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Influence of dietary conjugated linoleic acid on growth, fatty acid composition and hepatic lipogenesis in large yellow croaker (*Pseudosciaena crocea* R.)*

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Abstract: We examined the effects of conjugated linoleic acid (CLA) on growth, fatty acid composition and enzyme activity of fatty acid oxidation in the liver of large yellow croaker. We divided 1600 fish (average initial weight 150 g) into 4 groups and reared them in 8 cages. Four dietary treatments were formulated to contain 0%, 1%, 2% and 4% (w/w) CLA, respectively. The fish were fed for 10 weeks ad libitum twice daily. We found that the dietary CLA had no effect on growth, biometric parameters and whole body proximate ($P>0.05$), but showed some significant effects on the fatty acid composition in both muscle and the liver. The activities of lipogenic enzymes were slightly depressed in fish fed with increasing levels of CLA when compared with control ($P>0.05$). Dietary CLA supplementation had no effects on liver lipid content, but significantly increased the contents of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) ($P<0.05$) and decreased monounsaturated fatty acid (MUFA) content in both muscle and the liver. Dietary CLA inclusion resulted in significant increases of the biologically active *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers in both tissues ($P<0.05$). The total accumulation of CLA was higher in the liver (3.83%, w/w) than in muscle (3.77%, w/w) when fed with 4% (w/w) CLA. This study demonstrates that large yellow croakers are capable of absorbing and depositing CLA and long-chain *n*-3 PUFA in the liver and muscle, showing that this species fed with CLA could be an important human food source for these healthful fatty acids.

Key words: Conjugated linoleic acid (CLA), Fatty acids, Lipid, Lipogenic enzymes, Large yellow croaker
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INTRODUCTION

Large yellow croaker (*Pseudosciaena crocea* Richardson) constitutes one of the most abundant species in the Northwest Pacific basin, inhabiting the continental shelf waters of China, Korea and Japan. Large yellow croaker is economically a very important aquaculture fish species in China (Tang *et al*., 2008). The aquaculture production of this species has become intensive but there are few researches in improving the quality of cultured large yellow croaker.

Conjugated linoleic acid (CLA) refers to a group of fatty acids, which exist as positional and stereoisomeric mixtures of octadecadienoic acids characterized by conjugated double bones. CLA is a geometric isomer of linoleic acid (C18:2 *n*-6; LA) with the two main naturally occurring isomers being *cis*-9, *trans*-11 and *trans*-10, *cis*-12. CLA is found naturally in many animal products, especially those from ruminant sources (Chin *et al*., 1992) where it is synthesized from linoleic acid by rumen bacteria (Kepler *et al*., 1966). It is well known that CLA is responsible for anticarcinogenic, antiatherosclerotic, antioxdative, immunomodulative and antibacterial properties in humans (Jahreis *et al*., 2000; Pariza *et al*., 2001; Roche *et al*., 2001) and protects against im-

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mune-induced cachexia (Cook and Pariza, 1998). Evidence from several experiments showed that dietary CLA decreases body fat, increases lean body mass (Thiel-Cooper *et al*., 2001; Tischendorf *et al*., 2002; Yamasaki *et al*., 2003), and enhances the rate-limiting enzyme in β-oxidation carnitine palmitoyltransferase (CPT) activity in skeletal muscle (Park *et al*., 1997). Other studies demonstrated that CLA exhibits several effects on hepatic lipid metabolism (Yotsumoto *et al*., 1999; Valente *et al*., 2007a; 2007b). The mechanisms of anti-obesity were proposed, which include decreased energy/food intake and increased energy expenditure (Ohnuki *et al*., 2001; Terpstra *et al*., 2002), decreased lipogenesis (Brown *et al*., 2001; Oku *et al*., 2003) and increased lipolysis and fatty acid oxidation (Evans *et al*., 2002).

The effects of dietary CLA have been studied in a number of fish species. When Atlantic salmon, rainbow trout and Atlantic cod were fed with CLA-containing diets (Berge *et al*., 2004; Kennedy *et al*., 2007a; 2007b), CLA in the diet showed no effect on growth parameters, body composition or feed conversion. Valente *et al*.(2007a; 2007b) found that the inclusion of CLA in the diet of sea bass reduced amounts of saturated and monounsaturated fatty acids and increased amount of polyunsaturated fatty acids (PUFA). Moreover, dietary CLA may also reduce tissue total lipids in some fish species (Twibell *et al*., 2000; 2001; Yasmin and Takeuchi, 2002).

The effects of dietary CLA on the growth performance and flesh quality of marine species of interest for Asian aquaculture have not been reported. Since fish are considered an important source of protein and *n*-3 PUFA, a further increase in their CLA content could be of interest to enhance the nutritional value of fish for human consumption. Thus, we evaluated the effects of graded dietary levels [0%, 1%, 2% and 4% (w/w)] of CLA on growth performance, body composition, tissue fatty acid deposition and lipogenic enzyme activities (malic enzyme, ME; glucose-6-phosphate dehydrogenase, G6PD; and fatty acid synthetase, FAS) of large yellow croaker.

MATERIALS AND METHODS

Fish and diets

Large yellow croakers, weighing 150 g in average and about 1 year old, were purchased from a private fish farm (Xiangshan Port, Zhejiang Province, China). The dietary experiment was performed at the fish farm between August and November in 2007. Collected from a single net-cage, 1600 fish were placed in 8 cages of 27 m^3 (3 m×3 m×3 m) in size and divided randomly into 4 groups (two replicates each group). Fish were held under optimal conditions and fed with the control diet (0% CLA) for 2 weeks before the beginning of the experiment. After the adaptation period, duplicated groups of fish for each treatment were fed by hand ad libitum twice a day (5:00 a.m. and 17:00 p.m.) for 10 weeks. Feed intake was monitored throughout the trial and waste feed was collected from the effluent water from each cage by a wire mesh. Dead fish were collected daily. During the experimental period, water temperature and salinity were 22~29 °C and 38 g/L, respectively.

Four diets (pellets with 6 mm diameter) containing 0%, 1%, 2% and 4% of CLA were prepared at the Tech-Bank Co., Ltd. (Ningbo, China). Fish oil was used either alone or in combination with CLA (FFA80, containing 80% (w/w) CLA free fatty acid in a 50:50 mixture of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers; Auhai Biotech Co., Ltd., Qingdao, China). The ingredients and proximate compositions of the experimental diets are given in Table 1, and the fatty acid compositions of the experimental diets in Table 2.

Table 1 Ingredient (percentage of dry ingredients) and proximate compositions (percentage of total diet) of experimental diets with different CLA incorporation levels (0%, 1%, 2% or 4%)

CLA level (%)	Ingredients (%)								Proximate composition (%)			
	Fish meal	Soybean meal	Blood meal	Yeast	Flour	Fish oil	CLA	Others[1]	Moisture	Lipid	Protein	Ash
0	46	7	4	4	26.3	9.20	0	3.5	7.96±0.07^{a}	9.28±0.15^{a}	39.30±0.35^{a}	11.20±0.01
1	46	7	4	4	26.3	7.95	1.25	3.5	8.14±0.02^{b}	9.66±0.39^{a}	38.61±0.37^{ab}	11.19±0.03
2	46	7	4	4	26.3	6.70	2.50	3.5	8.06±0.08^{b}	9.97±0.28^{ab}	38.52±0.20^{ab}	11.13±0.03
4	46	7	4	4	26.3	4.20	5.00	3.5	8.02±0.06^{b}	10.40±0.05^{b}	38.13±0.10^{b}	11.15±0.12

Data for proximate composition are mean±*SD* (n=3). Different superscript letters in the same column denote significant differences (P<0.05) between diets as determined by one-way analysis of variance (ANOVA) with Tukey's post-test; [1]Others include essential vitamins and minerals

Table 2 Main fatty acids and CLA isomers (percentage of total fatty acids) and total CLA (percentage of total fatty acids) of diets with different CLA incorporation levels (0%, 1%, 2% or 4%)

Fatty acids	Content (%)			
	0% CLA	1% CLA	2% CLA	4% CLA
C14:0	1.08±0.05[a]	1.09±0.04[a]	0.95±0.02[b]	0.59±0.01[c]
C16:0	18.48±0.27[a]	17.58±0.08[b]	16.44±0.16[c]	14.57±0.07[d]
C18:0	9.64±0.19[a]	9.37±0.03[ab]	9.24±0.11[b]	9.09±0.03[b]
Other saturated	2.67±0.06[a]	2.65±0.15[a]	2.50±0.04[a]	2.19±0.07[b]
∑saturated[1]	31.87±0.08[a]	30.69±0.13[b]	29.14±0.08[c]	26.44±0.04[d]
C16:1 *n*-7	2.43±0.32[a]	212±0.06[a]	2.09±0.02[a]	1.58±0.04[b]
C18:1 *n*-9	22.28±0.16	21.42±0.03	21.09±0.07	18.62±0.06
C22:1 *n*-9	0.78±0.09[a]	0.55±0.01[b]	0.48±0.03[b]	0.43±0.02[b]
Other monounsaturated	1.30±0.02[a]	1.62±0.03[b]	1.57±0.08[b]	1.30±0.05[a]
∑monounsaturated[2]	26.78±0.37[a]	25.72±0.07[b]	25.24±0.01[b]	21.92±0.12[c]
C18:2 *n*-6	3.48±0.04	3.42±0.08	3.45±0.01	3.45±0.02
C18:2 *cis*-9, *trans*-11	0[a]	2.42±0.03[b]	4.87±0.03[c]	9.73±0.02[d]
C18:2 *trans*-10, *cis*-12	0[a]	2.42±0.03[b]	4.89±0.02[c]	9.67±0.02[d]
C20:4 *n*-6	1.03±0.01[a]	1.00±0.05[a]	0.85±0.01[b]	0.82±0.02[b]
C20:5 *n*-3	6.87±0.10[a]	6.41±0.06[b]	5.74±0.07[c]	5.14±0.09[d]
C22:3 *n*-6	0.57±0.09[a]	0.59±0.10[a]	0.45±0.01[ab]	0.33±0.07[b]
C22:5 *n*-3	2.12±0.10	2.00±0.02	1.85±0.05	1.48±0.08
C22:6 *n*-3	14.70±0.32[a]	13.14±0.16[b]	11.66±0.02[c]	9.77±0.21[d]
Other polyunsaturated	10.58±0.04[a]	10.19±0.03[b]	10.09±0.13[b]	9.72±0.04[c]
∑polyunsaturated[3]	41.35±0.45[a]	43.59±0.14[b]	45.73±0.25[c]	51.64±0.16[d]
Total CLA[4]	0[a]	4.84±0.04[b]	9.77±0.03[c]	19.40±0.03[d]

The values are mean±*SD* (*n*=3). Different superscript letters in the same row denote significant differences ($P<0.05$) between diets as determined by one-way analysis of variance (ANOVA) with Tukey's post-test; [1]Saturated: C14:0, C15:0, C16:0, C17:0, C18:0, C20:0; [2]Monounsaturated: C15:1 *n*-7, C16:1 *n*-7, C17:1 *n*-9, C18:1 *n*-9, C20:1 *n*-9, C22:1 *n*-9, C24:1 *n*-9; [3]Polyunsaturated: C16:2 *n*-4, C20:2 *n*-6, C16:2 *n*-6, C18:1 *n*-6, C18:2 *n*-6, C18:2 *cis*-9, *trans*-11, C18:2 *trans*-10, *cis*-12, C20:4 *n*-6, C22:3 *n*-6, C16:3 *n*-3, C18:3 *n*-3, C18:4 *n*-3, C20:5 *n*-3, C22:5 *n*-3, C22:6 *n*-3; [4]Sum of CLA isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12

Sampling protocols

At the start and end of the trial, the fish in each tank were anaesthetized with 50 mg/L metacaine, individually weighed and measured. At the end of the trial, prior to sampling, fish fasted for 24 h and then were anaesthetized with 50 mg/L metacaine. Thirty-two fish per dietary treatment (16 fish per tank) were randomly captured from each replicate, and 6 fish (3 fish per tank) were frozen immediately on dry ice and subsequently stored at −20 °C for whole proximate body composition analysis. The remaining sampled fish were eviscerated and used for biometric determinations [hepato- and viscera-somatic indices (HSI and VSI)] and for the determination of liver lipid. Each fish was filleted from the left side after discarding the skin. Flesh and liver samples were taken from each fish, pooled in three samples of six fish each, then frozen immediately in liquid nitrogen (the livers) or dye ice (the flesh) and used for the determination of fatty acid compositions. All samples were subsequently stored at −20 °C prior to analyses.

Proximate composition and lipid analyses

Moisture content of whole fish was determined after drying in an oven at 80 °C for a minimum 72 h. The dried fish samples were then rigorously blended into a homogeneous crumble/meal and used for determination of whole body lipid, protein and ash contents. Lipid content in 1 g samples of dried fish crumb was determined using the Soxhlet method with extraction in petroleum ether at 120 °C (Avanti Soxtee 2050 Auto Extraction apparatus; Foss, Warrington, UK). Protein content ($N\times6.25$) was determined in the fish crumble using the automated Kjeldahl method (Tecator Kjeldahl Auto 1030 Analyzer; Foss, Warrington, UK). Ash content was determined after heating portions of the fish crumble at 160 °C for 48 h.

Total lipid was extracted from 1 g portions of the liver by homogenizing in 20 volumes of chloroform/methanol (2:1, v/v) in an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, UK). Total lipid was prepared according to the method of

Folch *et al*.(1957) and non-lipid impurities were removed by washing with 0.88% (w/v) KCl. The weight of lipid was determined gravimetrically after evaporation of solvent and overnight desiccation in vacuo.

Fatty acid analysis

Total lipids were extracted with chloroform/methanol (2:1, v/v) (Folch *et al*., 1957; Bligh and Dyer, 1959). Fatty acids were methylated with boron trifluoride (BF_3) in methanol (Kitts *et al*., 2004). The fatty acid methyl esters (FAME) were analyzed by gas chromatograph (Agilent 6890, USA) equipped with flame ionization detector and an auto injector (Agilent 7683, USA). Samples were injected into a capillary column (30 m×0.25 mm×0.25 μm, supplied by Supelco, Belleforte, PA), with helium (99.999%) as the carrier gas at 1.0 ml/min. The column temperature was initially set at 70 °C for 5 min, then increased to 270 °C at 8 °C/min and finally was kept at 270 °C for 5 min. Chromatographic peaks were integrated and identified using the software package, which were compared with known fatty acid methyl esters in the Omega Test standard supplied by Supelco (USA). Individual fatty acid is reported as weight percent of total fatty acids using mass response factors. The identification of fatty acid methyl esters was performed by external standards (all purchased from Sigma Chemical Co., USA) submitted to the same processes of manipulation as the biological samples analyzed. The values of fatty acids are presented as area percentage of total fatty acids.

Enzyme assays

For the enzyme assays, liver samples were homogenized in three volumes of ice-cold buffer [0.02 mol/L Tris-HCl, 0.25 mol/L sucrose, 2 mmol/L ethylene diamine tetraacetic acid (EDTA), 0.1 mol/L sodium fluoride, 0.5 mmol/L phenyl methyl sulphonyl fluoride, 0.01 mol/L β-mercaptoethanol, pH 7.4] and centrifuged at 30000×*g* at 4 °C for 20 min. Soluble protein content of the liver was determined on supernatant by the method of Bradford (1976) using bovine serum albumin (BSA) as standard. Selected lipogenic enzyme activities were assayed on the supernatant using spectrophotometric procedures: G6PD (EC 1.1.1.49) according to Bautista *et al*.(1988), ME (EC 1.1.1.40) according to Ochoa (1955) and FAS (EC 2.3.1.38) according to the methodology of Chang *et al*.(1967) as modified by Chakrabarty and Leveille (1969). One unit (U) of enzyme activity, defined as mmoles of substrate converted to product per min at assay temperature, expressed 1 mg of hepatic soluble protein specific activity.

Statistical analysis

The data were performed using the SPSS 15.0 for Windows statistical software package. Data were presented as mean±*SD*. The effects of dietary treatment were determined by one-way analysis of variance (ANOVA) with Tukey's post-test to determine significance of differences of means between groups. Differences were regarded as significant when $P<0.05$.

RESULTS

Growth and biometry

Weight gain, feed conversion ratio (FCR), specific growth rate (SGR), and condition factor (*K*) were not significantly affected by dietary CLA (Table 3). HSI and VSI were both slightly increased in fish fed with high inclusion of CLA ($P>0.05$).

Table 3 Effects of dietary CLA incorporation levels (0%, 1%, 2% and 4%) on growth and biometric parameters in large yellow croaker for 10 weeks

CLA level (%)	Initial weight (g)	Final weight (g)	*FCR*	*SGR* (%/d)	*K* (g/cm^3)	*HSI* (%)	*VSI* (%)
0	146.25±10.62	282.00±16.65	0.74	2.75	1.59±0.07	1.30±0.07	3.39±0.17
1	146.75±11.04	283.75±11.57	0.73	2.76	1.62±0.07	1.31±0.05	3.40±0.18
2	150.75±8.16	288.50±12.68	0.73	2.73	1.61±0.05	1.32±0.07	3.42±0.17
4	150.75±9.63	288.25±7.30	0.73	2.73	1.61±0.07	1.34±0.05	3.41±0.15

The values are mean±*SD* (n=20). Absence of superscript letters indicates no significant differences between treatments ($P>0.05$) as determined by one-way analysis of variance (ANOVA) with Tukey's post-test; Feed conversion ratio (*FCR*)=feed consumed (kg)/weight gain (kg); Specific growth rate (*SGR*)=[final weight (g)/initial weight (g)]×100%/days; Condition factor (*K*)=wet weight (g)×100/length^3 (cm^3); Hepato-somatic index (*HSI*)=liver weight (g)×100%/body weight (g); Viscera-somatic index (*VSI*)=viscera weight (g)×100%/body weight (g)

Whole body composition and lipid content

With an increasing of CLA concentration, protein, moisture, ash and lipid content in whole body increased gradually, but the difference was not significant between groups. The similar result was observed in liver lipid content (Table 4). Dietary CLA had no significant effects on whole body proximate composition and liver lipid content, although there was a trend of increasing lipid content in fish fed with high CLA.

Activities of lipogenic enzymes

The results of lipogenic activity in the liver of large yellow croaker during the experiment are presented in Table 5. G6PD evidenced a significantly higher (over 20-fold) activity than ME and FAS. This result confirms that G6PD is the main NADPH-generating enzyme. Although the levels of of lipogenic enzymes decreased slightly in CLA-fed fish, their activities were not significantly affected by dietary CLA (P>0.05).

Fatty acid profile in muscle and the liver

The fatty acid compositions in muscle (Table 6) and the liver (Table 7) reflected the fatty acid compositions of the diets. Dietary CLA had significantly effects on SFA, MUFA and PUFA in both muscle and the liver. MUFA decreased significantly, whereas SFA and PUFA showed a significant increase. The total accumulation of CLA was higher in the liver (3.83%, w/w) than in the flesh (3.77%, w/w) in fish fed with 4% CLA. Dietary CLA resulted in the deposition of the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers in both muscle and the liver. CLA supplementation also resulted in a significant increase of C16:0 and C18:0, whereas a significant decrease of C16:1 *n*-7 and C18:1 *n*-9 was observed in fish fed with 4% CLA. Concerning the PUFA fraction, a gradual increasing of C18:2 *n*-6 (LA), C20:5 *n*-3 (eicosapentaenoic acid, EPA) and C22:6 *n*-3 (docosahexaenoic acid, DHA) was observed in both muscle and the liver of fish fed with high CLA.

DISCUSSION

The primary aim of the present study was to determine if dietary CLA has any important effects on growth parameters and lipid metabolism. We found that increasing dietary CLA levels (0% to 4%) did not significantly affect large yellow croaker growth parameters. These results agree with previously studies in some fish species. In a recent study, CLA had no effect on growth performance or feed conversion

Table 4 Effect of dietary CLA on proximate composition (percentage) and liver lipid content (percentage of wet tissue weight) of large yellow croaker

CLA level (%)	Proximate composition in whole body (%)				Liver lipid content (%)
	Moisture	Lipid	Protein	Ash	
0	12.14±0.11	4.34±0.06	68.74±0.68	5.77±0.10	2.94±0.06
1	12.16±0.13	4.36±0.07	69.01±0.88	5.78±0.10	2.98±0.04
2	12.16±0.18	4.39±0.07	69.74±0.83	5.81±0.13	3.03±0.07
4	12.17±0.15	4.42±0.09	69.94±1.00	5.81±0.10	3.05±0.10

The values are mean±*SD* (n=6). Absence of superscript letters indicates no significant differences between treatments (P>0.05) as determined by one-way analysis of variance (ANOVA) with Tukey's post-test

Table 5 Effects of dietary CLA incorporations levels (0%, 1%, 2%, or 4%) in hepatic lipogenic enzymes activities

CLA level (%)	ME (U/mg protein)	G6PD (U/mg protein)	FAS (U/mg protein)
0	17.75±0.82	475.52±37.57	46.15±2.27
1	17.13±1.28	454.28±42.32	44.73±2.60
2	16.70±0.33	428.47±36.21	42.66±3.85
4	16.33±0.45	407.63±44.91	41.52±1.86

The values are mean±*SD* (n=6). Absence of superscript letters indicates no significant differences between treatments (P>0.05) as determined by one-way analysis of variance (ANOVA) with Tukey's post-test; ME: Malic enzyme; G6PD: Glucose-6-phosphate dehydrogenase; FAS: Fatty acid synthetase

Table 6 Main fatty acids and CLA isomers (percentage of total fatty acids) and total CLA (percentage of total fatty acids) in muscle of large yellow croaker with different CLA incorporation levels (0%, 1%, 2% or 4%)

Fatty acids	Content (%)			
	0% CLA	1% CLA	2% CLA	4% CLA
C14:0	3.36±0.30	3.44±0.02	3.53±0.01	3.63±0.03
C16:0	29.15±0.18^{a}	29.25±0.03^{a}	29.41±0.06^{ab}	29.60±0.06^{b}
C18:0	4.45±0.11^{a}	4.58±0.03^{ab}	4.64±0.02^{b}	4.81±0.02^{c}
Other saturated	2.08±0.12	2.13±0.06	2.16±0.04	2.21±0.09
∑saturated[1]	39.04±0.14^{a}	39.40±0.04^{b}	39.74±0.04^{c}	40.25±0.08^{d}
C16:1 *n*-7	11.53±0.15^{a}	10.56±0.08^{b}	9.19±0.02^{c}	7.49±0.02^{d}
C18:1 *n*-9	28.05±0.33^{a}	27.21±0.14^{b}	26.29±0.07^{c}	25.10±0.17^{d}
C22:1 *n*-9	0.43±0.01^{a}	0.39±0.02^{a}	0.33±0.02^{b}	0.30±0.02^{b}
Other monounsaturated	3.42±0.06^{a}	3.32±0.08^{a}	3.03±0.13^{b}	2.90±0.09^{b}
∑monounsaturated[2]	43.42±0.14^{a}	41.49±0.07^{b}	38.84±0.22^{c}	35.79±0.15^{d}
C18:2 *n*-6	0.29±0.04^{a}	0.33±0.03^{ab}	0.37±0.02^{bc}	0.41±0.02^{c}
C18:2 *cis*-9, *trans*-11	0.04±0.01^{a}	0.56±0.02^{b}	0.99±0.02^{c}	1.89±0.02^{d}
C18:2 *trans*-10, *cis*-12	0.02±0.01^{a}	0.51±0.02^{b}	0.97±0.02^{c}	1.87±0.02^{d}
C20:4 *n*-6	0.73±0.06^{a}	0.79±0.08^{a}	0.90±0.07^{ab}	1.08±0.02^{b}
C20:5 *n*-3	5.00±0.02^{a}	5.09±0.03^{a}	5.22±0.15^{ab}	5.45±0.08^{b}
C22:3 *n*-6	0.26±0.04	0.28±0.02	0.30±0.01	0.31±0.02
C22:5 *n*-3	1.24±0.07^{a}	1.30±0.04^{ab}	1.38±0.05^{b}	1.43±0.01^{b}
C22:6 *n*-3	6.11±0.13^{a}	6.28±0.08^{a}	7.21±0.06^{b}	7.34±0.05^{b}
Other polyunsaturated	3.86±0.11	3.97±0.09	4.08±0.09	4.18±0.19
∑polyunsaturated[3]	17.54±0.19^{a}	19.11±0.03^{b}	21.42±0.23^{c}	23.96±0.23^{d}
Total CLA[4]	0.06±0.01^{a}	1.08±0.01^{b}	1.96±0.02^{c}	3.77±0.03^{d}

The values are mean±*SD* (*n*=3). Different superscript letters in the same row denote significant differences ($P<0.05$) between diets as determined by one-way analysis of variance (ANOVA) with Tukey's post-test; [1]Saturated: C14:0, C15:0, C16:0, C17:0, C18:0, C20:0; [2]Monounsaturated: C15:1 *n*-7, C16:1 *n*-7, C17:1 *n*-9, C18:1 *n*-9, C20:1 *n*-9, C22:1 *n*-9, C24:1 *n*-9; [3]Polyunsaturated: C16:2 *n*-4, C20:2 *n*-6, C16:2 *n*-6, C18:1 *n*-6, C18:2 *n*-6, C18:2 *cis*-9, *trans*-11, C18:2 *trans*-10, *cis*-12, C20:4 *n*-6, C22:3 *n*-6, C16:3 *n*-3, C18:3 *n*-3, C18:4 *n*-3, C20:5 *n*-3, C22:5 *n*-3, C22:6 *n*-3; [4]Sum of CLA isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12

Table 7 Main fatty acids and CLA isomer (percentage of total fatty acids) and total CLA (percentage of total fatty acids) in the liver of large yellow croaker with different CLA incorporation levels (0%, 1%, 2% or 4%)

Fatty acids	Content (%)			
	0% CLA	1% CLA	2% CLA	4% CLA
C14:0	6.59±0.03^{a}	6.61±0.02^{ab}	6.64±0.01^{bc}	6.68±0.01^{c}
C16:0	28.76±0.09^{a}	29.02±0.05^{b}	29.10±0.04^{b}	29.17±0.06^{b}
C18:0	5.23±0.03^{a}	5.26±0.04^{a}	5.32±0.04^{ab}	5.41±0.03^{b}
Other saturated	2.91±0.01	2.91±0.04	2.94±0.04	2.93±0.07
∑saturated[1]	43.48±0.03^{a}	43.79±0.08^{b}	44.00±0.03^{c}	44.20±0.03^{d}
C16:1 *n*-7	13.01±0.06^{a}	12.14±0.03^{b}	11.87±0.03^{c}	11.16±0.08^{d}
C18:1 *n*-9	24.03±0.08^{a}	22.84±0.08^{b}	22.22±0.11^{c}	21.09±0.03^{d}
C22:1 *n*-9	0.52±0.03^{a}	0.45±0.03^{b}	0.43±0.02^{b}	0.41±0.01^{b}
Other monounsaturated	1.60±0.02^{a}	1.55±0.07^{a}	1.31±0.04^{b}	1.09±0.06^{c}
∑monounsaturated[2]	39.16±0.14^{a}	36.98±0.02^{b}	35.82±0.09^{c}	33.75±0.15^{d}
C18:2 *n*-6	0.22±0.03^{a}	0.24±0.02^{ab}	0.25±0.01^{ab}	0.29±0.03^{b}
C18:2 *cis*-9, *trans*-11	0.05±0.02^{a}	0.68±0.02^{b}	1.05±0.03^{c}	1.93±0.01^{d}
C18:2 *trans*-10, *cis*-12	0.04±0.01^{a}	0.65±0.02^{b}	1.05±0.03^{c}	1.90±0.02^{d}
C20:4 *n*-6	0.31±0.07^{a}	0.41±0.02^{ab}	0.45±0.01^{b}	0.46±0.00^{b}
C20:5 *n*-3	4.18±0.06^{a}	4.31±0.06^{ab}	4.32±0.04^{ab}	4.49±0.11^{b}
C22:3 *n*-6	0.21±0.02^{a}	0.22±0.02^{ab}	0.24±0.01^{bc}	0.27±0.01^{c}
C22:5 *n*-3	1.00±0.02	1.13±0.07	1.13±0.07	1.14±0.01
C22:6 *n*-3	7.19±0.03^{a}	7.35±0.04^{b}	7.35±0.06^{b}	7.37±0.08^{b}
Other polyunsaturated	4.16±0.13	4.25±0.02	4.34±0.06	4.21±0.12
∑polyunsaturated[3]	17.35±0.15^{a}	19.23±0.07^{b}	20.18±0.07^{c}	22.05±0.13^{d}
Total CLA[4]	0.09±0.01^{a}	1.32±0.02^{b}	2.10±0.01^{c}	3.83±0.02^{d}

The values are mean±*SD* (*n*=3). Different superscript letters in the same row denote significant differences ($P<0.05$) between diets as determined by one-way analysis of variance (ANOVA) with Tukey's post-test; [1]Saturated: C14:0, C15:0, C16:0, C17:0, C18:0, C20:0; [2]Monounsaturated: C15:1 *n*-7, C16:1 *n*-7, C17:1 *n*-9, C18:1 *n*-9, C20:1 *n*-9, C22:1 *n*-9, C24:1 *n*-9; [3]Polyunsaturated: C16:2 *n*-4, C20:2 *n*-6, C16:2 *n*-6, C18:1 *n*-6, C18:2 *n*-6, C18:2 *cis*-9, *trans*-11, C18:2 *trans*-10, *cis*-12, C20:4 *n*-6, C22:3 *n*-6, C16:3 *n*-3, C18:3 *n*-3, C18:4 *n*-3, C20:5 *n*-3, C22:5 *n*-3, C22:6 *n*-3; [4]Sum of CLA isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12

efficiency in juvenile yellow perch (*Perca flavescens*) or catfish (*Ictalurus punctatus*) fed with diets containing up to 1% CLA (Twibell *et al*., 2001; Twibell and Wilson, 2003). Similarly, CLA does not seem to affect growth rate or feed conversion efficiency in Atlantic salmon fed with diets containing up to 4% CLA (Leaver *et al*., 2006). Higher levels of dietary CLA, up to 5% of diet, also had no effect on growth rate or feed conversion efficiency in juvenile tilapia (*Oreochromis niloticus*) (Yasmin *et al*., 2004). Twibell and Wilson (2003) observed that dietary CLA had little potential for improving growth responses in channel catfish. In contrast, Chio *et al*.(1999) reported that weight gain and feed conversion efficiency were reduced in Nile tilapia, rockfish and common carp fed with diets containing 2% CLA.

Dietary CLA has some beneficial effects on body composition in mammals; decreased body fat and increased lean body mass were observed in mice (Ohnuki *et al*., 2001; Terpstra *et al*., 2002), rats (Yamasaki *et al*., 2003) and pigs (Thiel-Cooper *et al*., 2001; Tischendorf *et al*., 2002). CLA also decreased whole body triacylglycerol (TAG) accumulation in hamsters (Bouthegourd *et al*., 2002) and reduced liver TAG levels in rats (Rahman *et al*., 2002). However, we did not observe the similar effects in large yellow croaker in the present trail, which agrees with the redults from a few previous studies, such as the findings in salmon fry (Berge *et al*., 2004), rainbow trout juveniles (Figueiredo-Silva *et al*., 2005), and smolts (Kennedy *et al*., 2005). The inconsistence of results might be caused by the differences of animal species and feeding environments.

No difference was observed in total lipid level in muscle and the liver of fish fed with the four diets. Fat content (w/w) of muscle ranged between 4.34% and 4.42%, whereas in the liver the minimum and maximum levels were 2.94% and 3.05%, respectively. HSI was not significantly increased by dietary CLA in the present trial. The increased HSI was associated with increased lipid content in the liver. In an earlier research, CLA supplementation did not affect total lipid in the liver and muscle of rainbow trout juveniles (Bandarra *et al*., 2006). In contrast, Kennedy *et al*.(2005) reported that dietary CLA showed a clear trend of increase of total lipid and TAG contents in both the liver and the flesh of Atlantic salmon, particularly fed with high oil diets, and Twibell *et al*.(2000; 2001) and Yasmin *et al*.(2004) reported that in yellow perch and tilapia, increased HSI was not associated with increased lipid content and, indeed, liver lipid content was reduced by CLA in striped bass. Protein content in muscle varied between 68.74% and 69.94%. There were no significant differences between the control and any other treatment. This observation is in agreement with previous studies in Atlantic salmon (Berge *et al*., 2004) and channel catfish juveniles (Twibell and Wilson, 2003), where protein level in muscle was not significantly affected by dietary CLA. The present results correspond well with earlier observations in rainbow trout that reported an absence of differences in whole body composition among fish fed with increasing levels of CLA (Figueiredo-Silva *et al*., 2005).

In the present work, large yellow croaker fed with various dietary levels of CLA did not display any significant changes in the activities of the analyzed lipogenic enzymes, which corresponds well with the lack of differences in fat gain or body composition. Dias *et al*.(1998) reported that G6PD had higher activity (over 25-fold) than ME in juvenile European sea bass, and our result confirms that G6PD is the main NADPH generating enzyme in the species. Dietary CLA seemed to slightly depress both ME and G6PD activities, whereas liver lipid content tended to increase in large yellow croaker fed with high CLA. The similar results were evidenced in rainbow trout (Valente *et al*., 2007a) and sea bass (Valente *et al*., 2007b), in which the malic enzyme activity decreased with CLA supplementation. Other studies showed a clear increase in both mRNA expression and activities of various enzymes involved in lipogenesis (including ME, G6PD and FAS) and fatty acid oxidation in mice liver (Takahashi *et al*., 2003; Ide, 2005). In rats, the ingestion of a CLA mixture increased ME liver activity, whereas both G6PD and FAS activities remained unaffected (Faulconnier *et al*., 2004). In the present study, ME and G6PD activities were negatively correlated with PUFA and total CLA contents in the liver. Although regulation of fatty acid synthesis and lipogenic enzyme expression has been unexplored, the relative amount of polyunsaturated fatty acids has been shown to directly modulate the expression of lipogenic enzymes in rats (Stabile *et al*., 1998) and rainbow trout (Alvarez *et al*., 2000). These various results suggest that the effect of CLA on he-

patic lipogenic enzymes is dependent on the species and may possibly be related to the various nutritional and physiological conditions.

In large yellow croaker, the total percentages of the main group of fatty acids in the liver and muscle were affected by dietary inclusion of CLA. Dietary CLA had significantly increased SFA and decreased MUFA in both muscle and the liver. These results corroborate previous studies in hybrid striped bass (Twibell *et al*., 2000), yellow perch (Twibell *et al*., 2001), Atlantic salmon (Berge *et al*., 2004; Kennedy *et al*., 2005), rainbow trout (Bandarra *et al*., 2006; Valente *et al*., 2007a; 2007b), and several mammal species (Bretillon *et al*., 1999; Bee, 2000; Lauridsen *et al*., 2005). In the liver and muscle, both C16:0 and C18:0 were responsible for increasing the saturated fraction in fish fed with CLA. Concomitantly to the increase in the saturated fraction, a general decrease of the MUFA (C16:1 *n*-7 and C18:1 *n*-9) with CLA supplementation was evidenced. The results are related to the inhibition of stearoyl coenzyme A (CoA) desaturase (SCD) activity. The dietary incorporation of CLA led to a increasing of PUFA content in muscle and the liver due mainly to the higher deposition of these conjugated fatty acids in the lipid fraction. Moreover, increases of DHA and EPA were observed in fish fed with higher CLA (2% and 4%) compared with the control group. However, the present results differ from the findings in hybrid striped bass (Twibell *et al*., 2000) that showed a decrease of DHA in muscle. In the liver and muscle, most of PUFA, particularly *n*-3 PUFA, were affected by dietary CLA supplementation. Berge *et al*.(2004) reported that the dietary incorporation of CLA clearly affected heaptic lipid metabolism contributing to a more efficient fatty acids with a prominent biosynthesis and deposition of long-chain *n*-3 fatty acids. Pereira *et al*.(2003) pointed out that this elevation might be a result of higher Δ5 and Δ6 fatty acyl desaturase activity in this species.

In conclusion, in the present study we found that CLA had no effect on growth parameters and feed conversion efficiency, but had a trend of increase of total lipid contents in both the liver and the flesh in CLA-fed large yellow croaker. Moreover, the fish were capable of absorbing and depositing CLA and long-chain *n*-3 PUFA in the liver and muscle, showing that this species fed with CLA could be an important human food source for these healthful fatty acids.

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