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Conjugated linoleic acid in diets for lambari (*Astyanax altiparanae*) (Garutti & Britski, 2000)

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Abstract

This study aimed to evaluate the effect of different levels of conjugated linoleic acid (CLA) on growth performance, carcass chemical composition and fatty acid profile of lambari (*Astyanax altiparanae*). A completely randomized experimental design with six treatments, diets with graded levels of CLA (0.0, 5.0, 10.0, 15.0, 20.0 and 25.0 g kg^{-1}) and five repetitions. Fish (n = 570; weight: 1.58 ± 0.23 g) were distributed into 30 (70-L) aquaria and fed the experimental diets during 90 days. No effects of dietary supplementation with CLA on fish performance and carcass chemical composition were observed. CLA influenced carcass fatty acid profile, a positive relationship was found for 16:1 n-9, 18:1 n-9, 18:2(cis-9, trans-11), 18:2(trans-10, cis-12) and 20:1 n-9, and a negative relationship was found for 15:1 n-7, 16:0, 16:1 n-5, 18:2 n-6, 18:3 n-3, 20:5 n-3 and 22:4 n-6. Total CLA and monounsaturated fatty acids (MUFA) linearly increased with the increase in dietary CLA, while saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) of n-6 and n-3 series linearly decreased. Dietary CLA can be incorporated into the lambari (*A. altiparanae*) muscle, and the fish can be used as functional foods, because CLA is related to the prevention of various diseases in humans.

KEY WORDS: *Astyanax altiparanae*, carcass chemical composition, conjugated linoleic acid, fatty acid profile, fish diets, functional foods

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Introduction

Lambari, *Astyanax altiparanae*, is a freshwater, omnivorous and native species in South America. The specie distinguished by short production cycle, good acceptance of processed diets, high reproductive rate and fry production without hormonal induction (Gonçalves *et al.* 2014). It is distributed from Brazilian Northeast to the La Plata Basin (Vilela & Hayashi 2001), located between Brazil, Paraguay, Argentina, Bolivia and Uruguay. In Brazil, the improvement in lambari (*A. altiparanae*) production has important economic implications to the commercial market (Porto-Foresti *et al.* 2010). This species is quite popular as snack and as bait for sport fishing; moreover, it has potential to be marketed canned or as raw material for manufacturing of fishmeal (Porto-Foresti *et al.* 2010). The small size of the lambari (*A. altiparanae*) allows its use as an experimental model for other large-sized species (Gonçalves *et al.* 2014).

Conjugated linoleic acid (CLA) is a term used to designate a mixture of geometric isomers of linoleic acid (18:2 n-6), where the double bonds are separated by a carbon–carbon single bond instead of a methylene group. Among the isomers of CLA, cis-9, trans-11 and trans-10, cis-12 have biological activity (Pariza *et al.* 2001), whereas cis-9, trans-11 is considered the primary form of CLA, found naturally in some foods (Chin *et al.* 1992) including ruminant meats, cheese, butter and milk (Pariza *et al.* 2001). Like functional foods, CLA is getting momentum in inhibiting some diseases such as cancer, atherosclerosis and diabetes in humans (Park & Pariza 2007; Benjamin & Spener 2009). CLA also reduces inflammatory, improves immune responses (Song *et al.* 2005; Bhattacharya *et al.* 2006), prevents cardiovascular diseases (Park & Pariza 2009), provides improvements in asthmatics patients (MacRedmond

et al. 2010) and reduces rheumatoid arthritis (Aryaeian *et al*. 2009).

Besides the effects as a functional food, CLA can also reduce whole-body lipids in yellow catfish (*Pelteobagrus fulvidraco*; Tan *et al*. 2010), increase whole-body protein in Nile tilapia (*Oreochromis niloticus*; Santos *et al*. 2011) and in Atlantic salmon (*Salmo salar*; Leaver *et al*. 2006) and improve feed efficiency in rockfish (*Sebastes schlegeli*; Twibell *et al*. 2000). However, dietary CLA supplementation has shown no effect on growth performance in rainbow trout (*Oncorhynchus mykiss*; Bandarra *et al*. 2006), Atlantic salmon (*S. salar*; Kennedy *et al*. 2005) and Nile tilapia (*O. niloticus*; Yasmin *et al*. 2004) or negative effects on growth performance in yellow catfish (*P. fulvidraco*; Tan *et al*. 2010). On the other hand, CLA shows a positive effect on growth and feed utilization in channel catfish (*Ictalurus punctatus*) after 3 weeks (Peterson *et al*. 2003). Therefore, CLA effects can vary among species, probably due to different physiological responses between animals to its inclusion in the diet (Tan *et al*. 2010). These physiological responses are related to the animal's ability to metabolize fatty acids in the liver and its use as energy source or essential fatty acids (Twibell *et al*. 2001).

This study was conducted to evaluate the effects of different inclusion levels of CLA in the diet for lambari (*A. altiparanae*) through the growth performance, chemical composition and fatty acid profile of fish carcass.

Materials and methods

This experiment was approved by the Ethics Committee of Department of Animal Science, UFV (Protocol n° 19/2011).

Experimental diets

All experimental diets were formulated to be isonitrogenous (320 g kg^{-1}) and isoenergetic (15.7 MJ kg^{-1}) with increasing levels of CLA (0.0, 5.0, 10.0, 15.0, 20.0 and 25.0 g kg^{-1} of diet). Commercial CLA (LUTA-CLA® 60-BASF, São Paulo, SP, Brazil), containing 600 g kg^{-1} CLA methyl esters as a 50: 50 mixture of cis-9, trans-11 and trans-10, cis-12 isomers, was used. Diets were grounded in hammer mill (0.8-mm sieve), manually mixed and pelleted in electric meat grinder in a 1-mm die, dried in a forced air oven at 50 °C for 24 h, crushed in a manual mill and manually passed through granulometric sieves (Tecnal, Piracicaba, SP, Brazil) to obtain pellets of 0.5–1.5 mm. The proximate composition and fatty acid analyses of the diets are shown in Tables 1 and 2, respectively.

Table 1 Formulations and proximate compositions of the experimental diets

	Levels of CLA in experimental diets (g kg^{-1})					
Ingredient	0.0	5.0	10.0	15.0	20.0	25.0
Soybean meal	400.0	400.0	400.0	400.0	400.0	400.0
Cottonseed meal	100.0	100.0	100.0	100.0	100.0	100.0
Corn gluten meal	127.0	127.0	127.0	127.0	127.0	127.0
Corn meal	80.5	80.5	80.5	80.5	80.5	80.5
Wheat bran	100.0	100.0	100.0	100.0	100.0	100.0
Rice meal	90.0	90.0	90.0	90.0	90.0	90.0
Soybean oil	50.0	41.0	32.0	23.0	14.0	5.0
CLA[1]	–	9.0	18.0	27.0	36.0	45.0
L-lysine	4.0	4.0	4.0	4.0	4.0	4.0
DL-methionine	2.0	2.0	2.0	2.0	2.0	2.0
Dicalcium phosphate	35.0	35.0	35.0	35.0	35.0	35.0
Mineral and vitamin mix[2]	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin C	1.0	1.0	1.0	1.0	1.0	1.0
Salt	5.0	5.0	5.0	5.0	5.0	5.0
BHT[3]	0.2	0.2	0.2	0.2	0.2	0.2
Dry matter (g kg^{-1})[4]	940.0	939.0	948.0	945.0	937.0	935.0
Crude Protein (g kg^{-1})[4]	330.0	327.0	335.0	334.0	328.0	319.0
Crude lipid (g kg^{-1})[4]	90.0	89.0	91.0	95.0	92.0	94.0
Ash (g kg^{-1})[4]	94.0	95.0	100.0	95.0	100.0	102.0
Gross energy (Mj kg^{-1})[4]	15.65	15.79	15.12	15.42	16.14	15.85

Soybean meal, corn meal, corn gluten meal, (Cargill Inc., Brazil), cottonseed meal (Maeda S.A., Brazil), wheat bran (Vilma Ailmentos, Brazil), rice meal (Rozcampo, Brazil), dicalcium phosphate (Serrana S.A., Brazil), salt (National Refinery S.A., Brazil), Soybean oil (ADM Ltda, Brazil), DL-methionine (Evonik Ind., Brazil), L-lysine (Ajinomoto Ind. and Com. Ltda, Brazil) and Vitamin C (Saint Charbel Farm., Brazil).

[1] Conjugated linoleic acid (CLA) oil (LUTA-CLA 60®, BASF, São Paulo, SP, Brazil, containing 600 g kg^{-1} CLA).

[2] Assurance levels per kilogram of product: Vit. A, 1 200 000UI; Vit. D3; 200 000UI; Vit. E, 12 000 mg; Vit. K3, 2400 mg; Vit. B1, 4800 mg; Vit. B2, 4800 mg; Vit. B6, 4000 mg; Vit. B12, 4800 mg; Ac. Folic, 1200 mg; calcium pantothenate, 12 000 mg; Vit. C, 48 000 mg; biotin, 48 mg; cholin, 65 000 mg; niacin, 24 000 mg; Fe, 10 000 mg; Cu, 6000 mg; Mg, 4000 mg; Zn, 6000 mg; I, 20 mg; Co, 2 mg; Se, 20 mg (Guabi Nutrição Animal, Brazil).

[3] Butylated hydroxytoluene (antioxidant) (ISOFAR Ind., Brazil).

[4] Values determined at Laboratory of Animal Science of Federal University of Viçosa – MG, Brazil.

Fish and culture conditions

Fry lambaris (*A. altiparanae*) with 1.58 ± 0.23 g (mean weight ± SD), weighed on a precision scale (model MB45 Toledo ® 0.01 g, Brazil), were randomly distributed into 30

Table 2 Fatty acid composition (mg g^{-1} of total identified fatty acids) of experimental diets

	Levels of conjugated linoleic acid (CLA) in experimental diets (g kg^{-1})					
Fatty acids[1]	0.0	5.0	10.0	15.0	20.0	25.0
14:0	1.4	1.4	1.5	1.6	1	1.5
16:0	141.0	134.3	126.9	120.5	115.4	110.2
18:0	30.3	29.2	30.2	30.9	31.9	31.7
18:1 n-9	280.2	286.9	293.5	299.1	303.5	308.6
18:2 n-6	500.9	451.2	399.4	351.3	295.6	245.3
18:3 n-6	2.5	2.5	2.1	1.7	0.8	0.7
18:3 n-3	43.7	37.7	32.1	26.1	22.4	16.3
18:2 (c9,t11)	0.0	22.6	47.5	69.9	95.3	118.2
18:2 (t10,c12)	0.0	34.2	67.0	99.0	133.8	167.4
SFA	172.6	164.8	158.5	153.0	148.8	143.5
MUFA	280.2	286.9	293.5	299.1	303.5	308.6
PUFA	547.1	548.2	548.1	547.9	547.8	548.0
PUFA/SFA	31.7	33.3	34.6	35.8	36.8	38.2
n-6	503.4	453.7	401.5	352.9	296.4	246.0
n-3	43.7	37.7	32.1	26.1	22.4	16.3
n-6/n-3	115.3	120.3	125.2	135.5	132.1	150.8
Total CLA	0.0	56.8	114.5	168.9	229.0	285.6

[1] Values are given as mg g^{-1} of total fatty acids identified in the sample and determined. Values determined at Cromalimentos Laboratory (State University of Maringá, PR, Brazil). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; PUFA/SFA, polyunsaturated/saturated fatty acids ratio.

aquaria (70 L of water), at a density of 19 fish per aquarium, each aquarium being an experimental unit. Aquaria were maintained in a recirculated system with mechanical and biological filter and UV sterilization lamp. Water temperature was maintained at 27.0 °C during the trial. Dissolved oxygen was maintained at 6.5–7.5 mg L^{-1}, the pH at 6.5–6.8 and ammonia at 0.00–0.02 mg L^{-1}, throughout the experimental period. Water parameters were measured with Multiparameter Meter (model HI 9828, Hanna Instruments, Baureri, SP, Brazil). Photoperiod was adjusted to 12 h by fluorescent lamps (60W).

Fish were fed manually, four times daily until satiation, over a period of 90 days. The amount of feed consumed was recorded at the end of the trial.

At the end of the experiment period, after 1 day of starvation, all of the fish from each aquarium unit were counted and weighed on a precision scale (0.01 g) to evaluate the productive performance: survival rate, weight gain, specific growth rate and feed conversion ratio. After that, they were slaughtered with a lethal dose of anaesthetic (0.1 g benzocaine L^{-1}). It was recorded whole-body, carcass, gonads, viscera and liver weights, to determination of carcass yield, gonadosomatic indices, viscerosomatic indices and hepatosomatic indices. Viscerosomatic index included the weight of the stomach, intestine, pyloric caeca, gonads, heart, liver, gallbladder and swimming bladder. Nine fish from each aquarium were randomly sampled and pooled, being six fish for carcass chemical composition analysis and three for fatty acid analysis. Carcass was considered fish without scales and viscera.

Chemical analysis

To proceed with carcass chemical composition analysis, including dry matter, crude protein, crude lipid, ash and gross energy content, fish were previously lyophilized and ground in a ball mill. Crude protein content was determined according to the methodology described by Silva & Queiros (2002), crude lipid, dry matter and ash according to Association of Official Analytical Chemists (AOAC) (2000), and gross energy obtained in a calorimetry bomb in the Food Analysis Laboratory, Department of Animal Science, Federal University of Viçosa, Brazil.

To determine the fatty acid profile of carcasses, three fish from each aquarium were pooled and ground in a blender, homogenized and subjected to lipid extraction with a mixture of chloroform, methanol and water (2 : 2 : 1.8 v/v), according to the Bligh & Dyer (1959) method. Preparation of methyl esters of fatty acids was performed according to the Hartman & Lago (1973) method. The methyl esters were separated by gas chromatography CP-3380 (Varian, EUA), equipped with a flame ionization detector and a fused silica capillary column, CP – 7420 (Select FAME, with 100 m length, 0.25 mm internal diameter and 0.25 μm

cyanopropyl). The gas fluxes (White Martins) were 1.2 mL min^{-1} for the carrier gas (H_2), 30 mL min^{-1} for the make-up (N_2), and 30 and 300 mL min^{-1} for the H_2 and for the synthetic air flame, respectively. The division ratio of the sample (split) was 1 100^{-1}. The chromatographic conditions adopted for separation of fatty acids were injection point and detector temperatures maintained at 220 and 240 °C, respectively. The initial column temperature was programmed at 165 °C, maintained for 12 min, increased to 165–235 °C at a rate of 5 °C min^{-1} and kept at 235 °C for 9 min. The fatty acid identification was made comparing the relative retention times of the sample peaks and a mixture of FAME standards and methyl esters containing linoleic acid geometric isomers c9,t11 and t10,c12 (189-19 and O5632 Sigma, Saint Louis, MO, USA) and standard spiking along the sample. The injections were given in triplicates with volumes of 2 μL. The software Star 5.0 (Varian, Palo Alto, CA, USA) was employed to determine peak areas, and data were calculated as normalized area of fatty acids in mg g^{-1}. The analyses were performed at State University of Maringá, Brazil.

Experimental design and statistical analysis

A completely randomized design with six treatments (six supplementation levels of CLA in the diet) and five replicates was used. Statistical analysis of data of growth performance, carcass chemical composition and fatty acid profile was submitted to Lilliefors test to verify the assumption of normality of errors and Bartlett test to verify the homogeneity of variances, after this, was performed one-way analysis of variance (ANOVA) at 5% significance. In case of differences, a linear or quadratic (second order) regression analysis was used. To choose the most suitable regression model, the significance of the regression coefficients, the magnitude of the coefficients of determination and the biological response of each variables under study were considered. Statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA).

Results

Growth performance of lambaris (*A. altiparanae*) fed graded levels of dietary CLA is shown in Table 3. Dietary levels of CLA did not significantly affect ($P > 0.05$) survival rate, weight gain, specific growth rate, feed conversion ratio, carcass yield, body indices (viscerosomatic, gonadosomatic and hepatosomatic indices) of fish (Table 3) and chemical composition of carcasses (Table 4).

Dietary CLA levels influenced ($P < 0.05$) carcass fatty acid profiles of fish (Table 5). Carcass content of 16:1 n-9, 18:1 n-9 and 20:1 n-9 showed a linear increase with increasing levels of CLA in the diet ($y = 0.0376x + 2.318$, $R^2 = 0.72$; $y = 1.7506x + 368.82$, $R^2 = 0.77$; $y = 0.2587x + 6.014$, $R^2 = 0.82$, respectively). On the other hand, carcass 18:2 n-6 (linoleic acid), 18:3 n-3-(α-linolenic acid) and 16:0 linearly decreased with increasing levels of dietary CLA ($y = -2.8418x + 235.51$, $R^2 = 0.82$; $y = -0.1568x + 9.452$, $R^2 = 0.86$; $y = -0.9289x + 204.05$, $R^2 = 0.72$, respectively). A quadratic relationship between dietary CLA levels and

Table 3 Growth performance of lambari (*Astyanax altiparanae*) fed diets containing graded levels of conjugated linoleic acid (CLA) for 90 days

	CLA levels (g kg^{-1})					
Growth performance parameters	0.0	5.0	10.0	15.0	20.0	25.0
SR^{ns} (%)	90.0 ± 11.39	95.6 ± 6.09	94.4 ± 3.93	95.6 ± 4.65	95.6 ± 4.65	98.9 ± 2.49
WG^{ns} (g)	2.6 ± 0.66	2.0 ± 0.39	2.0 ± 0.36	2.1 ± 0.28	2.4 ± 0.46	1.9 ± 0.31
SGR^{ns}	0.9 ± 0.19	0.8 ± 0.18	0.8 ± 0.12	0.8 ± 0.08	0.9 ± 0.21	0.8 ± 0.22
FCR^{ns}	2.3 ± 0.97	2.5 ± 0.53	2.4 ± 0.28	2.2 ± 0.05	2.1 ± 0.21	2.4 ± 0.35
CY^{ns} (%)	85.6 ± 1.83	86.0 ± 1.75	85.4 ± 0.56	85.3 ± 1.78	84.4 ± 2.28	86.3 ± 2.28
VSI^{ns} (%)	14.4 ± 1.83	14.0 ± 1.75	14.7 ± 0.56	14.7 ± 1.78	15.6 ± 2.28	13.7 ± 2.28
$GSIm^{ns}$ (%)	4.1 ± 2.25	4.0 ± 0.81	4.4 ± 2.37	4.3 ± 1.30	6.3 ± 3.44	4.1 ± 0.91
$GSIf^{ns}$ (%)	15.3 ± 2.07	15.9 ± 1.69	15.6 ± 1.81	17.7 ± 6.14	18.5 ± 6.12	14.6 ± 9.00
HSI^{ns} (%)	0.4 ± 0.13	0.4 ± 0.10	0.3 ± 0.10	0.3 ± 0.11	0.4 ± 0.12	0.5 ± 0.14

ns, not significant by analysis of variance, *F* test ($P > 0.05$); SR (survival rate), (final number of fish/initial number of fish) × 100; WG (weight gain), final mean biomass − initial mean biomass; SGR (specific growth rate), (ln final weight − ln initial weight) $days^{-1}$) × 100; FCR (feed conversion ratio), dry feed intake/wet weight gain; CY (carcass yield), (eviscerated fish weight/whole fish weight) × 100; VSI (viscerosomatic index), (viscera weight whole-body $weight^{-1}$) × 100; GSIm (gonadosomatic index of males), (gonad weight whole-body $weight^{-1}$) × 100; GSIf (gonadosomatic index of females), (gonad weight whole-body $weight^{-1}$) × 100; HSI (hepatosomatic index), (liver weight whole-body $weight^{-1}$) × 100.
Data are presented as means ± SD ($n = 5$).

Table 4 Carcass proximate composition (g kg^{-1} dry matter) of lambaris (*Astyanax altiparanae*) fed diets containing graded levels of conjugated linoleic acid (CLA) for 90 days

	CLA levels (g kg^{-1})					
Chemical composition[1]	0.0	5.0	10.0	15.0	20.0	25.0
Crude protein[ns]	581.6 ± 8.9	557.4 ± 48.0	578.3 ± 13.6	575.0 ± 9.4	574.4 ± 20.1	572.6 ± 24.1
Crude lipid[ns]	177.9 ± 10.9	184.7 ± 40.7	227.8 ± 26.0	207.7 ± 66.2	183.6 ± 23.5	186.0 ± 28.0
Dry matter[ns]	289.1 ± 10.0	291.1 ± 11.7	296.4 ± 12.9	294.5 ± 9.0	288.3 ± 12.2	293.5 ± 13.8
Ash[ns]	138.3 ± 10.6	140.7 ± 11.6	141.2 ± 6.7	141.7 ± 6.5	140.3 ± 6.5	139.0 ± 10.3
Gross energy (kj g^{-1})[ns]	17853 ± 1619	18595 ± 1926	18124 ± 1567	19945 ± 3691	19082 ± 793	16873 ± 252

ns = not significant by analysis of variance, *F* test ($P > 0.05$).
[1] Determined at Laboratory of Animal Science of Federal University of Viçosa – MG, Brazil. Data are presented as means ± SD ($n = 5$).

Table 5 Carcass fatty acid profile (mg g^{-1} of total identified fatty acids) of lambaris (*Astyanax altiparanae*) fed diets containing graded levels of conjugated linoleic acid (CLA) for 90 days

	CLA levels (g kg^{-1})					
Fatty acids[1]	0.0	5.0	10.0	15.0	20.0	25.0
12:0[ns]	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
14:0[ns]	3.6 ± 0.1	3.9 ± 0.2	3.8 ± 0.1	3.8 ± 0.4	4.0 ± 0.1	4.0 ± 1
15:1n-7	3.1 ± 0.1	2.5 ± 0.2	2.2 ± 0.2	2.1 ± 0.0	2.1 ± 0.0	1.9 ± 0.1
16:0	201.7 ± 3.0	200.5 ± 8.6	197.5 ± 1.09	191.7 ± 1.7	182.1 ± 2.8	181.3 ± 3.4
16:1n-9	2.4 ± 0.1	2.4 ± 0.2	2.6 ± 0.2	3.1 ± 0.1	3.1 ± 0.0	3.2 ± 0.2
16:1n-7[ns]	6.0 ± 0.1	6.0 ± 0.3	6.0 ± 0.5	5.1 ± 0.5	5.3 ± 0.3	5.5 ± 0.3
16:1n-5	4.8 ± 0.0	4.4 ± 0.3	3.8 ± 0.2	3.4 ± 0.3	3.3 ± 0.1	3.3 ± 0.1
17:0[ns]	3.7 ± 0.2	3.6 ± 0.2	3.5 ± 0.3	4.1 ± 0.2	3.9 ± 0.3	3.7 ± 0.1
18:0[ns]	66.3 ± 2.1	74.6 ± 0.6	70.0 ± 4.3	64.3 ± 0.6	70.6 ± 1.6	65.8 ± 0.9
18:1n-9	360.9 ± 0.8	387.6 ± 3.5	386.7 ± 1.2	390.5 ± 3.8	411.5 ± 1.7	407.0 ± 10.0
18:2n-6	245.0 ± 4.3	209.0 ± 15.9	206.2 ± 4.4	198.5 ± 0.9	171.6 ± 5.8	169.5 ± 9.1
18:3n-6[ns]	5.0 ± 0.1	4.7 ± 0.2	5.1 ± 0.5	5.0 ± 0.2	3.8 ± 0.2	3.9 ± 0.1
18:3n-3	9.5 ± 0.4	8.3 ± 0.6	8.1 ± 0.5	7.6 ± 0.3	6.1 ± 0.3	5.4 ± 0.4
18:2(c9,t11)	0.0 ± 0.0	10.1 ± 0.8	14.4 ± 1.4	23.7 ± 0.8	30.6 ± 1.7	35.2 ± 2.8
18:2(t10,c12)	0.0 ± 0.0	7.9 ± 0.6	10.2 ± 0.3	17.3 ± 1.0	23.9 ± 1.5	28.8 ± 2.6
20:1n-9	6.0 ± 0.1	8.5 ± 0.7	7.3 ± 0.6	9.5 ± 0.3	11.5 ± 0.9	12.8 ± 1.0
20:2n-6[ns]	8.0 ± 0.8	6.9 ± 0.2	6.6 ± 0.6	8.1 ± 0.4	8.8 ± 0.6	9.0 ± 0.8
20:3n-6[ns]	4.4 ± 0.2	1.7 ± 0.2	2.2 ± 0.2	3.0 ± 0.2	3.2 ± 0.3	2.8 ± 0.3
20:3n-3[ns]	8.0 ± 0.5	5.5 ± 0.3	6.8 ± 0.5	6.3 ± 0.5	6.0 ± 0.4	5.9 ± 0.4
22:1n-9	10.1 ± 0.6	9.2 ± 0.7	11.8 ± 1.2	10.4 ± 0.7	8.5 ± 0.3	10.1 ± 0.9
20:4n-6[ns]	10.0 ± 0.6	8.9 ± 0.4	9.1 ± 0.4	9.1 ± 0.2	9.6 ± 0.8	9.6 ± 0.9
20:5n-3	9.5 ± 0.2	7.8 ± 0.5	7.6 ± 0.4	7.4 ± 0.2	6.8 ± 0.6	6.6 ± 0.2
22:4n-6	23.8 ± 0.7	18.8 ± 1.0	17.8 ± 0.7	16.6 ± 0.4	14.9 ± 1.3	14.0 ± 0.4
24:1n-9[ns]	2.9 ± 0.2	2.3 ± 0.2	4.1 ± 0.3	3.6 ± 0.2	3.6 ± 0.2	4.5 ± 0.3
22:6n-3[ns]	5.0 ± 0.5	4.9 ± 0.5	6.5 ± 0.6	5.6 ± 0.2	4.9 ± 0.2	5.9 ± 0.5

[1] Values are given as mg g^{-1} of total fatty acids identified in the sample. ns = not significant by analysis of variance, *F* test ($P > 0.05$). Significance of differences between CLA levels was determined by one-way ANOVA followed by a regression analysis. Values determined at Cromalimentos Laboratory (State University of Maringá, PR, Brazil). Data are presented as means ± SD ($n = 5$).

carcass content of 15:1 n-7, 16:1 n-5, 20:5 n-3 (eicosapentaenoic acid) and 22:4 n-6 (docosatetraenoic acid) was observed ($y = 0.0023x^2 - 0.0979x + 3.002$, $R^2 = 0.87$; $y = 0.0028x^2 - 0.1325x + 4.835$, $R^2 = 0.85$; $y = 0.0042x^2 - 0.2041x + 9.223$, $R^2 = 0.75$; $y = 0.0128x^2 - 0.6739x + 23.153$, $R^2 = 0.85$, respectively), being highest in the non-supplemented diet. Lower levels of 15:1 n-7, 16:1 n-5, 20:5 n-3 and 22:4 n-6 for carcass composition were estimated with 22.0, 24.4, 24.9 and 26.5 g kg^{-1} CLA dietary level, respectively.

A positive linear effect of CLA levels incorporated on the diets and its isomers in fish carcass was observed ($y = 2.5498x + 1.793$, $R^2 = 0.98$, for Total CLA; $y = 1.4115x + 1.350$, $R^2 = 0.97$, for 18:2(c9, t11) isomer; $y = 1.1383x + 0.444$; $R^2 = 0.97$, for 18:2(t10, c12) isomer).

The inclusion of the 18:2(c9, t11) isomer was greater than 18:2(t10, c12) isomer (Table 5). The sum of saturated fatty acids (SFA) linearly decreased ($y = -1.0265x + 281.79$, $R^2 = 0.62$) when increasing CLA in the diet. Similarly, the concentration of the n-6 and n-3 fatty acids series in fish carcasses linearly decreased ($y = -3.1855x + 282.21$, $R^2 = 0.81$; $y = -0.2912x + 30.63$, $R^2 = 0.66$, respectively) with the increase in CLA concentration in the diet (Table 6). For the monounsaturated fatty acid (MUFA) content, a positive linear effect ($y = 1.9535x + 403.58$, $R^2 = 0.81$) of levels of dietary CLA was observed (Table 6). The sum of polyunsaturated fatty acids (PUFAs), PUFA/SFA ratio and n-6/n-3 ratio did not differ between fish fed increasing levels of dietary CLA levels (Table 6).

Discussion

At the end of the trial, no significant effect of dietary supplementation with CLA on the growth performance and feed efficiency of lambaris (*A. altiparanae*) was observed. For Nile tilapia (*O. niloticus*), dietary supplementation with 50.0 g kg^{-1} CLA did not affect weight gain, feed intake, feed conversion ratio and hepatosomatic index (Yasmin *et al.* 2004). Also, other previous reports agree with the results obtained in this study, as obtained for juvenile rainbow trout (*O. mykiss*; Bandarra *et al.* 2006), juvenile Atlantic salmon (*S. salar*; Kennedy *et al.* 2005) and juvenile European sea bass (*Dicentrarchus labrax*; Valente *et al.* 2007a). However, other authors reported that supplementation with 0–100 g kg^{-1} of CLA in the diet of common carp (*Cyprinus carpio*), Nile tilapia (*O. niloticus*) and rockfish (*S. schlegeli*) resulted in significant differences in growth rates (Choi *et al.* 1999). The authors observed that fish fed 10.0 g kg^{-1} CLA showed improvement in growth rate only for common carp (*C. carpio*), while dietary CLA concentration of 100.0 g kg^{-1} reduced growth rates in all of the three species (Choi *et al.* 1999). In yellow catfish (*P. fulvidraco*) fed with 0.0, 5.0, 10.0, 15.0 or 20.0 g kg^{-1} CLA, Tan *et al.* (2010) observed that fish fed dietary with higher CLA levels showed lower weight gain, specific growth rate, feed intake and poor feed conversion ratio. Similar results were also observed in hybrid striped bass (*Morone saxatilis* × *Morone chrysops*) fed diet containing 10.0 g kg^{-1} CLA (Twibell *et al.* 2000). The effect of dietary CLA supplementation on fish growth and feed utilization seems to be species dependent and may be related to particular fatty acids metabolism in the liver and their use as energy source or as essential fatty acids (Twibell *et al.* 2001).

In this study, no significant difference in carcass chemical composition of fish fed graded levels of CLA was observed and is in accordance with the results observed with other species, such as juvenile channel catfish (*I. punctatus*) fed diets containing 5.0 or 10.0 g kg^{-1} CLA (Twibell & Wilson 2003); Atlantic salmon (*S. salar*) fingerlings fed diets with 0.0, 5.0, 10.0 and 20.0 g kg^{-1} CLA (Berge *et al.* 2004); juvenile Atlantic salmon (*S. salar*) fed diets with 0.0, 10.0 or 20.0 g kg^{-1} CLA (Kennedy *et al.* 2005); and juvenile rainbow trout (*O. mykiss*) fed diets with 0.0, 5.0, 7.5 or 10.0 g kg^{-1} CLA (Valente *et al.* 2007b). However, other studies showed increased protein content in the whole-body composition of Atlantic salmon (*S. salar*; Leaver *et al.* 2006) and Nile tilapia (*O. niloticus*; Santos *et al.* 2011). Others showed decrease in lipid content of whole-body yel-

Table 6 Sum and relations of fatty acids of the carcasses (mg g^{-1} of total identified fatty acids) of lambaris (*Astyanax altiparanae*) fed diets containing graded levels of conjugated linoleic acid (CLA) for 90 days

Sum and relationships of fatty acids[1]	CLA levels (g kg^{-1})					
	0.0	5.0	10.0	15.0	20.0	25.0
Total CLA	0.0 ± 0.0	17.9 ± 1.7	24.6 ± 1.9	41.0 ± 0.3	54.5 ± 0.5	64.0 ± 6.3
SFA	275.7 ± 6.0	282.8 ± 10.6	275.1 ± 5.3	264.2 ± 2.4	260.8 ± 6.3	255.1 ± 5.1
MUFA	396.1 ± 1.5	422.8 ± 6.1	424.4 ± 0.2	427.6 ± 4.8	448.9 ± 1.3	448.2 ± 12.0
PUFA^{ns}	328.3 ± 7.2	294.3 ± 16.7	300.5 ± 5.2	308.2 ± 2.6	290.3 ± 5.7	296.7 ± 6.9
PUFA/SFA^{ns}	11.9 ± 0.5	10.4 ± 1.0	10.9 ± 0.4	11.7 ± 0.1	11.1 ± 0.5	11.6 ± 0.1
n-6	296.3 ± 5.6	249.9 ± 18.3	247.0 ± 5.7	240.3 ± 1.7	211.9 ± 5.4	208.9 ± 11.8
n-3	32.0 ± 1.7	26.5 ± 1.1	28.9 ± 1.2	27.0 ± 0.7	23.8 ± 0.7	23.8 ± 1.5
n-6/n-3^{ns}	92.3 ± 3.1	94.6 ± 7.8	85.5 ± 2.2	89.2 ± 1.8	89. ± 3.5	87.9 ± 0.9

[1] Values are given as mg g^{-1} of total fatty acids identified in the sample. ns, not significant by analysis of variance, *F* test ($P > 0.05$). Significance of differences between CLA levels was determined by one-way ANOVA followed by a regression analysis. Values determined at Cromalimentos Laboratory (State University of Maringá, PR, Brazil). Data are presented as means ± SD ($n = 5$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; PUFA/SFA, polyunsaturated/saturated fatty acids ratio.

low catfish (*P.fulvidraco*; Tan *et al.* 2010), Nile tilapia (*O. niloticus*) fillet (Santos *et al.* 2011) and rainbow trout (*O. mykiss*) viscera (Bandarra *et al.* 2006) fed diets supplemented with CLA.

The total CLA concentration on the carcass reflected the dietary levels of CLA, corroborating the idea that fatty acid profiles of fish reflect the fatty acid profiles of the diet consumed (Asdari *et al.* 2011). The values of CLA found in the fatty acid profile of the lambari (*A. altiparanae*) were higher than those found in juvenile yellow perch (*Perca flavescens*; Twibell *et al.* 2001) and European sea bass (*D. labrax*; Makol *et al.* 2013) fed diets with 10.0 g kg^{-1} CLA, and both authors use CLA mixture containing 600 g kg^{-1} CLA. However, in juvenile hybrid striped bass (*M. saxatilis* × *M. chrysops*), a higher concentration of CLA in the muscle was observed by Twibell *et al.* (2000), as well as in juvenile European sea bass (*D. labrax*; Valente *et al.* 2007a), but the last authors use CLA mixture containing 750 g kg^{-1} CLA. These differences should be related to the differences in CLA concentrations, fish species and size (Twibell *et al.* 2001).

The incorporations of 18:2(cis-9, trans-11) and 18:2 (trans-10, cis-12) isomers were proportional to their levels in the diet, as previously observed (Bandarra *et al.* 2006; Valente *et al.* 2007b; Tan *et al.* 2010). These results suggest that, like other freshwater fish species (Tan *et al.* 2010), *A. altiparanae* can successfully incorporate biologically active isomers of CLA into the edible parts. The major incorporation of 18:2(cis-9, trans-11) relative to 18:2(trans-10, cis-12) isomers may be related to differences in digestibility and metabolism of each isomers (Tan *et al.* 2010). However, until now, the digestibility of each biologically active isomer of CLA is unknown.

In the present study, carcass content of eicosapentaenoic acid (EPA) and docosatetraenoic acid (22:4 n-6) was significantly reduced in fish fed CLA supplemented diets. Despite the quadratic effect observed for these fatty acids, with the use of CLA levels higher than 25 g kg^{-1}, an asymptotic effect could be obtained. Similar results were observed in Atlantic salmon (*S. salar*; Kennedy *et al.* 2005) and hybrid striped bass (*M. saxatilis* × *M. chrysops*; Twibell *et al.* 2000). However, in other studies, CLA supplementation did not affect EPA levels in muscle (Twibell *et al.* 2001; Berge *et al.* 2004; Kennedy *et al.* 2007; Valente *et al.* 2007a,b). Also, dietary CLA did not affect DHA and ARA content in lambaris (*A. altiparanae*) muscles, as previously observed in hybrid striped bass (*M. saxatilis* × *M. chrysops*; Twibell *et al.* 2001), Atlantic salmon (*S. salar*; Berge *et al.* 2004), Atlantic cod (*Gadus morhua L.*; Kennedy *et al.* 2007) and rainbow trout (*O. mykiss*; Valente *et al.* 2007b). On the other hand, Valente *et al.* (2007a) observed ARA reduction in European sea bass (*D. labrax*) fed diets containing 20 g kg^{-1} CLA.

The effect of dietary CLA on the reduction in carcass eicosapentaenoic acid of lambari (*A. altiparanae*) may be explained by regulatory effect of CLA on the activity of enzymes that participate in the formation of PUFA, such as Δ5 and Δ6 desaturase and elongase, as observed in Atlantic salmon (*S. salar*; Kennedy *et al.* 2006). The CLA can also regulate expression of genes involved in the PUFA biosynthetic pathway (Takahashi *et al.* 2003). Leaver *et al.* (2006) observed an increase in Atlantic salmon (*S. salar*) liver PUFA synthesis and a decrease in muscle EPA relative proportion, but it cannot be explained by changes in Δ6 desaturase mRNA, which were not significantly altered by CLA feeding; in contrast, the author found that fatty acid elongase expression was increased at 20 or 40 g kg^{-1} CLA.

The reduction in SFA and the increase in MUFA in carcass of lambari (*A. altiparanae*) fed diets with increasing levels of CLA can be attributed to the fatty acid profile of the experimental diets, which can be attributed to the substitution of soybean oil for CLA. However, several authors have reported an increase in saturated fatty acids (SFA) and a reduction in monounsaturated acids (MUFA) in fish fed diets containing CLA (Bandarra *et al.* 2006; Valente *et al.* 2007a; Tan *et al.* 2010; Luo *et al.* 2012), but these authors included the CLA replacing fish oil.

Similarly, the total fatty acids of the series n-6 and n-3 content in lambari (*A. altiparanae*) carcasses reflect the PUFA composition of the diets. Indeed, the replacement of soybean oil by the CLA induced a reduction in dietary and carcass content of some PUFAs. A reduction in muscle PUFA concentration was observed in Nile tilapia (*O. niloticus*) fed diet containing 50.0 and 100.0 g kg^{-1} CLA (Santos *et al.* 2011), hybrids striped bass fed 10.0 g kg^{-1} CLA in the diet (Twibell *et al.* 2000) and juvenile yellow perch (*P. flavescens*) that also received 10.0 g kg^{-1} CLA diet (Twibell *et al.* 2001), when compared with fish fed with a CLA free diet. Kennedy *et al.* (2005) suggest that deposition of CLA in the muscle of Atlantic salmon (*S. salar*) occurs at the expense of other PUFA, such as EPA and DHA. Thus, the increased levels of CLA can lead to reduced levels of PUFAs in animal muscle. In contrast, Berge *et al.* (2004) observed in juvenile Atlantic salmon (*S. salar*) an increase in total PUFA, especially DHA, in fish fed diets with higher levels of CLA (5.0, 10.0 and 20.0 g kg^{-1}).

The results of this study indicate that CLA can be included in diets for lambari (*A. altiparanae*), up to 25.0 g kg^{-1}, without negative effect on growth performance and carcass chemical composition, even showing a low reduction on EPA and 22:4 n-6 levels. It was also demonstrated that supplemented dietary CLA was incorporated into carcass of lambari (*A. altiparanae*). Fish flesh containing high concentrations of CLA can be used as a functional food, because CLA is related to the prevention of various diseases such as arteriosclerosis, cancer and diabetes in humans (Milner 1999; Evans *et al*. 2002; Park & Pariza 2007).

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References

Aryaeian, N., Shahram, F., Djalali, M., Eshragian, M.R., Djazayeri, A., Sarrafnejad, A., Salimzadeh, A., Naderi, N. & Maryam, C. (2009) Effect of conjugated linoleic acids, vitamin E and their combination on the clinical outcome of Iranian adults with active rheumatoid arthritis. *Int. J. Rheum. Dis*., **12**, 20–28.

Asdari, R., Aliyu-Paiko, M., Hashim, R. & Ramachandran, S. (2011) Effects of different lipid sources in the diet for *Pangarsius hypophthalmus* (Sauvage, 1878) juvenile on growth performance, nutrient utilization, body indices and muscle and fatty acid composition. *Aquacult. Nutr*., **17**, 44–53.

Association of Official Analytical Chemists (AOAC) (2000) Official Methods of Analysis of Official Analytical Chemists International. 17th edn. Association of Official Analytical Chemists Inc., Gaithersburg, MD.

Bandarra, M., Nunes, M.L., Andrade, A.M., Prates, J.A.M., Pereiraa, S., Monteiroa, M., Remac, P. & Valente, L.M.P. (2006) Effect of dietary conjugated linoleic acid on muscle, liver and visceral lipid deposition in rainbow trout juveniles (*Oncorhynchus mykiss*). *Aquaculture*, **254**, 496–505.

Benjamin, S. & Spener, F. (2009) Conjugated linoleic acids as functional food: an insight into their health benefits. *Nutr. Metab*., **6**, 36.

Berge, G.M., 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.

Bhattacharya, A., Banu, J., Rahman, M., Causey, J. & Fernandes, G. (2006) Biological effects on conjugated linoleic acids in health and disease. *J. Nutr. Biochem*., **17**, 789–810.

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

Chin, S.F., Liu, W., Storkson, J.M., Ha, Y.L. & Pariza, M.W. (1992) Dietary sources of conjugated dienoic isomers of linoleic acid a newly recognized class of anticarcinogens. *J. Food Compost. Anal*., **5**, 185–197.

Choi, B.D., Kang, S.J., Ha, Y.L. & Ackman, R.G. (1999) Accumulation of conjugated linoleic acid (CLA) in tissues of fish fed diets containing various levels of CLA. In: Quality Attributes of Muscle Foods, 1st edn, Vol. 1 (Xiong, Y.L., Ho, C.T. & Shahidi, F. eds), pp. 61–71. Kluwer Academic/Plenum Publishers, New York, NY, USA.

Evans, M.E., Brown, J.M. & McIntosh, M.K. (2002) Isomer-specific effects of conjugated linoleic acid (CLA) on adiposity and lipid metabolism. *J. Nutr. Biochem*., **13**, 508–516.

Gonçalves, L.U., Parisi, G., Bonelli, A., Sussel, F.R. & Viegas, E.M.M. (2014) The fatty acid compositions of total, neutral and polar lipids in wild and farmed lambari (*Astyanax altiparanae*) (Garutti & Britski, 2000) broodstock. *Aquacult. Res*., **45**, 195–203.

Hartman, L. & Lago, R.C.A. (1973) Rapid preparation of fatty acid methyl ester from lipids. *Lab. Pract*., **22**, 475–476.

Kennedy, S.R., Campbell, P.J., Porter, A. & Tocher, D.R. (2005) Influence of dietary conjugated linoleic acid (CLA) on lipid and fatty acid composition in liver and flesh of Atlantic salmon (*Salmo salar*). *Comp. Biochem. Physiol. B Biochem. Mol. Biol*., **141**, 168–178.

Kennedy, S.R., Leavera, M.J., Campbellb, P.J., Zhenga, X., Dicka, J.R. & Tochera, D.R. (2006) Influence of dietary oil content and conjugated linoleic acid (CLA) on lipid metabolism enzyme activities and gene expression in tissues of Atlantic Salmon (*Salmo salar* L.). *Lipids*, **41**, 423–436.

Kennedy, S.R., Bickerdike, R., Berge, R.K., Porter, A.R. & Tocher, D.R. (2007) Influence of dietary conjugated linoleic acid (CLA) and tetradecylthioacetic acid (TTA) on growth, lipid composition and key enzymes of fatty acid oxidation in liver and muscle of Atlantic cod (*Gadus morhua L.*). *Aquaculture*, **264**, 372–382.

Leaver, M.J., Tocher, D.R., Obach, A., Jensen, L., Henderson, R.J., Porter, A.R. & Krey, J. (2006) Effect of dietary conjugated linoleic acid (CLA) on lipid composition, metabolism and gene expression in Atlantic salmon (*Salmo salar*) tissues. *Comp. Biochem. Physiol., Part A Mol. Integr. Physiol*., **145**, 258–267.

Luo, Z., Tan, X.Y., Liu, C.X., Li, X.D., Liu, X.J. & Xi, W.Q. (2012) Effect of dietary conjugated linoleic acid levels on growth performance, muscle fatty acid profile, hepatic intermediary metabolism and antioxidant responses in genetically improved farmed Tilapia strain of Nile tilapia *Oreochromis niloticus*. *Aquacult. Res*., **43**, 1392–1403.

MacRedmond, R., Singhera, G., Attridge, S., Bahzad, M., Fava, C., Lai, Y., Hallstrand, T.S. & Dorscheid, D.R. (2010) Conjugated linoleic acid improves airway hyper-reactivity in overweight mild asthmatics. *Clin. Exp. Allergy*, **40**, 1071–1078.

Makol, A., Torrecillas, S., Fernández Vaquero, A., Rincón, L., Ginés, R. & Izquierdo, M. (2013) Deposition of conjugated linoleic acid in market size sea bass (*Dicentrarchus labrax*) and its effects on performance, composition and fillet sensory and texture attributes. *Aquacult. Nutr*., **19**, 1–116.

Milner, J.A. (1999) Functional foods and health promotion. *J. Nutr*., **129**, 1395–1397.

Pariza, M.W., Park, Y. & Cook, M.E. (2001) The biologically active isomers of conjugated linoleic acid. *Prog. Lipid Res*., **40**, 283–298.

Park, Y. & Pariza, M.W. (2007) Mechanisms of body fat modulation by conjugated linoleic acid (CLA). *Food Res. Int*., **40**, 311–323.

Park, Y. & Pariza, M.W. (2009) Bioactivities and potential mechanisms of action for conjugated linoleic acids. *Food Sci. Biotechnol*., **18**, 586–593.

Peterson, B.C., Manning, B. & Li, M. (2003) CLA-enriched diets for channel catfish. *Glob. Aquac. Advocate*, **6**, 48–50.

Porto-Foresti, F., Castilho-Almeida, R.B., Senhorini, J.A. & Foresti, F. (2010) Biologia e criação do lambari-do-rabo-amarelo (*Astyanax altiparanae*). In: Espécies Nativas Para Piscicultura No Brasil, 2nd edn, Vol. 1 (Baldisserotto, B. & Gomes, L.C. eds), pp. 101–115. Editora UFSM, Santa Maria, RS, Brasil.

Santos, L.D., Furuya, W.M., Silva, L.C.R., Matsushita, M. & Castro Silva, T.S. (2011) Dietary conjugated linoleic acid (CLA) for finishing Nile tilapia. *Aquacult. Nutr.*, **17**, 70–81.

Silva, D.J. & Queiros, A.C. (2002) Analise de Alimentos (Métodos Químicos e Biológicos), 3rd edn. 235p. Universidade Federal de Viçosa, Viçosa, MG, Brasil.

Song, H.J., Grant, I., Rotondo, D., Mohede, I., Sattar, N., Heys, S.D. & Wahle, K.W.J. (2005) Effect of CLA supplementation on immune function in young healthy volunteers. *Eur. J. Clin. Nutr.*, **59**, 508–517.

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

Tan, X.Y., Luo, Z., Xie, P., Li, X.D., Liu, X.J. & Xi, W.Q. (2010) Effect of dietary conjugated linoleic acid (CLA) on growth performance, body composition and hepatic intermediary metabolism in juvenile yellow catfish *Pelteobagrus fulvidraco*. *Aquaculture*, **310**, 186–191.

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

Twibell, R.G., Watkins, B.A. & Rogers, L. (2000) Effects of dietary conjugated linoleic acids on hepatic and muscle lipids in hybrid striped bass. *Lipids*, **35**, 155–161.

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

Valente, L.M.P., Bandarra, N.M., Figueiredo-Silva, A.C., Cordeiro, A.R., Simoes, R.M. & Nunes, M.L. (2007a) Influence of conjugated linoleic acid on growth, lipid composition and hepatic lipogenesis in juvenile European sea bass (*Dicentrarchus labrax*). *Aquaculture*, **267**, 225–235.

Valente, L.M.P., Bandarra, N.M., Figueiredo-Silva, A., Rema, P., Vaz-Pires, S., Martins, J.A.M. & Pratese Nunes, M.L. (2007b) Conjugated linoleic acid in diets for large size rainbow trout (*Oncorhynchus mykiss*): effects on growth, chemical composition and sensory attributes. *Br. J. Nutr.*, **97**, 289–297.

Vilela, C. & Hayashi, C. (2001) Desenvolvimento de juvenis de lambari *Astyanax bimaculatus* (Linnaeus, 1758), sob diferentes densidades de estocagem em tanques-rede. *Acta. Sci. Biol. Sci.*, **23**, 491–496.

Yasmin, A., Takeuchi, T., Hayashi, M., Hirota, T., Ishizuka, W. & Ishida, S. (2004) Effect of conjugated linoleic acid and docosahexaenoic acids on growth of juvenile *Oreochromis niloticus*. *Fish. Sci.*, **70**, 473–481.