Comparative Biochemistry and Physiology, Part A 152 (2009) 552–559

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

A vegetable oil feeding history affects digestibility and intestinal fatty acid uptake in juvenile rainbow trout *Oncorhynchus mykiss*

Inge Geurden [a,*], Fredrik Jutfelt [b], Rolf-Erik Olsen [c], Kristina S. Sundell [b]

[a] INRA UMR1067 Laboratory of Nutrition, Aquaculture and Genomics, NuAGe, INRA Hydrobiology Station, F-64310 Saint Pée-sur-Nivelle, France
[b] Fish Endocrinology Laboratory, Department of Zoology, University of Gothenburg, Sweden
[c] Institute of Marine Research, Matre Aquaculture Research Station, N-5984 Matredal, Norway

ARTICLE INFO

Article history:
Received 20 November 2008
Received in revised form 29 December 2008
Accepted 30 December 2008
Available online 8 January 2009

Keywords:
Fish oil substitution
Plant oils
Salmonid fish
In vitro fatty acid absorption
Transepithelial resistance
Intestinal integrity
Ussing chamber

ABSTRACT

Future expansion of aquaculture relies on the use of alternatives to fish oil in fish feed. This study examined to what extent the nature of the feed oil affects intestinal lipid uptake properties in rainbow trout. The fish were fed a diet containing fish (FO), rapeseed (RO) or linseed (LO) oil for 8 weeks after which absorptive properties were assessed. Differences in digestibility due to feed oil history were measured using diet FO with an indigestible marker. Intestinal integrity, paracellular permeability, *in vitro* transepithelial fatty acid transport (^{3}H-18:3*n*−3 and ^{14}C-16:0) and their incorporation into intestinal epithelia were compared using Ussing chambers. Feed oil history did not affect the triacylglycerol/phosphatidylcholine ratio (TAG/PC) of the newly synthesized lipids in the segments. The lower TAG/PC ratio with 16:0 (2:1) than with 18:3 (10:1) showed the preferential incorporation of 16:0 into polar lipids. The FO-feeding history decreased permeability and increased transepithelial resistance of the intestinal segments. Transepithelial passage rates of 18:3*n*−3 were higher when pre-fed LO compared to RO or FO. Similarly, pre-feeding LO increased apparent lipid and fatty acid digestibilities compared to RO or FO. These results demonstrate that the absorptive intestinal functions in fish can be altered by the feed oil history and that the effect remains after a return to a standard fish oil diet.

1. Introduction

Due to the imminent shortage of fish oil for the rapidly expanding aquaculture industry and its upward price trend, oils of vegetable origin gained huge interest in fish feed production (FAO, 2007). Vegetable oils are absent from the natural diet of salmonids and other carnivorous fish. Their fatty acid (FA) composition differs from that of fish oils by chain length (not more than 18 carbons) and degree of unsaturation (not more than 3 double bonds). This change in dietary FA may modify the absorptive functions of the fish intestine, which is the first organ directly exposed to the ingested FA. This is suggested i) by the presence of lipid droplet accumulations in enterocytes of fish fed vegetable oils, indicating a higher uptake than export rate (Olsen et al., 1999, 2000; Caballero et al., 2002), and ii) by the differences in the apparent digestibility of a FA according to the nature of the feed oil. For instance, depending on its concentration in the oil, the apparent digestibility of 16:0 in Atlantic salmon (*Salmo salar*) decreased from 85% when fed capelin oil to 78% and 16% when supplied in the form of sunflower oil or palm oil, respectively (Torstensen et al., 2000). The levels of 16:0 were 13%, 6% and 38% (% total FA) in the diets (isolipidic) with capelin, sunflower and palm oil, respectively. The same study further revealed differences in FA digestibility which were independent of the concentration of the FA in the diet; e.g. oleic acid, present in the diet at a range of 36–40% (% total FA) had a digestibility of 97% in a capelin/sunflower oil diet and only 60% in a palm oil diet (Torstensen et al., 2000), suggesting an interfering effect of the other accompanying FA on the digestibility of the considered FA. In line with this, higher levels of dietary saturated FA not only decreased the digestibility of the saturated FA, but also of the accompanying monoenes and polyunsaturated FA (PUFA) in Atlantic salmon (Menoyo et al., 2003).

It remains uncertain what explains the observed differences in FA digestibility according to the oil source. High levels of saturated FA, with low amphiphilic properties, may negatively affect the formation of micelles in the intestinal lumen and hence reduce the apical FA uptake by the enterocyte (Menoyo et al., 2003). The different FAs in the oil may also compete for the same transporter in case of protein-mediated transport, as shown with human Caco-2 cells where the presence of palmitic, linoleic or oleic acid in the culture medium reduced uptake rates of linolenic acid (Tranchant et al., 1997). The dietary FA supply may further affect intracellular events of the lipid absorption process. In this respect, lipid droplet accumulations, observed in enterocytes of fish fed linseed oil with high levels *n*−3 PUFA, were found to be prevented by co-feeding 16:0, possibly

* Corresponding author. Tel.: +33 559515951; fax: +33 559545152.
E-mail address: geurden@st-pee.inra.fr (I. Geurden).

1095-6433/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2008.12.016

because 16:0 enhanced the synthesis of chylomicron PC and thus the efflux of the lipid from the enterocytes (Olsen et al., 2000).

In addition to aforementioned direct interactions between the ingested FA during digestion and absorption, intestinal nutrient uptakes might also be altered in a more indirect manner by modifications in the composition of the intestinal membranes. Because of their rapid turnover, intestinal membrane FA are highly influenced by changes in dietary FAs. In mammals or birds such changes have been reported to modify intestinal function and uptake properties of a large variety of passively and actively transported nutrients, including FA (Thomson et al., 1987; Tranchant et al., 1997, 1998; Perin et al., 1999; Ferrer et al., 2003). In fish, only limited information is available on whether FA intake history affects intestinal FA absorption (Caballero et al., 2006; Jutfelt et al., 2007), in spite of the habitually long feeding periods with the different feed oils prior to the FA digestibility measurements. The feeding trials often exceed 4 weeks (*e.g.* Torstensen et al., 2000; Caballero et al., 2002; Menoyo et al., 2003; Francis et al., 2007), a time period sufficient to modify the fish enterocytes membrane FA composition (Olsen et al., 2003; Björnsson et al., 2004), which in turn can affect membrane fluidity or functioning of membrane bound enzymes, such as fatty acid binding proteins (Poirier et al., 1996) and thereby alter absorptive functions.

The purpose of the present study was to examine to what extent the nature of the feed oil history affects the (*in vivo*) FA digestibility and the (*in vitro*) intestinal FA absorption capacities in juvenile rainbow trout.

2. Materials and methods

2.1. Diets

The diets were prepared at the INRA experimental fish farm (Donzacq, France). They had similar levels of crude protein, crude fat and gross energy and contained fish meal and soybean concentrate as protein source. They differed solely by the oil supplement: fish oil (control diet FO), rapeseed oil (diet RO) or linseed oil (diet LO). Diet FO had low levels of linoleic acid (18:2*n*−6) and was relatively rich in palmitic acid (16:0), oleic acid (18:1*n*−9), long chain monounsaturated FA (20:1 and 22:1) and *n*−3 highly unsaturated fatty acids (HUFA), EPA (20:5*n*−3) and DHA (22:6*n*−3). The *n*−3 HUFA present in the 100% vegetable oil diets RO and LO originated from the residual fat content (approximately 15%) in the fish meal. Diet RO contained high levels of 18:1*n*−9 (40%) and diet LO high levels (33%) of linolenic acid (18:3*n*−3). The two vegetable oil diets (RO and LO) had similar levels of total *n*−6 PUFA and total saturated FA. The ratio of PUFA/saturates was 0.9 for diet FO, 1.5 for diet RO and 2.7 for diet LO (Table 1).

Table 1
Analysed proximate composition and fatty acid profile of the three diets fed for 8 weeks to rainbow trout prior to the digestibility and fatty acid uptake trials

	Diet FO	Diet RO	Diet LO
Proximate composition (% diet DM)			
Dry matter (DM, % diet)	94.0±0.0	92.0±0.0	95.3±0.0
Crude proteins (% DM)	49.0±0.3	49.4±0.3	49.6±0.1
Crude lipid (% DM)	24.3±0.1	25.0±0.1	24.2±0.2
Gross energy (kJ/g DM)	24.3±0.1	24.2±0.1	24.1±0.1
Fatty acid composition (% total fatty acids)			
14:0	6.4	2.9	2.8
16:0	21.1	12.6	12.4
18:0	3.9	2.6	3.7
20:0	0.3	0.1	0.1
Sum saturates	34.3	20.3	20.6
16:1	8.0	3.3	3.3
18:1	16.8	40.4	15.8
20:1	4.7	2.0	2.0
22:1	4.5	1.9	1.9
Sum monoenes	34.5	49.8	24.4
18:2 *n*−6	4.2	12.4	10.7
20:4 *n*−6	0.9	0.4	0.4
Sum *n*−6 PUFA	7.3	14.0	12.2
18:3 *n*−3	1.1	6.4	33.2
18:4 *n*−3	2.5	1.1	1.1
20:5 *n*−3	7.7	3.2	3.2
22:5 *n*−3	0.8	0.3	0.3
22:6 *n*−3	11.0	4.6	4.6
Sum *n*−3 PUFA	23.8	16.8	43.5
PUFA/Sat	0.91	1.52	2.70
n−3/*n*−6 PUFA	3.26	1.20	3.57

Ingredients (% diet): fishmeal: 49.1%, soybean concentrate: 11.1%, extruded wheat: 23.2%, Vitamin and mineral premix: 0.7%, binder (alginate): 1.7%, oil: 14.3% (fish oil, rapeseed oil and linseed oil in diets FO, RO and LO, respectively).

2.2. Pre-feeding trial

The pre-feeding trial lasted 8 weeks. Each diet was fed twice a day by hand to visual satiation to triplicate groups (80 fish/group) of rainbow trout *Oncorhynchus mykiss* (55 g initial body weight). The fish were kept in 150 l flow-through tanks supplied with water at 16±1 °C (INRA experimental fish farm, Donzacq, France). Mortality, if any, was recorded daily. Each group (24-h feed deprived) was bulk-weighed and counted bi-weekly for the determination of the average body weight. Voluntary feed intakes were calculated by weekly weighing of the feed remains in a pre-tarred feed bucket. At the end of the trial, six 24-h feed deprived fish per tank were eviscerated and weighed individually for the calculation of the viscero-somatic index (100*visceral weight/body weight). The viscera were kept separated from the eviscerated bodies, pooled per tank and stored at −20 °C for further analyses. At the same time, 25 fish were randomly sampled from each tank and transported to the INRA fish rearing unit (St Pée-sur-Nivelle, France) for digestibility and absorption studies. The fish were deprived of food 36–40 h before the start of the digestibility or FA uptake trials in order to clear the gut from faeces and excess lipids.

2.3. In vivo nutrient and fatty acid digestibility trial

For the digestibility trial, the three groups of fish with different nutritional (feed oil) history were placed in 60 l cylindro-conical tanks supplied with water of 17.5 °C (15 fish per tank, 3 tanks per feed oil history). The fish received the diet FO (Table 1) enriched with 1% Cr_2O_3 and were fed by hand twice a day to visual satiation. The faeces were collected automatically by a sieving system as described by Choubert et al. (1982), allowing a rapid (5–10 s) recovery of the faeces from the water without manipulating the fish. The faeces, collected over a 10-day period, were stored daily at −20 °C, pooled per tank and freeze-dried for further biochemical analyses. The apparent digestibility (AD, %) coefficients were calculated as: AD dry matter = 100−(100*%Cr_2O_3 diet/%Cr_2O_3 faeces); AD of protein, lipid or energy (*X*) = 100−(100*(%*X* in faeces/%*X* in diet)*(%Cr_2O_3 diet/%Cr_2O_3 faeces)). The AD of the FA was calculated as: 100−(100*(%FA in faeces*faeces lipid content/%FA in diet*diet lipid content)*(%Cr_2O_3 diet/%Cr_2O_3 faeces)). For evaluating the effect of the feed oil history on FA uptakes, we compared the level of non-absorbed FA in the faeces (%), *i.e.* '100−%AD of each FA'.

2.4. In vitro fatty acid uptake trial

The effect of the feeding history on the uptake of two selected free FA as well as on intestinal epithelial viability and integrity were assessed using the Ussing chamber protocol, modified from Sundell et al. (2003) and Jutfelt et al. (2007).

2.4.1. Preparation of intestinal segments

The fish were killed by anaesthetic overdose (2-phenoxyethanol, 1 mg/l) and decapitated. The peritoneal cavity of the fish was opened laterally, and mesenteries and adipose tissue removed. The intestine