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Echium oil is better than rapeseed oil in improving the response of barramundi to a disease challenge

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ABSTRACT

Pathogen infection stimulates the fatty acid (FA) metabolism and the production of pro-inflammatory derivatives of FA. Barramundi, *Lates calcarifer*, was fed on a diet rich in preformed long-chain ($\geqslant C_{20}$) polyunsaturated fatty acids (LC-PUFA) from fish oil (FO), to compare with diets containing high levels of C_{18} precursors for LC-PUFA – stearidonic (SDA) and γ-linolenic acid (GLA) – from *Echium plantagineum* (EO), or rapeseed oil (RO) rich in α-linolenic acid (ALA), but a poor source of LC-PUFA and their precursors. After 6 weeks, when growth rates were similar amongst the dietary treatments, a sub-lethal dose of *Streptococcus iniae* was administered to half of the fish, while the other half were maintained unchallenged and were pair-fed with the infected fish. Under a disease challenge situation, the tissue FA depots depleted at 3 days post-infection (DPI) and were then restored to their previous concentrations at 7 DPI. During the infection period, EO fish had a higher content of n3 and n6 PUFA in their tissues, higher n3:n6 PUFA ratio and reduced levels of the eicosanoids, TXB_2 and 6-keto-$PGF_{1\alpha}$, in their plasma compared with RO fish. Fish fed on FO and EO had a longer lasting and enduring response in their FA and eicosanoid concentrations, following a week of bacterial infection, compared with those fed on RO. EO, containing SDA and GLA and with a comparatively higher n3:n6 PUFA ratio, proved more effective than RO in compensating for immunity stress.

1. Introduction

The functional roles of fatty acids (FA) are largely and selectively attributed to the n6 and n3 long-chain ($\geqslant C_{20}$) polyunsaturated fatty acid (LC-PUFA) families and their modulation of immunity and cytokine secretion (Galli & Calder, 2009; Wymann & Schneiter, 2008). LC-PUFA are precursors and modulators for eicosanoid production, including prostaglandins (PG) and thromboxanes (TX), via a synthesis pathway involving cyclooxygenase (COX) and lipoxygenase (LOX) enzyme activity (Gravaghi et al., 2010; Wang & Dubois, 2010). However, when compared to arachidonic acid (ARA, 20:4n6), n3 LC-PUFA are considered poor substrates for COX and LOX (Jump, 2004; Lee & Hwang, 2008).

Vegetable oils (VO) lack eicosapentaenoic acid (EPA, 20:5n3), docosahexaenoic acid (DHA, 22:6n3) and ARA, and are generally rich in C_{18} monounsaturated FA (MUFA) and C_{18} PUFA such as linoleic acid (LA, 18:2n6) and α-linolenic acid (ALA, 18:3n3) which, compared to LC-PUFA, may compromise some biological functions in many vertebrates (Guil-Guerrero, 2007; Vedtofte, Jakobsen, Lauritzen, & Heitmann, 2011). Oil from the seeds of *Echium plantagineum* (EO) is rich in stearidonic acid (SDA, 18:4n3) and γ-linolenic acid (GLA, 18:3n6) compared with other terrestrial vegetable oils (Guil-Guerrero, Maroto, & Gimenez, 2001). SDA and GLA are considered superior to ALA and LA, respectively, as precursors of LC-PUFA as they are beyond the initial rate-limiting desaturation step in the LC-PUFA biosynthesis pathway (Venegas-Caleron, Sayanova, & Napier, 2010), and therefore have more potential to induce eicosanoid production compared to ALA and LA (Horia & Watkins, 2005; Kirkup, Cheng, Elmes, Wathes, & Abayasekara, 2010). Dietary EO enhanced LC-PUFA biosynthesis and accumulation in the tissues of birds (Kitessa & Young, 2009), rodents (Ishihara, Komatsu, Saito, & Shinohara, 2002; Yang & O'Shea, 2009) and humans (Harris et al., 2008; Surette, Edens, Chilton, & Tramposch, 2004).

A well-balanced dietary FA profile is a determinant of animal health and welfare, and thus there is the potential that the replacement of fish oil (FO) by VO in feeds for cultured fish will affect this balance (Montero et al., 2010). Feeding EO to

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barramundi, *Lates calcarifer*, a widely farmed tropical fish, did not lead to increased accumulation of LC-PUFA in the whole body or separate tissues (Alhazzaa, Bridle, Carter, & Nichols, 2012; Alhazzaa, Bridle, Nichols, & Carter, 2011a, 2011b). However, it is still of considerable interest to investigate the modulation of LC-PUFA composition and immunity response in barramundi, fed on EO, followed by a bacterial infection. The malleability of the lipid and FA profile of barramundi, in response to alterations in ambient conditions, makes this species a valuable model to explore the pathways of induced and compensatory lipid metabolism (Alhazzaa et al., 2013; Alhazzaa et al., 2011b; Carter, Glencross, Katersky, & Bermudes, 2010). Since *Streptococcus iniae* is known to cause serious diseases and mortality in this fish species (Bromage & Owens, 2002), it was used in the present study to infect barramundi fed on diets made with EO, rapeseed oil (RO) or fish oil (FO) to explore the compositional and compensatory changes in tissue FA, focusing on PUFA and LC-PUFA, and to also link these changes with possible changes in the eicosanoid production in response to bacterial infection. Therefore, the overarching aim of the current study is to evaluate the capacity of barramundi to biosynthesise LC-PUFA, and their metabolites, from different dietary precursors following a disease challenge.

2. Materials and methods

2.1. Fish and diet

Barramundi (50 ± 2.4 g) were randomly stocked into twelve 80 l tanks, at a density of 12 fish per tank, in brackish water (15 ppt). The semi-flow through tanks systems were held at 30 °C, with a 24 h light photoperiod and 20% water change every day. Experimental diets, differing only in their lipid source, compared EO (Crossential SA14, Croda Chemicals), RO (Canola Oil, Woolworths Ltd.) and FO (South American anchovy oil, Skretting Australia) at a 17% supplementation level in the diet (Table 1) and were made in 4 mm pellets, as reported previously (Alhazzaa et al., 2011a, 2011b). A ratio of 4% of body weight per day (% BW/d) was hand-fed to fish at 09:00 and 17:00. Food consumption (FC, g) was recorded and specific growth rate (SGR% per day) was calculated as = $100 \times (\ln(W_f/W_i))/d$ where W_f and W_i are the final and initial masses (g), respectively, and d is the number of days of the experiment. Feed efficiency ratio (FER) was calculated as FER (g/g) = total mass gain (g)/FC (g). In barramundi, the apparent digestibility (AD) of FA in diets of similar composition to the ones used in this experiment were not significantly different regardless of the salinity (Alhazzaa et al., 2011a). Therefore, in this experiment the AD was not measured before infection.

Table 1
Ingredients and lipid and fatty acid composition of experimental diets (g/kg of dry matter), (FO, fish oil; EO, Echium oil; RO, rapeseed oil).

Ingredient composition	FO	EO	RO
Fishmeal	690	690	690
Echium oil	0	170	0
Fish oil	170	0	0
Rapeseed oil	0	0	170
Gelatinised starch	59	59	59
Vitamin mix[a]	15	15	15
Mineral mix[b]	15	15	15
Vitamin C[c]	20	20	20
Choline chloride	10	10	10
CMC	10	10	10
Sodium mono-phosphate	10	10	10
Yttrium oxide[d]	1	1	1
Chemical composition			
Dry matter	872	858	864
Crude protein	605	602	600
Total lipid	203	205	203
Energy (MJ/kg dry matter)	20.9	21.6	21.1
Fatty acid			
16:0	24.7	7.2	12.3
Total SFA	34.6	12.5	21.0
16:1n7	12.4	1.1	2.3
18:1n9	15.8	16.1	105
∑ MUFA	41.3	20.5	140
18:3n3	1.2	25.5	13.1
18:4n3	2.7	10.2	0.5
20:5n3	20.4	2.9	3.2
22:6n3	14.5	2.4	2.1
∑ n3 PUFA	39.3	42.6	18.9
18:2n6	3.4	18.5	27.5
18:3n6	0.5	10.1	0.1
20:4n6	1.4	0.2	0.2
∑ n6 PUFA	5.3	28.2	28.1
n3:n6	7.4	1.5	0.7
∑ PUFA	62.4	61.5	47.2

CMC, carboxymethyl cellulose.
[a] Vitamin mix (mg/kg): vitamin A (22·5), vitamin D (27), rovimix E50 (450), menadone sodium bisulphate (9), riboflavin (18), calcium D-pantothenate (97·83), nicontinic acid (45), vitamin B-12 (0·045), D-biotin (2·025), folic acid (4·5), thiamin HCl (5·04), pyridoxine HCl (16·47), *myo*-Inositol (1350) and α-cellulose (2452·59) as a filler.
[b] Mineral mix: (mg/kg): $CuSO_4 \cdot 5H_2O$ (106·111), $FeSO_4 \cdot 7H_2O$ (1633·95), $MnSO_4 \cdot H_2O$ (276·84), Na_2SeO_3 (2·97), $ZnSO_4 \cdot 7H_2O$ (593·73), KI (6·48), $CoSO_4 \cdot 7H_2O$ (42·93) and α-cellulose (1836·99) as a filler.
[c] Rovimix Stay-C 35.
[d] Digestibility marker, results for digestibility not shown.

2.2. Bacteria preparation, fish infection and sampling

Starter inoculums of *Streptococcus iniae*, strain TCFB 1950, isolated from barramundi in South Australia, were grown for 24 h at 37 °C with continuous shaking in a brain–heart infusion (BHI) broth. When the broth reached an optical density of ~1 at A_{600}, successive serial dilutions were spread onto brain–heart infusion agar plates and colony forming units (cfu) were counted after overnight incubation. To test the potency of the inoculum, a sub-lethal dose of 5×10^3 cfu/l was administered individually to 24 fish via a 2 min immersion in a 4 l aerated bath. Those fish were not used later in the feeding and challenging experiment. Infection was confirmed by streaking a kidney onto a BHI plate and picking a growing colony after 24 h of incubation, at 37 °C, and running a PCR assay as in Mata, Blanco, Dominguez, Fernandez-Garayzabal, and Gibello (2004). Infection resulted in less than 20% mortality, after 24 h, and no mortality afterwards.

Before distribution into tanks, the blood plasma was collected from six anaesthetised (benzocaine 100 mg/l) intact fish by drawing blood into a heparinised syringe and centrifuging the collected blood at 300g and collecting the supernatant. Skeletal muscle and liver were collected to measure the lipid content and composition from intact fish. After feeding each of the three experimental diets to fish in eight tanks for 6 weeks, fish from four tanks were then removed and infected individually with the sub-lethal dose. Samples were collected from three infected fish per tank after 3 and 7 days post infection (3 and 7 DPI, respectively). Control samples (three per tank, six per treatment) were taken, as described above, from non-infected tanks for each dietary treatment, at the same sampling times. As the feed intake decreased in infected fish, the control treatments were pair-fed based on the ration size during week 7. A ration-size pair-feeding approach is considered ideal for studying the interaction between fish physiology and nutrition under stress (Brett, Shelbourn, & Shoop, 1969; Foster, Hall, & Houlihan, 1993; Foster, Houlihan, Hall, & Burren, 1992) and was used to avoid introducing another unplanned random factor to the experiment, such as an unequal feed intake.