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Effects of dietary medium-chain triacylglycerols (tricaprylin and tricaproin) and phospholipid supply on survival, growth and lipid metabolism in common carp (*Cyprinus carpio* L.) larvae

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Abstract

The present study investigated the interaction of dietary medium-chain fatty acids (MCFA) and phospholipids (PL) on survival, growth and lipid metabolism in common carp larvae. Nine diets based on casein and dextrin and with a variable lipid part were tested in triplicate for 22 days post first feeding. The 3 × 3 design consisted of three triacylglycerols (3% of diet) combined with three different lipid supplements. Tested triacylglycerols were triolein (TOL), tricaprylin (TC8) and tricaproin (TC6), and lipid supplements were 2% soybean oil (low-fat diets without PL), 2% soybean lecithin (low-fat diets with 2% PL) or both 2% soybean lecithin and 6% TOL (high-fat diets with 2% PL).

In the first step, both TC6 and TC8 resulted in improved survival and growth rates compared to TOL, irrespective of the PL supply. In the second step, TC8 decreased survival and growth rates, whereas the difference between TC6 and TOL became less. Histological signs of impaired intestinal absorption of neutral lipids were evidenced in larvae fed TOL without PL and also in high-fat diets with 2% PL. The latter diets also resulted in poorer growth rates compared to low-fat diets with 2% PL. These results suggest that the quantitative PL requirement of larvae increases as the dietary level of long-chain triacylglycerols increases. Larvae fed TC6 or TC8 showed enlarged liver and hepatocyte volume and a decreased level of body neutral lipids. Based on β-hydroxybutyrate (β-HBA) measurements in whole larvae, TC8 was found to be more ketogenic than TC6. TC6 and TC8 affected differently the fatty acid profile of larval body neutral

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lipids. TC6 did not induce the appearance of MCFA, whereas TC8 feeding resulted in a low level of 8:0 and relatively high levels of 10:0 (3.8% of total fatty acids). Neither 8:0 nor 10:0 were found in larval polar lipids.

This study confirmed the essentiality of PL in common carp larval diets and underlines differences in the utilization of TC6 and TC8, which both initially stimulate growth during the first week, but only temporarily in the case of TC8.

Keywords: *Cyprinus carpio*; Common carp; Larva; Medium-chain triacylglycerols; Phospholipids

1. Introduction

A nutritional requirement for phospholipids (PL), especially for phosphatidylinositol and phosphatidylcholine, has been reported for fish larvae (Kanazawa, 1993; Geurden et al., 1995). Phosphatidylcholine appears to be crucial in the formation of very low density lipoproteins (VLDL) during the intestinal absorption of neutral lipids and thus increases the amount of energy available for growth (Field and Mathur, 1995; Fontagné et al., 1998). Another way of intestinal absorption of neutral lipids, which does not require formation of VLDL, is known in mammals. This way concerns medium-chain triacylglycerols (MCT), which are composed of fatty acids with a chain length comprised between 6 and 12 carbon atoms. MCT proved to be an efficient energy source especially for young mammals as they are rapidly hydrolyzed, absorbed and little deposited in body fat stores (Bach and Babayan, 1982; Megremis, 1991). The ability of first feeding carp larvae to utilize MCT supplied either as coconut oil or tricaprylin (TC8) has been investigated in a previous experiment (Fontagné et al., 1999). Coconut oil proved to be an efficient energy-yielding nutrient for common carp larvae unlike TC8, which induced decreased survival and growth rates. Likewise, Craig and Gatlin (1995) have shown that dietary TC8 resulted in depressed weight gain and elevated plasma ketone bodies in juvenile red drum. These results suggest that fish response depends on the dietary MCT chain length. Indeed, another pure MCT, tricaproin (TC6), has been found to be efficiently utilized by common carp larvae, contrary to TC8 (unpublished results).

Therefore, the objective of the present study was to examine the interaction of two dietary MCT (TC8 and TC6) and PL on survival, growth and lipid metabolism in first feeding common carp larvae. In addition to the effects of MCT on fatty acid composition of larvae, ketone body levels were evaluated. The effects of MCT and PL supplementation on liver and intestine histology of larvae were also investigated to assess intestinal lipid absorption and liver lipid deposition.

2. Materials and methods

2.1. Experimental fish and diets

Common carp (*Cyprinus carpio* L.) larvae were reared in the experimental facilities of the INRA Hydrobiology Station (Saint-Pée-sur-Nivelle, France) as described in

Fontagné et al. (1999). First feeding (day 0 of the experiment) started 2 days after hatching. The trial lasted 22 days. The water temperature was monitored and increased from 19.5 to 24°C within 3 days, whereupon it remained at 24°C. Experimental diets were tested in triplicate with 400 first-feeding larvae per 6-l tank. A duplicate group was starved and served as a negative control.

Nine semi-purified diets were formulated (Table 1). All dietary ingredients of the common casein and dextrin basal had the same origin as in Radünz-Neto et al. (1994). The 3×3 design consisted of three triacylglycerols sources (3% of diet) combined with three different lipid supplements. The triacylglycerols were triolein (TOL), TC8 and TC6 and were obtained from Sigma (Saint-Quentin-Fallavier, France). The lipid supplements were composed of 2% soybean oil (low-fat diets without PL, diets L0), 2% soybean lecithin (low-fat diets with 2% PL, diets L2) or both 2% soybean lecithin and 6% TOL (high-fat diets with 2% PL, diets H2). Soybean oil and lecithin were obtained from Bouton d'Or (Chaulnes, France) and S.D.A. (Marne-la-Vallée, France), respectively.

Table 1
Formulation and composition of experimental diets (g/100 g dry weight)

	Experimental diets								
	TOL_{L0}	$TC8_{L0}$	$TC6_{L0}$	TOL_{L2}	$TC8_{L2}$	$TC6_{L2}$	TOL_{H2}	$TC8_{H2}$	$TC6_{H2}$
Ingredients									
Casein basal[a]	56.4	56.4	56.4	56.4	56.4	56.4	56.4	56.4	56.4
Vitamin mixture[b]	9.4	9.4	9.4	9.4	9.4	9.4	9.4	9.4	9.4
Mineral mixture[c]	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
Dextrin	23.5	23.5	23.5	23.5	23.5	23.5	17.5	17.5	17.5
Peanut oil	1	1	1	1	1	1	1	1	1
Soybean oil	2	2	2	–	–	–	–	–	–
Soybean lecithin	–	–	–	2	2	2	2	2	2
Triolein	3	–	–	3	–	–	9	6	6
Tricaproin	–	3	–	–	3	–	–	3	–
Tricaprylin	–	–	3	–	–	3	–	–	3
Proximate composition									
Dry matter (%)	92.4	93.9	93.0	93.7	94.6	91.9	94.2	95.0	94.1
Crude protein	54.2	54.1	54.4	55.4	55.6	55.9	54.7	55.2	55.8
Total lipids	5.1	6.6	6.1	5.6	5.4	5.9	11.2	10.9	10.9
Ash	4.8	4.9	4.9	5.0	5.0	5.2	5.4	5.5	5.7
Gross energy (kJ/g dry matter)	21.2	21.5	21.4	21.5	21.1	21.3	22.5	22.0	22.3

[a] Casein basal: 40% casein; 11.7% casein sodium salt; 4.7% casein hydrolysate.

[b] Vitamin mixture (g/kg vitamin mix): retinyl acetate, 1; cholecalciferol, 2.5; DL-α-tocopheryl acetate, 5; menadione, 1; thiamin–HCl, 0.1; riboflavin, 0.4; D-calcium panthothenate, 2; pyridoxine–HCl, 0.3; cyanocobalamin, 1; niacin, 1; choline, 200; ascorbic acid (L-ascorbyl-2-polyphosphate), 5; folic acid, 0.1; D-biotin, 1; meso-inositol, 30. All ingredients were diluted with α-cellulose.

[c] Mineral mixture (g/kg mineral mix): KCl, 90; KI, 0.04; $CaHPO_4 \cdot 2H_2O$, 500; NaCl, 40; $CuSO_4 \cdot 5H_2O$, 3; $ZnSO_4 \cdot 7H_2O$, 4; $CoSO_4$, 0.02; $FeSO_4 \cdot 7H_2O$, 20; $MnSO_4 \cdot H_2O$, 3; $CaCO_3$, 215; MgOH, 124; Na_2SeO_3, 0.03; NaF, 1.

Chemical analysis of the diets was conducted according the following procedures: dry matter after drying at 105°C for 24 h, protein (N × 6.25) by the Kjeldahl method after acid hydrolysis, ash by incineration at 550°C for 16 h and gross energy in an adiabatic bomb calorimeter. Total lipids and fatty acid composition of diets were analyzed as described below for larvae. Fatty acid composition of diets is given in Table 2. Dietary levels of $n-3$ and $n-6$ fatty acids were comprised between 1.8% and 3.4% of total fatty acids and 11.6% and 24.8%, respectively, even for high-fat diets containing 12% lipids and thus met the essential fatty acid requirements as defined by Radünz-Neto et al. (1996) for common carp larvae. In diets, TC8 and TC6, caprylic and caproic acids amounted to 58–61% of total fatty acids in low-fat diets L0 and L2 (6% lipids) and 26–28% in high-fat diets H2 (12% lipids). In diets TOL, levels of oleic acid were lower than expected due to low purity of the TOL, which was determined by gas chromatography analysis to contain only 64% oleic acid with 13% linoleic acid and some saturates and monoenes from 12:0 to 18:0. In contrast, TC6 and TC8 were highly purified products ($\geq$ 99%).

2.2. Sample collection

The final survival was calculated from daily mortality and from the final number of surviving larvae recorded in each tank. Ten larvae were sampled and anaesthetized in

Table 2
Fatty acid composition of experimental diets (% of total fatty acids)[a]

	Experimental diets								
	TOL_{L0}	$TC8_{L0}$	$TC6_{L0}$	TOL_{L2}	$TC8_{L2}$	$TC6_{L2}$	TOL_{H2}	$TC8_{H2}$	$TC6_{H2}$
6:0	0.3	0.4	58.9	0.2	1.5	60.7	0.1	0.2	26.1
8:0	1.0	58.3	0.9	1.4	61.4	0.5	0.1	28.2	0.2
14:0	2.3	0.3	0.4	2.6	0.6	0.4	2.6	2.0	2.1
16:0	9.8	5.6	5.5	10.1	6.0	7.1	6.8	6.3	6.2
18:0	2.3	1.5	1.4	2.1	1.5	1.3	1.6	1.2	1.3
Total saturates[b]	16.7	66.7	67.8	17.7	72.1	71.2	12.3	38.7	36.2
16:1	3.5	0.2	0.2	3.9	0.3	0.1	4.9	3.5	3.5
18:1	47.6	14.4	13.6	52.0	13.1	12.8	60.5	40.3	41.8
20:1	0.6	0.2	0.1	0.8	0.2	0.1	1.0	0.5	0.6
Total monoenes[c]	52.8	14.9	14.0	58.0	13.8	13.2	68.0	45.3	47.0
18:2$n-6$	24.8	14.9	14.4	20.1	11.6	13.0	14.9	11.8	12.0
Total $n-6$[d]	24.8	14.9	14.4	20.2	11.6	13.0	15.0	11.9	12.2
18:3$n-3$	3.1	1.8	1.8	2.0	1.4	1.6	1.7	1.5	1.4
Total $n-3$[e]	3.4	2.1	2.0	2.5	2.1	2.1	2.1	1.8	1.9

[a] Values are means of two replicate analyses.
[b] Includes 10:0, 12:0, 15:0, 17:0 and 20:0.
[c] Includes 14:1, 17:1 and 22:1.
[d] Includes 20:2$n-6$.
[e] Includes 18:4$n-3$, 20:5$n-3$, 22:5$n-3$ and 22:6$n-3$.

diluted 2-phenoxyethanol on day 0 and from each tank on day 7, 14 and 21 for length measurements performed with a semi-automatic image analyzer (VIDS, Systèmes Analytiques, France). Four larvae were sampled from two rearing tanks of each dietary treatment on day 8 after 8 h of food delivery for histological analyses. Thirty larvae were sampled from two rearing tanks of each dietary treatment on day 15 for ketone body determination. At the end of the experiment (day 22) and after starvation for 24 h, all remaining larvae from each tank were collected, anaesthetized for wet weight determination and stored at −80°C for lipid analyses.

2.3. Histology

Sampled larvae were anaesthetized, immersed in the Serra fixative (ethanol/formalin/acetic acid, 6:3:1, v/v/v), dehydrated and embedded in paraffin. Serial sections (10 μm) of longitudinally oriented larvae were stained by alcian blue, periodic acid Schiff reagent, Groat hematoxylin and orange G. Anterior intestine and liver were examined as described by Fontagné et al. (1998) in order to assess intestinal lipid absorption and eventual liver hypertrophy. The sections were examined using a semi-automatic image analyzer (VIDS, Systèmes Analytiques, France). The volumes of liver and eye were estimated by multiplying the sum of the surface areas measured on every 10 serial sections by 100 μm. The mean eye volume was used as an internal standard of larval size as the mean eye volume determined on sections was shown to be related to the total length of fresh larva (Alami-Durante, 1990). To estimate mean enterocyte height and hepatocyte volume, the same anatomical location was used for the 36 larvae. For the enterocyte height estimation, 50 measurements were made per larva. For the hepatocyte volume estimation, 10 microscopic fields were examined per larva. The calculation of hepatocyte volume was based on the number and size of hepatocyte nuclei, and on the proportion of constituents other than hepatocytes determined for each sample as detailed in Escaffre and Bergot (1986).

2.4. Ketone body and lipid analyses

Whole larvae collected on day 15 were analyzed for the presence of β-hydroxybutyrate (β-HBA) using the enzymatic method described by Williamson and Mellanby (1974). Collected samples were chilled and immediately homogenized and deproteinized using a Potter homogenizer held on ice with ice-cold 6% perchloric acid. The final weight of homogenate was adjusted to three times the weight of larval samples. The homogenate was centrifuged (10 min at $2000 \times g$). The supernatant was neutralized with potassium carbonate to pH 8.5 and centrifuged (10 min at $2000 \times g$). The supernatant was used to assess larval β-HBA levels.

Total lipids of larvae were extracted and measured gravimetrically according to Folch et al. (1957) using dichloromethane instead of chloroform. Total lipids were separated into neutral and polar fractions according to Juaneda and Rocquelin (1985). Fatty acid methyl esters of neutral and polar lipids were prepared as previously described in Fontagné et al. (1999).

2.5. Statistical analyses

Results are given as means ± standard error (SE). The feeding experiment was analyzed according to the 3×3 design with three triacylglycerols and three lipid supplements. Two-way ANOVA was used to test the effects of triacylglycerol and lipid supplement and their interaction. The Newman–Keuls multiple range test was used to compare means when a significant difference was found. Percentage data were arcsin-transformed. Weight and volume data were log-transformed before analysis. The theoretical biomass of each group, calculated as the product of survival by the mean weight of 100 larvae, was also log-transformed before analysis. Occurrence of intestinal steatosis was analyzed by χ^2 tests and mean enterocyte height by the contrast test. All the statistical analyses were performed with the computing program STAT-ITCF (ITCF, 1988) and differences were considered significant when P values were < 0.05.

3. Results

3.1. Survival and growth rates

Dietary triacylglycerol supplementation significantly affected survival (Table 3). In the first step (from day 6 to day 14), both TC6 and TC8 resulted in improved survival compared to TOL. In the second step (from day 17 to the end of the experiment), TC8

Table 3
Survival, total length and final growth parameters of common carp larvae fed diets containing different triacylglycerols (TOL, TC8 or TC6) with different lipid supplements (low-fat diets without PL or with 2% PL and high-fat diets with 2% PL)[a]

	Triacylglycerol			Lipid supplement			Interaction
	TOL	TC8	TC6	L0	L2	H2	
Survival (%)							
Day 7	95[b]	98[a]	99[a]	98	98	96	NS
Day 14	74[b]	84[a]	90[a]	86[a]	86[a]	77[b]	NS
Day 22	67[b]	67[b]	83[a]	69	77	70	NS
Total length (mm)							
Day 7	9.5[b]	10.0[a]	9.9[a]	9.8	9.9	9.7	NS
Day 14	12.7	12.7	12.5	12.4[b]	13.5[a]	12.0[b]	NS
Day 21	16.9[a]	15.2[c]	15.9[b]	15.6[b]	16.6[a]	15.8[b]	NS
Final growth parameters							
Mean wet weight (mg)	50[a]	30[c]	37[b]	34[b]	44[a]	39[b]	*
Theoretical biomass (g)	3.4[a]	2.0[b]	3.1[a]	2.3[b]	3.5[a]	2.7[ab]	NS

[a]Values are means of nine rearing tanks (three diets). Within rows and for each diet-related effect (triacylglycerol or lipid supplement), means not sharing a common superscript letter are significantly different ($P < 0.05$). Interactions between triacylglycerols and lipid supplements indicated by NS or * are not significant ($P \geq 0.05$) or significant ($P < 0.05$), respectively.

decreased survival. Final survival of groups fed TC8 was not significantly different from that of groups fed TOL and was lower than that of groups fed TC6 (67 ± 10% vs. 83 ± 9%, respectively). Lipid supplement significantly affected survival from day 9 to day 15. On day 15, survival of larvae fed low-fat diets L0 and L2 was higher compared to larvae fed high-fat diets H2 (84 ± 9% vs. 75 ± 11%, respectively, on day 15). Final survival of larvae fed low-fat diets with 2% PL (77 ± 13%) was not significantly higher than that of the two other groups (70 ± 11%). Among TOL-diets, TOL_{L2} resulted in higher survival compared to TOL_{L0} and TOL_{H2} (74 ± 5% vs. 64 ± 12%, respectively). Among diets L2, final survival of TOL-fed larvae was not significantly different from that of TC8 and remained significantly lower than that of TC6 (Fig. 1).

Both dietary triacylglycerol and lipid supplement affected growth rates of larvae (Table 3). During the first week, both TC6 and TC8 resulted in improved growth rates compared to TOL, irrespective of the lipid supplement, with total lengths of 9.9 ± 0.3 vs. 9.5 ± 0.3 mm, respectively. During the second week, the advantage of TC8 and TC6 over TOL disappeared and all groups showed the same mean total length (12.6 ± 1.0 mm). During the third week, TOL-fed larvae grew faster than TC6-fed larvae, whereas TC8 resulted in significantly decreased total length on day 21 (16.9 ± 0.8, 15.9 ± 0.9 and 15.2 ± 0.7 mm, respectively). The final mean wet weight also discriminated the three groups TOL, TC6 and TC8 (50 ± 10, 37 ± 7 and 30 ± 6 mg, respectively). From the second week, low-fat diets with 2% PL displayed significant higher growth rates than high-fat diets with 2% PL and low-fat diets without PL and resulted in higher final mean wet weight (44 ± 15 vs. 36 ± 8 mg). The significant interaction in final mean larval weight was due to a difference between TC8 and the two other diets. TC8 resulted in higher growth rates when associated in diet H2 than when associated in diet L2 (35 ± 6 vs. 28 ± 1 mg, respectively), unlike TOL and TC6.

The final theoretical biomass was thus affected by both triacylglycerol and lipid supplement (Table 3). Both TOL and TC6 resulted in significantly higher final theoreti-

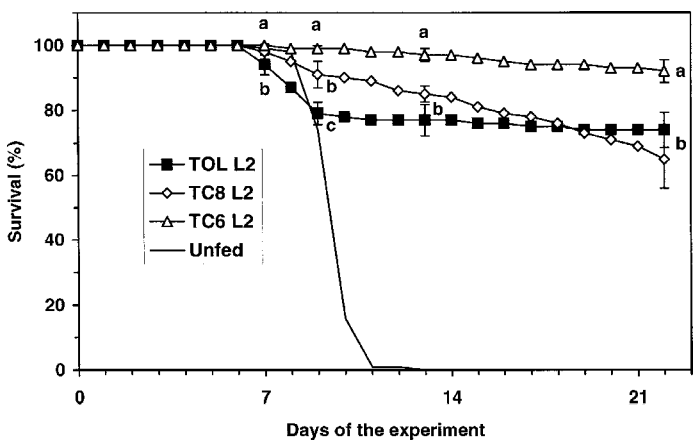

Fig. 1. Survival of common carp larvae fed the three low-fat diets containing 2% PL (L2). Values are means ± SE ($n = 3$). Means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by a Newman–Keuls test.

cal biomass (3.3 ± 0.9 g) compared to TC8 (2.0 ± 0.5 g). Larvae fed diets L2 displayed significantly higher final theoretical biomass (3.5 ± 1.3 g) compared to larvae fed diets L0 (2.3 ± 0.6 g), larvae fed diets H2 being intermediate (2.7 ± 0.8 g).

3.2. Intestine and liver histology

Occurrence of intestinal steatosis was associated with a high mean enterocyte height due to accumulation of lipid droplets in enterocytes of 8-day larvae (Table 4). These histological signs of impaired intestinal absorption of neutral lipids were more frequent in larvae fed diets L0 and also diets H2 than in larvae fed diets L2 (9/24 intestinal steatoses vs. 0/12 associated with an enterocyte height of 29.1 ± 2.5 vs. 26 ± 1.1 μm, respectively). This result was more marked for larvae fed TOL (5/8 intestinal steatoses vs. 0/4 associated with an enterocyte height of 31.3 ± 2.1 vs. 25.5 ± 0.0 μm, respectively) compared to larvae fed TC8 or TC6 (only 4/16 intestinal steatoses vs. 0/8 associated with an enterocyte height of 28.0 ± 1.8 vs. 26.2 ± 1.3 μm, respectively). Due to the significant dietary interaction in mean enterocyte height, measurements of the nine dietary groups were compared by a contrast test (Fig. 2). Larvae fed TOL without PL exhibited the highest mean enterocyte height (32.9 ± 1.0 μm). Larvae fed high-fat diets H2 displayed higher mean enterocyte height than larvae fed low-fat diets L2 and diets $TC8_{L0}$ and $TC6_{L0}$ without PL (29.4 ± 0.9 vs. 26.2 ± 1.3 μm, respectively).

Larvae fed TC6 or TC8 showed significantly larger liver and hepatocyte volumes compared to larvae fed TOL ($188 \pm 52 \times 10^{-3}$ mm^3 and $4.4 \pm 0.7 \times 10^3$ μm^3 vs. $132 \pm 52 \times 10^{-3}$ mm^3 and $3.6 \pm 0.8 \times 10^3$ μm^3, respectively). Likewise, larvae fed

Table 4
Histological parameters measured on common carp larvae sampled at day 8 of the experiment and fed diets containing different triacylglycerols (TOL, TC8 or TC6) with different lipid supplements (low-fat diets without PL or with 2% PL and high-fat diets with 2% PL)[#]

	Triacylglycerol			Lipid supplement			Interaction
	TOL	TC8	TC6	L0	L2	H2	
Intestine histology							
Number of steatosis[##]	5	3	1	4[a]	0[b]	5[a]	–
Enterocyte height (μm)	29.4[a]	27.2[b]	27.6[b]	28.8[a]	26.0[b]	29.4[a]	**
Eye volume (10^{-3} mm^3)	60	73	62	59[b]	75[a]	60[b]	NS
Liver histology							
Liver volume (10^{-3} mm^3)	132[b]	208[a]	168[ab]	177[a]	203[a]	128[b]	NS
Liver volume/Eye volume ratio	2.2	2.8	2.7	3.0[a]	2.7[a]	2.1[b]	NS
Hepatocyte volume (10^3 μm^3)	3.6[b]	4.2[a]	4.6[a]	4.3[a]	4.6[a]	3.5[b]	NS

[#] Values are means of six rearing tanks (three diets). Within rows and for each diet-related effect (triacylglycerol or lipid supplements), means not sharing a common superscript letter are significantly different ($P < 0.05$) according to a Newman–Keuls test. Interactions between triacylglycerols and lipid supplements indicated by NS or ** are not significant ($P \geq 0.05$) or highly significant ($P < 0.01$), respectively.

[##] For each treatment, 12 larvae were examined and values are numbers of larvae exhibiting an intestinal steatosis. Data of intestinal steatosis not sharing a common superscript letter are significantly different ($P < 0.05$) according to a χ^2 test.

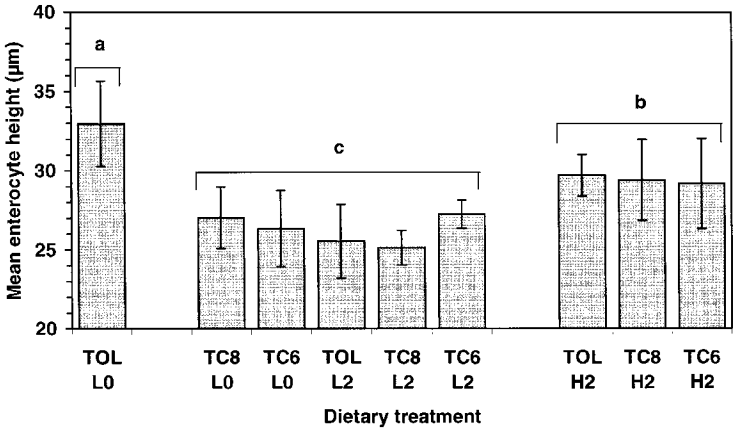

Fig. 2. Mean enterocyte height of common carp larvae fed nine different diets. Values are means ± SE ($n = 3$). Groups not sharing a common superscript letter are significantly different ($P < 0.05$) according to contrast test.

low-fat diets L0 and L2 displayed significantly larger liver and hepatocyte volumes than larvae fed high-fat diets H2 ($190 \pm 46 \times 10^{-3}$ mm^3 and $4.4 \pm 0.7 \times 10^3$ µm^3 vs. $128 \pm 58 \times 10^{-3}$ mm^3 and $3.5 \pm 0.6 \times 10^3$ µm^3, respectively). Larval size of sampled larvae fed the different lipid supplements was different as shown by the mean eye volume measurements. Comparison of larvae with a similar mean eye volume (60 ± 11

Table 5
Ketone body levels and lipid composition of common carp larvae fed diets containing different triacylglycerols (TOL, TC8 or TC6) with different lipid supplements (low-fat diets without PL or with 2% PL and high-fat diets with 2% PL)[a]

	Triacylglycerol			Lipid supplement			Interaction
	TOL	TC8	TC6	L0	L2	H2	
Day 15							
Ketone body levels (nmol/g)							
β-HBA	158^c	202^a	174^b	177^{ab}	190^a	167^b	NS
Day 22							
Lipid composition (% wet weight)							
Dry matter	17.2^a	15.6^c	16.2^b	15.6^b	15.9^b	17.4^a	**
Total lipid	5.3^a	4.2^b	4.1^b	4.1^b	4.0^b	5.3^a	NS
Neutral lipid	3.9^a	2.8^b	2.8^b	2.7^b	2.7^b	3.9^a	NS
Polar lipid	1.4	1.4	1.4	1.4	1.4	1.4	NS

[a]Values are means of six (ketone body levels) or nine rearing tanks (lipid composition) representing three diets. Within rows and for each diet-related effect (triacylglycerol or lipid supplement), means not sharing a common superscript letter are significantly different ($P < 0.05$). Interactions between triacylglycerols and lipid supplements indicated by NS or ** are not significant ($P \geq 0.05$) or highly significant ($P < 0.01$), respectively.

$\times 10^{-3}$ mm^3) showed that larvae fed diets L0 without PL displayed higher liver volume than larvae fed high-fat diets H2 ($177 \pm 57 \times 10^{-3}$ vs. $128 \pm 58 \times 10^{-3}$ mm^3, respectively).

3.3. Ketone bodies measurements

Based on measurements in whole larvae sampled on day 15, both triacylglycerol and lipid supplements significantly affected β-HBA levels (Table 5). TC8 was found to be significantly more ketogenic than TC6, which was more ketogenic than TOL (202 ± 24, 174 ± 12 and 158 ± 11 nmol/g, respectively). Significantly higher levels of β-HBA were displayed in larvae fed diets L2 compared to larvae fed diets H2 (190 ± 31 vs. 167 ± 18 nmol/g, respectively), larvae fed diets L0 exhibiting intermediate β-HBA levels (177 ± 21 nmol/g).

Table 6
Fatty acid composition of neutral lipid from common carp larvae fed diets containing different triacylglycerols (TOL, TC8 or TC6) with different lipid supplements (low-fat diets without PL or with 2% PL and high-fat diets with 2% PL)[#]

	Triacylglycerol			Lipid supplement			Interaction
	TOL	TC8	TC6	L0	L2	H2	
8:0	t[b]	1.4[a]	t[b]	0.5	0.4	0.6	*
10:0	2.1[b]	3.8[a]	2.5[b]	3.1	3.1	2.2	NS
12:0	0.3[b]	0.5[a]	0.3[b]	0.3[ab]	0.4[a]	0.3[b]	NS
14:0	2.3	2.1	2.1	2.0	2.2	2.3	NS
16:0	15.4[b]	16.9[a]	17.3[a]	17.6[a]	18.4[a]	13.4[b]	NS
18:0	4.1	4.0	4.1	4.2[a]	4.2[a]	3.9[b]	NS
Total saturates[##]	24.7[c]	29.2[a]	26.9[b]	28.2[a]	29.2[a]	23.3[b]	NS
16:1	6.8	7.5	7.5	7.7[a]	7.8[a]	6.3[b]	NS
18:1	50.5[a]	46.3[b]	47.8[b]	44.4[c]	46.5[b]	53.7[a]	NS
20:1	1.4	1.4	1.4	1.4	1.3	1.5	NS
Total monoenes[###]	59.5[a]	55.8[b]	57.4[b]	54.0[c]	56.2[a]	62.5[a]	NS
18:2$n-6$	8.5	8.0	8.0	8.5	8.1	8.0	NS
20:4$n-6$	0.5	0.5	0.5	0.6[a]	0.5[b]	0.4[b]	NS
Total $n-6$[†]	11.2	10.9	10.9	11.7[a]	10.7[b]	10.5[b]	NS
18:3$n-3$	1.1	1.0	0.9	1.1	1.0	1.0	NS
Total $n-3$[‡]	1.7	1.6	1.3	1.7	1.5	1.4	NS

[#] Values are means of nine rearing tanks (three diets). Within rows and for each diet-related effect (triacylglycerol or lipid supplement), means not sharing a common superscript letter are significantly different ($P < 0.05$). Interactions between triacylglycerols and lipid supplements indicated by NS or * are not significant ($P \geq 0.05$) or significant ($P < 0.05$), respectively.

[##] Includes 15:0 and 17:0.

[###] Includes 14:1 and 17:1.

[†] Includes 18:3$n-6$, 20:2$n-6$, 20:3$n-6$ and 22:5$n-6$.

[‡] Includes 18:4$n-3$.

3.4. Lipid and fatty acid composition

The dry matter contents of larvae at the end of the experiment were related to mean wet weight (Table 5). Significantly higher amounts of total lipid were found in larvae fed high-fat diets H2 compared to other diets (5.3 ± 0.6% vs. 4.1 ± 0.6%, respectively). Larvae fed TOL, which were bigger, contained more total lipid than larvae fed TC8 and TC6, which were smaller (5.3 ± 0.6% vs. 4.2 ± 0.7%, respectively). The increase in total lipid was due to an increase in neutral lipid whereas no significant difference in polar lipid levels was noted.

Both triacylglycerol and lipid supplements affected the fatty acid profile of larval neutral lipid (Table 6). TC8 and TC6 resulted in higher levels of 16:0 and lower levels of 18:1 compared to TOL (17.1 ± 2.5% of total fatty acids and 47.1 ± 4.6% vs. 15.4 ± 2.8% and 50.5 ± 4.3%, respectively). TC8 and TC6 affected differently the saturated fatty acid levels. TC8 feeding resulted in relatively high levels of 8:0

Table 7
Fatty acid composition of polar lipid from common carp larvae fed diets containing different triacylglycerols (TOL, TC8 or TC6) with different lipid supplements (low-fat diets without PL or with 2% PL and high-fat diets with 2% PL)[#]

	Triacylglycerol			Lipid supplement			Interaction
	TOL	TC8	TC6	L0	L2	H2	
14:0	1.0	0.9	0.8	0.9	0.8	1.0	NS
16:0	21.6	22.3	21.8	22.6	22.3	20.8	NS
18:0	7.3	7.5	7.5	8.1[a]	7.2[a]	7.2[a]	NS
Total saturates[##]	30.6	31.5	30.7	32.2	30.9	29.7	NS
16:1	5.4	5.5	5.4	5.6	5.6	5.2	NS
18:1	27.8	26.0	27.2	25.5[b]	26.9[b]	28.7[a]	NS
20:1	1.3	1.2	1.3	1.2	1.3	1.3	NS
Total monoenes[###]	35.0	33.3	34.4	32.6[b]	34.4[ab]	35.7[a]	NS
18:2$n-6$	5.7	5.3	5.5	5.2[b]	5.4[ab]	5.9[a]	NS
20:2$n-6$	4.6	4.7	4.7	4.9	4.9	4.1	NS
20:3$n-6$	2.6	2.6	2.7	2.6	2.6	2.7	NS
20:4$n-6$	6.7	6.8	7.1	7.0	7.0	6.6	NS
22:5$n-6$	3.2	3.6	3.7	3.7	3.5	3.3	NS
Total $n-6$[†]	24.6	25.1	25.6	25.1	25.6	24.5	NS
18:3$n-3$	0.7	0.6	0.9	0.8	0.6	0.8	NS
22:6$n-3$	2.4	2.9	2.8	2.9	2.6	2.5	NS
Total $n-3$[‡]	4.0	4.7	4.9	5.0	4.3	4.3	NS

[#] Values are means of nine rearing tanks (three diets). Within rows and for each diet-related effect (triacylglycerol or lipid supplement), means not sharing a common superscript letter are significantly different ($P < 0.05$). Interactions between triacylglycerols and lipid supplements indicated by NS are not significant ($P \geq 0.05$).

[##] Includes 15:0, 17:0 and 20:0.

[###] Includes 17:1.

[†] Includes 18:3$n-6$, 22:2$n-6$ and 22:4$n-6$.

[‡] Includes 18:4$n-3$, 20:5$n-3$ and 22:5$n-3$.

(1.4 ± 0.3%) and 10:0 (3.8 ± 1.5% vs. 2.3 ± 0.7%), whereas TC6 did not induced the appearance of more medium-chain fatty acids (MCFA) than TOL. Levels of $n-6$ and $n-3$ fatty acids were not significantly different between TOL, TC8 and TC6 (11.0 ± 1.0% and 1.5 ± 0.4%, respectively). Diets H2 containing 6% TOL resulted in higher levels of 18:1 (53.7 ± 1.9% vs. 45.4 ± 3.3%) and lower levels of 16:0, 18:0, 16:1 and $n-6$ fatty acids compared to low-fat diets L2 and L0.

Lipid supplement significantly affected the fatty acid profile of polar lipid of larval lipids unlike triacylglycerol source (Table 7). Higher levels of 18:1 and 18:2$n-6$ were found in diets H2 compared to low-fat diets L2 and L0 (28.7 ± 1.2% and 5.9 ± 0.5% vs. 26.0 ± 2.0% and 5.3 ± 0.6%, respectively). Other fatty acid levels in the polar lipid of whole larvae were not significantly different between dietary treatments. Levels of saturates, $n-6$ and $n-3$ fatty acids amounted for 31.0 ± 2.3%, 25.1 ± 1.7% and 4.6 ± 1.0% of total fatty acids, respectively.

4. Discussion

In the present experiment, both the nature of the dietary triacylglycerol (TC6, TC8 or TOL) and the PL supplementation affected the survival and growth rates of larvae. Moreover, the larval response changed according to the period, which suggests a change of the limiting nutritional factors between the beginning and the end of the experiment.

During the first period, up to 7 days, TC6 and TC8 showed a significant superiority over TOL, for both survival and growth, irrespective of the PL supply. This result is in accordance with the idea that MCFA, being more rapidly absorbed, transported and oxidised than long-chain fatty acids, can represent an advantageous energy source for small larvae. No beneficial effect of TC8 over TOL has been noted in a previous experiment with diets containing 10% dextrin (Fontagné et al., 1999). Possibly, efficient use of TC8 is favoured by a relatively high level of carbohydrate in the diet (17.5–23.5% dextrin in the present experiment), as suggested by Bach et al. (1996) and Papamandjaris et al. (1998) in mammals. The apparent lack of effect of the PL supply during the first week is perhaps explained by the availability of residual endogenous PL from yolk.

During the second period (from day 8 to day 14) the nutritional priorities of larvae clearly changed. On the one hand, PL became a major limiting factor and, on the other hand, a marked divergence appeared between TC6 and TC8 fed larvae. Compared to TOL group, TC6-fed larvae kept their initial advantage of a better survival, but lost their relative advantage for growth, whereas TC8 resulted in the poorest final performances of growth and survival. The final low results with TC8 were in agreement with previous observations in common carp larvae (Fontagné et al., 1999) as in juvenile red drum (Craig and Gatlin, 1995).

Histological detection of large fat droplets and enlarged enterocytes in the anterior intestine discriminated the PL-deficient from the PL-supplemented TOL diets, in accordance with previous observations on the role of phosphatidylcholine in lipoprotein synthesis (Fontagné et al., 1998). The absence of steatosis with TC6 and TC8 diets, with 2% PL or without PL, conforms to what could be expected based on the properties of MCFA which, in contrast to long-chain fatty acids, can be exported directly as

non-esterified fatty acids from enterocytes into the blood and therefore do not need to be incorporated into VLDL (Bach and Babayan, 1982). A new information provided by the present work is that 2% PL appears insufficient for an optimal absorption of neutral lipids in high-fat diets (with a total lipid level amounting to 12% of diet). This opinion is based on the histological measurements, which show an enlargement of the enterocytes due to accumulation of large lipid droplets, and on the global reduction of larval survival and growth associated with the fat enrichment of diets. Previous investigations in common carp larvae indicated that a 2% level of PL supplementation was sufficient for similar casein-based diets with total lipid levels amounting to only 6–8% of dry diets (Geurden et al., 1995). Present observations with higher fat levels suggest that the PL requirement increases when the dietary neutral lipid level increases, at least as regards phosphatidylcholine needed for the intestinal absorption of long-chain fatty acids.

Low-fat diets (6% of diet) containing 23.5% dextrin (vs. 17.5% for high-fat diets) resulted in enlarged livers compared to high-fat diets. According to observations of liver hypertrophy in common carp larvae fed pregelatinized starch by Szlaminska et al. (1991), this increase of liver volume may be related to a higher dietary level of digestible carbohydrate rather than to a lower level of lipid. The tendency toward a liver enlargement noted in larvae fed TC6 and TC8 on day 8 may be interpreted as indicating either a better energy provision by MCT than by TOL, as expressed also by the higher growth, or a specific effect of MCT as reported in mammals (Newport et al., 1979) although not systematically (Gondret et al., 1998). Another common feature observed for TC6- and TC8-fed larvae, despite their final growth divergence, is the lower amount of body neutral fat compared to TOL-fed larvae. A similar effect of MCT has been described in larger fish (Nakagawa and Kusunoki, 1990; Mustafa et al., 1991; Davis et al., 1999). It is also known in mammals (Lavau and Hashim, 1978; Geliebter et al., 1983) and assayed for the treatment of obesity in human (Bach et al., 1996).

Decreased growth of fish fed TC8 has been reported in juvenile red drum and related to elevated ketone bodies levels in the blood (Craig and Gatlin, 1995). In the present study, levels of β-HBA in whole larvae fed TC8 and sampled on day 15, when growth of this group began to decline, were higher than in the other groups. The reasons for 8:0 being more ketogenic than 6:0 are not clear and possibly due to an impaired further utilisation of ketone bodies rather than to an increased rate of formation. Lower levels of ketone bodies were detected in larvae fed high-fat diets, which exhibited also a poor growth rate and altered intestinal lipid absorption, compared to larvae fed low-fat diets supplemented with 2% PL. In the present case, the low ketone bodies levels of larvae fed high-fat diets may reflect a decreased dietary energy supply.

A peculiarity of TC8 feeding is the appearance of 8:0 and the increase of 10:0 and 12:0 in larval body neutral lipid, which are not seen after TC6 feeding. The increase of 10:0 following 8:0 feeding was also noted in a previous experiment with common carp larvae (Fontagné et al., 1999). Such elongation suggests an impaired functioning of the fatty acid synthase complex which produces usually 16:0 without release of shorter chain acyl intermediates (Gurr and Harwood, 1991). As previously observed for phosphatidylcholine (Geurden et al., 1999), the fatty acid composition of the polar lipid fraction of larvae appears fixed within strict limits, likely due to strong constraints for membrane integrity and proper functioning of membrane-anchored enzymes. A typical

example is the absence of fatty acids shorter than 14 carbon atoms in the polar fraction. In contrast, the neutral lipid fraction appears more affected by diet.

In conclusion, TC6 and TC8 appear to be efficient energy sources for first feeding common carp larvae, but only for a short time in the case of TC8 which induces metabolic alterations. Two different roles of PL are also confirmed: the one for normal growth of larvae fed either long-chain fatty acids or MCFA and the other specific for the intestinal absorption of long-chain fatty acids.

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References

Alami-Durante, H., 1990. Growth of organs and tissues in carp (*Cyprinus carpio* L.) larvae. Growth Dev. Aging 54, 109–116.

Bach, A.C., Babayan, V.K., 1982. Medium-chain triglycerides: an update. Am. J. Clin. Nutr. 36, 950–962.

Bach, A.C., Ingenbleek, Y., Frey, A., 1996. The usefulness of dietary medium-chain triglycerides in body weight control: Fact or fancy? J. Lipid Res. 37, 708–726.

Craig, S.R., Gatlin, D.M. III, 1995. Coconut oil and beef tallow, but not tricaprylin, can replace menhaden oil in the diet of red drum (*Sciaenops ocellatus*) without adversely affecting growth or fatty acid composition. J. Nutr. 125, 3041–3048.

Davis, D.A., Lazo, J.P., Arnold, C.R., 1999. Response of juvenile red drum (*Sciaenops ocellatus*) to practical diets supplemented with medium chain triglycerides. Fish Physiol. Biochem. 21, 235–247.

Escaffre, A.-M., Bergot, P., 1986. Morphologie quantitative du foie des alevins de truite arc-en-ciel (*Salmo gairdnerii*) issus de gros ou de petits oeufs: incidence de la date du premier repas. Arch. Hydrobiol. 107, 331–348.

Field, F.J., Mathur, S.N., 1995. Intestinal lipoprotein synthesis and secretion. Prog. Lipid Res. 34, 185–198.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Fontagné, S., Geurden, I., Escaffre, A.-M., Bergot, P., 1998. Histological changes induced by dietary phospholipids in intestine and liver of common carp (*Cyprinus carpio* L.) larvae. Aquaculture 161, 213–223.

Fontagné, S., Pruszynski, T., Corraze, G., Bergot, P., 1999. Effect of coconut oil and tricaprylin vs. triolein on survival, growth and fatty acid composition of common carp (*Cyprinus carpio* L.) larvae. Aquaculture 179, 241–251.

Geliebter, A., Torbay, N., Bracco, E.F., Hashim, S.A., Van Itallie, T.B., 1983. Overfeeding with medium-chain triglyceride diet results in diminished deposition of fat. Am. J. Clin. Nutr. 37, 1–4.

Geurden, I., Radünz-Neto, J., Bergot, P., 1995. Essentiality of dietary phospholipids for carp (*Cyprinus carpio* L.) larvae. Aquaculture 131, 303–314.

Geurden, I., Bergot, P., Van Ryckeghem, K., Sorgeloos, P., 1999. Phospholipid composition of common carp (*Cyprinus carpio*) larvae starved or fed different phospholipid classes. Aquaculture 171, 93–107.

Gondret, F., Mourot, J., Lebas, F., Bonneau, M., 1998. Effects of dietary fatty acids on lipogenesis and lipid traits in muscle, adipose tissue and liver of growing rabbits. Anim. Sci. 66, 483–489.

Gurr, M.I., Harwood, J.L., 1991. Lipid Biochemistry. An Introduction. 4th edn. Chapman & Hall, London, UK, 406 pp.

ITCF, 1988. STAT-ITCF. 4th edn. Service des Etudes Statistiques, Paris, France.

Juaneda, P., Rocquelin, G., 1985. Rapid and convenient separation of phospholipids and non phosphorus lipids from rat heart using silica cartridges. Lipids 20, 40–41.

Kanazawa, A., 1993. Essential phospholipids of fish and crustaceans. Fish Nutrition in Practice. In: Kaushik, S.J., Luquet, P. (Eds.), Les Colloques 61 Ed. INRA, Paris, France, pp. 519–530.

Lavau, M.M., Hashim, S.A., 1978. Effect of medium chain triglyceride on lipogenesis and body fat in the rat. J. Nutr. 108, 613–620.

Megremis, C.J., 1991. Medium-chain triacylglycerols: a non-conventional fat. Food Technol. 45, 108–114.

Mustafa, M.G., Nakagawa, H., Ohya, S., Shimizu, T., Horikawa, Y., Yamamoto, S.I., 1991. Effects of various level of dietary medium chain triglycerides on growth and lipid reservation in ayu. Nippon Suisan Gakkaishi 57, 2327–2331.

Nakagawa, H., Kusunoki, T., 1990. Influence of dietary medium chain triglycerides on lipid accumulation in Tilapia, *Oreochromis niloticus*. Suisanzoshoku 38, 353–359, (in Japanese with English abstract).

Newport, M.J., Storry, J.E., Tuckley, B., 1979. Artificial rearing of pigs: 7. Medium chain triglycerides as a dietary source of energy and their effect on live-weight gain, feed:gain ratio, carcass composition and blood lipids. Br. J. Nutr. 41, 85–93.

Papamandjaris, A.A., MacDougall, D.E., Jones, P.J.H., 1998. Medium chain fatty acid metabolism and energy expenditure: obesity treatment implications. Life Sci. 62, 1203–1215.

Radünz-Neto, J., Corraze, G., Charlon, N., Bergot, P., 1994. Lipid supplementation of casein-based purified diets for carp (*Cyprinus carpio* L.) larvae. Aquaculture 128, 153–161.

Radünz-Neto, J., Corraze, G., Bergot, P., Kaushik, S.J., 1996. Estimation of essential fatty acid requirements of common carp larvae using semi-purified artificial diets. Arch. Anim. Nutr. 49, 41–48.

Szlaminska, M., Escaffre, A.-M., Bergot, P., 1991. Utilization of dietary pregelatinized starch by common carp (*Cyprinus carpio* L.) larvae. J. Anim. Physiol. Anim. Nutr. 65, 65–71.

Williamson, D.H., Mellanby, J., 1974. D-(−)-3-Hydroxy-butyrate. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis. Academic Press, London, UK, pp. 1836–1839.