Aquaculture 433 (2014) 164–172

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effect of ration level and dietary docosahexaenoic acid content on the requirements for long-chain polyunsaturated fatty acids by juvenile barramundi (*Lates calcarifer*)

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ARTICLE INFO

Article history:
Received 19 March 2014
Received in revised form 30 May 2014
Accepted 31 May 2014
Available online 8 June 2014

Keywords:
Asian seabass
Docosahexaenoic acid (DHA)
Eicosapentaenoic acid (EPA)
Essential fatty acid (EFA)
Long-chain polyunsaturated fatty acid (LC-PUFA)

ABSTRACT

Juvenile barramundi were fed one of six diets containing differing docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) levels. Fish were restricted fed on a pair-fed feeding regime to eliminate variability in feed intake, with two diets fed to satiety to examine the effects of fixed or variable feed rations on EFA requirements. Weight gain, feed intake, feed utilisation, and physical clinical signs were monitored. No effect of dietary DHA and EPA concentration, DHA:EPA ratio or total LC-PUFA level was observed on weight gain, growth rate, feed conversion ratio (FCR), survival or physical clinical health signs ($P > 0.05$). Satiety fed fish had higher feed intake, final weight, weight gain and growth rate compared to their respective restrictively fed treatments ($P < 0.05$). No effect of ration level on the responses to DHA concentration was observed. Body fatty acid composition was affected by diet, increasing dietary DHA resulted in higher body tissue DHA concentration, and a similar relationship was observed for EPA. Plasma haemoglobin increased with increasing DHA + EPA levels ($P < 0.05$) while glutamate dehydrogenase increased for fish fed DHA + EPA in a 1:1 ratio, regardless of total dietary LC-PUFA ($P < 0.05$). Juvenile barramundi may be fed diets containing as low as 1 g kg^{-1} DHA without compromising growth or health status.

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1. Introduction

Traditionally, commercial aquaculture diets have relied on marine fishery oils for their lipid component as they were readily available, competitive in price and contained the essential fatty acids required by fish (reviewed by Turchini et al., 2009). Increasing aquaculture production continues to place demands on static global wild fish resources, making their continued use unsustainable (Tacon and Metian, 2008). To meet global aquaculture demands, a number of alternative lipid resources have been trialled including vegetable oils (e.g. palm oil, soybean oil, canola oil and rapeseed oil) and animal fats (e.g. lard, poultry oil and tallow; Turchini et al., 2009). However, all of these alternative lipid sources are non-marine and lack long-chain polyunsaturated fatty acids (LC-PUFA).

Some freshwater species have little or no requirement for dietary LC-PUFA. However, the majority of aquatic marine species have a defined dietary requirement for LC-PUFA (reviewed by Glencross, 2009). This probably results from an evolutionary adaptation to naturally LC-PUFA rich diets, resulting in many marine species losing their ability to convert C18 n−6 and n−3 PUFA into the corresponding C20 and C22 n−3 and n−6 PUFA in vivo by alternating cycles of desaturation and elongation (Tocher, 2010). Marine and catadromous species, including barramundi (*Lates calcarifer*), have an absolute dietary requirement for the longer, and more unsaturated 20:5n−3 (EPA) and 22:6n−3 (DHA; Buranapanidgit et al., 1988 cited by Boonyaratpalin, 1997; Glencross and Rutherford, 2011; Williams et al., 2006). A total n−3 LC-PUFA inclusion level that is between 10 and 17 g kg^{-1} (as a combination of DHA and EPA) was sufficient to support growth of barramundi (Buranapanidgit et al., 1988, 1989 cited by Boonyaratpalin, 1997). Later work by Williams et al. (2006) found little effect of dietary LC-PUFA level on growth but observed that some fish exhibited a 'fainting' response, a reported sign of EFA deficiency in rainbow trout when fed an LC-PUFA free diet containing 2% lipid (made up of varying amounts of lauric, linoleic and linolenic acids; Castell et al., 1972). In a subsequent study, Glencross and Rutherford (2011) hypothesised a quantitative requirement of around 10 g kg^{-1} DHA for barramundi. The authors did not observe an effect of DHA inclusion levels on growth parameters, even at inclusion levels as low as 1 g kg^{-1}. Although in that study all diets were pair-fed restrictively and it is unclear as to what effect that this feed ration regime had on the outcomes of the study. In that study,

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http://dx.doi.org/10.1016/j.aquaculture.2014.05.047
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a subcutaneous haemorrhaging, observed as a reddening of the skin, occurred as dietary DHA levels increased from 1 to 18 g kg^{-1}, which was ameliorated by the inclusion of 10 g kg^{-1} EPA. These results highlighted the importance not only of the absolute dietary inclusion level, but also of the influence of other LC-PUFAs in the diet. The authors concluded, based on their study and that of Williams et al. (2006), that the total LC-PUFA inclusion level for barramundi could be reduced to at least 12 g kg^{-1} provided that the DHA and EPA are held in balance. However, it was suggested that further research was required to better define the EPA requirements, and the optimal ratio of DHA:EPA in the diets of barramundi.

To address some of these questions an experiment was conducted to examine the effects of different DHA and EPA inclusion levels, total LC-PUFA inclusion levels and the effects of fixed or satietal rations, to further refine the understanding of the requirements of LC-PUFA by barramundi.

2. Materials and methods

2.1. Experiment overview

This study consisted of six dietary treatments with a range of DHA inclusion levels both with and without EPA (Table 1). Each of these diets was restrictively pair-fed to eliminate effects of feed intake variation as a secondary variable. Two additional treatments consisted of those diets containing 1 g kg^{-1} DHA + 1 g kg^{-1} EPA (D1E1) and 10 g kg^{-1} DHA + 1 g kg^{-1} EPA (D10E10), which were each fed to apparent satiety. These additional treatments were included to measure the effect of varying feed intake on the results achieved relative to the pair-fed strategy. All the procedures described herein were approved by CSIRO's Animal Ethics Committee (A3/2012) and performed in accordance with the Australian Code of Practice for Care and Use of Animals for Scientific Purposes.

Table 1
Formulations, chemical composition (g kg^{-1}) and fatty acid composition (as % of total fatty acids) of the experimental diets.

	Formulated DHA:EPA content (g kg^{-1})					
	D1E1	D5E1	D10E1	D10E10	D5E5	D3E3
Ingredient						
Pregelled wheat starch	50	50	50	50	50	50
Wheat gluten	80	80	80	80	80	80
Wheat flour	182	182	182	182	182	182
Casein	100	100	100	100	100	100
Soy protein isolate	200	200	200	200	200	200
Fish oil	0	0	0	75	30	15
Olive oil	41	37	31	4	26	34
DHASCO	0	8	20	0	0	0
Defatted fish meal	300	300	300	300	300	300
Butter fat	41	37	31	4	26	34
Yttrium oxide	1	1	1	1	1	1
Pre-mix vitamins[a]	5	5	5	5	5	5
Diet composition						
Dry matter (g kg^{-1})	871	963	873	964	967	968
Ash (g kg^{-1})	55	65	57	65	66	66
Crude protein (g kg^{-1} DM)	578	613	574	609	609	632
Lipid (g kg^{-1} DM)	98	85	94	99	105	85
Gross energy (MJ kg^{-1} DM)	22.6	22.0	22.3	22.2	22.3	22.2
Fatty acids as a percent of total fatty acids						
14:0	5.8	5.9	6.2	7.1	6.4	6.1
16:0	19.8	21.4	20.4	20.2	20.9	21.4
18:0	7.0	6.0	5.6	4.4	5.3	5.7
SFA	38.4	38.4	36.3	35.2	36.6	36.5
16:1n−7	1.8	2.2	2.1	7.4	4.7	3.7
18:1n−9	43.9	36.6	35.6	18.9	29.7	33.4
MUFA	46.3	39.3	37.9	26.9	34.7	37.4
18:2n−6	8.7	10.4	9.0	8.6	10.1	11.6
18:3n−3	1.0	1.2	1.0	1.2	1.2	1.3
PUFA	15.3	22.3	25.9	37.9	28.7	26.2
20:4n−6	0.2	0.3	0.0	0.9	0.6	0.0
20:5n−3	1.8	2.7	2.7	12.2	7.3	5.4
22:6n−3	2.6	5.9	9.8	11.9	7.5	6.5
LC-PUFA	4.6	9.8	15.2	25.0	15.4	11.8
n−3	5.9	10.4	14.1	27.3	17.4	14.2
n−6	9.1	11.8	11.8	9.5	10.7	11.5

[a] Vitamin and mineral premix includes (IU kg^{-1} or g kg^{-1} of premix): Vitamin A, 2.5 MIU; Vitamin D3, 0.25 MIU; Vitamin E, 16.7 g; Vitamin K3, 1.7 g; Vitamin B1, 2.5 g; Vitamin B2, 4.2 g; Vitamin B3, 25 g; Vitamin B5, 8.3; Vitamin B6, 2.0 g; Vitamin B9, 0.8; Vitamin B12, 0.005 g; Biotin, 0.17 g; Vitamin C, 75 g; Choline, 166.7 g; Inositol, 58.3 g; Ethoxyquin, 20.8 g; Copper, 2.5 g; Ferrous iron, 10.0 g; Magnesium, 16.6 g; Manganese, 15.0 g; and Zinc, 25.0 g.

2.2. Animal management

Juvenile barramundi (*L calcarifer*) were sourced from Betta Barra (Cairns, QLD, Australia) and on-grown in a 10,000 L tank and fed a commercial diet (Marine Float, Ridley Aquafeed, Narangba, Qld, Australia). At the commencement of the experiment, fish (n = 480; 16.5 ± 0.1 g, mean ± S.D.) were anesthetised with AQUI-S™ (0.02 mL L^{-1}; AQUI-S New Zealand, Lower Hutt, New Zealand), weighed on an electronic top-loading balance to 0.1 g accuracy and allocated to one of 24 aquaria (300 L; n = 20 fish per aquaria) housed at the Aquaculture Feed Technology Laboratory at the Bribie Island Research Centre, Qld, Australia. Aquaria were supplied with flow-through seawater (salinity = 35 PSU; dissolved oxygen 7.0 ± 0.01 mg L^{-1}) at a rate of 4 L min^{-1}, maintained at 28.9 ± 0.6 °C (mean ± S.D.) for the duration of the experiment (28 days). Treatments were randomly allocated to 24 tanks, with each treatment having three replicates.

2.3. Diet preparation and management

A series of six diets, three with different DHA and three with different DHA + EPA inclusion levels were created by blending a range of ingredients including defatted fishmeal, an algal derived DHA source, fish oil, olive oil and butter fat to provide the lipid (Table 1).

Diets were formulated to provide approximately 540 g kg^{-1} protein and 100 g kg^{-1} of lipid at a gross energy level of 20.0 MJ kg^{-1} (estimated digestible protein and energy of 500 g kg^{-1} and 17.0 MJ kg^{-1} respectively; Tables 1 and 2). Diets were manufactured by making a single 40 kg batch of extruded pellets before an oil coating was applied under vacuum to each treatment. Dry ingredients were mixed using an upright planetary mixer (Robo Coupe, Vincennes Cedex, France).

Following dry mixing, the base diet was extruded using an APV MFP24 laboratory scale twin-screw extruder (APV-Baker, Peterborough, UK) using the operational configuration as described in Glencross et al. (in press). The pellets were produced by delivering the dry mash into the barrel at a feed rate of around 20 kg h^{-1}. Barrel temperatures were set for each of the four zones from drive to die at 50, 80, 100 and 110 °C respectively. Water was peristaltically pumped (Baoding Longer Precision Pump Co., Ltd, Hebei, China) into the barrel at 82 mL min^{-1}. Feeds were extruded through a 4 mm Ø die with the machine running at c. 470 rpm. Pellets were cut into 6–7 mm lengths using a two-bladed variable-speed cutter and collected on large aluminium oven trays (650 × 450 × 25 mm, length × width × depth). Approximately 40 kg of a single basal formulation of the resulting pellets was collected and dried at 65 °C prior to coating with oil.

Specific lipid allocations, consisting of blends of butter, olive oil, DHA oil and fish oil (Table 1), were vacuum-infused into the pellets after warming both the oils and pellets in an oven at 60 °C for 1 h. Warmed pellets were then placed in a Hobart mixer and the prescribed oil blend for each treatment was poured over the pellets whilst mixing. After mixing, the pellets were then exposed to a vacuum through the application of a perspex lid with a rubber seal to the mixing bowl and a vacuum pump connected to a valve in the lid. After all pellets were vacuum-infused with their respective oil blend, the pellets were stored at −20 °C until required for feeding.

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