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Dietary medium chain fatty acids from coconut oil have little effects on postprandial plasma metabolite profiles in rainbow trout (*Oncorhynchus mykiss*)



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ABSTRACT

This study examined the effect of dietary medium-chain triglycerides supplied by coconut oil on postprandial plasma metabolite profiles in rainbow trout. The fish (initial body weight 71.3 ± 0.3 g, 17 °C) were fed one of four practical diets containing either 5% fish oil (FO low-fat, FL), 15% fish oil (FO high-fat, FH), 5% coconut oil (CO low-fat, CL) or 15% coconut oil (CO high-fat, CH) for 3 weeks. At the end of the trial, the fish were weighed and plasma sampled to determine glucose, non-esterified fatty acids (NEFA), triglyceride (TG), cholesterol, high density lipoprotein-cholesterol (HDL-cholesterol), and myeloperoxidase (MPO) at 3, 6, 9, 12, 15 and 24 h after the last meal. Plasma total ketone bodies (KB) were determined at 6, 12 and 24 h after meal. Blood nitroblue tetrazolium (NBT) tests were also performed in samples withdrawn at 24 h after meal.

Plasma glucose was higher in fish fed the low fat level diet than those fed high fat level, and peaked at postprandial 9–12 h. Fish fed CH showed higher plasma TG than CL at 3 h after meal, and there was no significant difference in plasma TG at the other time points. The peak of TG appeared 12 h after the meal. No clear pattern was found for cholesterol and HDL-cholesterol (HDL-C) in any of the groups. However, fish fed diet FH had the highest postprandial plasma HDL-cholesterol level and HDL-C/cholesterol ratio. The peak of NEFA was observed at 12–15 h after meal and plasma NEFA of fish fed CH was the highest. Plasma total KB decreased with postprandial time, and fish of FH groups had higher KB than that of CL group at 6 h. Besides, NBT in fish fed FH was significantly higher than that of CH, but there were no differences in MPO between groups. In summary, time-course changes in plasma profiles related to dietary fat level were as expected whereas those related to dietary fat source were relatively small.

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1. Introduction

The trend in aquaculture diets, especially in salmon and trout farming, during the last decades has been to increase the energy content in the diets by increasing the dietary fat content to improve feed or protein utilization efficiency and to reduce nitrogen losses. Fish oil (FO) is the most extensively used fat source in salmon feeds because of its relatively rich $n\!-\!3$ long-chain polyunsaturated fatty acid (LC-PUFA) levels to ensure optimal growth and reproduction (Sargent et al., 1995, 1999). However, marine resources in general and FO in particular might be in shortage for future feed production due to an increasing aquaculture production and at best stable or declining catches for FO production (Tacon and Metian, 2008). In search for alternatives to FO, several studies undertaken with salmonids have shown

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that substitution of FO with vegetable oil may not affect the growth and survival of fish (Bell et al., 2003, 2004; Greene and Selivonchick, 1990; Richard et al., 2006; Torstensen et al., 2004; Turchini et al., 2009).

Medium chain fatty acids (MCFA) are defined by a fatty acid chain length from 6- to 12-carbon atoms (Marten et al., 2006). Coconut oil (CO) is a natural plant source, rich in MCFA with lauric acid (C12:0) representing 40-50% of total fatty acids. CO is rich in saturated fat content with a high melting point making it heat stable and resistant to peroxidation, allowing its long-term storage (Bruce, 2005). It might be supposed that in warm blooded mammals, the utilization of CO will be easier than in fish reared at low temperatures. However, CO has been proven to be an efficient fat source in compound diets for firstfeeding larvae of African catfish (Heterobranchus longifilis) (Legendre et al., 1995) and of common carp (Cyprinus carpio L.) (Fontagne et al., 2000). Similarly, no negative effects were reported following dietary inclusion of CO on growth of Ayu (Plecoglossus altilelis) (Nakagawa and Kimura, 1993), red drum (Sciaenops ocellatus) (Craig and Gatlin, 1995), milkfish (Chanos chanos) (Alava, 1998) and even, the cold water species, rainbow trout (Figueiredo-Silva et al., 2012b), or on

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reproductive performances in rainbow trout (Ballestrazzi et al., 2006). On the contrary, Hsieh et al. (2007) found that CO inclusion led to lower growth than with FO in hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*). Also for polka-dot grouper (*Cromileptes altivelis*), fed diets containing high levels of CO (15%) displayed significantly reduced growth compared to those fed FO (Williams et al., 2006). Although growing similarly well with FO or CO containing diets, there were significant effects on the respiratory and cardiovascular physiology in the European eel (*Anguilla anguilla* L.) and Adriatic sturgeon (*Acipenser naccarii*) (McKenzie, 2001).

The role of MCFA as energy-yielding substrates has been largely investigated in terrestrial animals (Aurousseau et al., 1989; Bozzolo et al., 1993). In mammals, MCFA have been reported to directly enter the portal vein, bypassing the intestinal re-acylation and chylomicron packaging process, resulting in a more rapid absorption and plasma appearance. Moreover, MCFA do not need carnitine palmitoyltransferase-1 (CPT-1) mediated transport to enter mitochondria which may accelerate their oxidation. As a result, only small amounts of MCFA are recovered in peripheral tissues (Johnson et al., 1990; Lhuillery et al., 1988). Also in fish, dietary inclusion of MCFA (mainly 8:0 and 10:0) resulted in decreased fat deposition in the muscle, liver and in intra-peritoneal fat with similar or improved growth rates (Nakagawa and Kimura, 1993). Negative effects on the efficiency of energy or lipid retention were also seen in Atlantic salmon (Salmo salar) fed purified MCFA (Nordrum et al., 2003) and in polka-dot grouper fed CO, suggesting high levels of C12-\(\beta\)-oxidation (Williams et al., 2006). By comparison, Figueiredo-Silva et al. (2012b) revealed high C12:0 retention from CO, with C12 representing over 20% of total body fatty acids compared to less than 1% in trout fed FO. Other studies in mammals (Allan et al., 2001; Mohamed et al., 2002; Nagao and Yanagita, 2010) documented the different effects of CO compared to LCFA oils on plasma parameters related with the metabolic syndrome (glycemia, cholesterol, lipoprotein levels, ketone bodies, etc.). In fish, little is known on the effect of feeding CO on these plasma parameters.

The dietary model used in this study was to feed rainbow trout high-fat (21%) or low-fat diets (11%) containing either a highly saturated fat (CO) or a highly unsaturated fat (FO) as the only fat source. The aim of this study was to compare the effects of the source and level of these dietary fats on postprandial plasma parameters known to be affected by dietary fat source i.e. glucose, triglycerides, NEFA, cholesterol, HDL, LDL, ketone bodies and on parameters related with health status (NBT and MPO).

2. Material and methods

2.1. Experimental diets

Four fishmeal based diets (Table 1) were formulated to be isonitrogenous, but to contain two levels of crude fat supplied either by fish oil (FO) or coconut oil (CO). Diet FH (fish oil high fat) contained 15% FO, whereas diet FL (fish oil low fat) contained 5% FO and 10% extra gelatinized starch. Diet CH (coconut oil high fat) contained 15% CO, whereas diet CL (coconut oil low fat) contained 5% CO and 10% extra gelatinized starch. The four diets were manufactured using a twinscrew extruder (Clextral, France) at the experimental feed unit (Donzacq, France) of the French National Institute of Agronomy Research (INRA, France). The analyzed composition is provided in Table 1. Table 2 gives the details of the fatty acid profile of the diets.

2.2. Fish and experimental conditions

The experiment was carried out in a flow-through fish rearing system (INRA Donzacq facilities, France) using natural spring water of constant temperature (17 °C) and under natural photoperiod conditions (October). Rainbow trout juveniles (71.3 \pm 0.3 g initial body weight) were randomly distributed among sixteen 150-liter tanks

Table 1Composition (%) of the four experimental diets fed to rainbow trout.

	Experimental diets					
	FL	FH	CL	СН		
Ingredients						
Fish meal	38	38	38	38		
Wheat gluten	20	20	20	20		
Whole wheat	12	12	12	12		
Gelatinized starch, maize	21	11	21	11		
Coconut oil			5	15		
Fish oil	5	15				
Vitamin mixture ^a	1	1	1	1		
Mineral mixture ^b	1	1	1	1		
Binder (Sodium alginate)	2	2	2	2		
Proximate composition (% dry matter)						
Dry matter (% diet)	91.6	91.8	91.7	90.9		
Crude protein	48.0	47.5	48.0	47.8		
Crude fat	10.8	21.3	11.1	21.1		
Ash	6.1	6.1	6.3	6.1		

^a Vitamin mixture (IU or mg/kg diet): DL-a-tocopherol acetate, 60 IU; sodium menadione bisulfate, 5 mg; retinyl acetate, 15,000 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; B12, $0\cdot05$ mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium panthotenate, 50 mg; choline chloride, 2000 mg (Unité de Préparation des Aliments Expérimentaux, Jouy-en-Josas, INRA, France).

b Mineral mixture (g or mg/kg diet): calcium carbonate (40% Ca), 2.15 g; magnesium oxide (60%Mg), 1.24 g; ferric citrate, 0.2 g; potassium iodide (75% I), 0.4 mg; zinc sulfate (36% Zn), 0.4 g; copper sulfate (25% Cu), 0.3 g; manganese sulfate (33%Mn), 0.3 g; dibasic calcium phosphate (20% Ca, 18% P), 5 g; cobalt sulfate, 2 mg; sodium selenite (30% Se), 3 mg; KCl, 0.9 g; NaCl, 0.4 g (Unité de Préparation des Aliments Expérimentaux, Jouy-en-Josas, INRA, France).

(sixty fish per tank). Each of the four experimental diets was fed by hand (twice per day) to visual satiation to four replicate groups of fish for 3 weeks. The fish in each tank were weighed at the start and end of the trial in order to calculate the initial and final daily body weight. Weight gain (%) = 100% * (final body weight — initial body weight) / initial body weight; daily food intake (FI, %BW per d) = 100% * dry

Table 2Fatty acid composition of the diets (mg/g of diet).

Diets	FL	FH	CL	CH
10:0	ND	0.20	2.32	6.48
12:0	0.20	1.41	20.65	62.40
14:0	5.82	11.66	11.27	28.45
16:0	16.65	32.17	13.59	22.96
18:0	3.07	6.64	2.74	5.49
20:0	0.20	0.40	0.11	0.20
\sum Saturates	25.95	52.48	51.09	126.57
16:1	4.70	9.85	2.42	2.35
18:1	15.02	31.97	11.38	19.62
20:1	6.13	10.86	4.11	4.32
22:1	7.15	9.05	6.53	6.67
∑ MUFA	33.00	61.73	24.54	33.16
18:2n-6	5.82	7.64	6.64	9.62
18: 3n-6	0.10	0.20	0.11	ND
20: 2n-6	0.20	0.40	0.21	0.20
20: 3n-6	0.10	0.20	ND	ND
20:4n-6	0.61	1.41	0.32	0.39
$\sum n-6$	6.85	9.85	7.27	10.40
18:3n – 3	1.02	1.61	0.84	0.98
18:4n – 3	1.63	3.22	1.05	0.98
20:3n-3	0.10	0.20	0.11	ND
20:4n-3	0.72	1.61	0.42	0.39
20:5n-3	8.28	18.90	4.21	4.32
22:5n-3	1.84	4.42	0.84	0.39
22:6n-3	12.57	26.34	7.69	3.73
$\sum n-3$	26.16	57.51	15.17	10.99
PUFA	36.68	74.40	23.81	22.76
SFA:PUFA	0.7	0.7	2.2	5.6
FA mg/g diet	102.17	201.07	105.34	196.23

ND: not detected; FA, fatty acid.

Diets: FL: fish oil, low-fat; FH: fish oil, high-fat, CL: coconut oil, low-fat; CH: coconut oil, high-fat.

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