Effects of dietary polyunsaturated fatty acids on growth and lipid metabolism of Arctic charr, Salvelinus alpinus (L)

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EFFECTS OF DIETARY POLYUNSATURATED FATTY ACIDS ON GROWTH AND LIPID METABOLISM OF ARCTIC CHARR, Salvelinus alpinus L.

BY

XIUWEI YANG

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Abstract

While polyunsaturated fatty acids (PUFAs) primarily govern the fluidity of cellular membranes, fish, like other vertebrates, cannot synthesize de novo two of the n-3 and n-6 PUFAs, namely, 18:2n-6 (linoleic acid) and 18:3n-3 (linolenic acid). As a result of this, freshwater fish must rely on their diets to provide these PUFAs for maintenance of normal development, growth, reproduction and physiological status and these PUFAs are therefore considered essential fatty acids (EFA). The metabolism and nutritional values of PUFAs have received little attention in Arctic charr, Salvelinus alpinus, the most northerly distributed freshwater species.

This thesis examined growth, physiological status and lipid metabolism of cultured juvenile Arctic charr fed diets containing levels of dietary n-3 and n-6 PUFAs. Experimental results indicated that charr on a treatment low in n-3 or n-6 PUFAs generally had low growth rate and feed efficiency. At the same time there were marked biochemical changes in tissues, such as fatty livers (increased total and neutral lipids), elevated water content in muscle and substantial accumulations of 20:3n-9. However, the level of total lipids from fish fed the PUFA-free diet was comparable to those of fish given a diet with high PUFA content, contrary to that reported for other salmonids. The PUFA-deficiency symptoms were least apparent in charr given diets containing more than 2.0% of 18:3n-3 or 1.6% 18:3n-3 in combination of 0.5%-1.0%

18:2n-6.

Data on tissue fatty acid composition also indicated an extensive desaturation and elongation of 18:3n-3 and 18:2n-6 to long-chain PUFAs through a common set of D^6, D^5 and D^4 desaturases and elongases in the livers of Arctic charr. The products from these processes were selectively incorporated into liver phospholipid. By comparison, there was a small increase in muscle long-chain PUFAs which was attributed to low D^5 conversion. It was also clear that dietary 18:3n-3 inhibited the conversion of dietary 18:2n-6 and incorporation of the long-chain PUFAs into tissue phospholipids of Arctic charr. In addition, data from an experiment which compared Arctic charr and rainbow trout indicated that charr were more sensitive to PUFA deficiency but less efficient in the desaturation and elongation of PUFAs compared to trout, particularly at the D^5 desaturation step. The experimental data on Arctic charr also suggested that n-6 PUFAs were inferior to n-3 PUFAs in the competition for common set of desaturases and elongases or incorporation into tissue phospholipids.

It is concluded that Arctic charr require 18:3n-3 as an essential fatty acid and their requirement for dietary 18:3n-3 is approximately 2.0% and 1.6% in the absence and presence of 0.5-1.0% dietary 18:2n-6, respectively.

To:

My parents

Deping & Helen

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General Introduction

Polyunsaturated fatty acids (PUFAs) are groups of individual fatty acids containing two or more double bonds in the cis- or trans- configuration separated by methylene groups. In vertebrate tissues the most common PUFAs include two of the n-6 PUFA family, 18:2n-6 and 20:4n-6, and three of the n-3 PUFA family, 18:3n-3, 20:5n-3 and 22:6n-3. While n-6 PUFAs are more common than n-3 PUFAs in terrestrial vertebrates, the most abundant PUFAs in fish are n-3 PUFAs, especially 20:5n-3 and 22:6n-3. It is generally accepted that knowledge of the nutrient composition in the diets of wild animals provides clues in the formulation of diets for farmed animals. From this, it appears that fish may require n-3 PUFAs, while terrestrial vertebrates may need 18:2n-6 or 20:4n-6.

While it has long been recognized that 18:2n-6 is an essential fatty acid (EFA) for humans (Burr and Burr, 1930), early evidence that fish require EFAs came from the study on two coldwater species, brook trout (Salvelinus fontinalis) and rainbow trout (Oncorhynchus mykiss) (Phillips et al., 1963; Higashi et al., 1966; Lee et al., 1967). Brook and rainbow trout fed diets with corn oil (rich in 18:2n-6) as the sole lipid source exhibited poor growth and high mortalities but they grew better and had a higher survival rate on diets supplemented with salmon oil or 18:3n-3 (Phillips et al., 1963; Higashi et al., 1966; Lee et al., 1967). Additional evidence of the EFA requirements of fish came from the studies

with radio-labelled PUFAs (Owen et al., 1975; Kanazawa, 1985). Fish, like other vertebrates, are incapable of synthesizing de novo n-3 and n-6 PUFAs unless exogenous 18:2n-6 and 18:3n-3 are provided for freshwater species or 20:5n-3 and 22:6n-3 for marine species (Owen et al., 1975; Kanazawa, 1985). Overall, these studies suggested that 18:2n-6 and 18:3n-3 are EFA for freshwater fish and 20:5n-3 and 22:6n-3 for marine fish.

The first convincing evidence that fish require EFAs came from an extensive investigation of growth, feeding and physiological responses of rainbow trout to varying levels of dietary 18:2n-6 and 18:3n-3 (Castell et al., 1972 a, b, c). These findings were further supported by the studies of Watanabe et al. (1974 a, b, c). By 1993, there have been over a dozen freshwater and marine species studied with respect to EFA requirements.

The EFA requirements of freshwater fish may not correlate with their feeding habits and the requirement for n-3 PUFAs may not be as great for warmwater fish as for coldwater fish. In summarizing the EFA studies on fish, Kanazawa (1985) suggested that the EFA requirement of freshwater fish can be divided into three types: tilapia type (only 18:2n-6 as EFA); carp-type (both 18:2n-6 and 18:3n-3 as EFA); rainbow trout type (only 18:3n-3 as EFA). Further, the channel catfish (Ictalurus punctatus), a warmwater species, has an exclusive requirement for 18:3n-3 (Satoh et al., 1989b), while chum salmon (Oncorhynchus keta), a coldwater species, seems to

require both 18:2n-6 and 18:3n-3 which is the same as the requirements of the common carp (Cyprinus carpio) (Takeuchi et al., 1979). Takeuchi and Watanabe (1982) also suggested that the growth and lipid metabolism responses to dietary PUFAs differed between rainbow trout and coho salmon (Oncorhynchus kisutch), even though both species require 18:3n-3 as their EFA. Overall, this information suggests that the EFA requirements are species-specific for freshwater fish and that n-3 and n-6 PUFAs are important in diets of coldwater and warmwater fish.

Arctic charr, Salvelinus alpinus (L.), a coldwater species introduced to aquaculture for over a decade in Canada, has been primarily cultured up to their mature stage using regular trout or salmon commercial diets (Baker, 1983; de March and Baker, 1991). While the growth, feeding and genetic aspects of cultured Arctic charr were extensively investigated (Baker, 1983; Wallace and Aasjord, 1984; Tabachek, 1988; Jorgensen and Jobling, 1989; Tompkins, 1989), there is limited information on nutritional aspects of Arctic charr and their requirement for individual nutrients, such as EFA, essential amino acids, vitamin and minerals. The available information on the nutritional aspects of Arctic charr generally suggest that the trout or salmon diets available commercially are suitable but not optimum for the growth of Arctic charr and implies that the nutritional requirement of Arctic charr may differ from those of trout or salmon. Jobling and Wandsvik (1983) reported

that the protein requirement of Arctic charr ranged from 35 to 45% which is similar to the range for rainbow trout. While comparing effects of several commercial diets on growth and body composition of Arctic charr, Tabacheck (1984) noted that not all commercial trout or salmon diets produced are optimal for growth of Arctic charr. It was also shown that dietary lipids had an apparent protein-sparing effect on Arctic charr regardless of levels of dietary protein. These findings suggested that charr may be similar to coho salmon but different from trout in terms of the optimum ratio of dietary protein to lipids (Tabachek, 1986). Ringø (1989) also reported that the conventional commercial salmon diets supplemented with linoleic acid had negative effects on growth and protein and lipid digestibility of charr.

It is conceivable from available EFA studies on many fish species that the PUFA requirement of fish may be highly correlated with their geographical distribution. In nature Arctic charr have the most northern distribution, while the majority of salmonids are found in temperate regions (Johnson, 1980). Some charr migrate to sea on an annual basis during part of their life, while other salmonids only migrate once throughout their entire life. Therefore it is possible that charr may require higher dietary levels of PUFAs compared to other salmonids.

While this study was under way, the study by Ringø (1989) was published which suggested that the inclusion of a high

level of 18:2n-6 in charr diets led to a marked reduction in growth and digestibility of dietary protein and lipid, and possibly this was a consequence of altered intestinal microflora (Ringø, 1993). Olsen et al. (1991) using casein-based semi-purified diets examined the metabolism of dietary PUFAs in Arctic charr. Their results suggest that charr are capable of converting 18:2n-6 and 18:3n-3 to long-chain PUFAs and this species appears to require higher amounts of dietary n-3 PUFAs than other salmonids. At the end of this feeding experiment, Olsen and Ringø (1992) injected radio-labelled PUFAs into charr and they noted that these fish can extensively desaturate and elongate n-3 and n-6 PUFAs which corroborated the findings from their feeding experiment. However, the effect of dietary PUFAs on growth and the quantitative requirement of EFA are still unknown for Arctic charr.

Although the aquaculture industry relies on fish oil to provide dietary lipids for farmed fish, the traditional fisheries catch is rapidly decreasing. Consequently, there is a need to explore alternative lipid sources. Studies have suggested that the fat or oil from farmed terrestrial animals and plants are appropriate alternatives to fish oil (Yu et al., 1977). Since these lipid sources are diverse in their PUFA composition, the replacement of marine lipid sources with these alternative lipid sources depends to a large extent on the EFA requirements of cultured fish. Although Arctic charr

is a recently new cultured salmonid in Canada and Europe, an understanding of its EFA requirement is important to the aquaculture industry.

This study was undertaken to determine the EFA requirement of Arctic charr, mainly through examination of growth and feeding response and lipid metabolism of Arctic charr fed diets containing different levels of n-3 and n-6 PUFAs. The specific objectives of this study were:

- to examine symptoms of induced PUFA-deficiency in Arctic charr
- to investigate effects of varying levels of dietary n-3 PUFAs including 18:3n-3, 20:5n-3 and 22:6n-3 on growth and feed utilization
- to examine metabolism of dietary PUFAs in Arctic charr
- to compare the metabolism of dietary 18:3n-3 and 18:2n-6 for Arctic charr and rainbow trout
- to evaluate the interaction of dietary 18:2n-6 and 18:3n-3
- to examine the effects of selected dietary minerals, vitamin and amino acid on the assessment of EFA requirements of Arctic charr

Chapter 1.

Literature Review

1.1. Lipids of biological importance

Lipids include fatty acids and their derivatives and substances related biosynthetically or functionally to these compounds (Christie, 1989). The fatty acids are carboxylic acids, which consist of a hydrocarbon chain and terminal carboxylate group. Waxes and sterols, which are common in animal bodies, are also classed as lipids.

1. Fatty acids and eicosanoids

Fatty acids isolated from animal tissues generally contain even numbers of carbon atoms in straight chains varying in number from 14 to 22, but on occasion the chain length spans from 2 to 36 or even more (Christie, 1982). According to the number of double bonds, fatty acids are divided into two broad groups: saturated and unsaturated. The saturated fatty acids have no double bonds and their most abundant forms are straight-chain compounds with 14, 16 and 18 carbon atoms. The unsaturated fatty acids contain cis-double bonds in straight hydrocarbon chain and may be called monoenoic, dienoic, trienoic, ... hexanoic if one, two, three, ... six double bonds are present. The unsaturated fatty acids, containing more than one double bond separated by methylene groups, are often called polyunsaturated fatty acids (PUFA). According to the carbon position of the first double bond numbered from the methyl end, PUFA can be further divided into a series of PUFA families. For instances, n-3, n-6 and n-9 PUFA families, the

most common PUFA in animal tissues, contain the first double bond at number 3, 6 and 9 carbon counted from the methyl end, respectively.

Eicosanoids, derived from n-3, n-6 and n-9 eicosa- (C_{20}) PUFA, are comprised of the prostanoids and leukotrienes. Prostanoids include prostaglandins (PGs), prostacyclins (PGIs) and thromboxanes (TXs). Compared to saturated fatty acids, unsaturated fatty acids and eicosanoids have low melting points and are extremely vulnerable to oxidative deterioration or autoxidation because of the presence of double bonds.

2. Neutral and polar lipids

Lipids are frequently divided into two broad classes on the basis of their relative polarity: neutral and polar lipids. Neutral lipids are less polar and yield at most two types of primary products, fatty acids and glycerol on hydrolysis (Christie, 1982). Principal neutral lipids include free fatty acids, monoacylglycerols (MGs), diacylglycerols (DGs), triacylglycerols (TGs), cholesterol and cholesterol esters. The MGs, DGs and TGs contain one glycerol and one, two and three fatty acid components, respectively. In vertebrates TGs usually contain saturated fatty acids on the first and third carbon of glycerol, while another saturated or unsaturated fatty acid may be attached to the second carbon of glycerol.

Polar lipids include phosphoglycerides, glycolipids, sphingomyelins (or cardiolipin) and plasmolagens. Individual

polar lipids other than glycolipids contain a single phosphate group and are often termed as phospholipids. Principal phospholipids include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI). The saturated and monounsaturated fatty acids are frequently present in the first carbon position of the glycerol backbone in polar lipids with an PUFA in the second carbon position. It is thought that when phospholipids are exposed to oxidation, PUFA are protected, even if the saturated fatty acids or monoenes are oxidized.

2. Biological and nutritional roles

Phospholipids and cholesterol are part of cell membranes. Fatty acyl groups in phospholipids and cholesterol provide the hydrophobic interior part of cell membranes, which form an impermeable barrier to water and polar molecules. To a large extent, the physical properties of the membrane are determined by the fatty acid composition of individual phospholipids and their interactions with cholesterol and proteins, which may be enzymes or part of the cytoskeleton material. In fish and other poikilotherms, the degree of unsaturation of membrane fatty acids is directly correlated to the animal's adaptation to environmental temperature and salinity, ion permeability and function of membrane-bound enzymes such as Na^+- and K^+-ATPase (Bell et al., 1986).

In addition to being structural components, certain membrane phospholipids and their constituent fatty acids are

metabolically very active and involved in the cell signal transduction or biosynthesis of physiologically important eicosanoids (Mayes, 1990). Lipids are also the main energy sources in growth and development of animals. Excess energy in animal tissues can be converted into fatty acids and stored in adipose or other tissues in TG form, while tissue TGs can always be readily mobilized and converted back to energy to meet animal energy demand. Moreover, lipids are necessary for the blood transport of fat-soluble vitamins, such as vitamin A, E and K.

The majority of lipids in animal diets act as an energy source. In addition, n-6 and n-3 PUFA are essential to the normal development and growth of vertebrates and they are considered to be EFAs (Henderson and Tocher, 1987; Simopoulos, 1991).

1.2. Lipid metabolism in fish

1. Digestion and absorption of dietary lipids

Approximately 80 to 90% of lipids in fish and mammalian diets are TGs, while the remaining lipids are either free and esterified cholesterol or phospholipids. In mammals the entry of food into the duodenum stimulates the secretion of bile and pancreatic juice. Lipase and colipase from the pancreatic juice, forms a complex with bile salts, which acts on TGs and other lipids (Johnston, 1977). Similar processes are thought to occur in fish but the lipase seems to come from more diverse sources, such as pancreas, pyloric caeca, stomach and intestine (Henderson and Tocher, 1987). There are two types of lipase in fish, colipase-dependent and bile-salt-dependent lipase, which attack micellar and emulsified lipids, respectively (Patton et al., 1977; Leger, 1985). In teleost, the digestion of dietary TGs results primarily in free fatty acids and glycerol (Robinson and Mead, 1973; Leger, 1985), while a similar process in mammals produces free fatty acids and 2-MGs (Johnston, 1977).

In mammals the micelles containing the lipolytic products and bile salt are passively diffused or actively transported to the intestinal villus, where fatty acids and monoacylglycerols penetrate the brush border of the enterocyte and the bile salt is left in the lumen (Mayes, 1990). The free fatty acids from hydrolysis of dietary TGs are absorbed in the

pyloric caeca and proximal intestine in fish but the importance of these locations are mainly dependent on fish feeding habits. For instance, the primary site of lipid absorption is the pyloric caeca in carnivorous fish including salmonids (Ezeasor and Stokoe, 1981) and the midgut regions in herbivores such as goldfish (Iwai, 1968). The mechanism of lipid absorption in fish is unknown.

The absorbed dietary fatty acids in fish are esterified as TGs or phospholipids through the glycerol 3-P pathway within the intestinal cells (Sire et al., 1981; Patton et al., 1978). The esterified lipids subsequently combine with protein to form chylomicron-like particles in the endoplasmic reticulum and Golgi vesicles. These particles are excreted into intracellular space and carried to tissues or organs through blood or by the lymph (Leger, 1985). The lipoproteins reaching the liver are likely transported via the portal vein in carp, and by both the portal vein and lymph in trout (Henderson and Tocher, 1987).

The absorption of fatty acids in fish is affected by the nature of the fatty acids. The long-chain saturated fatty acids are less readily, or less completely absorbed than the shorter-chain or the more highly unsaturated fatty acids (Leger, 1985). Other factors such as temperature, fish size and level of dietary lipid also influence the digestibility of dietary lipids. For instance, in both channel catfish (Ictalurus punctatus) and rainbow trout, lipid digestibility

increases with increasing temperature (Atherson and Aitken, 1970; Andrews et al., 1978).

2. Biosynthesis of lipids

Vertebrates are capable of endogenously synthesizing fatty acids, in addition to incorporating dietary fatty acids into body lipids. The principal sites of lipogenesis vary among fish species (Henderson and Tocher, 1987).

The sites for lipid uptake and storage vary among fish species. For instances, in salmonids, the peri-visceral adipose tissue is the main site for lipid uptake and storage (Lin et al., 1977; Henderson and Sargent, 1981). Other species such as herring and capelin appear to have more obvious sub-cutaneous lipid depots (Sargent et al., 1989). Demersal fish species like cod generally store lipids in their livers (Henderson and Tocher, 1987).

1.) Saturated fatty acids

Fish are capable of de novo synthesis of saturated fatty acids. The process begins with the carboxylation of a two carbon acetyl-CoA unit to malonyl-CoA by acetyl-CoA carboxylase and biotin (as a cofactor). The malonyl-CoA is then converted to fatty acids by fatty acid synthetase complex via a series of condensation and reduction reactions involving the utilization of NADPH (Henderson and Tocher, 1987). Since propenyl-CoA can replace acetyl CoA as a primer of fatty acid synthesis, odd-numbered fatty acids may also be produced by the same enzyme complex. However, the primary products from

these pathways are fatty acids with an even number of carbon units. For example, the radioactivity from ^{14}C-acetate incorporated into fatty acids by trout liver is mainly in 16:0, while it is primarily distributed in 16:0 and 18:0 in plaice (Pleuronectes platessa) (Henderson and Tocher, 1987).

The study by Voss and Jankowski (1986) found that the rates of fatty acid synthesis are similar between trout and rat hepatocytes. However, the rate of fatty acid synthesis from alanine in fish is markedly greater than that from glucose when the two substrate are supplied at equal concentrations. This finding suggested that protein is preferentially utilized over carbohydrate for lipogenesis (Henderson and Sargent, 1981). This differences may reflect the dietary requirement of the fish, i.e., diets should contain low carbohydrate and high protein content.

2.) Unsaturated fatty acids

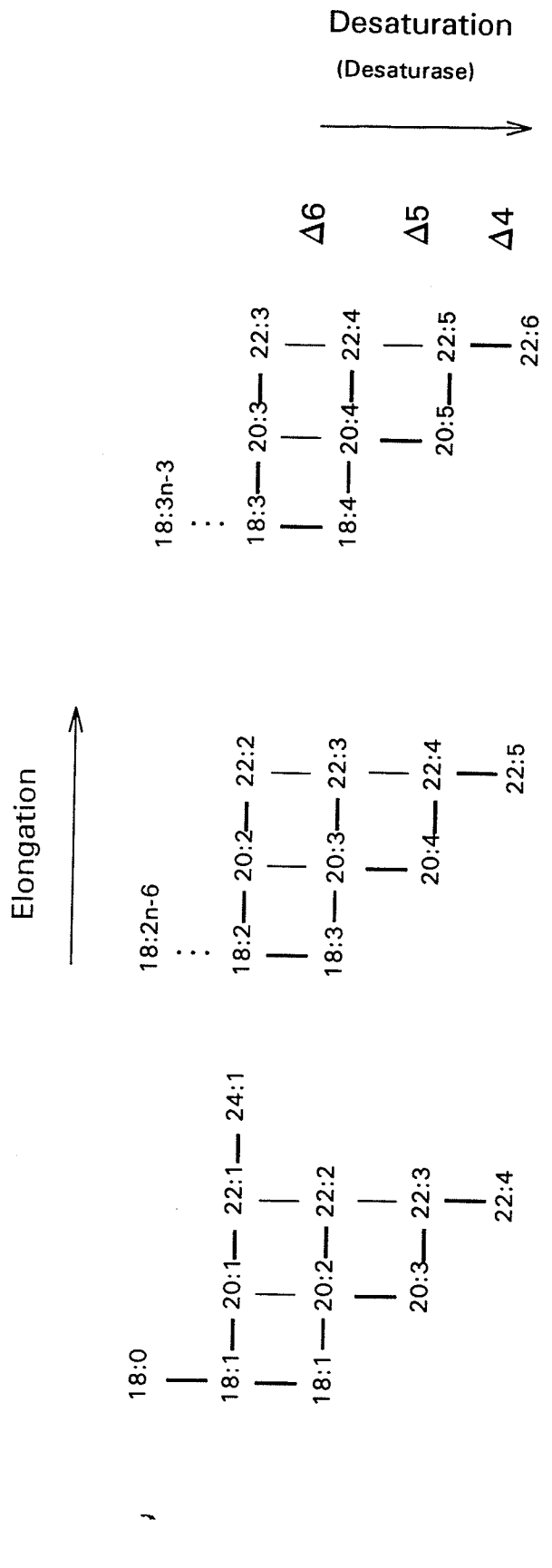
While the fatty acids formed de novo through the fatty acid synthetase complex are saturated fatty acids, fish lipids are characterized by their abundant monoenes and PUFA. Extensive studies have revealed that, in addition to incorporating dietary unsaturated fatty acids into body lipids, fish are capable of modifying both dietary and endogenously synthesized unsaturated fatty acids through desaturation and elongation (Henderson and Tocher, 1987).

The desaturation and elongation pathway established for mammals, may operate in fish tissues as well (Henderson and

Tocher, 1987) (Fig. 1.). Like other vertebrates fish do not possess Δ^{12} and Δ^{15} desaturases, the enzymes required for the synthesis of 18:2n-6 and 18:3n-3, respectively but they do possess a series of desaturases and elongases which include Δ^{9}, Δ^{6}, Δ^{5} and Δ^{4} desaturases for the metabolism of 18:1n-9, 18:2n-6 and 18:3n-3 to long-chain PUFA. Each desaturase may contain three integral components: desaturase, cytochrome b_5 and cytochrome b_5 reductase. The reaction at the Δ^{6} desaturase step is considered rate-limiting (Henderson and Tocher, 1987). Although the presence of Δ^{9}, Δ^{6}, Δ^{5} desaturases have been confirmed for mammalian tissues, there has been no evidence for the presence of Δ^{4} desaturases. According to Voss et al. (1991), 22:6n-3 in mammalian liver may be generated by the β oxidation of 24:6n-3.

Studies with fish also indicate that the Δ^{6} desaturase has preference for substrate: 18:3n-3 > 18:2n-6 > 18:1n-9. On the other hand, the subsequent products from these reactions, i.e., long-chain PUFA, can exert competitive or feedback inhibition on desaturation (Yu and Sinnhuber, 1976, 1979). When 18:1n-9 becomes the only substrate available for Δ^{6} desaturase, enzymes involved in this pathway metabolize n-9 PUFA which leads to increased synthesis of 20:3n-9 in animal tissues, particularly livers. Similarly, there are elevated levels of 20:3n-9 or a high ratio of 20:3n-9 to 22:6n-3 in tissues of fish fed diets without both n-6 and n-3 PUFA (Castell et al., 1972c; Watanabe et al., 1974b). This is of

Fig. 1. Pathways for desaturation and elongation of polyunsaturated fatty acids in fish


Desaturation
(Desaturase)
Δ6
Δ5
Δ4
Elongation
Family
n-9
n-6
n-3
18:0
18:1
20:1
22:1
24:1
18:1
20:2
22:2
20:3
22:3
22:4
18:2n-6
18:2
20:2
22:2
18:3
20:3
22:3
20:4
22:4
22:5
18:3n-3
18:3
20:3
22:3
18:4
20:4
22:4
20:5
22:5
22:6

significance for assessment of EFA requirements of fish (Watanabe, 1982).

Comparative studies with rats and trout revealed that when trout hepatocytes were incubated at 12 °C and those from rat at 37 °C, both species had similar Δ^6 desaturase activity, but trout had twice the Δ^5 activity of the rat (Hagve et al., 1986). Fish differ from other vertebrates by having a large variation in Δ^6 desaturase activity. Extensive studies involving feeding or injection of labelled PUFA clearly show that the activity of Δ^6 desaturase in marine fish is much lower than freshwater fish, (Castell et al, 1972a, c; Owen et al., 1975; Kanazawa et al., 1979). Among freshwater fish, coldwater species such as rainbow trout appear to have a higher Δ^6 desaturase activity than warmwater species such as ayu and eel (Kanazawa, 1985).

While 22:6n-3 to 22:5n-3 and 20:5n-3 may be retroconverted to other fatty acids in mammals, fish are incapable of retroconverting 22:6n-3 (Schlenk et al., 1969; Henderson and Tocher, 1987).

3.) TGs and phospholipids

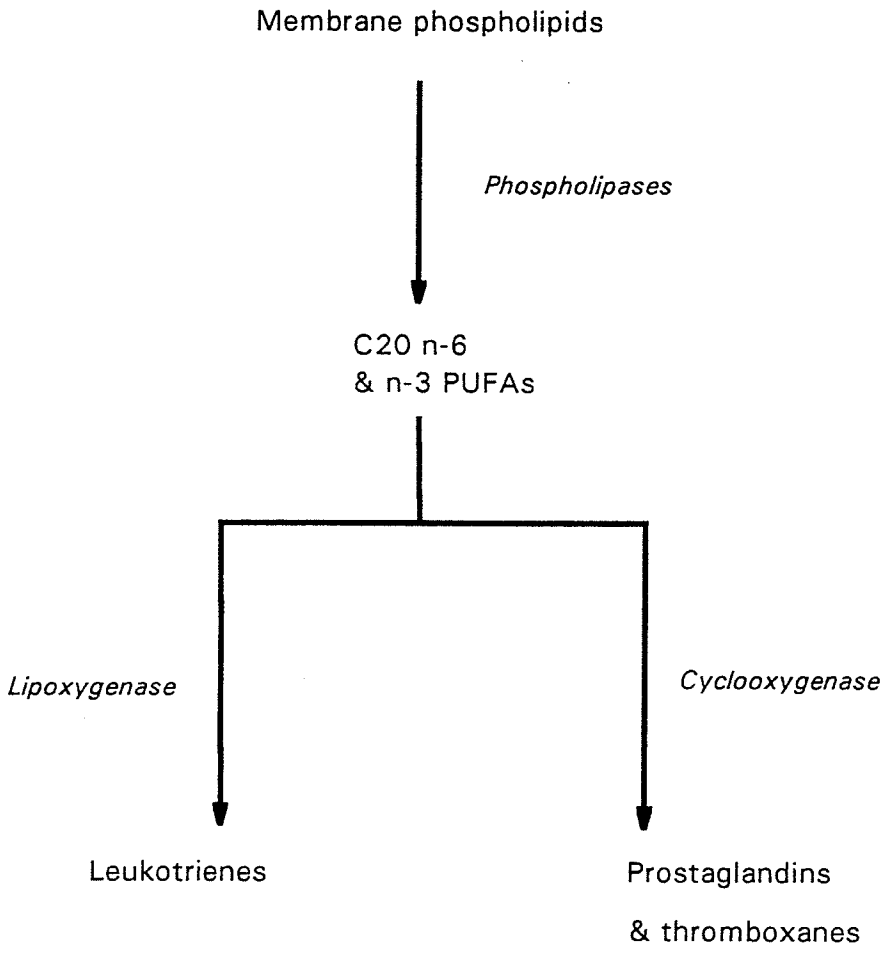
Fatty acid products from the hydrolysis of dietary lipids are re-esterified into TGs in the intestinal epithelium of fish (Sire et al., 1981). This also appears to be the case in fish adipose tissue and muscle (Henderson and Tocher, 1981). The synthesis of TGs and phospholipids, to a large extent, occurs by the glycerol-3-phosphate and CDP-choline pathways in

fish, respectively (Greene and Selivonchick, 1987). The enzymes involved in phospholipid synthesis are located in the microsomal membrane fraction in fish (Holub et al., 1975; Leslie and Buckley, 1975).

4.) Eicosanoids

Certain fatty acids derived from cell membrane phospholipids give rise to eicosanoids which include physiologically active compounds known as prostaglandins (PGs), thromboxanes (TXs), and leukotrienes (LTs). These eicosanoids are synthesized through cyclooxygenase and lipoxygenase pathways from the essential fatty acids, i.e., linoleate, arachidonate, and linolenate, respectively (Fig. 2.). The major pathway is usually from arachidonic acid which gives rise to the group two eicosanoids. Like mammals fish can synthesize 1, 2 and 3 series of PGs, TXs and LTs through cycloxygenase and lipoxygenase (Bell et al., 1986; Henderson and Tocher, 1987). The PG synthesis from exogenous or endogenous fatty acids is detectable in virtually every fish tissue so far studied. Despite the preponderance of long-chain n-3 PUFA in fish tissues, 20:4n-6 appears to be the dominant substrate for the eicosanoid synthesis in fish (Bell et al., 1986). It is possible that the relative rates of PG synthesis from 20:4n-6 and 20:5n-3 in fish are governed by their rates of release from phospholipids, particularly phosphatidylinositol (Henderson and Tocher, 1987).

Fig. 2. Pathways for eicosanoid formation in fish


Membrane phospholipids
Phospholipases
C20 n-6
& n-3 PUFAs
Lipoxygenase
Cyclooxygenase
Leukotrienes
Prostaglandins
& thromboxanes

Available data indicate that eicosanoids perform similar physiological functions in fish as they do in mammals (Greene and Selivonchick, 1987). PGs appear to be directly involved in ovulation and act on the brain to elicit behavioral changes such as spawning activities (Sargent et al., 1989; Sveinsson, 1992). There is also evidence that the TX_2/PG_2 balance plays a role in the control of thrombocyte aggregation and blood clotting in fish (Henderson and Tocher, 1987).

3. Lipid transport

In general, vertebrates transport lipids obtained from their diet or endogenously synthesized, to extrahepatic tissues through the circulatory system in complexes with protein, called lipoproteins. The main classes of lipoproteins include chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). Two routes are known to be responsible for transport of lipoproteins in mammals (Mayes, 1990). One is responsible for the transport of absorbed dietary lipids to adipose tissue where they are converted into storage lipids or to other tissues where they are utilized as energy. The second route is for the transport of lipids mobilized from adipose tissue or newly synthesized in the liver.

Fish also transport lipids in lipoproteins (Babin and Vernier, 1989) and the intestine and liver are the primary sites for production of lipoproteins (Sire and Vernier, 1979;

Rogie and Skinner, 1985; Babin and Vernier, 1989). Free fatty acids from mobilization of lipids or from the hydrolysis of lipoproteins are transported in association with an albumin-like protein (Henderson and Tocher, 1987). However, the lipoproteins in the majority of fish species investigated are markedly higher in PUFAs, particularly 20:5n-3 and 22:6n-3, compared to those of mammals (Leger et al., 1985).

Excessive deposition of lipids may occur in the liver of vertebrates and this is often regarded as a pathological sign of an essential fatty acid deficiency (Mayes, 1990). When lipid accumulations become chronic (i.e., fatty liver), fibrotic changes may progress to cirrhosis and impaired liver function. In mammals, the fatty livers are considered to be due to the depressed synthesis of phospholipids and competition by other components such as cholesterol and EFAs for esterification (Mayes, 1990). By contrast, the development of fatty liver in fish seems to be correlated to impaired lipoprotein biosynthesis (Henderson and Tocher, 1987).

4. Catabolism and mobilization of lipids

Fatty acids transported to extrahepatic tissues are used either for provision of energy through oxidation or storage after esterification. When they are used for energy, free fatty acids are oxidized in the mitochondria to produce ATP by using NAD and FAD as coenzymes. Depending upon the position of the double bonds, the CoA esters of unsaturated fatty acids

are degraded by the enzymes responsible for β-oxidation to Δ^3-cis-acyl-CoA or Δ^4-cis-acyl-CoA. The oxidation of long-chain fatty acids (eg, C20, C22) is aided by a modified form of β-oxidation in peroxisome. However, fatty acid oxidation is reduced when animals are fed high-carbohydrate diets.

The fatty acid oxidation in fish seems to proceed mainly via a β-oxidation system, similar to that operating in mammals (Henderson and Sargent, 1985). However, fish are capable of utilizing a broader spectrum of fatty acid derivatives for β-oxidation than mammals (Henderson and Tocher, 1987). The rate of β-oxidation in fish is substantially lower in peroxisome than in mitochondria, which is also the case in mammals under normal physiological conditions. In addition, the long-chain PUFA such as 22:6n-3 are oxidized at a much lower rate than saturates or monoenes such as 18:1 and 16:1 (Henderson and Sargent, 1985). Perhaps this ensures that PUFA are specifically retained to fulfil their role as a major constituent of biomembrane phospholipids.

When animals pass from a fed to a fasting state, lipids from the adipose tissue are mobilized as free fatty acids and glycerol through the action of lipoprotein lipase. During prolonged starvation, the increasing oxidation of fatty acids results in an increase of ketone bodies in the liver. The ketone bodies are transported from the liver to extrahepatic tissue where they are converted back to acetyl-CoA for further oxidation or lipogenesis.

Many fish species go through a period of fasting in winter or during spawning. Periods of gonad maturation and migration of anadromous fish, usually occurs without feeding but relies on the energy supplies from their lipid reserves (Sheridan, 1988). In general, fish can survive much longer periods of starvation than mammals. Visceral adipose tissue is the most mobile store of lipids during starvation for most fish species.

The release of fatty acids from storage lipids is selective. TGs are always mobilized before phospholipids from lipid depots. Saturated fatty acids are preferentially released from visceral lipids and monounsaturated acids, including 16:1, 18:1n-9 and 20:1n-9, from muscle and liver lipids (Henderson and Tocher, 1987). The subsequent decrease in 18:1n-9 from body and liver lipids usually leads to an increase in the relative proportions of 22:6n-3 in the remaining TGs and phospholipids of fish tissues (Takeuchi and Watanabe, 1982).

4. Protective mechanisms against autoxidation of PUFA

PUFA, the predominant component in fish body lipids, are highly susceptible to oxidative deterioration (Sargent et al., 1989). This autoxidation affects cell membrane fluidity and hence cell function. The compounds derived from PUFA autoxidation are highly toxic and harmful to fish but autoxidation is usually avoided with the cellular self multi-level defence system. Factors involved in the defense system

include: i) Copper, zinc and manganese as functional components of the superoxide dismutases, ii) selenium as a component of peroxidase and iii) vitamin E as a scavenger of free radicals (Bell and Cowey, 1985).

1.3. Essential fatty acid requirements of fish

1. General aspects

Requirements of vertebrates for essential fatty acids (EFAs) were first obtained from studies using mammals. Burr and Burr (1930) discovered that rats fed diets devoid of fat developed poor growth, scaly skin, alopecia, impaired energy metabolism, excess water loss, bleeding and other symptoms. By comparison, the first discovery of EFA requirement in fish occurred in the 1960's. Two coldwater fish species, brook trout (Salvelinus fontinalis) and rainbow trout fed diets with corn oil (rich in 18:2n-6) as the sole lipid source, exhibited poor growth and high mortality, while those fed diets with salmon oil or 18:3n-3 showed better growth and higher survival rate (Philipis et al., 1963; Higashi et al., 1966; Lee et al, 1967). These results suggested that these two fish species required n-3 PUFA as their EFAs.

To date, extensive studies on the EFA requirements of more than a dozen fish species indicate that the EFA requirement is species-specific (Sargent et al., 1989). The lipids for the aquaculture feed industry come from a variety of sources including plants and aquatic and terrestrial animals, and differ markedly in EFA values. Consequently there is a need to accurately assess the EFA requirement of each cultured fish species.

2. Approaches to define EFA requirement

Two approaches have been widely employed to estimate EFA requirements of fish. One approach is to feed fish with varying levels of n-3 and n-6 PUFAs and assess subsequent growth and feed utilization of fish, along with their histological and tissue lipid changes (Watanabe, 1982). Another approach is the in vivo administration of labelled PUFA to examine the animal's ability to desaturate and elongate n-3 and n-6 PUFA (Kanazawa, 1985).

1.) Feeding

This is the most definitive approach to determine the EFA requirements of animals. It begins with the acclimation of subjects to fat-free or PUFA-free diets and is followed by the feeding of experimental diets varying in PUFA composition (usually semi-purified). Casein and gelatin are usually included in diets as protein sources and starch, dextrin or glucose as carbohydrate source, while dietary lipids are given in the form of highly pure methyl or ethyl esters of fatty acids. Young animals are usually preferred because the metabolism of fatty acids in adult animals is slower than in young animals and the cost of feeding young animals is much less. Feeding trials with experimental diets may last until certain deficiency symptoms or differences in growth or other responses become evident among treatments.

The length of acclimation of fish varies from weeks to months, while feeding trials usually last 10 to 24 weeks

(Castell et al., 1972a; Watanabe et al., 1974; Throngrod et al., 1992). It appears that the 10-12 weeks of feeding with experimental diets is sufficient for detecting the EFA requirements of young fish (Takeuchi and Watanabe, 1982). The majority of recent studies has adopted a protocol of 2 to 4 weeks of acclimation and 8 to 16 weeks of feeding experimental diets (Satoh et al., 1989; Throngrod et al., 1990a, b).

Responses monitored by the EFA studies include animal growth, feed intake and efficiency, and health. At the end of the feeding trial animals are usually sacrificed to determine tissue protein, carbohydrate, water content, lipid and fatty acid composition. However, conclusions on the qualitative and quantitative EFA requirements of the animals tested are primarily based on factors such as growth, feed utilization and histological changes (Cowey, 1988).

2.) Labelled PUFA <u>in vivo</u>

The EFA requirements of animals are governed by their abilities to metabolize PUFA to a large extent. The animal's ability to convert dietary PUFA can be directly evaluated by monitoring the metabolites of labelled PUFA. To date, the conversion of 18:2n-6 and 18:3n-3 into long-chain PUFAs has been analyzed for both marine and freshwater species. However, labelling studies do not provide quantitative information on EFA requirements (Kanazawa et al., 1979; Yamada et al., 1980; Kanazawa, 1985).

3. EFA deficiency symptoms

Deficiencies of 18:2n-6 or 18:3n-3 are rare in mammals but may be of concern in premature infants where catabolism and utilization of EFAs are rapid while their reserves are limited (Innis, 1991). The general symptoms of n-6 PUFA deficiency in mammals are listed as follows (Kinsella et al., 1990):

- reduced growth rates and increased water loss via skin
- increased susceptibility to bacterial infections
- male and female sterility and decreased eicosanoid synthesis
- reduced contraction of myocardial tissue
- abnormal platelet aggregation and impaired monocyte and macrophage function

It is also apparent that a low intake of 18:3n-3 induces poor physical activity and ability to learn, lower visual acuity thresholds and abnormal electroretinograms in monkeys (Anderson and Conndor, 1989) and a low level of 22:6n-3 in rat brains (Walker, 1967). It is clear that the synthesis of eicosanoids from linoleic acid does not account for all essential fatty acid requirements. The majority of these symptoms may reflect different roles of linoleic acid, i.e., as a membrane component, as an integral component of skin acylglucoceramides, as precursor of arachidonic acids and eicosanoids.

Feeding fish with PUFA-deficient diets often results in the following adverse changes (Watanabe, 1982):

- Poor growth and low feed efficiency
- Increased water content in whole body, muscle and viscera
- Pale and swollen liver and increased hepatosomatic index
- vertebrate column curvature
- Increased respiration rate and swelling rate of mitochondria
- Elevated ratio of neutral lipid / polar lipid, and 20:3n-9/22:6n-3 in polar lipids
- Fin erosion and loss of consciousness after sudden shocks

In addition, low fecundity and fertilization and poor hatchability of fish eggs may occur where fish broodstock are EFA-deficient (Watanabe, 1982).

The ratio of 20:3n-9/20:4n-6 was originally proposed as an index of EFA status and linoleic acid deficiency for mammals by Holmman (1960). Although a complete profile of tissue fatty acid composition is preferred (Holman et al., 1991), the ratio of 20:3n-9/22:6n-3 appears to be a useful measure of mammalian n-6 EFA deficiency. It has been widely adopted for fish to monitor EFA deficiency but it has often been critically reviewed (Cowey, 1979; Watanabe, 1982; Henderson and Tocher, 1987). To a large extent the use of this index is complicated by the competitive interaction among n-3, n-6 and n-9 PUFAs and other biochemical and nutritional factors. For instance, the factors affecting Δ^5 desaturase may affect desaturation of 20:2n-9 to 20:3n-9 and 20:3n-6 to 20:4n-6 equally, while the ratio remains constant. Consequently, caution is recommended

in applying this ratio as a sole index of EFA status. To date it is often applied for assessing the n-3 PUFA requirement of fish, along with the growth response and histological and biochemical parameters.

4. Qualitative and quantitative requirements of EFA

The melting points of n-3 PUFAs are generally lower than n-6 PUFA of equal carbon chain length. Fish (poikilotherms) have higher percentages of tissue n-3 PUFA but lower body temperatures than mammals (homeotherms), suggesting that the PUFA requirements of fish are different from mammals. To date, more than a dozen fish species have been studied and results suggest that freshwater fish display three types of EFA requirements, namely, rainbow trout type requiring n-3 PUFAs; carp type requiring both n-3 and n-6 PUFAs; tilapia type requiring n-6 PUFA. Marine fish on the other hand, have an exclusive requirement for n-3 long-chain PUFAs (Table 1.).

Among freshwater fish requiring n-3 PUFAs only, rainbow trout is the most extensively studied for its EFA requirements and has the most efficient desaturase and elongase system for converting 18:3n-3 to long-chain PUFAs (Kanazawa, 1985). Dietary n-3 long-chain PUFAs appear to be more than twice as effective as 18:3n-3 in enhancing growth for several species compared to dietary 18:3n-3 (Watanabe et al., 1977; Satoh et al., 1989b). In fish requiring both n-3 and n-6 PUFAs, the 1.0% dietary n-3 long-chain PUFA have the same growth-

Table 1. Published information on the essential fatty acid (EFA) requirements of fish species

Type	Species	Requirement		Total lipids (% dry diet)	Reference
		EFA	% (dry diet)		
Freshwater					
Trout	Rainbow trout, Oncorhychus mykiss	18:3n-3	1.0	2.0	Castell et al., 1972.
			0.83-1.66	5.0	Watanabe et al., 1974.
		or n-3 LCP[1]	0.5	5.0	Takeuchi and Watanabe, 1977.
	Coho salmon, Oncorhychus kisutch	18:3n-3	1.0-2.5	10.0	Yu and Sinnhuber, 1979.
	Cherry salmon, Oncorhychus masou	18:3n-3	1.0	5.0	Throngrod et al., 1990b.
	White fish, Coregonus lavaretus maraena	18:3n-3	> 2.0	5.0	Throngrod et al., 1990a.
		or n-3 LCP	1.0		
	Channel catfish, Ictalurus punctatus	18:3n-3	1.0	5.0	Satoh et al., 1989.
		or n-3 LCP	0.5		
	Ayu, Plecoglossus altivelis	18:3n-3	1.0	?	Kanazawa et al., 1985.
		or 20:5n-3	1.0		
	Milkfish, Chanos chanos	18:3n-3	2.0	7.0	Borlongan, 1992
Carp	Common carp, Cyprinus carpio	18:2n-6	1.0	5.0	Takeuchi and Watanabe, 1977.
		+ 18:3n-3	1.0		
	Grass carp, Ctenopharyngodon idella	18:2n-6	1.0	5.0	Takeuchi et al., 1991.
		+ 18:3n-3	0.5-1.0		

... Continued

Table 1 (continued)

	Eel, Anguilla japonica	18:2n-6 + 18:3n-3	0.5 0.5	7.0	Takeuchi et al., 1980.
	Chum salmon, Oncorhychus keta	18:2n-6 + 18:3n-3	1.0 1.0	5.0	Takeuchi et al., 1979.
Tilapia	Tilapia zillii	18:2n-6 or 20:4n-6	1.0 1.0	5.0	Kanazawa et al., 1980
	Tilapia nilotica	18:2n-6	1.0	5.0	Takeuchi et al., 1983.
Marine					
	Red sea bream, Chrysophrys major	20:5n-3 + 22:6n-3	1.0 0.5	≈ 11.0	Takeuchi et al., 1992b, c
	Striped jack, Longirostris delicatissimus	20:5n-3 + 22:6n-3	< 0.8 1.7	10.8	Takeuchi et al., 1992a.
	Turbot, Scophthalmus maximus	n-3 LCP	0.8	10.0	Gatesoupe et al., 1977.
	Red drum, Sciaenops ocllatus	n-3 LCP	0.5	7.0	Lochmann and Gatlin III, 1993.

[1]Long-chain PUFAs including 20:5n-3 and 22:6n-3

enhancing effect as 1.0% dietary 18:3n-3 (Takeuchi and Watanabe, 1977c; Takeuchi et al., 1980). Supplementing diets with n-3 PUFA may lead to vertebral column curvature (Takeuchi et al., 1991). There is evidence that two tilapia species may require only 18:2n-6 as their EFAs (Kanazawa et al., 1980; Takeuchi et al., 1983).

It was always thought that marine fish require C20 or C22 n-3 PUFA. However, recent studies indicate that marine fish can convert 20:5n-3 to 22:5n-3 and to a limited extent only to 22:6n-3 but they have limited ability to retroconvert 22:6n-3 to 22:5n-3 or 20:5n-3 (Takeuchi et al., 1992 a, b). Moreover, 22:6n-3 is superior to 20:5n-3 in enhancing growth and the requirement for 20:5n-3 by marine fish is lower than for 22:6n-3 (Takeuchi et al., 1992 a, b). These facts suggest that marine species require both 20:5n-3 and 22:6n-3. Consequently, the ratio of 20:3n-9 to 22:6n-3 is not suitable for the assessment of the EFA status in marine fish. Instead, a ratio of total 18:1 monoenes to n-3 long-chain PUFA may be a more suitable index for EFA status of marine fish and their EFA requirements are considered satisfied once the ratio is below 1.0 (Takeuchi et al, 1992a, b).

Although n-6 PUFA are not considered to be EFA for marine fishes, there is a substantial amount of 20:4n-6 in their tissue phosphotidylinositol which is metabolically important to animals (Bell et al., 1986). This finding suggests that marine fishes may also need C20 n-6 PUFA.

Most of the EFA requirements summarized above come from the studies on juvenile fish. By comparison, there have been fewer studies on newly hatched larvae. Data suggest that fish require certain types of phospholipid for growth, development and survival at their larval stages which is different from the requirements of juveniles and adults. For instance, ayu and striped jack had better growth and survival rates when they were fed diets supplemented with phosphatidylcholine (Kanazawa, 1985; Takeuchi et al., 1992b). However, the reason behind this remains unclear.

The EFA requirements of anadromous fish are similar for the freshwater and marine life stages, although body fatty acid composition changes during parr-smolt transformation (Takeuchi and Watanabe, 1982).

Watanabe (1982) suggested that the precise EFA requirement of fish should be defined on the basis of a given level of dietary lipid. This is largely based on that fact that the EFA requirement of fish changes with levels of dietary lipids. Consequently, the requirement of rainbow trout for 18:3n-3 is defined at about 20% of dietary lipids (Takeuchi and Watanabe, 1977b).

Results from feeding studies on EFA requirements are sometimes misleading. For example, several studies have suggested that channel catfish require 18:2n-6 but have a low requirement for 18:3n-3 (Stickney and Hardy, 1989). However, a recent study by Satoh et al. (1989b), suggests that catfish

have an exclusive requirement for 18:3n-3. Kanazawa et al. (1980) found that tilapia grew well on diets rich in menhaden oil (rich in n-3 PUFA) but Takeuchi et al. (1983) reported that n-3 long-chain PUFA had no EFA value for tilapia. It is evident that the EFA requirements of these species need clarification and the prevention of oxidation of dietary lipids appears to be important (Satoh et al., 1989a).

Comparisons of the fatty acid compositions of wild and cultured fish are also useful for obtaining information on EFA requirements. For instance, Atlantic salmon (Salmo salar) caught in the wild, have markedly higher levels of 20:4n-6 than those reared in the hatchery, despite the availability of 18:2n-6 in the artificial diets (Ackman and Takeuchi, 1986). This difference may account for the poor health (fin erosion) of these fish when released to the wild.

1.4. Life stages and feeding of wild Arctic charr

Freshwater Arctic charr have the most northern distribution of all freshwater fish species and they are widespread in Arctic and subarctic regions of North America, Europe, and Asia (Johnson, 1980). In Canada, Arctic charr distribute in Newfoundland, Quebec, throughout the Northwest Territories and many other regions (Johnson, 1980). The charr populations used in this study were collected originally from two locations within the sub-Arctic region: Fraser River, Labrador (56°39'N, 63°10'W) and Nauyuk lake, Northwest Territories (68°22'N; 107°35'W). In many sub-Arctic regions, Arctic charr coexists with other fish species. For instance, charr coexists with lake trout (Salvelinus namaycush) and lake whitefish (Coregonus clupeaformis) in Nauyuk lake and with lake trout and brook trout in the Labrador region. Arctic charr in northern Labrador may also hybridize with lake trout and brook trout (Hammar et al., 1989; Hammar et al., 1991).

Charr in the wild are extremely plastic with respect to morphology and life history. Two forms of Arctic charr have been identified, i.e., a completely freshwater (landlocked) form and an anadromous form, which migrate to sea on an annual basis. Charr do not spawn each year, but on an intermittent basis, once every two or even four years (Dempson and Green, 1985; Johnson, 1989). The mean age of charr at maturation generally increases with latitude. This age has been reported

to be 6.9 years for the Labrador population (Dempson and Green, 1985) and 13.2 years for the Nauyuk Lake population (Johnson, 1989). Maturing anadromous charr usually move upstream and overwinter in a lake and then spawn the following fall. While landlocked and anadromous forms may be distinguishable morphologically, there is a high probability of reciprocal pairing between these groups in nature.

1. Life stages of anadromous charr

The fertilized eggs of Arctic charr overwinter on gravel in lakes. The fry emerge about 8 months later and live in the shallow waters. Juvenile anadromous charr grow in freshwater until they reach certain size or age and then migrate to sea in late spring (Johnson, 1980). After summer feeding in the sea, charr return to freshwater in the fall. Rapid growth of Arctic charr is characteristic of the brief summer periods of intensive feeding in the lake or inshore marine waters (Johnson, 1980).

2. Feeding in freshwater

The types of food consumed by Arctic charr in sub-Arctic freshwater fluctuates seasonally and annually and are to a large extent correlated with the availability of their food sources. In Europe charr feed on hatching and flying insects, mainly chironomids during the summer and fall and on Gammarus, Plecoptera larvae and chironomid larvae during the winter

(Johnson, 1980; Amundsen and Klemetsen, 1988). In sub-Arctic lakes feeding of Arctic charr appears to be related to the presence or absence of other Salvelinus species. Arctic charr in the absence of other fish species consume a variety of food items ranging from fish to invertebrates (aquatic insect larvae and pupae, gastropods, gammarids and zooplankton) (Fraser and Power, 1984). In contrast, when lake trout and brook charr are present, charr were noted to consume a much higher proportion of gastropods, gammarids and zooplankton (Fraser and Power, 1984). In addition, the composition of the charr diet changes with their size. For example, during the summer riverine Arctic charr < 10 cm in the Arctic region prey mainly on larval chironomidae (Eukiefferiella bavaria), while those longer than 10 cm feed on other charr (Moore and Moore, 1974; Sparholt, 1985). During the winter these small charr have empty stomachs, while the large charr feed on other fish species (Moore and Moore, 1974). This suggests that Arctic charr are opportunistic feeders in environments where food sources are limited.

3. Feeding in the sea

Arctic charr in the marine environment seem to be less opportunistic feeders than in freshwater. In the sub-Arctic regions, anadromous charr mainly feed on invertebrates, including amphipods (Parathemisto libellula), euphausiid shrimp (Megsnyctiphanes norvegica) and fish such as capelin

(Mallotus villosus), the young mailed sculpin (Triglops pingeli) and sandlance (Ammodytes americanus) (Andrews and Lear, 1956; Adams et al., 1989). In the Arctic region, Arctic charr primarily prey on amphipods, copepods and fish. Their predominant food species is Parathemisto libellula, often accounting for more than 50% of charr stomach content by dry weight (Moore and Moore, 1974). Small charr (447 mm mean length) feed mainly on amphipods and Mysis oculata and large charr (665 mm) feed on polar cod (de March et al., 1978). Changes in species composition of stomach contents with the size of Arctic charr reflect their selection of prey size.

1.5. Lipids of wild coldwater species and their prey

1. General aspects

The lipid and fatty acid compositions of wild fish are influenced by water temperature, their diets and life stages. It has been reported that there is an increase in the proportion of tissue unsaturated fatty acids in fish during their acclimation to cold environment (Hazel, 1979; Sellner and Hazel, 1982). To date available data also indicate that fish selectively incorporate and modify PUFAs of their food items such as phytoplankton, zooplankton or other fish species. In addition, profiles of fatty acid composition of fish differ largely between their life stages.

2. Seasonal change

A number of coldwater fish species including several salmonid and coregonid species, along with their prey, have been investigated for seasonal variation in lipid and fatty acid composition (Agren et al., 1987; Muje et al., 1989; Ringø and Burkow, 1990; Linko et al., 1992). In general, these species have marked seasonal fluctuation in levels of total lipids and proportions of polar and neutral lipids. In the flesh of Arctic charr and vendace (Coregonus albula, L.), total lipid content and proportions of triacylglycerols gradually increase in the early summer, peak at the end of summer and decline rapidly thereafter (Ringø and Burkow, 1990;

Linko et al., 1992). It is believed that the rapid increase in lipid content is highly correlated with increased feeding activity of fish and availability of food with rising water temperature and an autumn decrease appears to be associated with declining food sources as water temperature decreases (Ringø and Burkow, 1990). In addition, the free fatty acid content has been found to be higher in Arctic charr caught in early spring and late fall than those sampled from the summer (Ringø and Burkow, 1990). Likely this results from mobilization of fish body lipids during periods of starvation.

There is less variation in levels of individual fatty acids of the wild coldwater fishes, in contrast to their lipid composition. In north temperate regions, freshwater insects and crustacea are major food items for many fish species including Arctic charr. While these invertebrates are generally rich in 18:2n-6, 18:3n-3, 20:4n-6 and 20:5n-3 and low in 22:6n-3, fish preying on them tend to have higher levels of tissue n-3 and n-6 long-chain PUFA but a lower ratio of n-6 PUFA to n-3 PUFA (Hanson et al., 1985; Muje et al., 1989; Yurkowski, 1989; Linko et al., 1992). However, there are few studies on seasonal changes in PUFA composition of coldwater fishes. Available data suggest that seasonal variations in levels of 18:2n-6 in muscle lipids occur in Arctic charr but not vendace (Ringø and Burkow, 1990; Linko et al., 1992). Perhaps, the correlation between season and degree

of unsaturation of fish body lipids, determined largely by PUFA composition, is low in wild fishes due to influences of other factors such as feeding, maturation and individual variation (Muje et al., 1989; Ringø and Burkow, 1990; Linko et al., 1992).

3. Changes during parr-smolt transformation

The majority of salmonid species, including Arctic charr are anadromous, i.e., they spend the early part of their lives in freshwater before they migrate to sea. Extensive studies indicate that during the parr-smolt transformation, parr shift their lipid and fatty acid composition towards the composition noted for marine fish (a decreased level of total lipids, 16:1n-7 and 18:1n-9 but elevated n-3 PUFA in muscle) (Ota and Yamada, 1974; Sheridan et al., 1985; Ackman and Takeuchi, 1986; Li and Yamada, 1992). In other words, the fatty acid compositions of parr are typical of freshwater fish, while those of smolt are more similar to marine than freshwater fish. This change in tissue fatty acid composition, along with other physiological and biochemical changes, has been correlated with an increased capacity for osmoregulation by these fish during parr-smolt transformation (Li and Yamada, 1992).

4. Comparison of lipid and fatty acid composition of cultured and wild salmonids

Castell (1979) proposed that information on lipid and fatty acid composition of fish caught in the wild helps to determine the dietary lipid requirements of cultured fish. This is supported by a recent study on the lipids of Atlantic salmon at the parr stage. Atlantic salmon caught in the wild, like other coldwater species, were found to be rich in arachidonic acid (20:4n-6) (Ackman and Takeuchi, 1986; Yurkowski, 1989; Ringø and Burlow, 1990). By comparison, Atlantic salmon reared in a hatchery had much lower levels of tissue 20:4n-6, although diets were supplemented with adequate 18:2n-6, suggesting that high concentrations of dietary 20:5n-3, 22:5n-3 and 22:6n-3 may inhibit the conversion of 18:2n-6 to 20:4n-6 (Ackman and Takeuchi, 1986). Since 20:4n-6 is related to the production of eicosanoids by cycloxygenases, the fin erosion associated with smolt released from the hatchery may be correlated with a lower level of tissue 20:4n-6 than those from the wild. This suggest that Atlantic salmon may require 20:4n-6. Additional evidence on this aspect may be obtained through the determination of EFA requirements of Atlantic salmon under culture conditions.

Chapter 2.

General Materials and Methods

2.1. Experimental fish

Two strains of Arctic charr were selected as experimental animals to investigate their essential fatty acid (EFA) requirements. In addition, one strain of rainbow trout was used for comparison. All experimental fish and their parents were cultured at the Rockwood Aquaculture Research Centre (RARC), Department of Fisheries and Oceans (Gunton, Manitoba). Charr used for the 1989 and 1991 experiments, belonged to the Labrador strain (Fraser River, Labrador), and were bred randomly in late 1988 and 1990, respectively. Charr used for the 1990 experiments belonged to the Nauyuk strain originally collected from Nauyuk Lake (NWT, Canada), which were spawned at RARC in early 1990. Rainbow trout used were the offspring of Mount Lassen × Tagwerker crosses.

Arctic charr often develop marked variation in size under culture conditions (Papst et al., 1992), which is different from many other cultured fish species. Considering this, experimental fish were selected randomly from their populations without grading since this approach was considered to be more representative of their genetic makeup.

2.2. Experimental Diets

1. Composition of basal casein-based semi-purified diet

Casein-based semi-purified diets have been used widely to determine the nutrient requirement of fish and were also used in this study. Before this study, one casein-based semi-purified diet was tested on Arctic charr. However, this diet was found to be unsuitable for studies determining the nutritional requirements of this species (Yurkowski, 1986). Moreover, there has been little information on the requirements of Arctic charr fingerling for individual nutrients, while a number of studies reported that this species grew as well as rainbow trout on either salmon or trout diets (Baker, 1983; Tabachek, 1984). With this background, a casein-based semi-purified basal diet was formulated, primarily by referring to previous studies on the nutrient requirements of other salmonids (Halver, 1989; Wilson, 1989; Sargent et al., 1989; Lall, 1989). The main ingredients of the basal diet included 52.0% casein, 15.2% starch, 10.0% dextrin, 4.0% gelatin, 5.0% lipid, 5.5% mineral premix and 2.0% vitamin premix (Table 2). The amino acid composition of casein-based basal diet were calculated with conversion factors reported previously (Lovell, 1989; Wilson, 1989) and the values generally fell within the ranges reported by NRC (1981).

Table 2. Composition of casein-based basal diet

Ingredient	% (dry)
Casein	52.0
Gelatin	4.0
Starch	15.2
Dextrin	10.0
α-cellulose	2.0
Carboxymethylcellulose	1.5
Methionine	0.5
Arginine	1.3
Mineral premix[1]	5.5
Vitamin premix[2]	2.0
Choline Chloride premix[3]	1.0
Lipid	5.0

[1]Containing the following concentrations (mg/kg): $CaHPO_4.2H_2O$, 37222; Na_2HPO4, 1543; K_2HPO_4, 3671; $CaCO_3$, 1063; NaCl, 1139; K_2SO_4, 3655; $MgSO_4$, 2500; $FeSO_4.7H_2O$, 167; $MnSO_4.H_2O$, 39; $ZnSO_4.7H_2O$, 89.8; $CuSO_4.5H_2O$, 12; $CoCl_2.6H_2O$, 7.8; KI, 0.3; Na_2SeO_4, 0.6; starch, 3890.8. [2]Containing the following concentrations (mg/kg): biotin, 1.4; folic acid, 13; thiamin.HCl, 15.8; pyridoxine.HCl, 22; riboflavin, 30; D-Ca pantothenate, 150; ascorbic acid, 300; myo-inositol, 450; niacin, 200; BHA, 1000; starch, 167749; vitamin K, 26.3; vitamin B_{12}, 0.2; Vitamin A, D_3 and E were added at 5000, 2600 and 250 IU/kg, respectively [3]Containing the following concentrations (mg/kg): choline chloride, 3000; a-cellulose, 7000;

2. Formulation of experimental diets

All casein-based experimental diets were formulated by varying the lipid component of the basal diet described above. In addition to this, one casein-based diet with 8.0% lipid was made by replacing starch of the basal diet proportionally with lipids. The commercial trout diet regularly used for culturing Arctic charr in the hatchery was also used as a reference diet in this study.

3. Preparation of experimental diets

Prior to the manufacture of semi-purified diets, several main ingredients including casein, starch and dextrin, were extracted with hot ethanol to remove trace amounts of lipids. The extraction was carried out as follows: > 99% pure ethanol was heated to boiling in a large container and then ingredients of about 1/3 volume of heated ethanol were added and stirred thoroughly for half an hour and allowed to settle and the ethanol removed. This procedure was repeated twice with additional ethanol but the mixture of ingredients and ethanol from the final extraction was allowed to settle overnight. The extracted ingredients were then dried with circulating air at room temperature, to ensure complete evaporation of ethanol.

For experimental diets, all dry ingredients except gelatin, lipid and water, were weighed individually and mixed thoroughly. Lipids accurately weighed were then added and

mixed with the other ingredients thoroughly. The 6.7% gelatin solution (in distilled water, at 30-40 °C) was then added to the mixture. The mixture was mixed and extruded through a food grinder with a 3 mm die in diameter. The sphagetti-like extrusions were cut into pieces about 2 cm long, air-dried for about 10 hours at room temperature and then stored in a sealed and nitrogen flushed plastic bag at -20 to -50 °C until required at which time the feed was crumbled. The moisture content in experimental diets was determined by drying diets at 100 °C for 16 hours. Throughout this study, the level of moisture varied from 8.0 to 11.0% among diets. However, the effect of this difference on the estimate of feed efficiency may be less apparent than other factors such as performance of feeding.

Before the crumbling, appropriate particle sizes were estimated by referring to the study by Tabachek (1986). About 30 g of the non-crumbled feed was crumbled using a roller mill and sieved. The size distribution of food particles was assessed for the size of Arctic charr used through sieving and compared with the recommended by Tabachek (1988). This process was repeated three to five times until appropriate distribution of feed particles was obtained (Append. 1). The resulting feed particles were stored in zipbloc plastic bags under nitrogen gas. Throughout this study no marked difference occurred between fish fed the high PUFA casein-based diets and the commercial diet, indicating minimal lipid oxidation of

diets throughout my study.

4. Lipid composition of experimental diets

Experimental diets contained various levels and types of fatty acids. The unsaturated fatty acids in these diets were either pure methyl esters purchased from SIGMA (St. Louis, MO, USA) or they originated from flax oil, which was analyzed for its fatty acid composition (Chapter 3, 5 and 6). To assess amounts of polyunsaturated fatty acids (PUFAs), lipids in the diet containing 5.0% 12:0 (lauric acid, a 12-carbon saturated fatty acid), were extracted and analyzed for fatty acid composition (Chapters 3, 5 and 6). The lipid content and fatty acid composition were also determined for the reference diet (Chapters 3 and 5). Forms of 12:0 were methyl and ethyl esters for the 1989, and 1990 and 1991 experiments.

2.3. Experimental Design and Performance

1. Design

The experimental diets differed in their types and percentages of n-3 and n-6 PUFAs. They were assigned randomly to duplicate or triplicate groups of 75 charr in the experiments. Since rearing tanks were arranged in banks of 24 with an upper and lower row of 12 tanks each, each row generally contained one replicate of each diet treatment. Sometimes one row contained two replicate groups if the treatment was triplicated.

2. Performance

At the beginning of each experiment, fish of approximately 1 g, were taken randomly from the populations of the designated strains and assigned to rearing tanks in groups of 100 individuals. These fish were then fed on a commercial trout diet for 2 weeks. Subsequently, the PUFA-free acclimation diet as described above was applied for 2 to 4 weeks to condition the fish to eat casein-based diets and reduce their tissue stores of PUFAs. Groups of 75 individual charr were then fed experimental diets for 12-14 weeks.

3. Rearing regime

In all experiments of this study, fish were maintained under similar rearing regimes. In general, each rearing tank contained 60 litres of aerated well water with 95% recirculation and at a flow rate of approximately 2.4 litre.min^{-1}. Water temperature was maintained at constant 10

°C, which is close to the optimum temperature range for growth of Arctic charr (Baker, 1983; Tompkins, 1989). The photoperiod was 12 hours dark : 12 hours light. Fish were fed 5 times per day to apparent satiation.

2.4. Sampling

1. Assessment of fish growth and feed utilization

Throughout my study, fish growth was assessed by measuring temporal wet weight of fish and feed utilization was examined by monitoring the change in wet weight of fish and amount of feed used. In the 1989 experiment, fish were individually weighed bi-weekly and fork lengths were recorded for 30 individuals sampled at random. In the 1990 and 1991 experiments, batch weights were taken at 2 week intervals for adjusting feed amount and fish were weighed individually at 4 week intervals for estimating growth.

2. Analyses of biochemical and physiological parameters

Ten individual fish from each replicate of each treatment were sampled randomly for determining haematocrit, haemoglobin values and water content of whole body, liver and muscle and calculating hepatosomatic index (HSI). To collect blood, fish were anaesthetized with 2-phenoxyethanol and the tail severed posterior to the anal fin, blood was drawn in micro-haematocrit capillary tubes and 25 μl micro-sampling pipettes (heparinized). Whole liver and upper lateral muscle (without

skin) of 5 to 10 individuals were also sampled and pooled for analyses of lipid and fatty acid composition. Samples were stored at -75 °C prior to chemical analyses.

2.5. Chemical Analyses

1. Water content, haematocrit and haemoglobin

Water content was determined by freeze-drying samples to a constant weight. The drying lasted 30-40 hours for livers and muscle and 65-80 hours for whole fish. Haematocrit values were determined by centrifuging micro-haematocrit tubes with blood for 5 minutes on a clinical centrifuge. Blood samples taken in micro-sampling pipettes were assayed spectrophotometrically (wavelength = 540 nm) for haemoglobin content (cyanmethaemoglobin) using the standard purchased from SIGMA (St. Louis, MO, USA) according to the method described by Wedemyer and Yasutake (1977).

2. Lipid analyses

Throughout my study lipids were extracted from livers, muscle and diets. Most of the lipid extracts were subjected to further analyses including separation into polar and neutral classes and isolation of triacylglycerols from neutral lipids. Solvents used for these analyses were either HPLC or analytical grade.

1.) Lipid extraction

Total lipids in livers and diets were extracted according to the Folch et al. method given by Christie (1982). Briefly, about 1 gram of frozen liver or diet samples (stored at -70 °C) was accurately weighed and homogenized for 1 min with 10

ml methanol and then for another 2 min. after adding 20 ml chloroform. The homogenate was filtered though a sintered glass funnel with slight suction and the solid residue left was suspended in 30 ml of 2:1 chloroform: methanol and homogenized for another 3 min. and filtered. The residue was then washed with 10 ml methanol and 20 ml chloroform. All filtrates were combined and transferred to a 100 ml glass cylinder where they were mixed thoroughly with a 0.88% potassium chloride solution of 1/4 volume of the combined filtrates, and allowed to form biphases at 4 °C over 4 hours. After the removal of the upper layer, the lower layer containing lipids was washed two to three times with 1:1 methanol : water of 1/4 the volume of the lower layer. The washed lower layer, together with 5-10 ml benzene, was evaporated to a volume of about 2-4 ml in a rotary evaporator. These concentrated lipid extracts were then transferred to a pre-weighted culture tube for the determination of total lipids.

Lipids from muscle tissue were extracted according to the Bligh and Dyer method described by Christie (1982). Briefly, about 5 gram of frozen fish muscle were weighed and homogenized for 50-60 secs with 15 ml of 1:2 chloroform : methanol and filtered through a sintered glass funnel with slight suction. The residue was further homogenized for 20-30 secs with 8 ml chloroform and the homogenate was filtered. Subsequently, the filtrates were combined and transferred to

a 50 ml glass cylinder where they were mixed thoroughly with 5-7 ml 0.88% potassium chloride and allowed to form biphases at 4 °C, over 4 hours. After removing the upper layer, the lower layer was washed 3 times with 1 : 1 methanol : water at 1/4 volume of the lower layer. The washed lower layer was evaporated together with benzene in a rotary evaporator and the remaining lipid extracts were transferred to a pre-weighed culture tube for determination of total lipids.

2.) Separation of lipids into classes

(a) Separation of polar and neutral classes

While thin-layer chromatography (TLC) remains a common technique for the separation of simple lipid classes, the pre-packed cartridges of silica gel (Sep-Pak, Millipore Corp., MA., U.S.A.) have recently been applied by researchers to separate lipids into neutral and polar fractions on a small scale (Bitman et al., 1984; Juaneda and Rocquelin, 1985; Satoh et al., 1989). In theory, both techniques are based on the differences in the degree to which lipid components are adsorbed to a solid support, relative to their solubility in an appropriate solvent. However, compared to the traditional TLC method, the Sep-Pak method is not only less time-consuming but allows further gravimetric determination of eluted lipid fractions.

In this study an aliquot of lipid extracts containing 30-70 mg lipids was dissolved in 500 μl chloroform and applied to a cartridge connected to the head of a glass syringe. The

neutral lipid fraction was eluted first by pumping 50 ml of 1 : 1 hexane : diethyl ether through the cartridge at approximately 5-10 ml/min. Then the polar lipid fraction was eluted with 25 ml methanol and 25 ml 1:1 chloroform : methanol. Both lipid fractions were then evaporated to dryness in a rotatory evaporator.

To validate the Sep-Pak method, the polar and neutral lipid fractions that were separated as described above were run on TLC (silica gel G) with two different developing solvent systems: benzene : diethyl ether : ethyl acetate : acetic acid (80:10:10:0.2), and hexane : diethyl ether : acetic acid (80:20:3). No overlapping was noted in either fraction, indicating complete separation of the neutral and polar lipids. In addition, the fatty acid compositions were compared for the polar lipids isolated from both the Sep-Pak cartridge and TLC procedures, and they were found to be very close to each other (Append. 2).

(b) Isolation of triacylglycerols

Triacylglycerols were also isolated from neutral lipids by using a TLC method. Initially, the neutral lipids recovered from Sep-Pak silica cartridges were applied on a TLC plate (silica gel G). The plates were then placed in a tank with hexane : diethyl ether : acetic acid (80:20:3). When the solvent was nearly at the top of the plate, the plate was removed from the tank, dried in air and sprayed with 0.02% Rhodamine 6G (aqueous solution) to render lipid classes

visible under UV light. The triacylglycerol band was identified by referring to a TG standard (triolein) from SIGMA (St. Louis, U.S.A.). The identified TG spot on the TLC plate was scraped off and collected in a glass pipette packed with glass wool (pre-washed with chloroform) and then eluted with chloroform.

3.) Quantification of total, polar and neutral lipids

Total, polar and neutral lipids were quantified gravimetrically. Briefly, lipid extracts or polar or neutral lipid fractions transferred to a pre-weighted culture tube, were dried to constant weight under a stream of nitrogen on a hot plate (37 °C) and then weighed on an analytical balance. Weight of lipid was determined by subtraction.

3. Analyses of fatty acid composition

In this study, total or separated lipids were also analyzed for their fatty acid composition. Two main procedures were employed for fatty acid analyses, i.e., preparation of fatty acid derivatives including transesterfication and purification of resulting methyl esters, and separation of fatty acid methyl esters by gas chromatography (GC). Solvents used for these analyses were either HPLC or analytical grade.

1.) Transesterfication

Before being analyzed by GC, fatty acids were converted into volatile esters through transesterfication.

An aliquot of about 2.5 mg lipids was dried in a tube under

a stream of nitrogen and dissolved in 1 ml toluene and 4 ml 5% HCl in methanol. After thorough mixing and flushing with nitrogen, the tube with the sample was capped tightly and then heated at 90-100 °C on a heating block for 90 minutes. The reaction proceeded as follows:

$$RCOOR' + CH_3OH \xrightarrow{H^+} RCOOCH_3 + R'OH$$

$$RCOOH + CH_3OH \xrightarrow{H^+} RCOOCH_3 + H_2O$$

After the completion of the reaction, a mixture of 5 ml of chloroform-extracted double distilled water and 2 ml of hexane were added to the reaction mixture and shaken thoroughly. The mixture was allowed to stand until the upper layer containing esters became transparent. The upper layer was then collected. The same extraction procedure was repeated twice, each with an additional 2 ml of hexane. The hexane extracts were combined for subsequent purification.

2.) Purification of methyl esters

The resulting esters from the transesterification were purified by use of the preparative TLC method. The ester mixtures, dissolved in hexane, were dried under a stream of nitrogen, re-dissolved in 150-200 μl of chloroform and then applied to a TLC plate (silica gel G, 25 mm thick). The plate was developed in a tank with toluene, and following this it was sprayed and the esters were identified according to the procedures described for isolation of triacylglycerols. The purified esters were scraped off the plate and transferred to

a glass pipette with glass wool (pre-washed with chloroform) and 4-6 ml of chloroform were used to elute the esters. The purified esters were then dried under nitrogen and re-dissolved in hexane and transferred to 1.5 ml glass vials, which were stored at -20 °C until GC injection.

3.) Composition of fatty acid methyl esters

(a). Principle

In most circumstances GC is the method of choice for fatty acid analysis (Christie, 1989). It allows a complete quantification of the fatty acid ester mixtures in a short time. Theoretically, the GC technique is based on the partitioning of ester components between gas and stationary phases to achieve the separation of mixtures of organic compounds. There are four major parts in GC: stationary or liquid phase in a column, mobile phase (carrier gas), detector, and recorder or integrator. The liquid phase is the principal factor determining the nature of the separations. Polar polyglycol as liquid phase (i.e. Carbowax 20™ and Supelcowax-10™) and helium or hydrogen as carrier gas have been applied commonly for the separations of esters of the saturated and unsaturated fatty acids (Christie, 1989). Both detector and recorder are used for detection and calculation of amounts of esters that emerged from the column.

(b). General procedures of GC analyses

At the beginning of GC analysis, fatty acid esters are dissolved in hexane and injected into a mobile phase (carrier

gas) where esters are evaporated and carried onto the liquid phase held in a column. Under constant gas flow, different ester components travel at different speeds between the mobile and liquid phases, depending on their relative affinities for the liquid phase. The esters that emerge from the column further travel to a detector where the concentration of ester components are converted into electrical signals, which are recorded as peaks by an integrator. Although final identification of a peak on a gas-liquid chromatogram rests on the physical properties of the isolated (collected) material, this is generally not feasible for complex mixtures of fatty acids (Christie, 1982). The identity of the peak of separated esters is obtained from its behaviour on different stationary phases, which have been calibrated with various standard compounds at the same temperature. Accordingly the chain lengths (i.e. carbon number) and number of double bonds are determined from the retention times of fatty acid esters. For reliable modern gas chromatography equipped with flame ionisation detectors, the areas under the peaks are linearly proportional to the amounts (by weight) of material eluting from the columns. Consequently, the weight percentage of resolved esters can be obtained.

C. Determination of fatty acid methyl esters

In my study the fatty acid compositions were analyzed by use of gas chromatography (Varian 3400) equipped with a fused silica capillary column (30 m, 0.32 mm ID, 0.25 μm film

thickness, Supelcowax™ 10) and flame ionization detector (FID). Temperatures for injection and flame ionization detector were 235 °C and 250 °C, respectively. The column temperature was programmed: to remain at 175 °C for 25 min, then increase to 210 °C at a rate of 1.0 °C/min and remain at that temperature for 30 min. For each GC run, 1 µl hexane containing 25 mg/ml ester was injected into the GC and the pressure for helium, hydrogen and air were maintained at constant 72, 40 and 60 PSI. Helium (carrier gas) was purified through a heated furnace (Supelco Canada Ltd.) to remove traces of oxygen and water.

The separated ester components were identified primarily according to the method described by Yurkowski (1989). Briefly, a series of standards containing known individual fatty acid esters were run under programmed conditions. The area under each peak was measured and converted to weight percentage by use of Varian Star Integrator Software Revision A (Varian, Ca, U.S.A.) (Append. 3).

2.6. Data analyses

Parameters associated with fish growth and feed utilization were calculated as follows:

1. Specific growth rate (SGR) = [Ln (final body weight) - Ln (initial body weight)] / 84 x 100, Ln: natural logarithm
2. Condition Factor = Body weight / (fork length)^3 x 10^5
3. HSI = Liver weight / fish weight x 100
4. Feed efficiency = Weight gain (wet) / food consumed (wet)

All Statistical analyses were performed using SAS as installed by the University of Manitoba Computer Service. Paired t-test, one way ANOVA analysis and Duncan's multiple range test were used to compare means between or among treatments. Results were considered significant at $p < 0.05$ level.

Chapter 3.

Effects of dietary n-3 polyunsaturated fatty acids on growth, feed efficiency and liver RNA and DNA content of Arctic charr, Salvelinus alpinus (L.).

Abstract

Arctic charr, Salvelinus alpinus (L.), initially weighing 1.6 g, were fed either commercial diet or one of 12 test semi-purified diets containing different concentrations and types of n-3 and n-6 polyunsaturated fatty acids (PUFA). The effects of dietary composition on growth, feed efficiencies, hepatosomatic indices, liver DNA and RNA concentrations and RNA/DNA ratios were investigated. Poorest growth occurred in charr fed diets deficient in PUFA and increasing dietary 18:3n-3 from 0.1% to 2.0% led to improvements in specific growth rates and feed efficiencies. The level of dietary 18:3n-3 required for adequate growth was noted to be 1.0 to 2.0%. Dietary 22:6n-3 was more effective for growth enhancement than dietary 18:3n-3 and dietary 18:3n-3 was more effective than dietary 18:2n-6. No significant differences in growth and feed efficiency were found for charr fed diets containing combinations of 2.26-2.82% 18:3n-3 with 0.56-0.70% 18:2n-6 or 2.0% 18:3n-3 only. This implies dietary 18:2n-6 has minimal effect in the presence of adequate amounts of dietary 18:3n-3. DNA concentrations were lower in livers from fish fed diets containing $\leq$ 1.0% n-3 PUFA and this suggests histological and biochemical changes. However, a higher DNA concentration was found in charr fed the PUFA-free diet. Hepatosomatic indices were correlated with DNA concentrations for charr ingesting casein-based diets. RNA concentrations in

livers appeared to be correlated to anabolic events and RNA/DNA ratios were of little value as an indicator of growth.

Introduction

Extensive studies have established that fish are incapable of de novo synthesis of the polyunsaturated fatty acids (PUFA), 18:3n-3 (linolenic acid) and 18:2n-6 (linoleic acid) (Kanazawa, 1985; Greene and Selivonchik, 1987). Indeed, one or both of these PUFAs are essential fatty acids (EFA) for normal growth and health in freshwater fish (Kanazawa, 1985; Henderson and Tocher, 1987). Dietary levels of 0.8% or more of 18:3n-3 or a combination of 1.0% 18:2n-6 and 1.0% 18:3n-3 are adequate to fulfil the EFA requirements of coldwater fish species including several salmonids and coregonids (Castell et al., 1972a; Watanabe et al., 1974a, 1989; Takeuchi and Watanabe, 1982; Takeuchi et al., 1979; Yu and Sinnhuber, 1979; Thongrod et al., 1990a, b). The long-chain n-3 PUFAs, 20:5n-3 and 22:6n-3, are however more effective than 18:3n-3 as EFA for majority of salmonids (Takeuchi and Watanabe, 1976, 1977b; Takeuchi et al., 1979).

Histological examination and a variety of other indicators, including the hepatosomatic index (HSI), have been used to assess EFA deficiency in fish (Henderson and Tocher, 1987). EFA-deficient fish may have degenerated nuclei and hyperplasia of connective tissue in the liver (Watanabe et al., 1974b, 1989) and it is possible that there are concomitant changes in RNA and DNA content. On the other hand, there are numerous studies (Bulow, 1970; Bulow et al., 1978; Wilder and Stanley,

1983; Miglavs and Jobling, 1989) indicating that RNA concentration and RNA/DNA ratios may correlate with fish growth or nutritional status, but to date, there are few studies which describe the effects of specific nutrients on RNA and RNA/DNA ratios (Rafael and Braunbeck, 1988).

Due to limited information about their specific nutrient requirements (Jobling, 1991), Arctic charr grown commercially are usually given commercial feeds developed for rainbow trout or salmon (Tabachek, 1984). To date there are few studies on the effects of dietary fatty acids on charr. Ringø (1989) reported that Arctic charr fed a commercial diet supplemented with a high level of 18:2n-6 had reduced body growth and digestibility of dietary protein, lipid and individual fatty acids and amino acids. More recently, Olsen et al. (1991) studied the effects of dietary n-6 and n-3 PUFAs on the lipid metabolism of charr using semi-purified diets. Olsen et al. (1991) suggested that n-3 PUFAs are EFA for charr and that higher amounts of PUFA may be required by charr than other salmonid species.

Based on the information about the EFA requirements of salmonid species, including Arctic charr, I hypothesized that n-3 PUFA were essential for Arctic charr. The objectives of this study were to determine the quantitative requirement for 18:3n-3, and to test whether 20:5n-3 and 22:6n-3 were more effective as EFAs than 18:3n-3. Assessments were made by measuring growth, feed conversion and liver parameters in

charr fed dietary n-3 PUFAs. In addition, we assessed the use of RNA, DNA and RNA/DNA ratios as indicators of somatic growth, and examined the value of using the hepatosomatic index as an indicator of liver condition.

Materials and Methods

Fish used in this study were the offspring of randomly bred Labrador anadromous Arctic charr, spawned at RARC (Gunton, Manitoba) in late 1988 and early 1989. The experiment was carried out using non-graded Arctic charr of Labrador strain. The formulation and manufacture of experimental diets were described in Table 2 and Chapter 2.

One commercial and 12 semi-purified diets varying in type and level of n-3 PUFA and 18:3n-3 were tested (Table 3). Treatments (diet) were assigned randomly to duplicate tanks according to a complete randomized block design. Each tank contained 75 charr of average initial weight 1.6 g. To reduce PUFAs stored in charr tissues and to condition them to eat the casein-based diets, charr were acclimated for two weeks on a casein-based diet which was the same as diet 12 in composition but dietary casein, starch and dextrin were not extracted with ethanol. During the 12 week feeding trial, fish were reared at constant 10 °C under a photoperiod of 12 h L : 12 h D.

Fish were weighed and measured bi-weekly and wet weights and fork lengths were recorded for 30 individuals selected at random from each tank. At the end of the feeding trial, 10 fish were sampled randomly from each tank for determination of RNA and DNA, and calculation of HSI.

The formulae used for calculation of specific growth rates (SGR), feed efficiency and HSI were provided in Chapter 2.

Table 3. Lipid composition of experimental diets

Diet	Fatty acids[1] (% dry weight)					
	18:3n-3	20:5n-3	22:6n-3	18:2n-6	12:0	Flax oil[2]
1	0.4	0.1	-	-	4.5	-
2	0.4	-	0.1	-	4.5	-
3	0.1	-	0.4	-	4.5	-
4	0.1	-	-	-	4.9	-
5	0.5	-	-	-	4.5	-
6	1.0	-	-	-	4.0	-
7	2.0	-	-	-	3.0	-
8	-	-	-	1.0	4.0	-
9	0.56[3]	-	-	0.14[3]	4.0	1.0
10	2.26[3]	-	-	0.56[3]	1.0	4.0
11	2.823	-	-	0.70[3]	-	5.0
12	-	-	-	-	5.0	-
13	Commercial[2,4]					

[1]The supplemented pure fatty acids were provided as methyl esters [2]Fatty acid composition is listed in Table 4 [3]Calculated on the basis of percentages of 18:2n-6 and 18:3n-3 in flax oil [4]Level of total dietary lipid was 19% on a dry weight basis

All pure fatty acid components were in the form of methyl esters purchased from Sigma (St. Louis, MO, USA) (Tables 3). Total lipids in the commercial and 5.0% 12:0 diets were extracted according to Folch et al. (1957) and determined gravimetrically. Fatty acid compositions of PUFA-free and commercial diets and flax oil were obtained using gas chromatography according to the methods described in Chapter 2, and the data have been presented in Table 4.

The RNA and DNA were extracted from livers by use of the Schmidt-Thannhauser method (Munro and Fleck, 1966) with the following modifications: samples were homogenized in ice-cold water with a homogenizer (Kinematica, CH-6010 Kriens-Lu, Switz) for 30 sec and centrifuged at 4 °C for 5 min using a microcentrifuge (10000 rpm). Samples were then incubated at 37 °C for 90 min. RNA extracted from livers was measured at 260 nm (wavelength) and it was found that protein contributed only 1-3% of the total Optical density (O.D.) measured by the method of Lowry et al. (1951). RNA concentrations were calculated by dividing the O.D. reading at 260 for the RNA by 0.03 (Buckley and Bulow, 1987). DNA was extracted from livers using 0.5 N perchloric acid at 90 °C for 30 min. DNA concentrations were determined according to the diphenylamine method (Buckley and Bulow, 1987) using salmon sperm DNA (Sigma, St. Louis, U.S.A.) as the standard. The liver from a 5 kg charr was used as a reference and aliquots extracted for RNA and DNA each time a batch of samples was run.

Table 4. Fatty acid compositions of seleceted diets and flax oil (weight %)

Fatty acid[2]	Diet[1]		Flax oil
	12	13	
12:0	93.8	tr[3]	tr
14:0	0.4	2.2	tr
16:0	0.9	13.9	5.4
18:0	tr	2.4	3.0
16:1n-7	tr	6.7	tr
18:1n-9[4]	0.8	16.9	19.8
18:1n-5	-	0.5	-
18:4n-1	_	0.2	-
20:1n-11	-	8.7	-
20:1n-9[5]	tr	0.6	0.2
22:1n-11[6]	tr	6.3	tr
24:1n-9	tr	1.0	tr
18:2n-6	tr	3.9	14.0
18:3n-6	tr	0.2	tr
18:3n-3	tr	0.2	56.4
18:4n-3	tr	0.3	tr
20:2n-6	tr	0.7	tr
20:3n-6	tr	0.9	tr
20:4n-6	tr	1.7	tr
20:4n-3	tr	0.2	tr
20:5n-3	tr	4.3	tr
22:5n-3	tr	0.8	tr
22:6n-3	tr	18.5	tr

[1]Lipid composition of the diets were listed in Table 3. [2]The unknown fatty acids making up < 10% of total fatty acids were not listed [3]Trace amount (<0.1%) [4]Includes 18:1n-7 and 18:1n-9 [5]Includes 20:1n-7 and 20:1n-9 [6]Includes 22:1n-7, 22:1n-9 and 22:1n-11

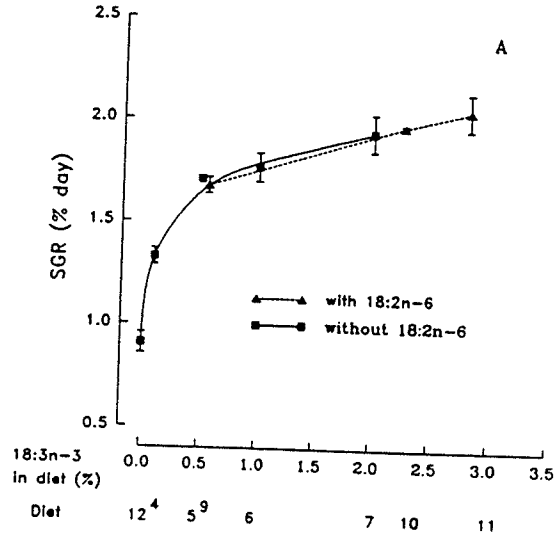
All statistical analyses were performed as described in Chapter 2.

Results

The relationship between percentage of dietary 18:3n-3 and specific growth rate (SGR) and feed efficiency is shown for charr fed diets varying in 18:3n-3 (Fig. 3). Growth rate and feed efficiency of charr increased markedly as dietary 18:3n-3 increased from 0 to 0.5% (diets 12, 4 and 5), but further increases in dietary 18:3n-3 to 2.0% (diets 5-7) only gave rise to small increases in these two parameters. Similar trends were noted in fish fed diets containing additional 18:2n-6 (diets 9-11) (Fig. 3). In addition, SGR and feed efficiency were 1.66 ± 0.04 and 1.00 ± 0.05 for charr fed diet 8 and 2.11 ± 0.11 and 0.82 ± 0.09 for those fed diet 13, respectively. Further, the effects of feeding diets containing different levels and types of n-3 PUFAs (diets 1, 2, 3, 5 and 12) on the growth and feed efficiency of the charr are shown in Fig. 4. Charr fed diets with 0.5% n-3 PUFA (diets 1, 2, 3 and 5) showed more rapid growth and feed efficiencies over 12 weeks than the fish fed the diet devoid of PUFA (diet 12) (Fig. 4). Moreover, the initial and final mean weights, and condition factor are shown for fish in all treatments (Append. 4).

Differences among treatments in HSI, RNA and DNA from livers, and RNA/DNA ratios were detected. There were no significant differences in RNA concentration among charr fed 0%-0.5% n-3 PUFA (diets 1-3, 5 and 12) (Fig. 5). Fish fed diet

Fig. 3. Change in specific growth rate (SGR) (A) and feed efficiency (B) of Arctic charr in relation to dietary level of 18:3n-3 (0, 0.1, 0.5, 0.56, 1.0, 2.0, 2.26 or 2.82). All curves were fitted by eye. Values = mean ± SEM (n = 2). •—• = diets with 18:3n-3 only; ▲---▲ = diets with both 18:3n-3 and 18:2n-6.


A
SGR (% day)
2.5
2.0
1.5
1.0
0.5
with 18:2n-6
without 18:2n-6
18:3n-3
in diet (%)
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
Diet
12 4 5 9 6 7 10 11

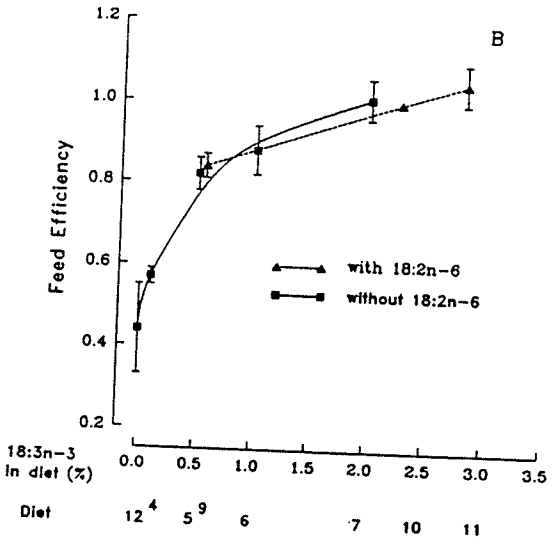

B
Feed Efficiency
1.2
1.0
0.8
0.6
0.4
0.2
with 18:2n-6
without 18:2n-6
18:3n-3
in diet (%)
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
Diet
12 4 5 9 6 7 10 11

Fig. 4. Specific growth rate (SGR) and feed efficiency of Arctic charr fed diets varying in n-3 PUFAs including 18:3n-3, 20:5n-3 and 22:6n-3. Values = mean ± SEM (n=2). Open bars = SGR, solid bars = Feed efficiency

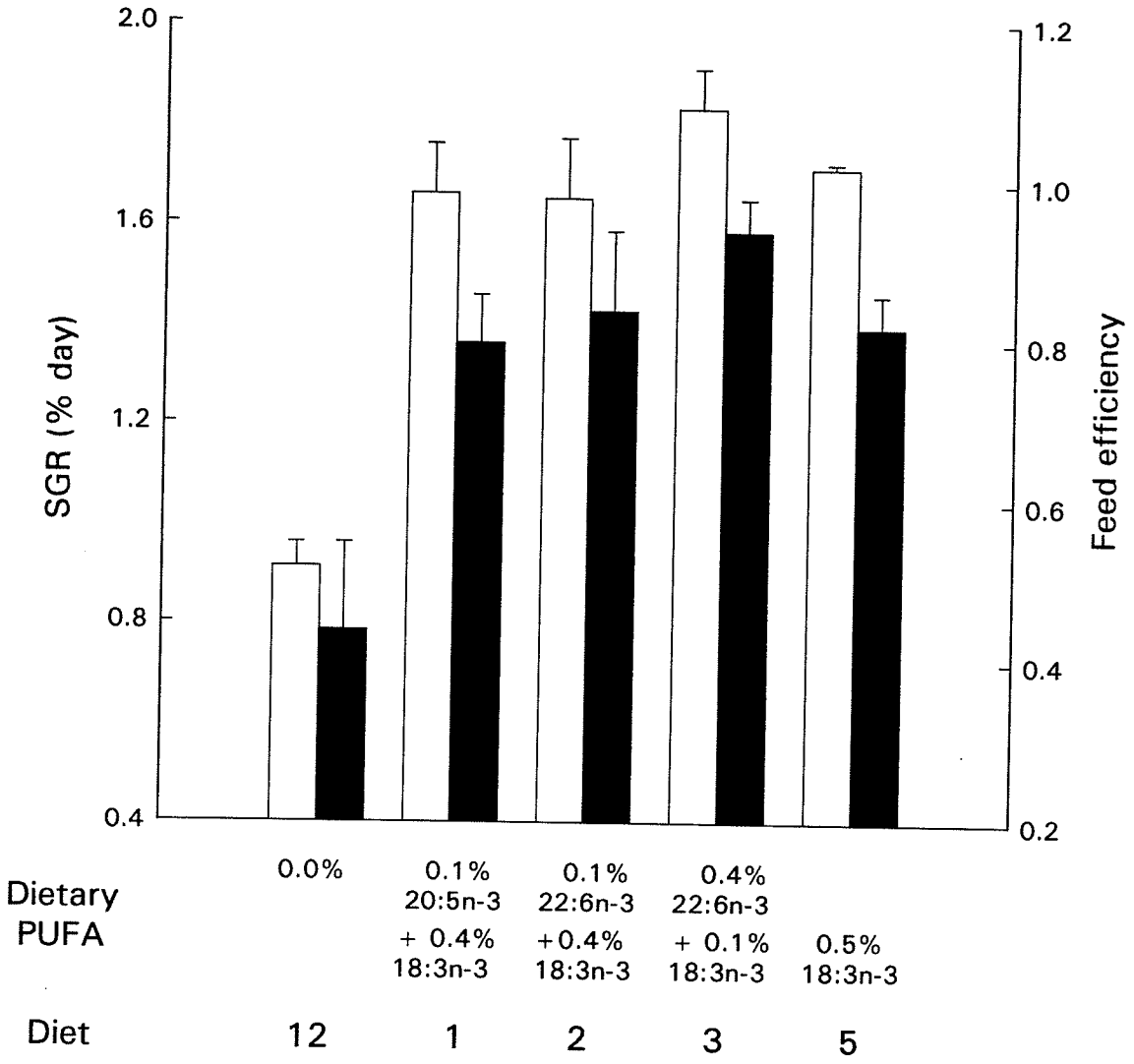
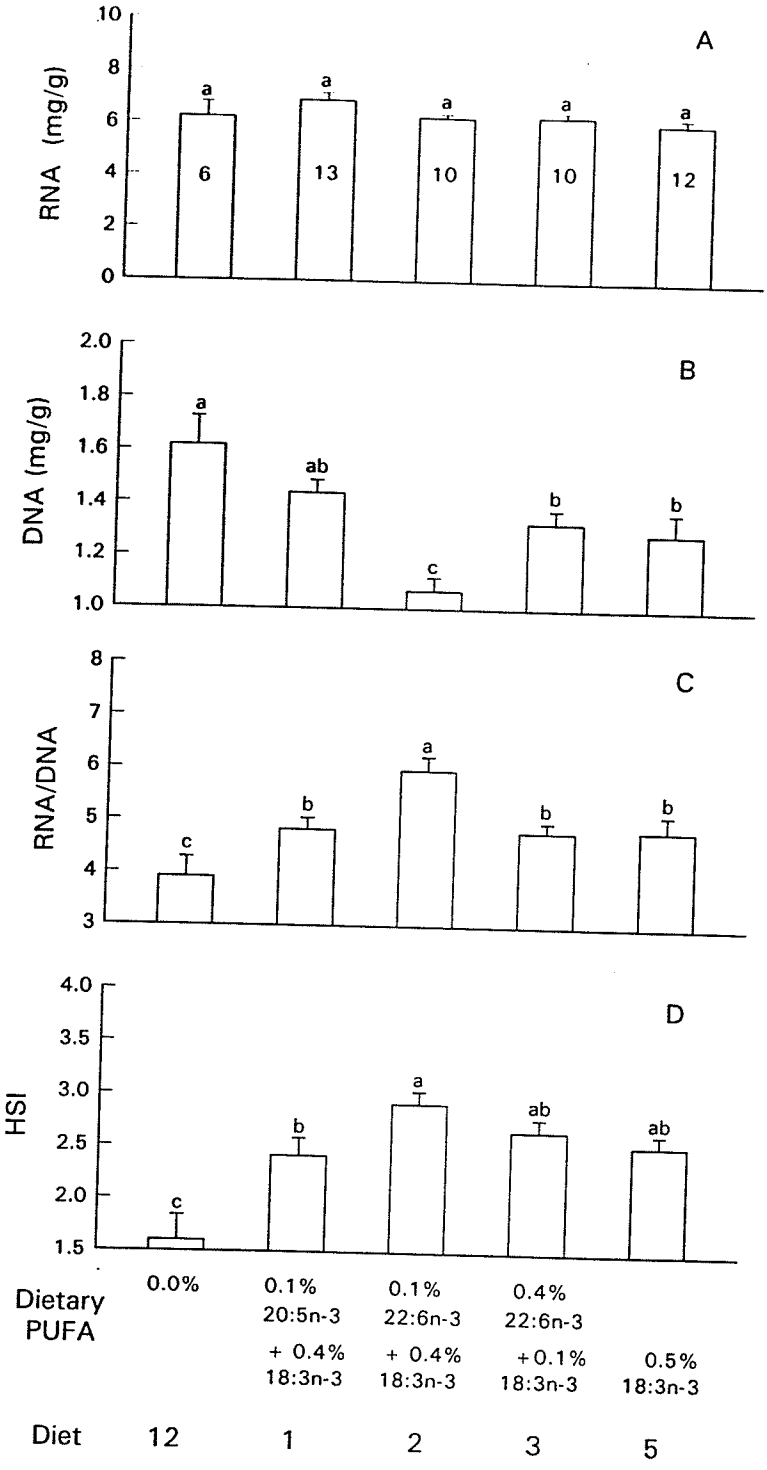
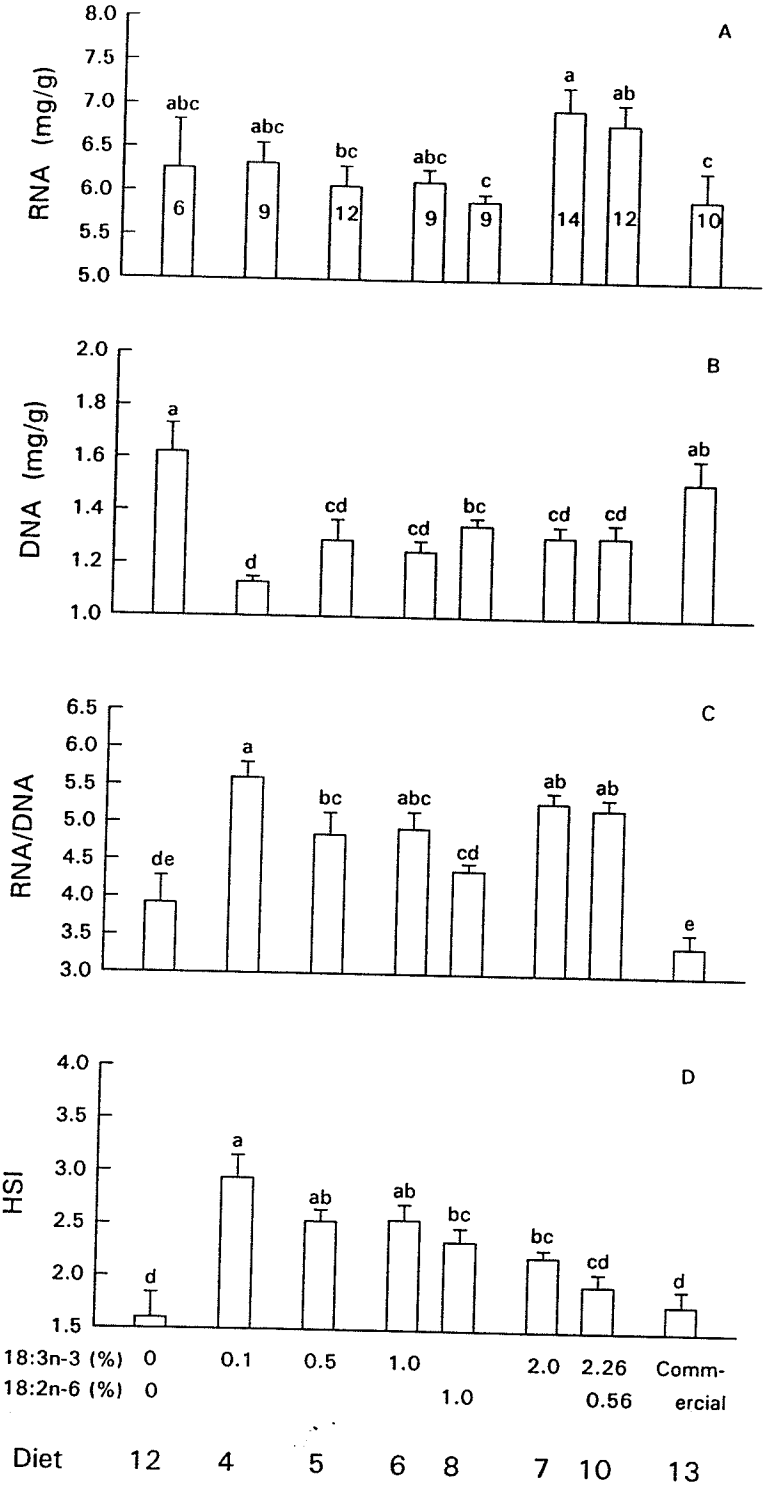

2.0
1.6
1.2
0.8
0.4
SGR (% day)
1.2
1.0
0.8
0.6
0.4
0.2
Feed efficiency
0.0%
0.1% 20:5n-3 + 0.4% 18:3n-3
0.1% 22:6n-3 +0.4% 18:3n-3
0.4% 22:6n-3 + 0.1% 18:3n-3
0.5% 18:3n-3
Dietary PUFA
Diet
12
1
2
3
5

Fig. 5. Comparison of total RNA (A), DNA (B) and RNA/DNA ratios (C) in liver and hepatosomatic index (HSI) (D) among groups of charr fed 0% or 0.5% PUFA including 18:3n-3, 20:5n-3 or 22:6n-3. Values = mean ± SEM. The numbers within the columns in (A) represent the number of individual fish and are the same for (B), (C) and (D). Bars with the same letters indicate no significant difference between groups.


A
RNA (mg/g)
10
8
6
4
2
0
a
a
a
a
a
6
13
10
10
12
B
DNA (mg/g)
2.0
1.8
1.6
1.4
1.2
1.0
a
ab
c
b
b
C
RNA/DNA
8
7
6
5
4
3
c
b
a
b
b
D
HSI
4.0
3.5
3.0
2.5
2.0
1.5
c
b
a
ab
ab
Dietary PUFA
0.0%
0.1% 20:5n-3 + 0.4% 18:3n-3
0.1% 22:6n-3 + 0.4% 18:3n-3
0.4% 22:6n-3 +0.1% 18:3n-3
0.5% 18:3n-3
Diet
12
1
2
3
5

2 (0.1% 20:5n-3 and 0.4% 18:3n-3) had the lowest DNA concentration but the highest RNA/DNA ratio and HSI. Among charr fed diets varying in the amount of 18:3n-3 or 18:2n-6 or combinations of 18:2n-6 and 18:3n-3 (diets 4-8, 10 and 12), those fed diet containing 2.0% 18:3n-3 (diet 7) and 2.26% 18:3n-3 and 0.56% 18:2n-6 (diet 10) had the highest RNA concentrations (Fig. 6). Furthermore, fish fed diet with 0% PUFA (diet 12) and commercial diet (diet 13) had significantly higher DNA concentrations but lower RNA/DNA ratios and HSI than charr fed other diets except diets 8 and 10. There were no significant differences in DNA concentration between charr fed diets containing 0.5-2.26% 18:3n-3 (diets 5-7, and 10). Charr fed the diet with 0.1% 18:3n-3 (diet 4) had the lowest liver DNA concentration but the highest RNA/DNA ratio and HSI. Values for HSI decreased as the levels of 18:3n-3 increased in the diet (diets 4-8, 10 and 12-13). Further, no mortality was found for charr in relation to their consumption of test diets.

Fig. 6. Comparison of total RNA (A), DNA (B) and RNA/DNA ratios (C) in liver and hepatosomatic index (HSI) (D) among groups of charr fed diets varying in 18:3n-3 and 18:2n-6. Values = mean ± SEM. The numbers within the columns in (A) represent the number of individual fish and are the same for (B), (C) and (D). Bars with the same letters indicate no significant difference between groups.


A
RNA (mg/g)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
abc
abc
bc
abc
c
a
ab
c
6
9
12
9
9
14
12
10
B
DNA (mg/g)
2.0
1.8
1.6
1.4
1.2
1.0
a
d
cd
cd
bc
cd
cd
ab
C
RNA/DNA
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
de
a
bc
abc
cd
ab
ab
e
D
HSI
4.0
3.5
3.0
2.5
2.0
1.5
d
a
ab
ab
bc
bc
cd
d
18:3n-3 (%) 0 0.1 0.5 1.0 2.0 2.26 Comm-
18:2n-6 (%) 0 1.0 0.56 ercial
Diet 12 4 5 6 8 7 10 13

Discussion

All the groups of charr fed diets containing 18:3n-3 and/or 18:2n-6 showed significant increases in mean weight, SGR and feed efficiency relative to the fish fed the PUFA-free diet (diet 12). Thus, charr require dietary PUFA for their normal growth. The correlation between increasing SGR and feed efficiency with the percentage of dietary 18:3n-3 in this study suggests that levels of dietary 18:3n-3 required for adequate growth and feed utilization in Arctic charr are 1.0 to 2.0%. This is consistent with the suggestion that charr require higher levels of PUFAs in their diet than other salmonids (Olsen et al., 1991). Studies on rainbow trout (Castell et al., 1972a; Watanabe et al., 1974a) demonstrated that food conversion or feed efficiency reached a plateau at 1.0% of dietary 18:3n-3, while the growth rate continued to increase. By contrast neither SGR nor feed efficiency reached plateau within the range of dietary 18:3n-3 used in my study (Fig. 3).

The EFA or PUFA requirement of fish is influenced by the level of total dietary lipids and the requirement for 18:3n-3 as an EFA for rainbow trout has been defined at 20% of total dietary lipids (Takeuchi and Watanabe, 1977a). Similarly, from my experiments the requirement of dietary 18:3n-3 by charr would be 20-40% of total dietary lipids. Furthermore, charr fed the diet with 1.0% 18:3n-3 had a higher SGR than those fed

1.0% 18:2n-6, suggesting that 18:3n-3 is more effective for growth enhancement. Charr fed diets supplemented with 2.26-2.82% 18:3n-3 plus 0.56-0.70% 18:2n-6 had slightly higher SGRs and feed efficiencies than those fed the diet containing 2.0% 18:3n-3. It appears that dietary 18:2n-6 had no adverse effects when diets contained adequate levels of n-3 PUFA. These results are consistent with those reported for rainbow trout (Yu and Sinnhuber, 1976).

Charr fed the diet containing 0.4% 22:6n-3 displayed better growth and feed efficiency than charr fed the diet containing 0.5% 18:3n-3. This indicates that 22:6n-3 is more effective as an EFA than 18:3n-3, and corroborates the findings for other salmonid species (Takeuchi and Watanabe, 1977b, 1982; Takeuchi et al., 1979). The non-significant differences for growth and feed efficiencies between charr fed diets containing 0.5% n-3 PUFA (diets 1-3, 5) may be due to the relatively low concentrations of 20:5n-3 or 22:6n-3 in these diets and/or my feeding period of 12 weeks. Nevertheless, these n-3 fatty acids in the diet clearly had a positive effect on growth relative to the diet devoid of n-3 or n-6 PUFAs.

One symptom of EFA deficiency in fish is fatty liver, which is characterized by increased total liver lipid, a high HSI, and atrophied hepatocytes with irregular-shaped nuclei (Watanabe et al., 1974b, 1989). Based on the assumption that liver DNA is a reasonable estimator of hepatocyte number (Rafael and Braunbeck, 1988), a change in liver DNA

concentration may reflect histological or cellular biochemical changes in the liver. This is likely the reason that the DNA content was relatively lower in charr fed diets containing 0.1-0.5% n-3 PUFA. In addition, from the studies by Watanabe et al. (1974b and 1989), fish on the PUFA-deficient diet (0% PUFA) would be exhibited more severe pathological changes including hyperplasia of connective tissue and excessive deposition of lipids in livers (Watanabe et al., 1974b). Consequently, one would predict that the DNA content in livers would be relatively high in fish fed a PUFA deficient diet if there was no excessive deposition of lipids. Interestingly this is what we found in charr but the findings appear to differ from those of rainbow trout (Watanabe et al., 1974b).

Charr fed the commercial diet had a markedly higher concentration of liver DNA relative to those fed most of the casein-based diets. This is not surprising as dietary ingredients are reported to affect animal tissue DNA content (Rafael and Braunbeck, 1988; Adeyeye et al., 1989) and it appears that the composition of the commercial feed used in my studies had a considerable effect on charr. My study also showed that changes in HSI corresponded to those in liver DNA concentrations for charr fed all casein-based diets. This suggests that both HSI and liver DNA concentrations are good indicators of liver condition.

There are several studies in which tissue RNA concentrations and RNA/DNA ratios have been used as indicators of recent

growth or nutritional status in fish (Bulow, 1970; Bulow et al., 1978; Wilder and Stanley, 1983; Miglavs and Jobling, 1989). The usual interpretation is that RNA concentrations in tissues (liver or muscle) give an estimate of protein synthesis with RNA concentration being highest in fast-growing fish and lowest in slow-growing fish. DNA concentrations are usually assumed to remain relatively constant. An examination of protein synthesis using labelled phenylalanine, together with an estimate of RNA concentrations, supports these assumptions (Foster et al., 1992), but the authors cautioned that initial fish size and environmental factors such as temperature might invalidate these assumptions. For this study, these assumptions appear to hold for charr fed ≥ 2.0% 18:3n-3 (diets 7,10, 11) and the commercial diet but not for those fed diets containing 0% or ≤ 1.0% PUFA. However, charr fed the commercial diet displayed a slightly higher SGR but significantly lower RNA concentration and RNA/DNA ratio, compared to those fed the other three diets. These findings suggest that if RNA/DNA ratios and RNA concentrations are used for assessing growth, the ingredients of the diet should be defined so that comparisons can be made among fish receiving test diets.

This study also found that there was a relatively low correspondence between the SGRs and RNA concentrations or RNA/DNA ratios for charr fed the casein-based diets and this corroborates the findings of Rafael and Braunbeck (1988).

However, levels of dietary n-6 and n-3 PUFAs varied between treatments and the conversion of 18:2n-6 or 18:3n-3 into long-chain PUFAs in the liver increased with either dietary 18:2n-6 or 18:3n-3. Rafael and Braunbeck (1988) suggested that the increase in RNA content of hepatocytes corresponds to liver anabolic activities. Perhaps, the differences in liver RNA concentrations among charr given the treatments in this study reflect to a large extent the influence of dietary fatty acids on liver anabolic activities.

In summary, data on growth, feed utilization and HSI in this study clearly indicate that at least 1.0% dietary 18:3n-3 is required for fast growth and efficient feed utilization. We also found that changes in liver DNA concentrations and HSI are good indicators of liver condition while RNA concentrations can be misleading as an indicator of growth. Data from charr fed the experimental diets suggested that liver RNA concentration may reflect anabolic events and the liver RNA/DNA ratio is of little value as an indicator of fish growth.

Chapter 4.

Effects of dietary n-3 polyunsaturated fatty acids on lipid and fatty acid composition and haematology of juvenile Arctic charr *Salvelinus alpinus* (L.)

Abstract

The effects of dietary n-3 and n-6 polyunsaturated fatty acids (PUFAs) on juvenile Labrador Arctic charr Salvelinus alpinus (L.) were investigated with respect to essential fatty acid (EFA) deficiency symptoms and lipid metabolism using one commercial and 12 casein-based test diets. Arctic charr with mean initial weight of 1.6 g were fed test diets for 12 weeks at 10 °C. At the end of the feeding period, blood, liver, muscle and whole fish were sampled to determine haematocrit, haemoglobin, water content and lipid and fatty acid composition. Charr fed diets containing 0-1.0% n-3 PUFAs showed typical EFA deficiency signs, i.e., fatty liver, elevated water content in whole body and substantial accumulation of 20:3n-9 in liver polar lipids. These signs were less apparent or absent when charr were fed diets containing ≥ 2.0% 18:3n-3. No correlation was found between dietary PUFAs and haematocrit or haemoglobin values. Significant changes in fatty acid composition of liver polar lipids noted in charr fed dietary PUFAs indicate that charr can convert 18:3n-3, 18:2n-6 and 20:5n-3 into long-chain PUFAs. While charr had a direct incorporation of dietary 22:6n-3 into liver and muscle there appeared to be preferential utilization of n-3 PUFAs for desaturation and elongation. The conversion of 18:4n-3 to 20:5n-3 was less apparent in muscle than in the liver. These findings, combined

with data on growth and feed efficiency, indicate that charr require 1.0 to 2.0% 18:3n-3 in their diet.

Introduction

Nutritional and biochemical aspects of n-9, n-3 and n-6 polyunsaturated fatty acids (PUFA) have been studied extensively in freshwater fish (Henderson and Tocher, 1987; Sargent et al., 1989). Fish fed diets deficient in two of these PUFAs, 18:2n-6 and 18:3n-3, usually develop deficiency signs such as retarded growth, low feed efficiency, fatty livers (elevated total lipids and percentage of neutral lipids in liver), increased water content in whole body or muscle, high hepatosomatic index (HSI) and substantial accumulation of 20:3n-9 in tissue polar lipids (Henderson and Tocher, 1987). These two fatty acids singly or in combination are considered to be essential fatty acids (EFA) for freshwater fish. Feeding diets with PUFAs or administering radio-labelled PUFAs *in vivo* have revealed that freshwater fish are incapable of synthesizing 18:3n-3 or 18:2n-6 *de novo* but they are able to desaturate and elongate these fatty acids to long-chain PUFAs, i.e., C20 or C22 PUFAs (Kanazawa, 1985; Henderson and Tocher, 1987). This process is influenced to a large extent by the levels of dietary PUFAs since n-9, n-6 and n-3 PUFAs share a common set of enzymes for desaturation and elongation (Henderson and Tocher, 1987).

Like other cultured salmonids, Arctic charr, *Salvelinus alpinus* (L.), require dietary 18:3n-3 for optimum growth and feed conversion (Chapter 3) and charr can convert 18:3n-3 or

18:2n-6 into long-chain PUFAs (Olsen et al., 1991; Olsen and Ringø, 1992). However, there is limited information on charr regarding symptoms relating to EFA deficiency and the effects of varying dietary levels of 18:3n-3, 20:5n-3 and 22:6n-3 on the metabolism of PUFAs.

The objectives of this study were to determine the requirements of charr for dietary 18:3n-3 by monitoring EFA deficiency signs and to investigate the desaturation and elongation of n-3 and n-6 PUFAs by examining the fatty acid composition of tissues.

Materials and methods

One commercial and 12 casein-based diets varying in levels of n-3 PUFAs and 18:2n-6 were tested using the Labrador strain of charr (Labrador, Newfoundland) (Table 3). The experimental design, feed formulations, performance parameters, rearing conditions and fish were the same as described in Chapter 3.

At the end of the feeding trial, 10 fish from each tank were sampled randomly for determination of the haematocrit and haemoglobin values and water content of the whole body. Livers and muscle from an additional 10 fish per tank were removed and pooled for lipid and fatty acid analyses.

Water content of livers and whole body and haematocrit and haemoglobin values were determined according to the methods described in Chapter 2. Liver and muscle lipids were extracted using the methods of Folch et al. (1957) and Bligh and Dyer (1959), respectively. An aliquot of the lipid extract from livers was separated into polar and neutral lipid fractions on Sep-Pak silica cartridges (Millipore Corp., MA., U.S.A.) using the method of Satoh et al. (1989b) (see Chapter 2 for details). Percentages of polar and neutral lipids were quantified gravimetrically. Fatty acid composition was analyzed for the total lipids of muscle and the polar and neutral lipids of liver using the methods given in Chapter 2.

Duncan's multiple range test was used to compare means using SAS (1985). Results were considered significant at $p < 0.05$.

Results

Haematocrit and haemoglobin values and the whole body water contents are given in Table 5. Generally, haemoglobin concentration increased as haematocrit values increased. The lowest haematocrit and haemoglobin values occurred in charr fed diets containing 0.1% 18:3n-3 or 1.0% 18:2n-6 (diets 4 and 8), while the highest values were found in charr fed the commercial diet (diet 13). Water content of whole body was lower in charr fed diets containing ≥ 2.0% 18:3n-3 (diets 7, 10 and 11) compared to those fed diets containing ≤ 1.0% 18:3n-3 (diets 1-3, 5, 6 and 9). Charr fed the commercial diet (diet 13) differed significantly in water content from those fed the diet containing 2.0% 18:3n-3 (diet 7) but not from those fed diets containing > 2.0% 18:3n-3 (diets 10 and 11).

The water contents and lipid compositions of livers are provided for groups given all test diets except diet 8 and 13 in Figures 7 and 8. Total lipids and water content (%) for charr fed the diet containing 1.0% 18:2n-6 (diet 8) were 8.7 ± 0.0 and 78.8 ± 0.1 and were 4.4 ± 0.1 and 76.9 ± 0.4 for the commercial diet (diet 13). The percentages for polar and neutral lipids for these two groups were 30.8 ± 2.1, 69.3 ± 2.1, 52.4 ± 5.3 and 47.6 ± 5.3, respectively. Percentages for water and polar lipids in livers increased as dietary 18:3n-3 increased, while total lipids and neutral lipids decreased.

Table 5. Haematocrit, haemoglobin concentration and whole body water content of Arctic charr in relation to diet treatment (Mean ± SEM, n[1] = 2)[2]

Diet	Haematocrit (%)	Haemoglobin (g/100 ml)	Water content (%)
1[3]	39.3 ± 2.2 [abc]	6.98 ± 0.44 [bc]	78.4 [cd]
2	41.3 ± 0.8 [abc]	6.88 ± 0.06 [bc]	78.2 ± 0.5 [cd]
3	40.5 ± 2.3 [abc]	6.92 ± 0.26 [bc]	78.3 ± 0.1 [cd]
4	35.6 ± 1.8 [c]	6.44 ± 0.15 [c]	80.1 ± 0.3 [b]
5	40.1 ± 3.4 [abc]	6.80 ± 0.29 [bc]	78.6 ± 0.3 [cd]
6	40.8 ± 1.4 [abc]	7.24 ± 0.42 [bc]	78.4 ± 0.4 [cd]
7	42.8 ± 0.6 [a]	6.72 ± 0.19 [bc]	77.9 ± 0.3 [de]
8	36.3 ± 0.5 [bc]	6.55 ± 0.18 [c]	78.8 ± 0.1 [c]
9[3]	41.0 ± 3.4 [abc]	6.68 ± 0.33 [bc]	78.1 [cd]
10	43.0 ± 0.4 [a]	7.38 ± 0.09 [abc]	77.3 ± 0.1 [ef]
11	42.5 ± 1.0 [ab]	7.83 ± 0.47 [ab]	77.0 ± 0.1 [f]
12	39.0 ± 1.6 [abc]	7.89 ± 0.30 [ab]	81.3 ± 0.2 [a]
13	45.0 ± 1.9 [a]	8.30 ± 0.84 [a]	76.9 ± 0.4[f]

[1]Value for each replicate was the mean of values determined from 10 individual fish

[2]The same letters within a column indicates no significant difference. [3]Value for water content was the mean of values determined from 10 fish in single replicate

Fig. 7. Total lipids, water content and polar and neutral lipids in the livers of charr fed diets containing 0.5% n-3 PUFA. Values = mean ± SEM, n = 2. Each replicate value was determined from 5-10 pooled fish livers

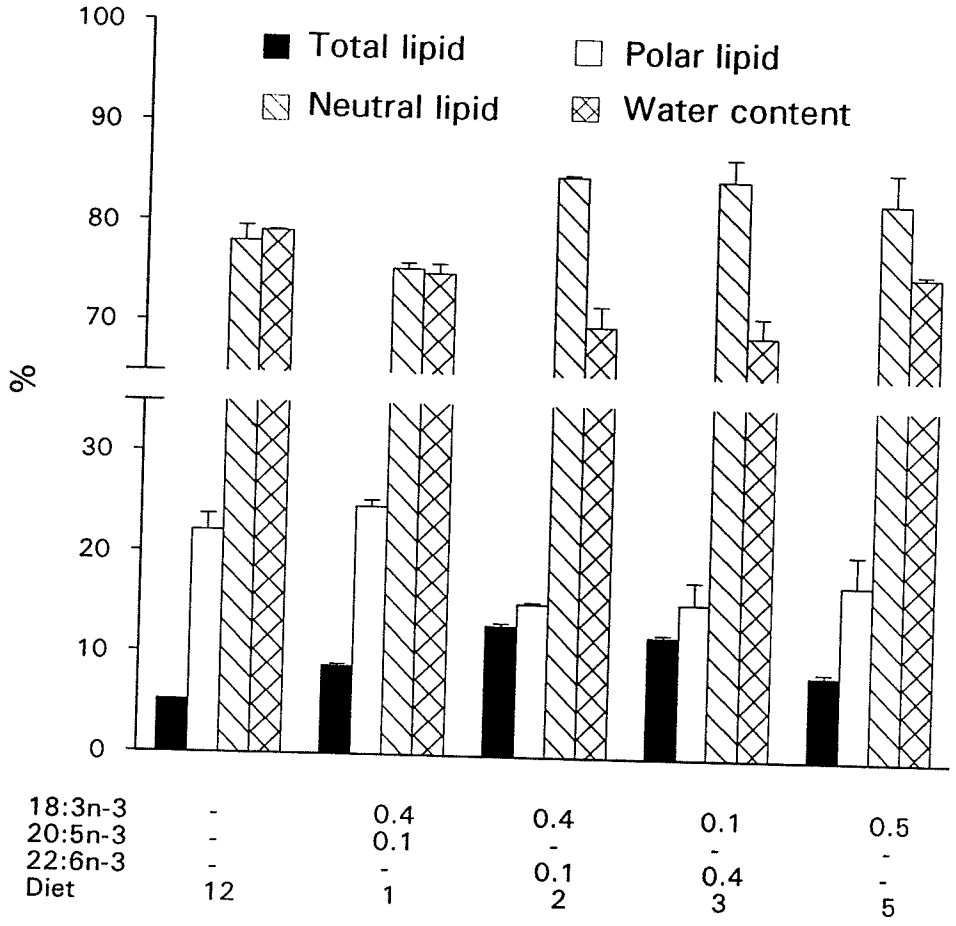
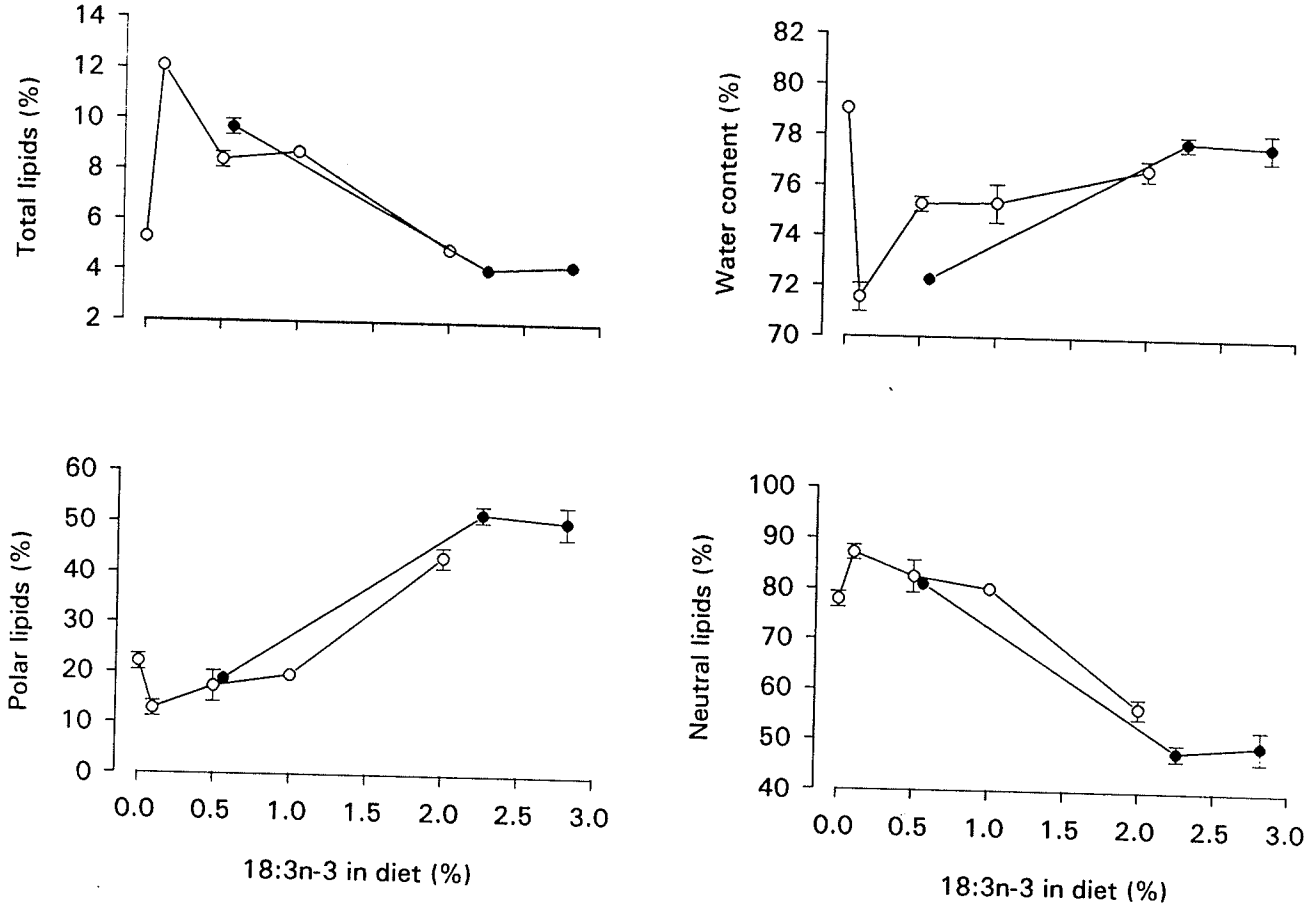

100
90
80
70
%
30
20
10
0
Total lipid
Polar lipid
Neutral lipid
Water content
18:3n-3 - 0.4 0.4 0.1 0.5
20:5n-3 - 0.1 - - -
22:6n-3 - - 0.1 0.4 -
Diet 12 1 2 3 5

Fig. 8. Total lipids, water content and polar and neutral lipids in the livers of charr fed diets varying in levels of 18:2n-6 and 18:3n-3. The open and solid circles represent groups fed diets containing 18:3n-3 only and both 18:2n-6 and 18:3n-3, respectively. Values = mean ± SEM, n = 2. Each replicate value was determined from 5-10 pooled fish livers


Total lipids (%)
14
12
10
8
6
4
2
Water content (%)
82
80
78
76
74
72
70
Polar lipids (%)
60
50
40
30
20
10
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
18:3n-3 in diet (%)
Neutral lipids (%)
100
90
80
70
60
50
40
0.0
0.5
1.0
1.5
2.0
2.5
3.0
18:3n-3 in diet (%)

Among charr fed diets containing 0.5% dietary n-3 PUFA (diets 1-3, and 5), the groups fed diets containing 22:6n-3 (diets 2 and 3) had the lowest percentages for water content and polar lipids but the highest percentages for total lipids (Fig. 7). Moreover, percentages for water and polar lipids generally increased as dietary 18:3n-3 was increased from 0.1 to 2.82% (diets 4-7 and 9-10), while those for total and neutral lipids declined (Fig. 8). The percentages of total and polar lipids in charr fed diets containing 0.56-0.7% 18:2n-6 and 2.26-2.82% 18:3n-3 (diets 10 and 11) differed significantly from those fed the diet with 2.0% 18:3n-3 (diet 7) but not from those given the commercial diet.

The fatty acid compositions of the liver polar lipids of charr in relation to diet treatments are given in Table 6 and Fig. 9. Generally, as the dietary level of 18:3n-3 increased from 0 to 2.0% (diets 4-7 and 12), the percentages of total n-3 PUFA in polar lipids increased in livers, while total n-9 and n-6 PUFA and the ratios of 20:3n-9/22:6n-3 declined. The percentages of 18:3n-3, 20:5n-3 and 22:6n-3 also increased while 20:3n-9 decreased in liver polar lipids. Similar trends were noted when charr were fed diets containing combinations of 18:2n-6 and 18:3n-3 (diets 9-11). The percentages of 18:2n-6, 20:3n-6 and 20:4n-6 in liver polar lipids from charr fed diets containing 18:3n-3 only (diets 5 and 7) were significantly lower than those noted in charr given diets with additional 18:2n-6 (diets 9 and 10).

Table 6. Percentages of selected fatty acids in liver polar lipids of Arctic charr (weight %, Mean ± SEM, n[1] = 2)[2]

Fatty acid	Initial[3]	Diet					
		1	2	3	4	5	6
14:0	1.9 ± 0.1	2.1 ± 0.1 ^{ab}	1.8 ± 0.2 ^{abc}	2.5 ± 0.1 ^{a}	2.0 ± 0.2 ^{ab}	2.3 ± 0.6 ^{ab}	1.8 ± 0.2 ^{abc}
16:0	18.2 ± 0.4	15.7 ± 0.5 ^{cde}	17.0 ± 0.9 ^{bcd}	16.2 ± 1.2 ^{cde}	13.5 ± 0.8 ^{e}	16.5 ± 0.6 ^{cde}	18.7 ± 0.2 ^{abc}
16:1n-9	0.7 ± 0.0	1.3 ± 0.1 ^{cd}	1.4 ± 0.1 ^{c}	1.6 ± 0.0 ^{c}	2.4 ± 0.3 ^{b}	1.5 ± 0.3 ^{c}	1.0 ± 0.1 ^{de}
16:1n-7	1.9 ± 0.1	4.4 ± 0.4 ^{bcd}	5.9 ± 1.1 ^{ab}	5.0 ± 0.7 ^{b}	6.9 ± 0.4 ^{a}	5.2 ± 0.5 ^{ab}	4.7 ± 0.6 ^{bc}
18:0	3.5 ± 0.0	4.5 ± 0.2 ^{ab}	4.4 ± 0.4 ^{ab}	4.1 ± 0.8 ^{abc}	3.5 ± 0.2 ^{bcd}	4.1 ± 0.3 ^{abc}	4.6 ± 0.3 ^{a}
18:1n-9	10.8 ± 0.1	27.0 ± 0.4 ^{ab}	30.0 ± 1.3 ^{a}	27.4 ± 0.6 ^{ab}	27.9 ± 1.7 ^{ab}	27.9 ± 0.9 ^{ab}	24.3 ± 0.3 ^{b}
18:1n-7	3.8 ± 0.1	3.6 ± 0.4 ^{cd}	3.6 ± 0.0 ^{cd}	3.9 ± 0.5 ^{cd}	4.8 ± 0.2 ^{ab}	4.1 ± 0.6 ^{bc}	3.1 ± 0.1 ^{def}
18:2n-6	2.3 ± 0.0	0.6 ± 0.1 ^{f}	0.2 ± 0.0 ^{g}	0.2 ± 0.0 ^{g}	0.4 ± 0.1 ^{g}	0.4 ± 0.1 ^{fg}	0.2 ± 0.0 ^{g}
18:3n-3	0.1 ± 0.0	0.7 ± 0.1 ^{c}	0.3 ± 0.1 ^{de}	0.1 ± 0.0 ^{e}	0.1 ± 0.0 ^{e}	0.5 ± 0.0 ^{cd}	0.8 ± 0.1 ^{c}
18:4n-3	0.1 ± 0.0	0.7 ± 0.0 ^{cd}	0.4 ± 0.1 ^{de}	0.1 ± 0.0 ^{ef}	0.2 ± 0.0 ^{ef}	0.7 ± 0.0 ^{cd}	1.0 ± 0.1 ^{bc}
20:1n-9	2.5 ± 0.1	3.5 ± 0.5 ^{bc}	3.1 ± 0.3 ^{bcd}	3.6 ± 0.2 ^{bc}	3.8 ± 0.3 ^{b}	3.4 ± 0.3 ^{bc}	2.4 ± 0.2 ^{de}
20:3n-9	0.2 ± 0.1	2.1 ± 0.2 ^{cd}	4.1 ± 1.4 ^{ab}	4.4 ± 0.8 ^{ab}	5.2 ± 0.5 ^{a}	3.2 ± 0.3 ^{bc}	2.4 ± 0.1 ^{cd}
20:3n-6	0.9 ± 0.0	0.8 ± 0.0 ^{fg}	0.3 ± 0.1 ^{i}	0.3 ± 0.1 ^{i}	0.5 ± 0.1 ^{hi}	0.6 ± 0.2 ^{gh}	0.3 ± 0.0 ^{hi}
20:4n-6	4.0 ± 0.0	0.8 ± 0.2 ^{de}	0.7 ± 0.1 ^{de}	0.6 ± 0.1 ^{e}	1.2 ± 0.1 ^{d}	0.8 ± 0.2 ^{de}	0.6 ± 0.1 ^{e}
20:3n-3	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1
20:4n-3	0.1 ± 0.0	0.9 ± 0.3 ^{bcd}	0.2 ± 0.1 ^{ef}	0.2 ± 0.1 ^{ef}	0.1 ± 0.0 ^{f}	0.5 ± 0.1 ^{cdef}	0.8 ± 0.0 ^{bcde}
20:5n-3	3.9 ± 0.2	3.7 ± 0.3 ^{c}	2.2 ± 0.2 ^{de}	1.0 ± 0.0 ^{f}	1.1 ± 0.0 ^{ef}	3.4 ± 0.1 ^{c}	5.2 ± 0.1 ^{b}
22:4n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	tr
22:5n-6	0.3 ± 0.0	0.2 ± 0.1 ^{c}	0.4 ± 0.1 ^{c}	0.3 ± 0.0 ^{c}	0.3 ± 0.0 ^{c}	0.2 ± 0.1 ^{c}	0.1 ± 0.0 ^{c}
22:5n-3	0.8 ± 0.1	2.1 ± 0.1 ^{bc}	1.3 ± 0.0 ^{d}	0.5 ± 0.1 ^{fg}	0.8 ± 0.1 ^{ef}	1.9 ± 0.3 ^{c}	2.8 ± 0.1 ^{a}
22:6n-3	32.7 ± 0.0	14.6 ± 0.2 ^{de}	12.8 ± 1.4 ^{ef}	16.6 ± 0.3 ^{d}	7.7 ± 0.1 ^{h}	12.0 ± 0.7 ^{f}	16.1 ± 1.0 ^{d}
∑ Saturates	24.0 ± 0.5	23.2 ± 0.6 ^{abcd}	23.9 ± 0.8 ^{abcd}	23.8 ± 1.4 ^{abcd}	19.6 ± 1.4 ^{e}	23.6 ± 0.1 ^{abcd}	25.6 ± 0.3 ^{ab}
∑ Monoenes	22.8 ± 0.0	41.7 ± 0.1 ^{ab}	45.7 ± 2.1 ^{a}	43.7 ± 1.8 ^{a}	48.4 ± 3.1 ^{a}	44.2 ± 2.4 ^{a}	36.8 ± 0.2 ^{b}
∑ PUFA	46.7 ± 0.3	30.2 ± 0.7 ^{b}	25.6 ± 2.3 ^{d}	27.7 ± 0.7 ^{cd}	21.0 ± 0.3 ^{e}	27.4 ± 1.5 ^{cd}	32.9 ± 0.9 ^{b}
∑ (n-9)	0.6 ± 0.1	4.1 ± 0.1 ^{d}	6.4 ± 1.4 ^{bc}	7.3 ± 0.4 ^{ab}	8.5 ± 0.4 ^{a}	5.7 ± 0.2 ^{c}	4.0 ± 0.1 ^{d}
∑ (n-6)	8.4 ± 0.1	3.4 ± 0.3 ^{f}	2.0 ± 0.1 ^{hi}	1.8 ± 0.0 ^{i}	2.6 ± 0.1 ^{gh}	2.6 ± 0.5 ^{gh}	2.2 ± 0.0 ^{ghi}
∑ (n-3)	37.8 ± 0.3	22.7 ± 0.9 ^{d}	17.2 ± 1.0 ^{e}	18.6 ± 0.3 ^{e}	10.0 ± 0.0 ^{f}	19.1 ± 1.2 ^{e}	26.8 ± 0.9 ^{c}
20:3n-9/22:6n-3	-	0.1 ± 0.0 ^{def}	0.3 ± 0.1 ^{cd}	0.3 ± 0.0 ^{cde}	0.7 ± 0.1 ^{b}	0.3 ± 0.0 ^{cde}	0.1 ± 0.0 ^{def}

...continued

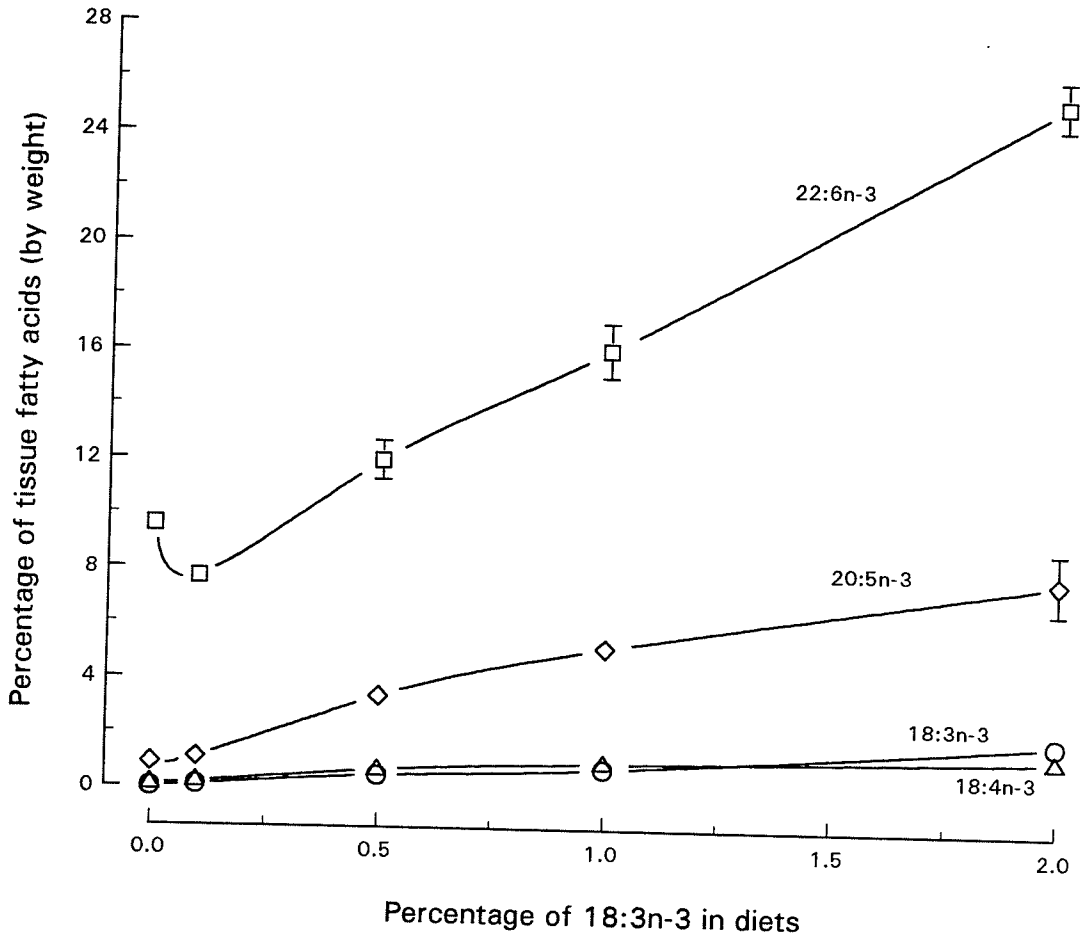
[1]Same letters in a row indicate no significant difference (Value for each replicate was determined from pooled livers of 5-10 fish) [2]Collected at the end of

Table 6. (continued)

Fatty acid	7	8	9	10	11	12	13
14:0	2.0 ± 0.0 ^{ab}	2.3 ± 0.3 ^{ab}	2.0 ± 0.2 ^{ab}	1.2 ± 0.3 ^{cd}	0.8 ± 0.1 ^{d}	2.5 ± 0.3 ^{a}	1.5 ± 0.2 ^{bc}
16:0	18.8 ± 0.8 ^{abc}	13.9 ± 1.2 ^{de}	18.0 ± 0.5 ^{bc}	20.3 ± 1.8 ^{a}	19.7 ± 0.6 ^{ab}	14.6 ± 1.6 ^{de}	18.1 ± 0.3 ^{abc}
16:1n-9	0.7 ± 0.1 ^{ef}	1.4 ± 0.1 ^{c}	1.4 ± 0.2 ^{c}	0.7 ± 0.1 ^{ef}	0.7 ± 0.1 ^{ef}	2.8 ± 0.1 ^{a}	0.4 ± 1.5 ^{f}
16:1n-9	2.8 ± 0.3 ^{de}	5.9 ± 0.1 ^{ab}	6.1 ± 0.4 ^{ab}	1.9 ± 0.2 ^{e}	2.3 ± 0.6 ^{e}	5.7 ± 0.2 ^{ab}	3.2 ± 0.4 ^{cde}
18:0	4.2 ± 0.2 ^{ab}	3.9 ± 0.0 ^{abc}	4.0 ± 0.3 ^{abc}	4.8 ± 0.3 ^{a}	4.8 ± 0.3 ^{a}	3.0 ± 0.1 ^{cd}	2.9 ± 0.3 ^{d}
18:1n-9	17.7 ± 1.7 ^{c}	27.8 ± 2.5 ^{ab}	27.8 ± 0.9 ^{ab}	17.0 ± 1.5 ^{c}	19.2 ± 2.1 ^{c}	23.8 ± 1.7 ^{b}	11.5 ± 1.2 ^{d}
18:1n-7	2.3 ± 0.1 ^{gfh}	3.2 ± 0.4 ^{cde}	3.6 ± 0.1 ^{cd}	1.9 ± 0.1 ^{hg}	1.7 ± 0.1 ^{h}	5.5 ± 0.2 ^{a}	2.7 ± 0.1 ^{efg}
18:2n-6	0.2 ± 0.0 ^{g}	2.7 ± 0.1 ^{b}	0.9 ± 0.1 ^{e}	2.0 ± 0.1 ^{d}	2.2 ± 0.0 ^{c}	1.0 ± 0.1 ^{e}	3.2 ± 0.1 ^{a}
18:3n-3	1.9 ± 0.2 ^{b}	tr	0.5 ± 0.1 ^{d}	1.9 ± 0.3 ^{b}	2.6 ± 0.1 ^{a}	tr	0.1 ± 0.0 ^{e}
18:4n-3	1.3 ± 0.3 ^{ab}	tr	0.7 ± 0.1 ^{cd}	0.9 ± 0.2 ^{c}	1.5 ± 0.2 ^{a}	0.1 ± 0.0 ^{ef}	0.2 ± 0.0 ^{ef}
20:1n-9	1.7 ± 0.3 ^{ef}	3.4 ± 0.2 ^{bc}	2.8 ± 0.3 ^{cd}	1.7 ± 0.1 ^{f}	1.4 ± 0.2 ^{f}	3.5 ± 0.1 ^{bc}	4.7 ± 0.2 ^{a}
20:3n-9	0.9 ± 0.2 ^{de}	2.2 ± 0.2 ^{cd}	2.9 ± 0.1 ^{bc}	0.3 ± 0.1 ^{e}	0.2 ± 0.1 ^{e}	4.8 ± 0.1 ^{a}	-
20:3n-6	0.3 ± 0.1 ^{i}	3.1 ± 0.2 ^{a}	1.0 ± 0.0 ^{ef}	1.8 ± 0.1 ^{b}	1.6 ± 0.2 ^{bc}	1.1 ± 0.1 ^{de}	1.4 ± 0.1 ^{cd}
20:4n-6	0.4 ± 0.1 ^{e}	7.3 ± 0.4 ^{a}	1.7 ± 0.2 ^{c}	2.9 ± 0.3 ^{b}	2.5 ± 0.1 ^{b}	2.6 ± 0.1 ^{b}	2.4 ± 0.1 ^{b}
20:3n-3	0.5 ± 0.0	tr	0.1 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	tr
20:4n-3	1.8 ± 0.6 ^{a}	tr	0.3 ± 0.0 ^{def}	1.1 ± 0.2 ^{bc}	1.2 ± 0.3 ^{ab}	tr	0.2 ± 0.0 ^{ef}
20:5n-3	7.8 ± 1.1 ^{a}	0.2 ± 0.1 ^{f}	3.0 ± 0.5 ^{cd}	5.0 ± 0.2 ^{b}	5.7 ± 0.2 ^{b}	0.9 ± 0.0 ^{f}	5.6 ± 0.5 ^{b}
22:4n-6	tr	1.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0
22:5n-6	0.1 ± 0.1 ^{c}	8.2 ± 0.4 ^{a}	0.4 ± 0.0 ^{c}	0.2 ± 0.0 ^{c}	0.2 ± 0.0 ^{c}	0.9 ± 0.1 ^{b}	0.3 ± 0.0 ^{c}
22:5n-3	2.8 ± 0.3 ^{a}	0.1 ± 0.0 ^{g}	1.8 ± 0.3 ^{c}	2.4 ± 0.0 ^{ab}	2.9 ± 0.1 ^{a}	0.6 ± 0.0 ^{ef}	1.0 ± 0.0 ^{de}
22:6n-3	25.3 ± 0.9 ^{bc}	1.5 ± 0.2 ^{i}	10.9 ± 0.5 ^{fg}	26.6 ± 0.5 ^{b}	24.1 ± 1.2 ^{c}	9.6 ± 0.0 ^{hg}	31.4 ± 0.1 ^{a}
∑ Saturates	25.6 ± 0.8 ^{ab}	21.8 ± 1.9 ^{cde}	24.8 ± 0.3 ^{abc}	26.6 ± 1.7 ^{a}	25.4 ± 0.8 ^{ab}	20.9 ± 1.1 ^{de}	22.6 ± 0.3 ^{bcde}
∑ Monoenes	26.5 ± 2.2 ^{c}	43.6 ± 2.9 ^{a}	43.3 ± 1.2 ^{a}	24.3 ± 1.9 ^{c}	26.5 ± 2.8 ^{c}	45.1 ± 1.6 ^{a}	26.5 ± 1.5 ^{c}
∑ PUFA	45.5 ± 1.6 ^{a}	28.9 ± 0.2 ^{cd}	27.6 ± 1.9 ^{d}	47.9 ± 0.1 ^{a}	48.2 ± 1.5 ^{a}	25.1 ± 0.1 ^{d}	46.8 ± 0.3 ^{a}
∑ (n-9)	1.4 ± 0.2 ^{e}	3.9 ± 0.3 ^{d}	5.2 ± 0.2 ^{cd}	0.6 ± 0.0 ^{e}	0.5 ± 0.1 ^{e}	7.7 ± 0.0 ^{ab}	0.2 ± 0.0 ^{e}
∑ (n-6)	2.9 ± 0.3 ^{fg}	23.1 ± 0.2 ^{a}	5.3 ± 0.4 ^{e}	9.3 ± 0.2 ^{b}	9.6 ± 0.1 ^{b}	6.0 ± 0.1 ^{d}	8.1 ± 0.1 ^{c}
∑ (n-3)	41.2 ± 1.5 ^{a}	1.9 ± 0.2 ^{g}	17.2 ± 1.4 ^{e}	38.1 ± 0.1 ^{b}	38.2 ± 1.5 ^{b}	11.5 ± 0.1 ^{f}	38.6 ± 0.4 ^{ab}
20:3n-9/22:6n-3	-	1.4 ± 0.3 ^{a}	0.3 ± 0.0 ^{cde}	-	-	0.5 ± 0.0 ^{bc}	-

aclimation period [3]Not detectable [4]Trace amount (<0.05)

Fig. 9. Production of n-3 PUFAs in liver polar lipids of Arctic charr fed diets containing various levels of 18:3n-3


Percentage of tissue fatty acids (by weight)
28
24
20
16
12
8
4
0
22:6n-3
20:5n-3
18:3n-3
18:4n-3
0.0
0.5
1.0
1.5
2.0
Percentage of 18:3n-3 in diets

Charr fed the diet containing 0.1% 20:5n-3 (diet 1) displayed significantly higher concentrations of total n-3 PUFA and 22:6n-3 but lower amounts of 20:3n-9 than charr fed the diet with 0.5% 18:3n-3 (diet 5), However, the groups given diets containing either low or high levels of 22:6n-3 (diets 2 and 3) had higher amounts of 20:3n-9 and lower amounts of 20:5n-3. In addition, charr fed the high 22:6n-3 diet (diet 3) had a significantly higher percentage of 22:6n-3 in the tissues.

In contrast to polar lipids, neutral lipids from livers differed less among treatments in their fatty acid composition (Table 7). As 18:3n-3 increased in the diets (diets 4-7 and 12), the percentages of total saturates, 12:0 and 16:0 gradually increased, while total monoenes and n-9 PUFA declined. There was a significant increase in tissue 18:3n-3 when 18:3n-3 reached 2.0% in the diet (diet 7). Total saturates, monoenes and total n-9 PUFA generally decreased with the increase of dietary 18:2n-6 and 18:3n-3 (diets 9-11). However, little difference was found in percentages of saturates, monoenes and PUFAs among groups fed diets containing 0.5% n-3 PUFAs (diets 1-3 and 5).

The fatty acid compositions of muscle lipids are shown in Table 8. Charr fed the 0% PUFA or commercial diet (diets 12 and 13) had significantly higher percentages of 22:6n-3 than those fed other casein-based diets. As 18:3n-3 in the diets increased from 0.1 to 2.82% (diets 4-7 and 9-11), 18:3n-3 and

Table 7. Percentages of selected fatty acids in liver neutral lipids of Arctic charr (weight %, Mean ± SEM, $n^1 = 2$)[2]

Fatty acid		Diet					
	Initial[3]	1	2	3	4	5	6
12:0	7.1 ± 0.8	6.2 ± 0.0 ^{ab}	7.1 ± 1.1 ^{a}	6.1 ± 0.9 ^{ab}	4.7 ± 0.3 ^{b}	5.8 ± 0.0 ^{ab}	6.9 ± 0.2 ^{a}
14:0	5.9 ± 0.6	4.6 ± 0.0 ^{abc}	5.2 ± 0.7 ^{a}	4.7 ± 0.2 ^{abc}	4.5 ± 0.0 ^{abc}	4.4 ± 0.2 ^{abc}	4.2 ± 0.3 ^{bc}
16:0	13.8 ± 1.4	8.8 ± 1.1 ^{bc}	11.1 ± 0.9 ^{abc}	9.5 ± 0.3 ^{abc}	8.4 ± 0.8 ^{c}	8.7 ± 1.1 ^{bc}	10.3 ± 0.1 ^{abc}
16:1n-9	0.5 ± 0.0	1.2 ± 0.0 ^{def}	1.6 ± 0.3 ^{cd}	1.8 ± 0.2 ^{bc}	2.3 ± 0.0 ^{b}	1.5 ± 0.1 ^{cd}	1.2 ± 0.1 ^{def}
16:1n-7	7.7 ± 0.5	8.1 ± 0.1 ^{bc}	10.0 ± 0.4 ^{a}	8.1 ± 0.6 ^{bc}	8.7 ± 0.5 ^{abc}	8.8 ± 0.3 ^{abc}	9.9 ± 0.2 ^{a}
18:0	2.8 ± 0.2	4.8 ± 0.3 ^{a}	4.6 ± 0.0 ^{ab}	4.7 ± 0.5 ^{ab}	3.8 ± 0.2 ^{cd}	3.9 ± 0.3 ^{bcd}	4.4 ± 0.1 ^{abc}
18:1n-9	15.2 ± 0.8	45.9 ± 1.6 ^{ab}	44.3 ± 2.8 ^{abc}	45.9 ± 1.1 ^{ab}	44.2 ± 0.0 ^{abc}	46.2 ± 0.3 ^{ab}	45.7 ± 0.2 ^{ab}
18:1n-7	3.5 ± 0.0	4.3 ± 0.1 ^{bcd}	3.8 ± 0.1 ^{de}	3.9 ± 0.1 ^{de}	5.2 ± 0.3 ^{ab}	4.8 ± 0.5 ^{bc}	3.9 ± 0.1 ^{cde}
18:2n-6	3.5 ± 0.1	0.3 ± 0.1 ^{d}	0.1 ± 0.0 ^{d}	0.1 ± 0.0 ^{d}	0.2 ± 0.1 ^{d}	0.2 ± 0.0 ^{d}	0.1 ± 0.1 ^{d}
18:3n-6	tr[5]	tr	-[4]	tr	tr	tr	tr
18:3n-3	0.3 ± 0.0	0.4 ± 0.1 ^{d}	0.1 ± 0.0 ^{d}	tr	tr	0.2 ± 0.1 ^{d}	0.4 ± 0.0 ^{d}
18:4n-3	0.5 ± 0.1	0.3 ± 0.1 ^{de}	0.2 ± 0.1 ^{de}	0.1 ± 0.0 ^{e}	0.1 ± 0.1 ^{e}	0.3 ± 0.1 ^{de}	0.4 ± 0.0 ^{de}
20:1n-9	4.7 ± 0.3	8.0 ± 0.4 ^{b}	5.6 ± 0.8 ^{d}	7.9 ± 0.3 ^{b}	8.4 ± 0.5 ^{b}	7.6 ± 0.7 ^{bc}	5.3 ± 0.3 ^{d}
20:2n-9	0.2 ± 0.0	1.3 ± 0.1 ^{abc}	1.1 ± 0.1 ^{bcd}	1.5 ± 0.2 ^{ab}	1.7 ± 0.2 ^{a}	1.5 ± 0.1 ^{a}	1.1 ± 0.1 ^{cde}
20:2n-6	0.5 ± 0.0	0.1 ± 0.0	tr	tr	tr	tr	-
20:3n-9	tr	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
20:4n-6	0.8 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
20:3n-3	0.1 ± 0.1	0.1 ± 0.0	tr	tr	tr	tr	0.1 ± 0.0
20:4n-3	0.2 ± 0.0	0.1 ± 0.0	-	tr	tr	0.1 ± 0.1	0.1 ± 0.0
20:5n-3	2.1 ± 0.1	0.2 ± 0.1 ^{c}	0.1 ± 0.0 ^{c}	0.1 ± 0.1 ^{c}	0.1 ± 0.1 ^{c}	0.1 ± 0.0 ^{c}	0.2 ± 0.0 ^{c}
22:5n-6	0.1 ± 0.0	tr	-	-	-	-	-
22:5n-3	0.6 ± 0.1	0.1 ± 0.1	tr	tr	tr	tr	0.1 ± 0.0
22:6n-3	6.1 ± 0.3	0.3 ± 0.2 ^{c}	0.3 ± 0.2 ^{c}	0.4 ± 0.1 ^{c}	0.7 ± 0.2 ^{c}	0.3 ± 0.1 ^{c}	0.3 ± 0.0 ^{c}
Σ Saturates	29.8 ± 2.8	24.8 ± 1.5 ^{abc}	28.1 ± 2.8 ^{a}	25.3 ± 1.9 ^{ab}	21.6 ± 0.8 ^{bcd}	23.0 ± 1.5 ^{abc}	26.1 ± 0.5 ^{ab}
Σ Monoenes	40.7 ± 1.9	70.1 ± 2.3 ^{ab}	67.5 ± 2.6 ^{abc}	69.7 ± 2.0 ^{ab}	72.2 ± 0.5 ^{a}	70.8 ± 0.7 ^{ab}	67.9 ± 0.4 ^{abc}
Σ PUFA	15.8 ± 0.1	3.5 ± 0.5 ^{bc}	2.3 ± 0.4 ^{c}	2.6 ± 0.3 ^{c}	3.6 ± 0.3 ^{bc}	3.1 ± 0.1 ^{bc}	3.2 ± 0.2 ^{bc}
Σ (n-9)	0.4 ± 0.1	1.7 ± 0.1 ^{cde}	1.5 ± 0.2 ^{de}	2.4 ± 0.2 ^{bc}	2.4 ± 0.2 ^{ab}	2.0 ± 0.2 ^{bc}	1.3 ± 0.0 ^{ef}
Σ (n-6)	5.6 ± 0.0	0.6 ± 0.2 ^{c}	0.2 ± 0.0 ^{c}	0.2 ± 0.0 ^{c}	0.5 ± 0.1 ^{c}	0.3 ± 0.0 ^{c}	0.3 ± 0.2 ^{c}
Σ (n-3)	9.8 ± 0.2	1.3 ± 0.4 ^{d}	0.6 ± 0.2 ^{d}	0.5 ± 0.0 ^{d}	0.8 ± 0.1 ^{d}	0.9 ± 0.0 ^{d}	1.6 ± 0.0 ^{d}

[1,2,3,4,5]Designations are the same as in Table 6.

....continued

Table 7. (continued)

Fatty acid	7	8	9	10	11	12	13
12:0	6.6 ± 1.6 ^{ab}	5.7 ± 0.1 ^{ab}	5.2 ± 0.6 ^{ab}	2.0 ± 0.0 ^{c}	0.2 ± 0.0 ^{c}	4.9 ± 0.0 ^{ab}	0.1 ± 0.0
14:0	4.1 ± 0.6 ^{c}	5.0 ± 0.1 ^{abc}	4.1 ± 0.1 ^{c}	2.6 ± 0.0 ^{de}	2.1 ± 0.2 ^{e}	5.2 ± 0.2 ^{ab}	3.0 ± 0.0 ^{d}
16:0	12.0 ± 1.2 ^{a}	11.5 ± 1.9 ^{ab}	10.1 ± 0.2 ^{abc}	11.0 ± 1.1 ^{abc}	10.8 ± 0.7 ^{abc}	8.7 ± 0.4 ^{bc}	10.2 ± 0.4 ^{abc}
16:1n-9	0.7 ± 0.0 ^{fg}	1.7 ± 0.1 ^{cd}	1.4 ± 0.1 ^{cde}	0.9 ± 0.0 ^{ef}	0.8 ± 0.1 ^{f}	2.9 ± 0.4 ^{a}	0.3 ± 0.1 ^{g}
16:1n-7	9.8 ± 0.4 ^{ab}	8.0 ± 0.4 ^{bc}	10.0 ± 0.6 ^{a}	8.0 ± 0.4 ^{c}	8.6 ± 0.3 ^{abc}	9.3 ± 1.1 ^{abc}	9.0 ± 0.7 ^{abc}
18:0	4.6 ± 0.4 ^{ab}	4.4 ± 0.1 ^{abc}	4.0 ± 0.2 ^{bcd}	3.7 ± 0.1 ^{cd}	3.6 ± 0.1 ^{d}	3.3 ± 0.0 ^{d}	1.8 ± 0.0 ^{e}
18:1n-9	41.2 ± 4.5 ^{abc}	39.3 ± 2.7 ^{abc}	46.8 ± 1.0 ^{a}	37.9 ± 3.9 ^{c}	41.1 ± 0.5 ^{abc}	39.0 ± 0.7 ^{bc}	21.1 ± 2.6 ^{d}
18:1n-7	3.2 ± 0.3 ^{ef}	4.3 ± 0.1 ^{bcd}	4.2 ± 0.4 ^{cd}	3.1 ± 0.3 ^{ef}	2.7 ± 0.4 ^{f}	5.9 ± 0.5 ^{a}	3.7 ± 0.1 ^{de}
18:2n-6	0.2 ± 0.0 ^{d}	1.8 ± 0.4 ^{c}	0.4 ± 0.1 ^{d}	3.3 ± 0.0 ^{b}	3.7 ± 0.3 ^{b}	0.3 ± 0.0 ^{d}	5.4 ± 0.2 ^{a}
18:3n-6	tr	tr	tr	tr	tr	0.1 ± 0.0	0.1 ± 0.1
18:3n-3	1.9 ± 0.1 ^{c}	tr	0.2 ± 0.0 ^{d}	4.5 ± 0.3 ^{b}	6.0 ± 0.6 ^{a}	tr	0.4 ± 0.0 ^{d}
18:4n-3	1.2 ± 0.1 ^{c}	0.1 ± 0.1 ^{e}	0.2 ± 0.0 ^{de}	2.5 ± 0.2 ^{b}	3.7 ± 0.4 ^{a}	tr	0.6 ± 0.1 ^{d}
20:1n-9	4.8 ± 0.8 ^{d}	8.1 ± 0.1 ^{b}	6.0 ± 0.3 ^{cd}	5.4 ± 0.6 ^{d}	4.5 ± 0.4 ^{d}	7.8 ± 1.1 ^{bc}	12.5 ± 0.5 ^{a}
20:2n-9	0.7 ± 0.1 ^{ef}	1.6 ± 0.1 ^{a}	1.4 ± 0.2 ^{abc}	0.8 ± 0.2 ^{def}	0.6 ± 0.1 ^{f}	1.6 ± 0.1 ^{a}	0.2 ± 0.0 ^{g}
20:2n-6	tr	0.5 ± 0.1	0.1 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.1 ± 0.1	0.7 ± 0.0
20:3n-9	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	-	-	0.3 ± 0.0	-
20:4n-6	tr	1.1 ± 0.6	0.1 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.5 ± 0.2
20:3n-3	0.3 ± 0.0	tr	tr	0.5 ± 0.0	0.5 ± 0.1	tr	0.1 ± 0.0
20:4n-3	0.5 ± 0.2	tr	tr	0.8 ± 0.0	1.1 ± 0.4	tr	0.3 ± 0.0
20:5n-3	0.6 ± 0.1 ^{c}	0.1 ± 0.0 ^{c}	0.1 ± 0.0 ^{c}	1.4 ± 0.5 ^{b}	1.3 ± 0.1 ^{b}	0.1 ± 0.0 ^{c}	2.8 ± 0.7 ^{a}
22:5n-6	tr	0.7 ± 0.7	tr	tr	tr	tr	0.1 ± 0.0
22:5n-3	0.2 ± 0.0	0.1 ± 0.1	tr	0.5 ± 0.2	0.5 ± 0.1	-	0.5 ± 0.1
22:6n-3	1.1 ± 0.0 ^{c}	0.5 ± 0.2 ^{c}	0.4 ± 0.0 ^{c}	3.5 ± 1.3 ^{b}	2.3 ± 0.3 ^{bc}	0.6 ± 0.0 ^{c}	6.7 ± 1.8 ^{a}
∑ Saturates	27.5 ± 3.8 ^{a}	26.8 ± 1.7 ^{ab}	23.7 ± 0.3 ^{abc}	19.4 ± 0.9 ^{cde}	16.9 ± 0.9 ^{de}	22.6 ± 0.1 ^{abc}	15.2 ± 0.4 ^{e}
∑ Monoenes	61.6 ± 5.9 ^{bcd}	62.5 ± 3.5 ^{abcd}	70.3 ± 0.4 ^{ab}	57.7 ± 4.1 ^{d}	59.3 ± 1.5 ^{cd}	69.0 ± 0.3 ^{ab}	59.3 ± 4.2 ^{cd}
∑ PUFA	6.8 ± 0.3 ^{bc}	7.5 ± 2.3 ^{b}	3.5 ± 0.2 ^{bc}	19.4 ± 2.4 ^{a}	21.0 ± 2.1 ^{a}	4.3 ± 0.1 ^{bc}	18.9 ± 2.7 ^{a}
∑ (n-9)	0.9 ± 0.2 ^{fg}	2.1 ± 0.2 ^{bc}	1.9 ± 0.3 ^{cd}	1.0 ± 0.2 ^{fg}	0.8 ± 0.1 ^{g}	2.5 ± 0.0 ^{a}	0.3 ± 0 h
∑ (n-6)	0.2 ± 0.0 ^{c}	4.8 ± 1.9 ^{b}	0.7 ± 0.1 ^{c}	4.9 ± 0.2 ^{b}	5.1 ± 0.4 ^{b}	1.1 ± 0.1 ^{c}	7.4 ± 0.2 ^{a}
∑ (n-3)	5.7 ± 0.0 ^{c}	0.7 ± 0.3 ^{d}	0.9 ± 0.0 ^{d}	13.6 ± 2.4 ^{ab}	15.2 ± 1.8 ^{a}	0.7 ± 0.0 ^{d}	11.3 ± 2.6 ^{b}

Table 8. Percentages of selected fatty acids in muscle lipids of Arctic charr (weight %, Mean ± SEM, n[1] = 2)[2]

fatty acid		Diet					
	Initial[3]	1	2	3	4	5	6
12:0	1.1 ± 0.0	16.2 ± 1.3 [a]	14.8 ± 1.2 [ab]	15.7 ± 1.6 [a]	9.3 ± 0.8 [d]	15.8 ± 1.1 [a]	13.8 ± 0.2 [abc]
14:0	2.8 ± 0.0	5.6 ± 0.1 [ab]	5.8 ± 0.0 [a]	6.1 ± 0.1 [a]	5.4 ± 0.1 [abc]	5.8 ± 0.6 [a]	4.8 ± 0.1 [cd]
16:0	15.0 ± 0.1	10.5 ± 0.3 [b]	11.1 ± 0.5 [b]	10.3 ± 0.0 [b]	11.1 ± 0.2 [b]	10.6 ± 0.1 [b]	11.3 ± 0.1 [b]
16:1n-9	0.4 ± 0.1	1.5 ± 0.0 [bc]	1.4 ± 0.1 [c]	1.8 ± 0.0 [b]	2.1 ± 0.0 [a]	1.5 ± 0.1 [bc]	1.1 ± 0.1 [d]
16:1n-7	4.8 ± 0.0	7.3 ± 0.2 [abc]	7.6 ± 0.4 [ab]	7.3 ± 0.5 [abc]	8.0 ± 0.6 [a]	7.7 ± 0.7 [a]	7.1 ± 0.3 [abc]
18:0	2.3 ± 0.0	2.2 ± 0.1 [ab]	2.3 ± 0.0 [ab]	2.0 ± 0.3 [b]	2.2 ± 0.2 [ab]	2.1 ± 0.4 [b]	2.3 ± 0.2 [ab]
18:1n-9	11.9 ± 0.1	25.7 ± 0.5 [a]	24.4 ± 1.4 [ab]	26.1 ± 0.1 [a]	24.4 ± 0.5 [ab]	25.0 ± 1.1 [ab]	21.9 ± 0.9 [bcd]
18:1n-7	3.0 ± 0.0	3.2 ± 0.4 [abc]	3.2 ± 0.3 [abc]	3.1 ± 0.5 [abc]	3.8 ± 0.3 [a]	3.2 ± 0.4 [abc]	2.8 ± 0.2 [bc]
18:2n-6	5.4 ± 0.0	1.6 ± 0.0 [cd]	1.9 ± 0.1 [cd]	1.3 ± 0.3 [d]	2.4 ± 0.1 [cd]	1.5 ± 0.4 [cd]	1.6 ± 0.1 [cd]
18:3n-6	tr[5]	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:3n-3	0.6 ± 0.0	2.2 ± 0.0 [ef]	1.6 ± 0.1 [ef]	0.5 ± 0.1 [ef]	0.7 ± 0.3 [ef]	2.2 ± 0.6 [ef]	4.3 ± 0.4 [d]
18:4n-3	0.7 ± 0.0	1.1 ± 0.1 [de]	1.0 ± 0.0 [de]	0.4 ± 0.0 [ef]	0.6 ± 0.2 [ef]	1.3 ± 0.1 [d]	2.3 ± 0.1 [c]
20:1n-9	6.4 ± 0.0	4.2 ± 0.0 [cd]	4.2 ± 0.1 [cd]	4.0 ± 0.4 [cd]	5.0 ± 0.4 [bc]	3.8 ± 0.3 [cd]	3.4 ± 0.3 [de]
20:2n-9	0.1 ± 0.0	0.9 ± 0.1 [ab]	0.8 ± 0.1 [ac]	1.0 ± 0.2 [a]	0.8 ± 0.0 [ab]	0.9 ± 0.1 [ab]	0.6 ± 0.1 [bcde]
20:2n-6	0.4 ± 0.0	0.7 ± 0.0 [bcd]	0.6 ± 0.0 [bcd]	0.7 ± 0.0 [bc]	0.8 ± 0.1 [b]	0.6 ± 0.1 [cd]	0.4 ± 0.1 [ef]
20:3n-6	0.4 ± 0.0	0.2 ± 0.0 [ef]	0.3 ± 0.1 [ef]	0.2 ± 0.1 [f]	0.3 ± 0.0 [def]	0.2 ± 0.0 [ef]	0.2 ± 0.0 [ef]
20:4n-6	0.8 ± 0.0	0.3 ± 0.0 [de]	0.4 ± 0.1 [de]	0.3 ± 0.1 [e]	0.6 ± 0.1 [c]	0.3 ± 0.0 [de]	0.3 ± 0.1 [e]
20:4n-3	0.4 ± 0.0	0.4 ± 0.2 [c]	0.3 ± 0.1 [c]	0.2 ± 0.1 [c]	0.2 ± 0.1 [c]	0.4 ± 0.1 [bc]	0.7 ± 0.3 [bc]
20:5n-3	6.6 ± 0.1	2.2 ± 0.1 [e]	2.3 ± 0.1 [e]	1.5 ± 0.1 [f]	2.6 ± 0.0 [de]	2.3 ± 0.2 [e]	3.1 ± 0.0 [cd]
22:1n-11	4.3 ± 0.1	1.1 ± 0.2 [c]	1.4 ± 0.1 [c]	0.7 ± 0.1 [c]	1.8 ± 0.2 [c]	0.6 ± 0.0 [c]	1.2 ± 0.3 [c]
22:5n-6	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
22:5n-3	1.1 ± 0.0	0.8 ± 0.1 [bc]	0.7 ± 0.0 [c]	0.5 ± 0.1 [d]	0.8 ± 0.1 [bc]	0.8 ± 0.1 [bc]	1.0 ± 0.1 [ab]
22:6n-3	21.0 ± 0.2	6.2 ± 0.7 [de]	7.5 ± 1.2 [cd]	10.5 ± 0.6 [abc]	8.2 ± 1.2 [cd]	6.3 ± 0.3 [de]	8.6 ± 0.9 [bcd]
∑ Saturates	21.3 ± 0.1	34.5 ± 1.1 [ab]	34.1 ± 0.8 [ab]	34.1 ± 1.7 [ab]	28.1 ± 0.7 [c]	34.3 ± 1.3 [ab]	32.2 ± 0.3 [ab]
∑ Monoenes	32.8 ± 0.1	44.3 ± 0.2 [ab]	43.7 ± 0.9 [ab]	44.1 ± 1.5 [ab]	47.3 ± 0.1 [a]	43.1 ± 0.3 [ab]	38.6 ± 0.9 [cd]
∑ PUFA	38.3 ± 0.0	17.2 ± 0.7 [g]	18.0 ± 1.4 [fg]	17.9 ± 0.3 [fg]	18.5 ± 1.0 [fg]	17.5 ± 1.7 [fg]	23.8 ± 0.6 [e]
∑ (n-9)	0.2 ± 0.0	1.2 ± 0.1 [ab]	1.0 ± 0.1 [abc]	1.3 ± 0.2 [a]	1.2 ± 0.2 [ab]	1.2 ± 0.0 [ab]	0.8 ± 0.0 [cd]
∑ (n-6)	7.5 ± 0.0	3.0 ± 0.0 [gh]	3.5 ± 0.1 [fg]	2.8 ± 0.3 [gh]	4.3 ± 0.0 [ef]	2.8 ± 0.4 [gh]	2.6 ± 0.0 [gh]
∑ (n-3)	30.6 ± 0.0	13.0 ± 0.6 [d]	13.6 ± 1.4 [d]	13.7 ± 0.7 [d]	13.1 ± 0.7 [d]	13.5 ± 1.3 [d]	20.5 ± 0.5 [c]

[1,2,3,4,5]Designations are the same as in Table 6 [6]Only one replicate was analyzed [7]20:2n-9 was not identified in one replicate

...continued

Table 8. (continued)

Fatty acid	7	8[6]	9	10	11	12[7]	13
12:0	11.3 ± 0.1 ^{cd}	16.2 ^{a}	12.2 ± 1.9 ^{bcd}	4.0 ± 0.2 ^{e}	0.3 ± 0.0 ^{f}	5.1 ± 0.4 ^{e}	0.2 ± 0.1 ^{f}
14:0	3.2 ± 0.2 ^{e}	5.5 ^{ab}	5.0 ± 0.1 ^{bcd}	1.7 ± 0.1 ^{f}	1.3 ± 0.0 ^{f}	4.5 ± 0.1 ^{d}	3.3 ± 0.1 ^{e}
16:0	10.9 ± 0.3 ^{b}	10.4 ^{b}	11.2 ± 0.0 ^{b}	11.2 ± 0.8 ^{b}	11.3 ± 1.3 ^{b}	11.8 ± 0.3 ^{ab}	13.1 ± 0.2 ^{a}
16:1n-9	0.9 ± 0.0 ^{de}	1.5 ^{bc}	1.4 ± 0.1 ^{c}	0.8 ± 0.1 ^{de}	0.8 ± 0.1 ^{e}	1.5 ± 0.0 ^{bc}	0.3 ± 0.1 ^{f}
16:1n-7	6.0 ± 0.2 ^{c}	6.2 ^{bc}	7.3 ± 0.3 ^{abc}	4.4 ± 0.3 ^{d}	3.6 ± 0.4 ^{d}	6.1 ± 0.3 ^{c}	6.9 ± 0.0 ^{abc}
18:0	2.3 ± 0.0 ^{ab}	2.5 ^{ab}	2.4 ± 0.3 ^{ab}	2.8 ± 0.2 ^{a}	2.8 ± 0.1 ^{a}	2.4 ± 0.2 ^{ab}	2.0 ± 0.1 ^{b}
18:1n-9	21.2 ± 1.5 ^{cd}	21.9 ^{bcd}	25.4 ± 0.0 ^{a}	23.7 ± 0.3 ^{abc}	23.2 ± 0.9 ^{abc}	19.0 ± 1.2 ^{d}	13.9 ± 0.7 ^{e}
18:1n-7	2.3 ± 0.1 ^{cde}	2.5 ^{cd}	3.0 ± 0.3 ^{abc}	1.9 ± 0.1 ^{de}	1.6 ± 0.2 ^{e}	3.5 ± 0.1 ^{ab}	3.2 ± 0.3 ^{abc}
18:2n-6	1.3 ± 0.3 ^{d}	7.4 ^{a}	2.6 ± 0.0 ^{c}	6.3 ± 0.5 ^{a}	7.3 ± 0.7 ^{a}	4.2 ± 0.1 ^{b}	7.1 ± 0.1 ^{a}
18:2n-6	0.1 ± 0.1	1.2	0.2 ± 0.1	0.5 ± 0.1	0.6 ± 0.0	0.2 ± 0.0	0.3 ± 0.1
18:3n-3	9.8 ± 1.1 ^{c}	0.2 ^{f}	2.5 ± 0.0 ^{de}	13.0 ± 0.9 ^{b}	15.7 ± 1.2 ^{a}	0.5 ± 0.1 ^{ef}	0.7 ± 0.1 ^{ef}
18:4n-4	5.2 ± 0.3 ^{b}	0.2 ^{f}	1.4 ± 0.1 ^{d}	4.8 ± 0.4 ^{b}	7.2 ± 0.3 ^{a}	0.4 ± 0.0 ^{ef}	0.8 ± 0.1 ^{def}
20:1n-9	2.9 ± 0.1 ^{de}	3.7 ^{cd}	3.8 ± 0.0 ^{cd}	2.8 ± 0.4 ^{de}	2.2 ± 0.3 ^{e}	5.6 ± 1.1 ^{b}	9.2 ± 0.0 ^{a}
20:2n-9	0.4 ± 0.1 ^{efg}	0.7 ^{abcd}	0.7 ± 0.0 ^{abcd}	0.5 ± 0.2 ^{cdef}	0.2 ± 0.0 ^{fg}	0.4 ^{def}	0.1 ± 0.0 ^{g}
20:2n-6	0.3 ± 0.1 ^{f}	1.3 ^{a}	0.7 ± 0.1 ^{bc}	0.6 ± 0.1 ^{cd}	0.5 ± 0.0 ^{de}	0.7 ± 0.1 ^{bcd}	0.5 ± 0.0 ^{de}
20:3n-6	0.2 ± 0.1 ^{f}	1.8 ^{a}	0.5 ± 0.1 ^{cd}	0.6 ± 0.1 ^{bc}	0.7 ± 0.0 ^{b}	0.6 ± 0.1 ^{bc}	0.4 ± 0.1 ^{de}
20:4n-6	0.2 ± 0.0 ^{e}	2.3 ^{a}	0.6 ± 0.1 ^{c}	0.5 ± 0.1 ^{cd}	0.5 ± 0.1 ^{cd}	0.9 ± 0.1 ^{b}	0.6 ± 0.1 ^{c}
20:4n-3	1.2 ± 0.7 ^{ab}	-	0.4 ± 0.1 ^{bc}	1.6 ± 0.1 ^{a}	1.8 ± 0.0 ^{a}	0.2 ± 0.1 ^{c}	0.2 ± 0.2 ^{c}
20:5n-3	3.5 ± 0.4 ^{bc}	1.3 ^{f}	2.7 ± 0.1 ^{de}	3.2 ± 0.0 ^{cd}	3.6 ± 0.1 ^{bc}	4.0 ± 0.2 ^{b}	5.0 ± 0.5 ^{a}
22:1n-11	0.8 ± 0.3 ^{c}	1.3 ^{c}	1.1 ± 0.2 ^{c}	1.1 ± 0.5 ^{c}	0.7 ± 0.3 ^{c}	3.4 ± 0.9 ^{b}	8.8 ± 0.6 ^{a}
22:5n-6	0.1 ± 0.0	1.8	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
22:5n-3	1.2 ± 0.1 ^{a}	0.3 ^{d}	0.9 ± 0.0 ^{abc}	0.9 ± 0.0 ^{abc}	1.0 ± 0.0 ^{ab}	0.9 ± 0.0 ^{abc}	1.1 ± 0.2 ^{a}
22:6n-3	8.7 ± 0.2 ^{bcd}	3.5 ^{e}	7.8 ± 1.2 ^{cd}	8.1 ± 0.7 ^{cd}	8.7 ± 0.9 ^{bcd}	13.5 ± 2.2 ^{a}	11.8 ± 0.1 ^{ab}
∑ Saturates	27.7 ± 0.5 ^{c}	17.4 ^{a}	30.8 ± 2.0 ^{bc}	19.7 ± 1.0 ^{e}	15.7 ± 1.2 ^{f}	23.9 ± 0.7 ^{d}	18.7 ± 0.3 ^{ef}
∑ Monoenes	35.2 ± 1.3 ^{de}	19.3 ^{cd}	43.2 ± 0.4 ^{ab}	35.5 ± 0.4 ^{de}	32.5 ± 0.8 ^{e}	41.6 ± 3.2 ^{bc}	44.4 ± 1.3 ^{ab}
∑ PUFA	32.9 ± 1.8 ^{c}	22.7 ^{e}	21.4 ± 1.5 ^{ef}	41.5 ± 0.5 ^{b}	46.8 ± 0.7 ^{a}	25.0 ^{e}	29.0 ± 0.7 ^{d}
∑ (n-9)	0.6 ± 0.1 ^{de}	0.9 ^{bc}	1.0 ± 0.1 ^{abc}	0.6 ± 0.1 ^{de}	0.4 ± 0.0 ^{ef}	0.8 ^{cd}	0.3 ± 0.1 ^{f}
∑ (n-6)	2.1 ± 0.5 ^{h}	8.1 ^{a}	4.7 ± 0.0 ^{e}	8.5 ± 0.5 ^{c}	9.7 ± 0.8 ^{b}	6.9 ± 0.2 ^{d}	9.0 ± 0.2 ^{bc}
∑ (n-3)	30.2 ± 1.2 ^{b}	2.8 ^{e}	15.8 ± 1.4 ^{d}	32.4 ± 0.1 ^{b}	38.8 ± 0.0 ^{a}	19.7 ± 2.5 ^{c}	19.7 ± 0.4 ^{c}

18:4n-3 increased significantly in muscle, while 20:5n-3 and 22:6n-3 changed marginally. A similar change was noted as 18:3n-3 and 18:2n-6 were increased in the diets (diets 9-11). Among charr fed diets containing 0.5% n-3 PUFAs, charr fed the diet containing 0.4% 22:6n-3 (diet 3) had significantly higher levels of 22:6n-3 in the tissues but lower amounts of 20:5n-3 than charr fed diets containing 0.1% 20:5n-3 or 0.5% 18:3n-3 (diets 1 and 5).

Discussion

After 12 weeks of feeding the test diets, charr given diets containing 0.1-1.0% n-3 or 1% n-6 PUFAs displayed typical EFA deficiency signs: fatty livers, increased water content in whole body and high levels of 20:3n-9 in liver polar lipids. These signs are similar to reports for other coldwater species (Henderson and Tocher, 1987). The signs became less apparent or absent when charr were fed either the commercial diet or casein-based diets containing either 2.0% 18:3n-3 only or 0.56-0.7% 18:2n-6 and 2.26-2.82% 18:3n-3. Charr fed the diet without PUFA (0% PUFA) had high percentages for neutral lipids but low percentages for total and polar lipids in livers. These findings are consistent with that reported in Chapter 3 where the HSI was lower and the DNA concentration in livers was high but differs from the typical fatty livers reported for rainbow trout (Watanabe et al., 1974a). The low level of total liver lipid in charr fed the 0% PUFA diet is attributed to liver pathology (Chapter 3).

Despite the lack of correspondence between haematological values and dietary levels of PUFAs in this study, haematocrit and haemoglobin values for charr were generally within the range reported for other salmonids (Castell et al., 1972a; Greene and Selivonchick, 1990; Sandnes et al., 1988). This suggests an absence of anaemia (Cho, 1983). Charr fed diets containing 1.0% 18:2n-6 or 0.1% 18:3n-3 had much lower

haematocrit and haemoglobin values compared to those given diets containing > 0.1% n-3 PUFAs and these results differ from data reported for rainbow trout (Castell et al., 1972a). Whether this is due to the physiological and metabolic differences between these two species remains to be investigated.

It is generally accepted that freshwater fish are capable of converting 18:1n-9, 18:2n-6 and 18:3n-3 to long-chain PUFAs through a common set of enzymes, i.e., Δ^6, Δ^5 and Δ^4 desaturases and elongases (Henderson and Tocher, 1987). In my study charr had increased levels of 20:5n-3 and 22:6n-3 in liver polar lipids as the level of 18:3n-3 was increased in the diets. This finding suggests that charr can convert 18:3n-3 into 20:5n-3 and 22:6n-3. Similarly, charr fed the diet with 1.0% 18:2n-6 had significantly higher percentages of 20:4n-6 and 22:5n-6 than those fed casein-based diets without 18:2n-6, implying that charr can convert 18:2n-6 as well. The inclusion of 18:2n-6 in the diets led to an increase in tissue 18:2n-6 and 20:4n-6 but little change in tissue 18:3n-3 and 22:5n-6 when 18:3n-3 in diets was approximately 0.5% or 2.0% and the ratio of 18:3n-3/18:2n-6 remained at 4:1. This suggests that dietary 18:3n-3 partially inhibits the conversion of 18:2n-6 and n-3 PUFAs are preferred to n-6 PUFAs for desaturation and elongation. Furthermore, partial replacement of dietary 18:3n-3 with 0.1% or 0.4% 22:6n-3 resulted in a decrease in 20:5n-3, indicating that dietary 22:6n-3 may inhibit the conversion of

18:3n-3 in charr. These findings agree with those of Olsen and Ringø (1992). However, replacement of 0.4% dietary 18:3n-3 with 0.4% 22:6n-3 led to an increase in 22:6n-3. This suggests that Arctic charr is capable of directly incorporating dietary 22:6n-3 into its tissues. Moreover, the replacement of 0.1% dietary 18:3n-3 with 0.1% 20:5n-3 led to an increase in tissue levels of 22:6n-3 but little change in 18:3n-3 and 20:5n-3. This implies that dietary 20:5n-3 is converted to 22:6n-3 in the liver.

The levels of 20:3n-9 and 20:3n-9/22:6n-3 in charr polar lipids were inversely related to dietary level of 18:3n-3 and this trend is similar to that noted in previous studies on other salmonids (Castell et al., 1972b; Takeuchi and Watanabe, 1982). Compared to the data reported on other salmonids, using similar periods of acclimation and feeding (Takeuchi and Watanabe, 1982; Thongrod et al., 1990a, b), the percentages of 20:3n-9 and the 20:3n-9/22:6n-3 ratios in liver polar lipids from my study were lower but the percentage of 22:6n-3 was higher. The ability of fish to utilize PUFAs varies between species (Kanazawa, 1985) and fish raised at low temperatures generally have a higher content of 22:6n-3 in their polar lipids (Greene and Selivonchick, 1987). These differences are likely due to the species of fish tested and the lower rearing temperature (10 °C) used in my study compared to other studies (14-18 °C) (Takeuchi and Watanabe, 1982; Thongrod et al., 1990). Furthermore, my study showed that there was a small

amount of 20:3n-9 in charr fed the diet containing 2.0% 18:3n-3. This phenomenon has also been reported for cherry salmon by Thongrod et al. (1990b) and in my study on charr may have resulted from the length of the feeding trials (12 weeks). Interestingly, the percentages of n-3 and n-6 PUFAs in the tissues of charr fed diets containing 1.0% 18:2n-6 or 18:3n-3 from my study were similar to those reported by Olsen et al. (1991) but 20:3n-9 was lower in charr of this study. Perhaps these differences were due to rearing temperature, time for acclimation, and strain of charr.

By comaprison with polar lipids, there were much lower percentages of long-chain n-3 and n-6 PUFAs in the liver neutral lipids and these PUFAs changed little with the increase of dietary PUFA. These findings suggest that products from conversion of n-3 and n-6 PUFA are preferentially incorporated into polar lipids. This is consistent with published data on charr and other salmonids (Olsen et al., 1991; Henderson and Tocher, 1987). The substantial increase in 18:3n-3 in liver neutral lipids in charr fed diets containing $\geq$ 2.0% 18:3n-3 suggests that the Δ6 desaturase that converts 18:3n-3 into 18:4n-3 is already saturated in charr.

Ringø et al. (1990) reported that the amount of 22:6n-3 in the polar lipids of livers and muscle was specifically retained by charr following a starvation period of 25 or 50 days. Similar trends occurred in charr fed the diet with 0% PUFA in this study. In addition, a high percentage of 20:4n-6

was also noted for this group. These results suggest that n-3 and n-6 PUFAs are important fatty acids for Arctic charr. In contrast to liver polar lipids, the percentages of 20:5n-3 and 22:6n-3 increased marginally in muscle lipids but there was a significant increase in 18:3n-3 and 18:4n-3 as the percentage of 18:3n-3 was increased from 0.5 to 2.82% in the diets. In theory, 18:3n-3 is one of the substrates of Δ^6 desaturase, while 20:5n-3 results from the desaturation and elongation of 18:4n-3 through Δ^5 desaturase. Therefore, my results suggests that charr are efficient in converting 18:3n-3 to 18:4n-3 through Δ^6 desaturase in muscle but inefficient in converting 18:4n-3 to 20:5n-3 through Δ^5 desaturase. This also implies that the Δ^5 desaturase is limiting in the muscle of charr. Although Olsen et al. (1991) proposed that the low conversion of 18:3n-3 in muscle may be related to the adaptation of charr to an extremely cold environment it is still not clear why the activity of Δ^5 desaturase, and not other enzymes, is a limiting factor to the conversion of PUFAs in muscle. Furthermore, there was little conversion of 18:2n-6 to 18:3n-6 in muscle, suggesting that like livers, muscle preferentially utilizes n-3 PUFAs rather than for desaturation and elongation. On the other hand, charr fed the diet containing 0.4% 22:6n-3 or the commercial diet had significantly higher percentages of tissue 22:6n-3 than those fed other casein-based diets, except the diet with 0% PUFA. This suggests that charr can directly incorporate dietary 22:6n-3 in muscle and

this result is similar to what I found for liver in this chapter.

In conclusion, the Labrador strain of Arctic charr developed typical EFA deficiency signs when they were fed diets with 0-1.0% n-3 PUFAs: fatty liver, elevated water content in whole body and substantial accumulation of 20:3n-9 in liver polar lipids. These signs were less apparent or absent when charr were fed diets containing 2.0% 18:3n-3 or combinations of 2.3-2.8% 18:3n-3 and 0.6-0.7% 18:2n-6. My data on fatty acid composition of liver polar lipids also suggests that charr can efficiently convert 18:3n-3 and 18:2n-6 into long-chain PUFAs in livers. Although charr show a preference for utilization of n-3 PUFAs rather than n-6 PUFAs in both liver and muscle for desaturation and elongation, they appear to have little ability to convert 18:4n-3 to 20:5-3 in muscle. These findings, together with my observations on charr growth and feed efficiency when they were fed diets varying in levels of n-3 PUFAs (Chapter 3), suggest that 18:3n-3 is an EFA for Arctic charr and the requirements of charr for dietary 18:3n-3 is 1-2% (dry weight) or 20 to 40% of dietary lipids. Although feeding 18:2n-6 alone or in combination with 18:3n-3 has positive effects on Arctic charr, further studies will be needed to determine whether 18:2n-6 is an EFA for this species.

Chapter 5.

Comparison of the effects of dietary polyunsaturated fatty acids on growth and lipid metabolism of Arctic charr (*Salvelinus alpinus* L.) and rainbow trout (*Oncorhynchus mykiss*)

Abstract

Juvenile Arctic charr (Salvelinus alpinus) and rainbow trout (Oncorhynchus mykiss) were fed one commercial and three casein-based diets that varied in their amounts of 18:2n-6 and 18:3n-3 for 12 weeks at 10 ^{o}C. Fish growth and feeding responses, haematological values, tissue water content and lipid and fatty acid composition were examined. Both species on the diet without 18:2n-6 and 18:3n-3 (two polyunsaturated fatty acids; PUFAs) had lower specific growth rate (SGR), but higher percentages of liver neutral lipids and 20:3n-9 in liver polar lipids compared to those fed the diet with high PUFA content or the commercial diet. Regardless of the amount of 18:3n-3 in the diets, the percentages of 20:3n-9 and ratios of 20:3n-9/22:6n-3 in liver or muscle polar lipids were significantly lower in charr than in trout, while the values for trout were lower relative to other studies on this species. These findings put into question the usefulness of 20:3n-9/22:6n-3 as an indicator of essential fatty acid status for both species. Different levels of 20:3n-9, 22:6n-3 and other PUFAs in tissue polar lipids between two species suggested that D^6 and/or D^5 desaturases are less efficient in charr. While trout fed the test diets had slightly less 20:4n-6 than wild trout, charr had one tenth the level of 20:4n-6 than was noted in the in muscle polar and total lipids of wild charr. This result suggests that charr may require dietary n-6 and n-3 PUFAs for optimal performance.

Introduction

Essential fatty acids (EFA), key components in animal nutrition, have been studied extensively throughout the vertebrates (Henderson and Tocher, 1987; Simopoulos, 1991; Watkins, 1991). It is clear that vertebrates are incapable of synthesizing C18 or C20 n-3 and n-6 polyunsaturated fatty acids (PUFA) de novo and they require these PUFAs in their diets to maintain normal growth, development and physiological status. Fish on EFA-deficient diets usually develop a variety of symptoms, such as retarded growth, poor feed utilization, fatty livers and elevated levels of tissue 20:3n-9 (Sargent et al., 1989). Moreover, freshwater fish require one or both of C18 n-3 and n-6 PUFAs, i.e., 18:3n-3 and 18:2n-6, while marine fishes have an exclusive requirement for both C20 and C22 n-3 PUFAs (Kanazawa, 1985; Henderson and Tocher, 1987; Takeuchi et al., 1992).

Extensive studies suggest that EFA requirements of freshwater fish, particularly salmonids, may vary with species with respect to type and amount. Rainbow trout (Oncorhynchus mykiss) and coho salmon (O. kisutch) both require 18:3n-3 only (Castell et al., 1972a; Watanabe et al., 1974c; Yu and Sinnhuber, 1979), while chum salmon (O. keta) and chinook salmon (O. tshawytscha) appear to require both 18:3n-3 and 18:2n-6 (Takeuchi et al., 1979; Takeuchi and Watanabe, 1982; Dosanjh et al., 1988; Higgs et al., 1992). In addition,

rainbow trout and coho salmon differ markedly in survival, growth and lipid metabolism, when tested with diets varying in n-3 and n-6 PUFAs (Takeuchi and Watanabe, 1982).

Despite being cultured intensively throughout Canada and Europe, Arctic charr, Salvelinus alpinus, are raised on diets formulated for either trout or salmon (Jobling, 1991). It is unclear if trout or salmon diets contain optimal amounts of nutrients including EFAs for this species in terms of growth and feed conversion. Moreover, even within the same species, different populations or strains appear to differ with respect to growth and physiological parameters (Tompkins, 1989; Giles, 1991). There are also evidences that the Labrador strain of Arctic charr used in my previous experiments (Chapter 3) may contain hybrids of Arctic charr and other salmonids (Hammar et al., 1989, 1991). Consequently, it remains to be established whether my estimate for the EFA requirement of Labrador charr is true for other strains.

From my previous studies on the EFA requirement of the Labrador strain of Arctic charr (Chapters 3 and 4), it appears that this species might differ from rainbow trout in their metabolism of dietary PUFA. However, there has been no study which has directly compared Arctic charr and rainbow trout with respect to their growth, EFA deficiency symptoms and lipid metabolism when the fish have been fed varying levels of dietary PUFAs and they have been held under the same rearing conditions.

The main objective of this study was therefore to determine if the Nauyuk strain of Arctic charr, which likely does not include hybrids with other salmonids, differed from rainbow trout regarding EFA deficiency symptoms and metabolism of dietary 18:3n-3 and 18:2n-6. Accordingly both species were simultaneously fed diets varying in concentrations of 18:3n-3. The levels of 18:3n-3 were based on the EFA requirements of these species as determined from previous studies, i.e., values were selected to be below or within the range of the optimum requirement of each species. One diet did not contain any 18:3n-3 and 18:2n-6. The second objective was to estimate the EFA requirements of the Nauyuk strain of Arctic charr and to follow lipid metabolism in this strain of charr.

Materials and Methods

Cultured Arctic charr used in this study were the offspring of the stock originally collected from Nauyuk Lake (NWT, Canada). The sample of wild charr was collected in gill nets from an experimental lake at Saqvagjuac, 36 km north of Chesterfield Inlet (63°39'N), NWT, Canada. Fish were 12 to 15 year's old, 30.5 to 43.3 cm in fork length and 480 to 705 g in body weight. Rainbow trout selected for this study were hybrids of the Mount Lassen and Tagworker strains that had been maintained at the RARC (Department of Fisheries and Oceans, Winnipeg, Manitoba, Canada).

The four test diets that were fed to both species consisted of one reference diet and three casein-based diets. The formulations and methods of manufacture of the casein-based diets were the same as described in Chapter 3. Briefly, the casein-based diets had identical ingredient components except for varying levels of two PUFAs, 18:3n-3 and 18:2n-6 (Table 9.). The common ingredients included 52.0% casein, 15.2% corn starch, 10.0% dextrin, 4.0% gelatin, 5.0% lipids, 2.0% α-cellulose, 1.5% carboxymethylcellulose, 0.5% methionine, 1.3% arginine, 5.5% mineral premix, 2.0% vitamin premix and 1.0% choline chloride premix (Chapter 3). The sources of dietary lipids were >99% pure ethyl laureate (12:0) (Sigma, St. Louis, U.S.A.) and flax oil (Omega Nutrition, Vanc., Canada). The fatty acid composition was determined for the flax oil and the

Table 9. Lipid composition and 18:2n-6 and 18:3n-3 content in casein-based diets (% dry weight)

Diet	Lipids		PUFA content	
	12:0[1]	flax oil	18:2n-6	18:3n-3
PUFA-def.[2]	5	0	0	0
Low-PUFA	4	1	0.16	0.57
High-PUFA	2	3	0.48	1.71

[1]Ethyl ester [2]PUFA-deficient

PUFA-deficient and commercial diets and calculated for the low- and high-PUFA diets (Table 10). The main dietary ingredients for the casein-based diets, including casein, starch and dextrin, were extracted three times with hot 95% ethanol to remove trace amounts of lipids before diet manufacture. In addition, the reference diet is the commercial trout diet regularly used for culturing Arctic charr in the hatchery. The diet contained approximately 52% protein, 15% lipid and 2.5% fibre.

The diet treatments were each assigned randomly to replicate groups (n=2-5). The use of different numbers of replicates for the different treatments was largely based on the cost of the diets and the need to monitor the possible impact of size variation of fish on the experiments. Five month-old Arctic charr and rainbow trout at the beginning of the experiments were acclimated for 4 weeks on a PUFA-free casein-based diet (Chapter 3) to reduce the PUFA storage in fish tissues and to condition the fish to eat the casein-based diets. The feeding trial started with each tank (or replicate) containing 75 individual fish that had average initial weight of 2.3 g or 2.0 g for Arctic charr and rainbow trout, respectively. The spaghetti-like feed was crumbled and sieved. Feed sizes were selected as recommended by Tabacheck (1986). All fish were cultured at 10 ^{o}C under a photoperiod of 12 h light : 12 h dark for 12 weeks. A 95% recirculation system was used with each tank containing 60 L of aerated well water with 95%

Table 10. Fatty acid composition of experimental diets (% weight)

Fatty acid	Diet			
	PUFA-def.[1]	Low-PUFA[2]	High-PUFA[2]	Commercial[1]
12:0	98.0	80.0	40.0	0.1
14:0	0.1	0.0	0.0	5.1
15:0	-	-	-	0.4
16:0	0.3	1.1	3.3	15.5
16:1n-9	-	0.0	0.1	
16:1n-7	-	-	-	7.9
17:1n-9	-	-	-	0.8
18:0	0.1	0.7	2.2	2.5
18:1n-9	0.3	2.8	8.3	12.1
18:1n-7	-	0.1	0.4	2.7
18:1n-5	-	-	-	0.4
18:2n-6	0.4	3.6	10.9	7.3
18:3n-3	0.1	11.3	34.0	1.1
18:4n-3	-	-	-	1.8
18:4n-11	-	-	-	0.4
20:1n-11	-	0.0	0.1	0.4
20:1n-9	-	-	-	4.3
20:4n-6	-	-	-	0.4
20:4n-3	-	-	-	0.7
20:5n-3	-	-	-	9.2
22:1n-11	-	-	-	4.8
22:1n-9	-	-	-	0.6
21:5n-3	-	-	-	0.4
22:4n-6	-	-	-	0.1
22:6n-3	-	-	-	5.1
24:1n-9	-	-	-	0.5

[1]Determined by gas chromatography (GC) [2]Calculated on the basis of the fatty acid compositions of the PUFA-def. diet and flax oil analyzed by GC

recirculation at a flow rate of approximately 2.4 $L.min^{-1}$.Fish were fed 5 times a day to apparent satiation. A batch weight was taken at 2 week intervals to adjust amount of feed and all fish were weighed individually at 4 week intervals. Dissolved oxygen and ammonia levels were maintained above 8.0 mg/l and below 2.0 mg/l, respectively. All test diets were stored at -50 or -20 ^{o}C during the experiments.

At the end of the feeding trial, blood was collected from 10 fish sampled by netting without selection from each tank. Ten fish were also collected to determine hepatosomatic index (HSI) and water content in liver and muscle and 5-10 additional fish from each tank were sampled and pooled for lipid and fatty acid analyses. All biochemical analyses were performed as described in Chapter 4. In addition, specific growth rate (SGR), feed efficiency and HSI were calculated as described in Chapter 2.

The t-test and Duncan's multiple range test following ANOVA were performed on SAS (1985) to compare means between species and treatments, respectively. Differences were considered significant at $p < 0.05$.

Results

In both Arctic charr and rainbow trout, final mean weight differed significantly (Duncan's test) among treatments (Table 11). The groups fed the PUFA-deficient diet (0% PUFA) had the lowest SGR and feed efficiency. The two species had significantly different SGRs only when fed the PUFA-deficient or high-PUFA diet.

Haematocrit and haemoglobin values were lower in charr than in trout regardless of diet treatment (Table 12). There was a close correspondence between haematocrit and haemoglobin values in charr. Charr fed the commercial diet had significantly higher haematocrit and haemoglobin values than those fed the casein-based diets. No correspondence was found for haematocrit and haemoglobin values in trout.

The highest HSI values occurred in charr fed the low-PUFA diet and in rainbow trout fed the PUFA-deficient diet (Table 13). Charr fed the commercial or high-PUFA diet were significantly higher in HSI compared to trout, while charr fed the PUFA-deficient diet had significantly higher HSI than trout. Liver water content was significantly lower in charr fed the low-PUFA diet compared to those fed the other test diets, while liver water content differed little between treatments for trout. Muscle water content decreased in charr as the levels of PUFAs increased in casein-based diets but trout showed only slight differences between treatments. No

Table 11. Initial and final mean weights, specific growth rates (SGR) and feed efficiency for Arctic charr (AC) and rainbow trout (RT) [Mean ± SE (n[1])]

	Diet	AC	RT
Initial mean weight[2] (g)	PUFA-def.	2.29 ± 0.02 (3)	2.04 ± 0.01 (2)
	Low-PUFA	2.28 ± 0.02 (2)	2.03 ± 0.03 (2)
	High-PUFA	2.35 ± 0.06 (3)	2.11 ± 0.03 (2)
	Commercial	2.29 ± 0.03 (5)	2.00 ± 0.06 (2)
Final mean weight[2] (g)	PUFA-def.	3.97 ± 0.06 (3)	5.00 ± 0.43 (2)
	Low-PUFA	11.09 ± 0.13 (2)	7.80 ± 0.45 (2)
	High-PUFA	13.54 ± 0.51 (3)	10.41 ± 0.17 (2)
	Commercial	16.36 ± 0.94 (5)	16.06 ± 1.22 (2)
SGR	PUFA-def.	0.66 ± 0.02 (3)	1.06 ± 0.10 (2) *[3]
	Low-PUFA	1.88 ± 0.00 (2)	1.60 ± 0.08 (2)
	High-PUFA	2.09 ± 0.04 (3)	1.90 ± 0.03 (2) *
	Commercial	2.34 ± 0.06 (3)	2.48 ± 0.18 (2)
Feed efficiency	PUFA-def.	0.27 ± 0.03 (3)	0.44 ± 0.09 (2)
	Low-PUFA	0.81 ± 0.04 (2)	0.64 ± 0.05 (2)
	High-PUFA	0.85 ± 0.02 (3)	0.79 ± 0.05 (2)
	Commercial	0.69 ± 0.03 (3)	0.75 ± 0.08 (2)

[1]Number of rearing tanks [2]Not tested with *t*-test [3]Significant difference between charr and trout (*t*-test, $p < 0.05$)

Table 12. Haematocrit and haemoglobin values of Arctic charr (AC) and rainbow trout (RT) [Mean ± SE (n[1])]

	Diet	AC	RT
Haematocrit	PUFA-def.	35.5 ± 1.4 (3)	41.8 ± 0.9 (2)
	Low-PUFA	36.0 ± 0.7 (2)	44.4 ± 1.0 (2) *[2]
	High-PUFA	34.0 ± 0.9 (3)	40.3 ± 0.5 (2) *
	Commercial	39.3 ± 1.3 (5)	40.3 ± 0.0 (2)
Haemoglobin (g/100 ml)	PUFA-def.	7.66 ± 0.18 (3)	8.89 ± 0.40 (2) *
	Low-PUFA	7.83 ± 0.48 (2)	9.89 ± 0.28 (2)
	High-PUFA	7.30 ± 0.15 (3)	9.18 ± 0.24 (2) *
	Commercial	8.45 ± 0.13 (5)	9.95 ± 0.26 (2)

[1]Number of rearing tanks [2]Significant difference between charr and trout (*t*-test, $p < 0.05$)

Table 13. Hepatosomatic index (HSI) and water content in the livers and muscle of Arctic charr (AC) and rainbow trout (RT) (Mean ± SE, $n^1 = 2$)

	Diet	AC	RT
HSI	PUFA-def.	1.68 ± 0.08	2.28 ± 0.02 *[2]
	Low-PUFA	2.06 ± 0.18	1.58 ± 0.09
	High-PUFA	1.67 ± 0.03	1.46 ± 0.04 *
	Commercial	1.75 ± 0.08	1.20 ± 0.01 *
Liver water (%)	PUFA-def.	75.1 ± 0.3	74.8 ± 0.3 **[2]
	Low-PUFA	68.8 ± 0.2	75.0 ± 0.4
	High-PUFA	74.0 ± 0.7	74.8 ± 0.9
	Commercial	73.9 ± 0.3	74.7 ± 0.2 *
Muscle water (%)	PUFA-def.	79.5 ± 0.2	78.4 ± 0.2 *
	Low-PUFA	78.0 ± 0.0	78.4 ± 0.0 *
	High-PUFA	77.7 ± 0.3	78.0 ± 0.2
	Commercial	77.4 ± 0.7	77.8 ± 0.5

[1]Number of rearing tanks [2]Significant difference between charr and trout (*t*-test);

*, $p < 0.05$; **, $p < 0.005$

mortality, skin lesions or shock syndrome were found for Arctic charr or rainbow trout fed the test diets during the feeding trial.

The percentage of liver total lipids was highest in charr fed the low-PUFA diet and in trout fed the PUFA-deficient diet (Fig. 10). Levels of total lipids in muscle were lower for both species fed all test diets. Charr fed either the low-PUFA or commercial diet had higher levels of total lipids in muscle than those fed the other test diets. Levels of total muscle lipids were higher in rainbow trout fed the PUFA-deficient and low-PUFA diets. The level of polar lipids in liver was lowest in charr fed the low-PUFA diet while rainbow trout had an increase in polar lipids with dietary PUFA (Fig. 10). The level of polar lipids in muscle was highest in charr fed the PUFA-deficient diet. The change in percentages of neutral lipids in livers and muscle of both species were negatively correlated with those in polar lipids. Significant differences in percentages of polar or neutral lipids in liver or muscle occurred between charr and trout when fed the low-PUFA or PUFA-deficient diet.

The percentages of total PUFA and n-3 PUFA in liver polar lipids increased significantly in both species (Duncan's test) as PUFA increased in the casein-based diets, while total monoenes and n-9 PUFA decreased (Duncan's test) (Table 14 and Fig. 11). Individual fatty acids, including 16:0, 18:3n-3, 20:5n-3 and 22:6n-3, also showed a significant increase with

Fig. 10. Percentages of total, polar and neutral lipids in the liver (A-C) and muscle (D-F) of Arctic charr and rainbow trout fed five test diets

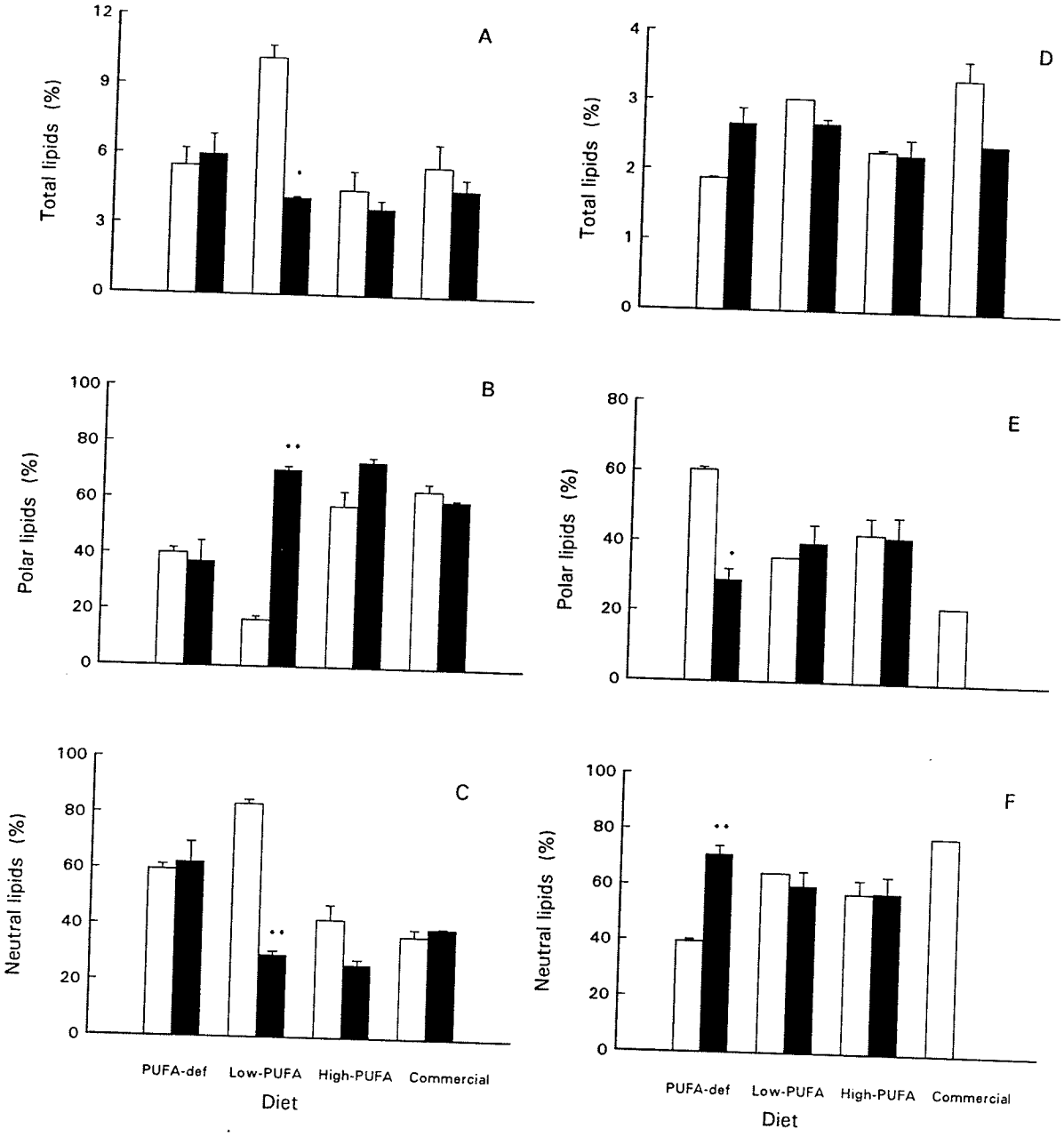

A
B
C
D
E
F
Total lipids (%)
Polar lipids (%)
Neutral lipids (%)
PUFA-def
Low-PUFA
High-PUFA
Commercial
Diet

Table 14. Fatty acid composition of liver polar lipids from Arctic charr (AC) and rainbow trout (RT) (weight %, Mean ± SE, n[1] = 2)

fatty acid	Diet							
	PUFA-def.		Low-PUFA		High-PUFA		Commercial	
	AC	RT	AC	RT	AC	RT	AC	RT
14:0	3.16 ± 0.23	2.76 ± 0.11	2.68 ± 0.18	3.13 ± 0.14	1.73 ± 0.09	2.07 ± 0.20	1.32 ± 0.02	1.20 ± 0.02 *[2]
16:0	13.50 ± 0.41	10.95 ± 1.27	17.79 ± 0.21	15.91 ± 0.81	19.33 ± 1.43	17.10 ± 0.14	17.04 ± 0.55	17.14 ± 0.60
16:1n-9	3.09 ± 0.07	2.86 ± 0.25	1.28 ± 0.12	2.09 ± 0.08 *	0.94 ± 0.11	1.40 ± 0.11	0.42 ± 0.03	0.44 ± 0.02
16:1n-7	5.27 ± 0.23	4.97 ± 0.14	4.47 ± 0.08	2.34 ± 0.14 *	1.71 ± 0.14	1.58 ± 0.12	2.01 ± 0.06	1.84 ± 0.17
18:0	2.84 ± 0.06	4.53 ± 0.12 *	3.71 ± 0.16	5.50 ± 0.18 *	4.27 ± 0.00	5.69 ± 0.27	2.52 ± 0.09	5.15 ± 0.43 *
18:1n-9	22.87 ± 0.38	23.08 ± 1.09	22.64 ± 0.22	14.83 ± 0.18 **[1]	15.20 ± 1.13	12.88 ± 0.19	9.51 ± 0.06	8.65 ± 0.33
18:1n-7	5.62 ± 0.11	5.06 ± 0.54	3.69 ± 0.18	2.35 ± 0.28	2.12 ± 0.04	1.43 ± 0.00 **[2]	2.84 ± 0.01	2.04 ± 0.04 **
18:2n-6	0.72 ± 0.09	0.42 ± 0.07	1.02 ± 0.02	0.71 ± 0.02 *	2.28 ± 0.09	1.60 ± 0.06 *	2.05 ± 0.00	1.69 ± 0.05 *
18:3n-6	0.10 ± 0.02	0.03 ± 0.00 *	0.14 ± 0.01	0.06 ± 0.00 *	0.12 ± 0.01	0.02 ± 0.01 *	0.14 ± 0.00	0.12 ± 0.00 *
18:3n-3	0.04 ± 0.01	0.03 ± 0.01	0.67 ± 0.06	0.28 ± 0.05 *	1.57 ± 0.17	1.07 ± 0.14	0.10 ± 0.00	0.08 ± 0.01
18:4n-3	0.05 ± 0.00	0.05 ± 0.01	0.64 ± 0.12	0.22 ± 0.04	0.55 ± 0.11	0.27 ± 0.06	0.11 ± 0.02	0.09 ± 0.03
20:1n-9	3.88 ± 0.13	2.32 ± 0.27 *	3.44 ± 0.19	1.63 ± 0.18 *	2.01 ± 0.30	1.26 ± 0.04	4.96 ± 0.07	3.88 ± 0.11 *
20:2n-9	1.82 ± 0.10	2.21 ± 0.03	1.37 ± 0.07	1.55 ± 0.04	0.33 ± 0.04	0.58 ± 0.10	0.08 ± 0.01	0.12 ± 0.00
20:2n-6	0.02 ± 0.02	0.02 ± 0.01	0.24 ± 0.03	0.01 ± 0.00 *	0.73 ± 0.00	0.43 ± 0.03 *	0.53 ± 0.03	0.51 ± 0.01

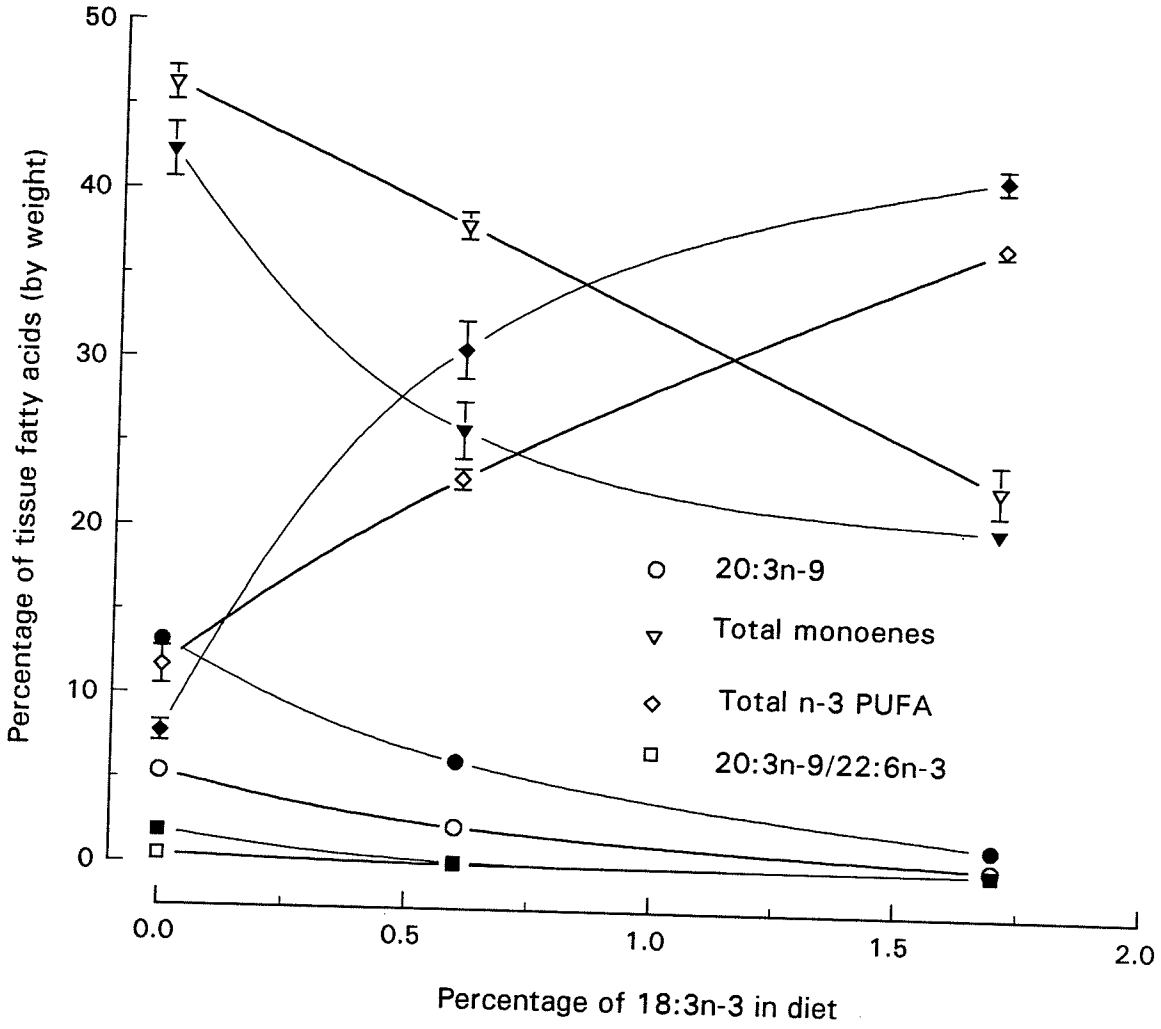
...continued

Table 14. (continued)

fatty acid	Diet							
	PUFA-def.		Low-PUFA		High-PUFA		Commercial	
	AC	RT	AC	RT	AC	RT	AC	RT
20:3n-9	5.44 ± 0.07	13.17 ± 0.20 **	2.27 ± 0.05	6.24 ± 0.46 *	0.33 ± 0.09	1.50 ± 0.00 **	0.00 ± 0.00	0.00 ± 0.00
20:3n-6	0.78 ± 0.10	0.37 ± 0.08	1.48 ± 0.12	0.87 ± 0.05 *	2.46 ± 0.01	1.42 ± 0.33	1.02 ± 0.07	0.43 ± 0.35
20:4n-6	2.03 ± 0.11	1.85 ± 0.11	1.40 ± 0.05	2.32 ± 0.29	2.84 ± 0.05	2.72 ± 0.28	2.61 ± 0.08	2.69 ± 0.45
20:4n-3	0.03 ± 0.00	0.03 ± 0.00	0.55 ± 0.00	0.25 ± 0.06 *	1.08 ± 0.12	0.66 ± 0.21	0.20 ± 0.00	0.17 ± 0.03
20:5n-3	0.73 ± 0.08	0.39 ± 0.02 *	3.64 ± 0.03	1.51 ± 0.09 **	3.89 ± 0.24	2.36 ± 0.06 *	5.90 ± 0.28	3.83 ± 0.33 *
22:4n-6	0.26 ± 0.00	0.16 ± 0.04	0.12 ± 0.00	0.17 ± 0.02	0.14 ± 0.02	0.12 ± 0.00	0.06 ± 0.01	0.04 ± 0.03
22:5n-6	0.85 ± 0.02	1.37 ± 0.18	0.27 ± 0.02	1.18 ± 0.25	0.24 ± 0.10	0.28 ± 0.03	0.32 ± 0.04	0.38 ± 0.00
22:5n-3	0.57 ± 0.07	0.23 ± 0.07	2.15 ± 0.00	1.15 ± 0.01 **	2.05 ± 0.22	1.15 ± 0.07	0.85 ± 0.04	0.92 ± 0.06
22:6n-3	10.11 ± 0.93	6.98 ± 0.54	15.21 ± 0.73	27.15 ± 1.47 *	27.72 ± 0.33	35.38 ± 1.15 *	34.67 ± 0.99	39.57 ± 0.27 *
Σ saturates	20.59 ± 0.23	19.28 ± 0.71	25.35 ± 1.20	25.44 ± 1.12	25.83 ± 1.42	25.55 ± 0.31	21.59 ± 0.67	24.06 ± 0.15
Σ monoenes	46.33 ± 0.95	42.31 ± 1.59	38.11 ± 0.83	25.92 ± 1.69 *	22.86 ± 1.50	20.36 ± 0.11	24.46 ± 0.01	19.83 ± 0.37 *
Σ PUFA	25.22 ± 1.15	29.14 ± 0.91	31.71 ± 0.89	44.45 ± 0.66 *	46.97 ± 0.32	50.16 ± 0.53 *	49.04 ± 1.34	50.98 ± 0.56
Σ (n-9)	8.81 ± 0.24	17.11 ± 0.17 **	4.06 ± 0.13	8.50 ± 0.52 *	0.83 ± 0.14	2.30 ± 0.12 *	0.23 ± 0.02	0.24 ± 0.02
Σ (n-6)	4.75 ± 0.31	4.22 ± 0.45	4.65 ± 0.20	5.31 ± 0.54	8.80 ± 0.07	6.58 ± 0.05 **	6.85 ± 0.08	5.97 ± 0.72
Σ (n-3)	11.67 ± 1.07	7.81 ± 0.63	23.00 ± 0.57	30.65 ± 1.72	37.34 ± 0.53	41.28 ± 0.71 *	41.97 ± 1.28	44.78 ± 0.18
20:3n-9/22:6n-3	0.54 ± 0.06	1.90 ± 0.18 *	0.15 ± 0.00	0.23 ± 0.03	0.01 ± 0.00	0.04 ± 0.00	-[3]	-

[1,2]Same as in Table 5 [3] < 0.005

Fig. 11. Percentages of 20:3n-9, total monoenes and n-3 PUFA and 20:3n-9/22:6n-3 in liver polar lipids of Arctic charr (empty symbol) and rainbow trout (filled symbol) fed diets varying in 18:2n-6 and 18:3n-3


50
40
30
20
10
0
Percentage of tissue fatty acids (by weight)
20:3n-9
Total monoenes
Total n-3 PUFA
20:3n-9/22:6n-3
0.0
0.5
1.0
1.5
2.0
Percentage of 18:3n-3 in diet

dietary PUFAs (Duncan's test), while C16, C18 and C20 monoenes, 20:2n-9 and 20:3n-9 decreased. Compared to n-3 PUFAs, levels of n-6 PUFAs showed little difference between treatments in both species. When charr and rainbow trout were fed the PUFA-deficient diet, charr had significantly lower levels of total n-9 PUFA and 20:3n-9 but higher levels of 22:6n-3 compared to trout. However, charr fed either the low- or high-PUFA diet had significantly lower levels of 20:3n-9 and 22:6n-3 but higher levels of 18:2n-6, 18:3n-6 and 20:5n-3 than trout. There were only small amounts of 22:6n-3 and 20:3n-9 (i.e., < 0.5%) in liver neutral lipids in both species when fed the PUFA-deficient diet, while n-6 or n-3 PUFAs increased as the levels of n-6 or n-3 PUFAs increased in casein-based diets (data not shown).

The percentages of tissue fatty acids at the beginning of the experiment were similar for charr and trout (data not shown). Muscle polar lipids, similar to liver polar lipids, showed significant differences in 20:3n-9 between species and treatments at the end of the feeding trials (Table 15). The percentage of tissue 18:3n-3 in the groups fed the low- or high PUFA diet was significantly higher (Duncan's test) than observed for the groups fed the PUFA-deficient diet for both species. However, charr fed either the low- or high-PUFA diets had lower levels of 22:6n-3 than those fed the PUFA-deficient diet, while trout had higher values. Charr fed either the commercial, low- or high-PUFA diets had lower levels of 20:4n-

6, 20:5n-3 and 22:6n-3, but higher levels of 18:3n-3 and 18:4n-3 compared to the wild charr (Table 15).

Both trout and charr fed the high-PUFA diet had higher percentages of 18:2n-6 and 18:3n-3 in total muscle lipids compared to those fed the PUFA-deficient diet (Table 16). In addition, charr fed the commercial diet or the high-PUFA diet had lower levels of 20:4n-6, 20:5n-3, 22:5n-6, 22:5n-3 and 22:6n-3 compared to wild charr but higher levels of 14:0 and monoenes, including 16:1n-7, 18:1n-9 and 20:1n-9. In contrast, trout from my study had lower levels of 16:0 and 16:1n-7 but had similar levels of 22:6n-3 to that reported for wild trout (Table 16).

Table 15. Comparison of fatty acid composition of muscle polar lipids from cultured and wild Arctic charr (AC), rainbow trout (RT) and Atlantic salmon (AS) (weight %, Mean ± SE, n[1] = 2)

Fatty acid	Diet								
	PUFA-def.		Low-PUFA		High-PUFA		Wild		
	AC[2]	RT	AC	RT	AC	RT	AC[3]	AC[4]	AS[5]
14:0	4.06 ± 0.10	4.43 ± 0.29	4.38	3.48 ± 0.11	2.06 ± 0.06	1.91 ± 0.12	0.51 ± 0.12	1.0 ± 0.2	1.1 ± 0.1
16:0	16.08 ± 1.11	11.34 ± 0.28	13.63	13.03 ± 0.48	16.16 ± 0.39	14.93 ± 0.16	18.75 ± 1.48	23.4 ± 2.0	18.7 ± 1.0
16:1n-9	2.25 ± 0.08	2.66 ± 0.01 *[6]	1.52	2.20 ± 0.02	1.03 ± 0.03	1.37 ± 0.04 *	0.30 ± 0.05	2.5 ± 0.3	†[7]
16:1n-7	5.75 ± 0.03	8.64 ± 0.73	6.67	6.76 ± 0.41	3.24 ± 0.58	3.66 ± 0.15	1.68 ± 0.04	†	†
18:0	2.52 ± 0.24	2.38 ± 0.12	2.02	2.39 ± 0.10	2.64 ± 0.16	3.25 ± 0.25	5.91 ± 0.16	2.3 ± 0.2	5.2 ± 0.0
18:1n-9	15.23 ± 0.25	20.54 ± 0.68 *	21.39	19.50 ± 1.00	15.10 ± 1.49	16.27 ± 0.10	6.57 ± 1.17	7.0 ± 0.5	9.0 ± 0.6
18:1n-7	3.34 ± 0.03	3.09 ± 0.39	3.09	2.06 ± 0.07	2.03 ± 0.06	1.74 ± 0.05	2.48 ± 0.11	†	†
18:2n-6	2.53 ± 0.03	1.91 ± 0.53	1.81	2.14 ± 0.22	4.74 ± 0.29	3.88 ± 0.01	3.55 ± 0.09	1.6 ± 0.2	1.7 ± 0.1
18:3n-6	0.13 ± 0.00	0.14 ± 0.05	0.22	0.06 ± 0.00	0.38 ± 0.03	0.22 ± 0.00	0.05 ± 0.03	†	†
18:3n-3	0.27 ± 0.02	0.47 ± 0.28	2.62	1.83 ± 0.01	6.90 ± 0.87	5.76 ± 0.14	1.18 ± 0.18	1.3 ± 0.2	1.5 ± 0.1
18:4n-3	0.27 ± 0.05	0.29 ± 0.13	2.02	1.14 ± 0.01	3.11 ± 0.12	2.01 ± 0.10 *	0.20 ± 0.05	1.3 ± 0.0	†
20:1n-9	2.42 ± 0.02	1.31 ± 0.05 **[6]	2.46	1.15 ± 0.04	1.42 ± 0.11	0.87 ± 0.06 *	0.18 ± 0.06	1.0 ± 0.1	0.4 ± 0.1
20:2n-9	0.59 ± 0.07	1.10 ± 0.05 *	1.14	1.67 ± 0.06	0.38 ± 0.07	0.90 ± 0.01 *	-[8]	†	†
20:2n-6	0.17 ± 0.00	0.00 ± 0.00	0.19	0.00 ± 0.00	0.78 ± 0.06	0.38 ± 0.05 *	0.25 ± 0.02	†	†

...continued

Table 15. (continued)

Fatty acid	Diet								
	PUFA-def.		Low-PUFA		High-PUFA		Wild		
	AC[2]	RT	AC	RT	AC	RT	AC[3]	AC[4]	AS[5]
20:3n-9	0.78 ± 0.14	3.35 ± 0.04 **	0.85	3.26 ± 0.08	0.16 ± 0.03	0.85 ± 0.06 *	-	†	†
20:3n-6	0.53 ± 0.02	0.40 ± 0.09	0.78	0.65 ± 0.05	1.45 ± 0.08	1.10 ± 0.03	0.47 ± 0.02	†	†
20:4n-6	1.04 ± 0.06	1.12 ± 0.13	0.72	1.02 ± 0.06	0.92 ± 0.02	0.98 ± 0.03	10.53 ± 0.19	6.9 ± 0.8	10.4 ± 0.1
20:4n-3	0.35 ± 0.00	0.29 ± 0.01 *	1.57	0.51 ± 0.06	2.31 ± 0.11	1.45 ± 0.11 *	0.39 ± 0.09	†	†
20:5n-3	5.53 ± 0.43	3.35 ± 0.48	4.55	2.72 ± 0.15	4.85 ± 0.19	3.41 ± 0.20 *	10.48 ± 2.04	14.1 ± 1.0	5.5 ± 0.1
22:4n-6	0.09 ± 0.01	0.16 ± 0.02	0.09	0.35 ± 0.02	0.08 ± 0.00	0.10 ± 0.00	0.61 ± 0.08	†	1.2 ± 0.3
22:5n-6	0.31 ± 0.03	0.56 ± 0.17	0.22	0.55 ± 0.04	0.19 ± 0.01	0.32 ± 0.06	1.23 ± 0.14	0.5 ± 0.1	2.6 ± 0.2
22:5n-3	1.21 ± 0.03	0.87 ± 0.03 *	1.87	1.12 ± 0.06	1.74 ± 0.09	1.26 ± 0.01 *	3.42 ± 0.14	2.2 ± 0.1	3.4 ± 0.4
22:6n-3	24.08 ± 0.25	17.91 ± 0.58 *	17.35	20.35 ± 0.72	21.17 ± 0.73	25.60 ± 0.11 *	25.58 ± 1.19	28.1 ± 2.0	21.8 ± 0.7
Σ saturates	24.70 ± 1.22	21.80 ± 0.61	22.84	22.83 ± 0.35	22.75 ± 0.25	21.87 ± 0.11	25.92 ± 1.48	26.7	27.9 ± 1.1
Σ monoenes	32.62 ± 0.06	39.74 ± 2.12	37.06	33.87 ± 1.63	24.14 ± 2.17	25.55 ± 0.04	13.55 ± 1.70	11.3	18.0 ± 0.7
Σ PUFA	38.80 ± 1.12	33.28 ± 1.33	36.90	38.53 ± 0.79	50.60 ± 1.77	49.43 ± 0.22	57.9 6± 0.41	56.0	48.7
Σ (n-9)	1.94 ± 0.26	5.56 ± 0.17 *	2.35	5.76 ± 0.14	0.66 ± 0.11	2.08 ± 0.11 *	-	†	†
Σ (n-6)	4.95 ± 0.09	4.39 ± 0.94	4.06	4.84 ± 0.39	8.56 ± 0.26	7.01 ± 0.11 *	16.72 ± 0.03	9.0	16.5 ± 0.6
Σ (n-3)	31.92 ± 0.77	23.33 ± 0.56 *	30.49	27.92 ± 1.04	41.38 ± 1.61	40.34 ± 0.44	41.24 ± 0.45	47.0	33.2 ± 0.9
20:3n-9†22:6n-3	0.03 ± 0.01	0.19 ± 0.00 **	0.05	0.16 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	-	†	†

[1]Number of rearing tanks for cultured charr and trout [2]Only one replicate was analyzed [3]Number of fish = 2 [4]From RingØ and Burkow (1990) (Number of fish = 10) [5]From Ackman and Takeuchi (1986) (Number of fish = 3) [6]Significant difference between charr and trout (*t*-test); *, $p < 0.05$; **, $p < 0.005$. [7]Already included in other fatty acids (e.g., monoenes) or not reported [8]Not detectable

Table 16. Comparison of fatty acid composition of total muscle lipids from cultured and wild Arctic charr (AC), rainbow trout (RT) and Atlantic salmon (AS) (weight %, Mean ± SE, n[1] = 2)

Fatty acid	Diet								
	PUFA-def.		High-PUFA		Commercial		Wild		
	AC	RT[2]	AC[2]	RT	AC	RT[2]	AC[3]	RT[4]	AS[5]
12:0	4.46 ± 0.40	8.31	5.03	6.34 ± 0.20	0.09 ± 0.02	0.09	-[6]	†[7]	†
14:0	4.98 ± 0.18	5.98	2.00	2.29 ± 0.08	3.40 ± 0.23	2.99	0.97 ± 0.16	3.8 ± 0.4	1.5 ± 0.01
16:0	13.10 ± 0.22	11.59	12.88	14.41 ± 0.36	14.44 ± 0.96	14.98	15.86 ± 0.60	22.8 ± 1.8	14.2 ± 0.14
16:1n-9	2.25 ± 0.20	2.41	0.86	1.25 ± 0.01	0.14 ± 0.01	0.10	0.41 ± 0.03	†	5.5 ± 0.10
16:1n-7	6.60 ± 0.18	10.10	4.66	5.36 ± 0.18	7.70 ± 0.27	6.50	3.66 ± 0.44	9.5 ± 0.8	†
18:0	2.20 ± 0.09	2.59	2.90	3.69 ± 0.00	2.68 ± 0.47	3.39	5.48 ± 0.12	4.3 ± 0.1	5.3 ± 0.04
18:1n-9	20.30 ± 0.54	31.73	21.92	24.70 ± 0.70	16.41 ± 1.55	7.37	11.27 ± 0.17	21.6 ± 2.8	12.6 ± 0.19
18:1n-7	3.83 ± 0.12	3.96	2.37	1.89 ± 0.10	3.19 ± 0.07	3.15	3.93 ± 0.35	†	†
18:1n-5	0.46 ± 0.02	0.65	0.09	0.24 ± 0.15	0.34 ± 0.00	0.33	-	†	†
18:2n-6	2.36 ± 0.07	0.99	6.25	5.09 ± 0.11	5.94 ± 0.07	5.11	6.76 ± 0.65	4.9 ± 1.3	3.1 ± 0.06
18:3n-6	0.10 ± 0.01	0.14	0.38	0.22 ± 0.02	0.23 ± 0.00	0.21	0.02 ± 0.01	†	†
18:3n-3	0.23 ± 0.01	0.10	10.35	8.76 ± 0.59	0.64 ± 0.02	0.48	1.81 ± 0.14	6.9 ± 1.6	2.2 ± 0.05
18:4n-3	0.30 ± 0.01	0.12	2.89	1.91 ± 0.11	0.81 ± 0.09	0.59	0.43 ± 0.07	†	†
20:1n-9	4.39 ± 0.32	1.93	2.48	1.23 ± 0.08	3.84 ± 3.84	0.78	0.53 ± 0.05	0.8 ± 0.3	0.8 ± 0.05
20:2n-9	0.65 ± 0.02	1.05	0.40	0.89 ± 0.03	0.11 ± 0.06	0.05	-	†	†

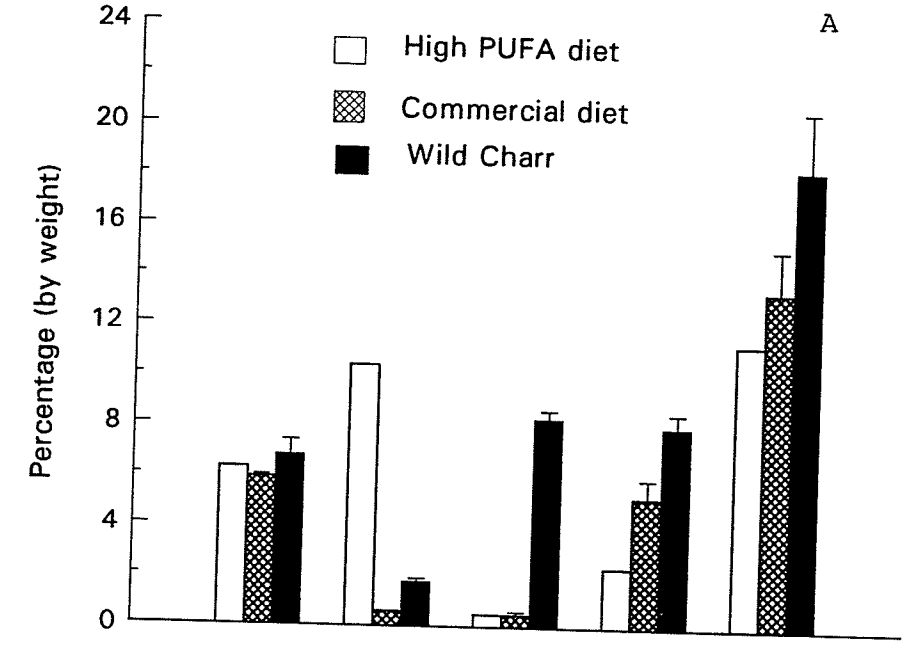
.... continued

Table 16. (continued)

Fatty acid	Diet								
	PUFA-def.		High-PUFA		Commercial		Wild		
	AC	RT[2]	AC[2]	RT	AC	RT[2]	AC[3]	RT[4]	AS[5]
20:2n-6	0.17 ± 0.07	†	0.79	0.32 ± 0.01	0.34 ± 0.13	0.37	0.54 ± 0.08	†	†
20:3n-9	0.61 ± 0.18	1.35	0.05	0.44 ± 0.02	0.16 ± 0.16	0.00	-	†	†
20:3n-6	0.39 ± 0.02	0.13	0.89	0.63 ± 0.01	0.30 ± 0.06	0.30	0.71 ± 0.03	†	†
20:4n-6	0.77 ± 0.04	0.38	0.51	0.44 ± 0.05	0.49 ± 0.06	0.53	8.26 ± 0.29	1.7 ± 0.3	8.0 ± 0.06
20:3n-3	0.04 ± 0.00	0.01	1.16	0.61 ± 0.00	0.08 ± 0.01	0.08	0.16 ± 0.01	†	†
20:4n-3	0.28 ± 0.00	0.12	1.47	0.91 ± 0.05	0.63 ± 0.02	0.54	0.46 ± 0.06	†	†
20:5n-3	4.01 ± 0.17	1.24	2.44	1.61 ± 0.01	5.25 ± 0.67	4.16	8.03 ± 0.54	5.3 ± 1.1	4.6 ± 0.01
22:1n-9	0.56 ± 0.04	0.21	0.32	0.09 ± 0.06	1.09 ± 0.06	0.37	0.09 ± 0.01	†	†
22:4n-6	0.09 ± 0.01	0.03	0.06	0.04 ± 0.02	0.06 ± 0.01	0.04	0.68 ± 0.09	†	1.3 ± 0.05
22:5n-6	0.23 ± 0.01	0.14	0.11	0.14 ± 0.00	0.14 ± 0.02	0.17	1.09 ± 0.13	†	2.0 ± 0.08
22:5n-3	0.88 ± 0.05	0.33	0.90	0.59 ± 0.04	1.12 ± 0.00	1.10	3.30 ± 0.27	†	3.3 ± 0.15
22:6n-3	16.25 ± 0.47	5.91	11.26	11.51 ± 0.76	13.45 ± 1.74	15.76	18.26 ± 2.28	11.7 ± 1.9	15.4 ± 0.27
24:1n-9	0.73 ± 0.01	0.32	0.34	0.31 ± 0.06	0.62 ± 0.03	0.60	0.76 ± 0.12	†	†
Σ saturates	25.15 ± 0.31	28.73	23.06	26.95 ± 0.24	21.30 ± 1.64	22.15	23.36 ± 0.95	†	24.4 ± 0.06
Σ monoenes	42.64 ± 0.84	53.60	33.88	35.80 ± 0.09	44.73 ± 1.06	37.58	22.26 ± 0.79	†	26.5 ± 0.13
Σ PUFA	28.11 ± 0.88	13.07	40.11	34.44 ± 0.13	30.42 ± 2.79	30.13	50.20 ± 1.87	†	45.3
Σ (n-9)	1.79 ± 0.27	3.31	0.58	1.67 ± 0.01	0.37 ± 0.11	0.18	-	†	†
Σ (n-6)	4.22 ± 0.07	1.87	9.02	6.87 ± 0.04	7.76 ± 0.34	6.98	18.14 ± 0.31	†	15.8 ± 0.04
Σ (n-3)	22.11 ± 0.68	7.89	30.51	25.91 ± 0.18	22.29 ± 2.56	22.97	32.06 ± 2.10	†	27.2 ± 0.40

[1,2]Same as in Table 7 [3]Number of fish = 3 [4]From Suzuki et al. (1986) (Number of fish =3) [5]From Ackman and Takeuchi (1986) (Number of fish = 3) [6]Not detectable [7]Already included in other fatty acids (e.g., monoenes) or not reported

Fig. 12. Percentages of n-6 PUFAs in total muscle lipids of Arctic charr (A) and rainbow trout (B) fed diets varying in 18:2n-6 and 18:3n-3. Data for wild trout were taken from Suzuki et al. (1986)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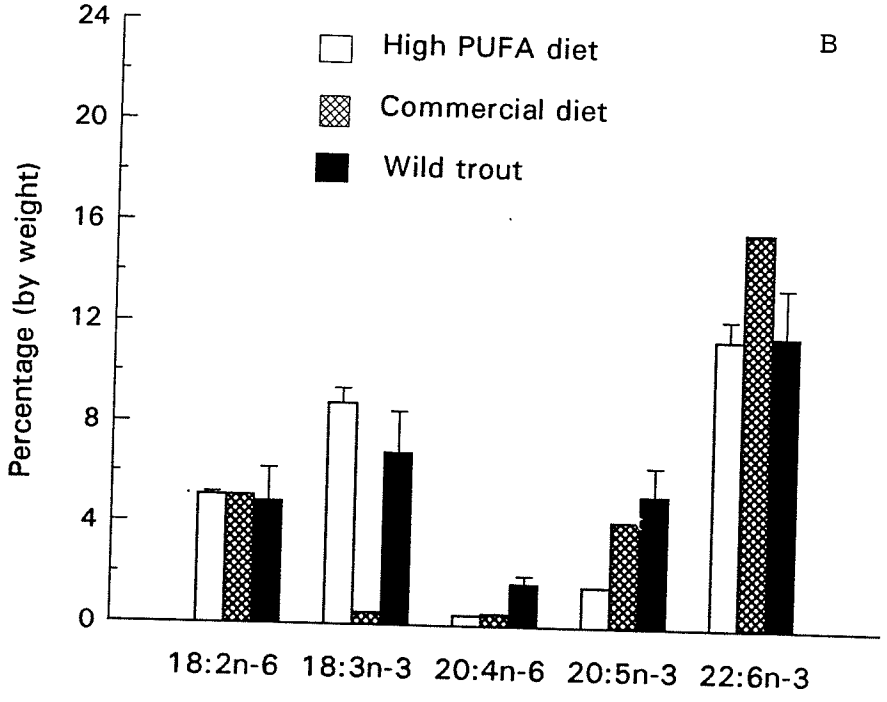
High PUFA diet
Commercial diet
Wild Charr
Percentage (by weight)
24
20
16
12
8
4
0


B
High PUFA diet
Commercial diet
Wild trout
Percentage (by weight)
24
20
16
12
8
4
0
18:2n-6
18:3n-3
20:4n-6
20:5n-3
22:6n-3

Discussion

Differences in sensitivity to a EFA deficiency have been reported for chum salmon and rainbow trout where chum salmon fed the PUFA-deficient diet (0% PUFA) were noted to have higher mortalities than trout (Takeuchi and Watanabe, 1982). In my study, Arctic charr (Nauyuk strain) displayed significantly lower SGRs than did rainbow trout when fed the PUFA-deficient diet, suggesting that charr are more sensitive to an EFA deficiency than trout. It is generally accepted that freshwater fish require minimum levels of dietary 18:3n-3 as their EFAs for optimum growth and normal lipid metabolism (Henderson and Tocher, 1987). Watanabe et al. (1974a) reported that the dietary requirement for rainbow trout cultured at 14-17 ^{o}C is 0.83% to 1.66%. My previous studies suggested that 1 to 2% of dietary 18:3n-3 is essential for the optimum performance of the Labrador strain of Arctic charr (Chapter 3 and 4). In this study using a different strain of charr (Nauyuk) and rearing temperature, I demonstrated that both charr and trout had the highest growth rates and feed efficiencies on the high-PUFA diet that contained 1.71% 18:3n-3 compared to respective groups fed other casein-based diets containing none or < 0.6% 18:3n-3.

The effects of increasing levels of dietary 18:2n-6 in the presence of sufficient dietary 18:3n-3 appear to vary among salmonids with respect to growth and feed utilization (Yu and

Sinnhuber, 1976; Takeuchi and Watanabe, 1982). In the present study charr fed either a low- or a high-PUFA diet had higher growth rates and feed efficiencies than trout. The levels of 18:2n-6 included in these diets (0.16 - 0.48%), were lower than those which had a negative effect on growth of rainbow trout (Yu and Sinnhuber, 1976) or Arctic charr (Chapter 3). Therefore, the dissimilar growth revealed for Arctic charr and rainbow trout likely can be attributed to the positive effects of this level of dietary 18:2n-6 on Arctic charr and/or other factors such as differences in other nutrient requirements and genetic backgrounds.

Numerous studies have shown that fish fed diets deficient in EFA develop a variety of symptoms including high HSI, fatty liver and substantial accumulation of 20:3n-9 in tissue polar lipids (Henderson and Tocher, 1987; Sargent et al, 1989). Most of these symptoms were also observed in this study when charr and trout were fed the PUFA-deficient diet (0% PUFA). However, trout fed this diet showed a high HSI and typical fatty livers (i.e., an increased percentage of neutral and total lipids in the livers) but no apparent change in muscle water content. By contrast, charr had higher muscle water content but no change in the HSI or the amount of total lipids in livers, indicating that charr and trout differ in their responses to an EFA deficiency. On the other hand, these results with Nauyuk charr are consistent with what I found for the Labrador strain of Arctic charr.

Haematological values have been widely used for assessing nutritional status of fish (Cho, 1985). In this study, the haematocrit and haemoglobin values of both species fell within the ranges reported by Castell et al. (1972b), Giles et al. (1984) and Chapter 4. Despite the marked differences between the two species with respect to haematocrit and haemoglobin values, the levels of dietary PUFAs had no effect on haematocrit and haemoglobin values for either species.

Most studies on fish support the theory that freshwater fish desaturate and elongate 18:1n-9, 18:2n-6 and 18:3n-3 to long-chain PUFAs through a common set of enzymes (Henderson and Tocher, 1987). In my study, charr and trout both showed a significant increase in the percentages of C20 and C22 n-3 PUFAs in liver polar lipids as 18:3n-3 increased in the diets, indicating that charr and trout can convert 18:3n-3 to the n-3 PUFAs. This agrees with previous reports on trout and charr (Castell et al., 1972c; Kanazawa, 1985; Olsen et al., 1991; Olsen and RingØ 1992; Chapter 4). When the low- and high-PUFA diets were fed to both species, charr had significantly lower percentages of 22:6n-3 and 20:3n-9 in liver polar lipids but slightly higher percentages of intermediates involved in the conversion of 18:3n-3 compared to trout. This suggests that D^6 and/or D^5 desaturases, key enzymes in the pathway for production of 20:3n-9 and 22:6n-3 from 18:1n-9 and 18:3n-3, may be less effective in charr than in trout. Furthermore, when the diet without PUFA (PUFA-deficient diet) was fed to

both species, the amount of 20:3n-9 in liver and muscle polar lipids from trout was nearly three times higher than noted for charr, while 22:6n-3 was lower in trout than in charr. This is a further indication that D^6 and/or D^5 desaturases are less active in charr than in trout. Moreover, these results suggest that charr fed the diet without PUFA retained higher levels of 22:6n-3 in tissues than did trout.

The ratio of 20:3n-9 to 22:6n-3 of tissue polar lipids, although often questioned, has been used widely to assess fish EFA requirements (Cowey, 1988; Satoh et al., 1989). The ratios of 20:3n-9 to 22:6n-3 calculated for trout in the present study were lower than those reported by Castell et al. (1972c) and Watanabe et al. (1974b, c). This may be attributed to differences in experimental conditions between studies, particularly the acclimation period and rearing temperature. Moreover, charr had significantly lower ratios of 20:3n-9/22:6n-3 in liver and muscle polar lipids than trout fed casein-based diets. The values for the ratio also differed between liver and muscle for charr and trout. Furthermore, the ratios calculated for charr and trout fed either the low- or high-PUFA diet were low (< 0.4), even though only the high-PUFA diet contained adequate amounts of 18:3n-3. Collectively, these data suggest that the 20:3n-9/22:6n-3 ratio in tissue polar lipids varies with species, tissue and experimental conditions. Consequently, the ratio of 20:3n-9 to 22:6n-3 may not be as sensitive an indicator of EFA status for Arctic

charr and rainbow trout as thought previously.

Castell (1979) proposed that the EFA requirement of freshwater fishes is highly correlated with water temperature of wild fish. Accordingly, fish living in a colder environment would have a higher requirement for 18:3n-3 than those from warmer waters. In relation to this hypothesis, the requirement for dietary 18:3n-3 would be expected to be higher for Arctic charr than for trout, since charr is a more northerly distributed species than rainbow trout (Johnson, 1980). However, data reported here showed little difference in growth, feed efficiency and other parameters between these two species when fed a diet containing 1.71% 18:3n-3. Previous studies (Chapters 3 and 4) indicated that charr required 1.0 to 2.0% dietary 18:3n-3, which overlap with the requirements of rainbow trout (Watanabe et al., 1974a).

To date there is little information on the relationship between n-6 PUFAs and the health and survival of Arctic charr. RingØ (1989) demonstrated that a commercial diet supplemented with 2.5% 18:2n-6 (the precursor of 20:4n-6) had adverse effects on digestibility of dietary fatty acids and amino acids for Arctic charr. However, results from Chapter 3 showed that 0.7 to 0.9% 18:2n-6 plus 2.3 to 2.9% 18:3n-3 in semi-purified diets did not adversely affect growth of the Labrador strain of Arctic charr. It appears that both the amount and the ratio of 18:2n-6 and 18:3n-3 may affect the growth and health of cultured charr.

Comparison of cultured and wild species in terms of their lipid and fatty acid composition has proved to be a useful approach to evaluate the n-6 PUFA requirements of fish (Ackman and Tackeuchi, 1986). Charr fed the experimental diets had one tenth the amount (%) of 20:4n-6 in muscle polar and total lipids than wild charr both in this study and that by RingØ and Burkow (1990). This is similar to smolting Atlantic salmon (Salmo salar) where the percentage of 20:4n-6 of whole body lipids of commercially cultured fish was one tenth of that recovered from wild Atlantic salmon (Ackman and Takeuchi, 1986). By contrast, there were only slight differences in tissue percentages of 20:4n-6 between the cultured and wild trout (Suzuki et al., 1986). On the other hand, it is clear that the level of tissue 20:4n-6 is highly correlated with the health and survival of smolting Atlantic salmon (Ackman and Takeuchi, 1986). Accordingly, the markedly high level of tissue 20:4n-6 in the wild charr from this study may imply that 20:4n-6 is important for Arctic charr.

In summary, this chapter compared EFA deficiency symptoms, growth and metabolism of dietary C18 n-3 and n-6 PUFA for cultured Arctic charr and rainbow trout simultaneously fed diets varying in dietary 18:2n-6 and 18:3n-3. This chapter demonstrated that charr and trout fed the PUFA-deficient diet differed not only in growth and feed efficiency but also in other universal EFA deficiency symptoms including HSI, muscle water content, fatty liver and the amount of 20:3n-9 which

accumulated in tissues. Furthermore, charr and trout significantly differed in their percentages of tissue 20:3n-9, 22:6n-3 and other PUFAs regardless of the concentrations of 18:3n-3 and 18:2n-6 in the diets, suggesting that D^6 and/or D^5 desaturase may be less efficient in charr than in trout at 10 ^oC. The major difference in amount of tissue 20:4n-6 between the cultured and wild Arctic charr suggests that juvenile Arctic charr may require n-6 PUFAs, which is similar to Atlantic salmon and chinook salmon but different from rainbow trout.

Chapter 6.

Dietary α-linolenic and linoleic acids competitively affect metabolism of polyunsaturated fatty acids in Arctic charr, *Salvelinus alpinus* (L.)

Abstract

The interaction of dietary 18:2n-6 (linoleic acid) and 18:3n-3 (α-linolenic acid) and their effects on the production of muscle n-3 long-chain polyunsaturated fatty acids (PUFAs) were investigated for Arctic charr, Salvelinus alpinus (L.). Fish were fed either 8 or 12 casein-based semi-purified diets in two separate experiments for 12 and 14 weeks, respectively.

Low levels of dietary 18:2n-6 and 18:3n-3 supplemented with pure methyl esters or flax oil had an additive effect on fish growth. High levels of dietary 18:2n-6 in the presence of 1.6-1.7% 18:3n-3 inhibited fish growth. While 18:2n-6 was converted to 20:4n-6 and 22:5n-6 in the absence or presence of dietary 18:3n-3, the dominant product of 18:3n-3 conversion was consistently 22:6n-3. Increasing dietary 18:3n-3 markedly inhibited the conversion of 18:2n-6, while the inhibition of dietary 18:2n-6 on 18:3n-3 conversion was noted only when dietary 18:2n-6/18:3n-3 increased from 1.0 to 1.5. Feeding diets rich in 18:3n-3 led to the marked accumulation of 18:3n-3 and 18:4n-3 in fish muscle but negligible changes in 20:5n-3 and 22:6n-3, regardless of the level of 18:3n-3 in the diets.

Introduction

Dietary polyunsaturated fatty acids (PUFAs), especially n-6 and n-3 PUFAs, have been studied extensively in relation to their essential roles in growth and development of vertebrates (Henderson and Tocher, 1987; Watkins, 1991; Innis, 1991). In addition to being sources of EFAs, dietary 18:2n-6 and 18:3n-3 interact biochemically and nutritionally in mammals. They compete with each other for the rate-limiting D^6 desaturase, and it is known that 18:3n-3 is preferred over 18:2n-6 as the substrate for desaturation (Brenner and Peluffo, 1966). The conversion of 18:2n-6 to 20:4n-6, especially at low levels of dietary 18:2n-6 intake, is inhibited by dietary 18:3n-3 (Mohrhauer and Holman, 1963). By contrast, dietary 18:2n-6 inhibits the conversion of 18:3n-3 to 20:5n-3 but to a less extent (Rahm and Holman, 1964).

Extensive studies with fish indicate that 18:3n-3 is an ubiquitous EFA in freshwater fish except those living in the tropical regions, while 18:2n-6 may or not be an EFA, depending on the species (Kanazawa, 1985). Various aspects of the interaction of dietary 18:2n-6 and 18:3n-3 have been examined for several fish species. Low levels of dietary 18:2n-6 and 18:3n-3 may or not have an additive effect on fish growth (Castell et al., 1972; Thongrod et al., 1990b). Increasing dietary 18:2n-6 may adversely affect fish growth when 18:3n-3 in the diet is adequate to meet EFA requirements

(Yu and Sinnhuber, 1976 and 1979). By comparison, there have been few studies on the influence of varying levels of dietary 18:2n-6 and 18:3n-3 on each other's metabolism.

Rainbow trout (Oncorhynchus mykiss) and coho salmon (O. kisutch), require 18:3n-3 as EFA but these species do not need or have very low requirements for dietary 18:2n-6 (Castell et al., 1972a; Takeuchi and Watanabe, 1982; Cowey, 1988). Despite this similarity in EFA requirement, it has been reported that dietary 18:2n-6 inhibits 18:3n-3 conversion in rainbow trout but not in coho salmon, while 18:3n-3 markedly inhibited the conversion of 18:2n-6 to long-chain PUFAs in both species (Yu and Sinnhuber, 1976 and 1979). This suggests that the interaction between dietary 18:3n-3 and 18:2n-6 varies among fish species. Additional studies are necessary to determine if similar trends occur in other salmonid species which require both 18:2n-6 and 18:3n-3.

Recent studies on Arctic charr, Salvelinus alpinus (L.), have shown that this species requires 18:3n-3 as its EFA and also there may be a requirement for 18:2n-6 (Chapters 3, 4 and 5). In addition, the metabolism of dietary PUFAs in charr is markedly different from that in rainbow trout (Chapter 5). However, the interaction between dietary 18:2n-6 and 18:3n-3 has not been fully investigated for charr.

The use of 18:3n-3-rich flax (linseed) oil is an attractive alternative to marine fish oil in the diets of cultured fish due to a declining marine fisheries, but there is limited

information on the effects of feeding high levels of dietary 18:3n-3 on the production of long-chain PUFAs in fish flesh (muscle). Feeding high levels of dietary lipids and 18:3n-3 to fish for 10 to 140 days resulted in substantial incorporation of 18:3n-3 into muscle but little change in levels of muscle 20:5n-3 and 22:6n-3 (Sowizral et al., 1990; Greene and Selivonchick, 1990). It was suggested that the limited conversion of 18:3n-3 in muscle may be attributed to the inhibition of dietary 20:5n-3 and 22:6n-3 and maintenance of membrane saturation by fish (Sowizral et al., 1990; Greene and Selivonchick, 1990). Whether increasing dietary 18:3n-3 in the absence of dietary 20:5n-3 and 22:6n-3 affects 18:3n-3 metabolism in muscle has not been thoroughly investigated.

The main objective of this study was to assess the interaction of dietary 18:2n-6 and 18:3n-3 with respect to fish growth, feed utilization, EFA requirement and lipid metabolism. The effect of increasing dietary levels of 18:2n-6 and 18:3n-3 on production of long-chain PUFAs was also examined for charr muscle.

Materials and Methods

Fish, Diets and Experimental Design

Two experiments were carried out with juvenile Arctic charr fed semi-purified diets varying primarily in levels of 18:2n-6 and 18:3n-3. Arctic charr used in Expt. I belonged to the Labrador population originally collected from Labrador (Canada). Charr used in Expt. II were offspring of the broodstock collected from Nauyuk lake (NWT, Canada).

The composition of the basal diet used in Expts. I and II was identical: 52.0% casein, 15.2% starch, 10.0% dextrin, 4.0% gelatin, 5.0% lipid, 5.5% mineral premix, 2.0% vitamin premix, 1.5% carboxymethylcellulose, 1.3% arginine, 1.0% choline premix and 0.5% methionine (Chapter 3). The level of total lipids was increased to 8.0% in one of the experimental diets, while the carbohydrate (starch) was decreased proportionally. The methods used for diet manufacture and storage condition of the diets were the same as described previously (Chapter 3).

Experimental design and rearing condition

One reference (commercial trout feed) and 11 casein-based diets were employed as treatments in Expt. I and 8 casein-based diets were tested in Expt. II (Table 17). The casein-based diets varied in type and amount of dietary fatty acids. The lipid sources selected for these diets were >99% pure esters of laurate (12:0), 18:1n-9, 18:2n-6 and 18:3n-3 (Sigma, St. Louis, U.S.A.) and flax oil (Omega Nutrition, Vanc.,

Table 17. Composition of dietary lipids and levels of polyunsaturated fatty acids (PUFA) in the test diets used in Experiment I and II (% in diet)

Diet	Lipid composition					PUFA content[3]		
	18:3n-3[1]	18:2n-6[1]	Flax oil	12:0[2]	18:1n-9[2]	18:3n-3	18:2n-6	Ratio[4]
Exp. I								
A	-	-	1.0	4.0	-	0.6	0.2	0.3
B	-	0.4	0.9	3.7	-	0.5	0.5	1.0
C	-	1.0	-	4.0	-	0	1.0	-
D	1.0	-	-	4.0	-	1.0	0	-
E	-	-	2.0	3.0	-	1.1	0.3	0.3
F	-	-	3.0	2.0	-	1.6	0.5	0.3
G	-	1.0	3.0	1.0	-	1.6	1.5	1.0
H	-	-	3.0	-	2.0	1.6	0.5	0.3
I	-	-	5.0	-	-	2.7	0.8	0.3
J	-	-	8.0	-	-	4.3	1.2	0.3
K	-	-	-	5.0	-	0	0	-
L	Reference diet							

... Continued

Table 17. (continued)

Diet	Lipid composition					PUFA content[3]		
	18:3n-3[1]	18:2n-6[1]	Flax oil	12:0[2]	18:1n-9[2]	18:3n-3	18:2n-6	Ratio[4]
Exp. II								
A[5]	-	-	1.0	4.0	-	0.6	0.2	0.3
B	0.5	0.5	-	4.0	-	0.5	0.5	1.0
C	-	1.0	-	4.0	-	0	1.0	-
D	1.5	-	-	3.5	-	1.5	0	-
E[5]	-	-	3.0	2.0	-	1.7	0.6	0.4
F	-	1.0	3.0	1.0	-	1.7	1.6	1.0
G	-	2.0	3.0	0	-	1.7	2.6	1.5
H	-	-	4.0	-	-	2.3	0.7	0.4

[1]Methyl ester [2]Ethyl ester [3]Calculated on the basis of lipids supplemented and fatty acid composition reported in Table 18 [4] 18:2n-6/18:3n-3 in diets [5]Shared with the experiment reported in Chapter 5

Canada). Percentages of dietary 18:2n-6 and 18:3n-3 were calculated on the basis of total dietary lipids including pure esters of 12:0, 18:1n-9, 18:2n-6 and 18:3n-3 and fatty acid composition of the PUFA-free diet and flax oil (Table 18).

All experiments were done at the Rockwood Aquaculture Research Centre, Department of Fisheries and Oceans (Manitoba, Canada). Prior to the administration of the experimental diets, fish were fed a casein-based PUFA-free diet for 4 weeks to condition the fish to eat the casein-based diets and to reduce the PUFAs stores in fish tissues. Replicated treatments (experimental diets) were then assigned randomly to rearing tanks, each of which held 75 individual fish with mean weights of 2.0 g for Expt. I and 2.3 g for Expt. II.

All fish were reared at constant 10 ^{o}C under a photoperiod of 12 hours light : 12 hours dark. Each rearing tank contained 60 litre of aerated well water with 95% recirculation at a flow rate of approximately 2.4 litre.min^{-1}.Fish were fed 5 times a day to apparent satiation and the amount of feed fed was recorded. To assess growth response and adjust feeding rate, fish were weighted individually at 28 day intervals and total weights of all fish in each tank were determined biweekly in order to adjust the amount of feed. The experimental duration was 14 weeks in Expts. I and 12 weeks in Expt. II. Water quality was maintained to be the same a sdecribed in chapter 3.

Table 18. Fatty acid composition of linseed oil and the PUFA-free diet

	Flax oil		Diet
	Exp. I	Exp. II.	K[1]
12:0	-	-	98.3
14:0	0.1	0.1	0.1
16:0	5.2	5.5	0.3
18:0	2.9	3.7	0.1
18:1n-9	20.4	13.8	0.3
18:1n-7	0.7	0.7	-
18:2n-6	15.4	18.2	0.4
18:3n-3	53.4	56.6	0.1

[1]From Exp. I

Chemical and Statistical Analyses

At the end of the feeding trials, 10 fish were randomly sampled from each tank to determine the hepatosomatic index (HSI) and the water content of liver and muscle. Livers and muscle from an additional 5 to 10 fish from each tank were collected and pooled for lipid and fatty acid analyses.

Lipids from the liver and muscle were extracted according to the procedures of Folch et al. (1957) and Bligh and Dyer (1959). An aliquot of the lipid extracts was separated into polar and neutral lipid fractions on Sep-Pak silica cartridges (Millipore Corp., MA, U.S.A.) according to Satoh et al. (1989). Percentages of polar and neutral lipids were determined gravimetrically. The fatty acid composition of flax oil, the PUFA-free diet and tissue lipids were determined by gas chromatography using the methods previously described (Chapter 5).

Growth response and feed utilization of fish were presented as specific growth rate (SGR) and feed efficiency and the parameters were calculated using the formula described elsewhere (Chapters 3 and 5). HSI was calculated as liver weight (wet) / body weight (wet) × 100.

One-way ANOVA and Duncan's multiple range test were performed on SAS to compare means between treatments (SAS, 1985). Differences between treatments were considered significant at $P < 0.05$.

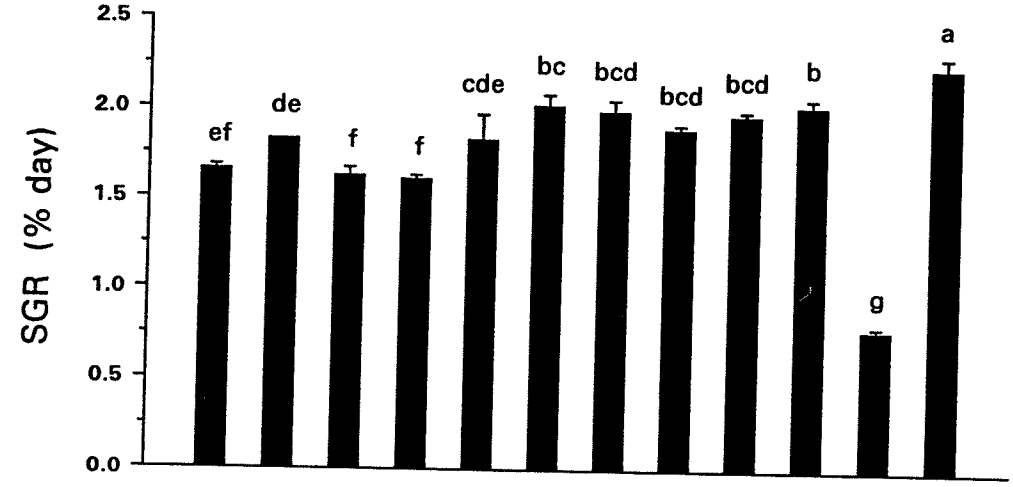
Results

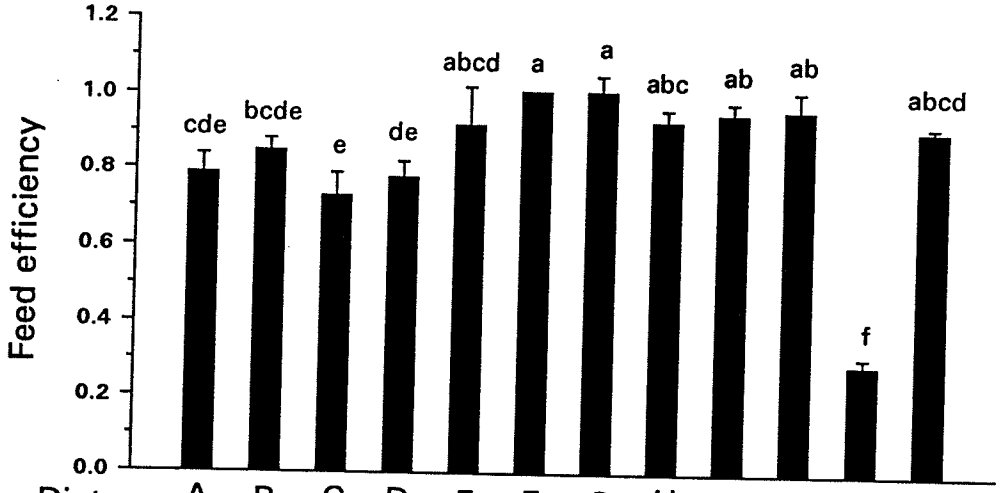
Growth and Feed efficiency

The influence of diet treatments on the growth performance and feed utilization for Arctic charr in Expt. I is shown in Fig. 13. Charr fed diets supplemented with PUFAs had significantly higher SGRs and feed efficiencies compared to those fed the PUFA-free diet (diet K). Fish fed the diet containing 0.5% 18:3n-3 and 18:2n-6 (diet B) had significantly higher growth rates than those fed diets containing 1.0% 18:2n-6 or 18:3n-3 alone (diets C, D). However, neither SGR nor feed efficiency showed significant change as the level of 18:2n-6 in the diets was increased from 0.5 to 1.5% and 18:3n-3 remained constant at 1.6% (diets F, G). When the ratio of 18:2n-6 to 18:3n-3 in diets remained below 1.0, an increase in dietary 18:3n-3 from 1.6 to 4.3% or total dietary lipids from 5.0 to 8.0% produced little change in SGR and feed efficiency (diets F, I, J). The replacement of 12:0 with 18:1n-9 resulted in a decline in SGR and feed efficiency but the change was statistically insignificant.

The specific growth rates (SGR) and feed efficiencies are also shown for charr fed casein-based diets from Expt. II (Fig. 14). Charr fed the diets containing high levels of 18:3n-3 (diets E, H) displayed significantly higher SGRs compared to those fed diets low in 18:2n-6 or 18:3n-3 (diets A-C). As dietary 18:2n-6 was increased from 1.6 to 2.6% and

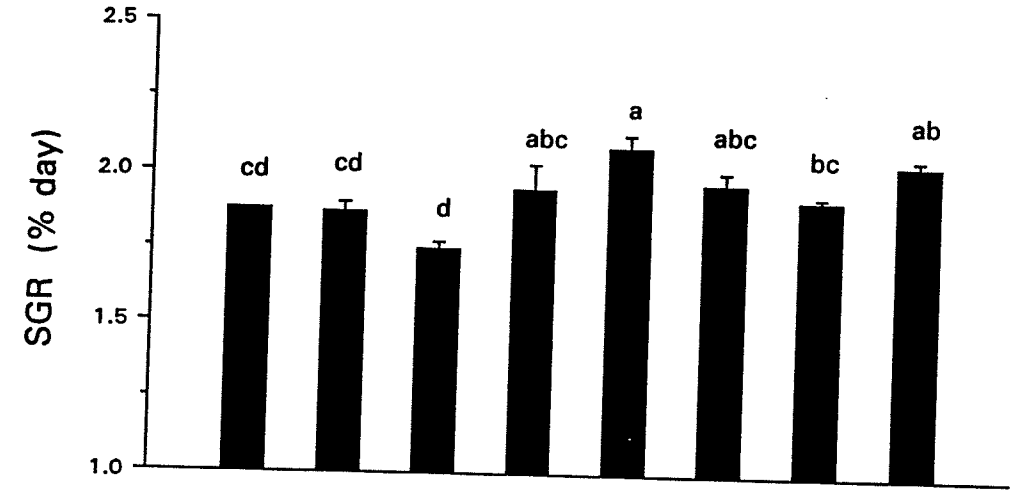
Fig. 13. Specific growth rates and feed efficiencies of Arctic charr fed diets varying in levels of 18:2n-6 and 18:3n-3 in Expt. I. Values = mean ± SEM, n = 2 (each replicate had 75 individual fish). Common letters on top of bars indicate no significant difference between treatments (diets) ($P > 0.05$)

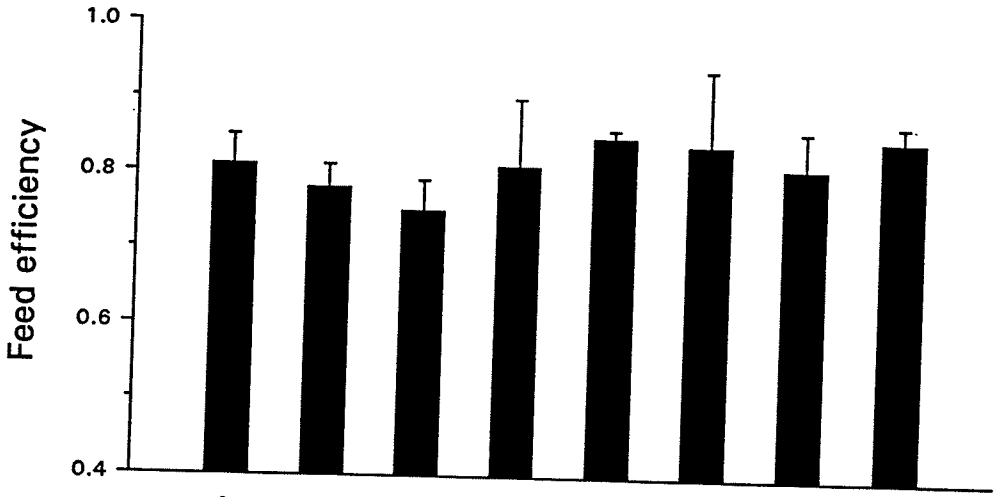




Diet	A	B	C	D	E	F	G	H	I	J	K	L
18:3n-3	0.6	0.5	0	1.0	1.1	1.6	1.6	1.6	2.7	4.3	0	-
18:2n-6	0.2	0.5	1.0	0	0.3	0.5	1.5	0.5	0.8	1.2	0	-

Fig. 14. Specific growth rates (SGR) and feed efficiencies of Arctic charr fed diets varying in levels of 18:2n-6 and 18:3n-3 in Expt. II. Values = mean ± SEM, n = 2 for all treatments (diets) except for treatments D and E where n = 3 (each replicate had 75 individual fish). Common letters on top of bars indicate no significant difference between treatments (or diets)





Diet	A	B	C	D	E	F	G	H
18:3n-3	0.6	0.5	0	1.5	1.7	1.7	1.7	2.3
18:2n-6	0.2	0.5	1.0	0	0.6	1.6	2.6	0.7

18:3n-3 was maintained at 1.7%, SGR gradually decreased (diets F, G). Diet C supported poorer growth and feed efficiency. However, no significant difference occurred in feed efficiency between treatments.

Water content, HSI and lipid composition

Water contents of the liver and muscle and hepatosomatic indices (HSI) are summarized for charr from both experiments (Table 19). Generally, charr fed casein-based diets low in PUFA had lower liver water content but higher muscle water content and HSI. The group fed the PUFA-free diet (diet K) in Expt. I had highest livers and muscle water content. Similar trends were also noted in Expt. II (Table 19).

The lipid composition of livers is presented in Figs. 15 and 16. The liver tissue of charr fed casein-based diets low in 18:n-6 and 18:3n-3 had high percentage of total lipids and neutral lipids but low percentages of polar lipids. Generally, no significant differences were noted for the parameters in charr fed diets containing high levels of 18:3n-3 but varying levels of 18:2n-6 in Expt. I (diets F-J) (Fig. 15). Percentages of total lipids, polar and neutral lipids in the charr fed diet D (Expt. II) containing 1.5% 18:3n-3 were significantly different from those fed diets containing $\geq$ 1.7% 18:3n-3 and 0.6% 18:2n-6 (Expt. II, diets E-H) (Fig. 16). Further, total lipids in muscle ranged from 2.0 to 3.0% (wet weight) for a majority of treatments in both experiments. No significant difference occurred in total muscle lipids for all

Table 19. Water content in livers and muscle of charr from experiments I and II (Mean ± SEM, $n^1 = 2$)[2]

Diet	Water content (%)		HSI
	Liver	Muscle	
Exp. I			
A	69.5 ± 0.0 [d]	78.5 ± 0.3 [b]	2.15 ± 0.05 [a]
B	69.5 ± 0.5 [d]	78.1 ± 0.3 [bc]	1.93 ± 0.08 [ab]
C	70.0 ± 0.1 [cd]	78.5 ± 0.1 [b]	1.85 ± 0.00 [abc]
D	71.9 ± 0.4 [abc]	78.2 ± 0.2 [b]	1.81 + 0.03 [abc]
E	70.5 ± 1.7 [bcd]	77.7 ± 0.3 [bcd]	1.81 ± 0.15 [abc]
F	71.7 ± 0.6 [abcd]	77.7 ± 0.1 [bcd]	1.58 ± 0.04 [cd]
G	72.2 ± 0.7 [abc]	77.7 ± 0.1 [bcd]	1.46 ± 0.12 [d]
H	72.2 ± 0.0 [abc]	77.6 ± 0.4 [bcd]	1.59 ± 0.04 [cd]
I	72.5 ± 0.4 [ab]	77.6 ± 0.1 [bcd]	1.38 ± 0.10 [d]
J	72.5 ± 1.0 [ab]	77.1 ± 0.2 [cd]	1.39 ± 0.05 [d]
K	73.2 ± 0.3 [a]	79.8 ± 0.6 [a]	1.65 ± 0.24 [bcd]
L	70.0 ± 0.2 [cd]	77.0 ± 0.2 [d]	1.86 ± 0.07 [abc]
Exp. II			
A	68.8 ± 0.2 [b]	78.0 ± 0.0	2.06 ± 0.18 [a]
B	69.4 ± 0.0 [b]	77.8 ± 0.4	2.06 ± 0.02 [a]
C	67.5 ± 1.2 [b]	78.2 ± 0.2	2.24 ± 0.04 [a]
D	69.4 [b]	78.3	2.02 [a]
E	74.0 ± 0.5 [a]	77.7 ± 0.2	1.68 ± 0.03 [b]
F	75.1 ± 0.1 [a]	77.9 ± 0.1	1.50 ± 0.08 [b]

... Continued

Table 19 (continued)

Diet	Water content (%)		HSI
	Liver	Muscle	
G	74.6 ± 0.4 [a]	77.6 ± 0.5	1.58 ± 0.07 [b]
H	74.4 [a]	77.9	1.42 ± 0.06 [b]

[1]Value for each replicate was the mean of values determined from 10 individual fish [2] n = 2 for all treatments in Exp I. In Exp. II, n = 2 for all treatments except treatments E, D and H. n = 3 for treatment E; n = 1 for treatment D; n = 1 for water content of treatment H [3]Same lettlers in a column indicate no significant difference between treatments

Fig. 15. Lipid composition of livers from charr fed diets varying in levels of 18:2n-6 and 18:3n-3 in Expt. I. Total lipids were expressed as the percentage of tissue weight (wet) and percentage of neutral and polar lipids were calculated from total lipids. Values = mean ± SEM, n = 2 (each replicate had 75 individual fish). Common letters on top of bars indicate no significant difference between treatments (diets) ($P > 0.05$)

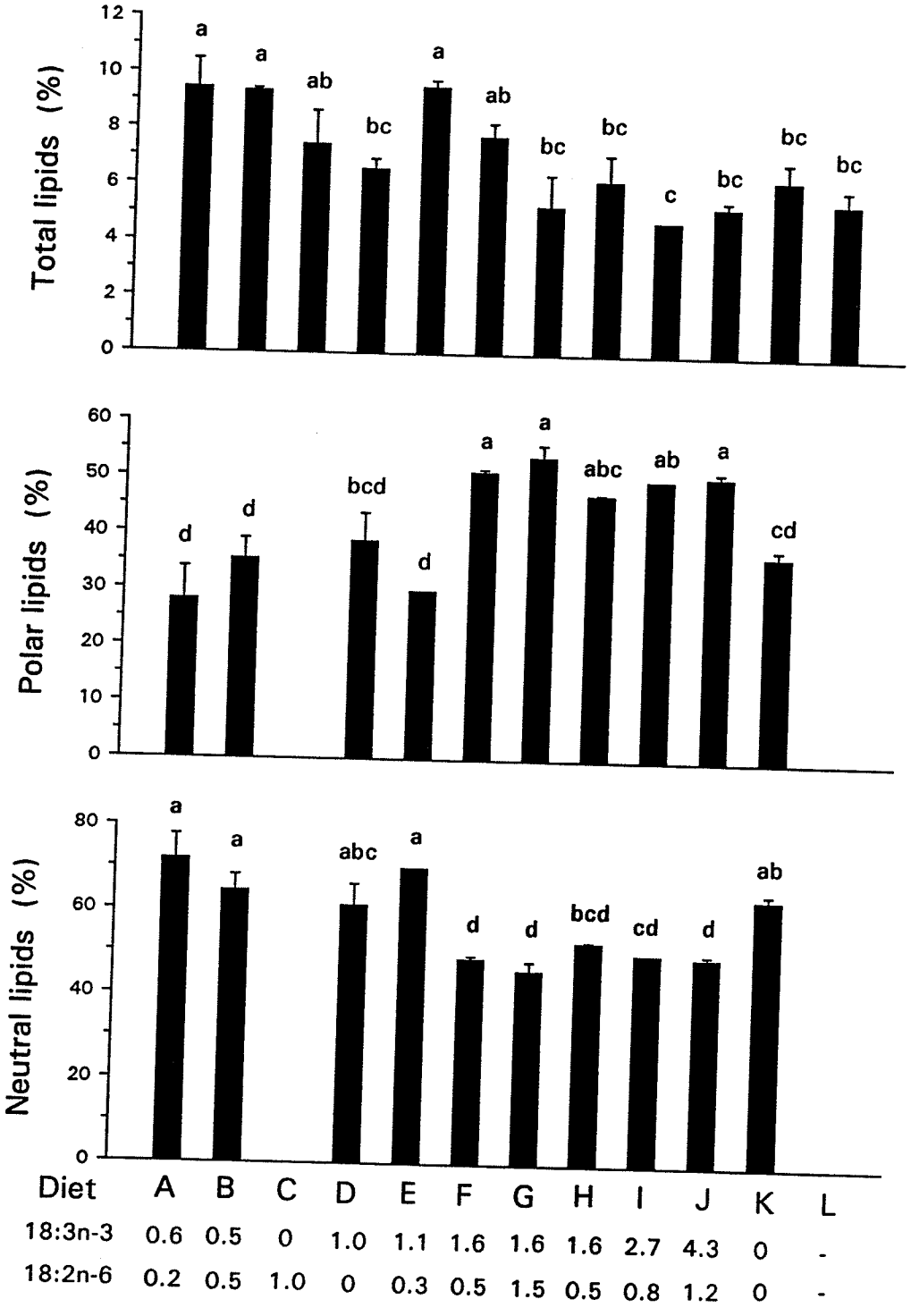
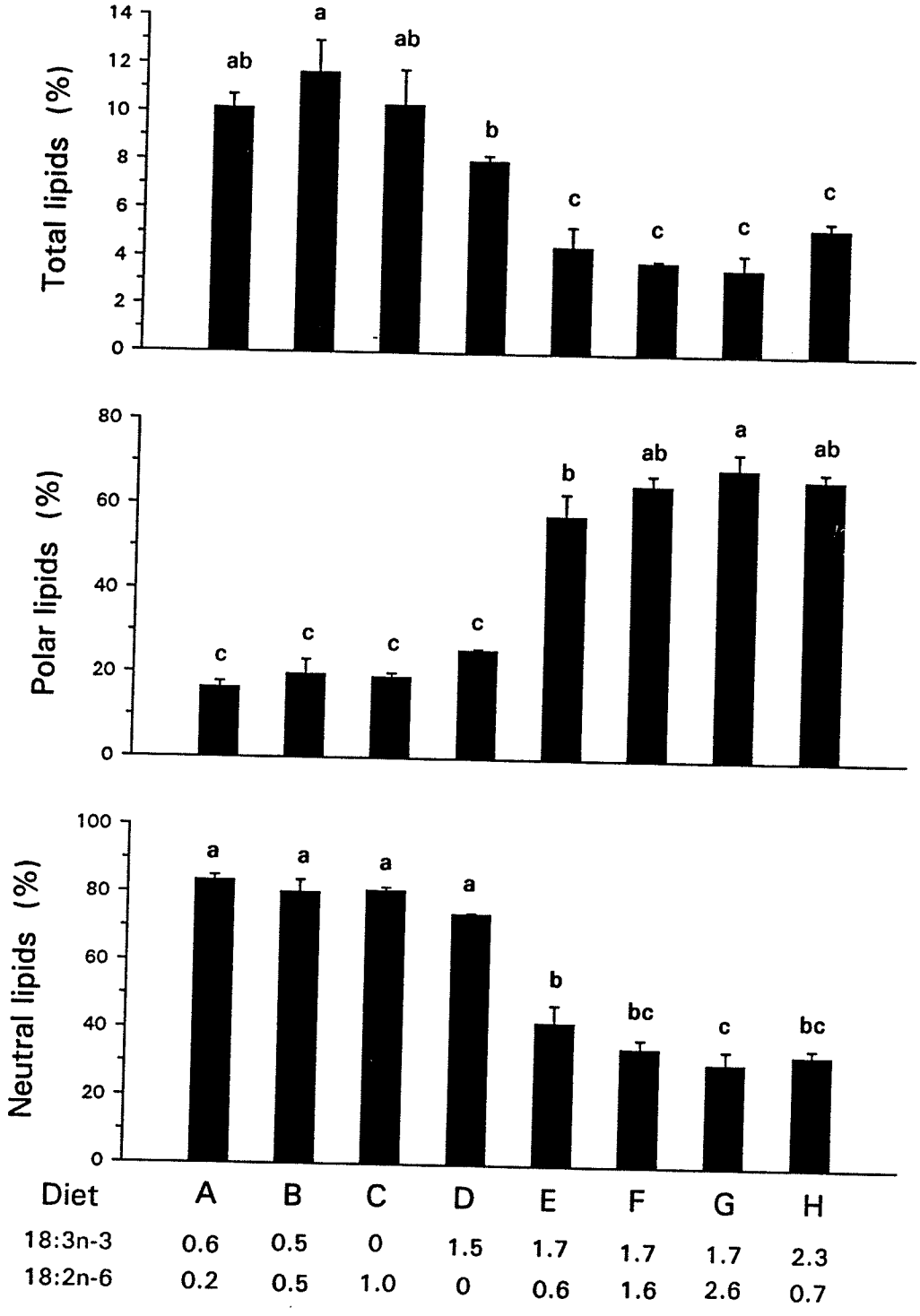

Total lipids (%)
Polar lipids (%)
Neutral lipids (%)
Diet A B C D E F G H I J K L
18:3n-3 0.6 0.5 0 1.0 1.1 1.6 1.6 1.6 2.7 4.3 0 -
18:2n-6 0.2 0.5 1.0 0 0.3 0.5 1.5 0.5 0.8 1.2 0 -

Fig. 16. Lipid composition of livers from charr fed diets varying in levels of 18:2n-6 and 18:3n-3 in Expt. II. Total lipids were expressed as the percentage of tissue weight (wet) and percentages of polar and neutral lipids were calculated from total lipids. Values = mean ± SEM, n = 2 (each replicate had 75 individual fish). Common letters on top of bars indicate no significant difference between treatments (diets) ($P > 0.05$)


Total lipids (%)
14
12
10
8
6
4
2
0
ab
a
ab
b
c
c
c
c
Polar lipids (%)
80
60
40
20
0
c
c
c
c
b
ab
a
ab
Neutral lipids (%)
100
80
60
40
20
0
a
a
a
a
b
bc
c
bc
Diet A B C D E F G H
18:3n-3 0.6 0.5 0 1.5 1.7 1.7 1.7 2.3
18:2n-6 0.2 0.5 1.0 0 0.6 1.6 2.6 0.7

treatments in both sets of experiments, except diets I and K from Expt. I.

Fatty acid composition of liver polar lipids

The fatty acid compositions of liver polar lipids are shown for all treatments from Expt. I (Table 20 and Fig. 17). Percentages of total n-3 or n-6 PUFA in tissue significantly increased as he level of 18:2n-6 or 18:3n-3 was increased in the diets. There were concomitant changes in individual PUFAs including n-3 or n-6 long-chain PUFAs and 20:3n-9. When the level of 18:2n-6 in the diets remained at 0.5%, increasing dietary 18:3n-3 from 0.5 to 1.6% (diets B, H) resulted in significant increases in 20:5n-3 and 22:6n-3 but decreases in 20:4n-6 and 22:5n-6. On the other hand, when dietary 18:3n-3 was unchanged, increasing dietary 18:2n-6 at three different levels of dietary 18:3n-3 (diets A, B, D, E-G) led to significant increases in both 20:4n-6 and 22:5n-6. Charr fed diets containing equal portions of 18:2n-6 and 18:3n-3 (diet B, G), had significantly higher percentages of 22:6n-3 than 20:4n-6 or 22:5n-6 PUFAs. Moreover, when the ratio of 18:2n-6 to 18:3n-3 was below 1.0 in the diets, increasing dietary lipids from 5.0% to 8.0% (diets I, J) resulted in little change in tissue PUFA composition, while the ratio of total n-6 PUFA to total n-3 PUFA in the tissues decreased. In addition, replacement of dietary 12:0 with 18:1n-9 in the diets containing 1.7% 18:3n-3 (diets F, H) resulted in a significant decrease in tissue saturated fatty acids but an

Table 20. Fatty acid composition of liver polar lipids of Arctic charr from Experiment I (% by weight) (Mean ± SEM, n[1] = 2)[2]

Fatty acid	Diet				
	A	B	C	D	E
14:0	1.95 ± 0.22 ^{ab}	2.15 ± 0.36 ^{ab}	1.93 ± 0.15 ^{ab}	2.24 ± 0.00 ^{a}	1.98 ± 0.37 ^{ab}
16:0	13.58 ± 2.18 ^{a}	15.12 ± 0.45 ^{a}	16.07 ± 2.54 ^{a}	16.35 ± 1.88 ^{a}	18.30 ± 2.81 ^{a}
16:1n-9	1.73 ± 0.21 ^{b}	1.52 ± 0.13 ^{bcd}	1.64 ± 0.08 ^{bc}	1.35 ± 0.22 ^{bcde}	1.27 ± 0.07 ^{bcde}
16:1n-7	2.86 ± 0.09 ^{bc}	2.62 ± 0.03 ^{bcd}	3.35 ± 0.34 ^{b}	3.25 ± 0.18 ^{b}	2.22 ± 0.29 ^{cd}
18:0	4.49 ± 0.77 ^{bcde}	4.85 ± 0.49 ^{abcde}	4.79 ± 0.33 ^{abcde}	3.81 ± 0.16 ^{cde}	5.05 ± 0.36 ^{abcd}
18:1n-9	25.42 ± 0.16 ^{a}	22.25 ± 1.75 ^{ab}	21.65 ± 1.15 ^{ab}	20.87 ± 0.58 ^{bc}	19.24 ± 3.00 ^{bcd}
18:1n-7	4.32 ± 0.47 ^{b}	4.31 ± 0.35 ^{b}	4.05 ± 0.28 ^{b}	4.00 ± 0.18 ^{b}	3.13 ± 0.15 ^{c}
18:2n-6	0.94 ± 0.08 ^{g}	2.25 ± 0.01 ^{de}	3.12 ± 0.09 ^{c}	0.25 ± 0.03 h	1.69 ± 0.04 ^{f}
18:3n-6	0.12 ± 0.00 ^{a}	0.24 ± 0.00 ^{a}	0.52 ± 0.01 ^{a}	0.02 ± 0.00 ^{a}	0.01 ± 0.01 ^{a}
18:3n-3	0.47 ± 0.10 ^{fgh}	0.46 ± 0.01 ^{fgh}	0.01 ± 0.01 ^{h}	0.94 ± 0.00 ^{efg}	1.20 ± 0.03 ^{def}
18:4n-3	0.43 ± 0.05 ^{b}	0.37 ± 0.08 ^{b}	0.01 ± 0.01 ^{b}	0.65 ± 0.04 ^{b}	0.53 ± 0.09 ^{b}
20:1n-9	4.70 ± 0.34 ^{a}	3.95 ± 0.27 ^{ab}	3.47 ± 0.65 ^{bc}	2.91 ± 0.14 ^{bcd}	2.70 ± 0.52 ^{cde}
20:2n-9	1.56 ± 0.16 ^{a}	1.11 ± 0.14 ^{b}	1.09 ± 0.19 ^{b}	0.90 ± 0.04 ^{bc}	0.62 ± 0.17 ^{cd}
20:2n-6	0.37 ± 0.04 ^{e}	0.65 ± 0.15 ^{bcd}	0.52 ± 0.01 ^{cde}	0.00 ± 0.00	0.45 ± 0.05 ^{de}
20:3n-9	4.75 ± 1.25 ^{b}	3.15 ± 0.25 ^{bc}	3.36 ± 0.25 ^{bc}	2.64 ± 0.29 ^{bc}	1.58 ± 0.16 ^{cd}
20:3n-6	1.08 ± 0.07 ^{cde}	2.26 ± 0.07 ^{b}	3.43 ± 0.17 ^{a}	0.21 ± 0.01 ^{e}	1.28 ± 0.11 ^{bcde}
20:4n-6	2.61 ± 0.27 ^{f}	5.77 ± 0.43 ^{c}	10.22 ± 0.25 ^{a}	0.59 ± 0.11 ^{h}	3.31 ± 0.13 ^{ef}
20:3n-3	0.09 ± 0.00 ^{efg}	0.09 ± 0.00 ^{efg}	0.01 ± 0.01 ^{g}	0.23 ± 0.02 ^{de}	0.27 ± 0.01 ^{d}
20:4n-3	0.38 ± 0.13 ^{def}	0.31 ± 0.04 ^{ef}	0.01 ± 0.01 ^{g}	0.61 ± 0.10 ^{cd}	0.64 ± 0.12 ^{cd}
20:5n-3	3.07 ± 0.10 ^{ef}	2.26 ± 0.31 ^{f}	0.13 ± 0.02 ^{g}	5.66 ± 0.04 ^{ab}	3.78 ± 0.34 ^{cde}
22:1n-9	0.23 ± 0.01 ^{a}	0.18 ± 0.01 ^{ab}	0.19 ± 0.05 ^{ab}	0.14 ± 0.02 ^{bcd}	0.15 ± 0.01 ^{bc}
22:4n-6	0.17 ± 0.01 ^{cd}	0.40 ± 0.07 ^{b}	1.16 ± 0.10 ^{a}	0.03 ± 0.01 ^{d}	0.15 ± 0.01 ^{cd}
22:5n-6	0.36 ± 0.22 ^{def}	1.82 ± 0.19 ^{b}	10.33 ± 0.07 ^{a}	0.07 ± 0.01 ^{f}	0.45 ± 0.01 ^{cde}
22:5n-3	1.42 ± 0.11 ^{abc}	1.20 ± 0.09 ^{abc}	0.11 ± 0.00 ^{d}	2.06 ± 0.34 ^{a}	1.68 ± 0.09 ^{ab}
22:6n-3	13.72 ± 0.80 ^{e}	12.44 ± 0.83 ^{e}	1.28 ± 0.01 ^{g}	22.37 ± 2.84 ^{cd}	20.75 ± 0.65 ^{d}
24:1n-9	0.88 ± 0.02 ^{a}	0.89 ± 0.03 ^{a}	0.90 ± 0.06 ^{a}	1.06 ± 0.09 ^{a}	0.91 ± 0.16 ^{a}
20:3n-9/22:6n-3	0.34 ± 0.07 ^{c}	0.25 ± 0.00 ^{cd}	2.63 ± 0.22 ^{a}	0.12 ± 0.00 ^{cd}	0.08 ± 0.01 ^{cd}
Σ[3]Saturate	20.57 ± 3.11	22.65 ± 0.34	23.20 ± 2.97	22.95 ± 1.94	26.02 ± 3.42
Σ Monoene	42.00 ± 0.56 ^{ab}	37.59 ± 2.19 ^{bc}	36.84 ± 1.95 ^{c}	34.88 ± 0.36 ^{cd}	31.01 ± 3.72 ^{de}
Σ PUFA	32.02 ± 2.38 ^{d}	35.31 ± 1.90 ^{d}	35.89 ± 0.61 ^{d}	37.61 ± 2.72 ^{d}	38.58 ± 1.63 ^{cd}
Σ n-9	6.81 ± 1.47 ^{b}	4.77 ± 0.15 ^{bc}	5.00 ± 0.45 ^{bc}	3.86 ± 0.24 ^{cd}	2.45 ± 0.33 ^{de}
Σ n-6	5.65 ± 0.39 ^{fg}	13.40 ± 0.47 ^{c}	29.30 ± 0.14 ^{a}	1.23 ± 0.05 ^{h}	7.32 ± 0.00 ^{def}
Σ n-3	19.57 ± 0.53 ^{f}	17.14 ± 1.27 ^{f}	1.60 ± 0.01 ^{h}	32.52 ± 2.43 ^{de}	28.81 ± 1.30 ^{e}
Σ n-6 / Σ n-3	0.29 ± 0.01 ^{e}	0.78 ± 0.03 ^{b}	18.37 ± 0.08 ^{a}	0.04 ± 0.00 ^{g}	0.26 ± 0.02 ^{ef}

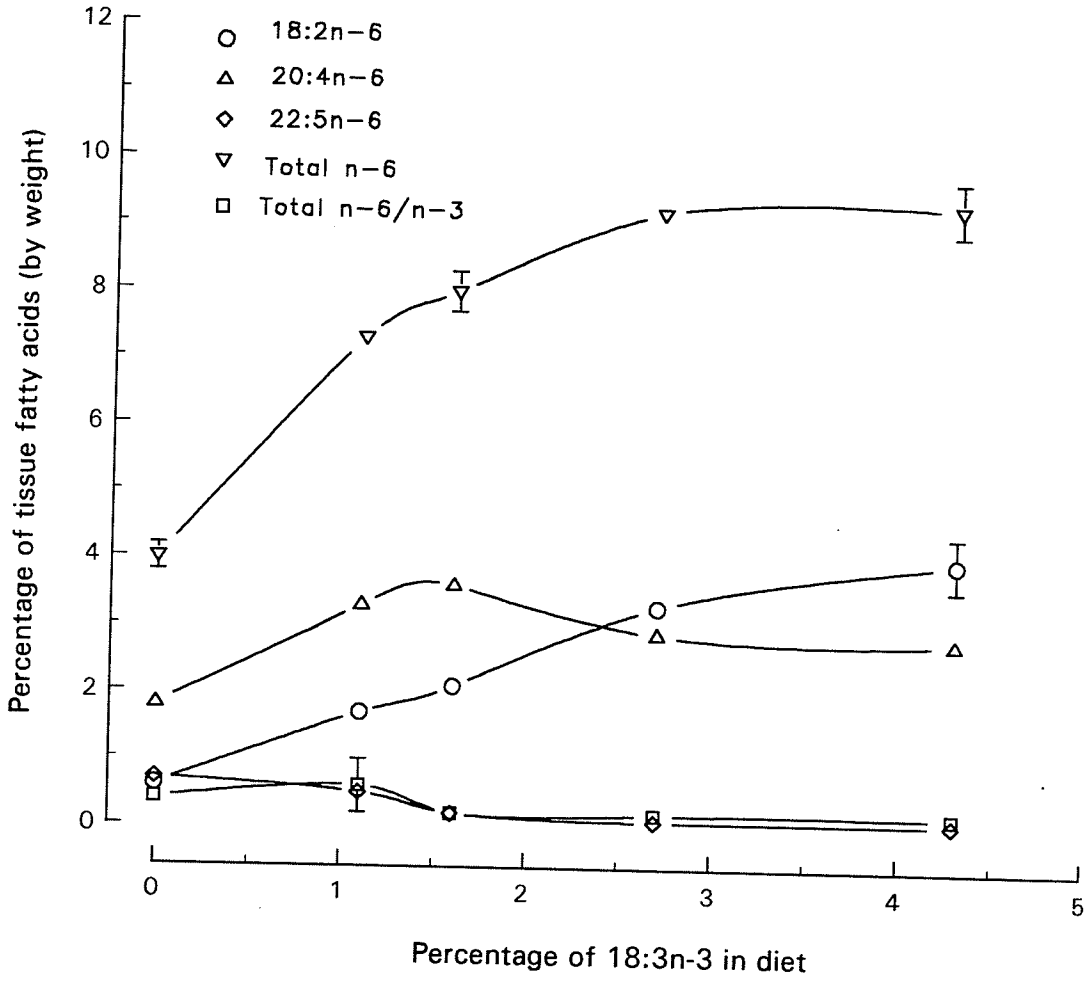
... Continued

[1]Livers from 5-10 fish were pooled in each replicate [2]Same letters in a row indicating no significant difference between treatments [3]Total [4]Single replicate was analyzed

Table 20. (continued)

F	G	H	I[4]	J	K	L
1.47 ± 0.33 abc	0.92 ± 0.08 cd	0.81 ± 0.07 cd	0.9 cd	0.53 ± 0.00 d	2.09 ± 0.33 ab	1.37 ± 0.14 bc
16.88 ± 1.33 a	16.99 ± 2.38 a	16.08 ± 1.23 a	18.74 a	15.10 ± 1.06 a	14.41 ± 1.24 a	17.64 ± 0.11 a
1.26 ± 0.09 bcde	1.04 ± 0.04 cde	1.58 ± 0.05 bcd	0.93 def	0.82 ± 0.00 ef	2.83 ± 0.52 a	0.35 ± 0.03 f
2.13 ± 0.39 cd	1.33 ± 0.19 ef	1.27 ± 0.12 ef	1.05 f	0.69 ± 0.02 f	5.45 ± 0.34 a	1.86 ± 0.19 de
4.79 ± 0.29 abcde	6.46 ± 1.03 a	4.26 ± 0.21 bcde	5.97 ab	5.62 ± 0.81 abc	3.09 ± 0.23 e	3.56 ± 0.26 de
16.68 ± 1.02 cde	14.41 ± 1.46 e	21.43 ± 0.48 ab	14.54 e	15.43 ± 0.46 de	22.87 ± 0.23 ab	9.76 ± 0.17 f
2.67 ± 0.24 c	1.84 ± 0.07 d	1.82 ± 0.04 d	1.77 d	1.10 ± 0.07 d	5.64 ± 0.19 a	3.01 ± 0.05 c
2.13 ± 0.03 def	5.26 ± 0.11 a	1.83 ± 0.04 ef	3.33 c	4.02 ± 0.37 b	0.56 ± 0.03 gh	2.50 ± 0.27 d
0.13 ± 0.01 a	2.65 ± 2.36 a	0.17 ± 0.01 a	0.12 a	0.20 ± 0.07 a	0.24 ± 0.10 a	0.17 ± 0.01 a
2.05 ± 0.16 c	1.87 ± 0.03 cd	1.44 ± 0.10 cde	3.06 b	4.91 ± 0.66 a	0.04 ± 0.02 h	0.16 ± 0.02 gh
0.70 ± 0.01 b	0.98 ± 0.27 ab	1.09 ± 0.17 ab	0.75 b	1.97 ± 0.85 a	0.06 ± 0.00 b	0.12 ± 0.03 b
1.82 ± 0.00 efg	1.08 ± 0.24 fg	2.10 ± 0.07 def	1.17 fg	0.78 ± 0.04 g	3.58 ± 0.17 bc	2.36 ± 0.18 de
0.29 ± 0.01 de	0.20 ± 0.05 e	0.33 ± 0.01 de	0.11 e	0.07 ± 0.01 e	1.81 ± 0.07 a	0.03 ± 0.03 e
0.56 ± 0.01 cde	1.07 ± 0.07 a	0.35 ± 0.03 e	0.8 b	0.71 ± 0.03 bc	0.04 ± 0.01 f	0.50 ± 0.02 de
0.44 ± 0.04 d	0.29 ± 0.02 d	0.51 ± 0.02 d	0.11 d	0.00 ± 0.00	8.13 ± 1.23 a	0.00 ± 0.00
1.17 ± 0.15 bcde	1.20 ± 0.96 bcde	1.37 ± 0.11 bcd	1.84 bc	1.48 ± 0.02 bcd	0.46 ± 0.05 de	0.56 ± 0.08 de
3.63 ± 0.08 e	7.18 ± 0.41 b	4.38 ± 0.04 d	2.92 ef	2.82 ± 0.07 f	1.82 ± 0.11 g	2.69 ± 0.09 f
0.50 ± 0.12 c	0.32 ± 0.03 d	0.19 ± 0.04 def	0.66 b	0.83 ± 0.00 a	0.10 ± 0.02 efg	0.06 ± 0.00 fg
0.74 ± 0.07 c	0.55 ± 0.04 cde	0.74 ± 0.04 c	1.47 b	1.94 ± 0.04 a	0.03 ± 0.01 g	0.28 ± 0.05 f
4.45 ± 0.23 c	3.21 ± 0.31 def	4.86 ± 0.24 bc	4.33 cd	6.21 ± 0.45 a	0.83 ± 0.01 g	6.74 ± 0.81 a
0.10 ± 0.02 cde	0.06 ± 0.02 e	0.09 ± 0.00 cde	0.07 de	0.05 ± 0.01 e	0.22 ± 0.02 a	0.14 0.03 bcd
0.09 ± 0.02 d	0.27 ± 0.02 bc	0.13 ± 0.02 cd	0.06 d	0.04 ± 0.01 d	0.18 ± 0.01 cd	0.10 ± 0.09 d
0.21 ± 0.08 ef	0.78 ± 0.10 c	0.33 ± 0.01 def	0.14 ef	0.07 ± 0.02 f	0.67 ± 0.08 cd	0.22 ± 0.01 ef
1.44 ± 0.32 abc	1.56 ± 0.02 abc	1.92 ± 0.26 a	1.77 ab	0.82 ± 0.81 bcd	0.58 ± 0.02 cd	1.09 ± 0.09 abcd
26.97 ± 0.97 b	24.90 ± 1.40 bc	25.55 ± 1.79 bc	27.64 b	28.64 ± 0.15 b	7.98 ± 0.01 f	35.72 ± 0.31 a
0.94 ± 0.11 a	0.80 ± 0.26 a	1.10 ± 0.23 a	1.11 a	0.99 ± 0.23 a	1.05 ± 0.12 a	0.16 ± 0.02 b
0.02 ± 0.00 d	0.01 ± 0.00 d	0.02 ± 0.00 d	0.00 d	0.00 ± 0.00 d	1.02 ± 0.16 b	0.00 ± 0.00 d
23.66 ± 1.32	24.78 ± 3.38	21.48 ± 1.15	25.98	21.58 ± 1.93	20.27 ± 1.03	23.13 ± 0.35
26.39 ± 1.44 e	21.15 ± 1.52 f	30.00 ± 0.01 de	21.40 f	20.19 ± 0.01 f	45.68 ± 0.26 a	19.96 ± 0.08 f
45.75 ± 0.38 bc	52.45 ± 3.88 ab	45.43 ± 1.56 bc	49.27 ab	54.82 ± 3.33 a	24.90 ± 1.40 e	51.27 ± 1.07 ab
0.95 ± 0.05 ef	0.61 ± 0.04 ef	1.01 ± 0.04 ef	0.32 ef	0.17 ± 0.01 f	11.18 ± 1.25 a	0.16 ± 0.05 f
7.96 ± 0.32 de	18.44 ± 1.89 b	8.63 ± 0.03 de	9.23 d	9.35 ± 0.45 d	4.04 ± 0.20 g	6.85 ± 0.40 ef
36.85 ± 0.75 cd	33.40 ± 1.94 de	35.80 ± 1.64 cd	39.72 bc	45.31 ± 2.89 a	9.68 ± 0.05 g	44.27 ± 0.72 ab
0.22 ± 0.01 ef	0.55 ± 0.02 c	0.24 ± 0.01 ef	0.23 ef	0.21 ± 0.00 ef	0.42 ± 0.02 d	0.15 ± 0.01 f

Fig. 17. The n-6 PUFA composition of liver polar lipids of Arctic charr fed diets containing varying levels of 18:3n-3


18:2n−6
20:4n−6
22:5n−6
Total n−6
Total n−6/n−3
Percentage of tissue fatty acids (by weight)
0
2
4
6
8
10
12
0
1
2
3
4
5
Percentage of 18:3n-3 in diet

increase in 20:4n-6.

The fatty acid compositions of liver polar lipids are shown for charr from Expt. II in Table 21 and Fig. 18. As PUFAs increased in the diets, the percentages of total tissue n-3 and n-6 PUFA showed a similar change to that observed in Expt. I. When dietary 18:2n-6 was increased from 0.6 to 2.6% and 18:3n-3 was maintained constant at 1.7% (diets E-G), there was a significant increase in percentages of C20 n-6 PUFAs in livers while those for 20:5n-3 and 22:6n-3 decreased. When approximately equal amounts of 18:2n-6 and 18:3n-3 were included in diets B and F, the n-3 long-chain PUFAs in the liver were also more abundant than n-6 long-chain PUFAs. However, an increase in percentages of both dietary 18:2n-6 and 18:3n-3 produced a decrease in the ratio of total n-6 PUFA to total n-3 PUFA.

Fatty acid compositions of muscle polar and total lipids

Fatty acid composition of muscle polar lipids are shown for charr given selected treatments in experiments I and II (Tables 22 and 23). Muscle polar lipids, compared to liver polar lipids, contained higher percentages of 18:3n-3 and 18:2n-6 in both experiments and they showed less fluctuation in the percentages of long-chain n-3 or n-6 PUFAs among treatments. As dietary 18:2n-6 increased from 0.5 to 1.5% and 18:3n-3 remained constant at 1.6% (Expt. I, diets F, G), levels of tissue 18:2n-6 and 20:4n-6 increased by more than 200%. By contrast, percentages of n-3 PUFAs changed little and

Table 21. Fatty acid composition of liver polar lipids of Arctic charr from Experiment II (% by weight) (Mean ± SEM, $n^1 = 2$)[2, 3]

	Diet							
	A	B	C	D	E	F	G	H
14:0	2.68 ± 0.18 [a]	2.67 ± 0.26 [a]	2.99 ± 0.49 [a]	3.08 ± 0.10 [a]	1.73 ± 0.09 [b]	1.35 ± 0.00 [b]	1.05 ± 0.13 [b]	1.21 ± 0.03 [b]
16:0	17.79 ± 0.21 [ab]	17.61 ± 0.61 [ab]	15.90 ± 0.18 [b]	19.26 ± 0.38 [a]	19.33 ± 1.43 [a]	18.68 ± 0.55 [ab]	19.08 ± 1.18 [a]	19.25 ± 0.95 [a]
16:1n-9	1.28 ± 0.12 [ab]	1.25 ± 0.13 [ab]	1.57 ± 0.16 [a]	1.11 ± 0.16 [bc]	0.94 ± 0.11 [bc]	0.91 ± 0.00 [bc]	0.81 ± 0.04 [c]	1.05 ± 0.12 [bc]
16:1n-7	4.47 ± 0.08 [a]	3.95 ± 0.11 [a]	3.55 ± 0.38 [ab]	2.34 ± 1.06 [bc]	1.71 ± 0.14 [cd]	1.24 ± 0.07 [cd]	0.72 ± 0.04 [d]	1.40 ± 0.03 [cd]
18:0	3.71 ± 0.16 [d]	4.08 ± 0.03 [cd]	4.12 ± 0.17 [cd]	3.89 ± 0.06 [d]	4.27 ± 0.00 [bcd]	4.85 ± 0.21 [b]	6.03 ± 0.22 [a]	4.64 ± 0.35 [bc]
18:1n-9	22.64 ± 0.22 [a]	19.93 ± 0.06 [b]	17.79 ± 1.72 [b]	18.35 ± 0.38 [b]	15.20 ± 1.13 [c]	12.04 ± 0.21 [d]	9.35 ± 0.01 [e]	13.55 ± 0.25 [cd]
18:1n-7	3.69 ± 0.18 [ab]	3.72 ± 0.39 [ab]	3.98 ± 0.02 [a]	3.33 ± 0.29 [b]	2.12 ± 0.04 [c]	1.58 ± 0.06 [cd]	0.99 ± 0.03 [d]	1.70 ± 0.06 [c]
18:2n-6	1.02 ± 0.02 [e]	2.27 ± 0.03 [d]	2.86 ± 0.10 [c]	0.56 ± 0.03 [f]	2.28 ± 0.09 [d]	4.98 ± 0.01 [b]	7.07 ± 0.28 [a]	2.93 ± 0.04 [c]
18:3n-6	0.14 ± 0.01 [cd]	0.33 ± 0.02 [bc]	0.50 ± 0.02 [b]	0.03 ± 0.02 [d]	0.12 ± 0.01 [cd]	0.38 ± 0.00 [bc]	1.01 ± 0.23 [a]	0.18 ± 0.02 [cd]
18:3n-3	0.67 ± 0.06 [d]	0.37 ± 0.05 [d]	0.02 ± 0.01 [e]	1.09 ± 0.15 [c]	1.57 ± 0.17 [b]	1.38 ± 0.09 [bc]	1.41 ± 0.01 [bc]	2.35 ± 0.13 [a]
18:4n-3	0.64 ± 0.12 [bc]	0.38 ± 0.09 [c]	0.02 ± 0.00 [d]	0.67 ± 0.14 [bc]	0.55 ± 0.11 [bc]	0.64 ± 0.06 [bc]	1.25 ± 0.12 [a]	0.83 ± 0.03 [b]
20:1n-9	3.44 ± 0.19 [a]	3.21 ± 0.35 [ab]	3.46 ± 0.23 [a]	2.62 ± 0.39 [bc]	2.01 ± 0.30 [c]	1.14 ± 0.03 [d]	0.59 ± 0.04 [d]	1.17 ± 0.00 [d]
20:2n-9	1.37 ± 0.07 [a]	1.11 ± 0.02 [b]	0.95 ± 0.14 [bc]	0.81 ± 0.05 [c]	0.33 ± 0.04 [d]	0.15 ± 0.01 [de]	0.11 ± 0.01 [e]	0.18 ± 0.02 [de]
20:2n-6	0.24 ± 0.03 [d]	0.70 ± 0.00 [c]	1.08 ± 0.08 [b]	0.17 ± 0.04 [d]	0.73 ± 0.00 [c]	1.43 ± 0.06 [a]	1.52 ± 0.02 [a]	0.78 ± 0.02 [c]
20:3n-9	2.27 ± 0.05 [ab]	2.31 ± 0.01 [a]	2.08 ± 0.09 [b]	1.40 ± 0.05 [c]	0.33 ± 0.09 [d]	0.00 ± 0.00 [e]	0.00 ± 0.00 [e]	0.14 ± 0.04 [de]
20:3n-6	1.48 ± 0.12 [d]	3.61 ± 0.05 [b]	5.08 ± 0.44 [a]	0.86 ± 0.04 [e]	2.46 ± 0.01 [c]	3.93 ± 0.10 [b]	4.53 ± 0.17 [a]	2.37 ± 0.14 [c]
20:4n-6	1.40 ± 0.05 [f]	4.88 ± 0.17 [d]	10.16 ± 0.77 [a]	0.79 ± 0.07 [f]	2.84 ± 0.05 [e]	6.32 ± 0.30 [c]	8.33 ± 0.24 [b]	2.72 ± 0.02 [e]
20:4n-3	0.55 ± 0.00 [c]	0.31 ± 0.02 [d]	0.01 ± 0.01 [e]	0.98 ± 0.11 [b]	1.08 ± 0.12 [b]	0.72 ± 0.04 [c]	0.66 ± 0.05 [c]	1.32 ± 0.04 [a]
20:5n-3	3.64 ± 0.03 [bc]	2.16 ± 0.04 [e]	0.13 ± 0.00 [f]	4.91 ± 0.04 [a]	3.89 ± 0.24 [b]	2.99 ± 0.19 [cd]	2.34 ± 0.07 [de]	4.34 ± 0.60 [ab]
22:4n-6	0.12 ± 0.00 [d]	0.49 ± 0.02 [b]	1.45 ± 0.03 [a]	0.05 ± 0.01 [e]	0.14 ± 0.02 [d]	0.30 ± 0.00 [c]	0.46 ± 0.04 [b]	0.08 ± 0.02 [de]
22:5n-6	0.27 ± 0.02 [c]	1.71 ± 0.07 [b]	12.25 ± 0.05 [a]	0.11 ± 0.00 [cd]	0.24 ± 0.10 [cd]	0.07 ± 0.01 [d]	1.82 ± 0.06 [b]	0.18 ± 0.03 [cd]
22:5n-3	2.15 ± 0.00 [ab]	1.56 ± 0.09 [c]	0.14 ± 0.04 [d]	2.40 ± 0.29 [a]	2.05 ± 0.22 [abc]	1.82 ± 0.09 [bc]	1.85 ± 0.14 [bc]	1.72 ± 0.09 [bc]
22:6n-3	15.21 ± 0.73 [e]	14.02 ± 0.21 [e]	1.87 ± 0.00 [f]	21.87 ± 1.47 [d]	27.72 ± 0.33 [ab]	26.75 ± 0.72 [bc]	24.63 ± 0.66 [c]	29.91 ± 0.40 [a]
24:1n-9	0.81 ± 0.19 [a]	0.66 ± 0.03 [a]	0.65 ± 0.15 [a]	0.84 ± 0.11 [a]	0.08 ± 0.03 [b]	0.11 ± 0.00 [b]	0.08 ± 0.02 [b]	0.76 ± 0.21 [a]
20:3n-9/22:6n-3	0.15 ± 0.00 [b]	0.16 ± 0.00 [b]	1.12 ± 0.05 [a]	0.06 ± 0.01 [c]	0.01 ± 0.00 [d]	0.00 ± 0.00 [d]	0.00 ± 0.00 [d]	0.00 ± 0.00 [d]
Σ [3]Saturate	25.35 ± 1.20	25.05 ± 0.55	23.59 ± 0.40	26.99 ± 0.51	25.83 ± 1.42	25.23 ± 0.33	26.48 ± 1.51	25.58 ± 1.41
Σ Monoene	38.11 ± 0.83 [a]	34.24 ± 0.75 [b]	33.01 ± 1.77 [b]	29.87 ± 0.55 [c]	22.86 ± 1.50 [d]	17.65 ± 0.07 [e]	12.98 ± 0.01 [f]	20.47 ± 0.08 [de]
Σ PUFA	31.71 ± 0.89 [e]	36.69 ± 0.09 [d]	39.10 ± 0.16 [d]	37.25 ± 2.19 [d]	46.97 ± 0.32 [c]	52.30 ± 0.34 [b]	57.31 ± 1.22 [a]	50.81 ± 0.85 [b]
Σ n-9	4.06 ± 0.13 [a]	3.84 ± 0.01 [ab]	3.51 ± 0.20 [b]	2.47 ± 0.12 [c]	0.83 ± 0.14 [d]	0.26 ± 0.01 [e]	0.23 ± 0.02 [e]	0.46 ± 0.05 [e]
Σ n-6	4.65 ± 0.20 [f]	13.97 ± 0.07 [d]	33.37 ± 0.33 [a]	2.56 ± 0.09 [g]	8.80 ± 0.07 [e]	17.40 ± 0.13 [c]	24.73 ± 0.56 [b]	9.24 ± 0.13 [e]
Σ n-3	23.00 ± 0.57 [d]	18.89 ± 0.01 [e]	2.23 ± 0.03 [f]	32.23 ± 2.22 [c]	37.34 ± 0.53 [b]	34.64 ± 0.23 [bc]	32.36 ± 0.64 [c]	41.12 ± 1.03 [a]
Σ n-6 / Σ n-3	0.20 ± 0.00 [d]	0.74 ± 0.00 [b]	14.96 ± 0.06 [a]	0.08 ± 0.00 [e]	0.24 ± 0.01 [d]	0.50 ± 0.00 [c]	0.76 ± 0.00 [b]	0.22 ± 0.01 [d]

[1, 2, 3]Same as in Table 20

Fig. 18. The n-3 PUFA composition of liver polar lipids of Arctic charr fed diets containing varying levels of 18:2n-6

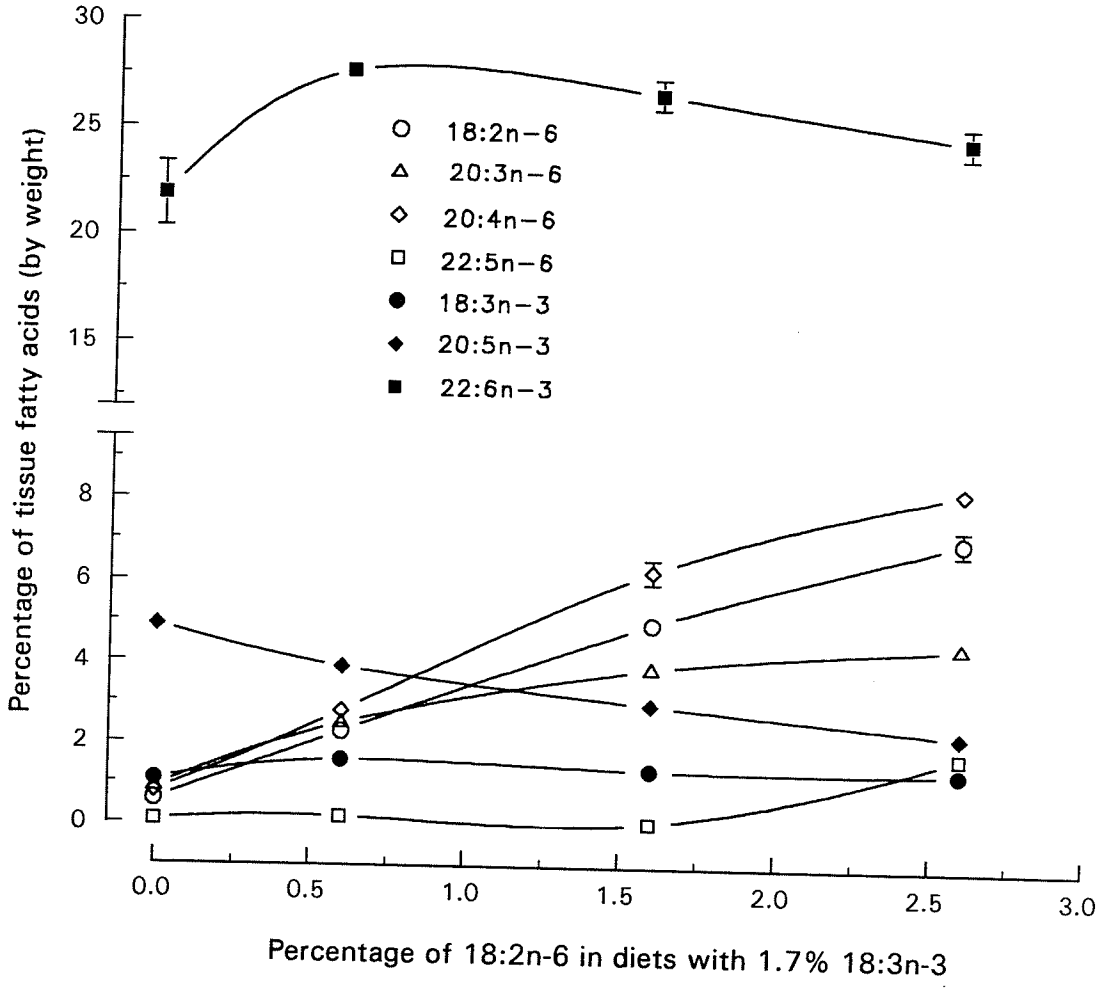

30
25
20
15
8
6
4
2
0
18:2n−6
20:3n−6
20:4n−6
22:5n−6
18:3n−3
20:5n−3
22:6n−3
Percentage of tissue fatty acids (by weight)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Percentage of 18:2n-6 in diets with 1.7% 18:3n-3

Table 22. Fatty acid composition of muscle polar lipids of Arctic charr given selected treatments in Exp. I (% by weight) (Mean ± SEM, n[1] = 2)[2]

	Diet					
	C	D	F	G	J[4]	K
18:2n-6	6.18 ± 0.10 [b]	0.62 ± 0.04 [e]	4.49 ± 0.04 [c]	9.18 ± 0.42 [a]	5.59 [b]	1.68 ± 0.13 [d]
18:3n-6	1.27 ± 0.05 [a]	0.09 ± 0.03 [c]	0.37 ± 0.03 [b]	1.31 ± 0.05 [a]	0.44 [b]	0.13 ± 0.02 [c]
18:3n-3	0.04 ± 0.03 [e]	3.14 ± 0.03 [d]	6.72 ± 0.08 [b]	5.58 ± 0.25 [c]	12.43 [a]	0.21 ± 0.02 [e]
18:4n-3	0.12 ± 0.01 [e]	2.11 ± 0.01 [d]	2.84 ± 0.23 [c]	3.89 ± 0.21 [b]	5.27 [a]	0.29 ± 0.02 [e]
20:3n-6	2.65 ± 0.25 [a]	0.17 ± 0.01 [c]	1.07 ± 0.07 [b]	2.46 ± 0.02 [a]	0.95 [b]	0.31 ± 0.03 [c]
20:4n-6	5.89 ± 0.25 [a]	0.38 ± 0.00 [d]	1.23 ± 0.05 [c]	3.00 ± 0.06 [b]	0.91 [c]	1.04 ± 0.06 [c]
20:4n-3	0.11 ± 0.00 [d]	1.26 ± 0.04 [c]	1.87 ± 0.08 [b]	1.64 ± 0.12 [b]	2.41 [a]	0.29 ± 0.02 [d]
20:5n-3	1.96 ± 0.07 [d]	5.40 ± 0.03 [bc]	4.96 ± 0.45 [bc]	4.80 ± 0.01 [c]	7.58 [a]	5.73 ± 0.01 [b]
22:4n-6	0.79 ± 0.02 [a]	0.04 ± 0.01 [e]	0.11 ± 0.01 [c]	0.26 ± 0.02 [b]	0.05 [d]	0.10 ± 0.00 [cd]
22:5n-6	7.44 ± 0.33 [a]	0.26 ± 0.01 [b]	0.25 ± 0.01 [b]	0.62 ± 0.01 [b]	0.11 [b]	0.29 ± 0.04 [b]
22:5n-3	0.45 ± 0.03 [c]	1.98 ± 0.04 [a]	1.75 ± 0.13 [a]	1.76 ± 0.16 [a]	1.77 [a]	1.24 ± 0.05 [b]
22:6n-3	6.60 ± 0.39 [b]	16.53 ± 0.54 [a]	18.04 ± 2.06 [a]	16.13 ± 0.18 [a]	18.41 [a]	18.23 ± 0.96 [a]
Σ[3] n-6	26.81 ± 0.84 [a]	1.59 ± 0.03 [e]	8.30 ± 0.01 [c]	18.08 ± 0.49 [b]	8.62 [c]	3.78 ± 0.10 [d]
Σ n-3	9.41 ± 0.52 [e]	31.00 ± 0.61 [cd]	37.33 ± 2.62 [b]	34.43 ± 0.28 [bc]	49.00 [a]	26.22 ± 0.95 [d]
Σ n-6 / Σ n-3	2.85 ± 0.07 [a]	0.05 ± 0.00 [d]	0.22 ± 0.02 [c]	0.53 ± 0.01 [b]	0.18 [cd]	0.15 ± 0.01 [cd]

[1]Muscle samples from 5-10 fish of each replicate were pooled for the analysis [2, 3]Same as in Table 21

[4]Single replicate was analyzed

Table 23. Fatty acid composition of muscle polar lipids of charr given selected treatments in Exp. II (% by weight) (Mean ± SEM, $n^1 = 2$)[2]

	Diet			
	A[4]	D	E	F
18:2n-6	1.81 [c]	1.25 ± 0.01 [c]	4.74 ± 0.29 [b]	9.19 ± 0.10 [a]
18:3n-6	0.22 [b]	0.11 ± 0.01 [b]	0.38 ± 0.03 [b]	1.20 ± 0.09 [a]
18:3n-3	2.62 [c]	3.99 ± 0.21 [bc]	6.90 ± 0.87 [a]	5.48 ± 0.03 [ab]
18:4n-3	2.02	2.80 ± 0.17	3.11 ± 0.12	3.67 ± 0.56
20:3n-6	0.78 [c]	0.39 ± 0.01 [c]	1.45 ± 0.08 [b]	3.30 ± 0.21 [a]
20:4n-6	0.72 [bc]	0.38 ± 0.01 [c]	0.92 ± 0.02 [b]	2.03 ± 0.14 [a]
20:4n-3	1.57 [b]	2.11 ± 0.13 [ab]	2.31 ± 0.11 [a]	2.02 ± 0.16 [ab]
20:5n-3	4.55	5.43 ± 0.14	4.85 ± 0.19	4.33 ± 0.45
22:4n-6	0.09 [b]	0.04 ± 0.01 [c]	0.08 ± 0.00 [b]	0.20 ± 0.01 [a]
22:5n-6	0.22 [b]	0.11 ± 0.01 [d]	0.19 ± 0.01 [c]	0.52 ± 0.00 [a]
22:5n-3	1.87 [ab]	2.23 ± 0.03 [a]	1.74 ± 0.09 [b]	1.72 ± 0.12 [b]
22:6n-3	17.35 [b]	20.58 ± 0.25 [a]	21.17 ± 0.73 [a]	18.64 ± 0.48 [ab]
Σ[3]n-6	4.06 [c]	2.44 ± 0.02 [d]	8.56 ± 0.26 [b]	17.99 ± 0.31 [a]
Σ n-3	30.49 [b]	38.00 ± 0.45 [a]	41.38 ± 1.61 [a]	36.70 ± 1.71 [ab]
Σ n-6 / Σ n-3	0.13 [c]	0.06 ± 0.00 [d]	0.21 ± 0.00 [b]	0.49 ± 0.01 [a]

[1, 2, 3, 4]Same as in Table 22

they were similar to those found for liver polar lipids (Table 22). There were also significant increases in 18:3n-3 and 20:5n-3 but no change in n-6 PUFAs and 22:6n-3 as 18:2n-6, 18:3n-3 and total lipids increased in diets F and J. Moreover, charr fed the reference diet had similar levels of n-3 PUFAs to those fed diets containing ≥ 1.6% 18:3n-3. As dietary 18:2n-6 increased from 0.6 to 1.6% in the presence of 1.7% 18:3n-3 (Expt. II, diets E, F), there were significant increases in tissue 18:2n-6 and 20:4n-6 but little change in n-3 PUFAs (Table 23).

The PUFA composition of fish total muscle lipids are presented for selected treatments from experiments I and II in Tables 24 and 25. The levels of tissue 18:2n-6 and 18:3n-3 were high in charr fed either low or high levels of dietary 18:2n-6 or 18:3n-3. As dietary percentages of 18:2n-6 or 18:3n-3 were increased, their levels in tissues showed proportional increases, but there were either relatively small or no changes in both n-3 and n-6 long-chain PUFAs. When the ratio of 18:2n-6 to 18:3n-3 was constant at 0.3, increasing dietary 18:3n-3 from 0.6 to 1.1% (Expt. I, diets A, E) resulted in slight increases in 20:5n-3 and 22:6n-3. A further increase of 18:3n-3 to 4.3% (diets E, F, I, J) produced only a marginal increase in 20:5n-3 and a decline in 20:4n-6 and 22:5n-6 (Table 24). An increase in 18:2n-6 from 0.5 to 2.6% with 18:3n-3 constant at 1.7% (Expt. II, diets E-G) led to a significant decrease in tissue 22:6n-3 and a larger increase

Table 24. Fatty acid composition of muscle total lipids of charr given selected treatments in Exp. I (% by weight) (Mean ± SEM, $n^1 = 2$)[2]

	Diet				
	A	E	F	I[4]	J
18:2n-6	2.02 ± 0.04 [d]	3.45 ± 0.17 [c]	5.58 ± 0.02 [b]	8.75 [a]	9.12 ± 0.09 [a]
18:3n-6	0.18 ± 0.00 [c]	0.26 ± 0.03 [c]	0.37 ± 0.02 [b]	0.69 [a]	0.69 ± 0.01 [a]
18:3n-3	2.18 ± 0.04 [e]	5.23 ± 0.13 [d]	9.98 ± 0.18 [c]	16.02 [b]	18.59 ± 0.60 [a]
18:4n-3	1.08 ± 0.03 [c]	1.91 ± 0.13 [bc]	3.12 ± 0.36 [b]	6.86 [a]	7.36 ± 0.56 [a]
20:3n-6	0.37 ± 0.02 [d]	0.49 ± 0.02 [cd]	0.61 ± 0.02 [bc]	0.76 [a]	0.67 ± 0.06 [ab]
20:4n-6	0.57 ± 0.01	0.62 ± 0.09	0.60 ± 0.08	0.45	0.42 ± 0.02
20:4n-3	0.40 ± 0.02 [d]	0.76 ± 0.08 [c]	1.12 ± 0.03 [b]	1.76 [a]	1.62 ± 0.05 [a]
20:5n-3	2.09 ± 0.06 [c]	2.59 ± 0.36 [bc]	2.57 ± 0.15 [bc]	3.35 [ab]	3.58 ± 0.06 [a]
22:4n-6	0.07 ± 0.00	0.06 ± 0.01	0.06 ± 0.00	0.04	0.04 ± 0.02
22:5n-6	0.16 ± 0.01 [a]	0.14 ± 0.02 [a]	0.14 ± 0.01 [a]	0.06 [b]	0.07 ± 0.01 [b]
22:5n-3	0.73 ± 0.02 [b]	0.88 ± 0.06 [ab]	0.87 ± 0.05 [ab]	0.96 [a]	1.00 ± 0.00 [a]
22:6n-3	6.17 ± 0.05	7.70 ± 1.46	8.29 ± 0.67	8.46	8.72 ± 0.71
Σ^3n-6	3.63 ± 0.04 [d]	5.46 ± 0.42 [c]	7.97 ± 0.16 [b]	11.35 [a]	11.51 ± 0.24 [a]
Σ n-3	12.87 ± 0.08 [d]	19.58 ± 2.29 [c]	26.85 ± 1.06 [b]	38.30 [a]	41.78 ± 0.38 [a]
Σ n-6 / Σ n-3	0.28 ± 0.00	0.28 ± 0.01	0.30 ± 0.01	0.30	0.28 ± 0.01

[1, 2, 3, 4]Same as in Table 22

Table 25. Fatty acid composition of total muscle lipids of charr given selected treatments in Exp. II (% by weight) (Mean ± SEM, $n^1 = 2$)

	Diet				
	B	D	E[2]	F	G[2]
18:2n-6	4.50 ± 0.45 [c]	1.10 ± 0.03 [d]	6.25 [c]	13.59 ± 0.47 [b]	18.59 [a]
18:3n-6	0.02 ± 0.01 [c]	0.03 ± 0.00 [c]	0.38 [c]	1.66 ± 0.23 [b]	3.57 [a]
18:3n-3	1.70 ± 0.18 [e]	5.52 ± 0.07 [d]	10.35 [a]	8.20 ± 0.18 [b]	6.74 [c]
18:4n-3	1.17 ± 0.03 [c]	2.89 ± 0.13 [b]	2.89 [b]	4.48 ± 0.41 [a]	4.99 [a]
20:3n-6	1.27 ± 0.04 [c]	0.19 ± 0.01 [e]	0.89 [d]	2.26 ± 0.08 [b]	3.05 [a]
20:4n-6	0.94 ± 0.02 [c]	0.18 ± 0.01 [e]	0.51 [d]	1.08 ± 0.04 [b]	1.77 [a]
20:4n-3	0.44 ± 0.00 [c]	1.28 ± 0.07 [a]	1.47 [a]	1.37 ± 0.07 [a]	0.96 [b]
20:5n-3	1.30 ± 0.04 [b]	2.37 ± 0.19 [a]	2.44 [a]	2.24 ± 0.13 [a]	1.98 [ab]
22:4n-6	0.15 ± 0.01 [b]	0.02 ± 0.01 [d]	0.06 [c]	0.13 ± 0.01 [b]	0.21 [a]
22:5n-6	0.41 ± 0.02 [a]	0.08 ± 0.04 [c]	0.11 [c]	0.28 ± 0.00 [b]	0.48 [a]
22:5n-3	0.55 ± 0.02	0.95 ± 0.08	0.9	0.90 ± 0.10	0.84
22:6n-3	6.12 ± 0.13 [c]	7.90 ± 0.66 [bc]	11.26 [a]	9.14 ± 0.09 [b]	8.07 [bc]
Σ[3]n-6	8.06 ± 0.63 [c]	1.75 ± 0.00 [d]	9.02 [c]	20.54 ± 0.57 [b]	29.06 [a]
Σ n-6	11.47 ± 0.40 [d]	21.56 ± 1.22 [c]	30.51 [a]	26.92 ± 0.78 [ab]	23.94 [bc]
Σ n-6 / Σ n-3	0.70 ± 0.03 [b]	0.08 ± 0.00 [d]	0.30 [c]	0.76 ± 0.00 [b]	1.21 [a]

[1, 2, 3, 4]Same as in Table 22

in 20:3n-6 than 20:4n-6 (Table 25).

Fish fed diets containing equal amounts of 18:2n-6 and 18:3n-3 had less tissue n-6 long-chain PUFAs than n-3 long-chain PUFAs in total and polar muscle lipids. This was similar to the trend noted for liver polar lipids (Tables 20 and 21). Furthermore, in the presence of high levels of dietary 18:3n-3 and 18:2n-6, tissue 18:3n-4 markedly increased with increasing dietary 18:3n-3. By contrast, tissue levels of 18:2n-6 changed slightly with increasing dietary 18:2n-6.

Discussion

Growth, feed utilization and EFA requirement

Charr fed casein-based diets supplemented with 18:2n-6 and 18:3n-3 had significantly faster growth and better feed utilization than those fed the PUFA-free diet. This suggests that charr require dietary PUFAs and this findings is consistent with results from my previous studies (Chapters 3 and 4). Low levels of dietary PUFAs had an additive effect on growth of Arctic charr, similar to that reported for rainbow trout (Castell et al., 1972a). However, at 1.6-1.7% dietary 18:3n-3, an increase in dietary 18:2n-6 from 0 to 1.6% had no effect on growth and feed utilization of charr. A further increase to 2.6% had a slight negative effect. When the ratios of 18:2n-6 to 18:3n-3 were below 1.0 in the diets, growth rate and feed efficiency of charr increased as dietary 18:3n-3 increased from 0.6 to 1.6% but further increases had no effect. These data suggest that the requirements of charr for dietary 18:3n-3 is 1.0 to 1.6% for both Labrador and Nauyuk strains of Arctic charr. In addition to having similar EFA requirements, the two strains of Arctic charr used in this study displayed similar patterns of interactions between dietary 18:2n-6 and 18:3n-3 (see discussion below).

Parameters such as tissue water content, HSI and liver lipid composition, have been used widely to monitor the EFA status of fish (Sargent et al., 1989). Data from my study

demonstrated that fish fed diets low in dietary 18:2n-6 and/or 18:3n-3 (≤1.0%) had high muscle water content, HSI and levels of total and neutral lipids in the liver. These findings further suggest that the optimum requirement of Arctic charr for dietary 18:3n-3 is above 1.0%. Additional evidence supporting these observations was the presence of substantial amounts of 20:3n-9 in tissues of charr fed diets containing ≤1.0% PUFAs.

Conversion of 18:2n-6 and 18:3n-3

It has been well documented that the livers of freshwater fish efficiently convert 18:2n-6 and 18:3n-3 to long-chain PUFAs and these in turn are preferentially incorporated into tissue polar lipids (Henderson and Tocher, 1987). Results from my study indicate that increasing dietary 18:3n-3 from 0.5 to 1.7% led to a significant increase in 22:6n-3 in liver polar lipids. This corroborates the findings of previous studies which showed that Arctic charr can efficiently desaturate and elongate 18:2n-6 and 18:3n-3 to long-chain PUFAs (Chapters 4 and 5; Olsen et al., 1991; Olsen and Ringo, 1992). On the other hand, further increases of dietary 18:3n-3 from 1.6 to 4.3%, produced only slight changes in tissue long-chain n-3 PUFAs, suggesting that the conversion of 18:3n-3 to long-chain PUFAs approaches a maximum at about 1.7% dietary 18:3n-3 in the liver of Arctic charr. It was also evident that charr fed diets supplemented with 18:2n-6 only, had increased levels of tissue n-6 long-chain PUFAs in the liver. In addition, 18:2n-6

in both livers and muscle was primarily converted to 22:5n-6 and 20:4n-6 in the absence and presence of dietary 18:3n-3, respectively, while 18:3n-3 was primarily converted to 22:6n-3 despite the availability of dietary 18:2n-6.

Competitive inhibition

The effects of dietary 18:2n-6 and 18:3n-3 on each other's metabolism in fish differs between species (Yu and Sinnhuber, 1976 and 1979). Increasing dietary levels of 18:3n-3 were found to inhibit the conversion of n-6 PUFAs and vice versa in rainbow trout but varying levels of dietary 18:2n-6 appeared to have no influence on the conversion of n-3 PUFAs in coho salmon (Yu and Sinnhuber, 1976 and 1979). This study investigated competitive inhibition in Arctic charr fed diets with varying ratios of 18:2n-6 to 18:3n-3 (18:3n-3 was primarily at three different dietary levels i.e., 0.5-0.6%, 1.0-1.1% and 1.6-1.7%).

An increase in dietary levels of 18:3n-3 when 18:2n-6 was maintained at 0.5-0.7% resulted in a significant decrease in n-6 long-chain PUFAs of liver polar lipids. The result suggests that dietary 18:3n-3 effectively inhibited the conversion of 18:2n-6 to long-chain n-6 PUFAs and is similar to that reported for trout and coho salmon by Yu and Sinnhuber (1976 and 1979). However, when equal levels of dietary 18:2n-6 and 18:3n-3 were fed to charr there were more products from 18:3n-3 conversion than from 18:2n-6. For instance, there was especially more 22:6n-3 than 20:4n-6 in liver or muscle polar

lipids, and the level of tissue 18:2n-6 was significantly higher than tissue 18:3n-3. This suggests that 18:3n-3 is preferentially desaturated and elongated to 22:6n-3 and 18:2n-6 to 20:4n-6 in Arctic charr, indicating that 18:3n-3 and 18:2n-6 compete for conversion at the D^6, D^5 and D^4 desaturase steps. The more pronounced preference for 18:3n-3 over 18:2n-6 appears to occur at the D^4 desaturase step. This is further supported by a comparison of levels of n-6 and n-3 long-chain PUFAs in liver polar lipids of charr given the treatments with 1.0% 18:2n-6 or 18:3n-3 and the treatment with a combination of these two PUFAs. Moreover, the pattern of the interaction between 18:2n-6 and 18:3n-3 appears to change with their levels in the diets. Feeding high levels of dietary 18:2n-6 and 18:3n-3 produced high percentages of 18:3n-3 in liver polar lipids but low levels of 20:4n-6.

High dietary levels of 18:2n-6 were noted to inhibit the conversion of 18:3n-3 in rainbow trout but not in coho salmon (Yu and Sinnhuber 1976, 1979). Data from the present study indicated that charr fed diets with high levels of dietary 18:2n-6 and ratios of <1.0 for 18:2n-6 and 18:3n-3 had significantly higher levels of 18:2n-6, 20:4n-6 and 22:5n-6 in the liver polar lipids but little change in total n-3 PUFA, 18:3n-3, 20:5n-3 and 22:6n-3. When the dietary ratio of 18:2n-6 to 18:3n-3 was 1.5, levels of 18:2n-6, 20:4n-6 and 20:5n-6 were markedly elevated in liver polar lipids or muscle total lipids, accompanied by a decline in tissue levels of 20:5n-3

and 22:6n-3. These findings suggest that there is competitive inhibition of 18:2n-6 on the metabolism of 18:3n-3 in Arctic charr and hence charr appears to be similar to rainbow trout in this regard (Yu and Sinnhuber, 1976). However, The EFA requirements of charr and rainbow trout differ in that charr appear to require both 18:2n-6 and 18:3n-3 whereas juvenile rainbow trout require 18:3n-3 only (Castell et al. 1972; Chapters 3, 4 and 5). Perhaps there is a difference in the extent of inhibition that 18:2n-6 exerts on 18:3n-3 metabolism between charr and rainbow trout that reflects differences in their EFA requirements. For example, the degree of inhibition of 18:2n-6 on the metabolism of 18:3n-3 may be less in Arctic charr than rainbow trout. Consequently, charr may be a better model to study the interaction of dietary 18:2n-6 and 18:3n-3 in cold water species of fish than rainbow trout.

Interestingly, the replacement of dietary 12:0 with 18:1n-9 resulted in a significant increase in total tissue monoenes, 18:1n-9 and 20:4n-6 but no change in total n-3 PUFAs, 20:5n-3 and 22:6n-3. These results suggest that shifting dietary saturates to monoenes affects the metabolism of 18:2n-6 but not 18:3n-3 in charr.

Enrichment of muscle long-chain n-3 PUFAs

Due to the decline of natural fish oils, use of 18:3n-3-rich flax oil as an alternate dietary lipid for cultured fish may become important. Sowizral et al. (1990) found that rainbow trout fed diets containing 20% lipids with increasing levels

of 18:3n-3 had limited the conversion of 18:3n-3 to 20:5n-3 and 22:6n-3. This limited conversion was attributed to the inhibition of D^6 desaturase activity by high levels of dietary 18:3n-3, 20:5n-3 and 22:6n-3 (Sowizral et al., 1990). Another study with rainbow trout, in which lower levels of dietary lipids and higher concentrations of dietary 20:5n-6 and 22:6n-3 were used, led to similar results. These were attributed to limited conversion of 18:3n-3 to enable the maintenance of optimum physiological status by fish (Greene and Selivonchick, 1990). Although charr in the current study were fed diets containing relatively lower amounts of dietary lipids and no 20:5n-3 or 22:6n-3, there was no apparent change in n-3 long-chain PUFAs in charr muscle, but instead a marked accumulation of 18:3n-3 as dietary 18:3n-3 increased similar to the findings of Sowizral et al. (1990). However, my previous studies also showed that there was a marked increase of 18:4n-3 in charr muscle as levels of dietary 18:3n-3 were increased. Collectively, these data suggest that the limited conversion of 18:3n-3 to long-chain PUFAs may be related to D^5 desaturation, in addition to D^6 desaturation. Further work is needed using the n-3 products of D^6 desaturase, such as 18:4n-3, to test whether the limited conversion is due to the limited action of D^6 desaturase alone or in concert with other desaturases. On the other hand, data from this study, together with other reports (Sowizral et al., 1990; Greene and Selivonchick, 1990), suggest that the enrichment of fish flesh

with long-chain n-3 PUFAs can not be achieved by feeding high levels of dietary 18:3n-3 to fish, regardless of the dietary level of lipids and availability of dietary 20:5n-3 or 22:6n-3.

This chapter has demonstrated that the effect of dietary 18:2n-6 and 18:3n-3 on growth of Arctic charr is additive at low levels of dietary PUFAs. High levels of dietary 18:2n-6 are inhibitory to growth of charr when their requirement for 18:3n-3 (about 1.6%) was met. While there is apparent inhibition of dietary 18:3n-3 on the metabolism of 18:2n-6, the influence of dietary 18:2n-6 on the metabolism of n-3 PUFAs appears to change with its dietary concentration. When both fatty acids were present in the diet in equal amounts, the conversion of 18:3n-3 to long-chain PUFA dominated that of 18:2n-6. Further, this study demonstrates that with increasing levels of dietary 18:3n-3, there is a concomitant increase of 18:3n-3 in the muscle. However, increasing dietary 18:3n-3 does not affect levels of 20:5n-3 and 22:6n-3 in the flesh of Arctic charr, regardless of concentrations of 18:3n-3 and ratios of 18:2n-6 to 18:3n-3 in the diets.

Chapter 7

Influences of dietary vitamin C, copper, iron, zinc, magnesium and arginine on growth, feed utilization and lipid metabolism of Arctic charr, Salvelinus alpinus (L.).

Abstract

The effects of varying levels of dietary nutrients including vitamin C, zinc, copper, iron, magnesium and arginine were investigated for cultured Labrador Arctic charr (Salvelinus alpinus, L.) with respect to growth, feed utilization and lipid metabolism. Five casein-based experimental diets were fed to charr weighing 2.0 g over a period of 14 weeks. Growth rates, feed efficiencies, hepatosomatic indices, haematological values, water content and lipid and fatty acid composition in liver and muscle were investigated. No significant differences were detected between treatments for these parameters with the exception of lipid and fatty acid composition. Charr fed the control diet had significantly higher level of total lipids in livers than those fed any of the other diets. No differences were detected for n-3 and n-6 fatty acids of liver polar lipids, but several C20 n-3 or n-6 fatty acids in muscle total and polar lipids differed significantly among treatments. The results suggested that dietary zinc or copper may affect the D^6 desaturation of n-9, n-6 and n-3 PUFAs. Overall, this study suggested that varying dietary levels of vitamin C, minerals including zinc, copper, iron and magnesium, and arginine had minimal effect on growth, feed utilization and lipid metabolism and consequently on the assessment of the essential fatty acid requirements of charr.

Introduction

Several studies have investigated the effects of vitamin C (ascorbic acid) and minerals on lipid metabolism of vertebrates (Davis and Mertz, 1986; Hambridge et al., 1986; Sandnes, 1991). It has been reported that levels of plasma free fatty acids and lipid deposition in fish can be influenced by dissimilar intakes of vitamin C (ascorbic acid) (John et al., 1979; WaagbØ et al., 1989). There is also evidence that copper and zinc may be involved directly in the desaturation and elongation of polyunsaturated fatty acids (Bettger et al., 1980; Cunnane, 1982; Davis and Mertz, 1986; Hambridge et al., 1986).

Prior to my investigation of the essential fatty acid requirements of Arctic charr there was limited information on the individual nutrient requirements of this species (Jobling, 1991). Since Arctic charr is a salmonid and grows reasonably well on either salmon or trout diets (Baker, 1983), the formulation of semi-purified diets in my previous experiments was largely based on published information on rainbow trout and other coldwater species (Chapter 3). However, the composition of semi-purified diets used in other studies on salmonids varied greatly so that intermediate levels were usually selected for minerals and vitamin (Chapter 3). Levels of certain nutrients supplemented in the semi-purified diets, such as vitamin C, copper, zinc and iron, therefore, are of

potential concern, particularly with respect to their effects on lipid metabolism of Arctic charr.

The objective of this experiment was to assess the impact of varying dietary levels of vitamin C, copper, zinc, iron and arginine on the EFA requirements of Arctic charr. Five semi-purified diets with different combinations of these components were fed to charr in order to assess possible effects on growth, feed utilization, haematological values and lipid metabolism of this species.

Materials and Methods

Fish used in this study were the offspring of the Labrador strain of Arctic charr originally collected from Labrador, Canada.

The test diets consisted of five casein-based diets varying in concentrations of arginine, vitamin C and minerals including Zn, Cu, Fe and Mg (Table 26). The diet on which charr grew the most (Chapter 6) was used as a control diet. All experimental diets had 3.0% flax oil and the same basic composition as reported in Table 2 (Chapter 2). Prior to formulation of these diets, casein, starch and dextrin were all extracted three times with hot 95% ethanol to remove trace amounts of lipids.

The diets were each assigned randomly to duplicated groups of 75 individual fish (average initial weight 2.0 g). Fish were acclimated on a PUFA-free diet as reported (Chapters 2 and 3) for 4 weeks. During the 14 week feeding trial, fish were reared at a constant 10 ^{o}C under a photoperiod of 12 h light : 12 h dark. Each tank contained 60 l of aerated well water with 95% recirculation at a flow rate of approximately 2.4 $l.min^{-1}$. Fish were fed 5 times per day, to apparent satiation and daily amounts of feed consumed were recorded.

Fish were weighed individually at 4 week intervals and feed was adjusted bi-weekly based on total fish weight in each tank. At the end of the 14 weeks of feeding, 10 fish from each

Table 26. Calculated concentrations of minerals, vitamin C and arginine in experimental diets and literature values (mg/kg feed)

Diet	Cu	Zn	Fe	Mg	Vitamin C	Arginine	
						FAA[1]	Total[2]
Control	3.1	20.4	33.6	504.5	300	13000	34900
Mod-MV	9.2	61.3	82.9	606.0	600	13000	34900
Mod-V	3.1	20.4	33.6	504.5	600	13000	34900
Mod-M	9.2	61.3	82.9	606.0	300	13000	34900
Mod-Arg	3.1	20.4	33.6	504.5	300	0	21900
Trout	3.0[3]	15-30[3]	-	500.0[3]	200[4]	-	18000[5]
Salmon	5.0[3]	-	60.0[3]	-	200[4]	-	24000[5]

[1]Free form of arginine supplemented [2]Total concentration of arginine estimated by adding arginine contained in casein, gelatin and free form of arginine supplemented [3]From Lall (1989) [4]From Halver (1989) [5]From Wilson (1989)

tank were sampled for a determination of haemoglobin and haematocrit values. An additional 10 fish were sampled for estimation of hepatosomatic index (HSI) and water content in the liver and muscle. Livers and muscle samples from an additional 5-10 fish were collected and pooled for analyses of lipid and fatty acid composition.

All Statistical analyses were performed using SAS as installed by the University of Manitoba Computer Service. One way ANOVA and Duncan's multiple range test were used to compare means among treatments. Results were considered significant at $P < 0.05$ level.

Results

The average weight of Arctic charr increased from about 2.0 g initially to 12.9 to 15.0 g by the end of the 14 week feeding trial (Table 27). However, no significant differences were detected for growth and feed utilization as reflected by SGR and feed efficiency among treatments (Table 27). Control fish had the highest values for SGR and feed efficiency.

There were no significant differences among groups in haematocrit and haemoglobin values between treatments and no correspondence was found between these two parameters (Table 28). The highest level of haemoglobin occurred in the group fed the diet that was high in both vitamin C and mineral content (Diet Mod-VM).

Percentages for water in liver and muscle also varied little between treatments (Table 28). HSI values for the groups fed the control diet or the diet high in both vitamin C and minerals (diet Mod-MV) were lower than those for charr fed diets high in vitamin C or minerals or low in arginine content (diets Mod-V, Mod-M and Mod-Arg).

Percentage of liver total lipids for charr fed the control diet was significantly higher than those fed other test diets, while percentage of muscle total lipids of fish fed the diet high in vitamin C only (diet Mod-V) were significantly higher relative to other test diets (Fig. 19A, D). In addition, significant differences were observed in liver polar and

Table 27. Initial and final mean weights, specific growth rates (SGR) and feed efficiency values for Arctic charr fed diets varying in levels of vitamin C, minerals and arginine (mean ± SEM, n[1] = 2)[2]

Diet	Initial weight (g)	Final weight (g)	SGR (% day)	Feed efficiency
Control	2.08 ± 0.04	15.03 ± 0.58 [a]	2.02 ± 0.06	1.01 ± 0.00
Mod-MV	1.90 ± 0.07	12.94 ± 0.45 [b]	1.96 ± 0.07	0.94 ± 0.06
Mod-V	1.85 ± 0.01	13.23 ± 0.13 [ab]	2.01 ± 0.02	0.94 ± 0.02
Mod-M	1.93 ± 0.02	13.70 ± 0.85 [ab]	2.00 ± 0.06	0.97 ± 0.06
Mod-Arg	1.96 ± 0.12	13.52 ± 0.35 [ab]	1.97 ± 0.03	0.97 ± 0.01

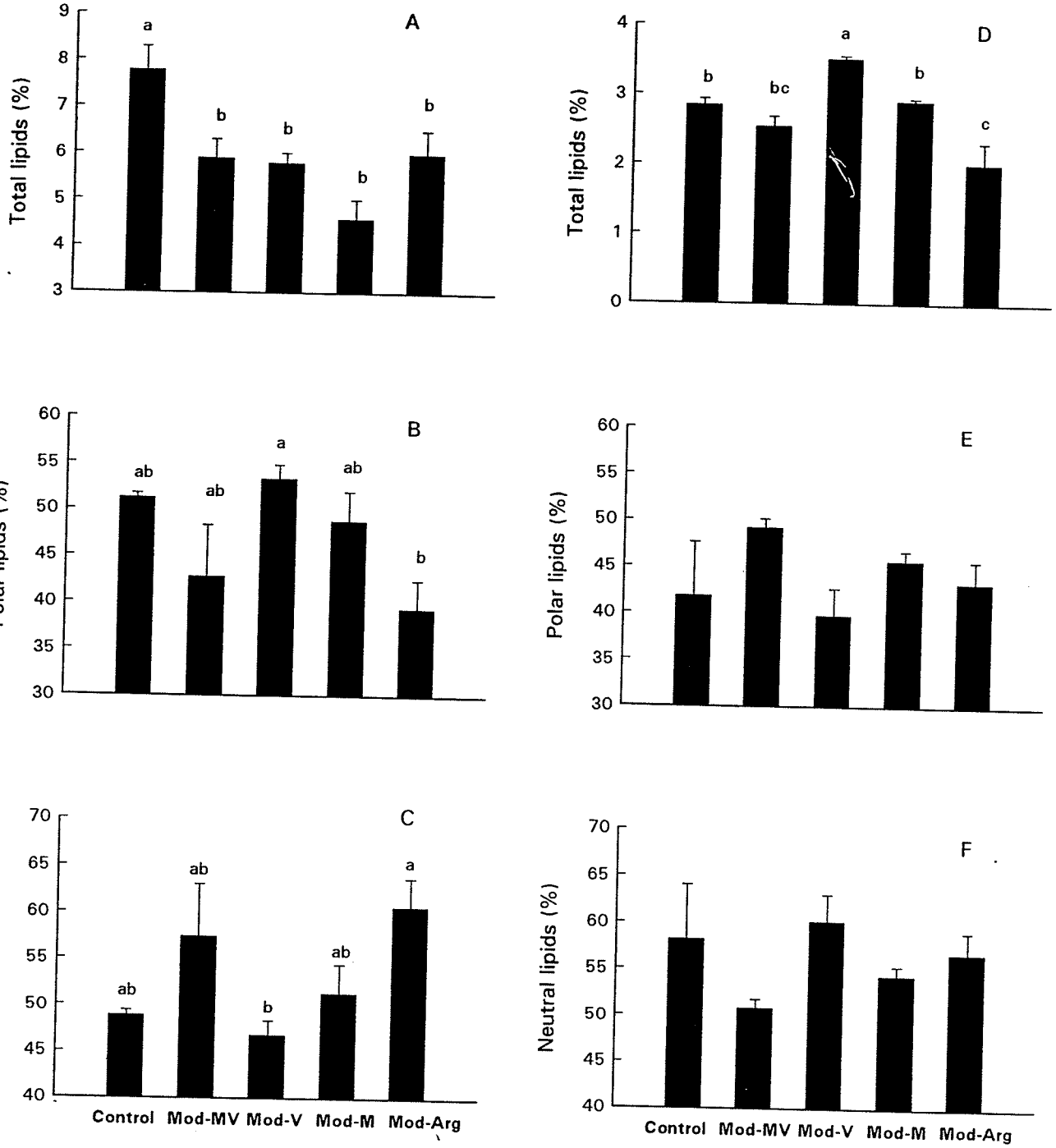
[1]Number of rearing tanks [2]Same letters in a column indicate no significant difference

Table 28. Hematocrit and hemoglobin values, water content in livers and muscle and HSI values for Arctic charr fed diets varying in levels of vitamin C, minerals and arginine (mean ± SEM, $n^1 = 2$)

Diet	Hematocrit (%)	Hemoglobin (g/100 ml)	Liver water	Muscle water	HSI
Control	37.3 ± 1.9	8.6 ± 0.2	71.7 ± 0.6	77.7 ± 0.2	1.58 ± 0.04
Mod-MV	36.8 ± 0.9	10.2 ± 1.1	71.9 ± 0.8	77.4 ± 0.0	1.56 ± 0.02
Mod-V	35.2 ± 2.6	8.7 ± 0.1	70.9 ± 1.5	77.4 ± 0.0	1.68 ± 0.00
Mod-M	37.7 ± 0.9	8.7 ± 0.2	71.1 ± 0.7	77.5 ± 0.3	1.66 ± 0.03
Mod-Arg	36.8 ± 1.2	9.2 ± 0.2	70.3 ± 0.9	77.4 ± 0.1	1.71 ± 0.08

[1]Value of each replicate was mean of values determined from 10 individual fish

Fig. 19. Total, neutral and polar lipids in the liver (A-C) and muscle (D-F) of Arctic charr fed diets varying in arginine, vitamin C or minerals including zinc, iron, copper and magnesium


A
Total lipids (%)
9
8
7
6
5
4
3
a
b
b
b
b
D
Total lipids (%)
4
3
2
1
0
b
bc
a
b
c
B
Polar lipids (%)
60
55
50
45
40
35
30
ab
ab
a
ab
b
E
Polar lipids (%)
60
55
50
45
40
35
30
C
Neutral lipids (%)
70
65
60
55
50
45
40
ab
ab
b
ab
a
Control
Mod-MV
Mod-V
Mod-M
Mod-Arg
F
Neutral lipids (%)
70
65
60
55
50
45
40
Control
Mod-MV
Mod-V
Mod-M
Mod-Arg

neutral lipids among groups fed the diets high in vitamin C or mineral content only (diets Mod-V and Mod-M) (Fig. 19, C). No differences were found for muscle polar or neutral lipids between treatments (Fig. 19E,F).

Only one of 26 fatty acids of liver polar lipids, i.e., 20:2n-6, showed significant differences between treatments (Table 29). The group fed the diet high in vitamin C and minerals (diet Mod-VM) had significantly higher level of 20:2n-6 than those fed the diet low in arginine (diet Mod-Arg).

Significant differences between treatments were detected for a number of unsaturated fatty acids of muscle polar lipids, including 20:1n-9, 20:2n-6, 20:3n-6, 20:3n-3 and 20:4n-3 (Table 30). Compared to control fish, charr fed the diet high in minerals (diet Mod-M) had significantly lower levels of 20:4n-3. Charr fed the diet high in vitamin C or low in arginine (diets Mod-V and Mod-Arg) had depressed percentages of 20:2n-6. The lowest level of 20:3n-3 occurred in the group fed the diet low in arginine content (diet Mod-Arg).

The percentage of several fatty acids of total muscle lipids were significantly affected by diet treatments (Table 31). Charr given the diet high in vitamin C (diet Mod-V) had significantly lower levels of 22:5n-6 and total n-9 PUFA than those fed the control diet. The group fed the diet high in both vitamin C and minerals (diet Mod-VM) was also higher in both 18:0 and 20:2n-6 relative to control fish.

Table 29. Fatty acid composition of liver polar lipids from Arctic charr fed diets varying in levels of vitamin C, minerals and arginine (weight %)

(mean ± SEM, $n^1 = 2$)[2]

Fatty acid	Diet				
	Control	Mod-VM	Mod-V	Mod-M	Mod-Arg
14:0	1.47 ± 0.33	1.58 ± 0.04	1.70 ± 0.38	1.37 ± 0.03	1.52 ± 0.38
16:0	16.88 ± 1.33	16.45 ± 0.42	17.86 ± 1.74	15.70 ± 1.33	17.54 ± 0.79
16:1n-9	1.26 ± 0.09	1.22 ± 0.01	1.17 ± 0.02	1.20 ± 0.07	1.18 ± 0.15
16:1n-7	2.13 ± 0.39	1.60 ± 0.32	1.60 ± 0.02	1.98 ± 0.13	2.14 ± 0.38
18:0	4.79 ± 0.29	5.39 ± 0.64	5.40 ± 0.23	4.84 ± 0.06	4.96 ± 0.00
18:1n-9	16.68 ± 1.02	16.37 ± 0.65	15.98 ± 0.40	17.28 ± 0.12	18.50 ± 2.16
18:1n-7	2.67 ± 0.24	2.44 ± 0.05	2.51 ± 0.07	3.01 ± 0.41	2.80 ± 0.11
18:2n-6	2.13 ± 0.03	2.31 ± 0.15	2.30 ± 0.04	2.37 ± 0.06	2.20 ± 0.08
18:3n-3	2.05 ± 0.16	2.25 ± 0.29	2.12 ± 0.04	2.29 ± 0.32	1.94 ± 0.22
18:4n-3	0.70 ± 0.01	0.59 ± 0.16	0.65 ± 0.03	0.67 ± 0.14	0.67 ± 0.08
20:1n-9	1.82 ± 0.00	1.87 ± 0.07	1.82 ± 0.25	1.89 ± 0.03	2.02 ± 0.37
20:2n-9	0.29 ± 0.01	0.29 ± 0.03	0.28 ± 0.05	0.29 ± 0.02	0.37 ± 0.11
20:2n-6	0.56 ± 0.01 [bc]	0.71 ± 0.03 [a]	0.66 ± 0.02 [ab]	0.63 ± 0.04 [abc]	0.51 ± 0.06 [c]
20:3n-9	0.44 ± 0.04	0.43 ± 0.02	0.46 ± 0.08	0.43 ± 0.03	0.59 ± 0.15
20:3n-6	1.17 ± 0.15	1.27 ± 0.08	1.44 ± 0.03	1.19 ± 0.12	1.43 ± 0.09
20:4n-6	3.63 ± 0.08	3.75 ± 0.06	3.44 ± 0.03	3.58 ± 0.40	3.19 ± 0.05
20:3n-3	0.50 ± 0.12	0.62 ± 0.01	0.57 ± 0.07	0.59 ± 0.04	0.46 ± 0.06
20:4n-3	0.74 ± 0.07	0.83 ± 0.04	0.93 ± 0.02	0.87 ± 0.01	0.83 ± 0.20
20:5n-3	4.45 ± 0.24	3.89 ± 0.38	4.15 ± 0.16	4.33 ± 0.29	3.88 ± 0.17
22:1n-9	0.10 ± 0.02	0.10 ± 0.01	0.10 ± 0.04	0.10 ± 0.01	0.11 ± 0.03
22:4n-6	0.09 ± 0.02	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.03
22:5n-6	0.21 ± 0.08	0.24 ± 0.04	0.28 ± 0.03	0.24 ± 0.02	0.28 ± 0.04
22:5n-3	1.44 ± 0.32	1.51 ± 0.02	1.64 ± 0.11	1.56 ± 0.15	1.50 ± 0.04

... Continued

Table 29. (continued)

Fatty acid	Diet				
	Control	Mod-VM	Mod-V	Mod-M	Mod-Arg
22:6n-3	26.97 ± 0.97	27.70 ± 0.01	27.00 ± 1.38	25.87 ± 2.28	25.60 ± 0.79
24:1n-9	0.94 ± 0.11	1.09 ± 0.30	0.99 ± 0.09	1.20 ± 0.34	0.93 ± 0.25
20:3n-9/22:6n-3	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01
Σ Saturates	23.66 ± 1.32	24.04 ± 0.94	25.49 ± 2.34	22.52 ± 1.32	24.67 ± 1.09
Σ Monoenes	26.39 ± 1.44	25.47 ± 0.41	24.87 ± 0.61	27.95 ± 1.35	28.49 ± 2.64
Σ PUFA	45.75 ± 0.38	46.66 ± 0.93	46.21 ± 1.70	45.27 ± 3.23	43.75 ± 1.36
Σ n-9	0.95 ± 0.05	0.89 ± 0.04	0.92 ± 0.12	0.95 ± 0.04	1.15 ± 0.25
Σ n-6	7.96 ± 0.32	8.37 ± 0.15	8.22 ± 0.01	8.13 ± 0.35	7.72 ± 0.24
Σ n-3	36.85 ± 0.75	37.41 ± 0.75	37.07 ± 1.57	36.19 ± 2.91	34.88 ± 1.37
Σ n-6 / Σ n-3	0.22 ± 0.01	0.22 ± 0.00	0.22 ± 0.01	0.23 ± 0.01	0.22 ± 0.00

[1]Values for each replicate were determined from pooled livers of 5-10 fish [2]Same letters in a row indicate no significant difference between treatments

Table 30. Fatty acid composition of muscle polar lipids from charr fed diets varying in vitamin C, minerals and arginine (weight %) (mean ± SEM, $n^1 = 2$)[2]

Fatty acid	Diet				
	Control	Mod-VM	Mod-V	Mod-M	Mod-Arg
12:0	1.52 ± 0.50	1.52 ± 0.29	1.40 ± 0.02	1.38 ± 0.47	1.57 ± 0.22
14:0	2.06 ± 0.23	1.98 ± 0.14	1.94 ± 0.01	2.17 ± 0.01	1.98 ± 0.15
16:0	15.08 ± 0.40	15.59 ± 0.27	15.23 ± 0.26	15.99 ± 0.29	15.66 ± 0.22
16:1n-9	1.20 ± 0.04	1.13 ± 0.02	1.05 ± 0.06	1.19 ± 0.07	1.06 ± 0.03
16:1n-7	3.62 ± 0.19	3.14 ± 0.24	3.42 ± 0.38	3.87 ± 0.27	3.94 ± 0.42
18:0	2.44 ± 0.04	2.83 ± 0.12	2.62 ± 0.15	2.49 ± 0.16	2.42 ± 0.02
18:1n-9	16.23 ± 0.36	16.38 ± 0.57	17.39 ± 0.20	17.04 ± 0.80	16.61 ± 0.27
18:1n-7	2.45 ± 0.11	2.26 ± 0.02	2.40 ± 0.29	2.43 ± 0.02	2.40 ± 0.04
18:2n-6	4.49 ± 0.04	4.66 ± 0.02	4.50 ± 0.18	4.31 ± 0.20	4.24 ± 0.34
18:3n-6	0.37 ± 0.03	0.37 ± 0.02	0.37 ± 0.02	0.38 ± 0.02	0.40 ± 0.02
18:3n-3	6.72 ± 0.08	7.02 ± 0.07	6.56 ± 0.11	6.29 ± 0.41	6.14 ± 0.58
18:4n-3	2.84 ± 0.23	2.97 ± 0.07	3.18 ± 0.16	2.98 ± 0.11	3.11 ± 0.12
20:1n-9	1.58 ± 0.07 [ab]	1.69 ± 0.00 [a]	1.49 ± 0.07 [ab]	1.47 ± 0.05 [b]	1.40 ± 0.05 [b]
20:2n-9	0.45 ± 0.01	0.44 ± 0.00	0.40 ± 0.04	0.48 ± 0.09	0.44 ± 0.04
20:2n-6	0.77 ± 0.04 [a]	0.71 ± 0.03 [ab]	0.52 ± 0.03 [b]	0.68 ± 0.11 [ab]	0.54 ± 0.02 [b]
20:3n-9	0.26 ± 0.02	0.29 ± 0.03	0.38 ± 0.04	0.32 ± 0.11	0.32 ± 0.03
20:3n-6	1.07 ± 0.07 [ab]	1.16 ± 0.01 [a]	1.03 ± 0.03 [ab]	1.00 ± 0.00 [b]	1.07 ± 0.01 [ab]
20:4n-6	1.23 ± 0.05	1.12 ± 0.13	1.13 ± 0.05	1.25 ± 0.08	1.11 ± 0.05
20:3n-3	1.12 ± 0.05 [ab]	1.23 ± 0.06 [a]	1.01 ± 0.04 [abc]	0.93 ± 0.07 [bc]	0.88 ± 0.08 [c]
20:4n-3	1.87 ± 0.08 [ab]	2.03 ± 0.01 [a]	1.90 ± 0.08 [ab]	1.64 ± 0.04 [c]	1.79 ± 0.01 [bc]
20:5n-3	4.96 ± 0.45	5.08 ± 0.09	5.60 ± 0.02	5.35 ± 0.35	5.34 ± 0.01
22:1n-9	0.09 ± 0.01	0.10 ± 0.00	0.13 ± 0.03	0.10 ± 0.00	0.09 ± 0.02
22:4n-6	0.11 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.05	0.10 ± 0.02

... Continued

Table 30. (continued)

Fatty acid	Diet				
	Control	Mod-VM	Mod-V	Mod-M	Mod-Arg
22:5n-6	0.25 ± 0.01	0.21 ± 0.03	0.20 ± 0.03	0.23 ± 0.01	0.21 ± 0.01
22:5n-3	1.75 ± 0.13	1.86 ± 0.00	1.81 ± 0.03	1.87 ± 0.16	1.87 ± 0.09
22:6n-3	18.04 ± 2.06	18.21 ± 0.88	19.05 ± 0.53	18.17 ± 1.21	18.50 ± 0.79
24:1n-9	0.51 ± 0.03	0.46 ± 0.01	0.55 ± 0.06	0.51 ± 0.05	0.49 ± 0.02
20:3n-9 / 22:6n-3	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00
Σ Saturates	21.23 ± 0.31	22.17 ± 0.53	21.35 ± 0.08	22.23 ± 0.06	21.88 ± 0.60
Σ Monoenes	26.60 ± 0.95	26.02 ± 0.72	27.29 ± 0.92	27.67 ± 1.29	27.25 ± 0.86
Σ PUFA	46.52 ± 2.61	47.65 ± 1.02	47.89 ± 0.45	46.20 ± 1.74	46.29 ± 1.43
Σ n-9	0.89 ± 0.00	0.88 ± 0.01	0.92 ± 0.08	1.01 ± 0.24	0.96 ± 0.12
Σ n-6	8.30 ± 0.01 [ab]	8.34 ± 0.14 [a]	7.84 ± 0.11 [ab]	7.94 ± 0.17 [ab]	7.68 ± 0.30 [b]
Σ n-3	37.33 ± 2.62	38.43 ± 0.88	39.13 ± 0.48	37.26 ± 1.81	37.66 ± 1.25
Σ n-6 / Σ n-3	0.22 ± 0.02	0.22 ± 0.00	0.20 ± 0.01	0.21 ± 0.01	0.20 ± 0.00

[1, 2]Same as in Table 29

Table 31. Fatty acid composition of total musule lipids from charr fed diets varying in vitamin C, minerals and arginine (weight %) (mean ± SEM, $n^1 = 2$)[2]

Fatty acid	Diet				
	Control	Mod-VM	Mod-V	Mod-M[3]	Mod-Arg
12:0	6.41 ± 0.43 [a]	5.26 ± 0.05 [b]	6.57 ± 0.07 [a]	5.72 [ab]	6.19 ± 0.09 [ab]
14:0	2.25 ± 0.15	1.88 ± 0.07	2.07 ± 0.05	2.26	2.20 ± 0.06
16:0	12.32 ± 0.21	12.43 ± 0.25	12.20 ± 0.44	12.98	12.96 ± 0.37
16:1n-9	0.94 ± 0.06	0.92 ± 0.04	0.82 ± 0.04	0.85	0.84 ± 0.02
16:1n-7	6.07 ± 0.29 [ab]	5.16 ± 0.08 [b]	6.04 ± 0.37 [ab]	6.05 [ab]	7.18 ± 0.78 [a]
18:0	2.48 ± 0.09 [b]	2.77 ± 0.08 [a]	2.67 ± 0.05 [ab]	2.69 [ab]	2.42 ± 0.01 [b]
18:1n-9	24.48 ± 1.11	25.76 ± 0.12	27.16 ± 0.39	24.27	25.64 ± 0.82
18:1n-7	2.52 ± 0.11	2.65 ± 0.08	2.44 ± 0.18	2.60	2.56 ± 0.05
18:2n-6	5.58 ± 0.02 [a]	5.39 ± 0.02 [ab]	5.51 ± 0.07 [ab]	5.60 [a]	5.00 ± 0.24 [b]
18:3n-6	0.37 ± 0.03	0.33 ± 0.01	0.37 ± 0.05	0.35	0.36 ± 0.03
18:3n-3	9.98 ± 0.18	8.61 ± 0.34	9.25 ± 0.45	9.07	8.45 ± 0.39
18:4n-3	3.12 ± 0.36	2.61 ± 0.12	3.18 ± 0.34	2.87	3.11 ± 0.16
20:1n-9	2.35 ± 0.07 [ab]	2.68 ± 0.05 [a]	2.39 ± 0.21 [ab]	2.30 [ab]	2.17 ± 0.04 [b]
20:2n-9	0.49 ± 0.03 [ab]	0.53 ± 0.00 [a]	0.45 ± 0.01 [b]	0.44 [b]	0.48 ± 0.01 [ab]
20:2n-6	0.60 ± 0.02 [b]	0.73 ± 0.01 [a]	0.54 ± 0.03 [b]	0.55 [b]	0.49 ± 0.05 [b]
20:3n-9	0.15 ± 0.03	0.12 ± 0.01	0.12 ± 0.01	0.12	0.13 ± 0.01
20:3n-6	0.61 ± 0.02	0.74 ± 0.03	0.60 ± 0.06	0.59	0.61 ± 0.04
20:4n-6	0.60 ± 0.08	0.62 ± 0.05	0.52 ± 0.01	0.57	0.54 ± 0.02
20:3n-3	0.88 ± 0.03 [ab]	1.01 ± 0.01 [a]	0.76 ± 0.05 [b]	0.77 [b]	0.68 ± 0.09 [b]
20:4n-3	1.12 ± 0.03 [ab]	1.20 ± 0.01 [a]	1.06 ± 0.05 [b]	1.05 [b]	1.08 ± 0.01 [ab]
20:5n-3	2.57 ± 0.16	2.57 ± 0.11	2.59 ± 0.06	2.99	2.65 ± 0.02
22:1n-9	0.25 ± 0.02	0.27 ± 0.02	0.27 ± 0.03	0.26	0.26 ± 0.01
22:4n-6	0.06 ± 0.01	0.12 ± 0.07	0.04 ± 0.00	0.06	0.06 ± 0.01

... Continued

Table 31. (continued)

Fatty acid	Diet				
	Control	Mod-VM	Mod-V	Mod-M[3]	Mod-Arg
22:5n-6	0.14 ± 0.01 [a]	0.12 ± 0.01 [ab]	0.09 ± 0.00 [b]	0.11 [ab]	0.11 ± 0.02 [ab]
22:5n-3	0.87 ± 0.05	0.99 ± 0.01	0.88 ± 0.03	0.90	0.92 ± 0.02
22:6n-3	8.29 ± 0.67	9.90 ± 0.07	8.12 ± 0.20	9.06	8.33 ± 0.70
24:1n-9	0.28 ± 0.02	0.26 ± 0.01	0.24 ± 0.04	0.31	0.27 ± 0.01
20:3n-9/22:6n-3	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01	0.02 ± 0.00
∑ Saturates	23.71 ± 0.11 [ab]	22.57 ± 0.33 [b]	23.67 ± 0.36 [ab]	24.22 [a]	24.31 ± 0.53 [a]
∑ Monoenes	37.73 ± 1.21	38.52 ± 0.13	39.92 ± 0.05	37.57	39.74 ± 1.35
∑ PUFA	35.61 ± 1.23	35.77 ± 0.63	34.18 ± 0.28	35.03	32.83 ± 1.37
∑ n-9	0.79 ± 0.00 [a]	0.79 ± 0.00 [a]	0.68 ± 0.03 [c]	0.71 [bc]	0.76 ± 0.02 [ab]
∑ n-6	7.97 ± 0.17 [a]	8.06 ± 0.09 [a]	7.67 ± 0.14 [ab]	7.56 [ab]	6.83 ± 0.35 [b]
∑ n-3	26.85 ± 1.06	26.92 ± 0.54	25.84 ± 0.17	26.76	25.24 ± 1.04
∑ n-6 / ∑ n-3	0.30 ± 0.01 [ab]	0.30 ± 0.00 [a]	0.30 ± 0.00 [ab]	0.28 [bc]	0.27 ± 0.00 [c]

[1, 2]Same as in Table 29 [3]Single replicate was analyzed.

Discussion

Fish fed diets inadequate in vitamin C, zinc, or copper or iron usually display retarded growth, poor feed utilization and anaemia (John et al., 1979; Halver, 1989; Lall, 1989). Charr fed diets varying in levels of these dietary components did not exhibit dissimilar growth rates and feed efficiencies. Hence, the amounts of these components included in the control diet appeared to be adequate for maximum growth and feed utilization of this species. Furthermore, haematological values revealed little difference among treatments and they fell within the range reported for other salmonids and Arctic charr fed the commercial diet (Castell et al., 1972b; Greene and Selivonchick, 1990; Chapters 4 and 5). These results suggest that the absence of anaemia in the experimental fish. Also, these data suggest that the previous basal diets contained adequate level of vitamin C and likely the trace minerals had minimal negative impact on the health of the experimental fish.

It is reported that dietary vitamin C and iron affect each other's uptake in fish (Hilton, 1989). Charr fed the diet containing high levels of iron, zinc and copper and magnesium and vitamin C had higher haemoglobin values than those fed other diets. Perhaps, this difference is associated with the increased absorption of iron in the presence of high levels of dietary vitamin C.

Effects of high dietary level of arginine is controversial with respect to fish growth. Some researchers suggest it stimulates growth of fingerling salmonids (Plisetskaya et al., 1991), while others suggest it inhibits growth of milkfish (Borlongan, 1991). The requirement of salmonids for dietary

The maximum requirement of salmonids for dietary arginine is between 1.2 to 2.5% (Wilson 1989; Kim et al. 1992). Interestingly, charr fed diets containing arginine ranging from 2.19 to 3.49% had same growth and feed efficiency. Little difference occurred in haematocrit, haemoglobin, liver and muscle water content and HSI between these two groups. There was also little difference in lipid classes and fatty acid composition of muscle and liver between groups fed diets differing in arginine content. However, total liver and muscle lipids of charr fed the diet high in arginine were significantly lower than those given the control diet. Perhaps, the level of arginine affects lipid synthesis or mobilization through its stimulation of insulin secretion. Although 20:2n-6 and 20:3n-3 from the muscle polar lipids were different between these groups, they were not on the main pathway of desaturation and elongation of 18:2n-6 and 18:3n-3. Overall my data suggest that changing levels of dietary arginine has a minimal effect on growth and the fatty acid metabolism of Arctic charr but may affect lipid synthesis or mobilization through its stimulation of insulin secretion.

Dietary ascorbic acid affects lipid mobilization in juvenile

rainbow trout, as reflected by changes in plasma triacylglycerols and free fatty acids (John et al., 1979). In addition, feeding diets devoid of ascorbic acid led to an increased deposition of lipids in the liver of maturing fish (WaagØ et al. 1989). No major differences were noted for individual saturated and unsaturated fatty acids in neutral and polar lipid fractions between diets high and low in vitamin C in this study. Although the level of total liver lipids in charr fed the control diet was higher than those fed the other test diets, it was also higher than the values reported from other experiments (Chapter 4, 5 and 6). In addition, charr fed the diets supplemented with the same amount of vitamin C as the control diet, did not show a difference in total liver lipids from those fed diets high in vitamin C. Perhaps, the small difference in the level of total liver lipids between the control and other treatments is unrelated to the level of vitamin C supplemented or it may be attributed to a slight reduction of the active form of vitamin C in the diet during storage or feeding of this diet. Nevertheless, data from these experiments suggest that changing levels of dietary vitamin C has a minimal effect on lipid metabolism and the assessment of EFA requirements of Arctic charr.

Dietary zinc and copper have been investigated in relation to the metabolism of polyunsaturated fatty acids (PUFA) in mammals, especially desaturation at the D^6 desaturase step

(Bettger et al., 1979; Cunnane, 1982; Cunnane et al., 1986). In this study the effects of diets containing varying levels of dietary zinc, copper, iron and magnesium were also investigated with respect to fatty acid composition of liver polar or muscle lipids. While an increase of Cu and Zn in the diet had no effect on liver polar lipids, a significant decrease was noted for 20:4n-3 in the muscle polar lipids, a product from the D^6 desaturation of 18:3n-3. This findings indicates that changing levels of dietary minerals such as zinc, copper and iron influences the metabolism of PUFA in fish. The effect of these minerals on lipid metabolism of charr are still unclear as 20:4n-3 is a relatively small proportion of total polyunsaturated fatty acids in muscle, and liver is the most active site for metabolism of polyunsaturated fatty acids.

Results from previous experiments suggest that levels of n-3 and n-6 PUFAs in the diet containing 3.0% flax oil are sufficient to meet the EFA requirement of Arctic charr (Chapter 6). Arctic charr fed diets with 3.0% flax oil and varying amounts of vitamin C and several minerals all had high growth rates and feed efficiencies but a low HSI, muscle water content and tissue 20:3n-9. In addition, varying levels of dietary vitamin C or minerals including copper, iron and magnesium had little influence on the metabolism of major polyunsaturated fatty acids of charr liver muscle. Overall my data suggest that the effects of dietary polyunsaturated fatty

acids on the essential fatty acid requirements of Arctic charr as described in above chapters is independent of the action of dietary arginine, vitamin C and minerals (copper, iron, magnesium and zinc), at least within the range of concentrations investigated in this study.

General Discussion

Little was known about the EFA requirement and metabolism of dietary PUFAs of Arctic charr prior to this study. The current study examined two strains of cultured Arctic charr regarding their qualitative and quantitative EFA requirements. More than 30 casein-based diets differing in PUFA or lipid composition were tested. Available biochemical and biological data from a series of feeding experiments suggest that Arctic charr require n-3 PUFAs and probably n-6 PUFAs as their EFA. Arctic charr are capable of converting dietary PUFAs to long-chain PUFAs or directly incorporating them into tissue lipids.

1. Development of semi-purified diet

Numerous studies have suggested that the formulation of a suitable semi-purified diet is a necessary and important step for assessing individual nutrient requirements of fish (Wilson, 1989; Lall, 1989; Sargent et al., 1989). While Arctic charr were reported to grow as well as rainbow trout on commercial trout or salmon diet, few researchers have used semi-purified diets to study the nutrient needs of this species (Baker, 1983; Tabachek, 1984, 1986). One study examined the growth and lipid metabolism of Arctic charr fed a semi-purified casein-based diet, but the experiment failed because fish fed the semi-purified diet had high mortality and poor growth (Yurkowski, 1986). More recently, Olsen et al.

(1991) successfully used semi-purified diets to investigate the lipid metabolism of Arctic charr.

Casein-based semi-purified diets were developed in this study for assessing the EFA requirement of juvenile Arctic charr (Chapter 2). The composition of these diets were primarily based on the nutritional requirements of other salmonids and the compositions of the semi-purified diets used in previous studies (Castell et al., 1972; Yu et al. 1976; NRC, 1981; Wilson, 1989; Sargent et al. 1989; Halver, 1989; Lall, 1989; Lovell, 1989). Available data indicate that the growth rates and feed efficiencies of Arctic charr fed these casein-based diets were acceptable and similar to those fed the commercial diets regularly used by hatcheries (Chapters 3-7). A close correspondence between dietary levels of PUFAs and EFA status was also noted for all experiments. Moreover, varying levels of vitamin C, zinc, copper, iron, magnesium and arginine in the basal diet, had no effect on growth, several of physiological parameters and the composition of tissue PUFAs for Arctic charr (Chapter 7). This suggests that determination of EFA requirement of charr with the basal diet was independent of the levels of those dietary components, at least for the levels tested.

2. Signs of EFA deficiency

Numerous signs and pathologies have been reported for a variety of fish species when they have been fed diets inadequate in n-3 and/or n-6 PUFAs over long periods of time

(8-16 weeks) (Watanabe, 1982; Sargent et al., 1989). These signs usually have included poor growth and feed utilization, elevated levels of liver total and neutral lipids, increased water content in muscle or whole body and substantial accumulation of tissue 20:3n-9. This study demonstrated that these signs are also prevalent in Arctic charr fed diets containing ≤ 1.0% PUFAs (Chapters 3-6). However, levels of total liver lipids were similar for charr fed the PUFA-free diet and the diets containing high concentrations of PUFAs. This is supported by the results from the determination of HSI and liver water, DNA and DNA content, and direct comparison of liver lipid levels for Arctic charr and rainbow trout (Chapters 3-6). This PUFA-deficiency feature of charr appears to differ from that of other salmonids (Watanabe et al., 1974a, b, c; Olsen et al., 1991). The difference may reflect dissimilar metabolism and ability to adapt to malnutrition among salmonids, rather than impaired lipid transport in PUFA-deficient fish (Henderson and Tocher, 1987).

3. Indicators of EFA status

Several indicators such as growth rate, feed conversion, and accumulation of tissue 20:3n-9, have been used widely to indicate the EFA status of freshwater fish (Takeuchi and Watanabe, 1979; Watanabe, 1982; Thongrod et al., 1990a). However, multiple indicators rather than single indicators for accurate evaluation of EFA status in fish may be necessary

(Cowey, 1988).

The most frequently used indictors for EFA status of fish are levels of 20:3n-9 and the ratio of 20:3n-9 to 22:6n-3 in tissue polar lipids. In principle, the percentage of 20:3n-9 and ratio of 20:3n-9 to 22:6n-3 decreases in fish tissues as levels of n-3 PUFAs increase in the diet. The levels of dietary n-3 PUFAs are considered sufficient to meet the EFA requirements of fish when ratios in tissues fall below 0.4 (Castell et al., 1972c; Watanabe, 1982). However, data from my study indicate that the values for the ratio change with species, tissues and experimental conditions (i.e., acclimation period and feeding duration) (Chapters 3-6). For example, when the ratios fall below 0.4, levels of dietary 18:3n-3 are considered to be inadequate for the EFA requirement of Arctic charr but sufficient for most of the other salmonid species (Castell et al., 1972c; Watanabe et al. 1974a, b and c). The ratio, therefore, does not appear to be a sensitive indicator of the EFA status of Arctic charr, although the ratio did change as the dietary levels of n-3 and n-6 PUFAs were altered. Recently, Thongrod et al. (1990a, b) and Satoh et al. (1989) have also indicated these difficulties in using this ratio. However, the ratio of 20:3n-9 to 22:6n-3 is useful to evaluate the ability of fish to desaturate and elongate PUFAs de novo.

Haematocrit and haemoglobin values have also been used as indicators of EFA status for freshwater fish (Castell et al.,

1972b; Chapters 4-6). While haematocrit and haemoglobin values from the current study generally fell within the normal range, reported for salmonids, no correlation was found between the dietary levels of n-3 or n-6 PUFAs and haematocrit or haemoglobin values for either Arctic charr or rainbow trout (Chapters 4-6). Castell et al. (1972b) suggested that the lack of correlation may be related to the large variation of these parameters among individual fish. This is a less likely interpretation in the current study as values were determined on the basis of 20 individuals for each treatment. Perhaps, these two parameters are insensitive to changes in EFA metabolism and may not be a useful indicator of fish EFA status. On the other hand, previous studies have suggested that haematocrit and haemoglobin values are sensitive indicators of nutritional adequacy with respect to levels of many minerals and vitamins (Cho, 1985; Halver, 1989; Lall, 1989). By contrast, Arctic charr from this study did not show any differences in haematocrit and haemoglobin values when fed diets varying in levels of vitamin C, iron, copper, iron, zinc and magnesium. The dietary levels of vitamin C and minerals used in this study, therefore, were likely above the minimal levels required by Arctic charr (Chapter 7) and hence the haematological data are consistent with those for growth rates, feed efficiencies, tissue water and lipid content and fatty acid composition.

4. Variation in EFA requirements

1.) n-3 PUFAs

The n-3 PUFAs are required by coldwater fish and the EFA values of different types of n-3 PUFAs are known to vary among fish species (Watanabe and Takeuchi, 1977; Thongrod et al., 1990a, b). The present study revealed that 1.0 to 2.0% of 18:3n-3 is optimal in the diet for both Labrador and Nauyuk strains of Arctic charr (Chapters 3 and 6). This conclusion is supported by data which show that levels of total and neutral lipids in livers and of water content in the whole body are similar between charr receiving diets containing the foregoing levels of 18:3n-3 and those fed the 22:6n-3-rich commercial diet (Chapters 4 and 6). The experimental results also indicate that charr require 1.0 to 1.7% of 18:3n-3 in the presence of 0.5% 18:2n-6. A dietary level of 18:3n-3 of less than 1.5% was insufficient to reduce excessive lipid deposition in livers of the Nauyuk strain of Arctic charr. Consequently, the optimal amounts of dietary 18:3n-3 required by Arctic charr lie between 1.0% and 2.0%, in the absence or presence of dietary 18:2n-6 (Chapters 4 and 6).

This study also investigated EFA values of different types of n-3 long-chain PUFAs for Arctic charr (Chapters 3 and 4). The experimental results suggest that dietary 22:6n-3 may be more effective in promoting charr growth than 18:3n-3. While dietary 20:5n-3 and 18:3n-3 at 0.1%, was found to be equally effective for charr growth and feed utilization, it appears

that an EFA value of ≥0.4% of 20:5n-3 and 22:6n-3 will be equivalent for Arctic charr.

2.) n-6 PUFAs

Different dietary concentrations of n-6 PUFAs are known to affect growth, health and survival of freshwater fish (Watanabe, 1982; Ackman and Takeuchi, 1986; Ringo et al., 1990). Salmonids fed diets containing more than 1.0% dietary 18:2n-6, along with 1.0 to 2.5% 18:3n-3, have been reported to have reduced growth and survival (Yu and Sinnhuber, 1976, 1979; Ringo, 1989). Atlantic salmon smolt fed a commercial diet, rich in n-3 PUFAs but low in n-6 PUFAs, were observed to have poorer health and lower survival rates than wild fish (Ackman and Takeuchi, 1986). Data from this study suggest that diets containing adequate amounts of 18:3n-3 and less than 2.0% 18:2n-6 did not affect growth and food conversion of Arctic charr. By contrast, diets with less than 1.0% 18:2n-6 and 18:3n-3 had an additive effect on growth (Chapters 3, 4, 5 and 6). The data also revealed that large amounts of 20:4n-6 were present in tissue lipids of wild Arctic charr, and the level of 20:4n-6 was noted to be only secondary to percentages of 22:6n-3 (Chapter 5). However, diets containing more than 2.0% 18:2n-6 markedly inhibited charr growth (Chapter 6). Collectively, these data suggest that Arctic charr require 0.5 to 1.5% of dietary 18:2n-6 for maximum performance. In other words, the EFA requirements of juvenile charr appear to be

more similar to Atlantic, chum and chinook salmon than to rainbow trout.

3.) EFA and life stages

The EFA requirements of fish differ between their larval and post-larval stages (Kanazawa, 1985; Sargent et al., 1989). Fish usually require specific individual fatty acids as their EFAs throughout their life. By contrast, dietary phospholipids high in 20:5n-3 and 22:6n-3 are required by fish larvae to reach maximum growth and normal development (Kanazawa, 1985).

Since the majority of studies have used fish fingerlings to investigate EFA requirement, the EFA requirement defined by these studies may still be valid for fish at post-larval stages. It is generally believed that small fish (fry or juvenile) respond faster than large fish to nutritional variables and are also more sensitive to diet differences (Lovell, 1989). If young fish grow well on an experimental diet, it is likely that old fish will do equally as well. Interestingly, anadromous fish do not appear to change their EFA requirements with their environment, i.e., from freshwater and seawater and vice versa (Takeuchi and Watanabe, 1982). Consequently, it appears that the EFA requirement of juvenile Arctic charr as determined by the current study, will apply to all their life stages except larval charr.

4.) General trends in EFA requirements of freshwater fish

Extensive studies on vertebrates other than fish have revealed that both n-3 and n-6 PUFAs are EFAs (Simopoulos,

1991; Walkins, 1992). Available data for more than a dozen freshwater fish, indicate that qualitatively there are three types of EFA requirements, i.e., tilapia-type (n-6 PUFAs), rainbow trout-type (n-3 PUFAs) and eel-type (both n-3 and n-6 PUFAs) (Table 1). Although EFA requirements of freshwater fish are determined largely on the basis of their growth and feeding response to dietary PUFAs, doubts remain about whether fish belonging to the rainbow trout-type require only 18:3n-3. Cowey (1988) suggested that rainbow trout may require 18:2n-6 but the dietary amount needed may be too low to be detected by current methods. If this hypothesis is proven to be correct, it is likely that fish displaying the tilapia-type of EFA requirement may need 18:3n-3 in their diets. Perhaps, all freshwater fish, like other vertebrates, require both 18:2n-6 and 18:3n-3 as their EFAs.

Castell (1979) proposed that the dietary levels of n-3 PUFAs required by coldwater species such as rainbow trout should not be less than those needed by warmwater species. Castell (1979) argued that coldwater species need more PUFAs to maintain cellular membrane fluidity as they adapt to cold water environments. It follows, then, that Arctic charr, a freshwater species that has a more northern distribution than rainbow trout, would require higher percentages of n-3 PUFAs in their diets (Olsen et al., 1991). A number of studies generally support this view (Thongrod et al., 1990; Olsen et al., 1991; Chapters 5 and 6). Results from this study indicate

that charr require about 1.6% of 18:3n-3 in their diets, but this value overlaps with those reported for rainbow trout (Chapters 3 and 6; Castell et al., 1972; Watanabe et al., 1974). The levels of dietary 18:3n-3 required by a coldwater species, at least in the case of Arctic charr, may not be highly related with its environment, or they may be related to some other variables such as feeding habits and minimal temperature rather than length of time spent at low temperature.

5. Metabolism of dietary PUFAs

Efficient desaturation and elongation of 18:2n-6 and 18:3n-3 to long-chain PUFAs have been noted for a number of cultured freshwater fishes (Henderson and Tocher, 1987) and has been suggested for Arctic charr (Olsen et al., 1991; Olsen and Ringo, 1992). Results from this study support these findings but in addition, they revealed that the de novo conversion of 18:3n-3 becomes saturated in Arctic charr when they are fed diets containing 1.0 to 2.0% of 18:3n-3 (Chapters 4-6).

Prior to this study the relationship of Arctic charr and rainbow trout was unclear with respect to their PUFA metabolism. Data on lipid and fatty acid composition of both species indicate that Arctic charr are less able to convert dietary 18:3n-3 and 18:2n-6 than are rainbow trout (Chapter 5). Two factors may account for this species difference, namely, dissimilar retention or mobilization of fatty acids

and activities of the enzymes involved in the synthesis of long-chain PUFAs. Further comparisons of the species revealed that Arctic charr fed the PUFA-free diet may have a longer retention time for tissue 22:6n-3 than rainbow trout.

It is generally felt that experiments using varying levels of dietary PUFAs will provide information on the activities of enzymes involved in the desaturation and elongation of PUFAs (Henderson, 1987; Greene and Selivonchick, 1987). This study revealed that the level of 18:4n-3 in muscle of charr were directly related to level of dietary 18:3n-3, while levels of tissue 20:5n-3 and 22:6n-3 changed very little (Chapter 4). This finding differs from the results for liver polar lipids, and suggests that D^5 desaturase activity may be much lower in the muscle of charr than in the liver. Furthermore, the levels of 18:4n-3 in muscle is markedly different between charr and trout (Chapter 5). Perhaps this difference between charr and trout was due to feedback inhibition of endogenous 20:5n-3 and 22:6n-3 on the conversion of 18:3n-3 to 18:4n-3 which was more severe in the muscle of charr than that of trout. It may also be related to the adaptation of Arctic charr to their extremely cold environment, where much more energy is needed to maintain their normal membrane fluidity.

Few studies using fish have examined competitive inhibition between dietary 18:2n-6 and 18:3n-3 (Yu and Sinnhuber, 1976, 1979). This study examined competitive inhibition in the Labrador and Nauyuk strains of Arctic charr (Chapters 3, 4 and

6). The data indicated that 18:3n-3 inhibited the desaturation and elongation of 18:2n-6 and vice versa when 18:3n-3 or 18:2n-6 was the dominant dietary PUFA (Chapter 6). However, the inhibition of dietary 18:2n-6 over 18:3n-3 was found to be less effective than that of 18:3n-3 over 18:2n-6. This was especially apparent when both fatty acids were included in the diets at equal amounts (Chapter 6). My data suggest that the inhibition of n-3 PUFAs on n-6 PUFAs is greater at the D^4 desaturase step than at either D^5 or D^6 step for Arctic charr.

6. Summaries and perspectives

Data from my experiments revealed important nutritional and biochemical aspects of Arctic charr regarding their EFA requirements and lipid metabolism. First, this study conclusively demonstrated that 18:3n-3 is an EFA for both Labrador and Nauyuk strains of Arctic charr. The quantitative requirement for dietary 18:3n-3 is about 1.6% for both of these strains. Second, 0.4% of dietary 22:6n-3 is more effective as EFA than a similar level of dietary 18:3n-3. Third, charr, particularly the Nauyuk strain, appear to also require 18:2n-6 as their EFA. Forth, the metabolic responses of Arctic charr to dietary PUFAs differ markedly from those of rainbow trout, particularly the activities of D^5 and D^6 desaturases which are involved in the desaturation of n-3 and n-6 PUFAs. Fifth, there is competitive inhibition between dietary 18:2n-6 and 18:3n-3 in Arctic charr. From a practical point of view these results imply that a diet where 18:3n-3 is

dominant over 18:2n-6, is suitable in terms of meeting EFA requirements for cultured Labrador and Nauyuk strains of Arctic charr. The 18:3n-3-rich flax oil appears to be an ideal lipid source for formulating practical diets for Arctic charr.

This thesis has given some interesting leads for further research on lipid nutrition and metabolism of Arctic charr. The EFA values of two n-3 long-chain PUFAs, namely, 20:5n-3 and 22:6n-3, were reported to vary among salmonids. Since results from this study are based on the use of relatively low levels of these PUFAs in charr diets no conclusive data are available for the comparison of the EFA values for 20:5n-3, 22:6n-3 and 18:3n-3. Further studies with diets containing > 0.5% of these two PUFAs, will help answer this question. Furthermore, data from this thesis suggest that charr may also require n-6 PUFAs as EFAs. Additional experiments are needed to test this suggestion and these could involve diets containing varying levels of 18:2n-6 and 20:4n-6 in the presence or absence of 18:3n-3. Data from my thesis also indicate that Arctic charr have less efficient D^5 desaturase in muscle and liver than rainbow trout. This question could be addressed more thoroughly using <u>in vitro</u> assays to determine the activities of these enzymes.

General Conclusion

- Arctic charr fed diets inadequate in either n-3 or n-6 PUFAs exhibited poor growth and feed utilization, fatty livers, high HSI, elevated water content in muscle and whole body and substantial accumulation of 20:3n-9 in tissue polar lipids
- Increasing dietary concentrations of n-3 PUFAs up to 2.8% led to a marked increase in growth, feed efficiencies and tissue n-3 and n-6 long-chain PUFAs and a decrease in tissue 20:3n-9
- Charr achieved maximum growth and feed utilization when they were fed diets containing about 1.6% 18:3n-3 in the presence or absence of 18:2n-6
- Both Labrador and Nauyuk strains of Arctic charr efficiently converted 18:3n-3, 20:5n-3 and 18:2n-6 to long-chain PUFAs and maximum conversion of 18:3n-3 occurred after they were fed diets containing 1.0 to 2.0% 18:3n-3
- Less than 1.0% of dietary 18:2n-6 and 18:3n-3 had an additive effect on growth and feed conversion of Arctic charr
- Charr may require 0.5 to 1.0% 18:2n-6 in their diets for maximum performance
- Increasing dietary 18:2n-6 above 2.0% in the presence of 1.5% dietary 18:3n-3, inhibited growth of Arctic charr

- Feeding dietary 18:2n-6 or 18:3n-3 competitively inhibited each other's de novo conversion in Arctic charr
- Muscle of Arctic charr was less efficient in converting 18:4n-3 to long-chain PUFAs compared to the liver
- Charr were more sensitive to EFA deficiency and less efficient in converting 18:2n-6 or 18:3n-3 compared to rainbow trout
- Determination of the EFA requirements of charr was not affected by levels of other dietary ingredients including vitamin C, zinc, iron, copper, magnesium and arginine, at least at the levels used in this study

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Appendix 1. An example of particles size distribution of casein based test diets[1]

Particle size (mm)	Percentage
0.60 — 0.85	17.9
0.85 — 1.00	36.7
1.00 — 1.18	45.4

[1]Applied for charr weighting 3-5 g on average

Appendix 2. Fatty acid composition of liver polar lipids of Arctic charr fed the commercial diet separated by using thin layer chromatography and Sep-Pak silica cartridges (weight %)

Fatty acid	Method	
	TLC	Sep-Pak
12:0	tr[1]	tr
14:0	2.4	1.6
16:0	23.6	21.4
16:1n-9	0.7	0.3
16:1n-7	2.8	2.0
16:1n-5	0.4	0.2
18:0	1.9	4.1
18:1n-9	9.5	10.7
18:1n-7	2.6	3.4
18:1n-5	0.5	0.6
18:2n-6	2.3	2.5
18:3n-9	0.1	tr
18:3n-6	0.2	0.2
18:4n-6	0.2	0.1
18:3n-3	0.2	0.2
18:4n-3	0.1	0.1
20:0	0.1	0.1
20:1n-11	0.1	0.2
20:1n-9	1.3	2.1
20:1n-7	0.2	0.4
20:2n-6	0.3	0.5
20:3n-6	0.4	0.5
20:4n-6	1.4	2.7
20:4n-3	0.3	0.2
20:5n-3	6.9	5.3
22:1n-1	0.2	0.3
22:1n-9	0.1	0.1
22:1n-7	0.1	0.1
22:1n-5	tr	0.1
21:5n-3	0.1	0.1
22:4n-6	0.1	tr
22:5n-6	0.2	0.2
22:5n-3	1.0	1.0
22:6n-3	29.8	31.4
24:1n-9	0.9	1.6
24:1n-7	0.2	0.2

[1]Trace amount (<0.05)

Appendix 3. Retention time (RT) and weight percentages (%) of fatty acids in two separate standards (Standard A and B) run on gas chromatography (GC)

Fatty acid	Standard A				Standard B			
	RT		%		RT		%	
	R[1]	RR[2]	K[3]	C[4]	R	RR	K	C
14:0	3.97	0.30	1.0	1.0	-[5]	-	-	-
14:1n-5	-	-		-	4.41	0.33	9.1	9.1
15:1n-5	-	-	-	-	5.79	0.43	9.1	9.2
16:0	6.89	0.52	4.0	4.3	-	-	-	-
16:1n-7	-	-	-	-	7.51	0.56	9.1	9.1
18:0	12.96	0.97	3.0	3.0	-	-	-	-
18:1n-9	14.18	1.06	60.0	60.6	13.84	1.04	9.1	9.1
18:2n-6	16.13	1.21	12.0	11.9	16.08	1.21	9.1	9.2
18:3n-3	19.79	1.48	5.0	5.0	19.87	1.49	9.1	9.0
20:0	25.31	1.90	3.0	2.9	-	-	-	-
20:1n-9	26.63	2.00	1.0	1.0	26.93	2.02	9.1	9.0
20:4n-6	-	-	-	-	34.63	2.60	9.1	9.1
22:0	42.05	3.15	3.0	2.8	-	-	-	-
22:1n-9	43.43	3.26	5.0	4.8	43.58	3.27	9.1	8.9
24:0	57.54	4.32	3.0	2.6	-	-	-	-
22:6n-3	-	-	-	-	58.08	4.36	9.1	8.9
24:1n-9	-	-	-	-	59.11	4.43	9.1	8.9

[1]Rentention time of fatty acid esters on GC

[2]Relative rentention time of fatty acid esters on GC
[3]Percentage of fatty acid esters known for the purchased standards
[4]Percentage of fatty acid esters estimated from GC analysis by this study
[5]Absent

Appendix 4. Initial and final weights, and condition factor of Arctic charr fed diets varying in levels of 18:2n-6 and 18:3n-3 (Mean ± SEM, n = 2)[1]

Diet	Initial weight (g)	Final weight (g)	Condition factor
1	1.65 ± 0.01	6.67 ± 0.53	1.03 ± 0.07
2	1.63 ± 0.04	6.52 ± 0.50	0.97 ± 0.02
3	1.51 ± 0.08	7.05 ± 0.85	1.04 ± 0.06
4	1.49 ± 0.04	4.55 ± 0.27	0.92 ± 0.03
5	1.61 ± 0.08	6.77 ± 0.39	0.97 ± 0.01
6	1.66 ± 0.03	7.35 ± 0.31	1.01 ± 0.02
7	1.60 ± 0.01	8.19 ± 0.60	1.01 ± 0.00
8	1.57 ± 0.09	6.31 ± 0.53	0.95 ± 0.00
9	1.59 ± 0.06	6.48 ± 0.03	1.01 ± 0.03
10	1.75 ± 0.04	9.13 ± 0.28	1.03 ± 0.02
11	1.60 ± 0.05	8.94 ± 0.42	1.03 ± 0.01
12	1.66 ± 0.05	3.59 ± 0.26	0.87 ± 0.01
13	1.62 ± 0.07	9.55 ± 0.55	1.00 ± 0.02

[1]Lipid and fatty acid content of diets were shown in Tables 3 and 4