Fish Physiol Biochem (2013) 39:1441–1456
DOI 10.1007/s10695-013-9798-2

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

Stefanie M. Hixson · Christopher C. Parrish · Derek M. Anderson

Received: 14 February 2013 / Accepted: 7 April 2013 / Published online: 13 April 2013

Abstract Camelina (*Camelina sativa*) oil was tested as a replacement for fish oil in diets for farmed Atlantic cod (*Gadus morhua*). Camelina differs from other plant oilseeds previously used in aquaculture with high lipid (40 %), α-linolenic acid (40 %), antioxidants and low proportions of saturated fats. Dietary treatments were fed to cod (19 g $fish^{-1}$ initial weight) for 9 weeks and included a fish oil control (FO), 40 % (CO40) and 80 % (CO80) replacement of fish oil with camelina oil. There was no effect of replacing fish oil with camelina oil included at levels up to 80 % on the growth performance. Cod fed CO80 stored more lipid in the liver ($p < 0.01$), including more neutral lipid ($p < 0.05$) and triacylglycerol ($p < 0.05$). Cod fed CO80 decreased in total polyunsaturated fatty acids (PUFAs) in muscle compared to CO40 and FO ($p < 0.05$), increased in monounsaturated fatty acids ($p < 0.01$), decreased in total ω3 fatty acids (FO > CO40 > CO80; $p < 0.01$) and increased in total ω6 fatty acids (FO < CO40 < CO80; $p < 0.01$). In the liver, long-chain (LC) PUFA such as 20:4ω6, 20:5ω3, 22:5ω3 and 22:6ω3 decreased when fish oil was removed from the diet ($p < 0.05$), and increased in 18-carbon fatty acids ($p < 0.01$). Camelina oil can reduce the amount of fish oil needed to meet lipid requirements, although replacing 80 % of fish oil reduced LC PUFAs in both tissues. A comparison of BF_3 and H_2SO_4 as catalysts to transmethylate cod liver and muscle lipids revealed small but significant differences in some fatty acid proportions.

Keywords *Camelina sativa* · Lipid class · Fatty acid · Atlantic cod

Introduction

Fish is a significant source of ω3 long-chain polyunsaturated fatty acids (PUFAs) in the human diet. Long-chain PUFA, particularly 20:5ω3 (eicosapentaenoic acid, EPA) and 22:6ω3 (docosahexaenoic acid, DHA), are important for a range of beneficial functions including cardiovascular and inflammatory diseases and neurological disorders (Siriwardhana et al. 2012; Stanley et al. 2012; De Felice et al. 2012; Gertsik et al. 2012). Pressure from commercial capture fisheries has left most fish stocks exploited, and as a result, aquaculture now produces nearly half of the world's seafood (FAO 2012). Atlantic cod (*Gadus morhua*) in Newfoundland, Canada, is a clear example of a wild

Electronic supplementary material The online version of this article (doi:10.1007/s10695-013-9798-2) contains supplementary material, which is available to authorized users.

S. M. Hixson (✉) · C. C. Parrish
Department of Ocean Sciences, Memorial University of Newfoundland, 1 Marine Lab Road, St. John's, NL A1C 5S7, Canada
e-mail: scolombo@mun.ca

D. M. Anderson
Department of Plant and Animal Science, Faculty of Agriculture, Dalhousie University, Truro, NS, Canada

stock that experienced so much pressure that the fishery collapsed in the early 1990s (deYoung and Rose 1993; Myers et al. 1997; Rose et al. 2000). Since then, Atlantic cod farming has been of interest in this part of the world (Brown and Puvanendran 2002; Brown et al. 2003; Booman et al. 2011), as well as Norway, the UK, Iceland, Spain and Chile (Rosenlund and Skretting 2006).

The expansion of aquaculture, particularly farming of carnivorous species like cod, is placing demands on fish meal and fish oil, which raises questions regarding sustainability (Naylor et al. 2000). Fish oil, produced from wild fisheries, is used more by aquaculture than any other industry (Tacon and Metian 2008). Dependency on a limited resource like fish oil will limit growth of aquaculture in the future. Therefore, research into alternative sources of lipid, especially of terrestrial origin, is necessary to both relieve pressure on wild fish stocks and to allow growth in aquaculture (Pickova and Mørkøre 2007). Several studies have shown that replacing fish oils with various terrestrial plant oils and animal fats in diets for different marine species does not compromise growth or overall health. However, most terrestrial plant oils are composed mainly of ω6 and ω9 fatty acids and lack the critical long-chain ω3 PUFA that are abundant in fish oil, resulting in lower levels of 20:5ω3 and 22:6ω3 in tissues (Jobling et al. 2008; Bell et al. 2010; Alhazzaa et al. 2011), potentially compromising health benefits that are normally provided to humans that consume fish. Therefore, research in terrestrial plant oils with significant amounts of ω3 is of particular interest.

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

Cod express some genes involved in the production of 22:6ω3 and 20:5ω3 from shorter ω3 fatty acid chains (Tocher et al. 2006). Based on this premise, we hypothesized that cod fed camelina oil, high in 18:3ω3, may be able to sustain sufficient levels of 22:6ω3 and 20:5ω3 in their tissues after feeding a diet with reduced fish oil. It is well known that cod liver is the primary site of lipid deposition and storage and is lipid rich with primarily triacylglycerol (TAG); therefore, it is most frequently studied and is a tissue of interest when determining changes in fatty acid composition due to diet. Although low in total flesh lipid (1 %), cod fillet contains a high proportion of phospholipid that is beneficial for membrane fatty acid function as well as being important for growth, prevention of skeletal deformities and stress resistance (Shahidi and Dunajski 1994; Jobling et al. 2008). Therefore, changes in both liver and muscle tissue fatty acids (particularly 18:3ω3, 22:6ω3 and 20:5ω3) due to dietary influence were examined.

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

The overall objective of the study was to evaluate camelina oil as a suitable lipid resource to replace fish

oil in diets for farmed Atlantic cod. Specifically, a nutritional feeding trial was conducted with diets containing camelina oil in order to analyze changes in growth performance and lipid and tissue fatty acid composition in cod.

Methods

Camelina oil

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

Experimental diets

Three practical diets were produced at the Faculty of Agriculture Campus, Dalhousie University (Table 1). Diets were formulated to meet the nutritional requirements of gadoids based on previous formulations (Tibbetts et al. 2004, 2006) and were isonitrogenous and isolipidic. Camelina oil was used as the test ingredient in each of the experimental diets, and herring oil was used in the control diet (FO). Camelina oil replaced 40 % (CO40) or 80 % (CO80) of the total fish oil in two experimental diets. The maximum replacement level of 80 % was chosen because marine fish have essential fatty acid requirements (NRC 2011), which would not have been met if 100 % of fish oil was replaced, and therefore, health and welfare of cod was considered when formulating the diets. Experimental diets were steam pelleted using a laboratory pelleting mill (California Pellet Mill, San Francisco, USA). The initial size of pellet was 1.5 mm and was increased to 2.5 mm as the fish grew larger throughout each trial. Diets were stored at −20 °C until needed.

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

Ingredient (% of diet)	FO	CO40	CO80
Herring oil	4.6	2.75	0.9
Camelina oil	–	1.85	3.7
Herring meal	50.9	50.9	50.9
Wheat gluten meal	5.0	5.0	5.0
CPSP-G[a]	5.5	5.5	5.5
Wheat middlings	14.6	14.6	14.6
Whey powder	7.0	7.0	7.0
Krill hydrolysate	2.5	2.5	2.5
Corn starch (pre-gel)	5.6	5.6	5.6
Vitamin mixture[b]	1.95	1.95	1.95
Mineral mixture[c]	1.95	1.95	1.95
Choline chloride	0.3	0.3	0.3
Proximate composition analyzed, as-fed basis (n = 3)			
Moisture	10.1	9.6	9.3
Ash	2.1	1.9	2.3
Protein	48.7	48.8	49.4
Lipid	11.8	11.9	12.0

[a] Concentre proteique soluble de poisson (soluble fish protein concentrate)

[b] See supplementary material

[c] See supplementary material

Experimental fish

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

Sampling

Sampling occurred at week 0 (before experimental diets were fed), week 1, 4, and 9. Fish were starved for 24 h prior to sampling. Ten fish per tank were randomly sampled and measured for length and weight. The whole liver was removed, weighed and sampled for dry matter and lipid analysis. The skin was removed on the left side of the fish, and muscle tissue was subsampled for dry matter and lipid analysis. Lipid samples were stored on ice during sampling of each tank and were processed within an hour. Samples were collected in 50-ml test tubes that had been rinsed three times with methanol followed by three rinses with chloroform. The tubes were allowed to dry completely before they were weighed. The tubes were weighed again following the addition of the sample. After wet weights were recorded, samples were covered with 8 ml of chloroform (HPLC-grade), the headspace in the tube was filled with nitrogen, the Teflon-lined caps were sealed with Teflon tape, and the samples were stored at −20 °C.

Lipid extracts

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

Lipid class separation

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

Fatty acid methyl ester (FAME) derivatization

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

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

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

Statistical methods

Statistical analysis followed methods outlined by Sokal and Rohlf (1994). The statistical approach is another critical part of any experiment. In these types of experiments, committing a type II error (accepting a false null hypothesis) may lead to misleading conclusions regarding the use of alternative feed ingredients to fish meal/oil. In order to guard against this risk, this study tested growth data in different ways to ensure that type II errors were not committed, and that conclusions made about camelina oil truly reflected the results. For analysis of growth data, lipid class data, and fatty acid data, where individual fish were weighed, measured and sampled, a three-way nested ANOVA was performed using the general linear model (Minitab 16 Statistical Software, State College, Pennsylvania, USA). The model was designed to test the effect of diet on the response variable and nested fish individuals within tanks to negate variability among tanks and individuals, while also testing for tank effects. For analysis of growth data that depend on comparison to an initial measurement and thus must be pooled per tank (i.e., mean weight gain and specific growth rate), a two-way ANOVA was performed to test the effect of diet and tank variability. In both cases, where significant differences occurred, treatment means were differentiated using the Tukey's HSD multiple comparison test and all residuals were evaluated for homogeneity and normality. For analysis of growth data, a *t* test was additionally performed between FO and CO80 (highest camelina replacement) to verify results from the ANOVA. For analysis of derivatization methods, a *t* test was used to compare the derivatization efficiency per tissue and a one-way ANOVA was used to compare tissue fatty acid proportions after derivatization by either method. To analyze differences between initial and final fatty acids in muscle and liver, a two-sample *t* test was performed to test between the initial and final CO80 fatty acids. For each model tested, the residuals were examined to evaluate the appropriateness of the model; therefore, normality, homogeneity and independence of residuals were considered. If a *p* value was close to 0.05 and residuals were not normal, a p-randomization was conducted >1,000 times to test the data empirically.

Results

Camelina oil fatty acid composition

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

Experimental diet composition

Diets ranged in moisture content from 9.3 to 10.1 % and ash from 1.9 to 2.3 % (Table 1). Protein was 49 % and lipid ranged from 11.8 to 12.0 %. The lipid was composed mainly of triacylglycerol (47 %) (Table 2). In terms of fatty acids, generally the control and CO80 diet differed significantly, mainly in terrestrial-type

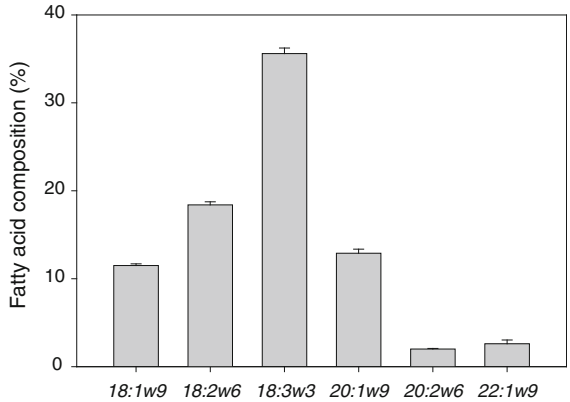

Fig. 1 Fatty acid composition (%) of *Camelina sativa* oil (mean ± SD; $n = 3$)

Table 2 Lipid class and fatty acid composition of control and experimental diets

Lipid class	FO	CO40	CO80	*F* stat	*p* value
(% ww^{-1})					
Neutral lipid	7.0 ± 0.4	7.4 ± 4.5	7.9 ± 1.1	3.01	0.13
Polar lipid	4.5 ± 0.6	4.5 ± 0.9	4.3 ± 0.2	2.96	0.13
(% total lipid)					
Triacylglycerol	46.6 ± 3.9	47.0 ± 3.9	47.0 ± 4.5	2.59	0.16
Free fatty acid	3.8 ± 0.3^{a}	5.1 ± 1.0^{a}	8.4 ± 1.6^{b}	21.1	0.02
Sterol	2.9 ± 1.0^{a}	4.7 ± 1.8^{b}	7.0 ± 0.8^{c}	7.48	0.02
AMPL[1]	3.8 ± 0.6^{a}	8.4 ± 1.4^{b}	3.7 ± 0.9^{a}	10.1	0.02
Phospholipid	29.7 ± 4.1	29.6 ± 5.9	24.1 ± 1.8	1.68	0.26
Fatty acid[2]					
14:0	4.4 ± 0.6	4.4 ± 0.6	3.9 ± 0.2	2.60	0.15
16:0	20.0 ± 0.4^{a}	19.4 ± 0.5^{a}	16.9 ± 1.3^{b}	10.6	0.01
16:1ω9	5.1 ± 0.5^{a}	5.0 ± 0.5^{a}	3.5 ± 0.2^{b}	3.80	0.01
18:0	3.1 ± 0.7	2.6 ± 0.03	2.4 ± 0.06	2.41	0.17
18:1ω9	11.5 ± 0.6^{a}	12.2 ± 0.5^{ab}	13.2 ± 0.1^{b}	5.11	0.04
18:2ω6	10.7 ± 1.0^{a}	13.0 ± 0.7^{ab}	14.3 ± 0.2^{b}	7.67	0.01
18:3ω3	1.1 ± 0.8^{a}	3.7 ± 0.6^{b}	7.3 ± 1.1^{c}	47.1	<0.001
20:1ω9	5.7 ± 1.4^{a}	5.3 ± 0.3^{a}	6.6 ± 0.4^{b}	42.5	<0.001
20:4ω6	0.6 ± 0.006	0.6 ± 0.004	0.5 ± 0.002	4.86	0.06
20:5ω3	8.9 ± 0.2	8.6 ± 0.4	7.0 ± 0.3	5.06	0.06
22:1ω9	5.7 ± 2.4	3.8 ± 0.6	4.4 ± 1.3	1.38	0.32
22:5ω3	0.9 ± 0.007	0.7 ± 0.008	0.7 ± 0.01	5.12	0.15
22:6ω3	10.5 ± 0.8	10.1 ± 0.2	9.3 ± 0.1	0.34	0.72
∑SFA[3]	27.9 ± 0.3^{a}	26.7 ± 0.5^{a}	23.5 ± 0.4^{b}	14.1	0.01
∑MUFA[4]	33.5 ± 1.2	31.2 ± 1.2	33.0 ± 0.8	0.55	0.60
∑PUFA[5]	37.3 ± 2.5	40.5 ± 0.9	41.2 ± 1.5	1.68	0.30
∑ω3	23.2 ± 1.4	24.6 ± 0.5	25.0 ± 0.9	0.56	0.60
∑ω6	11.7 ± 1.7^{a}	13.9 ± 0.8^{ab}	15.3 ± 0.8^{b}	7.08	0.03
ω3/ω6	2.0 ± 0.1^{a}	1.8 ± 0.1^{ab}	1.6 ± 0.2^{b}	5.44	0.04

Values are mean ($n = 9$) ± SD. Means within rows with different superscripts indicate significant differences

[1] Acetone-mobile polar lipid

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

[3] Saturated fatty acid

[4] Monounsaturated fatty acid

[5] Polyunsaturated fatty acid

fatty acids (18:3ω3, 18:2ω6, 18:1ω9). Total saturated fatty acids (SFAs) were significantly lower in the CO80 diet compared to CO40 and FO. Total ω3 fatty acids did not differ among diets, although 18:3ω3 increased significantly with the inclusion of camelina oil. Total ω6 fatty acids were significantly higher in the CO80 diet than the control diet; however, CO40 did not differ from either diet. The ω3/ω6 ratio varied significantly between CO80 and the control, but CO40 ω3/ω6 was not different from the FO and CO80 diets. There was no significant difference in total PUFA among diets.

Growth performance

The mean initial weight of the fish ranged from 18.9 to 19.5 g fish^{-1}, and there was no significant difference in fish weights among the treatment groups or tanks ($F = 0.41$; $p = 0.87$) (Table 3). The mean initial length of the fish ranged from 12.1 to 12.3 cm fish^{-1} and did not differ significantly among treatments or tanks ($F = 0.74$; 0.62). After the 9-week feeding trial, the fish more than doubled their weight (126–135 %), reaching a mean final mass of 45.4 g fish^{-1} (FO), 44.5 g fish^{-1} (CO40) and 44.0 g fish^{-1} (CO80). There was no significant difference in final weight among treatments or tanks ($F = 1.5$; $p = 0.18$). The fish gained 24.6–26.0 g fish^{-1}; however, the difference was not significant. Since weight gain showed the maximum change between diets, the minimum detectable difference was calculated. This determines the difference between means that could have been significantly different. Given the same variance and

Table 3 Growth performance of Atlantic cod fed experimental diets after 9 weeks

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

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

[a] *t* tests were conducted on final measurements between FO and CO80

[b] Specific growth rate = 100 × [ln (final body weight)—ln(initial body weight)]/days

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

[d] Hepatosomatic index = 100 × (liver mass/body mass)

sample size as in the original data, a two-sample *t* test was performed with two different means until a significant difference was observed. Each time a *t* test was performed the difference between the two means was increased. The difference between FO mean weight gain (26.0) and CO80 weight gain (24.6) was 1.4 g. If the weight gain of FO fish was 33.0 ± 2.9 and CO80 fish was 16.6 ± 5.8 ($n = 3$), the difference would be 16.4 g (*t* stat = 4.38; $p = 0.048$). A difference of 15.4 g between means was not significant (*t* stat = 4.11; $p = 0.054$). Even tripling the sample size from 3 tanks per diet to 9 tanks per diet, there would not be a significant difference given the same mean and variance (*t* stat = 0.65; $p = 0.53$). Therefore, we can conclude that the weight gain of fish is the same, and the difference would have to increase 16-fold before it would become significant. The mean final length was measured at approximately 16.0 cm, but there was no significant difference among groups or tanks ($F = 1.06$; $p = 0.39$). The mean specific growth rate (SGR) did not vary significantly among groups: it ranged from 1.23 to 1.29 % day^{-1}. The mean condition factor (CF) was 1.08 to 1.13; however, a significant difference was not detected among groups or tanks ($F = 1.24$; $p = 0.30$). The mean hepatosomatic index (HSI) ranged from 8.5 to 8.8 %, but the difference was not significant, and there were no significant differences among tanks ($F = 1.87$; $p = 0.10$). A two-sample *t* test was conducted between FO and CO80 for all growth parameters, since these groups tended to be the most different; however, there were no significant differences between these two treatments for any measurement. There were 2 mortalities recorded from separate tanks during the trial.

Lipid class tissue composition

Muscle

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

Liver

Initial liver total lipid was 45 %, composed of neutral lipid (29–32 %) and polar lipid (12–16 %) (Table 5). Lipid classes were TAG (72 %), AMPL (10 %), PL (7 %) and ST (3 %). After 9 weeks of feeding, liver

Table 4 Lipid class and fatty acid composition of juvenile Atlantic cod muscle tissue after 9 weeks of growth

Lipid class	Initial	FO	CO40	CO80	*F* stat	*p* value
(% ww^{-1})						
Total lipid	1.2 ± 0.1	0.7 ± 0.1	0.9 ± 0.3	0.7 ± 0.2	1.1	0.25
Neutral lipid	0.2 ± 0.01	0.1 ± 0.03	0.2 ± 0.1	0.1 ± 0.03	2.5	0.16
Polar lipid	1.1 ± 0.1	0.5 ± 0.01	0.6 ± 0.2	0.6 ± 0.2	1.1	0.34
(% total lipid)						
Steryl esters	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	0.3 ± 0.02	0.6	0.20
Triacylglycerol	3.4 ± 0.9	1.2 ± 0.5[a]	4.0 ± 1.4[b]	1.0 ± 0.3[a]	7.9	0.003
Free fatty acid	0.2 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.5 ± 0.3	0.3	0.71
Sterol	7.4 ± 2.2	8.9 ± 1.5	10.0 ± 2.0	10.0 ± 1.1	0.2	0.86
AMPL[1]	3.2 ± 1.3	2.5 ± 1.7[a]	6.1 ± 2.0[b]	2.9 ± 0.4[a]	6.9	0.004
Phospholipid	82.7 ± 4.8	79.4 ± 0.5	72.2 ± 4.4	80.0 ± 2.0	2.1	0.14
Fatty acid[2]						
14:0	1.2 ± 0.1	1.3 ± 0.2	1.2 ± 0.1	1.2 ± 0.4	0.3	0.73
16:0	17.1 ± 0.9	17.0 ± 0.6[a]	16.2 ± 0.7[b]	17.0 ± 1.6[a]	5.5	0.04
16:1ω7	2.1 ± 0.5	2.2 ± 0.3	2.1 ± 0.2	2.1 ± 0.7	2.6	0.15
18:0	3.9 ± 0.4	3.1 ± 0.3	3.3 ± 0.2	3.4 ± 0.3	1.7	0.26
18:1ω9	9.1 ± 0.8	8.7 ± 0.7[a]	9.5 ± 0.8[a]	11.2 ± 1.7[b]	10.9	0.01
18:2ω6	4.1 ± 0.7	5.1 ± 0.7[a]	6.3 ± 0.5[b]	8.5 ± 1.3[c]	54.1	<0.001
18:3ω3	0.6 ± 0.2	1.3 ± 0.6[a]	2.7 ± 0.3[b]	4.7 ± 0.7[c]	104	<0.001
18:4ω3	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.03	0.5 ± 0.1	0.3	0.75
20:1ω9	1.6 ± 0.2	1.6 ± 0.1[a]	1.7 ± 0.1[b]	1.9 ± 0.2[c]	17.3	0.003
20:4ω6	1.7 ± 0.1	1.6 ± 0.1[a]	1.5 ± 0.1[ab]	1.4 ± 0.1[b]	12.1	0.001
20:2ω6	0.3 ± 0.06	0.3 ± 0.06[a]	0.4 ± 0.06[a]	0.6 ± 0.03[b]	38.6	<0.001
20:3ω3	–	0.02 ± 0.06[a]	0.1 ± 0.04[b]	0.3 ± 0.1[c]	64.0	<0.001
20:5ω3	18.8 ± 0.8	19.0 ± 0.5[a]	17.5 ± 0.8[b]	15.5 ± 0.8[c]	123	<0.001
22:1ω9	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	3.4	0.1
22:5ω6	0.4 ± 0.04	0.4 ± 0.04[a]	0.4 ± 0.04[a]	0.2 ± 0.03[b]	21.9	0.001
22:5ω3	3.0 ± 0.6	0.4 ± 0.04	0.3 ± 0.04	0.3 ± 0.1	0.9	0.43
22:6ω3	27.7 ± 1.7	28.0 ± 2.0[a]	26.7 ± 1.2[a]	25.0 ± 2.0[b]	14.0	0.004
$\sum$SFA[3]	22.9 ± 1.7	25.7 ± 0.8	23.7 ± 0.7	24.0 ± 1.6	0.5	0.65
$\sum$MUFA[4]	17.2 ± 3.2	16.2 ± 1.2[a]	16.6 ± 0.8[ab]	18.5 ± 2.3[b]	14.0	<0.001
$\sum$PUFA[5]	58.8 ± 0.8	61.3 ± 0.8[a]	61.0 ± 0.7[a]	60.5 ± 2.6[b]	5.9	0.03
$\sum$ω3	51.7 ± 1.3	49.9 ± 1.2[a]	49.0 ± 0.6[b]	47.9 ± 1.2[c]	17.0	0.003
$\sum$ω6	6.5 ± 0.7	7.5 ± 0.6[a]	8.5 ± 0.5[b]	10.7 ± 1.2[c]	59.7	<0.001
ω3/ω6	8.0 ± 0.7	6.7 ± 0.7[a]	5.8 ± 0.4[b]	4.4 ± 0.9[c]	34.1	< 0.001
Terrestrial[6]	4.7 ± 0.6	6.4 ± 0.6[a]	8.7 ± 0.4[b]	10.7 ± 1.2[c]	77.7	< 0.001

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

[1] Acetone-mobile polar lipid

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

[3] Saturated fatty acid

[4] Monounsaturated fatty acid

[5] Polyunsaturated fatty acid

[6] Terrestrial = 18:2ω6 + 18:3ω3

Table 5 Lipid class and fatty acid composition of Atlantic cod liver tissue after 9 weeks of growth

	Initial	FO	CO40	CO80	*F* stat	*p* value
(% ww^{-1})						
Total lipid	44.8 ± 7.0	43.5 ± 16[a]	36.0 ± 10[a]	58.4 ± 17[b]	5.4	0.01
Neutral lipid	31.1 ± 9.7	38.7 ± 15[a]	32.7 ± 10[a]	50.9 ± 12[b]	4.1	0.02
Polar lipid	13.4 ± 4.2	4.7 ± 2.3	7.5 ± 5.1	3.4 ± 2.1	3.4	0.06
(*% total lipid*)						
Steryl esters	–	–	0.4 ± 0.1	0.1 ± 0.1	3.0	0.07
Triacylglycerol	72.1 ± 10	75.8 ± 11[a]	71.2 ± 11[a]	81.8 ± 4.0[b]	3.7	0.04
Free fatty acid	–	0.3 ± 0.1	1.0 ± 0.1	1.4 ± 1.0	2.1	0.14
Sterol	3.0 ± 1.1	10.7 ± 3.0[a]	7.8 ± 2.0[ab]	4.2 ± 1.3[b]	6.5	0.03
AMPL[1]	10.2 ± 4.0	8.5 ± 1.2[a]	6.4 ± 2.2[b]	4.0 ± 1.8[b]	10.0	0.001
Phospholipid	6.9 ± 3.0	2.7 ± 1.3	5.4 ± 1.4	3.5 ± 1.0	1.3	0.29
Fatty acid[2]						
14:0	3.2 ± 0.5	3.2 ± 0.15[a]	2.9 ± 0.3[b]	2.6 ± 0.3[c]	71.4	<0.01
16:0	14.3 ± 0.4	13.7 ± 0.7[a]	13.3 ± 0.5[ab]	12.6 ± 1.1[b]	9.6	0.01
16:1ω7	6.7 ± 0.2	6.2 ± 0.3[a]	5.6 ± 0.5[b]	4.5 ± 1.9[b]	27.7	<0.001
18:0	5.1 ± 0.5	4.5 ± 0.4	4.4 ± 0.6	3.9 ± 1.6	1.2	0.36
18:1ω9	18.9 ± 0.9	19.0 ± 0.6[a]	20.6 ± 1.1[b]	20.5 ± 1.0[b]	9.6	0.01
18:2ω6	5.7 ± 0.3	7.1 ± 0.7[a]	8.6 ± 1.2[b]	8.5 ± 1.2[b]	24.3	<0.001
18:3ω6	0.2 ± 0.01	0.1 ± 0.03[a]	0.2 ± 0.02[ab]	0.2 ± 0.06[b]	9.4	0.01
18:3ω3	0.8 ± 0.3	2.3 ± 0.9[a]	4.4 ± 1.6[b]	4.7 ± 1.9[c]	29.1	<0.001
18:4ω3	1.2 ± 0.1	1.2 ± 0.1[a]	1.3 ± 0.2[b]	1.3 ± 0.2[b]	8.2	0.02
20:1ω9	4.4 ± 0.5	6.1 ± 0.3	6.5 ± 0.8	6.6 ± 1.0	2.4	0.17
20:2ω6	–	0.3 ± 0.1[a]	0.4 ± 0.1[b]	0.5 ± 0.1[b]	7.1	0.03
20:4ω6	0.6 ± 0.1	0.5 ± 0.1[a]	0.3 ± 0.2[b]	0.4 ± 0.1[c]	5.9	0.04
20:3ω3	–	0.1 ± 0.04[a]	0.1 ± 0.1[a]	0.3 ± 0.1[b]	8.2	0.02
20:5ω3	12.3 ± 0.6	10.0 ± 0.7[a]	8.8 ± 1.2[b]	8.1 ± 1.5[c]	18.1	0.002
22:1ω9	3.4 ± 0.6	4.3 ± 0.4	3.6 ± 0.6	4.0 ± 0.8	3.8	0.09
22:5ω3	1.7 ± 0.3	1.6 ± 0.1[a]	1.3 ± 0.2[b]	1.2 ± 0.2[b]	32.7	<0.001
22:6ω3	7.2 ± 0.5	7.4 ± 0.4[a]	6.7 ± 0.7[ab]	6.5 ± 0.9[b]	11.7	0.01
∑SFA[3]	24.1 ± 0.7	22.2 ± 1.2[a]	21.1 ± 0.9[ab]	19.7 ± 2.9[b]	7.6	0.02
∑MUFA[4]	40.0 ± 1.0	42.5 ± 0.5	42.7 ± 1.0	42.8 ± 2.5	0.2	0.8
∑PUFA[5]	35.7 ± 1.4	34.9 ± 1.5	35.7 ± 0.7	35.2 ± 0.7	2.6	0.15
∑ω3	25.0 ± 1.0	23.5 ± 0.8	23.3 ± 0.9	22.8 ± 1.2	2.2	0.2
∑ω6	6.9 ± 0.5	8.7 ± 0.4[a]	9.8 ± 1.2[b]	9.7 ± 1.3[b]	23.3	<0.001
ω3/ω6	3.7 ± 0.3	2.8 ± 0.2[a]	2.4 ± 0.3[b]	2.4 ± 0.3[b]	22.6	0.001
Terrestrial[6]	6.6 ± 0.4	9.2 ± 1.9[a]	13.0 ± 2.8[b]	13.2 ± 1.3[b]	29.5	0.001

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

[1] Acetone-mobile polar lipid

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

[3] Saturated fatty acid

[4] Monounsaturated fatty acid

[5] Polyunsaturated fatty acid

[6] Terrestrial = 18:2ω6 + 18:3ω3

TAG was significantly higher in CO80 (82 %) than CO40 (71 %) and FO (76 %). Liver AMPL was significantly lower in CO40 (6 %) and CO80 (4 %) groups compared to the FO (9 %). There was a significant difference in ST, with a decreasing trend as fish oil was removed from the diets. There was no difference in PL between camelina-fed and fish oil-fed livers. Total lipid and neutral lipid increased significantly in CO80 compared to CO40 and FO. There were no significant differences among tanks for any of the lipid classes in liver tissue.

Fatty acid tissue composition

Muscle

Initial samples were taken after cod were fed the control diet for 1 week, and prior to the control diet, all fish were fed a fish meal/fish oil-based commercial diet of similar composition. Initially in the muscle, PUFA was the dominant fatty acid group, accounting for 59 % of total fatty acids, followed by SFA (23 %) and MUFA (17 %). Individual fatty acid proportions were typical for cod muscle after consuming a commercial-type diet (Shahidi and Dunajski 1994). Terrestrial-type fatty acids were relatively low, such as 18:1ω9 (9 %), 18:2ω6 (4 %) and 18:3ω3 (<1 %), whereas known marine-type fatty acids were higher, particularly 20:5ω3 (19 %), 22:5ω3 (3 %), 22:6ω3 (28 %).

After 9 weeks of feeding camelina diets, total PUFA decreased in CO80 compared to CO40 and FO, and MUFA increased with camelina inclusion (Table 4). SFA remained the same among groups after feeding the camelina diets. Total ω3 fatty acids decreased significantly as camelina oil was included in the diet (FO > CO40 > CO80) and ω6 fatty acids increased significantly. As a result, the ω3/ω6 ratio decreased significantly with inclusion of camelina oil from 7 to 4. The trend of dietary fatty acid affecting the corresponding fatty acid in the flesh was found for several individual fatty acids. The total "terrestrial" fatty acids (18:2ω6 + 18:3ω3) increased significantly in the muscle with inclusion of camelina oil; 20:5ω3 and 22:6ω3, as 22:6ω3 in CO80 fed cod, were significantly lower than the control and CO40. Long-chain ω6 fatty acids (≥20 carbons) significantly decreased in the CO80 diet compared to the control, with the exception of 20:2ω6; 20:3ω3 significantly increased and tripled its amount in the flesh in CO80 compared to the control and doubled in CO40. There were no significant differences among tanks for any of the fatty acids in muscle tissue.

Liver

Initially, MUFA was the predominant fatty acid group (40 %) in the liver, followed by PUFA (36 %) and SFA (24 %) (Table 5). The ω3 fatty acids were 25 % and ω6 were 7 %, which makes the ω3/ω6 ratio 7:1.

After 9 weeks of feeding, SFA was significantly lower in CO80 livers than FO from 22 to 20 % (Table 5). There was no difference in the total MUFA, PUFA, ω3 and the ω3/ω6 ratio. However, including camelina (both CO40 and CO80) significantly increased the total amount of ω6 from 9 to 10 % and terrestrial fatty acids (18:2ω6 + 18:3ω3) from 9 to 13 %; 18:3ω3 and 18:2ω6 both increased significantly with the inclusion of camelina. Generally in the liver, long-chain fatty acids (≥20 carbons) tended to decrease when fish oil was removed from the diet, for example, 20:4ω6, 20:5ω3, 22:5ω3 and 22:6ω3. There was one exception to this trend, however, 20:3ω3 was significantly higher in CO80 than the FO and CO40. There were no significant differences among tanks for any fatty acids in muscle tissue.

The difference between initial and final fatty acid compositions of cod fed CO80 in the muscle and liver for selected fatty acids was calculated by subtracting final fatty acid (%) by the mean initial fatty acid (%) (Table 6). All selected fatty acids in the muscle were significantly different from the initial fatty acid composition, except the sums of MUFA and PUFA. In the liver, all selected fatty acids were significantly different from the initial fatty acid composition, except 22:6ω3 and total PUFA.

Derivatization efficiency

Liver and muscle samples were derivatized using both BF_3 and H_2SO_4 methods. The derivatization efficiency using BF_3 or H_2SO_4 on either liver or muscle tissue ranged from 93 to 94 %, except when liver was derivatized using BF_3, and the efficiency was significantly lower (34 %) than using H_2SO_4 (Table 7). TAG remaining in liver BF_3 derivatives caused low efficiency ratios. There were small but significant differences in fatty acid proportions in liver tissue,

Table 6 Differences in fatty acid composition and the change ratio between initial and final samples of cod muscle and liver samples in fish fed CO80

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

Difference = Fatty acid % (Final)−Fatty acid % (Initial)

Fatty acids with +Δ Factor = Fatty acid % (Final)/Fatty acid % (Initial); Fatty acids with −Δ Factor = Fatty acid % (Initial)/Fatty acid % (Final)

depending on derivatization method (Table 8). Total PUFA ($F = 7.4$; $p = 0.02$), 20:5ω3 ($F = 14.0$; $p = 0.002$), 22:6ω3 ($F = 5.6$; $p = 0.03$) were significantly lower in BF_3 derivatized samples than H_2SO_4 samples. Muscle tissue fatty acids proportions, however, were not significantly affected by derivatization method.

Discussion

Up to 80 % of fish oil was replaced with camelina oil to evaluate this new potential lipid source in diets for farmed Atlantic cod. The most abundant fatty acids in camelina oil were 18:3ω3, 18:2ω6, 18:1ω9 and 20:1ω9. The comparable PUFA and ω3 content between camelina and fish oil gives camelina oil potential as a lipid source in diets for farmed cod. There was no effect of replacing fish oil with dietary camelina oil included at levels up to 80 % on the growth of Atlantic cod in this experiment. Several different types of vegetable oils and blends have been used to replace fish oil for Atlantic cod such as echium oil (Bell et al. 2006), soybean oil (Mørkøre et al. 2007), rapeseed, linseed, palm oil blend (Tocher et al. 2006; Jobling et al. 2008) and camelina oil blend in salmon (Bell et al. 2010), which did not show a significant difference in growth among dietary treatments. After the present study was conducted, a study was published that showed that cod fed camelina oil diets for 12 weeks did not show any significant differences in growth performance (Morais et al. 2012), which confirms growth results found in our experiment. Although growth performance results were similar between these two studies, the diets used

Table 7 Fatty acid methyl ester (FAME) derivatization efficiency comparing H_2SO_4 and BF_3 catalysts

Catalyst	Tissue	Extract acyl lipids[1]	Derivative acyl lipids[2]	% Derivatized	*t* stat	*p* value
H_2SO_4	Liver	13.8 ± 0.9	0.8 ± 0.04	93.8 ± 4.0[a]	23.5	<0.001
BF_3	Liver	14.8 ± 0.6	9.8 ± 0.5	34.2 ± 10[b]		
H_2SO_4	Muscle	12.1 ± 0.4	0.8 ± 0.1	93.4 ± 10	−0.2	0.84
BF_3	Muscle	11.7 ± 0.4	0.7 ± 0.2	94.1 ± 9.0		

Derivatization efficiency = [1−(derivative acyl lipids/extract acyl lipids)]×100 ($n = 18$)

[1] Extract acyl lipids is the total amount of extract acyl lipids prior to derivatization

[2] Derivative acyl lipids is the remaining amount of acyl lipid that did not transmethylate after the derivatization procedure

Table 8 Variation in proportions of fatty acids (%) in Atlantic cod muscle and liver tissue fed a fish oil diet using either H_2SO_4 or BF_3 as a fatty acid methyl ester (FAME) derivatization catalyst

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

Data expressed as area percentage of FAME (fatty acid methyl ester), values are mean ($n = 10$) ± SD. Means with different superscripts indicate significant differences

[1] Saturated fatty acid

[2] Monounsaturated fatty acid

[3] Polyunsaturated fatty acid

in the present study were more practical in comparison. The formulations were designed to be used in a commercial setting, with a reduction in fish meal and use of alternative ingredients (Crampton and Carr 2012), and consideration of essential fatty acid requirements of the fish without removing 100 % of fish oil (NRC 2011), compared to diets that would likely only be used in a research setting. In this study, growth rate, condition factor and hepatosomatic index of the fish were typical for farmed cod (Jobling 1988).

The lipid class profile of the feeds varied depending on camelina inclusion. The diets were formulated to be isolipidic, so the total lipid was the same among diets (12 %), composed of mainly TAG (47 %) and PL (29 %). Lipid storage differed in both liver and muscle tissue, depending on the lipid composition of the diet. In the liver, cod fed CO80 contained significantly more lipid, neutral lipid and TAG than CO40 and FO groups, despite all treatments being fed isolipidous diets. The HSI was the same among groups (8–9 %), which is a typical range for farmed cod (Lie et al. 1986), suggesting that the excess lipid stored in livers of CO80 did not affect the total liver weight. Cod are known to store excess dietary lipids in the form of TAG in the liver. Neutral lipids like TAG are known to more readily respond to changes in dietary lipid than polar lipids (Sargent et al. 1989; Higgs and Dong 2000). Although the diets in this study were not significantly different in the proportion of TAG, there are differences in the ways in which the lipids in fish oil and plant oil are digested, absorbed and metabolized (Jobling et al. 2008). Fish fed different vegetable oils have been found to store more neutral lipid than fish fed diets with fish oil (Wijekoon 2012). Salmon fed a vegetable oil blend containing 20 % camelina oil had significantly higher lipid deposition and increased TAG content (Leaver et al. 2011). The results in this study suggest that high amounts of camelina oil in the diet are stored as TAG in the liver rather than metabolized for energy compared to the control. Feeding this diet for extended time may have increased the liver weight. Fish fed CO80 had lower ST proportions compared to the fish fed FO, despite higher sterol proportions in the diet. The ST amount in CO80 was still lower than FO when considering higher total liver lipid in CO80. Cholesterol is the most

common ST in animal lipid (Tocher 2003) and is a major component of cell membranes, while some is stored together with neutral lipids. Phytosterols in plant oils like camelina are considered phytoestrogens, and they interfere with cholesterol synthesis. Several studies have investigated the efficiency of phytosterols in lowering cholesterol in fish (Pickova and Mørkøre 2007). A study by Mørkøre (2006) found that cod fed soy oil had significantly lower levels of cholesterol in the liver compared to the cod fed fish oil. It is most likely that the higher ST content found in CO80 lowered levels of cholesterol in cod liver fed CO80. Since cholesterol is a precursor of steroid hormones, the change in sterol content will impact on steroid synthesis in fish, affecting their reproductive development (Trembley and van der Kraak 1999). Whether this mechanism can be used in farming to delay reproductive maturation of cod has not yet been studied.

The changes observed in the fatty acid profile after feeding camelina oil to cod were expected, given that we know feeding vegetable oil to marine fish increases C_{18} fatty acids in the flesh and decreases in total ω3 fatty acids, 22:6ω3 and 20:5ω3 (Turchini and Mailer 2010). In this particular study, camelina oil is quite high in 18:3ω3; therefore, significant increases in this fatty acid were observed in liver and muscle, a result that was also observed in a recent study using camelina oil (Morais et al. 2012). Fatty acid profiles of the liver and muscle showed similar trends in terms of increases and decreases in particular fatty acids; however, the proportions were different as is expected for different tissues (Sargant et al. 1989; Tocher 2003). Camelina oil significantly increased C_{18} fatty acids, and the loss of fish oil decreased 20:5ω3, 22:6ω3 and total ω3, although uptake of 18:3ω3 was much greater than the loss of 20:5ω3 and 22:6ω3. Bell et al. (2003) and Stubhaug et al. (2007) have shown that fish are capable of sequestering of ω3 PUFA, mainly 20:5ω3 and 22:6ω3 when fed diets deficient of such fatty acids. Fish are capable of utilizing surplus dietary fatty acids for β-oxidation and energy production while preserving essential and limited ω3 PUFAs in membranes. A study in red sea bream (*Pagrus auratus*) found retention of essential long-chain PUFAs, like 20:5ω3 and 22:6ω3, when fed rapeseed or soy oil (Glencross et al. 2003). Fish can selectively mobilize or incorporate fatty acids in response to physiological demand. Therefore, subtle, yet significant changes in 20:5ω3 and 22:6ω3 are due to selective conservation, and more abundant fatty acids such as 18:1ω9 are used for energy production. However, it is unknown how long fish can sustain these levels of essential long-chain PUFA if it is not provided in the diet.

Differences between initial and final fatty acid composition of CO80 fish seemed to vary depending on fatty acid rather than tissue type. Diet appeared to have equally affected muscle and liver fatty acid compositions, contrary to the idea that neutral lipids (liver) tend to respond to dietary lipids more than polar lipids (muscle) (Tocher 2003). For example, 22:6ω3 loss in the muscle was 5 times greater than in the liver; however, the proportion of 22:6ω3 was over 25 % in the muscle and less than 7 % in the liver. Therefore, the ratio change of individual fatty acids should be considered since the change is relative to the initial composition. In general, the factor by which a fatty acid changes due to camelina inclusion was similar for both liver and muscle. The results suggest that storage and sequestering of fatty acids are more dependent on the fatty acid type rather than the tissue in which it is stored. Change ratios for 18:2ω6, 18:3ω3 and ω6 were greater than change ratios for 20:5ω3, 22:6ω3, PUFA and ω3, which supports the idea that some degree of selectivity and conservation of 22:6ω3, 20:5ω3 and PUFA occurred since the losses were small but still significant, and the loss was smaller than the uptake of C_{18} fatty acids. Sequestering ω3 fatty acids has been studied in cold water marine fish when essential ω3 fatty acids are limited in the diet and surplus fatty acids are used for energy production (Bell et al. 2003; Stubhaug et al. 2007; Leaver et al. 2011; Wijekoon 2012).

We find evidence that fatty acid synthesis has occurred, specifically fatty acid elongation, to produce 20:3ω3 which was not present in the diet or in initial tissue samples. In the CO80 group, muscle tissue contained 16 times more 20:3ω3 than FO tissue. The presence of this fatty acid suggests that elongation occurred from 18:3ω3 to 20:3ω3 but not to 20:5ω3. It is not likely that reaching 20:3 is a rate limiting step in the ω3 pathway, since 20:3ω3 does not accumulate in either tissue. This result has previously been observed with cod and salmon fed camelina oil (Morais et al. 2012; Bell et al. 2010). Cod expressed PUFA elongase in white muscle tissue, hepatocytes and enterocytes when fed a vegetable oil diet containing camelina; however, desaturase expression was low (Tocher et al.

2006). After injection of radiolabelled 18:2ω6 and 18:3ω3, radioactive 20:3ω3 and 20:2ω6 were recovered in Arctic charr (*Salvelinus alpinus*), rainbow trout, gilthead sea bream (*Sparus aurata)* and golden gray mullet (*Liza aurata*) (Olsen and Ringø 1992; Mourente and Tocher 1998). Elongation occurred, however, desaturation did not. The 22:6ω3 and 20:5ω3 provided from the minimal fish oil, and fish meal supplied in the CO80 was likely enough to sustain and conserve these essential fatty acids rather than encourage fatty acid synthesis to meet requirements. Bell et al. (2006) reasoned that the poor ability of marine fish to synthesize long-chain PUFA is not due to lack of delta 6 desaturase, but rather to deficiencies in other parts of the biosynthetic pathway.

No single procedure is suitable for derivatization of all types of lipids (Christie 1993), so investigation of the results of fatty acid analysis applying different techniques to different samples is necessary. This study found using certain methods for transmethylation can be inappropriate for certain sample types and will affect the amount of fatty acids that are actually derivatized, although it has little effect on the proportion. Using H_2SO_4 to derivatize cod liver was 94 % efficient in converting acyl lipids to fatty acid methyl ester lipids. However, using BF_3 to derivatize cod liver was only 34 % efficient, due to incomplete derivatization of TAG. In order to ensure equal opportunity for complete derivatization between both methods, similar amounts of acyl lipids were derivatized for each method and tissue. Also, the amount of reagent was increased from 1 ml reagent per 4–16 mg (Morrison and Smith 1964) to 1.5 ml reagent to ensure complete transmethylation for 14 mg of lipid, so it is unlikely that the low derivatization efficiency observed using BF_3 was due to methodology. Schlechtriem et al. (2008) found that H_2SO_4 transmethylation of salmon flesh resulted in a complete derivatization of TAG and all PL classes; however, after using the BF_3 method, the fatty acid methyl ester proportion was only 33.5 %. As for muscle tissue, derivatization efficiency was the same using either derivatization method. The method also did not affect the fatty acid proportions (%) of muscle tissue; however, there were significant differences between methods for liver fatty acid proportion. The BF_3 method tended to underestimate the proportion of long-chain PUFA, namely 20:5ω3, 22:6ω3 and total PUFA, and seemed to overestimate certain SFAs, for example, 14:0, 16:0. The results of this study suggest that using BF_3 to derivatize samples that are lipid rich and high in TAG is inefficient and will significantly underestimate both the amount and the proportion of the same fatty acids that are in the initial lipid sample. Christie (1993) stated that certain classes of simple lipids, such TAG, are not soluble in BF_3 alone, and an inert solvent must be added to ensure their solution and derivatization. This study shows that incomplete derivatization of a lipid sample significantly affects the amount of fatty acids in a sample, in this case when using BF_3 to derivatize cod liver, a sample which is high in lipid (50 % ww^{-1}) and high in TAG (80 %). The use of BF_3 is appropriate for samples that are low in lipid, such as cod muscle, and has also been verified with several other low lipid and proportionally lower TAG marine samples by Budge and Parrish (2003) such as blue mussels (*Mytilus edulis*), green algae (*Nannochloropsis sp*.) and flagellates (*Isochrysis galbana*). This study concludes that BF_3 is an inappropriate catalyst to transmethylate cod liver lipid and should be avoided for that purpose; therefore, all cod livers in this study were derivatized using H_2SO_4.

Although feeding high levels of CO did not prevent reductions in 20:5ω3 and 22:6ω3 compared to fish fed FO, the increased levels of 18:3ω3 in flesh are useful as it is an essential fatty acid for humans (Burdge 2006; Brenna et al. 2009; Morais et al. 2012). It is a concern, however, that currently there is no regulation to include nutrition labels on farmed seafood when sold to consumers. Alternative plant oils, like camelina, reduce the amount of 22:6ω3 and 20:5ω3 in fish fillets, which may be deceiving to consumers that purchase fish for health purposes. Selectively breeding different camelina lines will help improve amino acid and fatty acid profiles as well as reduce antinutritional factors for improved camelina meal and oil. It may also be possible to selectively breed fish families that can efficiently utilize dietary plant oils without significant losses of 22:6ω3 and 20:5ω3 levels in the flesh. Using camelina oil in commercial diets for cod is an appropriate lipid source and will help to reduce the amount of fish oil needed to meet lipid requirements, particularly since growth performance is unaffected; however, replacing 80 % of fish oil with camelina oil will reduce essential long-chain PUFAs. Long-term studies are needed to determine whether losses of 20:5ω3 and 22:6ω3 are enough to affect final amounts in fillets harvested for market.

Acknowledgments This study was supported by Genome Atlantic, Atlantic Canada Opportunities Agency (ACOA)—Atlantic Innovation Fund (AIF), and Research and Development Corporation of Newfoundland (RDC). The authors would like to acknowledge Dr. Matthew Rise for conceptual contribution to the project; the Joe Brown Aquaculture Research Building staff for rearing and maintenance of cod; Jeanette Wells, John Heath and Breanne Coady for technical support; Dr. Marije Booman, and Charles Feng for assistance with fish sampling.

References

Alhazzaa R, Bridle A, Nichols P, Carter C (2011) Replacing dietary fish oil with echium oil enriched barramundi with C18 PUFA rather than long-chain PUFA. Aquaculture 312:162–171

Bell J, McGhee F, Campbell P, Sargent J (2003) Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (Salmo salar): changes in flesh fatty acid composition and effectiveness of subsequent fish oil "washout". Aquaculture 218:515–528

Bell G, Strachan F, Good J, Tocher D (2006) Effect of dietary echium oil on growth, fatty acid composition and metabolism, gill prostaglandin production and macrophage activity in Atlantic cod (*Gadus morhua* L.). Aquacult Res 37:606–617

Bell G, Pratoomyot J, Strachan F, Henderson R, Fontanillas R, Hebard A, Guy D, Hunter D, Tocher D (2010) Growth, flesh adiposity and fatty acid composition of Atlantic salmon (*Salmo salar*) families with contrasting flesh adiposity: effects of replacement of dietary fish oil with vegetable oils. Aquaculture 306:225–232

Booman M, Borza T, Feng C, Hori T, Higgins B, Culf A, Leger D, Chute I, Belkaid A, Rise M, Gamperl K, Hubert S, Kimball J, Ouellette R, Johnson S, Bowman S, Rise M (2011) Development and experimental validation of a 20 K Atlantic cod (*Gadus morhua*) oligonucleotide microarray based on a collection of over 150,000 ESTs. Mar Biotechnol 13:733–750

Brenna J, Salem N, Sinclair A, Cunnane S (2009) α-Linolenic acid supplementation and conversion to $n-3$ long-chain polyunsaturated fatty acids in humans. Prostaglandins Leukot Essent Fatty Acids 80:85–91

Brown J, Puvanendran V (2002) Development of Atlantic cod (*Gadus morhua*) larviculture in Newfoundland. Bul Aquac Assoc Can 1:5–7

Brown J, Minkoff G, Puvanendran V (2003) Larviculture of Atlantic cod (*Gadus morhua*): progress, protocols and problems. Aquaculture 227:357–372

Budge S, Parrish C (2003) FA determination in cold water marine samples. Lipids 38:781–791

Burdge G (2006) Metabolism of α-linolenic acid in humans. Prostaglandins Leukot Essent Fatty Acids 75:161–168

Crampton V, Carr, I (2012) Spotlight 5: Fish Forever. EWOS publication. Bergen, Norway. pp 5–14. http://www.ewos.com/wps/wcm/connect/ewos-content-group/ewos-group/resources/ewos-spotlight. Accessed 14 Feb 2013

Christie W (1993) Preparation of derivatives of fatty acids for chromatographic analyses, inadvances in lipid methodology—two. In: Christie W (ed) Lipids. Oily Press, Ayr, Scotland, pp 62–112

De Felice C, Signorini C, Durand T, Ciccoli L, Leoncini S, D'Esposito M, Filosa S, Oger C, Guy A, Bultel-Ponce V, Galano J, Pecorelli A, De Felice L, Valacchi G, Hayek J (2012) Partial rescue of Rett syndrome by ω-3 polyunsaturated fatty acids (PUFAs) oil. Genes Nutr 7:447–458

deYoung B, Rose G (1993) On recruitment and distribution of Atlantic Cod (*Gadus morhua*) off Newfoundland. Can J Fish Aquat Sci 50:2729–2741

FAO (Food and Agriculture Organization) (2012) World review of fisheries and aquaculture. In: State of the world fisheries and aquaculture 2012. (FAO Fish Tech Paper). Rome, p 209

Gertsik L, Russell P, Bresee C, Rapaport M (2012) Omega-3 fatty acid augmentation of citalopram treatment for patients with major depressive disorder. J Clin Psychopharmacol 32:61–64

Glencross B, Hawkins W, Curnow J (2003) Evaluation of canola oils as alternative lipid resources in diets for juvenile red sea bream, *Pagrus auratus*. Aquac Nutr 9:305–315

Higgs D, Dong F (2000) Lipids and fatty acids. In: Stickney R (ed) Encyclopedia of aquaculture. Wiley, New York, pp 476–496

Jobling M (1988) A review of the physiological and nutritional energetics of cod, *Gadus morhua* L., with particular reference to growth under farmed conditions. Aquaculture 70:1–19

Jobling M, Leknes O, Saether B, Bendiksen A (2008) Lipid and fatty acid dynamics in Atlantic cod, *Gadus morhua*, tissues: influence of dietary lipid concentrations and feed oil sources. Aquaculture 281:87–94

Leaver M, Taggart J, Villeneuve L, Bron J, Guy D, Bishop S, Houston R, Matika O, Tocher D (2011) Heritability and mechanisms of $n-3$ long chain polyunsaturated fatty acid deposition in the flesh of Atlantic salmon. Comp Biochem Physiol D 6:62–69

Lie Ø, Lied E, Lambertson G (1986) Liver retention of fat and of fatty acids in cod (*Gadus morhua*) fed different oils. Aquaculture 59:187–196

Morais S, Edvardsen R, Tocher D, Bell G (2012) Transcriptomic analyses of intestinal gene expression of juvenile Atlantic cod (*Gadus morhua*) fed diets with Camelina oil as replacement for fish oil. Comp Biochem Physiol B 161:283–293

Mørkøre T (2006) Relevance of dietary oil source for contraction and quality of pre-rigor filleted Atlantic cod, *Gadus morhua*. Aquaculture 251:56–65

Mørkøre T, Netteberg C, Johnsson L, Pickova J (2007) Impact of dietary oil source on product quality of farmed Atlantic cod, *Gadus morhua*. Aquaculture 267:236–247

Morrison W, Smith L (1964) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res 5:600–608

Mourente G, Tocher D (1998) The in vivo incorporation and metabolism of [1-14C] linolenate (18:3n–3) in liver, brain and eyes of juveniles of rainbow trout Oncorhynchus mykiss L. and gilthead sea bream Sparus aurata L. Fish Physiol Biochem 18:149–165

Myers R, Hutchings J, Barrowman N (1997) Why do fish stocks collapse? The example of cod in Atlantic Canada. Ecol Appl 7:91–106

National Research Council (NRC) (2011) Nutrient requirements of fish and shrimp. National Academies Press, Washington, DC

Naylor R, Goldburg R, Primavera J, Kautsky N, Beveridge M, Clay J, Folke C, Lubchenco J, Mooney H, Troell M (2000) Effect of aquaculture on world fish supplies. Nature 405:1017–1024

Ni Eidhin D, Burke J, O'Beirne D (2003) Oxidative stability of ω3-rich camelina oil and camelina oil-based spread compared with plant and fish oils and sunflower spread. J Food Sci 68:345–353

Olsen R, Ringø E (1992) Lipids of Arctic charr, *Salvelinus alpinus* (L.) II. Influence of dietary fatty acids on the elongation and desaturation of linoleic and linolenic acid. Fish Physiol Biochem 9:393–399

Parrish C (1987) Separation of aquatic lipid classes by Chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. Can J Fish Aquat Sci 44:722–731

Parrish C (1999) Determination of total lipid, lipid classes and fatty acids in aquatic samples. In: Arts M, Wainman B (eds) Lipids in freshwater ecosystems. Springer, New York, pp 4–10

Pickova J, Mørkøre T (2007) Alternate oils in fish feeds. Eur J Lipid Sci Technol 109:256–263

Rose G, deYoung B, Kulka D, Goddard S, Fletcher G (2000) Distribution shifts and overfishing of the northern cod (*Gadus morhua*): a view from the ocean. Can J Fish Aquat Sci 57:644–663

Rosenlund G, Skretting M (2006) Worldwide status and perspective on gadoid culture. ICES J Mar Sci 63:194–197

Sargent J, Henderson R, Tocher D (1989) The lipids. In: Halver J (ed) Fish nutrition, 2nd edn. Academic Press, San Diego, pp 153–218

Schlechtriem C, Henderson R, Tocher D (2008) A critical assessment of different transmethylation procedures commonly employed in the fatty acid analysis of aquatic organisms. Limnol Oceanogr Methods 6:523–531

Shahidi F, Dunajski E (1994) Lipid fatty acids, growth and compositional characteristics of farmed cod (*Gadus morhua*). J Food Lipids 1:265–271

Siriwardhana N, Kalupahana N, Moustaid-Moussa N (2012) Health benefits of $n-3$ polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid. In: Kim S (ed) Advances in food and nutrition research: marine medicinal foods: implications and applications: animals and microbes, vol 65. Elsevier, Oxford, pp 211–219

Sokal R, Rohlf F (1994) Biometry. W. H. Freeman Company, New York, USA

Stanley W, Dabkowski E, Ribeiro R, O'Connell K (2012) Dietary fat and heart failure: moving from lipotoxicity to lipoprotection. Circ Res 110:764–776

Stubhaug I, Lie O, Torstensen B (2007) Fatty acid productive value and β-oxidation capacity in Atlantic salmon tissues (*Salmo salar* L.) fed on different lipid sources along the whole growth period. Aquac Nutr 13:145–155

Tacon A, Metian M (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. Aquaculture 285:146–158

Tibbetts S, Lall S, Milley J (2004) Apparent digestibility of common feed ingredients by juvenile haddock, *Melanogrammus aeglefinus* L. Aquac Res 35:643–651

Tibbetts S, Milley J, Lall S (2006) Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758). Aquaculture 261:1314–1327

Tocher D (2003) Metabolism and functions of lipids and fatty acids in teleost fish. Res Fish Sci 11:107–184

Tocher D, Zheng X, Schlechtriem C, Hastings N, Dick J, Teale A (2006) Highly unsaturated fatty acid synthesis in marine fish: cloning, functional characterization, and nutritional regulation of fatty acyl Δ6 desaturase of Atlantic cod (*Gadus morhua* L.). Lipids 41:1003–1016

Trembley L, van der Kraak G (1999) Comparison between the effects of the phytosterols β-sitosterol and pulp and paper mill effluents on sexually immature rainbow trout. Environ Toxicol Chem 17:679–686

Turchini G, Mailer R (2010) Rapeseed (canola) and other monounsaturated fatty acid-rich vegetable oils. In: Turchini G, Ng W, Tocher D (eds) Fish oil replacement and alternative lipid sources in aquaculture feeds. CRC Press, Boca Raton, Florida, p 161

Wijekoon M (2012) Effect of water temperature and diet on cell membrane fluidity and fatty acid composition of muscle, liver, gill and intestine mucosa of adult and juvenile steelhead trout, *Oncorhynchus mykiss*. Dissertation. Memorial University of Newfoundland. St. John's, Newfoundland, Canada

Zubr J (1997) Oil-seed crop: *Camelina sativa*. Ind Crop Prod 6:113–119