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Use of camelina oil to replace fish oil in diets for farmed salmonids and Atlantic cod

Stefanie M. Hixson [a,*], Christopher C. Parrish [a], Derek M. Anderson [b]

[a] Department of Ocean Sciences, Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, Canada
[b] Faculty of Agriculture, Dalhousie University, Truro, Nova Scotia, Canada

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ABSTRACT

In this study growth and lipid composition of Atlantic salmon, rainbow trout and Atlantic cod were compared after feeding a diet containing camelina oil (CO) as a full replacement of fish oil (FO). CO is an interesting candidate for replacement due to its high total lipid content (40%) and a high amount of the ω3 precursor, 18:3ω3; high levels of mono- and polyunsaturated fatty acids, as well as significant amounts of γ-tocopherol, a potent antioxidant. Diets were formulated to meet the nutritional requirements for each species, and either contained FO or CO as the main lipid source. Three separate experiments were conducted (triplicate tanks for FO and CO): Atlantic salmon (mean initial weight 242 ± 46 g) were fed experimental diets for 16 weeks in seawater at 14 °C, rainbow trout (44.9 ± 10 g) were fed for 12 weeks in freshwater at 14 °C, and Atlantic cod (14.4 ± 1.6 g) were fed for 13 weeks at 10 °C. Growth performance of Atlantic cod was significantly affected by dietary CO in comparison to cod fed the FO diet ($p < 0.05$); however, growth performance of the salmonids was generally unaffected by consuming dietary CO. The lipid class composition of salmonid muscle tissue compared with cod muscle tissue had an important effect on tissue fatty acid composition for each species. As such, the fatty acid profiles of salmonids fed CO were different to those in cod fed CO based on results from ANOSIM, SIMPER, cluster analysis and principal components analysis. These results exemplify selective retention of long chain ω3 polyunsaturated fatty acids in the phospholipid membranes in cod muscle tissue compared to salmonid muscle tissue, which also has a lipid storage function. Regression analysis including all species showed significant linear relationships between the tissue concentration and dietary concentration of 22:6ω3, 20:5ω3, 18:3ω3 and 18:2ω6 ($p < 0.001$). The fatty acid biosynthesis capacities of these species fed CO were estimated by a fatty acid mass balance method, which found that Atlantic salmon and rainbow trout were able to desaturate and elongate ~25% and 23%, respectively, of their own long chain polyunsaturated fatty acids from dietary 18:3ω3. This was significantly greater than the elongation of 18:3ω3 in Atlantic cod (6.1%) ($p < 0.001$). In terms of both growth performance and utilisation of CO towards fatty acid biosynthesis, it appears that dietary CO is more appropriate for salmonid species than gadids.

1. Introduction

Alternatives to using fish oil (FO) in aquaculture feeds have been researched intensively in the last decade. This research has influenced the commercial feed industry to reduce the level of FO in feeds and replace it with more sustainable, terrestrial plant oils (Crampton and Carr, 2012). To date, there are several lipid sources that have been the focus of research studies and could be used as a lipid source in commercial feeds, such as soybean, canola, linseed, sunflower, and palm oil (Turchini et al., 2009). Many of these options are excellent sources of energy in the diet, because substituting FO with these alternatives usually does not affect growth. However, most terrestrial plant oils lack sufficient levels of ω3 polyunsaturated fatty acids (PUFA), they tend to be high in ω6 PUFA, and they do not naturally contain eicosapentaenoic acid (20:5ω3, EPA) and docosahexaenoic acid (22:6ω3, DHA). This inevitably results in low levels of essential ω3 PUFA in the tissues, which can eventually compromise fish health (Turchini et al., 2009) and ultimately strips some of the health benefits for humans that are associated with consuming fish (Karakatsouli, 2012). Although this area has been researched extensively, significant work in this field is still needed to achieve the successful replacement of FO, in particular investigating FO alternatives that are high in ω3 PUFA and are appropriate for diverse species of farmed teleosts.

The oilseed *Camelina sativa* is of interest from both an aquaculture and agronomy perspectives. Camelina is a member of the Cruciferae (Brassicaceae) family, which includes mustards, rapes, broccoli, cabbage, collards, cauliflower and many weeds (Budin et al., 1995). It

* Corresponding author at: Department of Ocean Sciences, Memorial University of Newfoundland, 1 Marine Lab Road, St. John's, Newfoundland and Labrador, Canada A1C 5S7. Tel.: +1 709 864 2521.
E-mail address: scolombo@mun.ca (S.M. Hixson).

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requires minimal input for growth, grows well in semi-arid regions and in low-fertility and saline soils, is tolerant of insects and weeds and can also be cast at low seeding rates onto frozen ground and can survive frost and freeze-thaw cycles after emergence during late winter and spring (Putnam et al., 1993). The oilseed is particularly unique due to its high total lipid content (40%) and a high amount of the ω3 precursor α-linolenic acid (18:3ω3, ALA), comprising up to 30% of the total fatty acids (Ni Eidhin et al., 2003). Camelina oil (CO) contains greater levels of ω3 PUFA than most plant oils commonly used in aquaculture feeds, which could give it nutritional and commercial advantages over the currently available plant oils, such as canola, corn, soybean or palm oil. It also contains high levels of monounsaturated fatty acids (MUFA) and PUFA, as well as significant amounts of γ-tocopherol, a potent antioxidant. Camelina oil is sufficiently stable to constitute a technically and economically competitive alternative to FO as a source of ω3 PUFA (Ni Eidhin et al., 2003).

The use of CO in diets for farmed fish has been investigated for different fish species at different levels to replace fish oil (Bell et al., 2010; Leaver et al., 2011; Morais et al., 2012); however, like many studies in this area, each experiment is stand-alone and the breadth of information is accumulated based on a single species, and therefore lacks a complete synthesis of information on how a new lipid source will be utilised by different teleost families typical in the aquaculture industry. Based on their evolutionary history and lipid metabolism, salmonids and gadids are diverse teleost families. Therefore, their metabolism and utilisation of different dietary lipids should be different and this should be considered before including various alternative lipid sources in their diets, particularly if it is based on results from another teleost family. Species are rarely compared and contrasted in the same body of work in order to accomplish a full perspective on using a new lipid source in feeds such as CO. This information is useful as a model for species-specific lipid metabolism and also for industrial applications. This present study is a culmination of a series of individual experiments (Hixson and Parrish, 2014; Hixson et al., 2014a; Hixson et al., 2014b) presenting new data and findings which build upon the previous studies, including fully quantitative comparisons of lipid concentrations. The purpose of this study was to: 1) compare the use of CO in diets for different farmed fish species in terms of their evolutionary history and lipid metabolism, 2) compare differences in lipid storage and fatty acid metabolism and synthesis in salmonids and gadids, and 3) compare utilisation of CO between salmonids and gadids and their potential for fatty acid biosynthesis when fed a diet containing CO.

2. Methods

2.1. Experimental diets

Camelina (Calena cultivar) was grown and harvested by the Department of Plant and Animal Sciences, Faculty of Agriculture, Dalhousie University at an off-campus location (Canning, Nova Scotia, Canada). The seeds were single pressed using a KEK 0500 press at Atlantic Oilseed Processing, Ltd. (Summerside, Prince Edward Island, Canada) to extract the oil and ethoxyquin was added to the final product as an antioxidant. Camelina oil is composed of triacylglycerol (50%), free fatty acid (20%), and 10% each of sterol, acetone-mobile polar lipid and phospholipid; with ALA (30%), linoleic acid (18:2ω6; LNA) (24%), 18:1ω9 (15%), and 20:1ω9 (11%) as the major fatty acids (Hixson et al., 2014a).

Diets were formulated as isonitrogenous and iso-energetic, and were produced at the Faculty of Agriculture, Dalhousie University (Truro, Nova Scotia, Canada). The lipid component of the diets was either from FO (control diet) or from CO (100% of FO was replaced by CO). Diets were formulated to meet the nutritional requirements of gadids and salmonids based on previous formulations (Tibbetts et al. 2004, 2006) and the National Research Council (2011). All diets were steam pelleted using a laboratory size pellet mill (California Pellet Mill, San Francisco, USA). Diet formulations (Electronic supplementary material), proximate composition and lipid and fatty acid composition of the diets for rainbow trout (Hixson et al., 2014a), Atlantic salmon (Hixson et al., 2014b) and Atlantic cod (Hixson and Parrish, 2014) have been summarised previously.

2.2. Experimental fish – Atlantic salmon

An experiment was conducted with salmon smolts in seawater at 14 °C (233.5 ± 46 g fish^{-1} mean initial weight; 26.5 ± 1.8 cm mean initial length) at the Ocean Sciences Centre (Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, Canada). Fish were received from Cooke Aquaculture (St. Alban's, Newfoundland and Labrador, Canada). The salmon (Saint John River stock) were transferred from the hatchery (freshwater) to the Ocean Sciences Centre, Joe Brown Aquatic Research Building (JBARB) (seawater) to undergo smoltification. The guidelines for the ethical treatment of fish were according to the Canadian Council of Animal Care (Memorial University Institutional Animal Care Protocol Approved 12-50-MR). The smolts were randomly distributed (300 total) into experimental tanks (500 L), with triplicate tanks per treatment (6 total) and 50 fish per tank. The fish were acclimated on the control diet for one week prior to initial sampling. Throughout the trial, a flow-through system of filtered seawater was supplied to each tank at a rate of 12 L min^{-1} and the photoperiod was 12 hours. The dissolved oxygen (10 mg L^{-1}) and water temperature (14 °C) were monitored daily. Fish were fed to apparent satiation twice daily and feed consumption was recorded weekly, for a total of 16 weeks. Mortalities were weighed and recorded throughout the trial.

2.3. Experimental fish – rainbow trout

An experiment was conducted with juvenile rainbow trout (44.9 ± 10 g fish^{-1} mean initial weight; 15.7 ± 1.2 cm mean initial length) at the Faculty of Agriculture, Dalhousie University (CCAC, 2011–016). Fish were received from Fraser Mill's hatchery (Antigonish, Nova Scotia, Canada). Fish were randomly distributed (558 total) into experimental tanks (200 L capacity), with triplicate tanks per treatment (6 total) and 93 fish per tank. The fish were acclimated on the control diet for one week prior to initial sampling. A flow through system of freshwater was supplied to each tank at a rate of 10 L min^{-1} and the photoperiod was 12 hours. The dissolved oxygen (10 mg L^{-1}) and water temperature (14 °C) were monitored daily. Fish were fed to apparent satiation twice daily and feed consumption was recorded weekly, for a total of 12 weeks. Mortalities were weighed and recorded throughout the trial.

2.4. Experimental fish – Atlantic cod

An experiment was conducted with juvenile cod (14.4 ± 1.6 g fish^{-1}, mean initial weight; 11.3 ± 0.4 cm, mean initial length) at the Ocean Sciences Centre, Memorial University of Newfoundland where fish were cultured from hatch and reared to initial experimental size. Fish were randomly distributed (420 total) into experimental tanks (500 L capacity), with triplicate tanks per treatment and 70 fish per tank. Ethical treatment of fish in this experiment was followed the guidelines of the Canadian Council of Animal Care (Memorial University Institutional Animal Care Protocol Approved 12-50-MR). The fish were acclimated on the control diet for one week before initial sampling. A flow-through system of 1 μm filtered seawater was supplied to each tank at a rate of 8 L min^{-1} and the photoperiod was 12 hours. The dissolved oxygen (10 mg L^{-1}) and water temperature (10 °C) were monitored daily. Fish were fed to apparent satiation twice daily and feed consumption was recorded weekly, for a total of 13 weeks. Mortalities were weighed and recorded throughout the trial.

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