British Journal of Nutrition (2007), **97**, 289–297 DOI: 10.1017/S000711450733729X
© The Authors 2007

https://doi.org/10.1017/S000711450733729X Published online by Cambridge University Press

Conjugated linoleic acid in diets for large-size rainbow trout (*Oncorhynchus mykiss*): effects on growth, chemical composition and sensory attributes

L. M. P. Valente[1,2]*, N. M. Bandarra[3], A. C. Figueiredo-Silva[1], P. Rema[1,4], P. Vaz-Pires[1,2], S. Martins[3], J. A. M. Prates[5] and M. L. Nunes[3]

[1]*Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas, 177, 4050-123 Porto, Portugal*

[2]*Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Largo Prof. Abel Salazar, 2, 4099-003 Porto, Portugal*

[3] *Instituto de Investigação Agrária e das Pescas, Departamento de Inovação Tecnológica e Valorização dos Produtos da Pesca, Av. Brasília, 1449-006 Lisboa, Portugal*

[4]*Departamento de Zootecnia, Universidade de Trás-os-Montes e Alto-Douro, Apartado 1013, 5000-911 Vila Real, Portugal*

[5]*Faculdade de Medicina Veterinária, Rua Prof. Cid dos Santos, Pólo Universitário do Alto da Ajuda, 1300-477 Lisboa, Portugal*

(Received 14 December 2005 – Revised 20 July 2006 – Accepted 26 July 2006)

The effects of graded levels (0 %, 0·5 %, 0·75 and 1 %) of dietary conjugated linoleic acid (CLA) were assessed on 97 g rainbow trout. Fish were fed to satiation twice a day for 12 weeks. At the end of the experiment, all groups of fish weighed more than 250 g and no significant differences were detected in growth performance, feed conversion, nutrient or energy utilisation or body composition between treatments. A decrease in liver lipid content resulted from including CLA and was accompanied by a reduction in malic enzyme activity. The muscle saturated acid and PUFA content did not vary between dietary treatments, despite the increasing concentration of stearic acid and CLA. In the liver, however, both fractions increased significantly with dietary CLA. Moreover, the MUFA decreased significantly in both muscle and liver. CLA was incorporated into tissue lipids, with levels in flesh (2·1–4·2 %) being 2-fold higher than in liver (0·8–1·9 %). In muscle, the percentage of *cis*-9,*trans*-11 isomer ranged from 39·5 % to 41·8 % and that of *trans*-10,*cis*-12 isomer from 31·4 % to 33·4 % of total CLA. The incorporation of CLA isomers in the liver varied with dietary treatment, and the *cis*-9,*trans*-11 isomer seemed to be more efficiently incorporated than *trans*-10,*cis*-12. Sensory data indicated slight-to-moderate differences between the trout fed with and without CLA. The present results suggest that 250 g rainbow trout can incorporate CLA in both muscle and liver, contributing to the production of a functional food.

Rainbow trout: Conjugated linoleic acid: Growth: Lipid: Fatty acid composition: Lipogenesis

The current trend in fish production is to increase the lipid content in diets to spare proteins, improve feed conversion and decrease the amount of waste produced by fish. These diets, however, alter body composition and slaughter quality, particularly through an increase in lipid deposition (Cowey, 1993; Hillestad & Johnsen, 1994; Vergara *et al.* 1999). The farming industry is concerned with finding methods that control body lipid deposition and/or increase the market value of cultured fish when such diets are used.

Conjugated linoleic acids (CLA) is a collective term for isomers of $C_{18:2}$ fatty acids that differ in the geometry and position of two conjugated double bonds. Research with CLA indicates that these fatty acids are responsible for many biological properties that relate to health. CLAs have been shown to inhibit atherosclerosis (Lee *et al.* 1994), chemically induced carcinogenesis (Ha *et al.* 1990; Ip *et al.* 1999) and obesity (Park *et al.* 1997; West *et al.* 1998) and to enhance feed efficiency (Li & Watkins, 1998) in several rodent models. In addition, dietary CLA has been reported to decrease body fat in mice (Park *et al.* 1997), rats (Sisk *et al.* 2001), pigs (Ostrowska *et al.* 1999) and chickens (Szymczyk *et al.* 2001), although in man the results are limited and contradictory (Risérus *et al.* 2002, 2003; Gaullier *et al.* 2005).

In fish, studies with CLA are very scarce, but in certain species, CLAs have been shown to alter growth responses, feed efficiency and lipid deposition (Choi *et al.* 1999; Twibell *et al.* 2000, 2001; Twibell & Wilson, 2003). Dietary CLA did not, however, affect the growth performance or proximate composition of rainbow trout (Figueiredo-Silva *et al.* 2005) or Atlantic salmon juveniles (Berge *et al.* 2004; Kennedy *et al.* 2005), although the fatty acid profile of those fish was strongly modified (Berge *et al.* 2004; Bandarra *et al.* 2006). Fish are not a naturally rich source of CLA but are capable of incorporating high levels of CLA into tissue lipids after dietary supplementation (Twibell *et al.* 2000, 2001; Kennedy *et al.* 2005; Bandarra *et al.* 2006), this being a suitable route to produce ‘functional foods’.

High-lipid diets result in a marked increase in the flesh lipid content of fish, which is a component of organoleptic quality (Corraze, 2001). Fattening makes the raw flesh more tender

Abbreviation: CLA, conjugated linoleic acid.

* **Corresponding author:** L. M. P. Valente, tel +351 22 340 18 25, fax +351 22 339 06 08, email lvalente@icbas.up.pt

and slightly modifies the consumer's overall taste perception. The nature of the dietary lipids also affects the taste of the products (Boggio *et al.* 1985; Turchini *et al.* 2003; Izquierdo *et al.* 2005), but very little is known about the general effects of these alterations, particularly dietary CLA supplementation, on the sensory characteristics of rainbow trout.

The overall objective of the present study was to evaluate the effect of graded levels of CLA in high-fat diets fed to large-size rainbow trout. The growth performance, body composition, tissue fatty acid deposition, lipogenic enzyme activities (glucose-6-phosphate dehydrogenase, malic enzyme, fatty acid synthetase) and sensory characteristics of the flesh were then assessed using those fish.

Material and methods

Experimental diets

A commercial extruded diet for rainbow trout was supplied by Sorgal S.A. (Ovar, Portugal). The CLA mixture (containing 75 % CLA) was offered by Bioriginal Food and Science Corp. (Saskatoon, SK, Canada). Before oil-coating, the pellets (3 mm diameter) were analysed for proximate composition and then coated with 21·9 % oil containing the different CLA levels (0 %, 0·5 %, 0·75 %, 1 %). The CLA supplement was added to the diets at the expense of fish oil to maintain a constant energy level (25–26 kJ/g DM) between dietary treatments. The ingredients and proximate composition of the experimental diets are presented in Table 1 and the fatty acid profiles in Table 2.

Table 1. Ingredients and proximate composition of diets with different levels (0 %, 0·5 %, 0·75 %, 1 %) of conjugated linoleic acid (CLA)

	CLA dietary level (%)			
	0	0·5	0·75	1
Ingredients (%)				
Fish meal	46·99	46·99	46·99	46·99
Corn gluten meal	9·72	9·72	9·72	9·72
Extruded peas meal*	6·94	6·94	6·94	6·94
Brewer's yeast	0·97	0·97	0·97	0·97
Soyabean molasses	9·61	9·61	9·61	9·61
Fish oil	21·87	21·21	20·87	20·54
CLA†	0·00	0·67	1·00	1·33
Vitamin‡ and mineral mix§	3·89	3·89	3·89	3·89
Proximate composition				
DM (%)	92·63	92·71	92·75	92·86
Crude protein (% DM)	45·53	45·97	45·91	45·99
Crude fat (% DM)	29·79	29·82	29·83	29·50
Ash (% DM)	10·59	10·38	10·27	10·31
Gross energy (kJ/g DM)	25·86	26·37	26·11	25·85

* Aquatex (20·5 crude protein); Sotexpro, Bermericourt, France.
† CLA mixture: total CLA, 75 %; 18 : 2 (*cis*-9,*trans*-11), 33·6 %; 18 : 2 (*trans*-10,*cis*-12), 32·5 %.
‡ Vitamins (mg/kg diet or IU/kg diet): vitamin A, 8000 IU; vitamin D_3, 2000 IU; vitamin E, 100 mg; vitamin K, 10 mg; vitamin B_{12}, 0·02 mg; vitamin B_1, 15 mg; vitamin B_2, 25 mg; vitamin B_6, 15 mg; folic acid, 10 mg; biotin, 1 mg; vitamin C, 100 mg; betaine, 500 mg; inositol, 300 mg; nicotinic acid, 100 mg; pantothenic acid, 50 mg; choline chloride, 1000 mg.
§ Minerals (g/kg diet or mg/kg diet): $MnSO_4$, 20 mg; KI, 0·6 mg; $CuSO_4$, 5 mg; $CoSO_4$, 0·4 mg; $MgSO_4$, 500 mg; Zn (Bioplex; Alltech), 30 mg; Se (Sel-Plex 2000; Alltech), 0·3 mg; $FeSO_4$, 40 mg; $CaCO_3$, 2·15 g; dibasic calcium phosphate, 5 g; KCl, 1 g; NaCl, 0·4 g.

Growth trial

The trial was conducted with rainbow trout (*Oncorhynchus mykiss*) juveniles produced at the University of Trás-os-Montes and Alto Douro (UTAD, Vila Real, Portugal) rearing facilities. Fish were acclimated to the experimental conditions and fed the control diet (< 0·003 % CLA) for a period of 2 weeks before the beginning of the experiment. Homogenous groups of twenty-five fish with an average initial body weight of 97·8 (SD 0·02) g were then randomly distributed between twelve square fibre-glass tanks (250 litres) in an open flow-through system. Triplicate groups of fish for each treatment were hand-fed to apparent satiety twice a day (09.30 and 18.00 h) for 12 weeks. The pH, ammonia, nitrite, nitrate and phosphate levels were monitored during the entire trial and maintained at levels compatible with the species. The daily water temperature was 16 ± 1°C and fish were exposed to natural photoperiod. At the end of the growth trial, data on weight gain and feed intake were collected from all the fish. Prior to sampling, fish were fasted for 24 h and then anaesthetised by immersion in an ethylene glycol monophenyl ether (1:2500) bath. A pooled sample of six fish from the initial stock at the beginning of the experiment and three fish per tank at the end of the experiment were taken and stored at − 20°C for subsequent whole-body composition analyses.

At the end of the experiment, liver and viscera from nine fish per dietary treatment were removed and weighed for estimation of the hepatosomatic and viscerosomatic indices. The livers and muscle samples from nine fish per treatment were removed and frozen in liquid N and stored at − 80°C prior to fatty acid determination and analysis of lipogenic enzymes activity. Experiments were conducted according to the European Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

Analytical methods

Whole fish were ground, and their moisture content was determined (105°C for 24 h). Fish were subsequently freeze-dried before further analysis. Feed, whole-body samples and faeces were then analysed for DM (105°C for 24 h), ash by combustion in a muffle furnace (550°C for 12 h), crude protein (Micro-Kjeldahl; N × 6·25) after acid digestion, lipid content by petroleum ether extraction (at Soxhlet 40–60°C) and gross energy in an adiabatic bomb calorimeter (IKA, Staufen, Germany).

Determinations of the total lipid of the fish tissues were carried out following the Bligh & Dyer (1959) method with small modifications. Muscle samples were analysed individually, whereas in liver, owing to the scarcity of individual samples, the three livers per tank were sliced and divided into two samples that were analysed independently.

Fatty acid methyl esters (FAME) of diets were prepared after lipid extraction carried out according to the methodology of Bligh & Dyer (1959), followed by acid modified transesterification for 2 h at 80°C. To analyse the fatty acid methyl esters and CLA of trout muscle and liver, extracted total lipids were used for methyl ester preparation by base-catalysed transesterification, with 0·5 M-sodium methoxide solution in anhydrous methanol (2 h at 30 °C), as proposed by Park *et al.* (2001) and Kramer *et al.* (2002), in order to avoid the isomerisation

https://doi.org/10.1017/S000711450733729X Published online by Cambridge University Press

https://doi.org/10.1017/S000711450733729X Published online by Cambridge University Press

Table 2. Fatty acid profile (% total fatty acids) and total conjugated linoleic acid (CLA) content and CLA isomers (% total lipids) of diets with different levels of CLA (0 %, 0·5 %, 0·75 % and 1 %)

(Mean values and standard deviations for three determinations)

	CLA dietary level (%)								
	0		0·5		0·75		1		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	ANOVA *P* value
Fatty acids (%)									
14 : 0	5·95[a]	0·06	5·37[ab]	0·35	5·03[b]	0·06	5·23[ab]	0·03	0·044
16 : 0	17·33	0·12	16·35	0·99	15·49	0·21	16·45	0·03	0·055
18 : 0	3·42	0·02	3·56	0·22	3·52	0·05	3·55	0·01	0·250
Σ Saturated*	28·59	0·21	27·38	1·65	25·87	0·28	27·00	0·03	0·055
16 : 1	7·78[a]	0·04	5·43[ab]	0·41	5·04[b]	0·04	6·71[ab]	0·01	0·020
18 : 1*n*-9	14·59[ab]	0·05	15·64[a]	0·87	14·90[ab]	0·12	14·27[b]	0·08	0·025
20 : 1	4·77[ab]	0·02	5·15[ab]	0·28	5·31[a]	0·09	4·68[b]	0·03	0·021
Σ Monounsaturated†	32·54	0·10	32·20	1·94	31·32	0·54	30·64	0·08	0·190
16 : 2*n*-4	0·27	0·01	0·18	0·16	0·24	0·003	0·23	0·01	0·150
16 : 3*n*-3	0·32[a]	0·002	0·31[ab]	0·02	0·29[ab]	0·002	0·28[b]	0·001	0·024
18 : 2*n*-6	3·13	0·01	3·79	0·19	3·61	0·05	3·62	0·02	0·077
18 : 2 CLA	0·003[a]	0·001	2·50[b]	0·07	3·86[c]	0·10	4·59[d]	0·08	0·000
18 : 3*n*-3	1·05	0·003	1·21	0·10	1·17	0·09	1·02	0·01	0·025
18 : 4*n*-3	2·59	0·02	2·90	0·12	2·79	0·09	2·81	0·02	0·077
20 : 4*n*-6	1·03[a]	0·01	0·90[bc]	0·05	0·85[b]	0·02	0·94[c]	0·004	0·024
20 : 4*n*-3	0·92[a]	0·01	0·80[bc]	0·04	0·79[b]	0·02	0·85[c]	0·01	0·0002
20 : 5*n*-3	12·25[a]	0·10	7·99[b]	0·44	7·81[b]	0·19	11·33[c]	0·01	0·024
22 : 5*n*-6	0·31[ab]	0·01	0·33[a]	0·01	0·33[a]	0·01	0·29[b]	0·01	0·003
22 : 5*n*-3	1·55[a]	0·03	1·31[b]	0·08	1·31[b]	0·05	1·50[a]	0·01	0·0004
22 : 6*n*-3	10·13[ab]	0·10	10·63[ab]	0·58	10·82[a]	0·40	9·84[b]	0·04	0·045
Σ Polyunsaturated‡	35·76	0·23	34·27	1·89	34·71	0·87	39·30	0·19	0·025
Total CLA (%)§	0·07[a]	0·02	2·10[ab]	0·09	2·52[ab]	0·26	3·50[b]	0·75	0·016
18 : 2 *cis*-9,*trans*-11(%)‖	0·05[a]	0·00	0·35[b]	0·02	0·58[c]	0·10	0·85[d]	0·13	0·000
18·2 *trans*-10,*cis*-12(%)‖	0·02[a]	0·00	0·17[b]	0·03	0·30[c]	0·10	0·50[d]	0·06	0·000

[a,b,c,d] Mean values with unlike superscript letters were significantly different ($P<0·05$).
* Saturated: 12 : 0, 14 : 0, 14 : 0 isobr, 15 : 0, 16 : 0, 16 : 0 isobr, 17 : 0, 18 : 0, 19 : 0, 20 : 0, 22 : 0 and 24 : 0.
† Monounsaturated: 16 : 1*n*-7, 17 : 1*n*-8, 18 : 1*n*-9, 18 : 1*n*-7, 20 : 1*n*-9, 20 : 1*n*-7, 22 : 1*n*-11 and 22 : 1*n*-9.
‡ Polyunsaturated: 16 : 2*n*-4, 16 : 3*n*-3, 16 : 4*n*-3, 18 : 2*n*-6, 18 : 2CLA, 18 : 3*n*-6, 18 : 3*n*-3, 18 : 4*n*-3, 20 : 2*n*-6, 20 : 3*n*-3, 20 : 4*n*-6, 20 : 4*n*-3, 20 : 5*n*-3, 22 : 2*n*-6, 22 : 4*n*-6, 22 : 5*n*-6, 22 : 5*n*-3 and 22 : 6*n*-3.
§ Sum of all CLA isomers detected by HPLC.
‖ Isomers detected by HPLC.

of CLA. Fatty acid methyl esters prepared in triplicate were analysed in a Varian CP 3800 (Walnut Creek, CA, USA) GC, equipped with an autosampler and fitted with a flame ionisation detector at 250°C. The separation was achieved using a capillary column HP-Innowax (30 m length, 0·25 mm internal diameter, 0·25 (m film thickness) from Agilent (Albertville, MN, USA). After being kept constant at 180°C for 5 min, the temperature was raised by 4°C/min to 220°C and maintained at 220°C for 25 min with the injector at 250°C. The split ratio was 100:1, and quantification was carried out using an area of the internal standard of 21:0. All analytical determinations were done in triplicate.

In order to avoid some possible co-elution of individual CLA isomers using a short GC column (30 m), the methyl esters of the CLA isomers were individually separated by triple Ag ion columns in series (ChromSpher 5 Lipids; 250 mm′ 4·6 mm internal diameter, 5 μm particle size; Chrompack, Bridgewater, NJ, USA), using an HPLC system (Agilent 1100 Series; Agilent Technologies Inc., Palo Alto, CA, USA) equipped with an autosampler and diode array detector adjusted to 233 nm. The mobile phase was 0·1 % acetonitrile in *n*-hexane maintained at a flow rate of 1 ml/min, using injection volumes of 20 μl. The identification of the individual CLA isomers was achieved by comparing their retention times with commercial and prepared standards, as well as with values published in the literature In addition, the identification of each isomer was controlled by the typical UV spectra of CLA isomers from the diode array detector in the range 190 to 360 nm, using the spectral analysis of Agilent Chemstation for LC 3D Systems rev. A.09.01 (Agilent Technologies). The determination of total and individual CLA isomer content was based on the external standard technique (using 18 : 2 *cis*-9,*trans*-11, 18 : 2 *trans*-10,*cis*-12, 18 : 2 *cis*-9,*cis*-11 and 18 : 2 *trans*-9,*trans*-11 as representatives of each of the geometric groups of CLA isomers) and on the method of area normalisation (Association of Official Analytical Chemists, 2000). The CLA isomers were expressed in gravimetric contents as percentages of total lipids (g/100 g lipids).

Enzyme assays

Liver samples were homogenised in three volumes of ice-cold buffer 0·02 M-Tris–HCl, 0·25 M-sucrose, 2 mM-EDTA, 0·1 M-NaF, 0·5 m M-phenyl methyl sulphonyl fluoride and 0·01 M-β-mercaptoethanol at pH 7·4 and centrifuged (30 000 ***g*** at 4°C) for 20 min. The soluble protein content of the liver was determined in the supernatant by the method of Bradford (1976) using bovine serum albumin as a standard. Selected lipogenic enzyme activities were assayed on the supernatant

using spectrophotometric procedures: glucose-6-phosphate dehydrogenase (EC 1.1.1.49) according to Bautista *et al.* (1988), malic enzyme (ME, EC 1.1.1.40) according to Ochoa (1955) and fatty acid synthetase (EC 2.3.1.38) according to the methodology of Chang *et al.* (1967) as modified by Chakrabarty & Leveille (1969). Enzyme activity units (IU), defined as millimoles of substrate converted to product per minute at the assay temperature, were expressed per milligrams of hepatic soluble protein-specific activity. In order to take into account possible variations in the hepatosomatic index, the activities were also calculated per 100 g of fish.

Sensory analysis

At the end of the experiment, four fish per tank were killed by a sharp blow on the head and immediately put on ice. They were then stored for 24 h at 4°C before sensory analysis. The same protocol of slaughter and filleting was strictly applied to all the fish. The fillets were skinned, washed with tap water and hand-cut into 20 g portions. These were then put into small plastic cups previously coded with three-digit numbers and microwaved until homogeneous cooking (20 s at a maximum power of 850 W). Samples were randomised and served hot in a consumer-type test to thirty-one panelists who regularly ate fish, who were asked to compare unknown samples with an identified control (0 % CLA), in a room designed for sensory analysis. A hidden control was included among the samples to be classified. An explanation sheet with detailed instructions and questions to be answered was given to each panelist. The samples were evaluated using a scale from 0 (similar to control) to 6 (extremely different from control). If sensory differences were detected between the samples, panelists were asked to describe them to obtain a general idea of attributes of the different fish.

Statistical analysis

Statistical analyses followed methods outlined by Zar (1996). All data were tested for normality and homogeneity of variances by Kolmogorov-Smirnov and Bartlett tests, and were then submitted to a one-way ANOVA with the Statistica 6.0 package for Windows (StatSoft Inc., Tulsa, OK, USA). When data did not meet the assumptions of ANOVA, the non-parametric ANOVA equivalent (Kruskal–Wallis test) was performed. When these tests showed significance ($P<0·05$), individual means were compared using the Tukey or Dunn test. The results of sensory analysis were subjected to two-way ANOVA to test the effects of experimental diets and assessors, this being followed by a Dunnett test. Significant differences were considered when $P<0·05$.

Results

Rainbow trout fed the experimental diets during 12 weeks did not display significant differences in growth performance (2–2·3 %), feed conversion ratio (1·1–1·2), protein efficiency ratio (1·8–2·0) or feed intake (12·6–12·9 g/kg per day) between treatments (Table 3). Furthermore, neither the hepatosomatic (0·8–0·9) nor the viscerosomatic (9·8–11) index was affected by the dietary inclusion of CLA ($P>0·1$).

Data on the whole-body composition and retention of rainbow trout fed the different diets are presented in Table 4. By the end of the feeding trial, whole-body protein and lipid content were similar between treatments, ranging from 15·8 % to 16·5 % and 14·9 % to 16·5 %, respectively. Moreover, no significant differences were observed for protein, lipid and energy retention values between dietary treatments ($P>0·1$).

The fatty acid profile of the rainbow trout muscle is presented in Table 5. Total saturated fatty acid, MUFA and PUFA were not

Table 3. Effect of different dietary levels (0 %, 0·5 %, 0·75 %, 1 %) of conjugated linoleic acid (CLA) on growth, efficiency and feed intake in rainbow trout over 12 weeks

(Mean values and standard deviations*)

	CLA dietary level (%)								
	0		0·5		0·75		1		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	ANOVA *P* value
Growth									
Initial mean body weight	97·85	0·06	97·78	0·15	97·72	0·07	97·61	0·02	0·541
Final mean body weight	280·85	18·86	275·57	15·66	247·29	12·44	262·91	6·66	0·076
Hepatosomatic index†	0·88	0·11	0·81	1·11	0·89	0·12	0·88	0·08	0·361
Viscerosomatic index‡	9·79	1·61	10·53	1·94	10·98	1·77	10·44	1·54	0·539
Feed conversion ratio§	1·13	0·07	1·16	0·06	1·24	0·06	1·16	0·04	0·163
Daily growth index‖	2·31	0·18	2·26	0·15	1·99	0·12	2·14	0·06	0·076
Protein efficiency ratio¶	1·95	0·12	1·88	0·10	1·75	0·08	1·88	0·07	0·155
Intake (g/kg or kJ/kg average body weight/d)**									
DM	12·93	0·41	12·83	0·11	12·80	0·04	12·60	0·34	0·547
Protein	5·89	0·19	5·90	0·05	5·88	0·02	5·80	0·16	0·746
Lipid	3·85	0·12	3·82	0·03	3·82	0·01	3·72	0·10	0·266
Energy	334·40	10·55	338·32	2·82	334·26	1·02	325·55	8·85	0·232

Absence of superscripts letters indicates no significant differences between treatments ($P>0·05$).
* *n* 3 except for hepatosomatic index and viscerosomatic index, for which *n* 9.
† Hepatosomatic index = 100 × (liver weight/body weight), %.
‡ Viscerosomatic index = 100 × (viscera weight/body weight), %.
§ Feed conversion ratio = dry feed intake/weight gain.
‖ Daily growth index = 100 × ((final body weight)$^{1/3}$ − (initial body weight)$^{1/3}$)/days.
¶ Protein efficiency ratio = weight gain/crude protein intake.
** Average body weight = (initial mean body weight + final mean body weight)/2.

https://doi.org/10.1017/S000711450733729X Published online by Cambridge University Press

Table 4. Whole-body composition and retention of rainbow trout fed different levels of conjugated linoleic acid (CLA; 0 %, 0·5 %, 0·75 %, 1 %) for 12 weeks

(Mean values and standard deviations for three determinations)

	CLA dietary level (%)									
	Initial	0		0·5		0·75		1		
	Mean	Mean	SD	Mean	SD	Mean	SD	Mean	SD	ANOVA *P* value
Whole-body composition										
Moisture (%)	73·55	63·37	0·97	64·65	0·76	64·45	0·52	64·95	0·67	0·130
Protein (%)	16·21	16·34	0·18	15·76	0·56	16·51	0·73	15·94	0·20	0·273
Lipid (%)	5·61	16·49	1·65	16·04	0·40	14·87	0·19	14·78	1·07	0·173
Energy (kJ/g)	6·32	10·12	0·62	9·82	0·20	9·67	0·19	9·54	0·31	0·321
Ash (%)	3·67	3·34	0·30	2·90	0·32	3·60	0·22	3·02	0·24	0·051
Retention (% intake)										
Protein		32·02	1·46	29·09	1·87	29·33	3·42	29·73	1·54	0·402
Lipid		66·48	6·38	63·79	2·28	56·54	2·13	59·41	6·49	0·129
Energy		41·74	2·80	38·70	1·58	36·63	2·07	38·41	2·75	0·139

Absence of superscript letters indicates no significant differences between treatments ($P > 0.05$).

significantly affected by the incorporation of CLA. Moreover, muscle total lipids were similar between treatments. Within the saturated fraction, stearic acid (18 : 0) varied significantly with dietary CLA inclusion, although the expected decrease in 18 : 1 fatty acid was not seen. Among the PUFA, dietary CLA supplementation mainly resulted in a significant increase in

Table 5. Fatty acid profile (% total fatty acids), total conjugated linoleic acid (CLA; % total lipids) content, CLA isomers (% total lipids) and total lipids (% wet weight) in muscle of rainbow trout fed different dietary levels of CLA (0 %, 0·5 %, 0·75 %, 1 %) for 12 weeks

(Mean values and standard deviations for nine determinations)

	CLA dietary level (%)								
	0		0·5		0·75		1		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	ANOVA *P* value
Fatty acids (%)									
14 : 0	4·13	0·42	3·99	0·30	3·75	0·38	4·05	0·41	0·183
16 : 0	17·27	1·21	17·22	0·74	16·83	0·74	17·17	0·66	0·695
18 : 0	3·95[a]	0·18	4·53[b]	0·23	4·49[b]	0·56	4·50[b]	0·38	0·005
Other saturated	1·85	0·12	1·82	0·19	1·79	0·16	1·79	0·18	0·846
Σ Saturated*	27·20	1·77	27·56	0·96	26·85	1·27	27·51	0·84	0·618
16 : 1	5·11	0·38	4·74	0·21	4·49	0·77	4·90	0·38	0·156
18 : 1	18·25	1·11	17·03	1·57	17·43	1·22	17·55	0·58	0·193
20 : 1	4·14	0·15	4·13	0·22	4·07	0·20	3·95	0·18	0·132
Other monounsaturated	3·55	0·37	3·50	0·19	3·40	0·41	3·50	0·16	0·721
Σ Monounsaturated†	31·06	1·03	29·40	1·42	29·39	1·95	29·91	0·84	0·049
18 : 2*n*-6	4·56	0·28	4·62	0·34	4·60	0·52	4·68	0·34	0·918
18 : 2 CLA	0·17[a]	0·14	1·24[b]	0·21	1·60[c]	0·23	2·10[d]	0·37	0·000
18 : 3*n*-3	1·07	0·04	1·04	0·08	0·97	0·10	1·04	0·11	0·105
18 : 4*n*-3	1·61	0·12	1·64	0·05	1·52	0·10	1·63	0·12	0·055
20 : 4*n*-6	0·76	0·05	0·77	0·06	0·77	0·05	0·74	0·04	0·512
20 : 4*n*-3	1·27	0·11	1·31	0·07	1·23	0·09	1·22	0·07	0·083
20 : 5*n*-3	5·96	0·57	6·18	0·25	5·90	0·17	5·90	0·19	0·164
22 : 5*n*-6	0·35[a]	0·08	0·22[b]	0·11	0·32[ab]	0·07	0·28[ab]	0·09	0·024
22 : 5*n*-3	2·06	0·12	2·15	0·11	2·08	0·11	1·95	0·33	0·514
22 : 6*n*-3	19·19	1·81	19·71	1·61	19·94	2·67	18·56	2·07	0·514
Other polyunsaturated	1·82[a]	0·37	1·17[b]	0·35	1·47[ab]	0·30	1·60[ab]	0·39	0·005
Σ Polyunsaturated‡	38·82	2·54	40·10	1·50	40·41	2·49	39·70	1·92	0·443
Σ*n*-3	31·72	2·54	32·52	1·75	32·11	2·66	30·82	2·07	0·446
Total CLA (%)§	0·44[a]	0·15	2·10[ab]	0·35	2·74[bc]	0·66	4·16[c]	0·54	0·000
18 : 2 *cis*-9,*trans*-11(%)‖	0·05[a]	0·02	0·83[b]	0·14	1·13[c]	0·24	1·74[d]	0·15	0·000
18·2 *trans*-10 *cis*-12(%)‖	0·02[a]	0·01	0·66[b]	0·10	0·92[c]	0·25	1·39[d]	0·12	0·000
Total lipids (%)	4·84	0·61	5·11	0·71	4·92	1·24	4·22	0·50	0·293

[a,b,c,d] Mean values with unlike superscript letters were significantly different ($P < 0.05$).

* Saturated: 12 : 0, 14 : 0, 14 : 0 isobr, 15 : 0, 16 : 0, 16 : 0 isobr, 17 : 0, 18 : 0, 19 : 0, 20 : 0, 22 : 0 and 24 : 0.

† Monounsaturated: 16 : 1*n*-7, 17 : 1*n*-8, 18 : 1*n*-9, 18 : 1*n*-7, 20 : 1*n*-9, 20 : 1*n*-7, 22 : 1*n*-11 and 22 : 1*n*-9.

‡ Polyunsaturated: 16 : 2*n*-4, 16 : 3*n*-3, 16 : 4*n*-3, 18 : 2*n*-6, 18 : 2CLA, 18 : 3*n*-6, 18 : 3*n*-3, 18 : 4*n*-3, 20 : 2*n*-6, 20 : 4*n*-6, 20 : 3*n*-3, 20 : 4*n*-3, 22 : 2*n*-6, 20 : 5*n*-3, 22 : 4*n*-6, 22 : 5*n*-6, 22 : 5*n*-3 and 22 : 6*n*-3.

§ Sum of all CLA isomers detected by HPLC.

‖ Isomers detected by HPLC.

https://doi.org/10.1017/S000711450733729X Published online by Cambridge University Press

the biologically active *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA isomers. The total CLA content of the muscle reflected the dietary inclusion levels, the highest level being attained when fish were fed 1 % CLA. The *n*-3 PUFA, in particular 20 : 5*n*-3 (EPA), 22 : 5*n*-3 (docosapentaenoic acid) and 22 : 6*n*-3 (DHA), were not affected by dietary CLA inclusion.

Contrary to what was observed in muscle, the liver fat content was significantly reduced by the inclusion of 0·75 % or 1 % CLA (Table 6). The ingestion of increasing amounts of CLA induced significant changes in the fatty acid profile of the liver that were reflected in all fractions. Both saturated and polyunsaturated fractions increased significantly with CLA incorporation, whereas there was a significant reduction in MUFA. The most evident changes in saturated fatty acids and MUFA were related to the increase in 18 : 0 and decrease in 18 : 1 fatty acids. With regard to PUFA, the levels of 18 : 2 CLA were significantly raised as a result of both biologically active isomers, although the incorporation of the *cis*-9,*trans*-11 isomer was always greater. Moreover, the liver deposition of 20 : 5*n*-3 (EPA) and of 22 : 5*n*-3 (docosapentaenoic acid) showed the opposite trend: the level of the former increased significantly, whereas that of docosapentaenoic acid declined with dietary CLA incorporation. DHA deposition was similar in all treatments.

Activities of lipogenic enzymes

Data on the activities of the three lipogenic enzymes assayed are reported as both IU/mg protein and IU/100 g whole fish (Table 7). Glucose-6-phosphate dehydrogenase and fatty acid synthetase displayed the highest (7·8–8·9 IU/100 g) and lowest (60–90 mIU/100 g) activity values, respectively, but dietary CLA level had no significant effect on either of these two lipogenic enzymes. Malic enzyme activity in fish fed 0·75 % CLA was lower than that in fish fed the control diet (0 % CLA), although this difference was less evident when the results were expressed per 100 g fish.

Sensory analysis

All panelists correctly identified the hidden control, and non-significant differences were detected between the panelists' results ($P<0·01$). Sensory data indicated significant

Table 6. Fatty acid profile (% total fatty acids), total conjugated linoleic acid (CLA; % total lipids) content, CLA isomers (% total lipids) and total lipids (% wet weight) in the liver of rainbow trout fed different dietary levels of CLA (0 %, 0·5 %, 0·75 %, 1 %) for 12 weeks

(Mean values and standard deviations for six determinations)

	CLA dietary level (%)								
	0		0·5		0·75		1		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	ANOVA *P* value
Fatty acids (%)									
14 : 0	1·90	1·33	1·73	0·20	1·65	0·31	1·73	0·35	0·421
16 : 0	13·51	1·72	14·24	0·70	15·45	1·66	14·88	0·55	0·174
18 : 0	5·64[a]	0·34	8·26[b]	0·92	8·01[b]	0·70	8·44[b]	1·07	0·000
Other saturated	2·10	1·10	1·68	0·18	1·68	0·29	1·77	0·23	0·575
Σ Saturated*	23·15[a]	1·74	25·90[b]	0·72	26·79[b]	1·37	26·82[b]	1·00	0·000
16 : 1	2·51	1·20	1·13	0·82	2·88	2·93	1·77	0·78	0·069
18 : 1	17·14[a]	1·82	11·80[b]	2·13	11·38[b]	2·68	11·20[b]	2·68	0·001
20 : 1	4·00	0·68	3·17	0·13	2·79	0·65	2·98	0·38	0·102
Other monounsaturated	1·02	0·10	0·88	0·20	0·83	0·28	0·87	0·26	0·490
Σ Monounsaturated†	24·62[a]	3·12	16·98[b]	2·16	17·87[ab]	2·69	16·81[b]	3·86	0·008
18 : 2n-6	2·38	0·37	1·90	0·46	2·10	0·28	2·36	0·76	0·311
18 : 2 CLA	0·11[a]	0·14	0·57[b]	0·12	0·73[b]	0·24	1·07[c]	0·20	0·000
18 : 3*n*-3	0·38	0·22	0·46	0·09	0·44	0·08	0·50	0·13	0·487
18 : 4*n*-3	0·35	0·10	0·33	0·13	0·37	0·12	0·40	0·15	0·810
20 : 4*n*-6	2·39	0·71	3·13	0·56	2·85	0·90	3·37	0·51	0·177
20 : 4*n*3	1·03	0·08	0·85	0·25	0·85	0·23	0·99	0·08	0·339
20 : 5*n*-3	5·20[a]	1·36	6·25[ab]	1·52	7·28[b]	0·89	7·60[b]	0·30	0·002
22 : 5*n*-6	0·59	0·10	0·69	0·04	0·62	0·12	0·67	0·06	0·211
22 : 5*n*-3	2·85[a]	0·35	2·27[ab]	0·15	2·16[b]	0·10	2·06[b]	0·19	0·000
22 : 6*n*-3	29·98	2·45	32·15	1·20	32·91	1·83	31·67	2·32	0·109
Other polyunsaturated	1·58	0·30	1·59	0·53	1·25	0·21	1·25	0·26	0·167
Σ Polyunsaturated‡	47·17[a]	3·53	50·37[ab]	1·84	51·60[b]	1·95	51·99[b]	1·62	0·008
Σ*n*-3	40·16[a]	3·26	42·61[ab]	1·69	44·26[b]	2·08	43·45[ab]	1·95	0·035
Total CLA (%)§	0·29[a]	0·01	0·82[ab]	0·06	1·29[b]	0·26	1·98[b]	0·20	0·000
18 : 2 *cis*-9,*trans*-11 (%)‖	0·05[a]	0·00	0·35[b]	0·02	0·58[c]	0·10	0·85[d]	0·13	0·000
18 : 2 *trans*-10,*cis*-12 (%)‖	0·02[a]	0·00	0·17[b]	0·03	0·30[c]	0·10	0·50[d]	0·06	0·000
Total lipids (%)	5·80[a]	1·19	4·69[ab]	0·51	4·09[b]	0·51	4·09[b]	0·49	0·014

[a,b,c] Mean values with unlike superscripts letters were significantly different ($P<0·05$).

* Saturated: 12 : 0, 14 : 0, 14 : 0 isobr, 15 : 0, 16 : 0, 16 : 0 isobr, 17 : 0, 18 : 0, 19 : 0, 20 : 0, 22 : 0 and 24 : 0.

† Monounsaturated: 16 : 1*n*-7, 17 : 1*n*-8, 18 : 1*n*-9, 18 : 1*n*-7, 20 : 1*n*-9, 20 : 1*n*-7, 22 : 1*n*-11 and 22 : 1*n*-9

‡ Polyunsaturated: 16 : 2*n*-4, 16 : 3*n*-3, 16 : 4*n*-3, 18 : 2*n*-6, 18 : 2CLA, 18 : 3*n*-6, 18 : 3*n*-3, 18 : 4*n*-3, 20 : 2*n*-6, 20 : 4*n*-6, 20 : 3*n*-3, 20 : 4*n*-3, 22 : 2*n*-6, 20 : 5*n*-3, 22 : 4*n*-6, 22 : 5*n*-6, 22 : 5*n*-3 and 22 : 6*n*-3.

§ Sum of all CLA isomers detected by HPLC.

‖ Isomers detected by HPLC.

https://doi.org/10.1017/S000711450733729X Published online by Cambridge University Press

Table 7. Effects of different dietary levels (0%, 0·5%, 0·75% and 1 %) of conjugated linoleic acid (CLA) on the hepatic lipogenic enzyme activities of rainbow trout

(Mean values and standard deviations for nine determinations)

	CLA dietary level (%)								
	0		0·5		0·75		1		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	ANOVA *P* value
Malic enzyme									
IU/100 g fish	5·96	1·34	6·10	1·13	4·62	1·15	5·64	1·00	0·047
mIU/mg protein	62·10[a]	16·08	52·69[ab]	11·03	43·66[b]	11·29	52·23[ab]	10·47	0·032
Glucose-6-phosphate dehydrogenase									
IU/100 g fish	8·92	1·40	8·78	2·09	8·06	1·14	7·83	2·30	0·507
mIU/mg protein	92·34	14·65	73·93	19·47	78·15	18·01	72·47	21·07	0·110
Fatty acid synthetase									
mIU/100 g fish	63·83	31·55	66·47	57·03	87·70	86·42	66·90	37·53	0·799
mIU/mg protein	0·53	0·25	0·50	0·47	0·77	0·68	0·48	0·32	0·514

[a,b]Mean values with unlike superscript letters were significantly different ($P<0·05$).

differences in the organoleptic characteristics between trout fed the control diet and trout fed the different CLA levels (0·5 %, 0·75 %, 1 % CLA). These differences were classified as slight-to-moderate for 0·5 % and 0·75 % CLA, and between slight and slight-to-moderate for 1 % CLA, suggesting that CLA levels above 0·75 % were less perceptible in sensory terms than were lower values. Descriptors from the panelists included attributes of flavour and texture in the mouth, although panelists were divided concerning their preferences: approximately half of the panelists had the overall impression that the samples with CLA were better than the control, whereas the other half expressed the opposite view.

Discussion

The growth rates and nutrient utilisation of rainbow trout were good, and the incorporation of increasing dietary levels of CLA (0–1 %) led to similar final weights of the fish at the end of the 12-week trial. Moreover, the independency of growth performance upon dietary CLA level confirm earlier observations in juvenile rainbow trout growing from 5 to 70 g and fed low-fat diets with the same CLA level (Figueiredo-Silva *et al.* 2005), and are in agreement with results from other studies in various fish species fed 0·5–5 % CLA (Twibell *et al.* 2001; Twibell & Wilson, 2003; Berge *et al.* 2004; Kennedy *et al.* 2005). However, common carp fed 1 % CLA increased their weight gain (Choi *et al.* 1999), whereas hybrid striped bass reduced their feed intake and improved their feed efficiency (Twibell *et al.* 2000). Nevertheless, higher CLA inclusion levels reduced weight gain and feed efficiency in Nile tilapia, rockfish and common carp (Choi *et al.* 1999). It can be concluded that the effect of CLA on growth performance and feed efficiency is clearly dependent on the species considered.

In the present study, dietary CLA up to 1 % had no effect on the whole-body composition of rainbow trout, which is in agreement with previous findings in smaller-sized trout (Figueiredo-Silva *et al.* 2005), hybrid striped bass (Twibell *et al.* 2000), channel catfish (Twibell & Wilson, 2003) and Atlantic salmon (Kennedy *et al.* 2005). Several studies have, however, reported a decrease in lipid deposition in certain fish species (Choi *et al.* 1999; Twibell *et al.* 2000, 2001; Twibell & Wilson, 2003). The reduction in lipid deposition is particularly desirable when high-fat diets are used. In the present study, however, CLA administration did not affect the body composition of the fish.

With respect to the influence of CLA on tissue composition, a decrease in liver lipid content was obtained when high levels of CLA (>0·5 %) were fed. This is in accordance with earlier results from striped bass (Twibell *et al.* 2000) and yellow perch (Twibell *et al.* 2001). In contrast, dietary CLA had no significant effect on the liver lipid content of catfish (Twibell & Wilson, 2003), Atlantic salmon (Kennedy *et al.* 2005) or rainbow trout juveniles fed low-fat diets (Bandarra *et al.* 2006), whereas increased muscle lipid concentrations were observed in Atlantic salmon (Kennedy *et al.* 2005). In the present work, the hepatosomatic index was not affected by different CLA treatments, despite the reduction in liver lipid content by dietary CLA supplementation.

The reduction in liver lipid content was accompanied by a slight reduction in malic enzyme specific activity, whereas glucose-6-phosphate dehydrogenase and fatty acid synthetase were not significantly affected by the dietary CLA level. The positive correlation between malic enzyme specific activity and liver lipid content suggests an inhibition of malic enzyme activity and consequent liver lipid content by increasing dietary CLA levels. An inhibition of *de novo* lipogenesis by CLA isomers has been demonstrated in the mammary glands of sow (Bee, 2000) and mice (Lin *et al.* 2004). Moreover, other studies have demonstrated that CLA upregulates not only the mRNA expression, but also the activity of various enzymes involved in lipogenesis and fatty acid oxidation in mouse liver (Takahashi *et al.* 2003; Ide, 2005). In rats, the ingestion of a CLA mixture increased malic enzyme activity in the liver, whereas the activities of both glucose-6-phosphate dehydrogenase and fatty acid synthetase were unaffected (Faulconnier *et al.* 2004). Figueiredo-Silva *et al.* (2005) did not observe any significant changes in the activities of the lipogenic enzymes in rainbow trout juveniles fed low-fat diets (16 %) and increasing CLA levels, suggesting that the effect of CLA on hepatic lipogenic enzymes was dependent on the species considered and might be related to the various nutritional and physiological conditions involved in the regulation of hepatic fatty acid oxidation and synthesis.

https://doi.org/10.1017/S000711450733729X Published online by Cambridge University Press

Several studies have shown that the dietary CLA affects the fatty acid profile of fish independently of any effect on body fat level (Twibell *et al.* 2000; Berge *et al.* 2004; Bandarra *et al.* 2006). In the present work, the total percentage of the main fatty acids groups in muscle were not as strongly affected by the dietary inclusion of CLA as they were in rainbow trout juveniles fed low-fat diets (Bandarra *et al.* 2006). This suggests that the developmental stage of the fish and the dietary lipid content might affect the pattern of lipid metabolism. The saturated lipid fraction in muscle did not vary between dietary treatments despite the increasing concentration of stearic acid (18 : 0). In liver, however, the saturated fraction of the control group was significantly lower than that found in fish fed CLA, stearic acid concentration being mainly responsible for these differences. This was a clear effect of dietary CLA as the control diet contained higher amounts of saturated fatty acids. Moreover, the level of MUFA decreased significantly in both muscle and liver. The significant increase in 18 : 0 and the concomitant decrease in 18 : 1*n*-9 suggests an inhibition of Δ-9 desaturase activity by CLA. This was more evident in liver than muscle. Similar results have been reported in rainbow trout juveniles (Bandarra *et al.* 2006), hybrid striped bass (Twibell *et al.* 2000), yellow perch (Twibell *et al.* 2001) and Atlantic salmon (Berge *et al.* 2004; Kennedy *et al.* 2005). The dietary incorporation of CLA led to a significant increase in the polyunsaturated fraction in the liver. In muscle, besides CLA deposition, a reduction in 22 : 5*n*-6 was observed, whereas the *n*-3 fatty acid concentrations remained unchanged. Different results were previously reported in rainbow trout juveniles (Bandarra *et al.* 2006) and striped bass muscle (Twibell *et al.* 2000), for which CLA incorporation resulted in decreased levels of DHA (22 : 6*n*-3). In larger rainbow trout (>250 g), this negative effect was not observed in muscle and liver, where a prominent biosynthesis and deposition of DHA was reported.

CLA was successfully incorporated into tissue lipids, levels in flesh being 2-fold higher (2·1–4·2 %) than those in liver (0·8–1·9 %). Moreover, muscle CLA incorporation was not related to a decrease in DHA, as previously reported in juvenile rainbow trout fed low-fat diets (Bandarra *et al.* 2006), as well as other species (Twibell *et al.* 2000; Kennedy *et al.* 2005). In muscle and liver lipids, *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers were retained in proportions similar to those found in the diets. Results reported by Burdge *et al.* (2004) show that there was no differential incorporation of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 into the plasma phospholipids of healthy men. Nevertheless, the extent of incorporation of total CLA isomers seems to be tissue dependent, showing a higher deposition in muscle lipid than liver, as previously reported for rainbow trout juveniles (Bandarra *et al.* 2006), Atlantic salmon (Kennedy *et al.* 2005) and mammalian species (Ostrowska *et al.* 2003; Lauridsen *et al.* 2005).

It is well known that different dietary fat sources will affect the fatty acid composition of fish, and that this can be reflected in their sensory qualities (Boggio *et al.* 1985; Turchini *et al.* 2003; Izquierdo *et al.* 2005). In the present study, the sensory characteristics of trout fillet were slightly affected by the dietary supplementation with CLA. Schabbel *et al.* (2004) did not find any difference in trout previously fed with diets containing 0 %, 1 % and 3 % CLA, although the flavour of deep-frozen fillets with 3 % CLA and 600 mg vitamin E supplementation was considered to be positively distinct.

The results obtained in the present experiment clearly show that CLA can be incorporated in a proportion of up to 1 % in rainbow trout diets without altering fish performance, feed efficiency and whole-body composition. The effects of CLA in liver metabolism were more pronounced than reported in previous studies (Figueiredo-Silva *et al.* 2005; Bandarra *et al.* 2006), suggesting that the dietary oil content and developmental stage of the fish are important factors in determining the effects of CLA. The present results clearly suggest that CLA can be incorporated at a level of up to 1 % in rainbow trout diets, contributing to the production of a functional food. It is now important to determine the minimal administration period of dietary CLA to obtain the desirable deposition levels of CLA in the muscle of commercially sized rainbow trout.

Acknowledgements

Special thanks go to Sorgal, S.A., Ovar, Portugal, for supplying the extruded diet before coating, and to Bioriginal Food and Science Corp., Saskatoon SK, Canada, for offering the CLA mixture. We also thank António Júlio Pinto for his technical assistance during the growth trial and samplings, and Engs. Maria João Monteiro and Susana Teixeira, from Escola Superior de Biotecnologia, Universidade Católica Portuguesa, for their work and facilities for sensory testing. This study was supported by project POCTI/CVT/39 237/2001 (FCT, Portugal, with the support of the European fund FEDER).

References

Association of Official Analytical Chemists (2000) *Official Methods of Analysis of AOAC International: Method 963.22*. Washington, DC: AOAC.

Bandarra M, Nunes ML, Andrade AM, Prates JAM, Pereira S, Monteiro M, Rema P & Valente LMP (2006) Effect of dietary conjugated linoleic acid on muscle, liver and visceral lipid deposition in rainbow trout juveniles (*Oncorhynchus mykiss*). *Aquaculture* **254**, 496–505.

Bautista JM, Garrido-Pertierra A & Soler G (1988) Glucose-6-phosphate dehydrogenase from *Dicentrarchus labrax* liver: kinetic mechanism and kinetics of NADPH inhibition. *Biochim Biophys Acta* **967**, 354–363.

Bee G (2000) Dietary conjugated linoleic acids alter adipose tissue and milk lipids of pregnant and lactating sows. *J Nutr* **130**, 2292–2298.

Berge GM, Ruyter B & Asgard T (2004) Conjugated linoleic acid in diets for juvenile Atlantic salmon (*Salmo salar*); effects on fish performance, proximate composition, fatty acid and mineral content. *Aquaculture* **237**, 365–380.

Bligh E & Dyer W (1959) A rapid method of total lipid extraction and purification. *J Biochem Physiol* **37**, 911–917.

Boggio SM, Hardy RW, Babbitt JK & Brannon EL (1985) The influence of dietary lipid source and alpha-tocopheryl acetate level on product quality of rainbow trout (*Salmo gairdneri*). *Aquaculture* **51**, 13–24.

Bradford MM (1976) A rapid and sensitive method for the quantisation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann Biochem* **72**, 248–254.

Burdge GC, Lupoli B, Russell JJ, *et al.* (2004) Incorporation of cis-9, trans-11 or trans-10,cis-12 conjugated linoleic acid into plasma and cellular lipids in healthy men. *J Lipid Res* **45**, 736–741.

Chakrabarty K & Leveille GA (1969) Acetyl-CoA carboxylase and fatty acid synthetase activities in the liver and adipose tissue of meal-fed rats. *Proc Soc Exp Biol Med* **131**, 1051–1054.

https://doi.org/10.1017/S000711450733729X Published online by Cambridge University Press

Chang HC, Seidman I, Teebor G & Lane DM (1967) Liver acetyl-CoA carboxylase and fatty acid synthetase relative activities in the normal state and hereditary obesity. *Biochem Biophys Res Commun* **28**, 682–686.

Choi BD, Kang SJ, Ha YL & Ackman RG (1999) Accumulation of conjugated linoleic acid (CLA) in tissues of fish fed diets containing various levels of CLA. In *Quality Attributes of Muscle Foods*, pp. 61–71 [YL Xiong, CT Ho and F Shahidi, editors]. New York: Kluwer Academic/Plenum Publishers.

Corraze G (2001) Lipid nutrition. In *Nutrition and Feeding of Fish and Crustaceans*, pp. 111–130 [J Guillaume, S Kaushik, P Bergot and R Métailler, editors]. London: Springer Praxis.

Cowey CB (1993) Some effects of nutrition on the flesh quality of cultured fish. In *Fish Nutrition in Practice*, pp. 227–236 [SJ Kaushik and P Luquet, editors]. Paris: INRA Editions.

Faulconnier Y, Arnalb MA, Mirand PP, Chardignyc JM & Chilliarda Y (2004) Isomers of conjugated linoleic acid decrease plasma lipids and stimulate adipose tissue lipogenesis without changing adipose weight in post-prandial adult sedentary or trained Wistar rat. *J Nutr Biochem* **15**, 741–748.

Figueiredo-Silva AC, Rema P, Bandarra NM, Nunes M & Valente LMP (2005) Effects of dietary conjugated linoleic acid in growth, nutrient utilization, body composition and hepatic lipogenesis in rainbow trout juveniles (*Oncorhynchus mykiss*). *Aquaculture* **248**, 163–172.

Gaullier JM, Halse J, Hoye K, Kristiansen K, Fagertun H, Vik H & Gudmundsen O (2005) Supplementation with conjugated linoleic acid for 24 months is well tolerated by and reduces body fat mass in healthy, overweight humans. *J Nutr* **135**, 778–784.

Ha YL, Storkson J & Pariza MW (1990) Inhibition of benzo(a)pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. *Cancer Res* **50**, 1097–1101.

Hillestad M & Johnsen F (1994) High-energy/low-protein diets for Atlantic salmon: effects on growth, nutrient retention and slaughter quality. *Aquaculture* **124**, 109–116.

Ide T (2005) Interaction of fish oil and conjugated linoleic acid in affecting hepatic activity of lipogenic enzymes and gene expression in liver and adipose tissue. *Diabetes* **54**, 412–423.

Ip C, Banni S, Angioni E, Carta G, Mcginley J, Thompson HJ, Barbano D & Bauman D (1999) Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. *J Nutr* **129**, 2135–2142.

Izquierdo MS, Montero D, Robaina L, Caballero MJ, Rosenlund G & Gines R (2005) Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. *Aquaculture* **250**, 431–444.

Kennedy SR, Campbell PJ, Porter A & Tocher DR (2005) Influence of dietary conjugated linoleic acid (CLA) on lipid and fatty acid composition in liver and flesh of Atlantic salmon (*Salmon salar*). *Comp Biochem Physiol* **141**, 168–178.

Kramer JK, Blackadar CB & Zhou J (2002) Evaluation of two GC columns (60 m Supelcowax 10 and 100 m CP Sil 88) for analysis of milkfat with emphasis on CLA, 18:1, 18:2 and 18:3 isomers, and short and long-chain fatty acids. *Lipids* **37**, 823–835.

Lauridsen C, Mu H & Henckel P (2005) Influence of dietary conjugated linoleic acid (CLA) and age at slaughtering on performance, slaughter-and meat quality, lipoproteins, and tissue deposition of CLA in barrows. *Meat Sci* **69**, 393–399.

Lee KN, Kritchevsky D & Pariza MW (1994) Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* **108**, 19–25.

Li Y & Watkins BA (1998) Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo prostaglandin E2 biosynthesis in rats fed *n*-6 or *n*-3 fatty acids. *Lipids* **33**, 417–425.

Lin X, Loor JJ & Herbein JH (2004) *Trans*10,*cis*12–18:2 is a more potent inhibitor of de novo fatty acid synthesis and desaturation than *cis*9,*trans*11–18:2 in the mammary gland of lactating mice. *J Nutr* **134**, 1362–1368.

Ochoa S (1955) Malic enzyme. In *Methods in Enzymology*, vol. 1, pp. 739–753 [SP Colowick and NO Kaplan, editors]. New York: Academic Press.

Ostrowska E, Cross RF, Muralitharan M, Bauman DE & Dunshea FR (2003) Dietary conjugated linoleic acid differentially alters fatty acid composition and increases conjugated linoleic acid content in porcine adipose tissue. *Brit J Nutr* **90**, 925–928.

Ostrowska E, Muralitharan M, Cross RF, Bauman DE & Dunshea FR (1999) Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. *J Nutr* **129**, 2037–2042.

Park Y, Albright KJ, Cai ZY & Pariza MW (2001) Comparison of methylation procedures for conjugated linoleic acid and artefact formation by commercial (trimethylsilyl) diazomethane. *J Agric Food Chem* **49**, 1158–1164.

Park Y, Albright KJ, Liu W, Storkson JM, Cook ME & Pariza MW (1997) Effect of conjugated linoleic acid on body composition in mice. *Lipids* **32**, 853–858.

Risérus U, Arner P, Brismar K & Vessby B (2002) Treatment with dietary *trans*10*cis*12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. *Diabetes Care* **25**, 1516–1521.

Risérus U, Smedman A, Basu S & Vessby B (2003) CLA and body weight regulation in humans. *Lipids* **38**, 133–137.

Schabbel W, Maaβ D, Steinhart H, Reiter R & Schwarz FJ (2004) Performance, carcass quality and fatty acid composition of trout (*Salmo gairdnerii*) as affected by dietary conjugated linoleic acid and vitamin E supplementation. *Proc Soc Nutr Physiol* **13**, 49.

Sisk MB, Hausman DB, Martin RJ & Azain MJ (2001) Dietary conjugated linoleic acid reduces adiposity in lean but not obese zucker rats. *J Nutr* **131**, 1668–1674.

Szymczyk B, Pisulewski P, Szczurek W & Hanczakowski P (2001) Effects of conjugated linoleic acid on growth, feed conversion efficiency, and subsequent carcass quality in broiler chickens. *Br J Nutr* **85**, 465–473.

Takahashi Y, Kushiro M, Shinohara K & Ide T (2003) Activity and mRNA levels of enzyme involved in hepatic fatty acid synthesis and oxidation in mice fed conjugated linoleic acid. *Biochim Biophys Commun* **1631**, 265–273.

Turchini GM, Mentasti T, Frøyland L, Orban E, Caprino F, Moretti VM & Valfré F (2003) Effects of alternative dietary lipid sources on performance, tissue chemical composition, mitochondrial fatty acid oxidation capabilities and sensory characteristics in brown trout (*Salmo trutta* L.). *Aquaculture* **225**, 251–267.

Twibell RG & Wilson RP (2003) Effects of dietary conjugated linoleic acids and total dietary lipid concentrations on growth responses of juvenile channel catfish, *Ictalurus puncatus*. *Aquaculture* **221**, 621–628.

Twibell RG, Watkins BA, Rogers L & Brown PB (2000) Effects of dietary conjugated linoleic acids on hepatic and muscle lipids in hybrid striped bass. *Lipids* **35**, 155–161.

Twibell RG, Watkins BA & Brown PB (2001) Dietary conjugated linoleic acids and lipid source alter fatty acid composition of juvenile yellow perch, *Perca flavescens*. *J Nutr* **131**, 2322–2328.

Vergara JM, López-Calero G, Robaina L, Caballero MJ, Montero D, Izquierdo MS & Aksnes A (1999) Growth, feed utilization and body lipid content of gilthead seabream (*Sparus aurata*) fed increasing lipid levels and fish meals of different quality. *Aquaculture* **179**, 35–44.

West DB, DeLany JP, Camet PM, Blohm F, Truett AA & Scimeca J (1998) Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. *Am J Physiol* **275**, 667–672.

Zar JH (1996) *Biostatistical Analysis*. London: Prentice-Hall International.

https://doi.org/10.1017/S000711450733729X Published online by Cambridge University Press