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

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ABSTRACT

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

1. Introduction

Seafood is a major source of long chain (LC) ω3 polyunsaturated fatty acids (PUFA) in the human diet. Aquaculture supplies nearly half of the world's seafood supply, which requires a steady and sustainable supply of feed ingredients (FAO, 2012). Fish oil (FO) is a critical lipid source in feeds for aquaculture, which is highly dependent on wild fisheries. Availability, sustainability and cost are concerns for future use of FO in aquaculture. The FO supply is under severe pressure from a number of industries including pharmaceutical, agricultural, functional foods and aquaculture (Turchini, Torstensen, & Ng, 2009); therefore its availability and cost are inconsistent. Several different terrestrial oilseeds are commercially used in fish feeds (Turchini et al., 2009), however only a small proportion of FO can be replaced by these alternative oils due to their lack of LC ω3 PUFA. The ideal FO replacement should have a fatty acid composition that is highly digestible and should also provide high levels of precursor ω3 fatty acids for biosynthesis of LC ω3 PUFA and low levels of ω6 PUFA to maintain a high ω3/ω6 ratio which is beneficial for fish and human health (Torstensen et al., 2005).

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

Replacing FO with vegetable oils (VO) has been studied extensively in Atlantic salmon. These alternative oils can usually replace 100% of FO without compromising growth, feed intake and feed conversion; for example rapeseed oil, palm oil, linseed oil or soy oil. However levels of DHA and EPA in the fillet normally reduce

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to a fraction of that compared to salmon that were fed FO (Bell, Tocher, Henderson, Dick, & Crampton, 2003; Menoyo, Lopez-Bote, Obach, & Bautista, 2005). This affects the flesh nutritional quality for human consumption with implications for human health (Midtbø et al., 2013). Other quality aspects of Atlantic salmon fillet such as sensory quality, flesh texture and colour have been affected by dietary VO (Regost, Jakobsen, & Rora, 2004; Torstensen et al., 2005). Consideration of the final product is very important when investigating alternative diet sources in order to adhere to a general standard for consumer health and benefit.

Commercial feed companies replace portions of both fish meal (FM) and FO with vegetable sources (Crampton, Carr, & Norway, 2012). In order to test the effect of CO in a practical diet in a commercial setting, replacing FO with CO with the partial replacement of plant meal should be considered. Camelina meal (CM) is also considered as a fish meal replacement, on account of its crude protein level (45%), inclusion of some essential amino acids and its availability after oil extraction and has not yet been tested in diets for salmon. CO has replaced FO in diets for Atlantic cod (Hixson, Parrish, & Anderson, 2013; Morais, Edvardsen, Tocher, & Bell, 2012) and CO was also included in a VO blend (20%) for Atlantic salmon (Bell et al., 2010); however a full replacement has yet to be tested with Atlantic salmon. This was also the first study to evaluate the sensory quality of any fish species fed camelina oil. Therefore, the purpose of this study was to evaluate camelina oil as a suitable lipid source for farmed Atlantic salmon. A nutritional feeding trial was conducted with diets containing camelina oil and camelina meal in order to determine lipid and fatty acid composition in tissues and to assess the final fillet for flesh composition and quality for human consumption.

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, NS, Canada). The seeds were single pressed using a KEK 0500 press at Atlantic Oilseed Processing, Ltd. (Summerside, PEI, Canada) to extract the oil and ethoxyquin was added to the final product. The meal was ground with a hammer mill (screen size 8 mm) into a pre-pressed meal cake at Atlantic Oilseed Processing, then solvent extracted with petroleum ether at a concentration of 3 ml g^{-1} at the Faculty of Agriculture, Dalhousie University (Truro, NS, Canada).

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

2.2. Experimental fish

An experiment was conducted with salmon smolts in seawater (242 ± 46 g $fish^{-1}$ mean initial weight ± SD; 27 ± 1.8 cm mean initial length) at the Ocean Sciences Centre (Memorial University of Newfoundland, St. John's, NL, Canada). Fish were received from Cooke Aquaculture (St. Alban's, NL, 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. Ethical treatment of fish in this experiment was followed by guidelines according to the Canadian Council of Animal Care (Memorial University Institutional Animal Care Protocol Approved 12-50-MR). The smolts were randomly distributed (750) into 15 experimental tanks (500 L), each tank with 50 fish. The fish were acclimated on the control diet for one week prior to initial sampling. Throughout the duration of the trial, a flow through system of filtered seawater was supplied to each tank at a rate of 12 L min^{-1} and a photoperiod of 12 h. The dissolved oxygen (10 mg L^{-1}) and water temperature (14 °C) was monitored daily. Fish were fed to apparent satiation. Mortalities were weighed and recorded throughout the trial.

2.3. Tissue sampling

Sampling occurred at week 0 (the day before experimental diets were fed), week 1 (3 days of acclimation on the test diets plus 7 days on full test diet), 8, and 16. Individual fish were rapidly netted and euthanized by an overdose of anaesthetic (buffered tricaine methane sulfonate, TMS) and clinical signs of death were ensured prior to sampling. Three fish per tank were randomly sampled and measured for length and weight. The whole viscera was removed and weighed and sampled for lipid analysis. The skin was removed on the left side and white muscle tissue was subsampled for dry matter and lipid analysis. At the final sampling, additional tissues were sampled for lipid analysis, including dark muscle, belly flap and skin. 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 the wet weights of samples 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.

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