[2]Vitamin and mineral premix content (per kg): zinc, 77.5 mg; manganese, 125 mg; iron, 84 mg; copper, 2.5 mg; iodine, 7.5 mg; vitamin A, 5000 IU; vitamin D, 4000 IU; vitamin K, 2 mg; vitamin B12, 4 µg; thiamine, 8 mg; riboflavin, 18 mg; pantothenic acid, 40 mg; niacin, 100 mg; folic acid, 4 mg; biotin, 0.6 mg; pyridoxine, 15 mg; inositol, 100 mg; ethoxyquin, 42 mg; wheat shorts, 1372 mg.

[3]Pigment mix contains (per kg): selenium, 0.220 mg; vitamin E, 250 IU; vitamin C, 200 mg; astaxanthin, 60 mg; wheat shorts, 1988 mg.

Table 2.2. Fatty acid profiles (percentage of total fatty acids) of the FO control diet and the FO-free diet[1].

Fatty acid (%)	FO control	FO-free	F-value	*p*-value
14:0	3.2 ± 0.5	0.5 ± 0.02	94.9	0.001
14:1	0.09 ± 0.002	0.05 ± 0.001	1296	<0.001
16:0	16.3 ± 2.7	8.7 ± 0.2	23.8	0.008
16:1n-9	0.2 ± 0.04	0.1 ± 0.002	24.1	0.008
16:1n-7	4.7 ± 0.3	1.5 ± 0.03	280.6	<0.001
18:0	4.1 ± 1.0	2.8 ± 0.1	5.1	0.088
18:1n-9	25.2 ± 5.4	48.7 ± 0.3	57.3	0.002
18:1n-7	2.6 ± 0.1	2.6 ± 0.1	0.2	0.651
18:2n-6 (LNA)	13.2 ± 2.5	21.0 ± 0.2	29.5	0.006
18:3n-3 (ALA)	2.6 ± 0.6	6.3 ± 0.09	106.9	<0.001
18:4n-3	1.1 ± 0.1	0.1 ± 0.01	163.1	<0.001
20:0	0.3 ± 0.05	0.5 ± 0.01	24.2	0.008
20:1n-9	1.7 ± 0.4	1.7 ± 0.1	0.02	0.896
20:1n-7	0.2 ± 0.03	0.08 ± 0.006	34.2	0.004
20:2n-6	0.19 ± 0.04	0.09 ± 0.008	14.6	0.019
20:3n-6	0.16 ± 0.05	0.05 ± 0.002	10.0	0.034
20:4n-6 (ARA)	0.6 ± 0.02	0.2 ± 0.01	1251	<0.001
20:4n-3	0.4 ± 0.02	0.03 ± 0.002	942.6	<0.001
20:5n-3 (EPA)	7.3 ± 0.3	0.6 ± 0.04	1680.5	<0.001
22:0	0.3 ± 0.01	0.3 ± 0.01	3.26	0.145
22:1n-9	2.2 ± 0.01	1.9 ± 0.2	5.67	0.098
22:1n-7	0.09 ± 0.01	0.07 ± 0.01	7.5	0.052
22:5n-6	0.17 ± 0.004	0.016 ± 0.001	3307.3	<0.001
22:5n-3	0.9 ± 0.05	0.09 ± 0.004	888.5	<0.001
24:0	0.13 ± 0.05	0.16 ± 0.003	0.6	0.479
22:6n-3 (DHA)	6.6 ± 1.4	0.9 ± 0.04	50.1	0.002
24:1	0.5 ± 0.2	0.2 ± 0.02	8.7	0.042
ΣSFA	24.9 ± 3.2	13.0 ± 0.3	41.6	0.003
ΣMUFA	37.9 ± 6.5	57.2 ± 0.3	26.4	0.007
ΣPUFA	36.8 ± 3.3	29.7 ± 0.3	14.0	0.020
Σn-3	19.4 ± 0.3	8.0 ± 0.07	4094	<0.001
Σn-6	13.1 ± 0.07	21.5 ± 0.2	2315.3	<0.001
n-3/n-6	1.3 ± 0.2	0.37 ± 0.002	48.2	0.002
DHA/EPA	0.9 ± 0.2	1.5 ± 0.04	20.0	0.011
EPA/ARA	13.2 ± 0.2	3.0 ± 0.01	5052.9	<0.001

[1]Data expressed as mean ± SD ($n_{control} = 3$; $n_{FO\text{-}free} = 3$). Significant differences between diets were determined by one-way ANOVA with Bonferroni *post-hoc* correction test at a significant level of 0.05. LNA, linoleic acids (18:2n-6); ALA, α-linolenic acid (18:3n-3); ARA, arachidonic acid (20:4n-6); EPA, eicosapentaenoic acid (20:5n-3); DHA, docosahexaenoic acid (22:6n-3).

Table 2.3. Growth performance of the landlocked Grand Lake (GL) strain vs. farmed anadromous Saint John River (SJR) strain fed the FO control diet vs. the FO-free diet for 16 weeks[1].

	GL		SJR		*p*-value			
					One-way ANOVA	Two-way ANOVA		
	FO	FO-free	FO	FO-free	Treatment	Strain	Diet	Diet x Strain
Initial mean BW(g)	60.3 ± 2.2	56.8 ± 4.6	56.8 ± 3.6	58.4 ± 1.7	0.877	0.66	0.66	0.36
Final mean BW (g)	167.5 ± 2.8[b]	180.1 ± 3.3[ab]	199.2 ± 9.3[a]	202.6 ± 14.8[a]	0.002	0.10	0.41	0.63
Weight gain (g)	106.1±15.8[b]	123.3 ± 8.4[ab]	142.4 ± 4.7[a]	144.1 ± 6.9[a]	0.038	0.01	0.33	0.42
Weight increase (%)	147.2 ± 13.4[b]	228.0 ± 27.1[ab]	253.5 ± 10.9[a]	249.9 ± 29.9[a]	0.012	0.04	0.29	0.23
TGC[2]	1.16 ± 0.06[b]	1.58 ± 0.09[ab]	1.74 ± 0.03[a]	1.72 ± 0.16[a]	0.001	0.02	0.34	0.25
CF[3]	1.74 ± 0.19	1.61 ± 0.14	1.43 ± 0.10	1.66 ± 0.11	0.442	0.70	0.38	0.43
FCR[4]	0.96 ± 0.04	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02	0.636	0.10	0.72	0.77

[1]Mean ± SD (n = 5 per treatment). Different letters in the same row indicated significant differences ($p < 0.05$) among four treatments (GL/FO, GL/FO-free, SJR/FO, and SJR/FO-free). It was determined by a one-way ANOVA, followed by the Tukey's multiple comparison. BW, body weight.

[2]Thermal growth coefficient = [[(Final weight)$^{1/3}$ – (Initial weight)$^{1/3}$]/ Temperature in °C x Days] x 1000.

[3]Condition factor = Body mass/Length^3

[4]Feed conversion ratio = (Feed offered) / (Weight gain)

Table 2.4. Initial liver fatty acid profiles (percentage of total fatty acids) of the Grand Lake (GL) strain and Saint John River (SJR) strain Atlantic salmon prior to the trial[1].

Fatty acid (%)	GL	SJR	F-value	*p*-value
14:0	1.5 ± 0.2	1.2 ± 0.2	14.7	0.001
14:1	0.05 ± 0.01	0.04 ± 0.01	3.5	0.079
16:0	18.9 ± 1.1	20.7 ± 1.9	7.3	0.015
16:1n-9	0.4 ± 0.02	0.4 ± 0.07	0.5	0.510
16:1n-7	2.2 ± 0.5	1.8 ± 0.3	6.8	0.018
18:0	5.6 ± 0.4	5.7 ± 0.2	0.8	0.381
18:1n-9	12.5 ± 0.6	10.7 ± 1.1	7.5	0.014
18:1n-7	1.8 ± 0.2	1.7 ± 0.2	2.9	0.105
18:2n-6 (LNA)	6.0 ± 0.6	5.0 ± 0.3	19.0	<0.001
18:3n-3 (ALA)	0.5 ± 0.06	0.3 ± 0.05	22.2	<0.001
18:4n-3	0.2 ± 0.07	0.2 ± 0.02	0.3	0.574
20:1n-9	0.7 ± 0.1	0.4 ± 0.1	21.8	<0.001
20:1n-7	0.09 ± 0.02	0.06 ± 0.03	7.6	0.013
20:2n-6	0.7 ± 0.1	0.5 ± 0.1	20.9	<0.001
20:3n-6	0.8 ± 0.1	0.8 ± 0.1	0.7	0.410
20:4n-6 (ARA)	3.6 ± 0.4	4.2 ± 0.7	5.2	0.035
20:4n-3	0.4 ± 0.01	0.36 ± 0.05	16.7	0.001
20:5n-3 (EPA)	5.3 ± 0.5	6.1 ± 0.7	7.3	0.015
22:0	0.06 ± 0.02	0.19 ± 0.4	1.2	0.288
22:1n-9	0.1 ± 0.04	0.1 ± 0.04	0.9	0.356
22:1n-7	0.1 ± 0.09	0.1 ± 0.03	0.5	0.496
22:5n-6	0.5 ± 0.05	0.4 ± 0.07	1.4	0.246
22:5n-3	1.5 ± 0.5	1.6 ± 0.8	0.05	0.820
24:0	0.2 ± 0.5	0.5 ± 0.8	0.9	0.360
22:6n-3 (DHA)	30.7 ± 2.4	31.2 ± 2.0	0.3	0.596
24:1	1.4 ± 0.2	1.7 ± 0.4	4.3	0.043
ΣSFA	26.9 ± 1.1	28.9 ± 2.5	5.7	0.028
ΣMUFA	20.4 ± 2.4	18.0 ± 1.6	7.3	0.014
ΣPUFA	52.4 ± 2.5	52.9 ± 2.9	0.1	0.709
Σn-3	39.2 ± 2.8	40.3 ± 2.0	1.2	0.298
Σn-6	12.0 ± 0.4	11.5 ± 1.0	2.5	0.131
n-3/n-6	3.3 ± 0.3	3.5 ± 0.2	5.0	0.038
DHA/EPA	5.8 ± 0.6	5.2 ± 0.5	5.3	0.033
EPA/ARA	1.5 ± 0.2	1.5 ± 0.2	0.00	0.973

[1]Data expressed as mean ± SD (n_{GL} = 24; n_{SJR} = 24). Results were determined by one-way ANOVA with the Bonferroni *post-hoc* test (α =0.05). LNA, linoleic acids (18:2n-6); ALA, α-linolenic acid (18:3n-3); ARA, arachidonic acid (20:4n-6); EPA, eicosapentaenoic acid (20:5n-3); DHA, docosahexaenoic acid (22:6n-3).

Table 2.5. Initial muscle fatty acid content (% of total fatty acids) of the Grand Lake (GL) strain and Saint John River (SJR) strain prior to the trial[1].

Fatty acid (%)	GL	SJR	F-value	*p*-value
14:0	2.5 ± 0.3	2.6 ± 0.5	0.06	0.815
14:1	0.08±0.03	0.06 ± 0.04	1.8	0.195
16:0	20.3 ± 3.5	22.1 ± 3.5	1.2	0.283
16:1n-9	0.3 ± 0.03	0.3 ± 0.05	4.1	0.057
16:1n-7	4.2 ± 1.0	3.7 ± 1.1	1.1	0.309
18:0	5.6 ± 1.1	6.3 ± 1.2	2.0	0.170
18:1n-9	18.6 ± 3.5	16.5 ± 4.3	1.5	0.238
18:1n-7	2.5 ± 0.3	2.5 ± 0.3	0.2	0.637
18:2n-6 (LNA)	8.3 ± 1.5	7.4 ± 1.7	1.6	0.227
18:3n-3 (ALA)	0.8 ± 0.1	0.8 ± 0.1	0.9	0.357
18:4n-3	0.7 ± 0.1	0.7 ± 0.2	0.3	0.600
20:0	0.1 ± 0.02	0.1 ± 0.05	0.00	0.953
20:1n-9	1.1 ± 0.2	0.9 ± 0.3	0.9	0.346
20:1n-7	0.1 ± 0.02	0.1 ± 0.02	0.3	0.598
20:2n-6	0.5 ± 0.2	0.6 ± 0.08	1.4	0.245
20:3n-6	0.5 ± 0.03	0.5 ± 0.03	7.4	0.014
20:4n-6 (ARA)	1.2 ± 0.2	1.2 ± 0.08	0.3	0.576
20:4n-3	0.5 ± 0.07	0.5 ± 0.06	2.0	0.174
20:5n-3 (EPA)	4.8 ± 0.5	5.1 ± 0.8	0.9	0.345
22:0	0.09±0.05	0.08 ± 0.04	0.06	0.814
22:1n-9	0.2 ± 0.02	0.2 ± 0.04	1.3	0.277
22:1n-7	0.4 ± 0.3	0.6 ± 0.4	3.0	0.101
22:5n-6	0.4 ± 0.1	0.4 ± 0.1	0.00	0.977
22:5n-3	1.8 ± 0.8	1.8 ± 0.2	0.4	0.553
22:6n-3 (DHA)	17.7 ± 3.1	17.8 ± 4.3	0.01	0.939
24:1	0.4 ± 0.05	0.5 ± 0.05	2.2	0.158
ΣSFA	29.3 ± 4.6	32.1 ± 4.9	1.6	0.219
ΣMUFA	29.3 ± 5.0	26.8 ± 6.0	1.0	0.332
ΣPUFA	40.6 ± 2.7	40.3 ± 4.2	0.03	0.861
Σn-3	26.8 ± 3.4	27.1 ± 5.2	0.03	0.868
Σn-6	11.1 ± 1.2	10.5 ± 1.4	1.3	0.263
n-3/n-6	2.5 ± 0.5	2.7 ± 0.8	0.52	0.479
DHA/EPA	3.7 ± 0.5	3.5 ± 0.5	1.0	0.340
EPA/ARA	4.1 ± 0.6	4.2 ± 0.5	0.01	0.931

[1]Data expressed as mean ± SD (n_{GL} =24; n_{SJR} = 24). Results were determined by the one-way ANOVA with the Bonferroni *post-hoc* correction test at a significant level of 0.05. LNA, linoleic acids (18:2n-6); ALA, α-linolenic acid (18:3n-3); ARA, arachidonic acid (20:4n-6); EPA, eicosapentaenoic acid (20:5n-3); DHA, docosahexaenoic acid (22:6n-3).

Table 2.6. Liver fatty acid content (% of total fatty acids) of the Grand Lake strain and Saint John River strain after being fed FO- and FO-free diets for 16 weeks[1].

Fatty acid	GL		SJR		*p*-value			
					One-way ANOVA	Two-way ANOVA		
	FO	FO-free	FO	FO-free	Treatment	Diet	Strain	Strain x Diet
14:0	1.4 ± 0.1^{a}	1.0 ± 0.3^{b}	1.5 ± 0.6^{a}	0.7 ± 0.1^{b}	<0.001	<0.001	0.488	0.158
14:1	0.05 ± 0.02^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.02 ± 0.01^{b}	<0.001	0.009	<0.001	0.098
16:0	17.4 ± 1.5^{ab}	13.7 ± 2.0^{b}	22.0 ± 6.4^{a}	14.7 ± 3.0^{b}	<0.001	<0.001	0.003	0.140
16:1n-9	0.5 ± 0.05^{b}	0.6 ± 0.31^{b}	0.4 ± 0.08^{b}	0.8 ± 0.09^{a}	<0.001	<0.001	0.062	0.004
16:1n-7	1.8 ± 0.7^{a}	1.5 ± 0.5^{a}	1.4 ± 0.2^{ab}	0.9 ± 0.2^{b}	0.001	0.016	<0.001	0.407
18:0	4.7 ± 1.7	5.0 ± 1.9	6.6 ± 2.6	6.1 ± 0.7	0.142	0.805	0.017	0.515
18:1n-9	16.4 ± 5.5^{c}	39.2 ± 7.5^{a}	13.7 ± 2.0^{c}	27.1 ± 3.8^{b}	<0.001	<0.001	<0.001	0.007
18:1n-7	1.8 ± 0.9	1.7 ± 1.2	1.8 ± 0.3	2.0 ± 0.3	0.915	0.836	0.601	0.663
18:2n-6 (LNA)	4.4 ± 0.5^{b}	6.1 ± 2.5^{a}	3.8 ± 0.4^{b}	5.1 ± 0.5^{ab}	0.003	0.001	0.063	0.626
18:3n-3 (ALA)	0.6 ± 0.08^{ab}	0.8 ± 0.2^{a}	0.5 ± 0.1^{b}	0.5 ± 0.2^{b}	0.003	0.360	0.001	0.173
18:4n-3	0.2 ± 0.09^{b}	0.3 ± 0.05^{a}	0.2 ± 0.01^{b}	0.3 ± 0.05^{a}	0.001	<0.001	0.662	0.644
20:0	0.05 ± 0.05	0.06 ± 0.01	0.2 ± 0.30	0.1 ± 0.06	0.348	0.488	0.132	0.467
20:1n-9	2.1 ± 0.8^{ab}	3.1 ± 1.5^{a}	1.4 ± 0.6^{b}	2.5 ± 0.5^{ab}	0.004	0.002	0.044	0.945
20:1n-7	0.1 ± 0.06	0.6 ± 1.5	0.1 ± 0.06	0.1 ± 0.06	0.416	0.397	0.259	0.358
20:2n-6	1.0 ± 0.4	1.0 ± 0.6	0.9 ± 0.2	0.9 ± 0.3	0.845	0.672	0.440	0.868
20:3n-6	1.2 ± 0.2^{b}	3.4 ± 1.3^{a}	1.4 ± 0.6^{b}	4.4 ± 0.7^{a}	<0.001	<0.001	0.030	0.124
20:4n-6 (ARA)	3.4 ± 0.4^{b}	4.6 ± 1.7^{b}	3.4 ± 2.2^{b}	8.2 ± 3.0^{a}	<0.001	<0.001	0.004	0.004
20:4n-3	0.4 ± 0.07^{a}	0.3 ± 0.1^{b}	0.4 ± 0.1^{ab}	0.3 ± 0.02^{b}	0.004	0.001	0.098	0.394
20:5n-3 (EPA)	5.3 ± 0.6^{a}	1.2 ± 0.4^{b}	4.9 ± 1.4^{a}	1.6 ± 0.3^{b}	<0.001	<0.001	0.999	0.193
22:0	0.1 ± 0.06	0.1 ± 0.05	0.7 ± 1.8	0.1 ± 0.03	0.770	0.338	0.356	0.315
22:1n-9	0.3 ± 0.06	0.3 ± 0.08	0.3 ± 0.3	0.3 ± 0.05	0.639	0.715	0.824	0.225
22:1n-7	0.1 ± 0.08	0.09 ± 0.05	0.4 ± 0.6	0.06 ± 0.01	0.172	0.102	0.319	0.235
22:5n-6	0.5 ± 0.09^{c}	1.3 ± 0.2^{b}	0.6 ± 0.4^{c}	2.1 ± 0.4^{a}	<0.001	<0.001	<0.001	0.005
22:5n-3	1.4 ± 0.4^{a}	0.7 ± 0.3^{b}	1.4 ± 0.5^{a}	1.1 ± 0.2^{ab}	0.001	<0.001	0.152	0.102
24:0	0.15 ± 0.4	0.09 ± 0.02	0.24 ± 0.5	0.06 ± 0.05	0.633	0.251	0.791	0.588
22:6n-3 (DHA)	28.1 ± 3.8^{a}	10.2 ± 3.0^{b}	25.5 ± 8.1^{a}	15.6 ± 2.2^{b}	<0.001	<0.001	0.370	0.015
24:1	1.9 ± 0.7^{ab}	1.3 ± 0.3^{b}	2.2 ± 0.5^{a}	1.6 ± 0.5^{ab}	0.006	0.002	0.084	1.000
ΣSFA	24.4 ± 2.9^{b}	20.2 ± 3.3^{b}	31.7 ± 9.8^{a}	22.1 ± 3.5^{b}	<0.001	<0.001	0.015	0.138
ΣMUFA	26.5 ± 7.3^{c}	48.9 ± 7.7^{a}	22.7 ± 2.7^{c}	35.7 ± 4.4^{b}	<0.001	<0.001	<0.001	0.017
ΣPUFA	48.8 ± 5.2^{a}	30.6 ± 6.7^{b}	45.2 ± 9.2^{a}	42.1 ± 3.4^{a}	<0.001	<0.001	0.066	<0.001
Σn-3	36.6 ± 4.3^{a}	13.8 ±4.1^{b}	33.5 ± 8.8^{a}	19.6 ± 2.7^{b}	<0.001	<0.001	0.479	0.013
Σn-6	11.0 ± 1.2^{c}	15.9 ± 3.8^{b}	10.6 ± 1.9^{c}	21.9 ± 2.4^{a}	<0.001	<0.001	0.001	<0.001
n-3/n-6	3.4 ± 0.2^{a}	0.9 ± 0.3^{b}	3.2 ± 1.0^{a}	0.9 ± 0.2^{b}	<0.001	<0.001	0.628	0.600
DHA/EPA	5.4 ± 0.7^{c}	8.4 ± 1.0^{b}	5.1 ± 1.1^{c}	10.2 ± 1.3^{a}	<0.001	<0.001	0.036	0.005
EPA/ARA	1.6 ± 0.2	2.2 ± 6.1	1.4 ± 0.2	0.9 ± 2.3	0.865	0.934	0.496	0.6327

[1]Data expressed as mean ± SD, n=5 in each treatment (strain/diet). Different letters within a row indicated significant differences among four treatments determined by the one-way ANOVA with Bonferroni *post-hoc* correction test (α = 0.05). LNA, linoleic acids (18:2n-6); ALA, α-linolenic acid (18:3n-3); ARA, arachidonic acid (20:4n-6); EPA, eicosapentaenoic acid (20:5n-3); DHA, docosahexaenoic acid (22:6n-3).

Table 2.7. Muscle fatty acid content (% of total fatty acids) of the Grand Lake strain (GL) and Saint John River (SJR) strain after being fed FO and FO-free diets for 16 weeks[1].

Fatty acid	GL		SJR		*p*-value			
					One-way ANOVA	Two-way ANOVA		
	FO	FO-free	FO	FO-free	Treatment	Diet	Strain	Strain x Diet
14:0	2.3 ± 0.5^{a}	0.9 ± 0.2^{b}	2.8 ± 0.6^{a}	0.8 ± 0.2^{b}	<0.001	<0.001	0.142	0.037
14:1	0.03 ± 0.03	0.01 ± 0.01	0.03 ± 0.03	0.01 ± 0.01	0.058	0.008	0.990	0.663
16:0	22.1 ± 4.9^{ab}	18.1 ± 4.7^{b}	28.4 ± 10.8^{a}	17.7 ± 4.8^{b}	0.006	0.002	0.185	0.136
16:1n-9	0.3 ± 0.02^{b}	0.4 ± 0.02^{a}	0.2 ± 0.05^{c}	0.4 ± 0.03^{a}	<0.001	<0.001	<0.001	0.225
16:1n-7	2.8 ± 0.7^{a}	1.5 ± 0.3^{b}	2.5 ± 1.0^{a}	1.2 ± 0.3^{b}	<0.001	<0.001	0.175	0.943
18:0	6.6 ± 1.6^{ab}	5.0 ± 2.1^{b}	8.8 ± 4.0^{a}	6.6 ± 1.9^{ab}	0.035	0.027	0.032	0.709
18:1n-9	18.6 ± 4.1^{a}	31.4 ± 4.8^{b}	16.2 ± 6.0^{a}	29.7 ± 6.0^{b}	<0.001	<0.001	0.241	0.864
18:1n-7	2.7 ± 0.2	2.6 ± 0.1	2.5 ± 0.7	2.6 ± 0.09	0.532	0.474	0.328	0.341
18:2n-6 (LNA)	6.0 ± 2.5^{b}	10.2 ± 0.9^{a}	6.2 ± 2.2^{b}	10.1 ± 1.4^{a}	<0.001	<0.001	0.991	0.810
18:3n-3 (ALA)	1.3 ± 0.3^{b}	1.9 ± 0.2^{a}	1.1 ± 0.6^{b}	2.0 ± 0.3^{a}	<0.001	<0.001	0.698	0.373
18:4n-3	0.6 ± 0.2^{b}	0.9 ± 0.2^{a}	0.6 ± 0.3^{b}	0.9 ± 0.2^{a}	0.002	<0.001	0.866	0.847
20:0	0.15 ± 0.06^{b}	0.23 ± 0.04^{a}	0.21 ± 0.05^{ab}	0.23 ± 0.06^{a}	0.006	0.007	0.082	0.139
20:1n-9	1.3 ± 0.3^{ab}	1.6 ± 0.3^{a}	1.1 ± 0.4^{b}	1.5 ± 0.3^{ab}	0.029	0.007	0.126	0.695
20:1n-7	0.13 ± 0.03^{a}	0.08 ± 0.02^{bc}	0.11 ± 0.06^{ab}	0.07 ± 0.01^{c}	0.001	<0.001	0.219	0.800
20:2n-6	0.5 ± 0.09	0.7 ± 0.08	0.5 ± 0.17	0.6 ± 0.09	0.035	0.006	0.340	0.549
20:3n-6	0.5 ± 0.08^{b}	1.9 ± 0.3^{a}	0.4 ± 0.2^{b}	2.2 ± 0.3^{a}	<0.001	<0.001	0.321	0.046
20:4n-6 (ARA)	1.1 ± 0.3^{b}	2.4 ± 0.5^{a}	0.8 ± 0.3^{b}	2.9 ± 0.6^{a}	<0.001	<0.001	0.413	0.006
20:4n-3	0.4 ± 0.06^{a}	0.4 ± 0.05^{ab}	0.4 ± 0.2^{ab}	0.3 ± 0.04^{b}	0.012	0.021	0.027	0.509
20:5n-3 (EPA)	4.8 ± 1.1^{a}	1.9 ± 0.4^{b}	4.3 ± 1.8^{a}	2.2 ± 0.4^{b}	<0.001	<0.001	0.664	0.247
22:0	0.15 ± 0.05	0.17 ± 0.06	0.13 ± 0.08	0.18 ± 0.05	0.106	0.023	0.469	0.795
22:1n-9	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.564	0.581	0.333	0.333
22:1n-7	0.7 ± 0.7^{ab}	0.3 ± 0.3^{b}	1.6 ± 0.5^{a}	0.4 ± 0.2^{b}	0.003	0.002	0.064	0.093
22:5n-6	0.4 ± 0.1^{b}	0.7 ± 0.2^{a}	0.2 ± 0.2^{b}	0.7 ± 0.2^{a}	<0.001	<0.001	0.032	0.260
22:5n-3	1.6 ± 0.3^{a}	0.6 ± 0.3^{b}	1.3 ± 0.5^{a}	0.8 ± 0.1^{b}	<0.001	<0.001	0.946	0.017
22:6n-3 (DHA)	16.7 ± 4.8^{a}	9.7 ± 2.8^{b}	10.5 ± 5.5^{b}	9.4 ± 2.5^{b}	0.001	0.004	0.019	0.032
24:1	0.6 ± 0.1	0.5 ± 0.05	0.5 ± 0.2	0.6 ± 0.1	0.429	0.836	0.413	0.146
ΣSFA	32.2 ± 7.0^{ab}	24.9 ± 4.5^{b}	41.8 ± 11.5^{a}	25.9 ± 6.7^{b}	0.006	0.001	0.216	0.396
ΣMUFA	29.3 ± 5.1^{b}	40.3 ± 5.5^{a}	26.8 ± 7.5^{b}	38.2 ± 6.2^{a}	<0.001	<0.001	0.268	0.924
ΣPUFA	37.5 ± 6.4	34.2 ± 3.6	33.0 ± 7.0	35.3 ± 3.5	0.328	0.753	0.327	0.123
Σn-3	25.9 ± 6.0^{a}	15.8 ± 3.2^{b}	19.0 ± 8.1^{ab}	15.8 ± 3.1^{b}	<0.001	0.001	0.061	0.061
Σn-6	8.8 ± 2.3^{b}	17.3 ± 0.8^{a}	8.4 ± 2.7^{b}	17.8 ± 1.1^{a}	<0.001	<0.001	0.916	0.438
n-3/n-6	3.3 ± 1.7^{a}	0.9 ± 0.2^{b}	2.2 ± 0.6^{a}	0.9 ± 0.1^{b}	<0.001	<0.001	0.082	0.099
DHA/EPA	3.4 ± 0.3^{c}	5.0 ± 0.4^{a}	2.3 ± 0.4^{d}	4.3 ± 0.5^{b}	<0.001	<0.001	<0.001	0.180
EPA/ARA	4.3 ± 0.4^{b}	0.8 ± 0.08^{c}	5.1 ± 1.1^{a}	0.8 ± 0.09^{c}	<0.001	<0.001	0.035	0.015

[1]Data expressed as mean ± SD, n =5 in each treatment (strain/diet). Different letters within a row indicated significant differences among four treatments determined by the one-way ANOVA with Bonferroni *post-hoc* correction test (α = 0.05). LNA, linoleic acids (18:2n-6); ALA, α-linolenic acid (18:3n-3); ARA, arachidonic acid (20:4n-6); EPA, eicosapentaenoic acid (20:5n-3); DHA, docosahexaenoic acid (22:6n-3).

CHAPTER 3: LONG CHAIN POLYUNSATURATED FATTY ACID LEVEL BY THE SAINT JOHN RIVER ATLANTIC SALMON: A COMPARISON AMONG FIFTY FAMILIES

3.1 ABSTRACT

Family/genetic and diet effects affected Atlantic salmon n-3 LC-PUFA level. The capability of salmon n-3 LC-PUFA biosynthesis was inferred by analyzing the muscle and liver n-3 LC-PUFA content among selected SJR salmon families. Parr averaging 48 g and 15 cm among fifty SJR strain families were offered with two diets for 16 weeks. The FO control diet contained 10% FO and 11% canola oil, while the FO- free diet contained 17% camelina oil and 4% canola oil. Independent of family factor, the mean final body weight gain in the FO control diet group was significantly higher ($p < 0.001$) than the FO-free diet group, which was 121.5 ± 25.6 g vs 110.5 ± 22.4 g, respectively.

After 16 weeks, the best weight gain or the lowest weight gain families were selected in each diet group for tissue fatty acid analysis. Among fish fed the FO-free diet, the biosynthesis of n-3 LC-PUFA, particularly DHA level was upregulated in the muscle and liver. However, the improvement of n-3 LC-PUFA content differed among selective families fed either diet. Family #14 fed the FO-free diet had the highest ($p = 0.025$) DHA percentage at 16% in the liver among best weight gain families; additionally, the significantly increased n-3/n-6 ratio indicated the affinity to n-3 fatty acid. Among the families with the better growth performance, muscle EPA and DHA content among best weight gain families was similar, ranging from 1 to 1.4% and 3 to 4.5%, respectively, independent of the diet. Families within the lowest weight gain group did not significantly change their muscle and liver n-3 LC-PUFA percentage.

3.2 INTRODUCTION

Fish oil (FO) is considered the most nutritious and digestible lipid for farmed carnivorous fish and provides the major source of omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA), DHA and EPA (FAO, 2020. p.63; FAO, 2022. p.77). The heavy dependency on marine FO is a major challenge in the aquaculture (and animal) feed industry. Utilizing alternative sustainable, plant-based ingredients, with less inclusion of FO is the future trend in aquaculture production (Hixson et al., 2014a; Turchini et al., 2020). Dietary inclusion rates of FO as dietary lipids in salmon feeds has decreased from 24 to 11% between 1990 and 2019 in Norwegian

Atlantic salmon production (Ytrestøyl et al., 2015; Aas et al., 2019; MOWI, 2020). Terrestrial plant oils are used as alternative dietary lipid to substitute for FO in salmonid feed. However, the problem of terrestrial plant oils is their deficiency of essential n-3 LC-PUFA, EPA and DHA, which can cause adverse effects on the growth of salmonids and also reduce the nutritional value for human consumption (Turchini et al., 2009; Sprague et al., 2016; Bou et al., 2017a). Canola or rapeseed oil (*Brassica napus*) is rich in oleic acid (18:1n-9), followed by linoleic acid (LNA, 18:2n-6), and α-Linolenic acid (ALA, 18:3n-3), which represent about 60, 20, and 3 - 13% in total fatty acids, respectively (Przybylski and Eskin, 2011; Goyal et al., 2021). The progressive inclusion of rapeseed oil in the diet significantly increased 18:1n-9 in the muscle tissue from 15% to 30% and LNA from 5 % to 10% between 2010 and 2015 (Sprague et al., 2016). As a result, average absolute amount of EPA and DHA in the flesh of farmed Scottish Atlantic salmon decreased from 2.74 g/100g in 2006 to 1.36 g/100g in 2015 (Sprague et al., 2016).

Estimated minimum requirement of n-3 LC-PUFA for Atlantic salmon is 0.5% (5 g/kg) of EPA+DHA in the freshwater (Ruyter et al., 2000; Qian et al., 2020) where the ratio of EPA and DHA is 1:1 (Qian et al., 2020). Post-smolt Atlantic salmon need 1 to 1.5% (10 to 15g/kg) of dietary n-3 LC-PUFA for optimal growth (Glencross et al., 2014; Huyben et al., 2021a). Another study recommends the n-3 LC-PUFA (EPA: DHA = 1:1) inclusion between 1.6 and 2.6% for Atlantic salmon in seawater (Sissener et al., 2016). Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) can synthesize up to 25% of their own n-3 LC-PUFA from the n-3 C_{18} PUFA in dietary plant oils (Hixson et al., 2014b, c). Some non-anadromous Atlantic salmon have shown the potential to upregulate the n-3 LC-PUFA biosynthesis in fish tissues (Rollin et al., 2003; Betancor et al., 2016). Fillet LC-PUFA content is a heritable trait in farmed Atlantic salmon (Leaver et al., 2011; Horn et al., 2018). Therefore, it is hypothesized that the minimal requirement of dietary n-3 LC-PUFA for salmonids could be satisfied by n-3 LC-PUFA biosynthesis in the body, if n-3 fatty acid precursor (ALA) is sufficient in the diet.

When dietary FO is devoid, the upregulation of LC-PUFA biosynthesis in salmonid is too low to satisfy the basic level of LC-PUFA (Tocher, 2015; Sprague et al., 2019). To address this problem, it is feasible to selectively breed Atlantic salmon with the trait of high LC-PUFA biosynthesis (Horn et al., 2018). The relationship between diet, genetic variability, and LC-PUFA synthesis and storage has been exhibited in the Norwegian Atlantic salmon (Aquagen strain and SalmonBreed strain) (Berge et al., 2015; Betancor et al., 2016). A selected line of high

LC-PUFA Atlantic salmon (SalmonBreed strain, Norway) had a higher fillet n-3 LC-PUFA than the low LC-PUFA Atlantic salmon by 2 g/kg (Berge et al., 2015). Non-anadromous Atlantic salmon have the potential to upregulate the n-3 LC-PUFA biosynthesis in the liver compared with the farmed Aquagen strain (Betancor et al., 2016). The n-3 LC-PUFA level in Atlantic salmon muscle differed significantly between families when fed the same plant oils diet for 12 weeks; moreover, the heritability of n-3 LC-PUFA percentage and absolute amount of n-3 LC-PUFA in fillet lipid were 0.77 and 0.34 g/100g , respectively (Leaver et al., 2011). By comparison, the estimates of heritability for EPA, DHA percentages and absolute amount of n-3 LC-PUFA in Atlantic salmon (g/100g) flesh were 0.09, 0.26, and 0.35, respectively (Horn et al., 2018). Collectively, the n-3 LC-PUFA biosynthesis is a moderate heritable trait in Atlantic salmon, which is genetically correlated with muscle fat and visceral fat storage (Horn et al., 2018).

In North America, the improved nutrient utilization for n-3 LC-PUFA biosynthesis, has yet to be included in the farmed SJR strain broodstock programs. The objective of this study was to determine the phenotypic variability between fifty SJR strain Atlantic salmon families regarding their n-3 LC-PUFA tissue content and growth performance. The fatty acid profile in fish tissues is largely dependent on the dietary fatty acids (Bell et al., 2010; Hixson et al., 2014a; Alhazzaa et al., 2019). In this study, the FO control diet contained fish oil with a rich supply of EPA and DHA, while the FO-free diet had a mixture of canola and camelina oil that provided abundant LNA and ALA, respectively. Compared with canola oil, camelina oil has a significantly higher level of ALA (39% vs. 12%) (Przybylski and Eskin, 2011; Government of Canada, 2012) that can be used to balance the ratio of LNA and ALA close to 1 to 1, and can provide sufficient n-3 fatty acid precursor in the FO-free diet.

3.3 MATERIALS AND METHODS

3.3.1 Experimental Diets

Two isonitrogenous and isolipidic diets were produced at the Chute Animal Nutrition Lab, Faculty of Agriculture, Dalhousie University. A control diet contained 10% FO and 11% canola oil that matched the commercial salmon feed, while a FO-free diet had 17% camelina oil and 4% canola oil (Table 3.1). Ingredients were first mixed by a feed homogenizer for 20 minutes (Model 2010 Marion mixer made, Rapids Machinery Company, USA). Homogenized

ingredients were then prepared by blending steam and water at 96°C and 30% moisture before extrusion (type OEE 8 extruder, Amandus Kahl GmbH & Co. KG, Germany). The density of extruded pellets was between 430 and 500 kg/m^3. Pellets were then dried at 65°C for 4 - 5 hours in an industrial oven (JPW Design & Manufacturing, USA) until the moisture content was below 10%. The oils were heated to ~70°C before oil-coating. Dried pellets were oil-coated under the pressure of -0.9 bar at ~70°C with oil in a laboratory vacuum coater (Dinnissen Process Technology, Netherlands). To ensure optimal absorption of oils, pellets were soaked, stirred and held in the vacuum coater for 5 minutes at -0.9 bar, then the pressure was gradually increased to atmospheric pressure. Finished feed pellets were stored at -20°C to reduce the oxidization of fats. Diets were only exposed to room temperatures during periods of feeding.

The fatty acid compositions differed significantly between the FO control diet and FO-free diet. Total saturated fatty acids (SFA) were significantly higher in the control diet than the FO-free diet, which were 27% vs.14%, respectively (Table 3.2). Total monounsaturated fatty acids (MUFA) were 49% in the control diet and 39% in the FO-free diet. Total polyunsaturated fatty acids (PUFA) were significantly lower in the control diet than the FO-free diet, 23% vs. 46%, respectively (Table 3.2). Both total n-3 PUFA and total n-6 PUFA were high in the FO-free diet. The n-3/n-6 ratio in the FO-free diet was close to a suggested optimal n-3/n-6 ratio of 1:1 (Colombo et al., 2018), while the n-3/n-6 ratio was 0.7:1 in the control diet. The percentage levels of ARA (arachidonic acid, 20:4n-6), EPA, and DHA were 0.2%, 2.5%, and 1.6% in the control diet, while only 0.1%, 0.4%, and 0.4% in the FO-free diet, respectively (Table 3.2). The DHA/EPA ratio was 0.7:1 in the control diet and 1.1:1 in the FO-free diet. The EPA/ARA ratio in the control diet was 11.1:1, significantly higher than 3.2:1 in the FO-free diet (Table 3.2).

3.3.2 Experimental fish and husbandry

A commercial hatchery in Atlantic Canada selected fifty families of Atlantic salmon (family code #1 to #50) of the SJR strain from the 2018-year class of the breeding population whose parents were from the 2014-year class. The offspring were produced in a paternal half-sibling design where 50 male and 100 female broodstock with the best growth performance in the sea cage were cross bred (Liu et al., 2017; Horn et al., 2018).

Thirty parr from each of fifty families (n =1500) were individually identified by PIT (passive integrated transponder) tags and evenly distributed into two diet groups, where there were twelve tanks (120 L/tank) in each diet group (24 tanks total, with 62 or 63 fish per tank;

Table 3.3). Individuals were randomly distributed in tanks. At least one individual from each family was represented in each tank (50 + 12 or 13 other random individuals). The average initial fish body weight and fork length (mean ± SD) were 48.6 ± 7.3 g and 15.4 ± 0.8 cm in the FO control group; 47.3 ± 7.1 g and 15.1 ± 0.8 cm in the FO-free group.

Atlantic salmon parr were acclimated for a month in the experimental tanks prior to the trial. The experiment ran for 16 weeks from November 2019 to March 2020 in a commercial hatchery. The diets were distributed at 1% ration of biomass daily, using an automatic feeder. Fish were exposed to the natural daylength and provided with 11.0 mg/L dissolved oxygen and 11.5°C freshwater in the flow through system. Daily husbandry and system maintenance were administered by an experienced technician.

3.3.3 Sampling

Food was withheld a day before the sampling. All fish were anaesthetized (TMS, 0.1 g/L) for identification and body size measurement a day at week 0 and 16. In each diet group, seven out of twelve tanks were randomly selected for the fish muscle and liver collection (Table 3.3). A portion of skinless white dorsal muscle between the dorsal fin and the lateral line and a portion of liver were collected in individual cryogenic vials. Tissue samples were immediately flash frozen in liquid nitrogen and then stored in a -80°C freezer.

3.3.4 Growth Performance Analysis

After 16 weeks, fish weight gain (WG), specific growth rate (SGR), condition factor (CF), visceral somatic index (VSI), and feed conversion ratio (FCR) were calculated as follows:

Weight gain(WG)=Final body weight (g) - Initial body weight (g)

$$\text{SGR (\% per day)} = \frac{[\ln(\text{Final mean body weight, g}) - \ln(\text{Initial mean body weight, g})]}{\text{Number of days}} \times 100$$

$$\text{VSI(\%)} = \frac{\text{Viscera weight (g)}}{\text{Body weight (g)}} \times 100$$

$$\text{FCR} = \frac{\text{Feed offered (g)}}{\text{Weight gain (g)}}$$

$$\text{CF} = \frac{\text{Body weight (g)}}{\text{Length (cm)}^3} \times 100$$

Families with best and lowest weight gain after 16 weeks were selected for their tissue fatty acid analyses (Fig 3.2). Salmon from the same year had significant differences ($p < 0.001$) in initial body weight among the fifty families was due to the family effect (Table 3.5). Some salmon families with higher initial body weight kept the size advantage until the end (Fig 3.1). The specific growth rate is commonly used in aquaculture to estimate the percent increase in size per day (Lugert et al., 2016). In general, the SGR decreases as fish body size increases. The disadvantages of using SGR as the main criteria for ranking fish growth was that the fish long-term growth and the growth at different life stages are sometimes overestimated in practice (Lugert et al., 2016). Through observations, families having high mean weight gain also had high-ranking of final mean body weight. The SGR of the top five families regarding weight gain were same in either FO-free diet group or control diet group (Fig 3.3). Collectively, the weight gain among families highly reflected Atlantic salmon growth in this study.

3.3.5 Fatty Acid Extraction and Analysis

Five out of fifty families in each diet group which had the highest mean weight gain were selected for fatty acid analysis. Similarly, the five families with lowest body weight gain were also selected for fatty acid analysis. The lipid extraction and methylation were performed at the Nutrition lab, Haley Institute, DAL-AC. Muscle and liver samples were weighed and freeze-dried at -50ºC for 24 hours. Each dried sample was re-weighed to determine the water content, then was ground into a fine powder using small amounts of liquid nitrogen. The powder was stored at -80ºC. The procedure of lipid extraction followed the modified Folch method (Folch et al., 1957). Detailed procedures were introduced in Chapter 2.3.4.

3.3.6 Statistical Analysis

The statistical analyses were performed using Minitab 18.0 (Statistical Software, State College, PA, USA). Data were reported as mean values ± standard deviation. For fish growth performance analyses, family and diet were independent variables. Measures of fish growth were response variables - individual fish body weight, fork length, condition factor, specific growth rate, and feed conversion ratio. A nested analysis of variance (ANOVA) was performed using a mixed effect analysis, followed by Tukey's multiple comparisons test at a significant level of 0.05, to identify whether there were tank effects on response variables. Fish were nested within the tank factor, and then the tank factor was nested within the diet factor. A two-way

analysis of covariance (ANCOVA) was conducted using a general linear model, which included family and diet as the main effects, and initial fish weight as a covariate.

The analyses of tissue fatty acid composition were conducted by the two-way ANOVA, followed by the Bonferroni *post hoc* test at a significant level of 0.05, so that the effect of family and diet on fatty acid classes could be assessed. In all cases, the normal distribution and constant variances of sampled data were verified before accepting the statistical analyses. Individual outliers in the data set were excluded by the Grubbs' test with a significance level of 0.05.

3.4 RESULTS

3.4.1 Growth Performance

The two-way ANCOVA analyses indicated that the effect of initial fish body weight, and the interaction effect of family and diet, significantly influenced ($p \leq 0.004$) fish final mean body weight, fork length, weight gain, SGR, and FCR (Table 3.4). The mean initial fish body weight differed significantly ($p < 0.001$) among the fifty families due to the family effect, ranging from 37.1 g to 55.1 g in the FO-free diet group; 36.7 g to 58.7 g in the control diet group prior to the experiment (Fig 3.1). After 16 weeks, the mean final body weight increased over three-fold, to 170.1 ± 30.0 g in the FO control and 157.7 ± 25.9 g in the FO-free diet (Table 3.4). Salmon fed the control diet had a mean weight gain of 121.5 ± 25.6 g, while salmon fed the FO-free diet gained 110.5 ± 22.4 g in average (Table 3.4). Irrespective of family effect, Atlantic salmon fed the FO control diet had significantly higher ($p < 0.001$) mean final body weight, fork length, weight gain, and SGR than salmon fed the FO-free diet (Table 3.5). There were confounding tank effects ($p < 0.05$) in the final SGR, FCR, and VSI (Table 3.5).

Fish growth performance was primarily evaluated according to the mean weight gain after 16 weeks. Families with the leading mean weight gain highly reflected higher fish final body weight. Among the fish fed the FO-free diet, families including #38, 42, 06, 14, and 19, exhibited significantly higher weight gain than twenty nine families with the lowest weight gain (Fig 3.2). Family # 38, 42, 06, 14, and 19, were also in the top tier of the mean final body weight compared with other families; moreover, they were statistically similar regarding to the final body weight and SGR (Fig 3.1; 3.3). Similarly, among the fish fed the FO control diet, families including #34, 38, 11, 14, and 42, showed significantly higher mean weight gain and ranked in the top tier of the final body weight and the SGR (Fig 3.1; 3.3). In terms of lowest growth performance,

families #35, 45, 23, 27, and 32, had the lowest mean weight gain. The five families also exhibited lowest mean final body weight and inferior SGR. There was no significant difference on mean final body weight and SGR among families #35, 45, 23, 27, and 32 (Fig 3.1; 3.2; 3.3).

3.4.2 Muscle Fatty Acids Profiles

The major muscle fatty acid classes were 18:1n-9, 16:0, and 18:2n-6 (LNA), accounting for 26 - 30%, 11 - 15%, and 8 - 14% of total fatty acids, respectively, irrespective of diet. Families fed the FO-free diet contained significantly higher ($p < 0.001$) levels of muscle PUFA, total n-3 and total n-6 fatty acids than families fed the control diet, which were 39% vs. 22% in total PUFA, 20% vs 10% in total n-3 fatty acids, and 17% vs. 11% in total n-6 fatty acids, respectively. However, muscle MUFA level (mono-unsaturated fatty acids) was 40% in the FO-free group, significantly lower ($p < 0.001$) than 50% total MUFA in the control group (Table 3.6). Among the top 5 families fed FO or FO-free diet, muscle DHA, EPA, the DHA/EPA ratio and the EPA/ARA ratio did not differ significantly (Table 3.6). The muscle n-3/n-6 ratio was 1.25, significantly highest ($p < 0.001$) in the #14/FO-free, compared with other families in the FO-free diet group. Furthermore, the n-3 fatty acids including 18:3n-3 (ALA), 18:4n-3, and 20:4n-3 were significantly higher ($p < 0.001$) in salmon muscle in the FO-free diet group compared to the control diet group. The level of ALA was 9.2%, contributing nearly a half to the total n-3%, followed by 18:4n-3, DHA, EPA, 20:4n-3, and 20:3n-3. In comparison, the n-6 fatty acids, such as 18:2n-6 (LNA),18:3n-6, 20:2n-6 and 20:3n-6 were significantly higher in the muscle in the FO-free diet group than in the FO control diet group. In addition, muscle 20:4n-6 level (ARA, arachidonic acid) did not significantly differ among all families with the leading weight gain, irrespective of the diet (Table 3.6).

About 60% of the reported fatty acids in the salmon muscle were significantly affected by the diet effect, such as total SFA, MUFA, PUFA, n-3, n-6 and the n-3/n-6 ratio according to the two-way ANOVA analyses (Table 3.6). The family effect contributed to the significant increase of 20:2n-6 in salmon fed the FO-free diet, which increased from initially 0.4% to 1.2% (Table 3.6). There was no significant interaction effect on any fatty acid class higher than 1%.

Among the poorest weight gain families fed FO or FO-free diet, muscle 18:1n-9, 16:0, and 18:2n-6 (LNA) represented 30%, 13%, and 10 – 13% of total fatty acids, respectively (Table 3.8). The levels of total PUFA, n-3, and n-6 in the muscle increased significantly ($p < 0.001$) in the FO-free diet group, compared to the control diet group. Muscle DHA, EPA, the DHA/ EPA

ratio, and EPA/ARA ratio, in comparison, did not markedly differ among the lowest weight gain families in either diet group. About 60% of reported fatty acids were significantly influenced by the diet effect. Family effect contributed to the muscle ARA, 20:4n-3, 22:0, n-3/n-6 ratio. The interaction effect of diet and family on muscle fatty acids was not significant (not reported; Table 3.8).

3.4.3 Liver Fatty Acids Profiles

The highest fatty acid class in the liver was 18:1n-9, similar to muscle, which was 30% of total fatty acids; it was followed by 16:0, 18:2n-6, 20:1n-9 and DHA irrespective of diet (Table 3.7). The liver DHA content accounted for nearly one-half of the total n-3 fatty acids, followed by EPA, ALA, 18:4n-3, 20:4n-3, and 20:3n-3. The one-way ANOVA analyses indicated that among the best weight gain families fed FO or FO-free diet, liver contained a significantly higher (p = 0.025) DHA at 16 % in the #14/FO than the 5% liver DHA in both the #38/FO and the #38/FO-free. The liver EPA was at 5% in the #14/FO-free, significantly higher than 1% liver EPA in the # 38/FO (Table 3.7). There was no significant difference in the liver SFA level among the families offered either diet. The levels of MUFA, PUFA, and n-3 in the liver significantly differed between the #38/FO and #14/FO-free. Notably, #14/FO-free had a significantly lower (p = 0.16) total MUFA at 32%, and a significantly higher PUFA at 47% in the liver. In comparison, #38/FO had a significantly higher total MUFA at 60% and the lower PUFA at 21% in the liver. Total n-3 fatty acids in the liver were the markedly highest (p = 0.001) in the #14/FO-free, at 29%. The lowest liver n-3 fatty acids were in the #38/FO, at 9% (Table 3.7). Total liver n-6 fatty acids were 16 - 17% in the FO-free diet group, while they were significantly higher (p < 0.001) than 10 - 12% of n-6 in the FO control diet group. In addition, the n-3/n-6 ratio in the liver did not statistically differ between two diet groups; however, there was a significant family effect (p = 0.02) on the n-3/n-6 ratio (Table 3.7).

The two-way ANOVA demonstrated that one-half of the reported fatty acids were significantly affected by diet effect, while only a few fatty acids were highly associated with family effect, such as EPA, 22:5n-3, DHA, total PUFA, total n-3, and n-3/n-6 ratio (Table 3.7). The significant interaction effects were only found in 16:1n-9, 16:1n-11, and 21:5n-3 (less than 1%; not reported).

In terms of the families with lowest weight gain in each diet group, no significant increase of liver DHA and EPA were observed. Liver 18:1n-9 still represented the highest percentage, at

20 - 30% in total liver fatty acids. Next, liver 16:0 was the second highest, at 10 - 16%. Then it was followed by 18:2n-6, DHA, and 20:1n-9 (Table 3.9). The one-way ANOVA indicated that the levels of liver total SFA, MUFA, PUFA, n-3, and the n-3/n-6 ratio did not differ among the lowest weight gain families, independent of diet.

3.5 DISCUSSION

3.5.1 Growth Performance

Significant differences in fish average final body weight, fork length, weight gain, SGR, and FCR were attributable to the family x diet interaction effect in this study. Similarly, family x diet interaction effect also significantly influenced mean final body weight in Atlantic salmon; however, family effect alone made significantly differences on the initial weight, SGR, and FCR (Bell et al., 2010).In this study, salmon families were half-sibling and from the SJR strain, and the trial duration was 16 weeks. By comparison, Bell et al., (2010) used two strains of post-smolt Atlantic salmon and came up with three groups of salmon based on "fat or lean" and they conducted a 55-week trial, which was longer than this study. Diet effect (FO and FO-free diet) was not prominent on final growth performance in Bell et al., (2010).

Some SJR strain families grew equally as well on the FO-free diet as the control diet regarding fish final mean body weight, weight gain, and SGR. Previous studies evaluated the complete substitution of FO with a single canola oil or camelina oil for salmonids. When 16% FO in the diet was fully replaced by camelina oil, Atlantic salmon parr fed the camelina oil diet for 16 weeks had similar feed consumption, final mean body weight, weight gain, and FCR with the FO control diet (Ye et al., 2016). Rainbow trout (initially ~44 g) offered either the 14% FO control diet or the FO-free camelina oil diet for 12 weeks did not differ significantly in the final mean body weight, weight gain, and SGR (Hixson et al., 2014d). Full substitution of 19% FO in the diet with rapeseed oil did not significantly change the final mean body weight, SGR, and FCR of Atlantic salmon (initially ~54 g) (Bell et al., 2003). When Chinook salmon parr (*Oncorhynchus tshawytscha*) were fed the 14% FO control or the FO-free canola oil diet for 30 weeks, the final body weight, weight gain, and SGR were similar (Huang et al., 2008). Compared with the above studies, the current study used blended vegetable oils that canola oil and camelina oil was in a ratio of 1:4.3 in the FO-free diet. Despite this, the growth comparisons of some

families confirmed the usefulness of canola and camelina oil as alternative lipid sources for producing Atlantic salmon parr.

When family factor was not taken into consideration, Atlantic salmon fed the FO-free diet had significant reductions in the salmon final average body weight, weight gain, and SGR, compared with them being fed the FO control diet. Similarly, feeding post-smolt Atlantic salmon with the FO-free camelina oil diet for 16 weeks led to a markedly reduction in the fish final body weight, compared with 14% FO control diet (Hixson et al., 2014a). The lower final body weight in the camelina oil diet group was most likely due to the significantly less feed consumption and the lack of marine lipid in the diet (Hixson et al., 2014a). In contrast, Atlantic salmon parr from the Salmon Enterprises of Tasmania (SALTAS), Austrasia, fed a FO-free canola oil diet grew as well as the 13% FO control diet in the final body weight, weight gain, SGR, feed consumption, and FCR during the 6 weeks' trial (Miller et al., 2007). The rearing condition for the SALTAS salmon was at a 15℃ freshwater and a 16 hours daylight photoperiod. A warmer water temperature and a longer daylight would stimulate the salmon metabolic rate and promote feed intake of canola oil diet. In this study, SJR salmon grew at a 11.5 ℃ freshwater under a natural photoperiod for 16 weeks. Since the experiment applied automatic feeders to distribute feeds and the uneaten feed was not well estimated. It could result in a similar FCR between the two diet groups. Therefore, the inferior fish growth performance in the FO-free group could not be explained by FCR (Table 3.5).

The lower level of dietary EPA+DHA in the FO-free diet could suppress the early growth of Atlantic salmon. In freshwater, the minimal 0.5% (or 5 g/kg) of dietary EPA and DHA was required for the growth of Atlantic salmon parr (NRC, 2011; Qian et al., 2020); more specifically, the EPA+DHA (1:1) level was 3.3% of total fatty acids and the crude fat accounted for 22.6 % in the diet (Qian et al., 2020). In present study, the FO-free diet contained only 0.8% EPA+DHA (1:1), significantly lower than the minimum requirement of EPA+DHA and lower than 4.1% EPA+DHA in the FO control diet. Even though 18:3n-3 was largely provided to compensate the deficiency of EPA+DHA in the FO-free diet, salmon offered the FO-free diet still exhibited growth suppression, compared to the FO control diet (Table 3.5). It agrees with Ruyter et al., (2000) that direct inclusion of EPA and DHA in the diet satisfy the requirement of essential fatty acids at lower levels in comparison to dietary 18:3n-3 alone. Specially, some

families fed the FO-free diet performed a comparable growth with the control diet, such as family # 38, 42 and 14 (Fig 3.1).

3.5.2 Muscle Fatty Acid Content

Muscle fatty acid levels were analyzed according to the best and the lowest weight gain families fed FO control or FO-free diet. Among the best weight gain families, muscle DHA and EPA percentages were 3 - 4%, and 1 - 1.4%, respectively, regardless of diet. This demonstrated a similar level of n-3 LC-PUFA accumulation in the salmon muscle. The FO-free diet provided nearly zero of n-3 LC-PUFA intermediate products, 18:4n-3, EPA, and 22:5n-3. Significantly increases of muscle 18:4n-3 and 20:4n-3 content in the FO-free diet group indicated that the uptake of 18:3n-3 promoted the metabolic activities of n-3 LC-PUFA pathway. Despite this, the accumulation of muscle DHA and EPA level was limited and maintained at a similar level to the FO control diet group (Table 3.6). Specially, family #14 offered the FO-free diet exhibited an insignificantly higher DHA content of 4.5% and higher EPA content of 1.3 % in the muscle, compared with other top weight gain families fed the FO- free diet (Fig 3.4). Of the families with lowest weight gain, salmon in #32/FO-free had the highest muscle DHA of 5.0% and EPA of 1.5% in the FO-free diet group (Fig 3.5). Enhanced biosynthetic activities of intermediate n-3 and n-6 LC-PUFA were also observed regardless of diet. The muscle DHA and EPA percentage increased to a level at 3 - 5 % and 1 - 1.5%, respectively, in the FO-free diet group. Thus, the increase of muscle n-3 LC-PUFA was widely evident when dietary n-3 LC-PUFA was lower than basic requirement, regardless the large variations of weight gain among families.

Among the best weight gain families, salmon in the #14/FO-free exhibited a significantly higher n-3/n-6 ratio, implying a strong affiliation to n-3 fatty acids compared to other families in the FO-free diet group. Farmed Atlantic salmon had an affinity toward n-6 fatty acid, such as 18:2n-6 and 20:4n-6 (Betancor et al., 2016; Colombo et al., 2021). The incorporation of LNA in the muscle also stimulates the metabolic activities in the n-6 LC-PUFA pathway, specifically biosynthesis of18:3n-6 and 20:3n-6 when fed the FO-free diet; besides, LNA competes with the utilization of ALA in the n-3 LC-PUFA biosynthesis. To improve the use of 18:3n-3, the abundant dietary ALA was supplied to sustain the n-3/n-6 ratio at ~1:1 in the FO-free diet.

Except for the effect of LNA, the limited biosynthesis of n-3 LC-PUFA in the muscle could be that salmon muscle mainly plays the role of lipid storage and has a limited ability for producing n-3 LC-PUFA (Tocher, 2003). The deposition of DHA is generally more conserved

than EPA, since the catabolism of DHA requires more steps peroxisome oxidation compared with other PUFA. It is supported by the current results that the proportion of DHA was three-times higher than EPA, irrespective of diets and the of weight gain (Table 3.6; 3.8). Compared with DHA, dietary EPA actively participates in the catabolism (Murray et al., 2014), and eicosanoid synthesis (Serhan and Petasis, 2011; Colombo, 2022). As a result, the deposition of dietary EPA in the tissue is less efficient than DHA. A data synthesis study included Atlantic salmon, rainbow trout, and steelhead trout (*Oncorhynchus mykiss*) estimates that farmed salmonids can store an extra 23% of DHA in the muscle apart from the diet; in contrast, only a half of EPA in a diet is deposited in the muscle (Colombo et al., 2018).

The major fatty acids in the salmon muscle were 18:1n-9, 16:0, 18:2n-6, 20:1n-9, and 16:0 in both diet groups, which accounted for at least 60% of the total muscle fatty acids and highly reflected the diet fatty acid compositions (Bell et al., 2010; Hixson et al., 2014a; Betancor et al., 2015). Similarly, the predominant content of 18:1n-9, 18:2n-6, and 16:0 in the Atlantic salmon muscle were also reported by Horn et al., (2018) who fed fish with a commercial high FO feed.

Over 60% of the reported fatty acids were significantly influenced by diet. The family group did not have a profound effect for most muscle fatty acids, except for 20:2n-6 (Table 3.6) and ARA, 20:4n-3, 22:0 and n-3/n-6 ratio (Table 3.8). This is reasonable since the SJR strain Atlantic salmon have yet to be differentiated for the preference of certain fatty acid profile. Families with the affinity toward n-3 fatty acids might be an indicator for selectively breeding.

3.5.3 Liver Fatty Acid Content

Among the families regarding the top weight gain, salmon in #14/FO-free had relatively higher liver DHA and EPA content compared with other families in the FO-free group (Fig 3.4). There were significant increases of the n-3 intermedia products,18:4n-3 and 20:4n-3, indicating elevated activities toward the n-3 LC-PUFA biosynthesis, irrespective of diet. Among the families regarding the lowest weight gain, salmon fed either diet had a similar liver DHA and EPA percentage (Fig 3.5). Thus, the current results demonstrated that family regarding the poor weight gain did not indicate a lower n-3 LC-PUFA percentages in the liver.

The n-3 LC-PUFA level in the liver was higher than its level in the muscle, especially DHA. Liver is the main organ for lipogenesis and has higher metabolic activities in the biosynthesis of n-3 LC-PUFA. The upregulation of DHA and EPA in Atlantic salmon liver has been demonstrated in the percent level, when fish were fed a FO-free diet (Monroig et al., 2010;

Betancor et al., 2015, 2016). Family or genetic effects significantly differentiated the levels of liver DHA and EPA among the families of leading weight gain. In terms of hepatic desaturase and elongase gene expression, transcript expression involved in the LC-PUFA enzymatic reactions indicates a notable variability in post-smolt farmed Atlantic salmon when offered a FO-free camelina oil diet (Xue et al., 2015). Nevertheless, the upregulated transcript expression of LC-PUFA related enzymes in the liver and increased liver n-3 LC-PUFA level do not necessarily correspond to the increase of EPA and DHA storage in the salmon muscle (Xue et al., 2015; Hixson et al., 2017; Colombo et al., 2018). The liver LC-PUFA conversion rate to muscle is unknown and the mechanism requires more investigations.

The metabolic actives to n-6 LC-PUFA in the liver was also evident according to the increased level of 20:3n-6 and 20:4n-6. When the n-6 PUFA increased in the fish tissue, the stimulation of transcript expression was observed to create n-3 LC-PUFA in SJR strain Atlantic salmon (Colombo et al., 2021). The competition effect between n-3 and n-6 LC-PUFA pathway reflects a regulatory mechanism of LC-PUFA biosynthesis (Tocher, 2010), in response to dietary ratio of ALA to LNA in the diet (Thanuthong et al. 2011). In this sense, the n-3/n-6 ratio did not change among families in the FO-free group. Specially, in the # 14/FO-free, a relatively high n-3/n-6 ratio of 1.7:1 and significantly highest liver EPA, 22:5n-3, and DHA showed a better affinity to n-3 fatty acids, compared with other families in the FO-free diet group (Table 3.7).

Farmed Atlantic salmon exhibited a small improvement of n-3 LC-PUFA level to compensate for the deficiency of dietary LC-PUFA (Sprague et al., 2019; Mock et al., 2019). It was supported by the current study, especially in the muscle. A possible reason is that there is no long-term environmental pressure to stimulate the endogenous biosynthesis of n-3 LC-PUFA and result in excess deposition of DHA and EPA for fish growth (Leaver et al., 2008; Castro et al., 2012). On the other hand, fillet LC-PUFA content is a heritable trait in farmed Atlantic salmon (Leaver et al., 2011; Horn et al., 2018). There was a significant 2 – 3 % difference in fillet n-3 LC-PUFA % between Atlantic salmon families having the same level of fillet lipids (Leaver et al., 2011). There may be potential to increase the n-3 LC-PUFA level through selective breeding; however further investigation needs to be done.

3.6 CONCLUSIONS

The biosynthesis of n-3 LC-PUFA, particularly DHA, can be upregulated in the muscle and liver when Atlantic salmon were fed a diet devoid of n-3 LC-PUFA. This enhancement significantly differed by different salmon families. In general, liver DHA% storage in the liver was apparently higher than in the muscle. Compared with liver, muscle fatty acids more closely reflected the dietary fatty acids. Family #14 fed the FO-free diet was among the top 5 fast growing families and also had the highest DHA percentage of total fatty acid in the liver. The increased n-3/n-6 ratio indicates the affinity to n-3 fatty acids. Therefore, there is a potential of upregulating n-3 LC-PUFA content in salmon tissue by selective breeding of families with high tissue n-3 LC-PUFA% and n-3/n-6 ratio. Future study can consider the trait of high tissue n-3 LC-PUFA level in the commercial breeding program. Since this is the first time fatty acid composition has been evaluated on the SJR salmon population from this facility, this will serve as a benchmark for evaluating future generations.

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3.7 TABLES AND FIGURES

Table 3.1. Diet formulation and proximate composition of the FO control diet and the FO-free diet [1].

Ingredients (g/kg)	FO	FO-free
Fish meal	150	150
Fish (herring) oil	100	0
Ground wheat	173	173
Empyreal	150	150
Camelina oil	0	170
Canola oil	110	40
Poultry byproduct meal	150	150
Blood meal	110	110
Vitamin/Mineral mix	2	2
Northeast special pre-mix	20	20
Dicalcium phosphate	22	22
L-Lysine	0.5	0.5
L-Methionine	1	1
Choline chloride	10.5	10.5
L-Tryptophan	1	1
Total	1000	1000
Proximate analysis (as fed, g/kg)		
Dry Matter	964.1	962.6
Crude Protein	466.5	487.0
Crude Fat	235.6	215.0
Calcium	18.3	17.2
Potassium	4.9	5.1
Magnesium	1.2	1.2
Phosphorus	14.5	14.2
Sodium	3.4	3.5
Copper	0.01	0.01
Manganese	0.04	0.05
Zinc	0.15	0.15
Ash	73.8	75.3

[1] Ingredients were supplied by Northeast Nutrition (Truro, NS, Canada). Camelina oil was from Smart Earth Seeds (Saskatoon, SK, Canada). Canola oil was from Canolina™, Chabanel West Montreal (QC, Canada).

Table 3.2. Fatty acid profiles (% of total fatty acids) of the FO control and FO-free diet[1].

Fatty acids (%)	FO	FO-free	F-value	*p*-value
14:0	2.6 ± 0.1	0.4 ± 0.03	655.0	<0.001
14:1	0.1 ± 0.008	0.04 ± 0.002	394.3	<0.001
16:0	11.1 ± 0.7	8.8 ± 0.4	28.9	0.008
16:1n-7	4.0 ± 0.03	0.9 ± 0.04	7149.6	<0.001
18:0	2.5 ± 0.2	3.0 ± 0.1	28.3	0.006
18:1n-9	34.5 ± 0.4	26.0 ± 0.2	1207.1	<0.001
18:1n-7	2.4 ± 0.02	1.4 ± 0.01	5574.6	<0.001
18:2n-6 (LNA)	12.4 ± 0.2	19.8 ± 0.1	3855.0	<0.001
18:3n-3 (ALA)	3.6 ± 0.1	22.3 ± 0.5	4703.9	<0.001
18:4n-3	0.5 ± 0.02	0.1 ± 0.04	115.8	<0.001
20:0	0.4 ± 0.03	1.1 ± 0.003	1907	<0.001
20:1n-11	0.5 ± 0.01	0.06 ± 0.07	35.6	0.004
20:1n-9	6.1 ± 0.1	10.1 ± 0.1	1190.1	<0.001
20:1n-7	0.4 ± 0.01	0.3 ± 0.02	86.9	0.001
20:2n-6	0.09 ± 0.01	1.2 ± 0.06	1035.7	<0.001
20:4n-6 (ARA)	0.2 ± 0.001	0.1 ± 0.01	106.0	0.001
20:3n-3	0.03 ± 0.01	0.8 ± 0.02	2363.8	<0.001
20:5n-3 (EPA)	2.5 ± 0.10	0.4 ± 0.02	1218.2	<0.001
22:0	10.1 ± 0.2	0.7 ± 0.04	4330.7	<0.001
22:1n-7	0.5 ± 0.2	0.1 ± 0.04	12.8	0.023
21:5n-3	0.2 ± 0.03	0.04 ± 0.01	53.4	0.002
22:5n-6	0.07 ± 0.002	0.09 ± 0.07	0.2	0.676
22:5n-3	0.5 ± 0.004	0.04 ± 0.02	332.0	<0.001
24:0	0.1 ± 0.01	0.2 ± 0.02	24.2	0.008
22:6n-3 (DHA)	1.6 ± 0.06	0.4 ± 0.01	1193.3	<0.001
ΣSFA [2]	27.3 ± 0.8	14.4 ± 0.6	555.4	<0.001
ΣMUFA [3]	49.4 ± 0.4	39.3 ± 0.4	871.7	<0.001
ΣPUFA [4]	23.1 ± 0.5	46.3 ± 0.6	2748.8	<0.001
Σn-3	9.1 ± 0.3	24.3 ± 0.4	2950.3	<0.001
Σn-6	13.0 ± 0.1	21.2 ± 0.1	5301.8	<0.001
n-3/n-6	0.7 ± 0.01	1.1 ± 0.03	651.4	<0.001
DHA/EPA	0.7 ± 0.005	1.1 ± 0.03	1278.1	<0.001
EPA/ARA	11.0 ± 0.9	3.2 ± 0.4	178.9	<0.001

[1]Data expressed as mean ± SD ($n_{\text{FO control}} = 3$; $n_{\text{FO-free}} = 3$). This analysis was performed by the one-way ANOVA with Bonferroni *post hoc* correction test (α = 0.05). LNA, linoleic acid; ALA, α-linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Fatty acids level less than 0.1% in both diets were not reported in the table.
[2] contains 15:0 and 17:0.
[3] contains 16:1n-11, 16:1n-5, 17:1, 18:1n-11, 18:1n-5, and 22:1n-11.
[4] contains 16:3n-4, 18:2n-7, 18:2n-4, 18:3n-6, 18:3n-4, 20:3n-6, and 20:4n-3.

Table 3.3. The initial and final average body size of SJR Atlantic salmon fed either the FO control diet or FO-free diet[1].

FO	Tk 1	Tk 2	Tk 3	Tk 4	Tk 5	Tk 6	Tk 7	Tk 8	Tk 9	Tk 10	Tk 11	Tk 12
Initial BW, g	47.4 ± 6.8	47.6 ± 7.7	48.6 ± 7.1	47.4 ± 6.7	47.9 ± 6.9	47.5 ± 7.1	48.9 ± 7.7	51.8 ± 8.0	50.0 ± 7.8	49.0 ± 6.9	49.2 ± 7.6	48.0 ± 7.3
Final BW, g	164.1 ±26.2	167.7 ± 27.1	178.4 ± 32.0	160.6 ± 30.0	169.0 ± 26.1	170.2 ± 29.1	177.0 ± 34.2	181.5 ± 34.6	172.2 ± 32.1	165.8 ± 25.4	175.3 ± 28.2	159.0 ± 26.9
Initial FL, cm	15.2 ± 0.9	15.3 ± 0.9	15.7 ± 0.8	15.5 ± 0.8	15.5 ± 0.9	15.5 ± 0.8	15.6 ± 0.8	15.3 ± 0.8	15.4 ± 0.8	15.3 ± 0.6	15.2 ± 0.8	15.2 ± 0.6
Final FL, cm	23.7 ± 1.4	23.8 ± 1.2	24.3 ± 1.4	23.6 ± 1.6	24.0 ± 1.5	24.3 ± 1.6	24.4 ± 1.5	24.7 ± 1.5	24.1 ± 1.6	23.9 ± 1.3	24.3 ± 1.3	23.7 ± 1.6

FO-free	Tk 13	Tk 14	Tk 15	Tk 16	Tk 17	Tk 18	Tk 19	Tk 20	Tk 21	Tk 22	Tk 23	Tk 24
Initial BW, g	47.5 ± 6.8	43.1 ± 6.7	46.6 ± 6.8	48.1 ± 6.3	46.8 ± 7.2	49.5 ± 8.0	48.5 ± 7.4	46.8 ± 6.1	47.8 ± 7.7	47.1 ± 7.0	47.2 ± 6.9	48.2 ± 6.2
Final BW, g	162.7 ± 25.9	151.9 ± 22.8	158.5 ± 24.2	157.9 ± 23.1	164.0 ± 23.1	155.7 ± 30.3	165.8 ± 29.8	154.2 ± 23.0	151.5 ± 24.1	146.3 ± 20.1	161.7 ± 27.8	157.0 ± 28.4
Initial FL, cm	14.9 ± 0.8	14.8 ± 0.8	15.2 ± 0.9	15.3 ± 0.7	14.9 ± 0.8	15.1 ± 0.8	15.2 ± 0.8	15.0 ± 0.7	15.2 ± 0.9	15.0 ± 0.6	15.0 ± 0.7	15.1 ± 0.7
Final FL, cm	23.9 ± 1.7	23.0 ± 1.4	23.7 ± 1.4	23.6 ± 1.2	23.8 ± 1.3	23.6 ± 1.6	23.7 ± 1.5	23.3 ± 1.4	23.3 ± 1.3	23.1 ± 1.3	23.4 ± 1.5	23.5 ± 1.4

[1] Value was presented by mean ± SD. Each tank had 62 or 63 fish that represented all fifty families. Twelve or thirteen families in random had two representatives in a tank. In each diet group, seven tanks (grey) were randomly selected for liver and muscle collections. Tk, tank; BW, body weight; FL, fork length.

Table 3.4. The growth performance of Atlantic salmon fed the FO control diet or the FO-free diet at week 0 and after week 16[1]. There were significant differences in fish mean initial body weight and fork length among families.

			Initial BW		Family		Diet		Family x Diet	
Mean	FO	FO-free	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
Initial BW, g	48.6 ± 7.3	47.3 ± 7.1	/	/	15.6	<0.001	/	/	/	/
Final BW, g	170.1 ± 30.0	157.7 ± 25.9	706.7	<0.001	7.5	<0.001	70.6	<0.001	1.7	<0.001
Initial FL, cm	15.4 ± 0.8	15.1 ± 0.8	/	/	16.3	<0.001	/	/	/	/
Final FL, cm	24.1 ± 1.5	23.5 ± 1.4	642.8	<0.001	7.2	<0.001	17.2	<0.001	1.6	0.004
Weight gain, g	121.5 ± 25.6	110.5 ± 22.4	196.9	<0.001	7.6	<0.001	72.0	<0.001	1.7	<0.001
SGR % day^{-1}	1.11 ± 0.03	1.07 ± 0.04	112.2	<0.001	7.5	<0.001	68.6	<0.001	1.7	<0.001
CF	1.2 ± 0.02	1.2 ± 0.03	0.4	0.545	8.4	<0.001	0.3	0.620	0.81	0.817
FCR	1.0 ± 0.05	1.0 ± 0.06	217.1	<0.001	9.4	<0.001	0.6	0.443	1.61	0.005

[1] Values (mean ± SD) were based on the total number of fish ($n_{control}$ = 713 and $n_{FO\text{-}free}$ = 689). The two-way ANCOVA analyses were performed using the general linear model where family and diet were main effects, and initial fish BW was a covariate. BW, body weight; FL, fork length; SGR, specific growth rate; VSI, viscera somatic index; FCR, feed conversion ratio (dry matter).

Table 3.5. Tank and diet effects on the Atlantic salmon growth performance at week 0 and after week 16. The analyses did not include the family effect [1].

			Variance component		
			Tank		Diet
Mean	FO	FO free	% of total variance	*p*-value	*p*-value
Initial BW, g	48.5 ± 7.3	47.2 ± 7.1	1.6%	0.140	0.095
Final BW, g	170.6 ± 29.2	156.6 ± 25.4	2.0%	0.109	<0.001
Initial FL, cm	15.4 ± 0.9	15.1 ± 0.8	3.9%	0.051	0.017
Final FL, cm	24.1 ± 1.4	23.4 ± 1.4	1.6%	0.140	<0.001
Weight gain, g	123.1 ± 24.8	109.4 ± 21.7	3.2%	0.066	<0.001
SGR, % day^{-1}	1.11 ± 0.11	1.07 ± 0.13	5.6%	0.034	0.009
CF	1.2 ± 0.02	1.2 ± 0.03	2.0%	0.109	0.527
FCR	1.0 ± 0.2	1.0 ± 0.3	4.3%	0.046	0.594
VSI, %	8.4 ± 0.9	8.5 ± 1.2	11.6%	0.017	0.658

[1] Results were based on sampled fish and displayed as mean ± SD ($n_{control}$ = 350; $n_{FO\text{-}free}$ = 350). The analyses were conducted by the nested ANOVA, followed by the Tukey's multiple comparison test at the significant level of 0.05. BW, body weight; FL, fork length; SGR, specific growth rate; VSI, viscera somatic index; FCR, feed conversion ratio (dry matter).

Table 3.6. Muscle fatty acid profiles of Atlantic salmon fed the FO control diet or the FO-free diet for 16 weeks[1]. Five families with the highest weight gain were compared between the FO control diet and FO-free diet.

Family	38	34	11	14	42	38	42	6	14	19	*p*-value		
Diet	FO	FO	FO	FO	FO	FO-free	FO-free	FO-free	FO-free	FO-free	Treatment	Diet	Fam
14:0	2.6 ± 0.1a	2.5 ± 0.3a	2.6 ± 0.1a	2.5 ± 0.4a	2.5 ± 0.4a	1.1 ± 0.1b	1.2 ± 0.2b	1.0 ± 0.2b	1.4 ± 0.3b	1.4 ± 0.1b	<0.001	<0.001	0.740
16:0	14.7 ± 0.3	13.8 ±1.6	14.2 ± 0.3	13.4 ± 2.5	13.6 ±2.3	11.8 ±0.9	12.1 ± 0.9	13.8 ± 1.6	14.9 ± 3.3	13.5 ± 3.5	0.594	0.277	0.949
16:1n-7	3.7 ± 0.1a	3.8 ± 0.3a	4.0 ± 0.2a	3.8 ± 0.1a	3.8 ± 0.3a	1.8 ±0.1b	1.9 ± 0.1b	1.9 ± 0.01b	1.7 ± 0.2b	2.0 ± 0.4b	<0.001	<0.001	0.506
18:0	3.5 ± 0.1	3.3 ± 0.4	3.3 ± 0.1	3.1 ± 0.6	3.3 ± 0.6	3.5 ± 0.2	3.8 ± 0.2	4.3 ± 0.6	4.7 ± 1.1	4.2 ± 1.3	0.082	0.016	0.993
18:1n-11	1.0 ± 0.1a	1.1 ± 0.04a	0.9 ± 0.07a	0.9 ± 0.2a	0.9 ± 0.2a	0.09 ±0.01b	0.1 ± 0.02b	0.1 ± 0.01b	0.1 ± 0.02b	0.12 ± 0.01b	<0.001	<0.001	0.600
18:1n-9	31.8 ± 0.9abc	32.5 ± 2.1ab	32.6 ± 0.9ab	33.4 ± 1.5a	33.5 ±1.7a	27.9 ± 0.7cd	28.4 ± 0.9bcd	27.2 ± 0.3cd	26.0 ± 1.5d	27.5 ± 1.8d	<0.001	<0.001	0.819
18:1n-7	2.7 ± 0.1a	2.7 ± 0.1a	2.71 ± 0.1a	2.7 ± 0.1a	2.8 ± 0.2a	1.9 ± 0.06b	2.0 ± 0.1b	1.9 ± 0.1b	1.9 ± 0.2b	2.0 ± 0.3b	<0.001	<0.001	0.834
18:2n-6 (LNA)	8.8 ± 0.4b	9.0 ± 0.6b	8.9 ± 0.3b	9.3 ± 1.1b	9.2 ± 0.8b	14.2 ± 0.7a	14.0 ± 0.5a	12.8 ±1.0a	12.7 ± 1.3a	13.2 ± 1.1a	<0.001	<0.001	0.725
18:3n-6	0.5 ± 0.01b	0.6 ± 0.07b	0.7 ± 0.03b	0.7 ± 0.09b	0.5 ± 0.04b	1.6 ± 0.1a	1.3 ± 0.2a	1.4 ± 0.07a	1.5 ± 0.1a	1.4 ± 0.1a	<0.001	<0.001	0.152
18:3n-3 (ALA)	2.0 ± 0.2b	2.1 ± 0.2b	2.0 ± 0.2b	2.1 ± 0.4b	2.1 ± 0.3b	10.4 ± 0.6a	10.0 ± 0.6a	8.8 ± 1.1a	8.7 ± 1.6a	9.2 ± 1.4a	<0.001	<0.001	0.494
18:4n-3	0.8 ± 0.08b	0.9 ± 0.1b	1.0 ± 0.07b	1.0 ±0.2b	0.8 ±0.1b	4.9 ± 0.4a	4.0 ± 0.8a	3.8 ± 0.2a	3.8 ± 0.5a	4.0 ± 0.5a	<0.001	<0.001	0.706
20:1n-9	5.8 ± 0.4d	5.8 ± 0.4d	5.7 ± 0.4d	6.0 ± 0.4bcd	5.9 ± 0.3cd	8.0 ± 0.3a	7.6 ± 0.4a	7.1 ± 0.2abc	7.0 ± 0.2ab	7.3 ± 0.3a	<0.001	<0.001	0.523
20:2n-6	0.4 ± 0.02b	0.5 ± 0.05b	0.3 ± 0.04b	0.4 ± 0.04b	0.4 ± 0.08b	1.3 ± 0.03a	1.2 ± 0.03a	1.1 ± 0.01a	1.1 ± 0.07a	1.1 ± 0.06a	<0.001	<0.001	0.002
20:3n-6	0.4 ± 0.05b	0.5 ± 0.01b	0.4 ± 0.02b	0.4 ± 0.02b	0.4 ± 0.05b	0.7 ± 0.02a	0.6 ± 0.04a	0.7 ± 0.03a	0.6 ± 0.04a	0.7 ± 0.06a	<0.001	<0.001	0.082
20:4n-6 (ARA)	0.3 ± 0.09	0.4 ± 0.05	0.3 ± 0.10	0.3 ± 0.05	0.3 ± 0.03	0.3 ± 0.04	0.3 ± 0.02	0.4 ± 0.03	0.5 ± 0.1	0.3 ± 0.06	0.262	0.122	0.227
20:3n-3	0.6 ± 0.3	0.6 ± 0.5	0.4 ± 0.3	0.3 ± 0.3	0.4 ± 0.3	0.8 ± 0.1	1.0 ± 0.4	1.2 ± 0.5	0.8 ± 0.4	0.7 ± 0.2	0.159	0.007	0.832
20:4n-3	0.4 ± 0.06b	0.4 ± 0.08b	0.4 ± 0.06b	0.3 ± 0.02b	0.3 ± 0.02b	0.8 ± 0.03a	0.8 ± 0.09a	0.7 ± 0.01a	0.7 ± 0.05a	0.7 ± 0.03a	<0.001	<0.001	0.079
20:5n-3 (EPA)	1.1 ± 0.2	1.4 ± 0.2	1.2 ± 0.2	1.4 ± 0.3	1.2 ± 0.3	1.1 ± 0.09	1.0 ± 0.06	1.1 ± 0.2	1.3 ± 0.4	1.1 ± 0.4	0.765	0.420	0.651
22:0	7.7 ± 0.3a	7.2 ± 0.6a	7.7 ± 0.8a	8.0 ± 0.4a	7.8 ± 0.2a	0.9 ± 0.04b	1.0 ± 0.05b	1.0 ± 0.04b	0.9 ± 0.07b	1.0 ± 0.05b	<0.001	<0.001	0.364
22:1n-7	1.2 ± 0.2	0.7 ± 0.6	0.9 ± 0.1	0.7 ± 0.6	0.8 ± 0.8	0.1 ± 0.06	0.2 ± 0.1	0.5 ± 0.3	0.6 ± 0.8	0.5 ± 0.7	0.397	0.044	0.931
22:5n-3	0.5 ± 0.07	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.05	0.5 ± 0.07	0.6 ± 0.02	0.6 ± 0.1	0.6 ± 0.1	0.794	0.374	0.691
22:6n-3 (DHA)	3.8 ± 0.6	4.3 ± 0.1	3.6 ± 0.8	3.8 ± 0.6	3.8 ± 0.9	3.2 ± 0.5	3.3 ± 0.8	3.9 ± 0.7	4.5 ± 1.7	3.8 ± 1.1	0.829	0.613	0.795
ΣSFA[2]	29.3 ± 0.5a	27.7 ± 2.2abc	28.8 ± 0.4a	27.8 ± 3.2ab	28.1 ± 3.3ab	18.3 ± 1.3c	19.2 ± 1.2bc	21.6 ± 2.5abc	23.2 ± 5.2abc	21.3 ± 5.1abc	0.001	<0.001	0.912
ΣMUFA[3]	48.4 ± 1.3a	48.9 ± 2.2a	49.0 ± 1.2a	49.8 ± 1.2a	49.9 ± 1.4a	40.6 ± 0.9b	41.2 ± 1.1b	39.8 ± 0.2b	38.3 ± 1.2b	40.3 ± 1.4b	<0.001	<0.001	0.715
ΣPUFA[4]	22.0 ± 1.7b	23.1 ± 0.8b	21.9 ± 1.6b	22.1 ± 2.0b	21.7 ± 2.0b	40.8 ± 0.6a	39.4 ± 1.2a	38.3 ± 2.3a	38.2 ± 4.8a	38.1 ± 4.0a	<0.001	<0.001	0.862
Σn-3	9.6 ± 1.2b	10.6 ± 0.3b	9.5 ± 1.4b	9.7 ± 1.1b	9.5 ± 1.3b	21.9 ± 0.2a	20.8 ± 0.6a	20.5 ± 1.6a	20.7 ± 3.8a	20.4 ± 3.2a	<0.001	<0.001	0.846
Σn-6	10.6 ± 0.3b	11.0 ± 0.6b	10.7 ± 0.2b	11.2 ± 1.2b	10.9 ± 1.0b	18.1 ± 0.7a	17.5 ± 0.8a	16.5 ± 0.9a	16.4 ± 1.4a	16.8 ± 1.2a	<0.001	<0.001	0.935
n-3/n-6	0.9 ± 0.1cd	1.0 ± 0.04bcd	0.9 ± 0.1d	0.9 ± 0.05d	0.9 ± 0.05d	1.2 ± 0.05b	1.2 ± 0.05bc	1.2 ± 0.03b	1.3 ± 0.1a	1.2 ± 0.1b	<0.001	<0.001	0.086
DHA/EPA	3.5 ± 0.6	3.2 ± 0.4	2.9 ± 0.4	2.8 ± 0.3	3.3 ± 0.2	3.0 ± 0.3	3.2 ± 0.7	3.5 ± 0.06	3.4 ± 0.3	3.4 ± 0.1	0.395	0.892	0.855
EPA/ARA	3.4 ± 1.0	3.4 ± 0.9	4.2 ± 1.3	4.1 ± 1.0	4.0 ± 0.8	3.1 ± 0.1	3.0 ± 0.1	2.8 ± 0.7	2.8 ± 0.3	3.3 ± 0.5	0.213	0.027	0.801

[1] Data expressed as mean ± SD, n=3 per treatment (family/diet). Significant differences among ten treatments determined by the one-way ANOVA and Bonferroni *post hoc* correction test (α=0.05). The two-way ANOVA was used to determine diet, family and diet x family effect. The interaction effect was not reported due to no significant effect, except for 18:4n-1 and 22:5n-6.

Any fatty acid content less than 0.1% in both diets was not reported. Other fatty acids between 0.1% and 1% were listed below:

[2] contains 15:0, 17:0, 20:0, 23:0, and 24:0.

[3] contains 14:1, 16:1n-11, 16:1n-5, 17:1, 18:1n-5, 20:1n-11, 20:1n-7, 22:1n-11, and 24:1

[4] contains 16:3n-4, 16:4n-3, 16:4n-1,18:2n-7, 18:2n-4, 18:3n-4, 18:4n-1, 21:5n-3, 22:5n-6, and 22:4n-3.

Table 3.7. Liver fatty acid profiles of Atlantic salmon fed the FO control diet or the FO-free diet for 16 weeks[1]. Five families with the highest weight gain were compared between the FO control diet and FO-free diet.

Family	38	34	11	14	42	38	42	06	14	19	*p*-value		
Diet	FO	FO	FO	FO	FO	FO-free	FO-free	FO-free	FO-free	FO-free	Treatment	Diet	Fam
14:0	1.7 ± 0.4a	1.4 ± 0.3a	1.8 ± 0.01a	1.5 ± 0.2a	1.5 ± 0.2a	0.8 ±0.1b	0.7 ± 0.03b	0.7 ± 0.01b	0.7 ± 0.1b	0.8 ± 0.2b	<0.001	<0.001	0.501
16:0	11.5 ± 3.4	10.6 ± 6.5	10.5 ± 2.1	13.4 ± 5.0	13.8 ± 3.1	9.8 ± 0.9	8.6 ± 1.2	11.3 ± 3.7	13.4 ± 2.8	10.5 ± 3.1	0.676	0.213	0.688
16:1n-7	2.9 ± 0.6ab	2.8 ± 1.0abc	3.8 ± 0.4a	2.4 ± 0.8abc	2.4 ± 0.6abc	1.4 ± 0.4cd	1.4 ± 0.2cd	1.5 ± 0.08abcd	1.1 ± 0.2d	1.5 ± 0.2bcd	<0.001	<0.001	0.203
18:0	4.8 ± 1.1	4.1 ± 1.0	4.5 ± 1.2	4.7 ± 0.5	4.5 ± 0.5	5.5 ± 0.4	5.0 ± 0.4	5.9 ± 1.1	5.0 ± 0.4	5.8 ± 0.8	0.144	0.172	0.483
18:1n-11	2.9 ± 0.5a	2.1 ± 0.6a	2.3 ± 1.1a	2.3 ± 0.5a	2.5 ± 0.4a	0.3 ± 0.07b	0.3 ± 0.03b	0.3 ± 0.09b	0.3 ± 0.06b	0.3 ± 0.08b	<0.001	<0.001	0.518
18:1n-9	33.1 ± 4.4	31.6 ± 12.9	33.7 ± 3.2	26.3 ± 7.8	25.7 ± 5.9	28.8 ± 4.6	26.4 ± 3.4	26.6 ± 3.1	20.2 ± 6.5	29.1 ± 3.5	0.393	0.276	0.344
18:1n-7	3.0 ± 0.3a	2.9 ± 0.9ab	2.9 ± 0.04ab	2.5 ± 0.5ab	2.5 ± 0.4ab	1.8 ± 0.2abc	1.7 ± 0.3bc	1.8 ± 0.2abc	1.5 ± 0.3c	1.8 ± 0.2abc	<0.001	<0.001	0.454
18:2n-6 (LNA)	6.2 ± 0.5bcde	5.5 ± 1.4de	5.8 ± 0.5bcde	5.0 ± 0.6e	5.9 ± 0.6cde	9.7 ± 1.0a	8.8 ± 0.6ab	8.2 ± 0.2abcd	7.6±1.0abcde	8.6 ± 0.7abc	<0.001	<0.001	0.142
18:3n-6	0.5 ±0.1	1.3 ± 0.6	0.9 ± 0.1	1.0 ± 0.6	1.2 ± 0.7	1.4 ± 0.5	1.4 ± 0.3	1.2 ± 0.4	1.3 ± 0.3	1.5 ± 0.5	0.159	0.016	0.483
18:3n-3 (ALA)	1.0 ± 0.2b	0.9 ± 0.2b	1.0 ± 0.09b	0.8 ± 0.2b	1.0 ± 0.3b	3.9 ± 0.2a	4.0 ± 0.6a	3.3 ± 0.3a	3.0 ± 0.2a	3.3 ± 0.5a	<0.001	<0.001	0.057
18:4n-3	0.3 ± 0.03b	0.3 ± 0.1b	0.4 ±0.1b	0.3 ± 0.07b	0.3 ± 0.06b	1.7 ± 0.4a	1.9 ± 0.2a	1.6 ± 0.2a	1.8 ± 0.4a	1.9 ± 0.6a	<0.001	<0.001	0.182
20:1n-11	1.0 ± 0.1ab	1.1 ± 0.3ab	1.6 ± 0.6a	1.0 ± 0.4ab	0.8 ± 0.3abc	0.1 ± 0.08cd	0.3 ± 0.07bcd	0.07 ± 0.08d	0.1 ± 0.1d	0.2 ± 0.2cd	<0.001	<0.001	0.347
20:1n-9	6.9 ±0.9	6.5 ± 2.7	6.4 ± 1.2	5.2 ± 1.6	4.2 ± 1.5	7.4 ± 1.1	6.4 ± 1.0	5.5 ± 1.2	4.1 ± 1.5	6.7 ± 0.6	0.136	0.455	0.131
20:2n-6	1.0 ± 0.2bc	1.0 ± 0.4bc	0.9 ± 0.02bc	0.8 ± 0.1c	0.8 ± 0.2c	1.8 ± 0.09a	1.4 ±0.3ab	1.5 ± 0.07ab	1.2 ± 0.08abc	1.5 ± 0.1ab	<0.001	<0.001	0.105
20:3n-6	1.4 ± 0.2d	1.4 ± 0.2d	1.3 ± 0.1d	1.9 ± 0.4bcd	1.8 ± 0.3cd	2.7 ± 0.2ab	2.4 ± 0.4abc	2.9 ± 0.08a	2.9 ± 0.4a	2.7 ± 0.1ab	<0.001	<0.001	0.149
20:4n-6 (ARA)	1.2 ± 0.3b	1.3 ± 0.6b	1.1 ± 0.4b	2.1 ± 0.8ab	2.1 ± 0.8ab	1.7 ± 0.4ab	1.8 ± 1.2ab	2.6 ± 0.6ab	3.9 ± 0.9a	2.1 ± 0.2ab	0.012	0.168	0.062
20:3n-3	0.2 ± 0.01c	0.2 ± 0.2bc	0.2 ± 0.09bc	0.2 ± 0.04c	0.3 ± 0.2abc	0.9 ± 0.5a	0.6 ± 0.08ab	0.6 ± 0.09abc	0.4 ± 0.06abc	0.5 ± 0.05abc	<0.001	<0.001	0.680
20:4n-3	0.3 ± 0.07b	0.3 ±0.03b	0.3 ± 0.05	0.3 ± 0.04b	0.4 ± 0.07b	1.4 ± 0.06a	1.1 ± 0.2a	1.3 ± 0.2a	1.1 ± 0.3a	1.1 ± 0.1a	<0.001	<0.001	0.858
20:5n-3 (EPA)	1.2 ± 0.2c	1.4 ± 0.6bc	1.2 ± 0.08bc	2.5 ± 1.0abc	2.3 ± 0.5abc	1.6 ± 0.3abc	2.6 ± 1.5abc	3.1 ± 1.0ab	5.1 ± 2.0a	2.6 ± 0.6abc	0.002	0.046	0.006
22:0	0.3 ±0.2	1.6 ± 1.3	0.6 ± 0.06	1.0 ± 0.9	1.6 ± 1.1	0.7 ± 0.4	0.5 ± 0.5	0.6 ± 0.4	0.4 ± 0.3	0.7 ± 0.7	0.741	0.573	0.899
22:1n-9	1.1 ± 0.04	1.1 ± 0.3	1.1 ± 0.09	1.1 ± 0.2	0.9 ± 0.1	1.5 ± 0.4	1.2 ± 0.4	1.1 ± 0.3	0.8 ± 0.3	1.2 ± 0.2	0.153	0.481	0.436
22:1n-7	1.8 ± 1.0	1.4 ± 1.1	1.3 ± 0.4	1.5 ± 0.4	1.3 ± 0.6	0.7 ± 0.09	1.1 ± 1.1	0.8 ± 0.7	0.4 ± 0.3	0.6 ± 0.6	0.398	0.032	0.989
22:5n-3	0.4 ± 0.1b	0.6 ± 0.4ab	0.4 ± 0.1ab	0.9 ± 0.3ab	0.8 ± 0.4ab	0.7 ± 0.2ab	0.9 ± 0.4ab	1.0 ± 0.1ab	1.6 ± 0.5a	0.9 ± 0.08ab	0.011	0.023	0.048
22:6n-3 (DHA)	5.4 ± 1.3b	7.2 ± 3.9ab	5.0 ± 1.1ab	10.3 ± 3.9ab	10.5 ± 1.3ab	5.0 ±1.2b	8.5 ± 5.1ab	9.8 ± 3.0ab	15.7 ± 5.5a	7.9 ± 1.1ab	0.025	0.554	0.027
24:1	1.1 ± 0.2	2.0 ± 2.0	1.0 ± 0.01	1.7 ± 0.6	1.7 ± 0.5	1.8 ± 0.3	2.3 ± 0.9	1.9 ± 0.3	2.2 ± 0.1	2.0 ± 0.7	0.295	0.052	0.578
ΣSFA [2]	19.2 ± 5.1	18.3 ± 9.3	18.0 ± 3.2	21.5 ± 6.6	22.1 ± 4.7	17.7 ± 1.2	16.7 ± 1.5	19.4 ± 5.4	20.1 ± 3.1	18.5 ± 4.9	0.957	0.320	0.906
ΣMUFA [3]	59.6 ± 6.0a	56.9 ± 15.3ab	60.6 ± 5.3ab	49.5 ± 12.0ab	46.9 ±8.3ab	46.9 ± 4.0ab	43.1 ± 4.7ab	41.5 ± 4.0ab	32.4 ± 9.1b	45.2 ± 2.7ab	0.016	0.009	0.187
ΣPUFA [4]	21.0 ± 2.5d	24.6 ± 5.9cd	21.2 ± 2.0cd	28.8 ± 6.4bcd	30.8 ± 4.0bcd	35.3 ± 2.8abc	40.1 ± 5.0ab	39.1± 3.9ab	47.3 ± 7.8a	36.1± 2.4abc	<0.001	<0.001	0.026
Σn-3	9.0 ± 1.7c	11.3 ± 5.0bc	8.9 ± 1.3bc	15.6 ± 5.2abc	16.1 ± 2.7abc	16.2 ± 2.2abc	20.3 ± 6.1abc	21.1 ± 4.1ab	29.0 ± 7.9a	18.4 ± 2.4abc	0.001	0.001	0.027
Σn-6	10.5 ± 0.9b	10.7 ± 0.3b	10.3 ± 0.2b	11.2 ± 1.1b	12.1 ± 1.0b	17.5 ± 0.9a	16.3 ± 2.5a	16.9 ± 0.4a	17.6 ± 0.3a	16.7 ± 0.5a	<0.001	<0.001	0.890
n-3/n-6	0.9 ± 0.1	1.1 ± 0.5	0.9 ± 0.1	1.4 ± 0.4	1.3 ± 0.2	0.9 ± 0.1	1.3 ± 0.3	1.3 ± 0.2	1.7 ± 0.4	1.1 ± 0.1	0.055	0.595	0.020
DHA/EPA	4.7 ± 0.1a	4.8 ± 0.7a	4.1 ± 0.6ab	4.3 ± 0.3ab	4.7 ± 0.5a	3.1 ± 0.4b	3.3 ± 0.1b	3.1 ± 0.5b	3.2 ± 0.3b	3.1 ± 0.3b	<0.001	<0.001	0.605
EPA/ARA	1.0 ± 0.1	1.1 ± 0.05	1.2 ± 0.4	1.1 ± 0.07	1.1 ± 0.2	1.0 ± 0.2	1.5 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	0.213	0.164	0.225

[1]Mean ± SD, n=3 per treatment (family/diet). Significant differences among ten treatments determined by the one-way ANOVA and Bonferroni *post hoc* test (α=0.05). The two-way ANOVA was used to determine diet, family and diet x family effect. The interaction effect was not significant except for 21:5n-3. Fatty acid content less than 0.1% in both diets was mostly not reported. Other fatty acids between 0.1% and 1% were listed below:

[2] contains 15:0, 17:0, 20:0, and 23:0.

[3] contains 14:1, 16:1n-11, 16:1n-9, 16:1n-5, 17:1,18:1n-5, and 20:1n-7.

[4] contains 16:2n-4, 16:4n-1, 18:2n-7, 18:2n-4, 18:3n-4, 18:4n-1, 21:5n-3, 22:4n-6, 22:5n-6, and 22:4n-3.

Table 3.8. Muscle fatty acid profiles of Atlantic salmon fed the FO control diet or the FO-free diet for 16 weeks[1]. Five families with the lowest weight gain were shown between the FO control diet and FO-free diet.

Family	27	36	30	24	08	32	27	23	45	35	*p*-value		
Diet	FO	FO	FO	FO	FO	FO-free	FO-free	FO-free	FO-free	FO-free	Treatment	Diet	Fam
14:0	2.6 ± 0.3ab	2.4 ± 0.2abc	2.9 ±0.2a	2.4 ± 0.3abc	2.5 ± 0.3abc	1.6 ± 0.3cd	1.4 ± 0.3d	1.5 ± 0.09d	1.4 ± 0.3d	1.7 ± 0.3bcd	<0.001	<0.001	0.368
16:0	13.7 ± 1.8	12.7 ± 1.7	15.8 ± 1.6	12.7 ± 1.2	13.7 ± 1.4	13.0 ± 1.5	13.9 ± 2.9	13.1 ± 1.9	13.1 ± 2.1	13.7 ± 3.1	0.827	0.946	0.791
16:1n-7	4.0 ± 0.2a	4.0 ± 0.3a	3.8 ± 0.2a	3.8 ± 0.03a	4.0 ± 0.1a	2.3 ± 0.2b	1.9 ± 0.2b	2.1 ± 0.2b	2.0 ± 0.06b	2.2 ± 0.2b	<0.001	<0.001	0.151
18:0	3.2 ± 0.4	3.2 ± 0.2	3.6 ± 0.3	3.1 ± 0.3	3.2 ± 0.3	4.0 ± 0.5	4.4 ± 1.0	4.0 ± 0.6	4.1 ± 0.5	4.1 ± 1.0	0.030	0.017	0.910
18:1n-11	1.0 ± 0.1a	1.0 ± 0.07a	1.1 ± 0.1a	1.0 ± 0.04a	0.9 ± 0.2a	0.1 ± 0.01b	0.1 ± 0.02b	0.1 ± 0.02b	0.1 ± 0.01b	0.1 ± 0.01b	<0.001	<0.001	0.485
18:1n-9	32.5 ± 1.4a	33.3 ± 1.5a	31.4 ± 2.2ab	33.1 ± 0.5a	33.6 ± 0.7a	27.6 ± 0.9b	26.4 ± 2.3b	27.5 ± 0.7b	27.7 ± 0.9b	27.5 ± 1.8b	<0.001	<0.001	0.626
18:1n-7	2.74 ± 0.2a	3.0 ± 0.2a	2.6 ± 0.1a	2.9 ± 0.05a	2.7 ± 0.1a	2.1 ± 0.02b	1.9 ±0.2b	2.1 ± 0.07b	2.0 ± 0.03b	2.1 ± 0.2b	<0.001	<0.001	0.039
18:2n-6 (LNA)	9.3 ± 0.9b	9.8 ± 0.4b	8.4 ±0.6b	9.5 ± 0.5b	9.2 ± 0.5b	12.8 ± 0.6a	12.7 ± 1.4a	13.5 ± 0.4a	13.2 ± 0.5a	13.5 ± 1.3a	<0.001	<0.001	0.403
18:3n-6	0.6 ± 0.03b	0.6 ± 0.07b	0.6 ± 0.06b	0.5 ± 0.03b	0.6 ± 0.06b	1.3 ± 0.2a	1.3 ±0.1a	1.3 ± 0.1a	1.2 ± 0.2a	1.1 ± 0.2a	<0.001	<0.001	0.459
18:3n-3 (ALA)	2.1 ± 0.3b	2.3 ± 0.2b	1.8 ± 0.2b	2.2 ± 0.2b	2.0 ± 0.2b	8.5 ± 0.5a	8.9 ±1.3a	9.1 ± 0.3a	9.3 ± 1.1a	9.0 ± 1.2a	<0.001	<0.001	0.282
18:4n-3	0.9 ± 0.2b	1.0 ± 0.1b	0.9 ± 0.04b	0.9 ± 0.08b	0.9 ± 0.1b	3.8 ± 0.8a	3.7 ±0.9a	4.2 ± 0.3a	3.8 ± 0.6a	3.4 ± 0.9a	<0.001	<0.001	0.643
20:1n-9	5.4 ± 0.2d	5.8 ± 0.6bcd	5.7 ± 0.4cd	5.7 ± 0.2cd	5.9 ± 0.2bcd	6.5 ± 0.5abcd	6.9 ± 0.7abc	7.0 ± 0.3ab	7.2 ± 0.2a	7.2 ± 0.1a	<0.001	<0.001	0.367
20:2n-6	0.4 ± 0.01b	0.4 ± 0.06b	0.3 ± 0.02b	0.4 ± 0.01b	0.4 ± 0.03b	1.0 ± 0.05a	1.2 ± 0.09a	1.1 ± 0.07a	1.1 ± 0.12a	1.1 ± 0.04a	<0.001	<0.001	0.094
20:3n-6	0.4 ± 0.03cd	0.4 ± 0.03d	0.4 ± 0.03d	0.4 ± 0.05cd	0.4 ± 0.04bcd	0.6 ± 0.05a	0.7 ± 0.1a	0.6 ± 0.04ab	0.7 ± 0.2a	0.5 ± 0.01abc	<0.001	<0.001	0.135
20:4n-6 (ARA)	0.4 ± 0.01ab	0.4 ± 0.07ab	0.3 ± 0.05b	0.4 ± 0.06ab	0.3 ± 0.04ab	0.4 ± 0.06a	0.4 ± 0.04a	0.4 ± 0.04ab	0.4 ± 0.04ab	0.3 ± 0.02ab	0.004	0.353	0.006
20:3n-3	0.5 ± 0.5	0.2 ± 0.1	0.4 ± 0.4	0.6 ± 0.09	0.3 ± 0.3	0.7 ± 0.1	0.8 ± 0.3	0.7 ± 0.08	0.8 ± 0.02	0.7 ± 0.09	0.066	0.214	0.553
20:4n-3	0.4 ± 0.02cd	0.4 ± 0.03cd	0.3 ± 0.05cd	0.4 ± 0.01c	0.3 ± 0.02d	0.7 ± 0.05ab	0.8 ± 0.05a	0.7 ± 0.06ab	0.8 ± 0.1ab	0.6 ± 0.01b	<0.001	<0.001	0.002
20:5n-3 (EPA)	1.4 ± 0.3	1.7 ± 0.3	1.0 ± 0.1	1.4 ± 0.2	1.2 ± 0.3	1.5 ± 0.08	1.3 ±0.4	1.3 ± 0.2	1.3 ± 0.3	1.2 ± 0.2	0.111	0.476	0.082
22:0	7.1 ± 0.4a	7.0 ± 0.02a	7.6 ± 0.5a	7.2 ± 0.4a	7.9 ± 0.1a	1.0 ± 0.1b	1.0 ± 0.08b	1.0 ± 0.08b	0.9 ± 0.1b	1.1 ± 0.09b	<0.001	<0.001	0.032
22:1n-7	0.8 ± 0.6	0.5 ± 0.4	1.6 ± 0.6	0.5 ± 0.5	0.9 ± 0.7	0.5 ± 0.4	0.6 ± 0.6	0.3 ± 0.2	0.5 ± 0.6	0.6 ± 0.7	0.816	0.675	0.904
22:5n-3	0.6 ± 0.2ab	0.8 ± 0.2a	0.5 ± 0.02b	0.6 ± 0.08ab	0.6 ± 0.1ab	0.7 ± 0.03ab	0.6 ± 0.1ab	0.7 ± 0.06ab	0.6 ± 0.07ab	0.5 ± 0.05ab	0.038	0.296	0.027
22:6n-3 (DHA)	4.4 ± 1.0	4.9 ± 0.1	3.0 ± 0.7	4.2 ± 0.8	3.8 ± 0.5	5.0 ± 0.4	4.4 ± 1.2	4.3 ± 0.8	4.1 ± 0.7	3.6 ± 0.6	0.167	0.973	0.152
ΣSFA[2]	27.4 ± 2.9ab	27.3 ± 0.4ab	30.8 ± 1.6a	26.2 ± 1.5ab	28.0 ± 1.9ab	20.7 ± 2.3b	21.7 ± 4.5ab	20.5 ± 2.5b	20.5 ± 3.3b	21.9 ± 4.6ab	0.001	0.025	0.776
ΣMUFA[3]	48.6 ± 1.1a	48.5 ± 0.3a	48.5 ± 2.3a	49.2 ± 0.2a	50.1 ± 0.6a	40.3 ± 0.9b	38.8 ± 2.3b	40.1 ± 0.8b	40.4 ± 0.7b	40.7 ± 1.4b	<0.001	<0.001	0.579
ΣPUFA[4]	23.6 ± 1.9b	25.6 ± 3.1b	20.5 ± 0.9b	24.3 ± 1.4b	21.6 ± 1.3b	38.7 ± 1.8a	39.2 ± 2.4a	39.1 ± 1.9a	38.7 ± 3.0a	37.1 ± 3.5a	<0.001	<0.001	0.240
Σn-3	10.8 ± 1.5b	12.5 ± 2.1b	8.2 ± 0.6b	10.8 ± 1.0b	9.43 ± 0.9b	21.2 ± 1.4a	21.3 ± 2.2a	21.2 ± 1.5a	21.0 ± 2.4a	19.4 ± 2.2a	<0.001	<0.001	0.163
Σn-6	11.2 ± 0.9b	11.8 ± 0.8b	10.2 ±0.5b	11.5 ± 0.8b	11.0 ± 0.6b	16.4 ± 0.7a	16.4 ± 1.3a	17.0 ± 0.5a	16.8 ± 0.9a	16.7 ± 1.5a	<0.001	<0.001	0.577
n-3/n-6	1.0 ± 0.06cd	1.1 ± 0.1bcd	0.8 ± 0.1d	0.9 ± 0.04d	0.9 ± 0.03d	1.3 ± 0.04a	1.3 ± 0.07a	1.3 ± 0.07ab	1.3 ± 0.08ab	1.2 ± 0.04abc	<0.001	<0.001	0.014
DHA/EPA	3.1 ± 0.2	3.3 ± 0.2	3.0 ± 0.4	2.9 ± 0.1	3.2 ± 0.4	3.2 ± 0.1	3.5 ± 0.2	3.3 ± 0.07	3.2 ± 0.2	3.3 ± 0.04	0.221	0.054	0.524
EPA/ARA	3.5 ± 0.7	4.3 ± 0.5	3.7 ± 0.2	3.7 ± 0.3	4.1 ± 0.5	3.5 ± 0.5	3.0 ± 0.9	3.7 ± 0.2	3.1 ± 0.3	3.6 ± 0.7	0.158	0.262	0.432

[1]Mean ± SD, n=3 per treatment (family/diet). Significant differences among ten treatments determined by one-way ANOVA with Bonferroni *post hoc* correction test. The two-way ANOVAS was used to determine diet, family and diet x family. The interaction effect was not significant. Fatty acid content less than 0.1% in both diets was mostly not reported. Other fatty acids between 0.1% and 1% were listed below:

[2] contains 15:0, 17:0, 20:0, 23:0, and 24:0.

[3] contains 14:1, 16:1n-11, 16:1n-5, 17:1, 18:1n-5, 20:1n-11, 20:1n-7, 22:1n-11, and 24:1

[4] contains 16:3n-4, 16:4n-3, 16:4n-1,18:2n-7, 18:2n-4, 18:3n-4, 18:4n-1, 21:5n-3, 22:4n-6, 22:4n-3, and 22:5n-6.

Table 3.9. Liver fatty acid profiles of Atlantic salmon fed the FO control diet and the FO-free diet for 16 weeks[1]. Five families with the lowest weight gain were compared between the FO control diet and FO-free diet.

Family	27	36	30	24	8	32	27	23	45	35	*p*-value		
Diet	FO	FO	FO	FO	FO	FO-free	FO-free	FO-free	FO-free	FO-free	Treatment	Diet	Fam
14:0	1.7 ± 0.2a	1.6 ± 0.4ab	1.7 ± 0.01ab	1.5 ± 0.5ab	1.8 ± 0.1a	1.1 ± 0.3abc	0.9 ± 0.3bc	0.7 ± 0.1c	1.0 ± 0.2abc	0.9 ± 0.2bc	<0.001	0.001	0.496
16:0	12.9 ± 4.4	13.2 ± 5.2	15.6 ± 7.4	12.7 ± 2.6	11.9 ± 2.0	13.2 ± 4.7	13.5 ± 7.3	6.4 ± 1.9	12.3 ± 5.6	14.2 ± 2.5	0.575	0.995	0.565
16:1n-7	2.4 ± 0.5	2.3 ± 1.0	2.7 ± 1.7	2.3 ± 1.0	3.3 ± 0.1	1.2 ± 0.1	1.1 ± 0.2	1.7 ± 0.04	1.3 ± 0.3	1.0 ± 0.02	<0.001	<0.001	0.098
18:0	4.7 ± 0.8	4.5 ± 1.3	5.1 ± 1.7	4.5 ± 0.7	4.9 ± 0.9	6.3 ± 1.5	6.3 ± 2.2	4.4 ± 0.7	6.1 ± 1.9	6.7 ± 1.7	0.405	0.244	0.781
18:1n-11	2.7 ± 0.09a	2.0 ± 0.3a	1.9 ± 1.0a	1.7 ± 0.9ab	2.6 ± 0.4a	0.2 ± 0.09c	0.3 ± 0.07c	0.2 ± 0.08c	0.4 ± 0.09c	0.5 ± 0.07bc	<0.001	<0.001	0.098
18:1n-9	27.6 ± 6.5	26.0 ± 9.9	25.9 ± 13.8	24.5 ± 6.8	32.4 ± 3.0	26.6 ± 8.7	24.6 ±10.0	33.6 ± 2.3	26.1 ± 7.2	21.5 ± 4.4	0.765	0.649	0.696
18:1n-7	2.8 ± 0.4	2.5 ± 0.7	2.5 ± 0.8	2.5 ± 0.3	2.9 ± 0.2	1.8 ± 0.3	1.8 ± 0.5	2.0 ± 0.2	1.8 ± 0.3	1.6 ± 0.1	0.010	0.010	0.812
18:2n-4	1.6 ± 1.4	1.3 ± 1.4	0.5 ± 0.4	0.6 ± 0.2	0.6 ± 0.5	0.5 ± 0.7	0.3 ± 0.3	0.3 ± 0.3	0.4 ± 0.4	0.5 ± 0.4	0.569	0.069	0.866
18:2n-6 (LNA)	5.6 ±0.2ab	5.5 ± 0.7b	5.2 ± 1.2b	5.1 ± 0.5b	5.2 ± 0.2b	8.3 ± 1.7ab	8.7 ± 2.6ab	10.3 ± 1.4a	8.1 ± 2.4ab	8.0 ± 1.3ab	<0.001	0.016	0.792
18:3n-6	1.7 ± 1.6	1.6 ± 1.2	0.7 ± 0.3	0.6 ± 0.2	1.2 ± 1.1	1.6 ± 1.0	1.0 ± 0.5	1.3 ± 0.3	2.3 ± 1.5	2.0 ± 1.1	0.255	0.823	0.649
18:3n-3 (ALA)	0.9 ±0.09b	0.9 ± 0.06b	0.9 ± 0.3b	0.9 ± 0.3b	0.8 ± 0.1b	3.6 ± 0.2a	3.6 ± 1.5a	4.7 ± 1.2a	3.5 ± 1.3a	2.8 ± 0.5a	<0.001	0.004	0.393
18:4n-3	0.3 ±0.03b	0.4 ± 0.08b	0.3 ± 0.03b	0.4 ± 0.07b	0.4 ± 0.04b	1.3 ± 0.5a	1.0 ± 0.3a	2.0 ± 0.4a	1.2 ± 0.1a	1.3 ± 0.6a	<0.001	<0.001	0.106
20:1n-9	5.7 ± 1.4	5.0 ± 1.6	5.1 ± 2.8	5.0 ± 0.9	6.3 ± 0.7	6.4 ± 2.2	6.6 ± 2.9	8.1 ± 0.7	7.2 ± 2.3	5.4 ± 2.2	0.584	0.576	0.827
20:2n-6	1.1 ± 0.2	0.8 ± 0.2	0.8 ± 0.4	1.2 ± 0.5	0.9 ± 0.2	1.4 ± 0.3	1.7 ± 0.5	1.8 ± 0.3	1.8 ± 0.5	1.4 ± 0.3	0.009	0.056	0.609
20:3n-6	1.5 ± 0.1ab	1.8 ± 0.4ab	1.5 ± 0.3ab	1.9 ± 0.5ab	1.3 ± 0.1a	2.2 ± 0.6ab	2.3 ± 0.6ab	2.5 ± 0.2b	1.9 ± 0.2ab	2.4 ± 0.7ab	0.010	0.022	0.515
20:4n-6 (ARA)	1.7 ± 0.2	2.2 ± 0.3	2.0 ± 1.3	2.6 ± 0.9	1.2 ± 0.4	1.8 ± 0.7	2.1 ± 1.4	1.5 ± 0.7	1.5 ± 0.4	1.1 ± 0.2	0.519	0.911	0.530
20:4n-3	0.3 ± 0.07cd	0.3 ± 0.1cd	0.3 ± 0.04cd	0.3 ± 0.2cd	0.3 ± 0.03d	1.0 ± 0.5a	1.3 ± 0.4a	1.5 ± 0.4a	0.8 ± 0.3abc	0.9 ± 0.3ab	<0.001	<0.001	0.485
20:5n-3 (EPA)	1.6 ± 0.4	2.2 ± 0.3	2.1 ± 1.4	2.8 ± 1.2	1.4 ± 0.5	2.2 ± 0.8	2.6 ± 1.8	2.2 ± 0.9	1.6 ± 0.4	1.2 ± 0.2	0.577	0.370	0.491
22:0	0.9 ± 0.6	0.5 ± 0.5	0.6 ± 0.3	0.5 ± 0.2	0.7 ± 0.6	0.5 ± 0.3	0.6 ± 0.6	0.3 ± 0.2	0.7 ± 0.2	1.0 ± 0.9	0.907	0.325	0.867
22:1n-11	3.2 ± 0.9ab	2.5 ± 0.6abc	2.9 ± 1.5abc	3.1 ± 1.5ab	3.7 ± 0.6a	0.9 ± 0.4bcd	0.6 ± 0.2d	0.7 ± 0.2cd	1.1 ± 0.6abcd	0.8 ± 0.5cd	<0.001	<0.001	0.863
22:1n-9	1.2 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	1.0 ± 0.1	1.0 ± 0.2	1.5 ± 0.2	1.4 ± 0.7	1.2 ± 0.2	1.7 ± 0.3	1.4 ± 0.9	0.371	0.664	0.852
22:1n-7	1.6 ± 0.6	1.2 ± 1.0	1.0 ± 0.4	1.2 ± 0.7	1.9 ± 0.8	1.2 ± 0.5	1.2 ± 1.2	0.3 ± 0.3	1.7 ± 0.8	1.6 ± 1.4	0.544	0.382	0.562
22:5n-6	0.2 ± 0.03	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.05	0.2 ± 0.08	0.2 ± 0.1	0.2 ± 0.04	0.2 ± 0.05	0.2 ± 0.03	0.622	0.801	0.611
22:4n-3	0.2 ± 0.2	0.2 ± 0.1	0.06 ± 0.05	0.07 ± 0.04	0.06 ± 0.03	0.2 ± 0.1	0.2 ± 0.2	0.08 ± 0.02	0.2 ± 0.06	0.3 ± 0.2	0.661	0.400	0.966
22:5n-3	0.6 ± 0.05	0.8 ± 0.3	0.7 ± 0.4	0.8 ± 0.3	0.4 ± 0.1	0.8 ± 0.3	0.8 ± 0.5	0.6 ± 0.2	0.7 ± 0.1	0.8 ± 0.09	0.700	0.380	0.706
22:6n-3 (DHA)	8.5 ± 2.2	11.7 ± 2.0	8.9 ± 5.8	14.1 ± 7.0	5.9 ± 2.7	6.8 ± 3.1	7.9 ± 5.2	6.2 ± 2.3	5.3 ± 1.3	4.1 ± 0.4	0.287	0.656	0.551
24:1	1.5 ± 0.3	1.8 ± 0.7	1.6 ± 0.9	1.9 ± 0.7	1.1 ± 0.4	1.7 ± 1.5	2.1 ± 1.2	1.3 ± 0.2	2.4 ± 1.2	2.6 ± 0.6	0.573	0.372	0.673
ΣSFA [2]	20.9 ± 6.0	21.1 ± 7.8	18.6 ± 3.9	19.9 ± 3.3	19.9 ± 2.7	22.2 ± 6.7	22.3 ± 10.4	12.5 ± 2.7	22.4 ± 9.7	24.0 ± 5.5	0.677	0.907	0.593
ΣMUFA [3]	51.3 ± 8.8	46.9 ± 11.5	47.2 ± 20.6	46.1 ± 11.5	58.3 ± 5.1	43.5 ± 9.8	41.0 ± 11.6	50.5 ± 3.2	45.2 ± 7.7	37.7 ± 9.7	0.597	0.260	0.757
ΣPUFA [4]	27.5 ± 3.0	31.8 ± 5.4	25.7 ± 7.0	33.7 ± 11.0	21.5 ± 5.6	34.2 ± 5.6	36.5 ± 6.9	36.9 ± 1.7	32.2 ± 3.3	30.1 ± 0.8	0.090	0.076	0.297
Σn-3	12.7 ± 2.4	16.9 ± 2.5	13.7 ± 7.2	19.7 ± 8.9	9.6 ± 3.3	16.6 ± 4.2	18.5 ± 6.9	18.1 ± 2.4	14.4 ± 1.9	11.9 ± 0.05	0.306	0.191	0.306
Σn-6	11.9 ± 1.6bc	12.2 ± 1.5bc	10.5 ± 0.9c	11.8 ± 2.1bc	10.1 ± 1.6c	15.7 ± 1.4ab	16.2 ± 2.2ab	17.7 ± 0.6a	15.8 ± 1.5ab	16.3 ± 1.3ab	<0.001	0.003	0.475
n-3/n-6	1.1 ±0.3	1.4 ± 0.05	1.3 ± 0.7	1.6 ± 0.5	0.9 ± 0.2	1.1 ± 0.2	1.1 ± 0.4	1.0 ± 0.2	0.9 ± 0.1	0.8 ± 0.04	0.343	0.915	0.473
DHA/EPA	5.2 ± 0.5ab	5.3 ± 0.4a	4.1 ± 0.9abcd	4.8 ± 0.7abc	4.2 ± 0.8abcd	3.0 ± 0.5d	3.1 ± 0.3d	2.8 ± 0.1d	3.3 ± 0.5cd	3.4 ± 0.1bcd	<0.001	<0.001	0.191
EPA/ARA	0.9 ± 0.2b	1.0 ±0.09ab	1.1 ± 0.2ab	1.1 ± 0.2ab	1.2 ± 0.09ab	1.2 ± 0.09ab	1.3 ± 0.3ab	1.5 ± 0.1a	1.1 ± 0.2ab	1.2 ± 0.2ab	0.054	0.030	0.253

[1]Mean ± SD, n=3. Significant differences among ten treatments determined by the one-way ANOVA with Bonferroni *post hoc* test (α=0.05). The two-way ANOVA was used to determine diet, family and interaction effect. The interaction effect was not significant. Other fatty acids between 0.1% and 1% were listed below:

[2] contains 15:0, 17:0, 20:0, 23:0, and 24:0.

[3] contains 14:1, 16:1n-5, 16:1n-9, 16:1n-11, 17:1, 18:1n-5, 20:1n-11, and 20:1n-7.

[4] contains 16:2n-4,16:3n-4, 16:4n-3, 16:4n-1, 18:2n-7, 18:3n-4, 18:4n-1, 20:3n-3, 21:5n-3, 22:4n-3, 22:4n-6, and 22:5n-6.

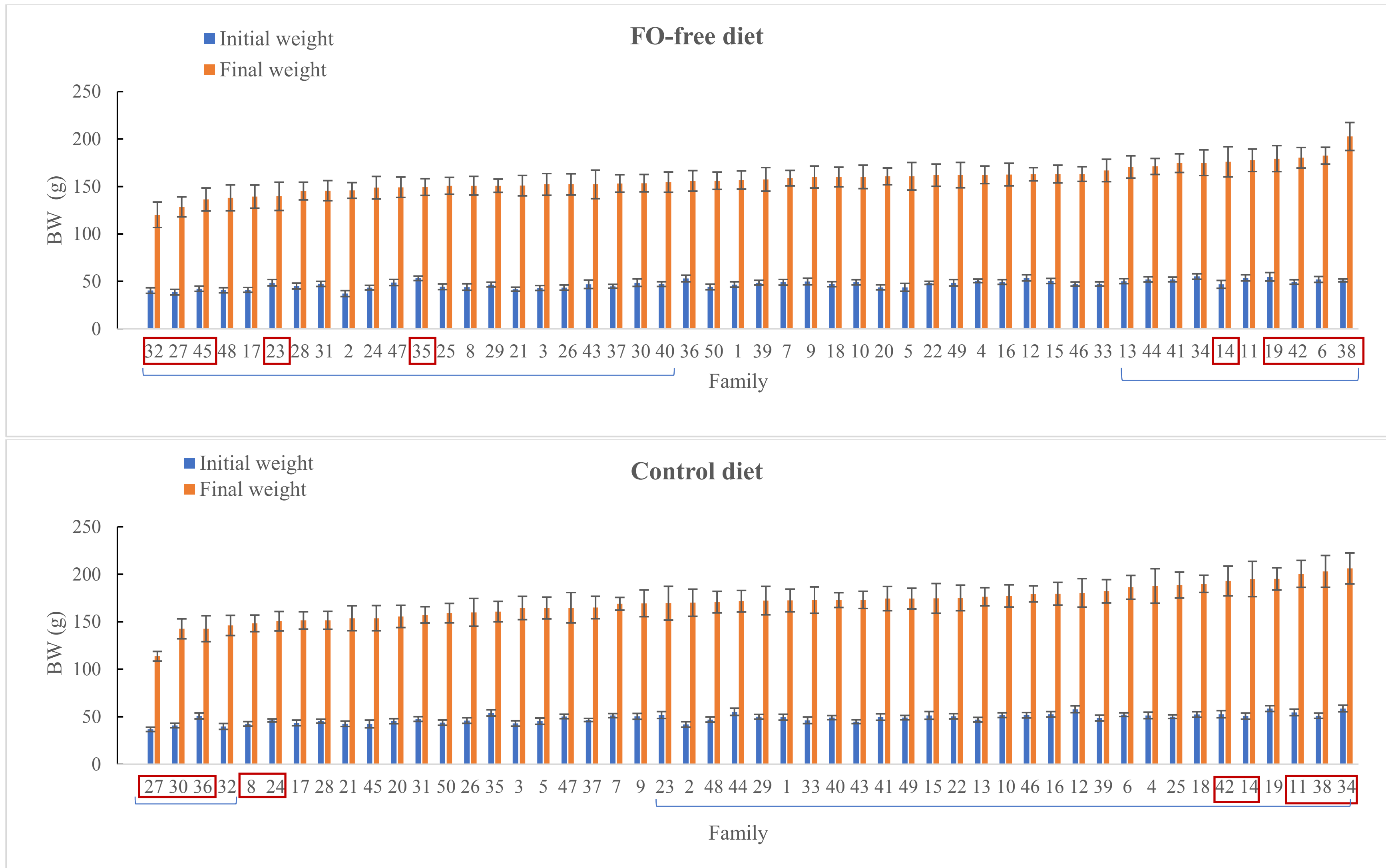

Figure 3.1. Comparison of SJR Atlantic salmon initial and final mean body weight (BW, mean ± SD) among fifty families in the FO control and FO-free diet group. Fish were fed the same commercial feed before the trial. SJR Atlantic salmon families selected for fatty acid analysis were highlighted in the red boxes. Families within the brackets had no significant differences (α = 0.05) in mean final body weight.

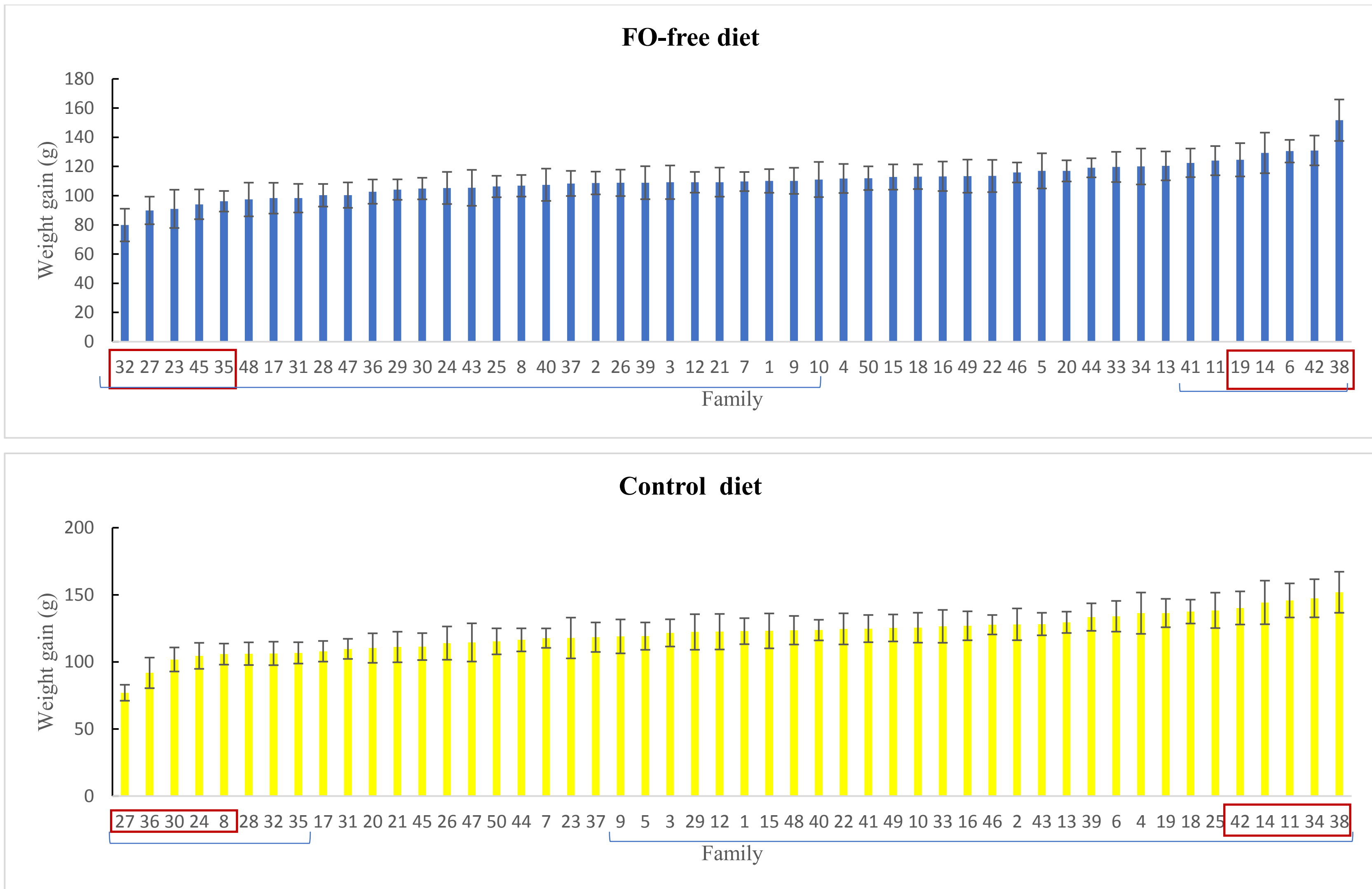

Figure 3.2. Comparison of the final mean weight gain (mean ± SD) of SJR Atlantic salmon among fifty families in the FO control and FO-free diet group after 16 weeks. Families selected for fatty acid analysis depended on the highest weight gain and lowest weight gain, which were highlighted in the red boxes. Families within the brackets showed no significant difference ($\alpha = 0.05$) in the final mean weight gain.

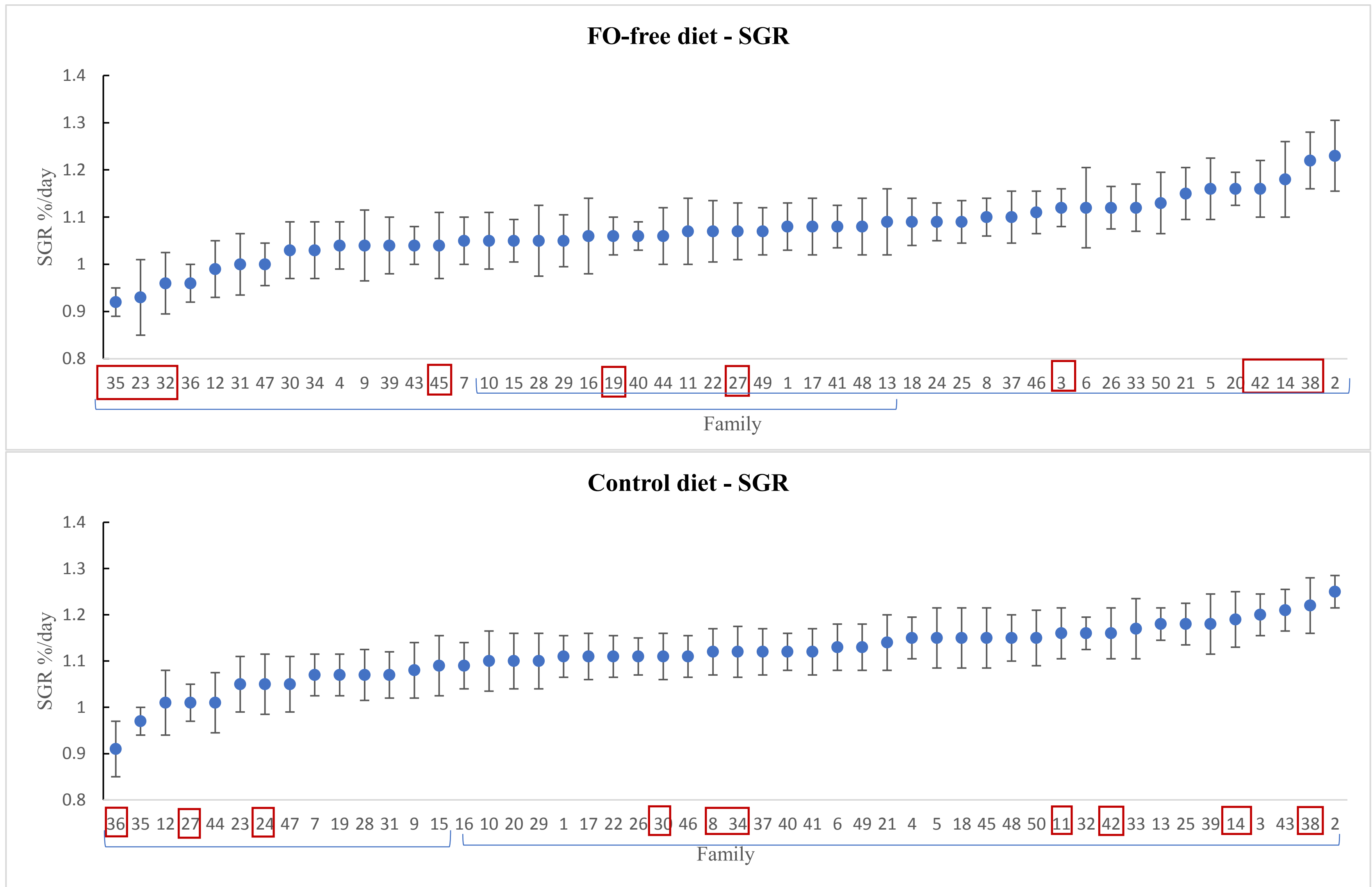

Figure 3.3. Comparison of SJR Atlantic salmon mean specific growth rate (SGR, mean ± SD) among fifty families in the FO control and FO-free diet group after 16 weeks. Families selected for fatty acid analysis was highlighted in the red boxes. Families within the brackets were not significantly different ($\alpha = 0.05$) in the final mean SGR.

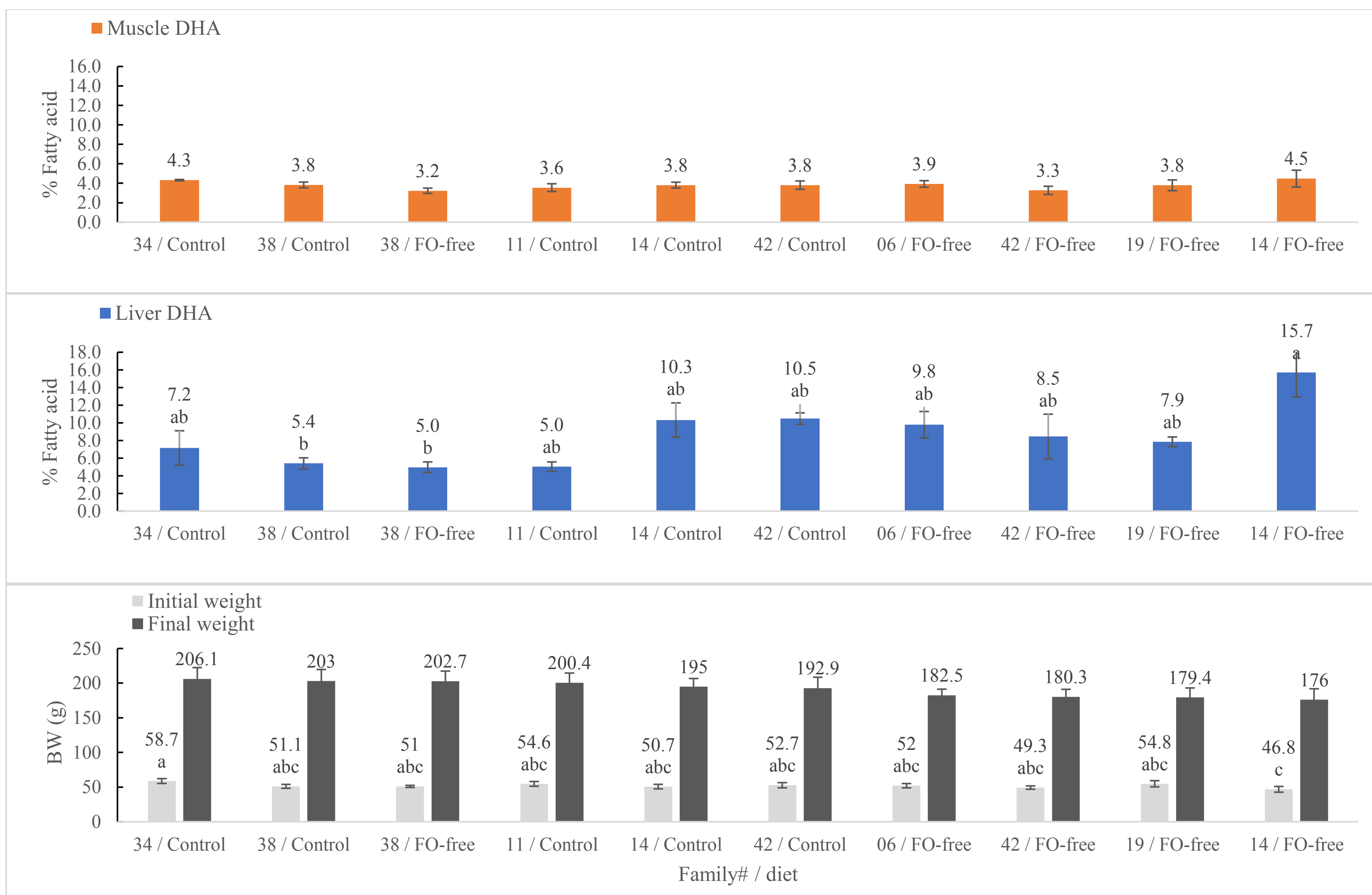

Figure 3.4. Atlantic salmon final mean DHA% in the muscle and liver and mean body weight (BW) among families regarding the leading weight gain. There was a significant difference (α = 0.05) in the initial mean BW due to the family effect.

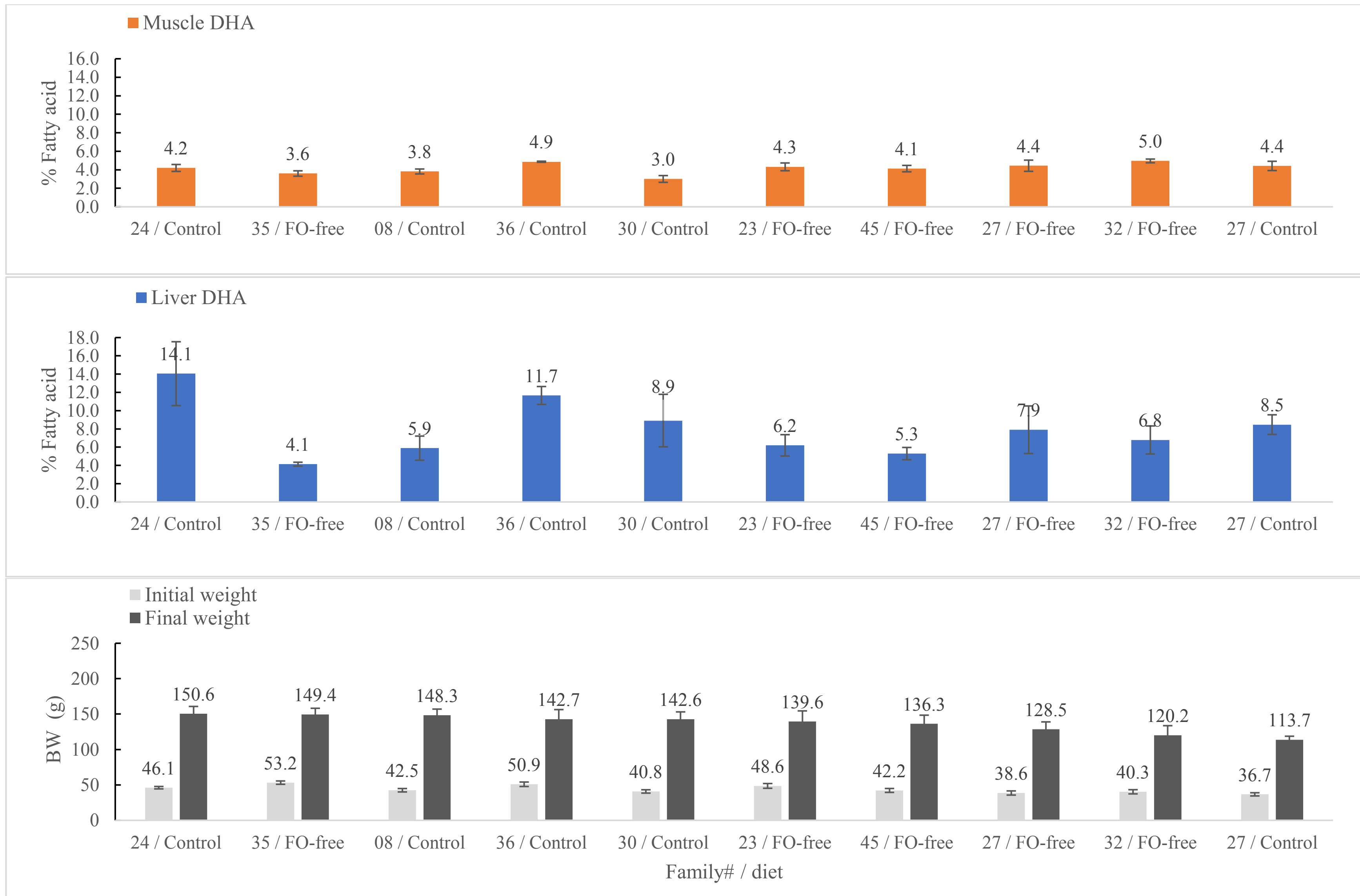

Figure 3.5. Atlantic salmon final mean DHA% in the muscle and liver, and mean body weight (BW) among families in terms of the lowest weight gain. No significant difference was found in the initial or final mean BW among the families fed either diet.

CHAPTER 4. CONCLUSIONS

There was clear evidence of the genetic effect on growth performance and tissue fatty acids in salmon from both studies in Chapter 2 and 3. For example, the growth performance of the GL strain fed the FO control diet was less than the SJR strain regardless of the diet. Freshwater landlocked GL strain salmon had a higher capacity for MUFA synthesis compared with SJR strain when fed the FO-free diet. The SJR strain had a higher capacity for n-6 PUFA synthesis and storage. Both strains can synthesize n-3 LC-PUFA when dietary n-3 LC-PUFA is low. Compared with the FO-free diet, muscle DHA in the landlocked salmon was significantly stored when fish were offered the control diet; it was less sensitive to the regulatory effect from high dietary DHA in the control diet. Nevertheless, muscle DHA and EPA content did not differ between strains within the FO-free diet group. Liver fatty acid composition were more affected by differences between strains, rather than diet alone, while the muscle fatty acid profile was mostly dependent on the dietary fatty acid profile. Additionally, to balance the competition effect from LNA, a higher content of ALA compared to LNA and minimum supply of DHA should be considered in future studies.

Fifty families of Atlantic salmon in SJR strain were compared regarding the weight gain and the n-3 LC-PUFA biosynthesis. Family #38, 42, and 14 were significantly higher in final body weight and weight gain, irrespective of diet. Compared with liver, muscle fatty acids more closely reflect the dietary fatty acid compositions. The biosynthesis of n-3 LC-PUFA, particularly DHA can be upregulated in the muscle and liver when Atlantic salmon were fed a diet devoid of n-3 LC-PUFA. Such enhancement was limited by the effect of 18:2n-6 and significantly differentiated by salmon family. Family #14 fed the FO-free diet had the highest DHA percentage of total fatty acid in the liver. The increased n-3/n-6 ratio in the SJR salmon family #14 indicates the affinity to n-3 fatty acids. The next step is to consider adding the trait of high n-3 LC-PUFA level in the salmon muscle and liver in the commercial breeding program.

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