Aquaculture 300 (2010) 148–155

Contents lists available at ScienceDirect

Aquaculture

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

Can dietary lipid source circadian alternation improve omega-3 deposition in rainbow trout?

Thomas D. Brown, David S. Francis *, Giovanni M. Turchini

School of Life & Environmental Sciences, Deakin University, PO Box 423, Warrnambool, Victoria 3280, Australia

ARTICLE INFO

Article history:
Received 30 October 2009
Received in revised form 16 December 2009
Accepted 17 December 2009

Keywords:
Fish oil replacement
Fatty acid deposition
Omega-3
Circadian patterns
Aquaculture
Canola oil
Rapeseed oil

ABSTRACT

With the salmonid industry currently exploiting the vast majority of globally available fish oil, there is the need to optimise fish oil utilisation by increasing its efficiency in terms of transferring the health-promoting long chain omega-3 fatty acids ($n-3$ LC-PUFA) into farmed fish flesh. The aim of this study was to evaluate if dietary fatty acid deposition is affected by the time of feeding, and hence identify possible innovative feeding strategies towards more efficient use of dietary fish oil. Over a period of 12 weeks, three diets with different lipid sources, canola oil (CO), fish oil (FO) or a 50/50 blend of the two oils (Mix), were alternated daily and fed to rainbow trout (*Oncorhynchus mykiss*). Six treatments were administered to fish, reference treatment (REF, continuously fed FO), control treatment (CT, continuously fed Mix), am canola oil ration (amCOR), pm canola oil ration (pmCOR), am canola oil satiation (amCOS) and pm canola oil satiation (pmCOS). Fish received either the CO diet in the am or pm feeds and received the FO diet at the opposite time. A significant increase in growth and feed consumption was noted in the pmCOS treatment. Fillet fatty acid profile was modified by associated feeding schedules and was generally reflective of dietary fatty acid profile. No significant increases in $n-3$ LC-PUFA deposition were observed. However, both linoleic acid (18:2$n-6$) and α-linolenic acid (18:3$n-3$) contents were significantly higher in pmCOR compared to amCOR and CT. The results of the present study suggest the existence of cyclical circadian patterns in fatty acid deposition in rainbow trout.

1. Introduction

Due to stagnating wild fisheries and a growing human population, aquaculture is expected to fill the gap in supplies of fish as food for humans, as demand continues to increase (Naylor et al., 2000; FAO, 2008; Turchini et al., 2009a). Fish oil (FO) in particular is heavily exploited globally for use in aquafeeds (Barlow, 2000; New and Wijkstrom, 2002; Jackson, 2006; FAO, 2008; Tacon and Metian, 2008). However, the rising cost, price volatility and sustainability concerns are driving the global search for alternatives (Bureau et al., 2008; Tacon and Metian, 2008).

The major issue regarding FO replacement in aquafeeds is the resultant modification of the fatty acid composition of the farmed fish, with significant reductions of the health-promoting long chain polyunsaturated fatty acids of the omega-3 series ($n-3$ LC-PUFA) (Caballero et al., 2002; Turchini et al., 2003; Bell et al., 2004; Turchini et al., 2006; Francis et al., 2007a; Trushenski and Boesenberg, 2009). Therefore, increasing the efficiency of FO utilisation is envisaged to be one of the fundamental steps required to progress towards a possible sustainable expansion of the aquaculture sector, alongside the identification of suitable alternative oils. Within this context, the use of finishing (wash-out) diets has been shown to be a possible strategy to increase the overall efficiency of FO utilisation in aquaculture (Glencross et al., 2003; Robin et al., 2003; Bell et al., 2004; Jobling, 2004a; Torstensen et al., 2005; Lane et al., 2006; Turchini et al., 2006, 2007; Trushenski and Boesenberg, 2009). However, large quantities of FO are still required during the finishing period to restore the fatty acid composition of fish previously fed with alternative oils (Robin et al., 2003; Bell et al., 2004; Trushenski and Boesenberg, 2009), and a suggestion by Francis et al. (2009) is that in order to optimise this strategy, it is best investigated from as many perspectives as feasible. In light of this suggestion, a new concept of alternative lipid source use in aquafeeds that has drawn recent research attention is the use of alternating feeding schedules in which dietary lipid source is routinely alternated over various time periods.

The impetus for investigating the effects of alternating feeding schedules on fish fatty acid profiles is derived from the observation of the existence of a cyclical pattern in the utilisation and retention of dietary fatty acids such as α-linolenic acid (ALA) and $n-3$ LC-PUFA in the Australian native freshwater fish Murray cod (*Maccullochella peelii peelii*) (Francis et al., 2009). This pattern was similar to that reported by De Silva (1985) for protein metabolism which triggered an intensive research effort around the possible uses of mixed feeding schedules (Nandeesha et al., 2002; Santiago and Laron, 2002; Patel and Yakupitiyage, 2003; Ali et al., 2005; El-Husseiny et al., 2008). Murray cod are able to grow faster and retain more eicosapentaenoic acid

* Corresponding author. Tel.: +61 03 5563 3002; fax: +61 03 5563 3462.
E-mail address: david.francis@deakin.edu.au (D.S. Francis).

0044-8486/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2009.12.020

(20:5$n-3$; EPA) and docosahexaenoic acid (22:6$n-3$; DHA) when reared on an alternating feeding schedule. In this scenario, a FO-based diet and a canola oil (CO) based diet were alternated over a 2-week, 4-week and a twice daily interval (Francis et al., 2009). This study, highlighted the added ability of Murray cod to deposit more $n-3$ LC-PUFA when fed a FO diet towards the end of the light phase of the photoperiod (i.e., dusk), in excess of what fish were capable of depositing when fed a FO diet at the beginning of the light phase of the photoperiod (i.e., dawn). This additional $n-3$ LC-PUFA deposition/retention ability suggests the existence of a cyclical circadian rhythm in the subject fish which promotes increased $n-3$ LC-PUFA deposition associated with the night/dark phase of a photoperiod. Although no direct mechanism was identified to explain the observed trends in fatty acid deposition, Francis et al. (2009) hypothesised that a cyclical circadian pattern of hormone production could be responsible.

The cyclical pattern evident in the ability of fish to deposit $n-3$ LC-PUFA was proposed to be mediated by endogenous rhythms, synchronised by exogenous stimuli including photoperiod as well as internal stimuli resultant of feed intake, feed composition and hormone secretion. Fully exploiting circadian rhythms in fish associated with lipid metabolism will allow for the further optimisation of FO use in aquafeeds, by promoting increased retention of $n-3$ LC-PUFA within fish tissues, in relation to the amount of $n-3$ LC-PUFA provided with the diet. Increased efficiency in $n-3$ LC-PUFA retention in cultured fish will ultimately help to reduce the volume of FO required in aquafeeds, whilst ensuring that the $n-3$ LC-PUFA content of cultured fish is not significantly diminished.

The aim of this study was to verify the existence of similar circadian patterns in $n-3$ LC-PUFA deposition in a salmonid species, as salmonid aquaculture currently accounts for the exploitation of over 55% of global fish oil supply (Tacon and Metian, 2008). Rainbow trout (*Oncorhynchus mykiss*) was chosen as the target species, and a feeding trial was implemented over the entire grow-out phase, up to commercial size, in which six individual dietary treatments, consisting of different sources and quantities of dietary lipids (namely FO and CO) were alternated daily.

2. Materials and methods

2.1. Animals and husbandry

Subject fish, 380 juvenile rainbow trout (23.5 ± 1.9 g at the commencement of the trial), were obtained from Fisheries Victoria—Department of Primary Industries Snobs Creek hatchery (Victoria, Australia). Trout were transported to Deakin University's Aquaculture Research Facility at the Warrnambool campus and acclimatised to the new environmental conditions for 7 weeks and maintained on a commercial diet.

The indoor recirculating aquaculture system (RAS) consisted of 18, 800-L, round conical bottomed polyethylene tanks, equipped with in-line biological and physical filtration (60-μm screen) (Hydrotech, Vellinge, Sweden), ultra violet (UV) disinfection, oxygen enrichment, temperature regulation and photoperiod control. Trout were subjected to a 12light:12dark (12 L:12D) photoperiod via direct illumination from fluorescent lighting and the water temperature was maintained at 15.3 ± 0.03 °C over the course of the feeding trial. Photoperiod contained both dawn and dusk phases, where light intensity gradually increased from complete darkness to full light and from full light to complete darkness over a 1.5-h period. Dissolved oxygen was monitored daily using an automatic temperature compensated Oxy Guard gamma probe (Oxy Guard International, Birkerød, Denmark) and metabolic waste products ammonia and nitrite, were monitored on a weekly basis, as was pH, using colorimetric test kits (Aquamerck, Merk, Darmstadt, Germany). A mean pH of 8.32 and mean nitrite and ammonia both below 0.16 mg/l^{-1} was recorded during the trial. Feed was supplied twice daily at 09:00 (am feed event) and 19:00 (pm feed event) with exception of bulk fish weighing days, when no fish were fed. Feed consumption was recorded weekly.

2.2. Experimental diets and study design

Three diets formed the basis of this trial and all were produced in 15-kg batches at Deakin University using existing feed production equipment as per standard protocol (Francis et al., 2006). All dry diet ingredients were combined in a commercial baker's mixer (MEC, Australia) and homogenised for 10 min until well mixed. Dietary oils were added slowly to the running mixer to assist in an even application of lipid to dry ingredients. Lipid and dry ingredients were further homogenised for 5 min before addition of 3–4 l of water (~80 °C) per 15 kg of diet and further mixing to attain a malleable doughy consistency. Finished diets were pelleted through a 5-mm die, dried to 2–3% moisture in a temperature controlled room at 35 °C over a 24-h period, and stored in airtight bags until needed. The three diets were practical diets (containing relatively large amount of fish meal) and formulated with the addition of different oil sources: fish oil (FO), canola oil (CO) and a 50/50 blend of fish and canola oils (Mix). Diets produced, their ingredients, proximate composition and fatty acid profile are reported in Tables 1 and 2, respectively.

Trout were weighed individually and randomly allocated (20 fish per tank) to one of the 18 tanks, and six treatments were randomly allocated in triplicate to the 18 tanks in the trial (Table 3). Both a control treatment (CT) and a reference treatment (REF) were used in this trial. CT replicates were fed to apparent satiety with the Mix diet in order for the fish in this treatment to receive a constant 50/50 blend of fish and canola oil during both am and pm feed events. REF treatment fish were fed to apparent satiety with the FO diet in both feeding events. Then, four treatments were implemented with a 2 × 2 factorial design consisting of daily alternation of the two diets (FO and CO) in the morning (am) and in the evening (pm), and the administration of feed to apparent satiety (S) or according to fixed rations (R). Therefore, the four treatments obtained were named amCOS and pmCOS (fish fed to satiety with the CO diet in the morning and FO diet in the evening, and vice versa, respectively) and amCOR and pmCOR (fish fed to fixed ration with the CO diet in the morning and FO diet in the evening, and vice versa, respectively) (Table 3). Rations administered to amCOR and pmCOR were calculated daily based on average feed consumption by CT replicates during the previous day and corrected for tank biomass. Ration allocation of diet resulted in amCOR and pmCOR replicates

Table 1
Experimental diet formulation (g kg^{-1}).

	Diets[a]		
	FO	CO	Mix
Fish meal[b]	338.3	338.3	338.3
Defatted soy meal[c]	338.3	338.3	338.3
Wheat Gluten[d]	56.4	56.4	56.4
Wheat flour[e]	110.5	110.5	110.5
Vit + min[b]	3.0	3.0	3.0
Choline cl[f]	5.0	5.0	5.0
Cr_2O_3[f]	2.0	2.0	2.0
Fish oil[g]	146.6	0.0	73.3
Canola oil[h]	0.0	146.6	73.3

[a] Diets abbreviations, FO = fish oil diet, CO = canola oil diet, Mix = 1:1 (fish oil: canola oil).
[b] Ridley AgriProducts, QLD, Australia.
[c] Mepunga grains, Mepunga, VIC, Australia.
[d] Gem of the West Vital Wheat Gluten, Manildra Starches Pty. Ltd., Altona, VIC, Australia.
[e] Black and Gold Pty. Ltd., Tooronga, VIC, Australia.
[f] Sigma, St. Louis, MO, USA.
[g] Sceney Chemicals Pty. Ltd., Sunshine, VIC, Australia.
[h] Canola oil = low erucic acid rapeseed oil, Crisco, Goodman Fielder, North Ryde, NSW, Australia.