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Effect of Dietary Conjugated Linoleic Acid on Growth, Lipid Class, and Fatty Acid Composition in Rainbow Trout (*Oncorhynchus mykiss*)

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The development of a fish that functionally provides both antioxidant and fat-reducing effects is an important goal in nutrition and aquaculture research. Dietary conjugated linoleic acid (CLA) can be successfully incorporated in fish muscle and viscera, but CLA and carotenoids have not been evaluated in such fish. An 8 weeks growth trial was conducted using rainbow trout, and all fish were fed twice daily with experimental diets containing graded amounts of CLA (1% and 5%). At the end of the experiment, the daily growth index, feed conversion rate, lipid class, and fatty acid composition were determined. Dietary CLA did not enhance the growth parameters of rainbow trout but did improve the feed conversion rate. The muscular polar lipid content decreased during the feeding period, while the content was stable in the viscera. In addition, a diet high in CLA decreased the polyunsaturated fatty acid content, but had no effect on the content of monounsaturated and saturated fatty acid in muscle.

Key words: CLA, Rainbow trout, Growth parameters, Lipid class, Fatty acid

Introduction

Conjugated linoleic acid (CLA) is a collective term used to describe one or more positional and geometric isomers of linoleic acid, an essential fatty acid. CLA is found naturally in a wide variety of food products in addition to those of ruminant origin, including seafood, turkey, and vegetables (Chin et al., 1992). Increasing the CLA content of food products (e.g., milk, meat, and fish) also has the potential of increasing their nutritional and therapeutic value. This enhancement could favorably influence the marketing of these value-added designed foods. Moreover, CLA isomers were demonstrated to possess antioxidant properties (Lee et al., 1994), inhibit carcinogen-DNA adduct formation (Josyula et al., 1998), induce apoptosis (Ip et al., 1999), modulate tissue fatty acid composition and eicosanoid metabolism (Sugano et al., 1998), and affect the expression and action of cytokines and growth factors (Turek et al., 1998). In addition, dietary CLA was reported to decrease body fat in humans, mice, rats, pigs, and chickens. Choi et al. (1999) reported an improvement in the growth rate of carp fed a diet containing 1% CLA. In contrast, Twibell et al. (2001) observed an opposite effect during the feeding of yellow perch with a diet containing the same level of CLA, and the results indicated that CLA affected muscle lipid content and fatty acid composition. This demonstrates that feeding CLA can enhance the tissue concentration of these fatty acid isomers in fish, such as Atlantic salmon (Berge et al., 2004), hybrid striped bass (Twibell et al., 2000), and tilapia (Yasmin et al., 2004). However, each species appears to differentially metabolize CLA. Fish are not a naturally rich source of CLA, but have the highest amount of muscle CLA deposition of any other animal, as a response to similar dietary supplementation levels. Thus, consumption of these fish may be a beneficial method of increasing human intake of CLA.

The objective of the present study was to test if dietary CLA can affect growth rates and modify fatty acid composition, and to determine the desirable deposition of CLA in both the muscle and viscera of

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rainbow trout.

Materials and Methods

Experimental diets and fish

Soybean oil (SO) was purchased from a local market (Jinju, Korea). CLA was chemically synthesized (56% purity) from SO by alkaline isomerization at high temperature (Park et al., 2000) and consisted of 46% c9,t11-CLA, 49% t10,c12-CLA, and 5% other CLA isomers (c9,c11-, c10,c12-, t9,t11-, and t10,t12-CLA) as analyzed by gas chromatography (GC). Carotenoids were extracted from ascidian tunics with acetone (Choi et al., 1994). After concentration under reduced pressure, the carotenoids were transferred to diethyl ether following the addition of distilled water. The total carotenoid content was calculated, assuming the $E^{1\%}_{1cm}$ value of diethylether to be 2,400 at 450 nm.

Rainbow trout were obtained from an aquaculture farm in Yeongdong, Korea. Experimental diets were prepared by mixing an appropriate amount of the chemically synthesized CLA and ascidian tunic extract or Carophyll Pink® with the commercial diet purchased from Woosung Feed Co. (Nonsan, Korea). The test diets were stored in a freezer at -30°C for 10 weeks before use. Rainbow trout (20 fish of 200±10 g) were placed in round glass fiber aquariums (1 ton) equipped with a water circulation and biological filtration system. The compositions of the four kinds of diet were CP12 (CLA 1%, Carophyll Pink® 0.2%), CP14 (CLA 1%, tunic extract 0.4%), CP52 (CLA 5%, Carophyll Pink® 0.2%), and CP54 (CLA 5%, tunic extract 0.4%) (Table 1).

Proximate composition

The moisture and ash content was determined according to standard AOAC (1990) methods. Moisture content was measured by constant-weight drying in an oven at 105°C. Protein levels were quantified by the micro-Kjeldahl method. Ash content analysis was performed in a muffle furnace at 550°C to constant weight. Total lipids were extracted and purified using the methods of Bligh and Dyer (1959), and the content was determined gravimetrically.

Lipid extraction and lipid class determination

Total lipids were extracted and analyzed from both the aquacultured rainbow trout and the diets. The extracted lipids were stored in 5 mL of 100% chloroform at -80°C until the lipid classes could be analyzed (Kang et al., 1997). Samples with a total lipid loading between 0.4 and 10.0 μg were applied onto Chromarods SIII (5 um silica gel-coated quartz rod; Iatron Laboratories, Inc., Tokyo, Japan) below the origin using a 2.0 μL blunt-tripped Hamilton syringe (Hamilton Co., Reno, NV, USA). The rods were developed with a one- or two-solvent system followed by partial or total scanning using an MK-5 TLC-FID analyzer (Iatron Laboratories, Inc.). The typical operational conditions for the Iatroscan were: 2 L/min airflow, 200 mL/min hydrogen flow, and 35 s/scan. The first development (hexane:diethyl ether: formic acid (80:20:1, v/v/v) for 40 min) mobilized the neutral lipid fraction (including hydrocarbons, wax esters, steryl esters, and fatty acid methyl esters) that migrate with the solvent front. The triacylglycerols (TAG), free sterols (ST), diacylglycerols (DAG), and free fatty acids (FFA) also mobilized but were partially separate from the rest during this development. The second development consisted of 35 min in chloroform:methanol (67:37), to move the phospholipids (PL) when the selective separation of this lipid class was necessary.

Analysis of fatty acid composition

The fatty acid methyl esters (FAME) from the diets and total tissue lipids were prepared by acid-catalyzed trans-estrification of the total lipids according to the method of Park et al. (2002). The FAME was separated and quantified by gas chromatography. The analysis was performed on a Shimadzu GC-17A (Shimadzu Seisakusho Co. Ltd., Kyoto, Japan) equipped with an Omegawax-320 fused-silica capillary column (30 m×0.32 mm i.d.; Supelco Co., Bellefonte, PA, USA). The oven temperature progressed from 180°C (initial time of 8 min) to 230°C at a rate of 3°C/min. The final holding time was 15 min. The temperature of both the injector and the detector was 250°C. Helium was used as the carrier gas with a constant column-inlet pressure of 1.0 kg/cm^2 and a split ratio of 1:50. Fatty acid methyl esters from the samples were identified by comparison with the equivalent chain length (ECL) standard (Sigma Chemical Co., St. Louis, MO, USA). Methyl tricosanate (99%; Aldrich Chemical Co., Milwaukee, WI, USA) was used as an internal standard.

Statistical analysis

All experiments were repeated at least three times. The data were analyzed for the degree of variation and significance of difference based on an analysis of variance (ANOVA), with Tukey's pair-wise compareson test to determine the differences (P<0.05) between treatment means. Analyses were performed using the JMP statistical discovery software (SAS

Institute Inc., Cary, NC, USA).

Results

Diet composition

The experimental diets were composed of about 50% protein, 20% lipid, and 9.5% ash (Table 1). The fatty acid composition of the five diets (control, CP12, CP14, CP52, and CP54) is shown in Table 2. The amounts of polyunsaturated fatty acid (PUFA) were 54.7%, 55.5%, 55.2%, 56.6%, and 56.4% in each diet, with amounts of eicosapentaenoic acid (20:5n-3; EPA) of 3.22%, 3.30%, 3.36%, 3.12%, and 1.98% and amounts of docosahexaenoic acid (22:6n-3; DHA) of 3.24%, 3.50%, 3.37%, 3.25%, and 2.77%, respectively. The amounts of total monounsaturated fatty acid (MUFA) in the experimental diets were 24.7%, 24.0%, 24.2%, 25.5%, and 27.1%, mainly 18:1n-9, and the amounts of saturated fatty acid were 20.3%, 20.1%, 20.3%, 17.6%, and 16.2%, respectively, mainly 16:0. Inclusion of CLA in the CP12, CP14, CP52, and CP54 diets resulted in CLA levels of 4.70%, 3.47%, 28.6%, and 30.9% of the total fatty acid, respectively. The total n-6 fatty acid content in the control, CP12 and CP14 diets were lower than that of the CP52 and CP54 diets, but the total n-3 fatty acid content was higher than in the control, CP12, and CP14 diets.

Proximate composition, growth, and biometry

The proximal amounts of CLA and pigment in the experimental fish were not affected by the 8 weeks feeding (Table 3). The growth parameters were not influenced by either the 1% or 5% CLA diets, and produced the following daily growth indices (DGI): control (3.55), CP12 (3.57), CP14 (4.02), CP52 (3.36), and CP54 (3.45). The feed conversion ratios (FCR) were the following: control (0.73), CP12 (0.73), CP14 (0.71), CP52 (0.76), and CP54 (0.74) (Table 4).

Lipid class composition

After 4 weeks of feeding, triacyleglycerols (TAG) in the muscles of the control group accounted for 73.6% of the total lipids, which was higher than that of the diets containing 1% and 5% CLA (67.0%, 66.0%, 64.8%, and 69.3%, respectively). The amount of polar lipids in the control diet was 25.7%, which was lower than in the 1% and 5% CLA diets (31.8%, 32.8%, 34.5%, and 30.0%, respectively). In contrast, after 8 weeks of feeding, the amount of TAG in the control group (77.1%) was slightly lower than in the CLA groups: CP12 (77.4%), CP14 (82.2%), CP52 (78.8%), and CP54 (79.1%). The component of polar

Table 1. Ingredients and proximate composition of the experimental diets. The values are mean±SD (n=3). Different superscript letters within rows represent significant differences between treatments ($P<0.05$). [1]Vitamin mixture (mg/kg diet or IU): thiamin 50 mg; riboflavin 60 mg; calcium pantothenate 200 mg; biotin 1 mg; folic acid 20 mg; pyridoxine 40 mg; cyanocobalamin 0.05 mg; niacin 250 mg; ascorbic acid 1,000 mg; inositol 400 mg; retinyl acetate 8,000 IU; DL-cholecalciferol 2,400 IU; DL-alpha tocopherol acetate 300 IU; sodium menadione bisulphate 5 mg. [2]Mineral mixture (mg/kg): calcium carbonate 850 mg; magnesium oxide 750 mg; copper sulfate 25 mg; manganese sulfate 100 mg; ferric citrate 150 mg; zinc sulfate 120 mg

	Dietary treatments				
	Control	CP12	CP14	CP52	CP54
Ingredients (g/kg)					
Fish meal	450	450	450	450	450
Soybean meal	70	70	70	70	70
Wheat flour	250	250	250	250	250
Vitamin mixture[1]	20	20	20	20	20
Mineral mixture[2]	10	10	10	10	10
Fish oil (squid liver)	64	64	64	64	64
Soybean oil	136	124	122	84	82
CLA	0	10	10	50	50
Pigments					
Carophyll pink®	0	2	0	2	0
Ascidian tunic extracts	0	0	4	0	4
Proximate composition(%)					
Moisture	6.93±0.5[a]	7.53±0.8[a]	7.63±0.7[a]	7.50±0.6[a]	7.57±0.9[a]
Crude protein	50.6±2.0[a]	51.0±2.4[a]	50.7±1.3[a]	50.3±1.9[a]	51.5±1.2[a]
Crude lipids	20.4±1.5[a]	20.5±1.2[a]	20.6±0.4[a]	20.5±1.1[a]	19.3±0.4[a]
Ash	9.47±0.1[a]	9.57±0.1[a]	9.67±0.1[a]	9.47±0.2[a]	9.63±0.2[a]

Table 2. Fatty acid compositions (percentage of area) of experimental diets. The values are mean±SD (n=3). Different superscript letters within rows represent significant differences between treatments ($P<0.05$). [1]Saturated: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0. [2]Monounsaturated: 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-9, 20:1n-7, 22:1n-11, 24:1n-9. [3]Polyunsaturated: 16:2n-4, 16:3n-4, 18:2n-6, 18:2n-4, 18:3n-3, 18:3n-1, 20:2n-6, 20:4n-6, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-3, 22:6n-3 and total CLA. [4]Sum of isomers c9,t11-; t10,c12-; c9,c11-; c10,c12-; c9,t11-; t10,t12-CLA

Fatty acids	Control	CP12	CP14	CP52	CP54
16:0	13.7 ± 0.1	13.7 ± 0.1	13.7 ± 0.1	11.8 ± 0.5	11.4 ± 0.1
18:0	3.90 ± 0.2	3.67 ± 0.2	3.81 ± 0.1	2.85 ± 0.1	2.86 ± 0.1
ΣSaturated[1]	20.3 ± 0.9[a]	20.2 ± 0.8[a]	20.3 ± 0.5[a]	17.6 ± 0.2[b]	16.2 ± 0.4[b]
18:1n-9	19.2 ± 0.2	19.2 ± 0.2	19.4 ± 0.2	21.6 ± 0.7	22.9 ± 0.4
18:1n-7	1.73 ± 0.1	1.67 ± 0.1	1.68 ± 0.2	1.38 ± 0.1	1.62 ± 0.2
ΣMonounsaturated[2]	24.7 ± 0.8[c]	24.0 ± 1.1[c]	24.2 ± 0.8[c]	25.5 ± 0.5[b]	27.1 ± 0.6[a]
18:2n-6	40.3 ± 3.2	34.5 ± 2.4	35.8 ± 2.2	17.0 ± 1.4	17.4 ± 1.1
18:3n-3	5.17 ± 0.1	4.25 ± 0.4	4.36 ± 0.1	1.98 ± 0.2	2.05 ± 0.1
20:4n-6	0.27 ± 0.1	0.28 ± 0.1	0.38 ± 0.0	0.88 ± 0.1	0.21 ± 0.0
20:4n-3	0.18 ± 0.0	0.19 ± 0.0	0.19 ± 0.0	0.19 ± 0.0	0.10 ± 0.0
20:5n-3	3.22 ± 0.2	3.30 ± 0.1	3.36 ± 0.2	3.12 ± 0.2	1.98 ± 0.1
22:6n-3	3.24 ± 0.1	3.50 ± 0.5	3.37 ± 0.1	3.25 ± 0.1	2.77 ± 0.1
ΣPolyunsaturated[3]	54.7 ± 2.9[a]	55.5 ± 3.9[a]	55.2 ± 2.2[a]	56.6 ± 3.0[a]	56.4 ± 2.4[a]
Total CLA[4]	0.00 ± 0.0[c]	4.70 ± 0.6[b]	3.47 ± 0.4[b]	28.6 ± 1.9[a]	30.9 ± 1.6[a]
Σn-6	40.8	39.5	39.9	46.7	48.6
Σn-3	12.4	11.9	12.0	9.14	7.40

Table 3. Proximate compositions of rainbow trout muscle after 8 weeks. The values are mean±SD (n=3). Different superscript letters within rows represent significant differences between treatments ($P<0.05$)

	Dietary treatments				
	Control	CP12	CP14	CP52	CP54
Moisture	71.5 ± 1.3[a]	71.4 ± 1.7[a]	71.3 ± 1.2[a]	71.5 ± 1.4[a]	70.1 ± 1.8[a]
Protein	21.6 ± 1.6[a]	22.1 ± 1.3[a]	22.3 ± 2.2[a]	22.1 ± 0.5[a]	22.7 ± 1.7[a]
Lipids	4.4 ± 0.6[a]	4.0 ± 0.3[a]	3.8 ± 0.5[a]	4.2 ± 1.1[a]	4.0 ± 0.3[a]
Ash	1.3 ± 0.2[a]	1.1 ± 0.1[a]	1.1 ± 0.4[a]	1.0 ± 0.3[a]	0.9 ± 0.1[a]

Table 4. Effect of dietary CLA on daily growth index (DGI), feed conversion ratio (FCR) for 8 weeks. The values are mean of two tanks±SD (n=4). Different superscript letters within rows represent significant differences between treatments ($P<0.05$). [1]DGI, daily growth index=100×[(Final body weight)$^{1/3}$-(Initial body weight)$^{1/3}$]/days. [2]FCR, feed conversion ratio=dry feed intake/weight gain

	Dietary treatments				
	Control	CP12	CP14	CP52	CP54
Initial body weight (g)	153.6 ± 13.3	153.2 ± 13.7	154.1 ± 12.4	155.2 ± 15.4	153.8 ± 14.8
Final body weight (g)	421.0 ± 67.2	422.6 ± 66.4	471.4 ± 67.0	404.4 ± 51.8	411.3 ± 57.7
DGI[1]	3.55 ± 0.4[a]	3.57 ± 0.9[a]	4.02 ± 0.7[a]	3.36 ± 0.5[a]	3.45 ± 0.4[a]
FCR[2]	0.73	0.73	0.71	0.76	0.74

lipids in the control diet (21.2%), however, was higher than in the experimental diets [CP12 (20.8%), CP14 (15.5%), CP52 (19.0%), and CP54 (19.0%)]. The TAG and polar lipids in the viscera were not strongly affected by CLA after 4 weeks of feeding. However, after 8 weeks, the TAG of the fish fed CLA was higher than that of the control group [CP12 (83.7%), CP14 (90.4%), CP52 (80.1%), CP54 (84.1%), and control group (78.5%)], while the polar lipids of the CLA groups were significantly lower than the control diet level of 20.7% (Table 5).

Fatty acid composition in muscle and viscera

In general terms, the fatty acid composition of the total lipids from the muscle and viscera reflected the fatty acid composition of the diets (Tables 6 and 7). Dietary inclusion of CLA decreased PUFA in both the muscle and viscera, although the effect was not obvious. In contrast, CLA had no influence on either monounsaturated fatty acids or saturated fatty acids. The percentages of CLA in muscle from the four kinds of diets (CP12, CP14, CP52, and CP54) were 0.84%, 0.98%, 4.01%, and 3.98%, respectively. In

Table 5. Effect of dietary CLA on lipid class composition of muscle and viscera in rainbow trout. The values are mean of two tanks±SD (n=4). Different superscript letters within rows represent significant differences between treatments ($P<0.05$)

Lipid class	Control	CP12	CP14	CP52	CP54
Muscle (4 week)					
Free fatty acids	trace	trace	trace	trace	trace
Triacylglycerols	73.6 ± 2.9^{a}	67.0 ± 0.9^{ab}	66.0 ± 0.9^{ab}	64.9 ± 5.8^{b}	69.3 ± 2.5^{ab}
Sterols	0.72 ± 0.1^{c}	1.21 ± 0.3^{ab}	1.25 ± 0.1^{a}	0.64 ± 0.1^{c}	0.83 ± 0.1^{bc}
Polar lipids	25.7 ± 3.0^{b}	31.8 ± 0.6^{ab}	32.8 ± 0.9^{ab}	34.6 ± 5.8^{a}	30.0 ± 2.3^{ab}
Muscle (8 week)					
Free fatty acids	trace	trace	trace	trace	trace
Triacylglycerols	77.1 ± 2.5^{a}	77.4 ± 3.5^{a}	82.2 ± 2.7^{a}	78.8 ± 5.2^{a}	79.1 ± 1.0^{a}
Sterols	1.68 ± 0.4^{a}	1.82 ± 0.3^{a}	2.25 ± 0.9^{a}	2.20 ± 0.4^{a}	1.83 ± 0.3^{a}
Polar lipids	21.2 ± 2.3^{a}	20.8 ± 3.7^{a}	15.5 ± 2.3^{a}	19.0 ± 4.9^{a}	19.1 ± 0.8^{a}
Viscera (4 week)					
Free fatty acids	trace	trace	trace	trace	trace
Triacylglycerols	82.4 ± 1.9^{a}	84.4 ± 2.3^{a}	82.0 ± 2.3^{a}	82.3 ± 4.2^{a}	77.5 ± 1.9^{a}
Sterols	9.02 ± 0.5^{ab}	4.81 ± 1.3^{c}	7.99 ± 1.7^{bc}	6.06 ± 1.2^{bc}	12.2 ± 1.9^{a}
Polar lipids	8.53 ± 2.1^{a}	10.8 ± 1.1^{a}	10.0 ± 0.7^{a}	11.6 ± 3.1^{a}	10.3 ± 1.0^{a}
Viscera (8 week)					
Free fatty acids	trace	trace	trace	trace	trace
Triacylglycerols	78.4 ± 3.4^{b}	83.7 ± 1.6^{ab}	90.4 ± 1.8^{a}	80.1 ± 3.1^{b}	84.1 ± 2.8^{ab}
Sterols	0.85 ± 0.1^{b}	1.84 ± 1.2^{b}	0.85 ± 0.3^{b}	8.63 ± 2.1^{a}	5.82 ± 1.8^{a}
Polar lipids	20.7 ± 3.4^{a}	14.4 ± 0.7^{ab}	9.12 ± 2.1^{b}	11.3 ± 2.8^{b}	10.1 ± 2.9^{b}

Table 6. Fatty acid compositions (percentage of area) of muscle lipid in rainbow trout after 8 week. The values are mean±SD (n=3). Different superscript letters within rows represent significant differences between treatments ($P<0.05$). [1]Saturated, [2]Monounsaturated, [3]Polyunsaturated, [4]Sum of isomers are same in Table 2

Fatty acid	Control	CP12	CP14	CP52	CP54
16:0	14.8 ± 1.1	14.4 ± 1.1	14.9 ± 0.8	14.6 ± 0.9	13.9 ± 0.1
18:0	4.23 ± 0.0	4.04 ± 0.6	4.67 ± 0.2	4.36 ± 0.1	4.03 ± 0.1
ΣSaturated[1]	22.5 ± 0.3^{a}	22.5 ± 0.3^{a}	23.0 ± 0.2^{a}	22.6 ± 0.2^{a}	21.3 ± 0.0^{a}
18:1n-9	20.3 ± 1.1	21.1 ± 1.0	20.1 ± 0.6	21.6 ± 0.5	22.3 ± 0.4
18:1n-7	2.43 ± 0.2	2.31 ± 0.1	2.58 ± 0.1	2.34 ± 0.2	2.42 ± 0.0
ΣMonounsaturated[2]	29.7 ± 0.2^{a}	30.4 ± 0.2^{a}	29.5 ± 0.1^{a}	32.0 ± 0.2^{a}	33.1 ± 0.1^{a}
18:2n-6	24.1 ± 1.2	24.0 ± 1.2	25.7 ± 1.6	16.8 ± 0.4	17.7 ± 0.2
18:3n-3	2.87 ± 0.3	2.90 ± 0.2	2.72 ± 0.1	1.84 ± 0.1	1.88 ± 0.0
20:4n-6	0.68 ± 0.1	0.63 ± 0.2	0.70 ± 0.0	0.62 ± 0.1	0.67 ± 0.0
20:4n-3	0.60 ± 0.1	0.66 ± 0.2	0.62 ± 0.2	0.54 ± 0.2	0.57 ± 0.0
20:5n-3	2.45 ± 0.3	2.51 ± 0.3	2.24 ± 0.2	2.47 ± 0.3	2.22 ± 0.0
22:6n-3	7.53 ± 0.6	7.85 ± 1.2	7.22 ± 0.4	7.03 ± 0.5	7.76 ± 0.3
ΣPolyunsaturated[3]	47.8 ± 0.2^{a}	45.9 ± 0.2^{a}	46.1 ± 0.2^{a}	38.7 ± 0.2^{b}	40.5 ± 0.1^{b}
Total CLA[4]	0.0 ± 0.0^{b}	0.84 ± 0.2^{b}	0.98 ± 0.1^{b}	4.01 ± 0.8^{a}	3.98 ± 0.3^{a}
Σn-6	29.0	27.8	29.6	23.0	24.2
Σn-3	15.7	16.3	14.7	14.0	14.6

addition, the composition of PUFA was 47.8%, 45.9%, 46.1%, 38.7%, and 40.5%, mainly ∑n-3 and ∑n-6 [∑n-3 (15.7%, 16.3%, 14.7%, 14.0%, and 14.6%) and ∑n-6 (29.0%, 27.8%, 29.6%, 23.0%, and 24.2%)]. An obvious decrease was observed in the CP12 and CP14 groups (Table 6). Total CLA in the viscera was affected by dietary treatment, attaining the highest level when the 5% diet was used. The depositions of CLA in the CP12, CP14, CP52, and CP54 diets were 0.73%, 0.87%, 5.17%, and 5.14%, respectively. Moreover, the respective PUFA contents were 40.7%, 36.4%, 38.5%, 40.7%, and 41.7%, mainly ∑n-6 and ∑n-3. The percentages of ∑n-6 were 22.3%, 16.4%, 18.6%, 19.7%, and 21.1%, respectively. Due to the enhancement of EPA and DHA, the percentage of the ∑n-3 component appeared to be similar (16.0%, 17.1%, 17.3%, 18.1%, and 17.8%, respectively) (Table 7).

Table 7. Fatty acid composition (percentage of area) of viscera in rainbow trout after 8 week. The values are mean±SD (n=3). Different superscript letters within rows represent significant differences between treatments ($P<0.05$). [1]Saturated, [2]Monounsaturated, [3]Polyunsaturated, [4]Sum of isomers are same in Table 2

Fatty acid	Control	CP12	CP14	CP52	CP54
16:0	16.3 ± 0.8	17.6 ± 0.7	17.6 ± 0.1	16.1 ± 0.6	16.9 ± 1.3
18:0	4.32 ± 0.3	4.66 ± 0.1	4.70 ± 0.1	4.13 ± 0.4	4.25 ± 0.5
ΣSaturated[1]	25.9 ± 1.0^{b}	29.1 ± 0.6^{a}	28.0 ± 0.1^{a}	25.8 ± 1.0^{b}	27.1 ± 1.8^{ab}
18:1n-9	18.3 ± 0.2	17.4 ± 0.3	19.3 ± 0.8	18.0 ± 0.9	18.5 ± 1.1
18:1n-7	3.14 ± 1.0	3.46 ± 0.3	2.62 ± 0.2	2.88 ± 0.7	2.91 ± 0.6
ΣMonounsaturated[2]	32.7 ± 1.2^{a}	34.3 ± 0.3^{a}	33.5 ± 0.8^{a}	33.9 ± 1.0^{a}	33.3 ± 0.9^{a}
18:2n-6	20.0 ± 0.7	13.4 ± 1.1	15.4 ± 1.1	12.3 ± 1.1	13.8 ± 2.3
18:3n-3	2.30 ± 0.0	1.52 ± 0.3	1.68 ± 0.1	1.63 ± 0.1	1.64 ± 0.2
20:4n-6	0.63 ± 0.1	0.85 ± 0.4	0.83 ± 0.1	0.72 ± 0.1	0.69 ± 0.1
20:4n-3	0.73 ± 0.1	0.77 ± 0.1	0.61 ± 0.1	0.87 ± 0.2	0.73 ± 0.1
20:5n-3	3.34 ± 0.3	3.31 ± 0.6	3.58 ± 0.4	3.76 ± 0.2	3.90 ± 0.6
22:6n-3	7.73 ± 0.5	7.89 ± 0.7	8.45 ± 0.3	8.23 ± 1.0	8.37 ± 0.2
ΣPolyunsaturated[3]	40.7 ± 0.5^{a}	36.4 ± 2.6^{b}	38.5 ± 0.6^{ab}	40.7 ± 1.1^{a}	41.7 ± 1.3^{a}
Total CLA[4]	0.0 ± 0.0^{b}	0.73 ± 0.1^{b}	0.87 ± 0.2^{b}	5.17 ± 0.7^{a}	5.14 ± 0.5^{a}
Σn-6	22.3	16.4	18.6	19.7	21.1
Σn-3	16.0	17.1	17.3	18.1	17.8

Discussion

Compared to the control group (0% CLA), dietary CLA (1% and 5%) had no significant effect on the daily growth rate or the feed conversion ratio of rainbow trout. These results were also found in previous studies on other fish species, such as Atlantic salmon (Berge et al., 2004), juvenile tilapia (Yasmin et al., 2004), yellow perch (Twibell et al., 2001), and channel catfish (Twibell and Wilson, 2003). However, CLA, in amounts of up to 1%, has also been reported to reduce feed intake and improve feed efficiency in hybrid striped bass (Twibell et al., 2000). Moreover, 1% CLA diets have been shown to increase weight gain in common carp (Choi et al., 1999) and other animals, such as rats (Szymczyk et al., 2000) and chickens (Latour et al., 2000). Nevertheless, when compared to fish fed no CLA, higher levels (2%) of CLA reduced weight gain and feed efficiency in Nile tilapia, rockfish, and common carp (Choi et al., 1999). From the various results of the above-mentioned studies, we can conclude that the effect of CLA on growth performance and feed efficiency is clearly dependent on the species of fish under consideration. Dietary CLA has been reported to have some beneficial effects on body composition in mammals, and decrease body fat and increase lean body mass in pigs, rats, and mice (Ohnuki et al., 2001; Terpstra et al., 2002; Thiel Cooper et al., 2001; Tischendorf et al., 2002; Yamasaki et al., 2003). CLA also decreases whole body TAG accumulation in hamsters (Bouthegourd et al., 2002) and reduces liver TAG levels in rats (Rahman et al., 2002). In contrast, similar effects have rarely been observed in fish. Dietary CLA had no significant effect on carcass lipid (Twibell and Wilson, 2003) or on tissue lipid content in tilapia (Yasmin et al., 2004). However, the intraperitoneal fat and liver lipid content were lowered by dietary CLA in striped bass. CLA suppressed TAG accumulation, and the t10,c12 isomer increased fatty acid oxidation in 3T3-L1 adipocytes (Evans et al., 2000). CLA inhibited fatty acid synthetase activity in rat liver (Oku et al., 2003), and the t10,c12 isomer attenuated lipogenesis in human AT cells by both decreasing synthesis and increasing oxidation (Brown et al., 2001). Unexpectedly, in rainbow trout, dietary CLA alone fed for 4 weeks reduced TAG in muscle, similar to previous results. Notably, CLA increased TAG and decreased the polar lipid content in both muscle and viscera. In addition, a 5% CLA diet contributed to the synthesis of sterols. However, to date, only gross effects have been measured in fish and the biochemical pathways have not been directly studied. This is an important point for future studies. Evidence suggests that CLA suppresses PUFA desaturase and elongase in cell systems and decreases C18 PUFA in pig muscle and fat, and DHA in chicken tissues. Decreased tissue PUFA levels after feeding with CLA was reported in yellow perch (Twibell et al., 2001) and tilapia (Yasmin et al., 2004). In striped bass, CLA increased PUFA levels in the liver but decreased PUFA in muscle. Dietary CLA increased total n-3 PUFA, especially DHA, in salmon fry, but had no effect on PUFA levels in the liver; in salmon smolts, it appears to be deposited in the flesh at the expense of EPA and DHA (Twibell et al., 2000). In the present study,

CLA was incorporated in tissue lipids to the same level in both the muscle and viscera, and the total amount of CLA was affected by the dietary treatment, attaining the highest level in the 5% diet. In addition, dietary CLA reduced PUFA in both the muscle and viscera, but had no significant effect on saturated and monounsaturated fatty acid. CLA deceased both ∑n-6 (18:2n-6) and ∑n-3 (18:3n-3) in muscle, while CLA increased ∑n-3 in the viscera due to enhanced EPA and DHA. This result was notable in the fish fed with 5% CLA. CLA can be efficiently delivered to humans via oily fish, with the accumulated level in salmon and trout fed a 2% CLA diet reaching 7% in the flesh (16-17% dietary lipid), or 4% in the flesh of salmon smolts (34% dietary lipid) and 7% in whole salmon fry (24% dietary lipid). Similarly, striped bass with high levels of lipid in the flesh (15%) accumulated CLA to over 7% of the total fatty acids in fish fed a 1% CLA diet, whereas the levels of CLA that accumulated in yellow perch, with flesh lipid levels of only 3%, were much lower. Consistent with the above, the incorporation of CLA into neutral lipids was about tenfold higher than incorporation into polar lipids in both the muscle and liver in tilapia (Twibell et al., 2004). In the present study, rainbow trout fed a 1% CLA diet deposited 0.8%-1% in muscle and 0.7%-0.9% in the viscera, and required a 5% CLA diet to incorporate 4% in muscle and 5% in the viscera. The percentages of CLA in the five different diets (control, CP12, CP14, CP52, and CP54) were 0%, 0.84%, 0.98%, 4.01%, and 3.98%, respectively. In addition, the respective percentages of PUFA were 47.8%, 45.9%, 46.1%, 38.7%, and 40.5%, mainly ∑n-3 and ∑n-6, with the following percentages of ∑n-3 (15.7%, 16.3%, 14.7%, 14.0%, and 14.6%) and ∑n-6 (29.0%, 27.8%, 29.6%, 23.0%, and 24.2%). This decreasing effect was obvious in the CP52 and CP54 groups. No significant difference was observed in the saturated fatty acid and MUFA content (Table 6). Total CLA in the viscera was affected by dietary treatment, attaining the highest level when the 5% diet was used. The percentages of CLA in the five kinds of diets (control, CP12, CP14, CP52, and CP54) were 0%, 0.73%, 0.87%, 5.17%, and 5.14%, respectively. The percentages for the PUFA content were 40.7%, 36.4%, 38.5%, 40.7%, and 41.7%, respectively, mainly ∑n-6 and ∑n-3. The respective amounts of ∑n-6 corresponded to 22.3%, 16.4%, 18.6%, 19.7%, and 21.1% (control>CP5>CP1). Due to the enhancement of EPA and DHA, the ∑n-3 component seems to have a similar result, with 16.0%, 17.1%, 17.3%, 18.1%, and 17.8% (control<CP1< CP5), respectively (Table 7).

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