Comparative Biochemistry and Physiology, Part D 14 (2015) 1–15

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part D

journal homepage: www.elsevier.com/locate/cbpd

Atlantic salmon (*Salmo salar*) liver transcriptome response to diets containing *Camelina sativa* products

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ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 26 January 2015
Accepted 26 January 2015
Available online 2 February 2015

Keywords:
Camelina
Lipid metabolism
Hepatic transcriptome
Nutrigenomics
Sustainable aquaculture diets

ABSTRACT

Due to increasing demand for fish oil (FO) and fish meal (FM) in aquafeeds, more sustainable alternatives such as plant-derived oils and proteins are needed. *Camelina sativa* products are viable feed ingredients given the high oil and crude protein content in the seed. Atlantic salmon were fed diets with complete or partial replacement of FO and/or FM with camelina oil (CO) and/or camelina meal (CM) in a 16-week trial [Control diet: FO; Test diets: 100% CO replacement of FO (100CO), or 100CO with solvent-extracted FM (100COSEFM), 10% CM (100CO10CM), or SEFM + 10% CM (100COSEFM10CM)]. Diet composition, growth, and fatty acid analyses for this feeding trial were published previously. A 44 K microarray experiment identified liver transcripts that responded to 100COSEFM10CM (associated with reduced growth) compared to controls, yielding 67 differentially expressed features (FDR < 5%). Ten microarray-identified genes [*cpt1*, *pcb*, *bar*, *igfbp-5b* (2 paralogues), *btg1*, *dnph1*, *lect-2*, *clra*, *klf9*, and *fadsd6a*], and three additional genes involved in lipid metabolism [*elovl2*, *elovl5* (2 paralogues), and *fadsd5*], were subjected to QPCR with liver templates from all 5 dietary treatments. Of the microarray-identified genes, only *bar* was not QPCR validated. Both *igfbp-5b* paralogues were significantly down-regulated, and *fadsd6a* was significantly up-regulated, in all 4 camelina-containing diet groups compared with controls. Multivariate statistics were used to correlate hepatic desaturase and elongase gene expression data with tissue fatty acid profiles, indicating the involvement of these genes in LC-PUFA biosynthesis. This nutrigenomic study provides molecular biomarkers for use in developing novel aquafeeds using camelina products.

1. Introduction

Fish products are a major source of ω3 long chain polyunsaturated fatty acids (LC-PUFA) in human diets (Tocher et al., 2006). Dietary LC-PUFA such as eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3), can benefit human health in several ways including enhancing cardiac health and reducing risk of inflammatory diseases (Calder and Yaqoob, 2009). The worldwide demand for seafood for human consumption, with approximately 50% coming from aquaculture, continues to climb due to flat or decreasing global wild fisheries in the face of rising human population (Agaba et al., 2005; Tocher et al., 2006; FAO, 2009; Bell et al., 2010). Finfish aquaculture, especially that of carnivorous fish such as Atlantic salmon (*Salmo salar*), has relied heavily on fish oil (FO) and fish meal (FM) from wild stocks for the production of feeds. Consequently, the increasing demand of FO and FM will exceed the wild fishery supplies, threatening the sustainability of fishery and aquaculture industries (Tocher et al., 2006).

The need to find alternatives to FO and FM in aquafeeds has been recognized as one of the most important areas of research in aquaculture (Bell et al., 2010). As an oilseed crop, camelina (*Camelina sativa*) has several characteristics that make it desirable for the aquaculture feed industry. Firstly, the oil content of camelina seed is about 40%, and camelina oil (CO) is especially rich in LC-PUFA precursors, α-linolenic acid (ALA, 18:3ω3) and linoleic acid (LNA, 18:2ω6). The levels of these fatty acids in CO are approximately 40% and 15%, respectively (Zubr, 1997; Hixson et al., 2013; Xue et al., 2014). Moreover, the ω3/ω6 ratio of CO, which is closely linked to both fish health as well as to the nutritional value of fish to human consumers, is higher than other plant oils such as soybean oil and palm oil (reviewed in Glencross, 2009). Some by-products of camelina from the oil extraction process, such as the seed meal, may also be used in the aquaculture feed industry. Camelina meal (CM) has a crude protein level of approximately 45%, similar to canola and other rapeseed meal (Acamovic et al., 1999; Frame et al., 2007). There are at least 18 amino acids found in camelina seed, and 9 of them are essential (Zubr, 2003). The most dominant essential amino acid in camelina seed is

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http://dx.doi.org/10.1016/j.cbd.2015.01.005
1744-117X/

arginine (8.2%), while the content of some other essential amino acids (e.g. glycine, proline, and valine) exceeds 5.0% (Zubr, 2003).

Research has previously been performed on FO substitutions with linseed oil (Torstensen et al., 2008), canola oil (Miller et al., 2007) and rapeseed oil (Jordal et al., 2005) for various fish species including Atlantic salmon. Studies have demonstrated that vegetable oil (either singly or as blends), which is high in C_{18} PUFA such as 18:3ω3 and 18:2ω6 but devoid of the LC-PUFA, can be used to replace up to 100% of FO without negatively influencing growth in salmonids and marine fish (Bell et al., 2001, 2010; Torstensen et al., 2005; Morais et al., 2012a; Hixson et al., 2013; Xue et al., 2014). However, the ω3 LC-PUFA content in fish fillets can be reduced significantly if FO is replaced by vegetable oil completely (Bell et al., 2010; Morais et al., 2012a). Moreover, the expression of genes involved in the LC-PUFA biosynthetic pathway is known to be altered following vegetable oil dietary treatments. Particularly, *delta-5 fatty acyl desaturase* (*fadsd5*) and *fatty acyl elongase* [*elongation of very long chain fatty acids* (*elovl*); e.g. *elovl2* and *elovl5*] genes are often up-regulated in the liver of Atlantic salmon fed diets containing vegetable oil (e.g. rapeseed oil) (reviewed in Leaver et al., 2008a). Compared to marine fish, freshwater fish and salmonids are better at producing 22:6ω3 and 20:5ω3 using 18:3ω3 (Santigosa et al., 2011). Last but not least, the changes in fatty acid profiles in the diets due to the replacement of FO by vegetable oil may alter fish metabolism, and could potentially affect various aspects of fish health including susceptibility to infectious diseases (Montero et al., 2003; Mourente et al., 2005).

CO-containing diets have been used in studies involving Atlantic cod (*Gadus morhua*) (Morais et al., 2012a; Hixson et al., 2013; Booman et al., 2014; Hixson and Parrish, 2014; Xue et al., 2014), Atlantic salmon (Bell et al., 2010; Leaver et al., 2011; Morais et al., 2011b; Hixson et al., 2014b), and rainbow trout (*Oncorhynchus mykiss*) (Hixson et al., 2014a). Previously in Atlantic salmon, CO was included in blends (20% CO) with other plant-based oils to study the effect of substituting FO with vegetable oil blends on growth (Bell et al., 2010), ω3 LC-PUFA deposition in the flesh (Leaver et al., 2011), and cholesterol and lipoprotein metabolism (Morais et al., 2011b). In salmon practical diets, both FM and FO have been partially replaced with plant materials simultaneously (Pratoomyot et al., 2010). Total replacement of FM with plant proteins in the diets of Atlantic salmon has been shown to result in reduced growth performance (Espe et al., 2006); however, partial replacement (~23%) of FM with soybean meal showed no reductions in weight gain and feed intake in Atlantic salmon, but caused reduced digestibility of various nutrients as well as abnormal morphology of the distal intestine (Øverland et al., 2009). Recently, an Atlantic salmon feeding trial was conducted to evaluate the growth performance, and the lipid and fatty acid composition in tissues, of fish fed with diets containing full replacement of FO with CO and/or partial inclusion of CM (Hixson et al., 2014b). In the current study, the impact of camelina-containing diets on salmon liver gene expression was investigated in parallel with Hixson et al. (2014b) by analyzing samples from the same individuals using DNA microarrays and quantitative reverse transcription–polymerase chain reaction (QPCR). While the diet ingredients and growth performance data for this feeding trial were previously published (Hixson et al., 2014b), we include them as supplementary information herein as they pertain to the current study as well (Supplemental Tables S1 and S2). Additional data arising from this feeding trial (e.g. diet and tissue fatty acid analyses) may be found in Hixson et al. (2014b).

Nutrigenomic approaches (e.g. involving DNA microarrays and QPCR) have been shown to be useful for the identification of genes that are differentially expressed in fish fed altered diet formulations, for example, with FO or FM replaced by plant-based ingredients (Jordal et al., 2005; Leaver et al., 2008b; Panserat et al., 2008a; Morais et al., 2011a). These previous studies focused on hepatic gene expression changes since the liver is the main organ involved in metabolizing carbohydrates, lipids, and proteins into biologically useful materials in vertebrates, and also plays key roles in detoxification and immunity (Vilhelmsson et al., 2004; Panserat et al., 2009). Therefore, the objective of this study was to use a 44,000 feature (44 K) salmonid oligonucleotide microarray (Jantzen et al., 2011; Sahlmann et al., 2013) and QPCR to assess the impacts of CO and/or CM containing diets on Atlantic salmon hepatic gene expression in order to identify candidate molecular biomarkers for responses to camelina-containing diets. In addition, the current study included: 1) assessment of the effect of different levels of dietary CO on the transcript expression of elongase- and desaturase-encoding genes involved in LC-PUFA biosynthesis; and 2) the correlation of tissue fatty acid levels with transcript expression levels of LC-PUFA-responsive genes. We anticipate that the molecular biomarkers (i.e. camelina product-responsive genes) identified in this study will be useful in the future development of camelina-containing diets that do not have deleterious effects on fish performance or physiology.

2. Material and methods

2.1. Experimental diets and animals

The feeding trial, involving Atlantic salmon post-smolts and test diets containing camelina products (e.g. CO and CM), was conducted at the Dr. Joe Brown Aquatic Research Building (Ocean Sciences Centre, Memorial University of Newfoundland, Canada). All diets were approximately iso-nitrogenous and iso-energetic on a crude protein and gross energy basis, according to the nutritional requirements of Atlantic salmon (National Research Council, 2011) (Supplemental Table S1). The experimental treatments in this feeding trial were as follows: a control diet with FO and FM (FO); 100% FO replacement with CO (100CO); 100% FO replacement with CO and including solvent-extracted FM (100COSEFM); 100% FO replacement with CO and including 10% CM (100CO10CM); 100% FO replacement with CO and including SEFM and 10% CM (100COSEFM10CM). SEFM was employed here to remove FO residue in the FM (about 8%) as much as possible in order to evaluate the full effect of total replacement of fish oil in the diet. Further details on formulation and fatty acid compositions of the diets are given in Hixson et al. (2014b).

Atlantic salmon post-smolts (242.1 ± 46.0 g mean initial weight ± SD; 27 ± 1.8 cm mean initial length ± SD) were randomly distributed among fifteen 500 L tanks (50 fish per tank) supplied with flow-through seawater (~14 °C, dissolved oxygen ≥ 10 mg L^{-1}), and all fish were kept on a photoperiod of 12 h. After the acclimation period (i.e. 1 week in the experimental tanks), all fish were gradually moved from the commercial diet (Nutra Transfer NP, 3 mm, Skretting Canada, St. Andrews, NB, Canada) to the control diet (i.e. FO) over 3 days, and were kept on control diet for one week prior to the initial sampling (week 0). Thereafter, fish were gradually weaned onto each assigned experimental or control diet over another 3 days. Triplicate tanks of fish were fed experimental or control diets to apparent satiety, twice each day for a period of 16 weeks. At week 0 and week 16 of the feeding trial, seven fish from each tank at each time point were euthanized by 400 mg L^{-1} tricaine–methane–sulfonate bath (TMS; Syndel Laboratories, Vancouver, BC) after 24 h of fasting. Body weight and fork length of fish were measured and recorded. Liver tissues (50–100 mg $sample^{-1}$) were collected, flash-frozen in liquid nitrogen, and stored at −80 °C until RNA extractions were performed. This study was carried out in accordance with Animal Care Protocol (12–50-MR) approved by the Institutional Animal Care Committee of Memorial University of Newfoundland. Further details on fish rearing conditions and sampling for lipid analyses are given in Hixson et al. (2014b).

2.2. RNA extraction, DNase treatment, and column purification

The above samples were homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA) with stainless steel beads (5 mm; QIAGEN, Mississauga,