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Dietary micronutrients and in vivo n − 3 LC-PUFA biosynthesis in Atlantic salmon

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ABSTRACT

Aquaculture, and in particular Atlantic salmon culture, is expected to deliver n − 3 long-chain polyunsaturated fatty acid (n − 3 LC-PUFA) rich products. Nevertheless, the availability of n − 3 LC-PUFA rich raw materials for aquafeed is dwindling, and at an ever increasing market price. Thus, there is the need to better understand the in vivo n − 3 LC-PUFA biosynthetic capabilities of cultured fish to enable the possible maximization of dietary 18:3n − 3 (ALA) bioconversion to 20:5n − 3 (EPA) and 22:6n − 3 (DHA). The cofactors and coenzymes involved in this metabolic pathway have so far received limited research attention. In this study, juvenile Atlantic salmon were fed an ALA-rich diet with no, normal, or over-fortified inclusion of those micronutrients reported to be essential cofactors (iron; zinc; magnesium) and coenzymes (riboflavin; biotin; niacin) for the fatty acid elongase and desaturase enzymes. The results showed that reduced dietary inclusion of these micronutrients impaired the normal n − 3 LC-PUFA biosynthetic capabilities of fish, whereas the over fortification did not provide any additional benefit. This study provides new knowledge on micronutrients and lipid metabolism interactions in a commercially important cultured species, and is envisaged to be a useful contribution towards developing more sustainable and commercially viable aquafeed for the future.

Statement of relevance

This work is the continuation and extension of a previous study (Lewis et al., 2013, Aquaculture 412/413, 215–222) in which we explored the physiological roles and potential effects of micronutrients on fatty acid metabolism in cultured fish. The present study differed from the previous in the blend of minerals and vitamins used, the species, the fatty acid composition of the test diet, and the inclusion also of a negative control. The results are most interesting, showing that riboflavin (B_2), biotin (B_7), and niacin (B_3), Iron (Fe), Magnesium (Mg) and Zinc (Zn) are all required for proper fatty acid bioconversion, but also that a dietary over-fortification does not translate into proportional improved bioconversion.

1. Introduction

Due to dwindling marine catches and increasing economic and environmental concerns, the aquaculture industry is increasingly replacing dietary fish oil with alternative oils in aquafeed. Fish oil is characterised by a high content of long chain polyunsaturated fatty acids (LC-PUFA, polyunsaturated fatty acids with 20 or more carbon atoms) and typically with the first double bond in omega-3 position (n − 3 LC-PUFA), such as eicosapentaenoic acid (EPA, 20:5n − 3) and docosahexaenoic acid (DHA, 22:6n − 3). n − 3 LC-PUFA are beneficial for the cultured fish and health-promoting for the final consumers of aquaculture products. The alternative oils used in aquafeed are commonly from plants, containing polyunsaturated fatty acids (PUFA) with 18 carbon atoms (C_{18} PUFA), saturated (SFA) and monounsaturated fatty acids (MUFA), but not LC-PUFA. The direct shifting from fish oil to vegetable oil in aquafeeds, results in progressively lesser n − 3 LC-PUFA being present in feed and in the tissues of cultured fish. For an in-depth discussion of this topic, the following recent reviews are recommended (Merino et al., 2012; Nasopoulou and Zabetakis, 2012; Olsen and Hasan, 2012; Pickova and Morkore, 2007; Shepherd and Jackson, 2013; Tacon and Metian, 2008; Turchini et al., 2009, 2011a).

As with all vertebrates, fish are unable to undertake de novo synthesis of PUFA and, resulting from adaptation and evolution, different finfish species possess different capabilities in bioconverting (elongating and desaturating) dietary C_{18} PUFA into LC-PUFA (Carmona-Antonanzas et al., 2013; Monroig et al., 2013; Torstensen and Tocher, 2011; Zheng et al., 2004). In general, the vast majority of the freshwater fish species, and the diadromous salmonids, are capable of bioconverting shorter chain (C_{18}) n − 3 fatty acid, such as α-linolenic acid (18:3n − 3, ALA) to n − 3 LC-PUFA (Buzzi et al., 1996, 1997; Hixson et al., 2014; Monroig et al., 2010; Morais et al., 2009; Wijekoon et al., 2014). Detailed

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information on the identification and functional characterisation of key LC-PUFA biosynthesis genes are also available for several species, including the Atlantic salmon (*Salmo salar*) (Hastings et al., 2004; Morais et al., 2009; Zheng et al., 2005, 2007). However, it has been well-documented that when fish oil is replaced by alternative oils, even by those vegetable oils rich in the precursor ALA, a substantial reduction of the final n − 3 LC-PUFA content of the fish fillet occurs (Cleveland et al., 2012; Francis et al., 2014; Friesen et al., 2013; Hixson et al., 2014; Miller et al., 2008b; Monroig et al., 2013; Pickova and Morkore, 2007; Torstensen and Tocher, 2011; Trushenski et al., 2011; Turchini and Francis, 2009; Turchini et al., 2009, 2011b).

For this reason, and because of anticipated expectation of consumers for high n − 3 LC-PUFA cultured fish products (Farrell et al., 2010; Henriques et al., 2014; Nichols et al., 2014), several strategies aimed at minimizing the reduction of n − 3 LC-PUFA in tissues of fish fed with little or no dietary fish oil have been attempted with, thus far, only mixed success. These strategies include, among others, the use of finishing diets (Bell et al., 2003, 2004; Codabaccus et al., 2012; Ng et al., 2004; Thanuthong et al., 2012; Trushenski et al., 2011), the use of specialty oils (Alhazzaa et al., 2013a, 2013b; Cleveland et al., 2012; Ganuza et al., 2008; Li et al., 2009; Miller et al., 2007; Raghukumar, 2008; Samocha et al., 2011; Song et al., 2007), the possible exploitation of circadian metabolic rhythms (Betancor et al., 2014; Brown et al., 2010), the selective breeding of cultured fish (Bicskei et al., 2014; Kamalam et al., 2013; Leaver et al., 2011; Morais et al., 2012; Yamamoto et al., 2014), the use of genetic engineered vegetable crops (Kitessa et al., 2014; Mansour et al., 2014; Miller et al., 2008a; Nichols et al., 2010; Petrie et al., 2010, 2014; Robert, 2006; Sayanova and Napier, 2011) or even the fish itself (Kabeya et al., 2014), and the possible use of bioactive compounds in aquafeeds towards improving n − 3 LC-PUFA biosynthesis or deposition/retention (Alhazzaa et al., 2012; Randall et al., 2013a, 2013b; Teoh and Ng, 2013; Trattner et al., 2007, 2008; Vestergren et al., 2012; Wagner et al., 2014).

In consideration that the bioconversion of C_{18} PUFA into LC-PUFA is fundamentally an enzymatic process, it is surprising that relatively little research has focused on the roles of micronutrients acting as cofactors and coenzymes for this process in fish. The role of iron (Fe) (Stangl and Kirchgessner, 1998; Zhou et al., 2011), zinc (Zn) (Eder and Kirchgessner, 1996; Eder et al., 1996) and magnesium (Mg) (Mahfouz et al., 1989) in acting as enzyme cofactors and supporting the bioconversion of ALA into EPA and DHA has been documented. Similar roles have also been shown for coenzyme vitamins, or their derivatives, such as niacin (B_3) (Guillou et al., 2010; Igarashi et al., 2007), riboflavin (B_2) (Duerden and Bates, 1985; Olpin and Bates, 1982) and biotin (B_7) (Mock et al., 1987, 1988). The effects of supplementing metal ions or vitamins on n − 3 LC-PUFA metabolism have been receiving some attention in both mammals (Bertrandt et al., 2004; Debski et al., 2007) and, to a minor extent, in fish (Senadheera et al., 2012a, 2012b). To address this gap, a recent study on rainbow trout (*Oncorhyncus mykiss*) focused specifically on the possible role of combined supplemental cofactors and coenzymes on LC-PUFA biosynthesis, with encouraging results (Lewis et al., 2013). In the latter study, a blend of vegetable oils (linseed and canola) was used, and the reference/control diet contained all the selected micronutrients at their recommended dietary intake. The study showed that the increase in their supplementation resulted in increased n − 3 LC-PUFA biosynthesis, albeit only minimally. To achieve further insight on the actual potential of targeted micronutrient fortification towards sustaining in vivo n − 3 LC-PUFA biosynthesis in cultured fish, the present study aimed at investigating the collective effects of graded inclusion of selected micronutrients (Fe, Zn, Mg, biotin, niacin and riboflavin) known to be involved in the n − 3 LC-PUFA biosynthesis, in an ALA-rich, linseed oil-based diet in Atlantic salmon (*S. salar*), and included also a dietary deficiency treatment. Atlantic salmon was used as a model species in the current experiment because of its global importance, and because the genes encoding the key enzymes involved in the n − 3 LC-PUFA biosynthetic pathway are known.

2. Material and methods

2.1. Fish husbandry and growth

All procedures implemented in the present experiment were approved by the Deakin University Animal Ethics Committee (ref. no. B12-2012). Atlantic salmon (*S. salar*) juveniles were procured from a private hatchery (Mountain Fresh Trout and Salmon Farm, Stoney Creek, VIC), and were acclimatized to experimental conditions in 1000 L tanks. Fish were fed on a commercial diet (Ridley Aquafeed) during an acclimatization period of two weeks. At the beginning of the experiment, two groups of 10 fish each, were euthanised (0.5 ml/L of AQUI-S, New Zealand) and stored frozen at −20 °C. Two hundred and eighty-eight fish (~15.3 g, average body weight) were randomly allocated to 12 1000 L tanks (24 fish per tank) in a fully controlled recirculation system, and maintained at 12 h light:12 h dark light cycle. The water temperature was maintained at 14.0 ± 1.0 °C with ultraviolet light disinfection and physical (60 μm screen drum filter) and biological filtration. Ammonia and nitrite levels were monitored using Aquamerck test kits (Merck, Germany) twice a week and maintained at a level below 0.5 and 0.05 mg/L, respectively, during the experiment. The 4 experimental dietary treatments were randomly allocated to triplicate tanks. Fish were carefully hand fed to apparent satiety, twice daily at 0900 and 1600 h for a grow-out period of 84 days. Feed consumption was recorded during the entire study period. At the end of the grow-out period, fish were fasted for 24 h for complete gut evacuation of feeds. All the fish were anesthetized, weighed, and three fish per tank were immediately dissected and a piece of liver was removed for gene expression analysis. The body of each fish was frozen for further analysis. During the last three weeks of the trial, faeces were collected as previously described (Huguet et al., 2015), and stored at −20 °C, until freeze-drying and subsequent analyses.

Growth and feed utilisation parameters over the experimental period were calculated according to the standard formulae described earlier (Norambuena et al., 2013); these included initial and final mean body weights, total feed consumption, gain in weight (%), food conversion ratio (FCR), specific growth rate (SGR, %), protein growth ratio (PGR) and fat deposition rate (FDR).

2.2. Diets

Four iso-nitrogenous, iso-lipidic and iso-energetic diets were designed with identical formulation, differing only in the levels of selected vitamins and minerals that are reported to act as n − 3 LC-PUFA biosynthesis coenzymes and cofactors (Table 1). A coenzyme and cofactor fortification mix, comprised of riboflavin (B_2), biotin (B_7), and niacin (B_3), Iron (Fe), Magnesium (Mg) and Zinc (Zn), at a concentration that provided 100% of known dietary requirements for Atlantic salmon (NRC, 2011) when included at 5 g/kg in the diet, was formulated (supplementary Table 1), and included at 0, 5, 15 or 30 g/kg in the four experimental diets. The diets are termed T-0 (0% coenzyme and cofactor fortification; negative control), T-100 (100% coenzyme and cofactor fortification; positive control), T-300 (300% coenzyme and cofactor fortification) and T-600 (600% coenzyme and cofactor fortification). Linseed oil was used as the sole supplemental lipid source in the diets, and soy protein concentrate, fishmeal, blood meal, soyabean meal, wheat gluten and whey protein were used for protein sources. Alpha-cellulose was used as inert filler to compensate for the addition of the coenzyme and cofactor mix added at increasing weights to the diets. Vitamin C (ascorbic acid) was added at a constant and high level in all diets to facilitate iron uptake and to minimize possible lipid peroxidation derived by the higher iron supply. Diets were pelleted as described previously (Brown et al., 2010), and stored in airtight sealed bags at 4 °C until use.