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Response of common carp (*Cyprinus carpio*) larvae to different dietary levels and forms of supply of medium-chain fatty acids

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Abstract − The effects of dietary medium-chain fatty acids (MCFA) on survival, growth and fatty acid composition of first-feeding carp larvae were investigated in two 21-day feeding trials. In trial 1, one diet CO was supplemented with 10% coconut oil, two diets, M1 and M2 with 1.7 or 4.2% interesterified medium-chain triacylglycerols (MCT), and two other diets T1 and T2 with 1% tricaprylin and 0.7% tricaprin, or 2.5% tricaprylin and 1.7% tricaprin. The five diets were made isolipidic (18% of dry diet) with triolein and compared to a control diet without MCFA. With a low dietary level (1% of dry diet) of caprylic acid (C8:0), final survival and mean weight of larvae fed CO, M1 and T1 were not significantly different from those of control (68 ± 6% and 62 ± 8 mg, respectively). In contrast, with a high dietary C8:0 level (2.5% of dry diet), final survival and mean weight of larvae fed M2 and T2 were markedly decreased (23 ± 2% and 40 ± 8 mg, respectively), without effect of the form of supply. In trial 2, larvae were fed six diets with graded tricaprylin contents (from 0 to 10% in diet). Survival was not significantly affected but growth was decreased by C8:0 levels higher than 2% of dry diet. Fatty acid composition of larval total lipids revealed high levels of lauric and myristic acids in larvae fed coconut oil. Deposition of C8:0 and capric acid (C10:0) was low after MCT feeding, but depended of the form of supply, being higher with pure than with interesterified MCT. Relatively high amounts of C8:0 and C10:0 were recorded in total lipids of larvae fed 10% tricaprylin. It is concluded that high levels of caprylic acid decrease growth but that low levels are well utilised by carp larvae, irrespective of the form of supply. © 2000 Ifremer/CNRS/INRA/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS

larval nutrition / diet / fatty acids / triglycerides / *Cyprinus carpio*

Résumé − Alimentation en acides gras des larves de carpe (*Cyprinus carpio*) avec différentes doses et formes d'apport en triglycérides à chaîne moyenne. Les effets des acides gras à chaîne moyenne (AGCM) sur la survie, la croissance et la composition en acides gras des larves de carpe ont été étudiés dans deux expériences d'alimentation de 21 jours. L'expérience n°1 a testé un régime supplémenté avec 10% d'huile de coprah, deux avec 1,7 ou 4,2% de triglycérides à chaîne moyenne (TCM) inter-estérifiés et deux autres avec 1% de tricapryline et 0,7% de tricaprine, ou 2,5% de tricapryline et 1,7% de tricaprine. Les régimes ont été rendus isolipidiques (18% de l'aliment sec) avec de la trioléine et comparés à un témoin sans AGCM. Avec des faibles teneurs alimentaires (1% de l'aliment sec) d'acide caprylique (C8:0), les survies et croissances n'ont pas été différentes de celles du témoin. En revanche, avec un niveau élevé de C8:0 (2,5% de l'aliment sec), les performances ont été diminuées quelle que soit la forme d'apport. Dans l'expérience n°2, les larves ont été nourries avec six régimes ayant des teneurs croissantes en tricapryline (de 0 à 10%). La survie n'a pas été affectée alors que la croissance a été diminuée par des taux de C8:0 supérieurs à 2% de l'aliment sec. La composition en acides gras des larves a été affectée par la taille des larves et l'aliment distribué. L'huile de coprah a entraîné un dépôt important d'acide laurique et myristique dans les larves. Les dépôts de C8:0 et d'acide caprique (C10:0) consécutifs aux TCM ont été faibles mais néanmoins plus importants avec des TCM homogènes qu'avec des TCM inter-estérifiés. Des proportions de C8:0 et de C10:0 relativement importantes ont été retrouvées dans les larves nourries avec 10% de tricapryline. Des niveaux élevés d'acide caprylique dans l'aliment diminuent la croissance mais des niveaux plus faibles sont bien utilisés par les larves de carpe. © 2000 Ifremer/CNRS/INRA/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS

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1. INTRODUCTION

In mammals, medium-chain fatty acids (MCFA) with chain length between 6 and 12 carbon atoms, are hydrolysed rapidly and utilised directly as an energy source with little or no deposition in body fat (Bach and Babayan, 1982). Coconut oil with lauric acid (C12:0) as its major fatty acid is the main natural source of MCFA. Besides coconut oil, other commercially available sources of MCFA are the medium-chain triglyceride (MCT) oils, which are derived from physical separation of coconut oil. These interesterified MCT consist of glycerol esterified by both caprylic (C8:0) and capric acids (C10:0) (Kaunitz et al., 1958). MCT oils have been used for various nutritional and medical purposes, especially in case of impaired or insufficient absorption of long-chain fatty acids (Bach and Babayan, 1982; Heydinger and Nakhasi, 1996). In addition, the role of MCT oils in the treatment of obesity has been investigated (Bach et al., 1996; Papamandjaris et al., 1998).

In fish as in mammals, dietary supplementation of MCT oil has been reported to decrease fat deposition and to improve growth rates (Mustafa et al., 1991; Nakagawa and Kimura, 1993). Coconut oil can replace other lipid sources in the diets of fish without impairing growth (Craig and Gatlin, 1995). In fish larvae, diets containing coconut oil also yielded high survival and growth rates when fed to fish larvae. In larvae of African catfish, *Heterobranchus longifilis*, coconut oil proved to be a better additive than either cottonseed oil, rich in n-6 fatty acids, or fish oil, rich in n-3 fatty acids (Legendre et al., 1995). In carp larvae, coconut oil yielded the same survival and growth rates as triolein, whereas purified tricaprylin, providing only C8:0 as MCFA, decreased both survival and growth (Fontagné et al., 1999). This negative effect of tricaprylin was also reported in juvenile red drum (Craig and Gatlin, 1995; Davis et al., 1999). The latter results suggested differences in metabolic utilisation of MCFA according to their chain length. This problem was further examined in the present study by comparing the response of carp larvae to different levels and form of supply of MCFA, especially C8:0, presented interesterified as coconut or MCT oils, or as pure triacylglycerol.

2. MATERIALS AND METHODS

2.1. Experimental diets

In each trial, artificial diets (*table I*) were formulated to be isoproteic (54–55% crude protein) and isolipidic (17–19% total lipids, including 10% supplied as triglycerides). The composition of the constant 90% basal mixture differed between trials only by the levels of soybean lecithin (5 or 7%) and vitamin mix (5 or 8%) compensated by the levels of dextrin (10 or 15%). The 5% lecithin level was assumed to be sufficient for satisfying the requirement for phospholipids of carp larvae (Geurden et al., 1997). Soluble fish protein concentrate and soybean lecithin provided mainly n-3 and n-6 fatty acids representing 4.5 and 18% respectively of total dietary fatty acids (*table II*), meeting the essential fatty acid requirements defined by Radünz-Neto et al. (1996) for carp larvae.

In both trials, the control diet (TO-diet) was formulated with 10% triolein. This lipid source, as determined by gas chromatography analysis, consisted of a blend of 64% oleic acid and linoleic acid (13% of total fatty acids) with some saturates and monoenes from C12:0 to C18:0. The five other diets were formulated with three different MCFA sources (either coconut oil in CO-diet, or MCT oil in M-diets or tricaprylin + tricaprin in T-diets) in order to obtain two levels of C8:0 (6–8 or 18% of total fatty acids). In T-diets, the level of C10:0 was adjusted to be the same as in M-diets (5 and 13% of total fatty acids) by addition of tricaprin. In trial 2, besides the control diet containing 10% triolein, five diets were supplemented with 2, 4, 6, 8 or 10% tricaprylin. In both trials, the diets were made isolipidic by addition of triolein.

All ingredient powders were finely ground below 100 µm and mixed before addition of the lipid emulsion of the triglyceride source, soybean lecithin and water. The moist blend was pelleted using a meat grinder. The pellets were dried in a ventilated oven at 36 °C for 48 h, ground and sieved to obtain microparticles with graded diameters (100–200, 200–400 and 400–630 µm).

2.2. Fish and experimental conditions

Two 21-day trials were carried out at the Hydrobiology Station in Saint-Pée-sur-Nivelle (France). First-feeding carp (*Cyprinus carpio*) larvae were obtained by induced spawning from one female and two males. One-day hatched larvae were randomly distributed into 20 tanks in a semi-recirculating system as described by Charlon and Bergot (1984) with 400 (trial 1) or 600 larvae (trial 2) per 6-L tank. The water temperature was monitored and increased from 21 to 24 °C within 3 days whereupon it remained at 24 °C. First-feeding (day 0) started 2 days after hatching when larvae exhibited an inflated swim-bladder. Automatic feed dispensers delivered food in excess throughout the 16-h artificial light period. Food particle size and water flow rates (from 0.3 to 0.8 $L \cdot min^{-1}$) were increased progressively in each tank every week. Diets were fed to triplicate groups of first-feeding larvae. A duplicate group was starved and served as a negative control in order to assess the possible availability of unwanted food in the recirculated water system and for comparison with the other experiments performed in similar conditions.

The final survival was calculated from daily mortality percentages and from the final record of surviving fry in each tank. In trial 1, the water flow stopped accidentally in the rearing system on day 5, which resulted in a temporary increase of the water temperature and in abnormal subsequent flow rates in four

Table I. Formulation and composition of the experimental diets.

	Trial 1						Trial 2					
Diets	TO	CO	M1	T1	M2	T2	TO	T2	T4	T6	T8	T10
Formulation (g·100 g^{-1})												
Basis 1[1]	90	90	90	90	90	90	–	–	–	–	–	–
Basis 2[2]	–	–	–	–	–	–	90	90	90	90	90	90
Triolein[3]	10	–	8.3	8.3	5.8	5.8	10	8	6	4	2	–
Coconut oil[4]	–	10	–	–	–	–	–	–	–	–	–	–
MCT oil[5]	–	–	1.7	–	4.2	–	–	–	–	–	–	–
Tricaprylin[6]	–	–	–	1	–	2.5	–	2	4	6	8	10
Tricaprin[7]	–	–	–	0.7	–	1.7	–	–	–	–	–	–
Proximate composition												
Dry matter (% DM)	96.1	96.5	96.3	95.6	96.1	96.1	92.0	91.7	91.6	92.8	91.7	92.4
Crude protein (% DM)	54.6	55.0	55.8	55.4	54.6	54.5	53.9	54.3	53.8	54.3	53.7	53.5
Total lipid (% DM)	18.8	18.0	18.7	18.3	18.6	18.8	18.9	18.3	17.4	17.2	19.0	18.6
Ash (% DM)	7.0	7.1	7.0	7.1	7.1	7.2	6.8	7.3	7.3	7.2	7.4	7.2
Gross energy (kJ·g^{-1} DM)	23.1	22.7	22.9	23.0	22.9	22.8	23.0	22.8	22.6	22.7	22.4	22.4

[1] Basis 1 (% diet): 30% soluble fish protein concentrate, CPSP 90, Sopropêche France; 30% casein, Prolabo 22544.292; 10% dextrin, Sigma D2256; 7% soybean lecithin, SAPA DAFA S.D.A., France; 8% vitamin premix; 5% mineral premix. [2] Basis 2 (% diet): 30% soluble fish protein concentrate, CPSP 90, Sopropêche France; 30% casein, Prolabo 22544.292; 15% dextrin, Sigma D2256; 5% soybean lecithin, Aquagran, Riceland Foods, Inc, AR; 5% vitamin premix; 5% mineral premix. [3] Prolabo 28789.297; composition (% total fatty acids): 64% C18:1, 5% C16:1, 1% C18:0, 7% C16:0, 4% C14:0, 2% C12:0, 13% C18:2n-6. [4] Saria® Industries, France; composition (% total fatty acids): 1% C6:0, 9% C8:0, 7% C10:0, 49% C12:0, 17% C14:0, 8% C16:0, 2% C18:0, 6% C18:1, 2% C18:2n-6. [5] Industrial origin; composition (% total fatty acids): 58% C8:0, 41% C10:0, 1% C6:0 + C12:0. [6] Fluka 91040 in trial 1 and Sigma T9126 in trial 2; composition (% total fatty acids): 99% C8:0. [7] Fluka 91022; composition (% total fatty acids): 99% C10:0.

neighbor tanks which were discarded (one replicate of larvae fed TO and T1 and two replicates of larvae fed M2).

Ten larvae were sampled on day 0 and from each rearing tank on days 7, 10, 15 and 21 and anaesthetised in diluted 2–phenoxyethanol for length measurements

Table II. Main fatty acid composition of dietary total lipid (g·100 g^{-1} total fatty acids)*.

	Trial 1						Trial 2	
Diets	TO	CO	M1	T1	M2	T2	TO	T10
C6:0	0.1	0.4	0.2	–	0.2	0.1	–	–
C8:0	0.1	5.6	7.6	7.6	17.6	18.3	0.2	58.8
C10:0	0.1	4.8	5.9	4.7	14.4	13.1	0.5	0.5
C12:0	1.2	37.0	0.8	0.7	0.9	0.5	0.7	0.1
C14:0	3.2	13.8	2.4	2.4	2.0	1.8	2.5	0.7
C16:0	11.6	11.9	10.0	11.2	10.5	10.1	11.1	8.6
C18:0	2.0	2.1	1.9	2.0	2.2	1.7	2.1	1.6
Σ saturates	18.7	75.8	29.2	29.0	48.2	45.9	18.9	71.5
C16:1n-7	4.4	0.9	3.7	3.9	3.0	2.7	4.3	1.3
C18:1n-7 + n-9	45.3	6.6	38.5	37.7	25.9	26.2	47.6	6.1
Σ monoenes	51.5	8.1	43.9	43.2	30.1	30.2	54.7	8.6
C18:2n-6	21.8	12.0	19.5	20.7	19.5	17.8	19.3	14.2
C20:4n-6	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.2
Σ n-6	21.9	12.2	19.6	20.8	15.7	17.8	20.1	14.5
C18:3n-3	2.7	1.8	2.9	2.6	2.0	2.5	1.9	2.0
C18:4n-3	0.4	0.1	0.3	0.3	0.2	0.2	0.2	0.2
C20:5n-3	0.9	0.6	0.8	0.8	0.6	0.7	1.0	1.1
C22:6n-3	1.0	0.8	1.1	0.8	1.1	0.7	1.4	1.3
Σ n-3	5.1	3.3	5.1	4.8	4.0	4.5	4.7	4.7

* Values are means of two replicates; –, not detected; Σ saturates includes C15:0 and C17:0; Σ monoenes includes C14:1, C17:1 and C20:1.

performed using a semi-automatic image analyser (VIDS version IV, Systèmes Analytiques, France). Larvae from the initial stock were collected on day 0 and from each tank at the end of the trial after starvation for 24 h, for wet weight determination and lipid analysis. Larvae were anesthetised, water-rinsed and blotted on absorbent tissue for wet weight determination and storage at –80 °C before lipid analyses.

2.3. Chemical analyses

Diets were analysed using the following procedures: dry matter after drying at 105 °C for 24 h, crude protein (total nitrogen × 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 IKA C4000.

Total lipids of diets and larvae were extracted and measured gravimetrically according to Folch et al. (1957) using dichloromethane instead of chloroform. Fatty acid methyl esters were prepared by acid-catalysed transmethylation of total lipids according to Shantha and Ackman (1990) and were analysed using a Varian 3400 gas chromatograph. The chromatograph was equipped with a DB Wax fused silica capillary column (30 m × 0.25 mm i.d., film thickness: 0.25 µm, J & W Scientific, Folsom, CA). Helium was used as carrier gas (1.4 $mL \cdot min^{-1}$). The thermal gradient was 100 to 180 °C at 8 $°C \cdot min^{-1}$, 180 to 220 °C at 4 $°C \cdot min^{-1}$ and a constant temperature of 220 °C during 20 min. Injector and flame ionisation detector temperatures were 260 and 250 °C, respectively. Fatty acid methyl esters were identified by comparison with known standard mixtures and quantified using a Spectra Physics 4270 integrator.

2.4. Statistical analyses

Differences between dietary groups were analysed using one-way ANOVA. The Newman–Keuls multiple range test was used to compare means when a significant difference was found. Percentage data were arcsin transformed and weight data and weight × survival product, which represents the final theoretical biomass obtained for an initial number of 100 larvae, were log-transformed before analysis. 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. Results are given as means ± standard error (SE).

3. RESULTS

In trial 1, the survival rates remained high (> 97%) by day 6 for all the treatments (*figure 1*). Mortality of the unfed negative control group began on day 8 and was almost complete on day 12. Between day 8 and day 13, high mortality rates were noted in larvae fed M2 and T2, the two diets with high levels of C8:0 (18% of total fatty acids), compared to larvae fed TO, CO, M1 and T1, the four diets with low levels of C8:0 (less than 8% of total fatty acids). The difference between these two groups was significant from day 12 (47 ± 7% vs. 84 ± 6%, respectively). At the end of the trial, survival of larvae fed TO, CO, M1 and T1 was 68 ± 6% vs. 23 ± 2% for larvae fed M2 and T2. No significant difference was found within groups.

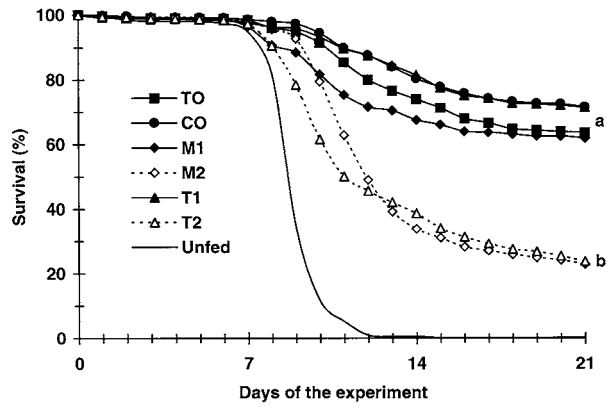

Figure 1. Survival of carp larvae fed diets supplemented with 10% triolein (TO), 10% coconut oil (CO), 1.7 or 4.2% MCT oil (M1 and M2), 1.7 or 4.2% tricaprylin + tricaprin (T1 and T2), and of unfed larvae. Final survival means ($n = 3$ for larvae fed CO, M2 and T2, $n = 2$ for larvae fed TO and T1 and $n = 1$ for larvae fed M1) sharing no common superscript letters are significantly different ($P < 0.05$).

Final length data of larvae fed high levels of C8:0 (diets M2 and T2) showed a lower growth than larvae fed the other diets (*figure 2*). Larvae fed M1 showed lower initial growth than larvae fed T1, but this difference was no more significant on day 21. The highest final mean wet weight was displayed by larvae fed CO and the lowest one by larvae fed M2 and T2 (*table III*). Final theoretical biomass of larvae fed low levels of C8:0 (diets TO, CO, M1 and T1) was significantly higher than that of larvae fed diets M2 and T2 with high levels of C8:0 (4.3 ± 0.8 g vs. 0.9 ± 0.2 g, respectively).

In trial 2, mortality of the unfed group began on day 9 and was almost complete on day 12. The final survival rates were not significantly different between dietary treatments (*table IV*). In contrast, growth rates were significantly decreased from day 11 by tricaprylin addition in the diet, even with the lowest tested level (total larval length: 10.0 ± 0.3 mm for larvae fed TO vs. 9.2 ± 0.2 mm for larvae fed T2, T4, T6, T8 and T10). The final mean wet weights were significantly different and decreased with the increase of dietary level of C8:0. The highest final theoretical biomass was recorded in larvae fed TO whereas the lowest one was found in larvae fed T10 (3.1 ± 0.2 g vs. 0.6 ± 0.3 g, respectively).

The fatty acid compositions of larval total lipids are given in *table III* for trial 1 and in *table V* for trial 2. In the two trials, initial larvae showed high levels of C16:0 (32 ± 3% of total fatty acids vs. 18 ± 1%),

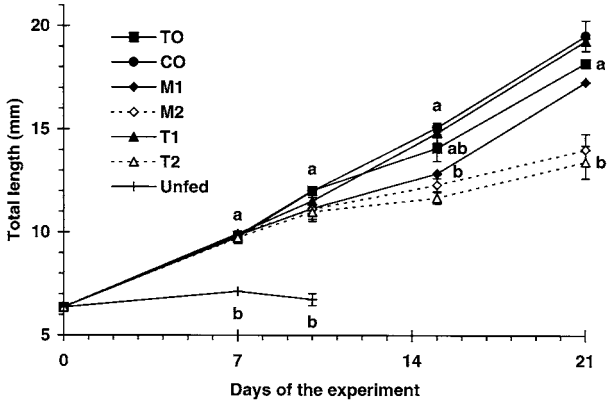

Figure 2. Growth of carp larvae fed diets supplemented with 10% triolein (TO), 10% coconut oil (CO), 1.7 or 4.2% MCT oil (M1 and M2), 1.7 or 4.2% tricaprylin + tricaprin (T1 and T2), and of unfed larvae. For a day of sampling, means ± se (n = 3 for larvae fed CO, M2 and T2, n = 2 for larvae fed TO and T1 and n = 1 for larvae fed M1) of total length measurements sharing no common superscript letters are significantly different ($P < 0.05$).

C20:5n-3 (3.8 ± 0.5% vs. 0.6 ± 0.4%) and C22:6n-3 (15 ± 2% vs. 3 ± 3%) and low levels of C18:2n-6 (2.0 ± 0.3% vs. 14 ± 3%) compared to 21-day fed larvae. In TO-fed control larvae, growth was associated with a decrease of saturates and n-3 fatty acids from 43 to 25% and from 23 to 4%, respectively, in trial 1. The large CO-fed larvae also contained low levels of n-3 fatty acids (3.8 ± 0.1% vs. an average of 4.5 ± 0.4% for the five other dietary treatments). At the end of trial 2, the small T10-fed larvae displayed high levels of n-3 fatty acids (14.4 ± 0.5% for larvae fed T10 vs. 3.6 ± 0.1% for larvae fed TO).

In trial 1, larvae fed CO contained high levels of C12:0 (26.1 ± 0.8% of total fatty acids) and C14:0 (11.9 ± 0.2%), reflecting the dietary fatty acid profile. M1 and T1 feeding resulted in higher larval levels of saturates (mainly MCFA but also saturates longer than C16:0) and lower levels of monoenes than in TO-fed larvae, which displayed a similar larval size. Larvae fed M1 and T1 contained lower MCFA than larvae fed M2 and T2 (5.0 ± 0.5% vs. 11.3 ± 1.8%, respectively). For a given dietary C8:0 level (6–8%), significant

Table III. Main fatty acid composition of larval total lipid in trial 1 (g·100 g^{-1} total fatty acids)*.

Dietary treatment	Initial	TO	CO	M1	T1	M2	T2
Dietary level of C8:0 (% total fatty acids)		–	6	8	8	18	18
Larval theoretical biomass (g)	0.2	4.0^{a}	4.8^{a}	2.9^{a}	4.4^{a}	0.9^{b}	1.0^{b}
Larval mean wet weight (mg)	2	62^{ab}	67^{a}	47^{ab}	62^{ab}	39^{b}	41^{b}
Larval dry matter (%)	16.0	19.4	19.0	19.0	20.1	18.8	18.5
Larval total lipid (%)	3.5	6.9	5.6	6.0	6.7	5.8	5.6
C8:0	0.2	t	t	0.8^{b}	1.0^{b}	1.2^{b}	2.1^{a}
C10:0	–	t	2.0^{d}	3.2^{c}	2.9^{cd}	7.3^{b}	9.0^{a}
C12:0	0.3	0.5^{d}	26.1^{a}	1.0^{c}	1.0^{c}	1.5^{b}	1.5^{b}
Σ MCFA	0.6	0.6^{e}	28.2^{a}	5.0^{d}	5.0^{d}	10.0^{c}	12.6^{b}
C14:0	1.5	2.5^{c}	11.9^{a}	2.5^{c}	2.8^{b}	2.6^{bc}	2.7^{bc}
C16:0	33.3	17.0^{b}	17.5^{ab}	17.1^{b}	18.0^{a}	17.6^{ab}	17.8^{ab}
C18:0	6.4	4.5^{b}	4.1^{c}	4.6^{ab}	4.6^{ab}	4.9^{a}	4.8^{ab}
Σ saturates ≥ C16:0	40.1	21.7^{b}	21.7^{b}	22.0^{ab}	22.8^{a}	22.8^{a}	22.7^{a}
Σ saturates	42.8	25.2^{f}	62.0^{a}	29.9^{e}	31.0^{d}	35.8^{c}	38.3^{b}
C16:1n-7	6.6	4.8^{a}	2.9^{d}	4.5^{b}	4.7^{a}	4.3^{c}	4.2^{c}
C18:1n-7	2.7	2.5	1.0	2.5	2.0	2.3	1.0
C18:1n-9	14.8	39.3^{a}	13.7^{d}	34.8^{b}	35.9^{b}	30.2^{c}	30.1^{c}
Σ monoenes	26.0	48.9^{a}	18.8^{d}	43.9^{b}	44.7^{b}	38.7^{c}	37.3^{c}
C18:2n-6	1.9	17.2^{a}	11.7^{e}	16.7^{b}	16.2^{c}	14.8^{d}	14.9^{d}
C20:4n-6	2.4	1.0^{c}	1.3^{b}	1.3^{b}	1.2^{bc}	1.6^{a}	1.6^{a}
Σ n-6	4.8	19.8^{a}	14.7^{c}	19.8^{a}	18.9^{b}	18.4^{b}	18.4^{b}
C18:3n-3	1.5	2.0^{ab}	1.4^{c}	2.2^{a}	1.7^{bc}	1.7^{bc}	1.5^{c}
C20:5n-3	4.1	0.4	0.4	0.4	0.4	0.5	0.5
C22:6n-3	15.8	1.6^{c}	1.8^{bc}	1.9^{b}	1.8^{bc}	2.3^{a}	2.3^{a}
Σ n-3	22.6	4.4^{ab}	3.8^{b}	4.7^{a}	4.1^{ab}	4.8^{a}	4.6^{a}

* Values are means of three replicates except for larval theoretical biomass and mean wet weight of larvae fed M1 (only one replicate), larval theoretical biomass and mean wet weight of larvae fed TO and M2 (two replicates) and fatty analyses of larvae fed M2 and T2 (two replicates). Within rows, means for fed groups not sharing a common superscript letter are significantly different; –, not detected; t, trace value < 0.1%; Σ saturates includes C15:0 and C17:0; Σ monoenes includes C14:1, C17:1 and C20:1; Σ n-6 includes C18:3n-6, C20:2n-6, C20-3n-6 and C22:5n-6; Σ n-3 includes C18:4n-3 and C22:5n-3.

Table IV. Survival and growth parameters of larvae in trial 2*.

Dietary treatment	TO	T2	T4	T6	T8	T10
Dietary level of C8:0 (% of total fatty acids)	–	12	24	36	48	60
Final survival (%)	92	77	69	72	78	84
Final mean wet weight (mg)	33.7^{a}	18.2^{bc}	22.6^{b}	17.2^{bc}	11.9^{c}	7.3^{d}
Final theoretical biomass (g)	3.1^{a}	1.4^{b}	1.5^{b}	1.3^{b}	0.9^{bc}	0.6^{c}
Total length (mm)						
day 7	8.4	8.2	8.1	8.3	8.4	8.2
day 11	10.0^{a}	9.1^{b}	9.4^{b}	9.2^{b}	9.0^{b}	9.1^{b}
day 15	11.5^{a}	10.0^{ab}	10.6^{ab}	10.6^{ab}	9.9^{ab}	9.4^{b}
day 21	14.2^{a}	12.2^{b}	11.6^{b}	12.4^{b}	10.5^{c}	9.7^{c}

* Values are means of three replicates. Within rows, means not sharing a common superscript letter are significantly different.

differences in levels of C8:0 in larval total lipids were found: larvae fed CO contained only traces of C8:0 vs. 0.9 ± 0.2% for larvae fed M1 and T1. For the high dietary C8:0 level (18%), larvae fed M2 contained lower amounts of C8:0 than larvae fed T2 (1.2 ± 0.0% vs. 2.1 ± 0.4%, respectively). Larvae fed M2 contained also lower amounts of C10:0 than larvae fed T2 (7.3 ± 0.2% vs. 9.0 ± 1.2%, respectively).

In trial 2, 21-day larvae fed T8, which exhibited significantly higher growth rates than larvae fed T10, contained higher levels of C8:0 than larvae fed T10 (1.2 ± 0.3% vs. 0.3 ± 0.0%, respectively). A significant increase of C10:0 level was recorded in 21-day larvae fed T10, which displayed poor growth rates, compared to larvae fed T8 or TO (6.4 ± 1.2% vs. 1.4 ± 0.5%, respectively).

Table V. Main fatty acid composition of total lipid of larvae in trial 2 (g·100 g^{-1} total fatty acids)*.

Day of sampling	day 0	day 13	day 13	day 13	day 21	day 21	day 21
Dietary treatment	initial	TO	T8	T10	TO	T8	T10
Dietary level of C8:0 (% of total fatty acids)	–	–	48	60	–	48	60
Larval theoretical biomass (g)	0.2	1.0	0.5	0.4	3.1^{a}	0.9^{b}	0.6^{b}
Larval mean wet weight (mg)	1.9	11.0	5.5	4.9	33.7^{a}	11.9^{b}	7.3^{c}
Larval dry matter (%)	12.3	14.9	13.1	12.6	17.7^{a}	15.0^{b}	12.4^{c}
Larval total lipid (%)	2.6	4.5	4.3	2.5	6.8^{a}	5.8^{b}	2.7^{c}
C8:0	–	–	0.1	0.1	–	1.2^{a}	0.3^{b}
C10:0	2.1	1.1	2.9	2.6	1.2^{b}	1.6^{b}	6.4^{a}
C12:0	–	0.2	0.1	0.1	0.4^{a}	0.4^{a}	0.1^{b}
Σ MCFA	2.1	1.3	3.2	2.8	1.5^{c}	3.2^{b}	6.8^{a}
C14:0	0.8	1.7	1.1	1.0	2.1^{a}	1.6^{b}	1.0^{c}
C16:0	28.0	16.6	20.3	22.3	15.8^{c}	18.1^{b}	19.8^{a}
C18:0	6.4	5.7	9.5	9.1	4.4^{b}	7.3^{a}	7.6^{a}
Σ saturates ≥ C16:0	35.0	22.8	30.3	32.3	20.5^{c}	25.8^{b}	28.6^{a}
Σ saturates	41.8	28.3	38.1	42.3	26.0^{c}	32.4^{b}	43.4^{a}
C16:1n-7	7.2	4.2	2.8	3.1	4.7^{a}	3.6^{b}	2.3^{c}
C18:1n-7 + 18:1n-9	21.3	40.8	25.6	14.4	43.2^{a}	33.6^{b}	12.4^{c}
Σ monoenes	32.4	48.5	31.9	20.7	51.2^{a}	40.9^{b}	18.2^{c}
C18:2n-6	2.4	12.9	9.1	6.4	14.2^{a}	13.9^{a}	6.8^{b}
C20:4n-6	2.2	1.8	4.0	4.2	1.1^{c}	2.6^{b}	3.9^{a}
Σ n-6	6.0	17.5	16.9	14.6	17.3^{b}	19.3^{a}	14.5^{c}
C18:3n-3	0.2	0.9	0.6	0.4	1.3^{a}	1.2^{a}	0.5^{b}
C20:5n-3	3.1	0.6	1.2	1.5	0.4^{b}	0.7^{b}	1.8^{a}
C22:6n-3	12.1	2.9	7.7	11.2	1.7^{c}	4.1^{b}	11.3^{a}
Σ n-3	16.3	4.7	10.0	13.9	3.6^{c}	6.4^{b}	14.4^{a}

* Values are means of three replicates except for larvae fed T10, only two replicates. Within rows, means of 21-day larvae not sharing a common superscript letter are significantly different; –, not detected; Σ saturates includes C15:0 and C17:0; Σ monoenes includes C14:1, C17:1, C20:1 and C22:1; Σ n-6 includes C18:3n-6, C20:2n-6, C20-3n-6 and C22:5n-6; Σ n-3 includes C18:4n-3, C20:4n-3 and C22:5n-3.

4. DISCUSSION

Present results confirm that coconut oil constitutes an excellent energy source in artificial diets for carp larvae (Fontagné et al., 1999), as also found for African catfish larvae (Legendre et al., 1995). Feeding coconut oil instead of triolein greatly altered the fatty acid profile of larval lipid but did not change the survival and growth performances.

Compared to coconut oil, which can be fed without negative effect up to 10% in diet, with C12:0 amounting up to 37% of total dietary fatty acids, use of MCT oil consisting of C8:0 and C10:0 appeared more limited without affecting growth or survival. In another experiment, no significant decrease of growth was noted in carp larvae fed C10:0 supplied as tricaprin as sole MCFA compared to larvae fed triolein (Fontagné et al., 1999). This suggests that the decreased growth presently observed is specifically due to C8:0. At the lowest MCT level tested (1.7% in diet) equivalent to 1% C8:0 in diet or 8% C8:0 in total fatty acids, MCT appeared as efficient as triolein whereas at the highest level tested (4.2% in diet) equivalent to 2.5% C8:0 in diet or 18% C8:0 in total fatty acids, survival and growth were decreased. Since in trial 2 the C8:0 level of 12% of total fatty acids (2% in diet) decreased significantly larval growth, results of both trials suggest a metabolic alteration when C8:0 exceeds about 10% of total fatty acids, at least for diets relatively rich in lipids and poor in carbohydrates as presently tested. Similar results were recorded by Davis et al. (1999) in juvenile red drum fed 2–8% MCT oil (14–38% 8:0 in total dietary fatty acids). Larger fish seem to tolerate higher levels, as no growth depression was reported in ayu and tilapia juveniles fed diets with up to 3.8 to 5.7% MCT oil (Mustafa et al., 1991; Nakagawa and Kimura, 1993; Nakagawa and Kusunoki, 1990; Nematipour et al., 1990). Besides, the fish response, as that of mammals, is likely influenced by the other dietary ingredients and especially by carbohydrates (Aurousseau et al., 1984).

General changes in the fatty acid composition of total lipid related to growth observed in the present trials are in agreement with the variations observed in the phosphatidylcholine fraction of carp larvae (Geurden et al., 1999), especially the increase of n-6 fatty acids compensating the decrease of n-3 fatty acids. Larvae fed coconut oil were characterised by large levels of C12:0 and C14:0, which are minor fatty acids in first-feeding larvae and in larvae fed diets without these fatty acids. Similar changes were reported in larger fish (Craig and Gatlin, 1995; Nematipour and Gatlin, 1993) and in mammals (Demarne et al., 1981; Jaturasitha et al., 1996). The deposition of C12:0 and C14:0 appears to be performed at the expense of oleic acid, and thus increases the global saturation of the depot fat without noticeable effect on the n-3 and n-6 fatty acid levels in body lipid. Demarne et al. (1978) reported a similar increase of saturates in rats fed trilaurin compared to rats fed a lipid-free diet. These authors attributed the decreased level of monoenes in trilaurin-fed rats to the inhibition by C12:0 of fatty acid *de novo* synthesis from acetyl units associated to a reduced elongation of C12:0. In the present study, however, it is not possible to check this hypothesis as the reference diet consisted of large amounts of monoenes, which are supposed to be directly deposited in larval total lipids.

Compared to the deposition of C12:0 observed with coconut oil, the deposition of C8:0 and C10:0 in larval lipids was modest, as was the associated reduction of monoenes. C8:0 was much less deposited than the C10:0 as shown in the different ratios in diet and larvae. This change can be explained partly by the lower incorporation of 8:0 into storage triacylglycerols, as evidenced in yearling carp (Shikata et al., 1994), piglets (Newport et al., 1979), goats (van den Top et al., 1995) and infants (Sarda et al., 1987). The level of C8:0 in larvae appeared affected by the form of dietary supply. Whereas C8:0 was not deposited at all in larvae fed coconut oil, feeding homogeneous instead of interesterified MCT, which did not affect growth, increased deposition of C8:0. Demarne et al. (1981) reported a higher deposition of C12:0 in rats fed trilaurin instead of coconut oil, and explained this increase by a preferential lymphatic absorption and deposition of the dietary MCFA located in the sn-2 position (Demarne et al., 1981; Ikeda et al., 1991). The small difference presently observed for C8:0 in carp larvae is also possibly related to a differential action of the pancreatic lipase and the absorption of a slightly higher amount of C8:0 as monoacylglycerol than as free fatty acid.

As previously observed (Fontagné et al., 1999), feeding purified tricaprylin results in the deposition of high amount C10:0, up to 6% of total fatty acids in 7 mg larvae fed 10% tricaprylin in trial 2. Lower levels of C10:0 found in larvae fed 8% tricaprylin (2.9% in 5.5 mg larvae on day 13 and 1.6% in 12 mg larvae on day 21) are likely related to a better nutritional status. In mammals fed with tricaprylin, C8:0 is completely degraded into acetyl units before eventual reutilisation of these units for resynthesis of fatty acids like palmitic acid (Carnielli et al., 1994; Demarne et al., 1978; Hwang et al., 1992) and no deposition of intermediate products such as C10:0 is expected. The high level of C10:0 presently observed in larvae fed high levels of tricaprylin, associated to a markedly decreased growth, can thus be interpreted as indicating a strongly altered fatty acid metabolism. In conclusion, the present work suggests that high levels of MCT oil rich in caprylic and capric acids should be avoided in practical diets for carp larvae, whereas no detrimental effects were observed for coconut oil, up to 10% in the diet.

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