Aquaculture 433 (2014) 74–81

Contents lists available at ScienceDirect

Aquaculture

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

Effect of dietary substitution of fish oil with flaxseed or sunflower oil on muscle fatty acid composition in juvenile steelhead trout (*Oncorhynchus mykiss*) reared at varying temperatures

Manjusri P.A. Wijekoon [a,1], Christopher C. Parrish [a,*], Atef Mansour [b]

[a] Department of Ocean Sciences, Memorial University of Newfoundland, Marine Lab Road, St. John's, NL A1C 5S7, Canada
[b] Fisheries and Oceans Canada, Northwest Atlantic Fisheries Centre, 80 East White Hills Rd., St. John's, NL A1C 5X1, Canada

ARTICLE INFO

Article history:
Received 30 January 2014
Received in revised form 21 May 2014
Accepted 22 May 2014
Available online 2 June 2014

Keywords:
Steelhead trout
Fish oil
Sunflower
Flaxseed
Fatty acid
Temperature

ABSTRACT

The effect of changing temperature on muscle fatty acid composition was examined in juvenile steelhead trout (~120 g) with complete substitution of fish oil (herring oil; HE) with either flax seed oil (FLX; also known as linseed oil) or sunflower oil (SF). The temperature was increased from ~10.0 °C to 18.0 °C following seasonal temperature changes in ~2 °C incremental steps with plateaus. Dietary lipid analysis showed the HE diet was rich in marine lipids, docosahexaenoic acid (DHA, 22:6ω3), eicosapentaenoic acid (EPA, 20:5ω3) and docosapentaenoic acid (DPA, 22:5ω3). In contrast FLX was rich in linolenic acid (ALA, 18:3ω3) and SF in linoleic acid (LA, 18:2ω6). Both diet and temperature had no significant effect on growth but significant effects were observed on muscle fatty acid composition. Proportions of marine fatty acids in HE fed fish were significantly higher than in both SF and FLX fish at the end (41, 31 and 31% respectively), with significantly lower levels of terrestrial plant fatty acids compared to the beginning of the experiment. Substitution of SF and FLX had a direct influence on muscle EPA:AA and ω6:ω3 ratios which could be detrimental to the health of the fish, as well as to the quality of the end product for human consumption.

1. Introduction

A number of studies have investigated limitations and cost benefits of replacing fish oil (FO) in marine fish feed with alternative lipid. Several replacements have been investigated including vegetable oils (VOs) (Bell et al., 2001; Caballero et al., 2002; Francis et al., 2006; Ng et al., 2007; Stubhaug et al., 2007), and rendered animal fat (AF) from beef, pork and poultry (Bureau and Gibson, 2004; Turchini et al., 2003; Watanabe, 2002). However, the primary focus of these studies was on growth and feed utilisation, with less emphasis on environmental stresses and its effect on muscle fatty acid composition.

Although several alternative oils have been explored in the past, both VO and AF or their blends have created the most interest due to availability, low cost and ability to be fully or partially replaced without affecting growth (Bell et al., 2004; Bureau and Gibson, 2004; Stubhaug et al., 2007; Torstensen et al., 2004, 2005; Turchini et al., 2003), flesh quality (Nanton et al., 2007), fatty acid composition (Bell et al., 2003; Regost et al., 2004; Torstensen et al., 2005), physical characteristics of the fillet (Mørkøre, 2006; Regost et al., 2003), metabolic disorders (Vegusdal et al., 2005) or disease resistance (Bransden et al., 2003; Thompson et al., 1996) in salmonids. However, further investigation is needed as most feeding trials are conducted under constant environment conditions.

FO differs from its main substitution contenders (VO and AF) in being rich in ω3 highly unsaturated fatty acid (HUFA, C_{20} or higher) such as docosahexaenoic acid (DHA, 22:6ω3) and eicosapentaenoic acid (EPA, 20:5ω3) (Menoyo et al., 2007). In contrast VO is rich in C_{18} fatty acids, mainly LA (18:2ω6), oleic (OL; 18:1ω9) and in the case of linseed or flax oil (FLX), α-linolenic acid (ALA; 18:3ω3). VO lacks EPA and DHA.

Marine carnivorous fish have a higher dietary requirement for ω3 HUFA due to a limited synthetic capacity compared to freshwater fish. The high requirement could be explained by the significant role played by DHA and EPA in maintaining the structure and function of cell membranes in marine fish (Sargent and Tacon, 1999). Sargent and Tacon (1999) also suggested that EFA ratios (ω3:ω6 as well as EPA:DHA:AA) in fish diets play a much bigger role in fish physiology and health than a minimum EFA content. Dietary FO replacement with VO or AF with a lower ω3:ω6 ratio could change the ratio of EPA:AA and the DHA level in tissue. In contrast freshwater fish have the ability to desaturate and elongate shorter chain ω3 fatty acids such as ALA to produce EPA and DHA (Arts et al., 2001; Castell et al., 1972; Menoyo et al., 2007; Ruyter et al., 2000).

* Corresponding author. Tel.: +1 709 864 3225.
E-mail address: cparrish@mun.ca (C.C. Parrish).
[1] Present address: Department of Clinical Studies, Ontario Veterinary College, University of Guelph, N1G 2W1.

http://dx.doi.org/10.1016/j.aquaculture.2014.05.028
0044-8486/

Water temperature has the potential to influence fatty acid composition of fish tissues (Farkas et al., 2001; Hochachka and Somero, 2002). Water temperature mostly influences polar lipids, although an increase could potentially decrease the unsaturated to saturated fatty acid ratio in muscle tissue (Farkas et al., 2001; Fodor et al., 1995; Hazel and Williams, 1990; Hochachka and Somero, 2002). Studies examining the effect of both diet and water temperature have usually kept the temperature stable at two different levels (Hazel, 1979, 5 and 20 °C; Jobling and Bendiksen, 2003, 2 °C and 8 °C; Skalli et al., 2006, 22 °C or 29 °C). Hazel (1979) found that rainbow trout acclimated to 20 °C had a higher composition of neutral lipid (NL), triacylglycerol (TAG) and free fatty acid (FFA) than at 5 °C, and the study conducted by Jobling and Bendiksen (2003) found that Atlantic salmon parr raised at 2 °C and 8 °C on FO or VO based diets until the fish doubled in mass showed significant changes in the polar and NL fractions. In the same study, feed had a pronounced effect on NL while the polar fraction was markedly influenced by the temperature with only a moderate change due to diet. In a study of European sea bass (*Dicentrarchus labrax*) juveniles (Skalli et al., 2006), fish were held at 22 °C or 29 °C and given a diet composed of ω3 HUFA either below or over the minimal required level for growth. The EFA deficient diet showed a marked drop in the NL fraction with a moderate influence on polar lipid of muscle, liver and gills and with a very low influence on eye and brain polar lipids at 29 °C. However, studies conducted to determine tissue fatty acid composition with simultaneous changes in water temperature are limited and give inconsistent results (Craig et al., 1995; Fracalossi and Lovell, 1995; Grisdale-Helland et al., 2002; Kelly and Kohler, 1999).

Steelhead trout (*Oncorhynchus mykiss*) is one of the species cultured in Bay d'Espoir, Newfoundland. Unexpected weather and temperature changes are always a challenge in intensive sea cage aquaculture in the northwest Atlantic. Sudden changes in environment could be stressful for caged fish, compromising growth, survival, quality of the final product, and timing of harvest. Therefore in this study we examined the effect of either complete substitution of FO (herring oil; HE) with flax seed oil (FLX) or sunflower oil (SF) together with changing environmental temperature on muscle lipid class and fatty acid composition in juvenile steelhead trout.

2. Materials and methods

2.1. Experimental fish

This study was conducted in the Dr. Joe Brown Aquatic Research Building (JBARB), Memorial University, St. John's, NL. Four hundred and eighty juvenile steelhead trout (transported from a Bay d'Espoir aquaculture site), 120 g in average body weight were moved to a 45 m^3 holding tank at ambient temperature (5 ± 1 °C). Dissolved O_2 and temperature were monitored twice daily and dead fish were removed and examined for external lesions. All wastes that accumulated at the bottom of the tank were removed daily. Fish were held in the holding tank for approximately a month prior to the experiment and fed with the same feed type used at the aquaculture site (Corey feed, Fredericton, NB, Canada).

2.2. Experimental tanks

Fifty-five fish were haphazardly picked and distributed to each experimental tank (6 × 6000 l tanks). Fish were held at 8.0 ± 1.0 °C water temperature with a flow of 6–7 l min^{-1} for approximately 2 weeks to acclimate to the experimental tanks. Fish were fed with experimental base diet (HE diet) and lighting was automatically controlled to follow the daily light and dark cycles. Dissolved O_2, salinity and temperature were monitored daily.

2.3. Experimental feed

Three experimental diets used in this study were formulated at the Marine Research Station, Sandy Cove, Halifax. The basal diet was formulated using the ingredients listed in Table 1. The origin of lipids was different among the three diets (herring oil, HE; flax seed oil, FLX; and sunflower oil, SF). The three diets were stored at −20 °C to minimise lipid oxidation and a portion of each diet was kept in a walk-in cooler and replaced as needed for daily feeding. All fish were fed with the experimental base diet (HE) during the acclimation period. Thereafter fish were fed with the previously assigned experimental diets in duplicate for a 12 week period.

2.4. Experimental temperature

The temperature was increased to 10.0 ± 1.0 °C after the initial acclimation period (8.0 ± 1.0 °C) in 1 to 2 days. The average temperature was increased in a stepwise manner, following seasonal temperature changes, from 10.0 °C (1st sampling) to a maximum of 18 °C (6th sampling) following ~2 °C incremental steps but with plateaus (Fig. 1). At each step, the temperature was increased gradually to the next level over an ~1–2 day period and left stable thereafter for 14 ± 1 days. Ambient water was heated and adjusted through an automatically controlled system at the header tank to obtain the required temperatures in the experimental tanks.

2.5. Sampling protocol

The first sampling was considered the baseline and was done at the end of the acclimation period prior to the feeding trial. However, feed was withdrawn a day prior to the day of sampling. A minimum of 3 fish per tank was randomly picked and lethally sampled with an overdose of anaesthetic TMS (MS-222) at the end of each thermal period. Muscle samples for lipid class and fatty acid analysis were obtained from the left epaxial region of the fish caudo-dorsal to the pectoral and ventral to the anterior base of the dorsal fin. Samples of muscle weighing approximately 1 g were collected in 50 ml glass vials previously cleaned for lipid residues by rinsing 3 times with methanol (MeOH) and chloroform ($CHCl_3$) respectively. Then each vial was flushed with nitrogen (N_2) after filling with 4 ml $CHCl_3$, sealed with Teflon lined caps and Teflon tape and stored at −20 °C. Samples collected to determine the muscle water content were weighed and wrapped in aluminium foil and stored in a fridge.

2.6. Lipid extraction

Total lipids from triplicate samples from each tank sampled at each temperature were extracted in $CHCl_3$/MeOH following Parrish (1999) using a modified Folch procedure (Folch et al., 1957). Fish muscle samples were quickly homogenised on ice (Brinkman Polytron blender) to a pulp and the metal rod of the blender was washed in 2:1 $CHCl_3$:

Table 1
Feed ingredients and composition of the three experimental diets: HE (herring oil), FLX (flax seed oil) or SF (sunflower oil).

Feed ingredient	Composition (%)
Herring meal	37
Soybean meal	15
Corn gluten meal	10
Dried whey	7
Wheat middling's	13.6
Vitamin premix	1.5
Choline chloride	0.4
Mineral premix	1.5
Lipid supplement[a]	14

[a] Herring, flax or sunflower oil was used as the lipid supplement in the three experimental diets.