Journal of Marine Science and Technology

Volume 30 | Issue 1 Article 1

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Recommended Citation

Chu, Jen-Hong; Hong, Yu-Cong; Chien, Angela; and Sheen, Shyn-Shin (2022) "Effects of dietary lipid, L-carnitine and L-lysine supplementation on the growth performance and body composition of Epinephelus lanceolatus larvae," *Journal of Marine Science and Technology*: Vol. 30 : Iss. 1 , Article 1.
DOI: 10.51400/2709-6998.2561
Available at: https://jmstt.ntou.edu.tw/journal/vol30/iss1/1

RESEARCH ARTICLE

Effects of Dietary Lipid, L-carnitine and L-lysine Supplementation on the Growth Performance and Body Composition of *Epinephelus lanceolatus* Larvae

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Abstract

The effects of different combinations of dietary lipid, L-carnitine and L-lysine on the growth performance and fatty acid profiles of larval grouper *Epinephalus lanceolatus* were evaluated. Eight treatment diets were formulated based on a 2 × 2 × 2 factorial design, namely 5 and 14% dietary oil mixture (3:1 fish oil/soybean oil), 0 and 0.5% L-carnitine and 0 and 2.83% L-lysine. Each diet was randomly assigned to triplicate groups of ten 0.08 g *E. lanceolatus* larvae for 42 days. The grouper larvae fed with diets containing 14% lipid had significantly higher weight gain percentages than those fed with diets containing 5% lipid. The weight gain percentage of the grouper larvae fed with a diet containing both 14% lipid and 0.5% L-carnitine without added L-lysine was found to be the highest, while the addition of dietary 2.83% L-lysine failed to have any additive effect on the growth performance. With the dietary lipid being 14%, not only dietary L-carnitine but also L-lysine is able to decrease lipid and increase protein levels in grouper muscle. The supplementation of dietary L-carnitine with a high level of lipid is also able to reduce liver lipid level of groupers. The levels of n-3 highly unsaturated fatty acid (n-3 HUFA) analyzed from muscle and liver of grouper larvae fed with diets supplemented with L-carnitine were significantly lower than those of grouper larvae fed with diets devoid of L-carnitine. This study indicated that the diets containing a high lipid level supplemented with L-carnitine are able to enhance the growth performance of grouper larvae.

Keywords: *Epinephelus lanceoratus*, L-carnitine, L-lysine, Lipid, Fatty acid

1. Introduction

Grouper culture is the most developed aquaculture industry in the Asia−Pacific regions, including Taiwan, Indonesia, Malaysia, Thailand, Hong Kong and Mainland China [39]. In Taiwan, grouper culture can be divided into four stages: the first stage, from eggs to 0.5 g larvae; the second stage, from 0.5 g larvae to 7 g fingerlings; the third stage, from 7 g fingerlings to adults; the fourth stage, a broodstocks [35]. In the first stage, the larvae live on their endogenous nutrition from egg sacs for 3 days after hatching (DAH) and then open their mouths to prey on living organisms [45,53], such as copepod nauplii and small-sized rotifer. The living organisms are crucial to survival and larvae growth in terms of essential nutrients [58]. Unfortunately, the live prey is inclined to accompany pathogens that could cause disease during larval cultivation. Thus, the artificial diets, known to be devoid of pathogens, become suitable substitutes for living organisms [5]. In order to enhance the growth performance of fish larvae, particularly *Epinephalus lanceolatus*, the evaluation of dietary nutritional requirements is necessary [32,36]. The lipid requirement for *E. lanceolatus* larvae ranged from 5 to 20%,

Received 30 December 2019; revised 27 October 2020; accepted 9 November 2021.
Available online 15 March 2022.

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https://doi.org/10.514/2709-6998.2561

with the optimum being 7.5% [14]. However, the diets containing 14% lipid would seem to enhance the growth performance of *E. lanceolatus* larvae compared with those containing 5% lipid [13]. Similarly [22,42], have shown that the high dietary lipid levels improve protein utilization and the growth performance of hybrid striped bass and African catfish larvae.

L-carnitine, a hygroscopic soluble component of 161.2 KDa, is not only a derivative from lysine but also a growth promoter synthesized in liver [24]. It is transported to skeletal and cardiac muscle tissues that utilize fatty acids as primary fuel through β-oxidation in mitochondria [24]. The dietary L-carnitine have been shown to enhance the growth performance of red sea bream [10,11], African catfish [54], hybrid striped bass [55], European sea bass [48], and tilapia [3,19,28]. However, negative effects on the growth performance has been found in channel catfish [7], rainbow trout [9,47], ornamental cichlid (*Pelvicachromis pulcher*) [25], European sea bass [17], hybrid striped bass [22,23], tilapia [49] and Atlantic salmon [29]. Apparently, the effects of dietary L-carnitine on the growth performance of European sea bass [17,48], tilapia [3,19,28,49] and hybrid striped bass [22,23,55] are contradictory. Such versatile effects of L-carnitine on fish growth may be attributed to several factors, such as the amount of L-carnitine intake, the presence of other dietary components, fish species, fish life stages and initial fish size [24,50]. The addition of dietary L-carnitine is able to decrease the liver and muscle lipid content of sea bass [10,11]. To increase the dietary lipid utilization, L-carnitine is likely to have positive effects on lipid metabolism of fish [9,57].

An important function of lysine serves as the precursor of carnitine that carries long chain fatty acid into mitochondria for β-oxidation [33,56]. At first, the carnitine in animals is biosynthesized from extrahepatic protein-bound trimethyllysine. After degradation, the free trimethyllysine is released and converted to γ-butyrobetaine, which is transported to liver for eventual hydroxylation into carnitine [2]. The lack of lysine not only limits the synthesis of trimethyllysine, but also reduces carnitine production in a body [6,16]. Thereafter, the reduced carnitine synthesis might contribute to an increase in lipid accumulation since the fatty acids fail to be transported into mitochondria for β-oxidation [16]. A decrease in muscle protein and weight gain could be observed in Atlantic salmon fed the diets containing a limited amount of L-lysine [20,46]. A high liver lipid deposition have also been found in Atlantic salmon fed low-lysine diets [46].

Compared with those fed diets containing 14% lipid without L-carnitine, the *E. lanceolatus* fed with a diet containing 14% lipid supplemented with 0.5% L-carnitine gained more weight [13]. Since lysine is a component that constitutes the backbone of carnitine, the addition of dietary lysine might be able to affect the growth and body composition of grouper larvae. In this study, a factorial design was undertaken to investigate the effects of lipid, L-carnitine and L-lysine on the growth performance, body composition and fatty acid profiles of 0.08 g *Epinephelus lanceolatus* larvae.

2. Materials and methods

2.1. Experimental diets

The ingredient compositions of the eight treatment diets formulated by a 2 × 2 × 2 factorial design are shown in Table 1. Three nutrient factors were tested at two levels: lipid, 5% and 14%; L-carnitine, 0% and 0.5%; and L-lysine, 0% and 2.83%. The lipid source is the addition of a mixture of 2:1 fish oil and soybean oil. To exclude other extra dietary lipid, the fish meal and shrimp meal were defatted using hot ethanol (1:1, w/v) for three successive times prior to formulation. Mixed ingredients were cold-extruded

Table 1. Feed formula and proximate analysis of experiment diets for grouper larvae, E. lanceolatus

Ingredients	Treatments							
	A	B	C	D	E	F	G	H
Basal mix[a]	68	68	68	68	68	68	68	68
Lipid[b]	5	5	5	5	14	14	14	14
α-Starch	27	26.5	24.17	23.67	18	17.5	15.17	14.67
L-Carnitine	0	0.5	0	0.5	0	0.5	0	0.5
L-Lysine	0	0	2.83	2.83	0	0	2.83	2.83
Analyzed composition (as fed)								
Moisture	8.68	8.41	8.03	8.02	9.22	8.92	7.92	8.30
Crude protein[c]	44.99	44.73	49.06	49.48	44.78	44.56	49.03	49.29
Crude fat[c]	5.06	5.34	5.64	5.60	14.67	14.71	14.68	14.52
Ash[c]	10.43	11.01	9.59	9.54	10.42	10.62	9.42	9.65
Crude fiber[c]	1.23	1.22	1.56	1.54	1.36	1.45	1.21	1.11
L-Carnitine[d]	0.056	5.046	0.056	5.046	0.056	5.045	0.056	5.041
L-Lysine[e]	3.32	3.30	6.45	6.48	3.30	3.28	6.44	6.46

[a] Lipid-extracted fish meal 54%, lipid-extracted shrimp meal (Jian-Bao Foods Co., Ltd., Taiwan) 6%, β-glucan 1%, yeast (Sun Right foods Co., Ltd., Taiwan) 4%, mineral mix [4] modified) 2%, vitamin mix [12] modified) (vitamin D_3 0.001%, vitamin A 0.06%, α-tocopheryl acetate 0.45%, vitamin K_3 0.4%, thiamine-HCl 0.5%, riboflavin 0.5%, calcium pantothenate 1%, niacin 2%, biotin 0.4%, pyridoxine-HCl 0.06%, folic acid 0.15%, B_{12} 0.001%, Inositol 20%, ascorbic-monophosphate-Mg 2.5%, choline chloride 40%, α-cellulose 31.978%) 1%.
[b] 75% fish oil and 24.8% soybean oil and ethoxyquin 0.2%.
[c] Expressed as percent dry weight.
[d] L-Carnitine (g/kg).
[e] L-Lysine (g/100 g protein).

through a chopper (0.1 cm die diameter) and then dried at 30 °C until the moisture content reduced to approximately 8%. Finally, the diets were ground into 0.2 mm particles using a Motor Grinder RM 100 (Retsch, Germany).

The grouper larvae at 25 days after hatching (DAH) were purchased and transported from a private hatchery farm (886 Apex Aquaculture Co., Ltd., Taiwan) to National Taiwan Ocean University, Taiwan. The 25 DAH larvae were fed with Artemia.in a 2000-L fiber reinforced plastic (FRP) tank. After a week of acclimation, the grouper larvae, which had initial weights of 0.08 g, were randomly distributed into the 24 FRP (45 × 30 × 30 cm), each of which contained 10 fish and 50 L of seawater. The experimental aquaria were supplied with continuous aeration connected to a central air compressor.

The three replicate groups of groupers were fed one of the eight treatment diets twice daily (9:30AM and 5:30PM) until satiation for 42 days. The water was exchanged for approximately 30% for each aquarium to remove the uneaten feed and feces. The water temperatures ranged from 24 to 27 °C. At the end of the feeding trial, feeding was stopped for 24 h prior to weighing, then each fish was individually weighed, sacrificed by placing in iced water and finally carefully dissected. Muscle tissue from each grouper of a given aquarium was isolated and pooled, so was the liver tissue from each grouper of a given aquarium. The weight gain percentage, survival and hepatosomatic index (HSI) were calculated using the following equations:

Weight gain percentage = 100% x $(W_t - W_0)/W_0$ whereW_0 is the initial mean body weight (g), W_t is the final mean body weight (g);

Survival = 100% x $(F_i - F_d)/F_i$ where F_i is the initial number of live fish and F_d is the number of dead fish;

HSI = 100% x (liver weight/fish weight).

2.2. Sample collection and chemical analysis

The proximate compositions of experimental diets, grouper muscle and grouper liver were analyzed according to the method of [1]. Crude protein was determined using a Kjeltec semi-auto-analyzer model 1007 (Tecator, Sweden) after acid digestion. Crude lipid was measured by the chloroform and methanol (2:1, v/v) extraction method [21]. Crude fiber was assessed by acid and alkaline digestion using the Fibertec system M1020 (Foss Tecator, Sweden). Ash and moisture were determined by conventional methods using a muffle furnace at 540 °C for 8 h and an oven at 110 °C for 4 h, respectively.

Dietary L-carnitine was extracted and analyzed using a high performance liquid chromatography (HPLC) equipped with a Hitachi L-6200 Intelligent Pump and a Hitachi L-4200 UV-VIS Detector based on the method of [8]. The mobile phase was 700 ml of 0.1 M ammonium acetate mixed with 300 ml of acetonitrile. The samples were separated on a C18 column (SUPELCO Ascentis™), and the wavelength of detector was set at 248 nm. L-lysine content of diets was extracted and assayed based on the method of Chinese National Standards (CNS) 12632 N 6221 at Food Industry Research and Development Institute, Taiwan.

The crude protein and crude lipid of the experimental diets were in the range of 44.56−49.48% and 5.06−14.71%, respectively. The diets supplemented with 0 and 0.5% L-carnitine actually contained 0.056 and 5.041 g kg^1 L-carnitine, respectively. The diets supplemented with 0 and 2.83% L-lysine actually contained 3.28 and 6.44 g/100 g protein L-lysine, respectively (Table 1).

The fatty acid profiles of the diets, fish muscle and liver were analysed. Muscle and liver tissues isolated from each grouper of a given aquarium were homogenized and analyzed in triplicate after sacrifice. In parallel, the treatment diets were also homogenized and analyzed in triplicate. The total lipid was extracted from homogenized samples [21] and refluxed in 50% KOH for 40 min. The fatty acids were then methylated by refluxing for 20 min in 14% boron trifluoride in methanol (BF_3MeOH) as described by [38] and then extracted with 50 ml ether and 20 ml distilled water in a separtory funnel. Fatty acid methyl esters (FAME) were analyzed using gas−liquid chromatography in a Trace GC 2000 instrument equipped with a flame ionization detector. The FAMEs were separated on a Restek's capillary column (30 m × 0.28 mm, 0.25 μm film thickness, Stabilwax) isothermally at 208 °C. Injection inlet and detector temperature were maintained at 250 °C and 200 °C, respectively. Nitrogen was used as the carrier gas.

Fatty acids were identified by comparison with retention times of a reference standard (GLC-68A, Nu-Check-Prep) consisting of a mixture of saturated and unsaturated fatty acids. In addition, the peaks of chromatograms were compared with identified peaks from a sample of cod liver oil that served as a secondary reference.

2.3. Statistical analysis

Data were analyzed in a completely randomized design using each aquarium as an experimental unit. The log transformation was perform to attain normality. The data were subjected to three-way ANOVA using the Statistical Analysis System (SAS-PC) [30]. If significant differences were indicated at or less than the 0.05 level, the Duncan's Multiple Range Test was used to identify significant differences between treatment means [52].

3. Results

The fatty acid compositions of the treatment diets are shown in Table 2. The levels of polyunsaturated fatty acid (PUFA) of the diets supplemented with 5% and 14% lipid were in the range of 4.38−4.48% and 8.64−8.87%, respectively. The levels of highly unsaturated fatty acid (HUFA) of the diets containing 5% and 14% lipid were 19.26−19.59 and 25.58−25.87%, respectively. The levels of eicosapentaenoic acid (EPA) of the diets supplemented with 5% and 14% lipid were 0.78−0.82% and 1.92−1.99%, respectively, while those of docosahexaenoic acid (DHA) of the diets supplemented with 5% and 14% lipid were 13.28−13.53% and 17.1−17.42%, respectively.

Table 2. The fatty acid composition (% of total fatty acid) of diets for E. lanceolatus

Fatty acid	Treatments							
	A	B	C	D	E	F	G	H
14:0	0.11	0.10	0.10	0.10	0.68	0.88	0.58	0.81
14:1	0.20	0.16	0.21	0.15	0.20	0.19	0.26	0.2
16:0	13.05	13.39	13.29	13.4	15.04	15.14	15.07	15.27
16:1	0.4	0.39	0.52	0.53	0.21	0.23	0.27	0.22
18:0	46.54	45.99	45.54	46.38	32.17	32.37	31.99	32.11
18:1	0.57	0.65	0.78	0.89	0.92	0.73	0.92	0.77
18:2	0.42	0.45	0.49	0.45	0.94	0.76	0.97	0.75
18:3	1.03	1.10	1.04	1.10	3.48	3.52	3.37	3.45
20:0	0.53	0.41	0.5	0.48	0.16	0.19	0.16	0.18
20:1	0.48	0.61	0.51	0.52	0.56	0.57	0.73	0.7
20:2	0.11	0.12	0.12	0.11	0.56	0.52	0.56	0.53
20:3 n-6	1.15	1.15	1.14	1.16	1.15	1.18	1.17	1.18
20:3 n-3	1.67	1.68	1.69	1.64	2.74	2.73	2.74	2.73
20:4	13.53	13.69	13.74	13.06	14.13	14.14	14.15	14.13
20:5n-3	0.78	0.81	0.82	0.79	1.92	1.99	1.98	1.96
22:0	0.20	0.21	0.22	0.23	0.72	0.82	0.74	0.72
22:1	0.53	0.53	0.52	0.54	0.47	0.45	0.47	0.46
22:5n-3	5.27	5.26	5.24	5.19	6.53	6.49	6.48	6.46
22:6 n-3	13.43	13.3	13.53	13.28	17.42	17.1	17.39	17.37
SFA	60.43	60.1	59.65	60.59	48.77	49.4	48.54	49.09
MUFA	2.18	2.34	2.54	2.63	2.36	2.17	2.65	2.35
PUFA	4.38	4.5	4.48	4.46	8.87	8.71	8.81	8.64
n-3 HUFA	19.48	19.37	19.59	19.26	25.87	25.58	25.85	25.79

SFA: saturated fatty acids.
MUFA: monounsaturated fatty acids.
PUFA: polyunsaturated fatty acids (18:2 + 18:3 + 20:2 + 20:3n-6 + 20:3n-3).
HUFA: highly unsaturated fatty acids (20:5n-3 +22:5n-3 + 22:6n-3).

The weight gain percentage, HSI and survival of grouper larvae fed with the experimental diets for 42 days are shown in Table 3. The grouper larvae fed with diets containing 14% lipid had significantly higher weight gain percentages than those fed with diets containing 5% lipid. The grouper larvae fed with the diet containing 14% lipid, 0.5% L-carnitine and 0% L-lysine had the highest weight gain percentage, which is not significantly different from the weight gain percentage of the grouper fed the diet containing 14% lipid, 0.5% L-carnitine and 2.83% L-lysine. Not only dietary lipid but also L-carnitine significantly affected the weight gain percentage and HSI of groupers, while the dietary lipid and L-carnitine together had additive effects on the weight gain percentage and HSI of groupers. Nevertheless, the dietary L-lysine showed no significant effects on the weight gain percentage and HSI of grouper larvae. The survival of the overall fish fed with the treatment diets ranged from 86.7 to 100%.

The muscle lipid, liver lipid and muscle protein levels of groupers fed with the treatment diets are shown in Table 4. The groupers fed with the diet containing 14% lipid, 0% L-carnitine and 0% L-lysine showed the highest muscle lipid level. Whether the L-lysine is included or not, the liver lipid levels of groupers fed with the diets containing 14% lipid without L-carnitine were significantly higher than those of groupers fed with the other treatment diets, while the muscle protein levels of groupers fed with the diets containing 14% lipid and 0.5% L-carnitine were significantly higher than those of groupers fed with the other treatment diets. Both dietary lipid and L-carnitine contributed significant effects on muscle lipid, liver lipid and muscle protein levels. The dietary L-lysine showed significant effects on muscle lipid and protein levels, while no significant effect of dietary L-lysine was observed on the liver lipid level. The dietary lipid and L-carnitine have additive effects only on the muscle protein level.

Fatty acid compositions of muscle of grouper larvae fed experiment diets for 42 days are shown in Table 5. The level of saturated fatty acid (SFA) of the muscle of grouper larvae fed with a diet containing 14% lipid, 0% L-carnitine and 2.83% L-lysine was significantly higher than that of fish fed with the other diets. The levels of mono-unsaturated fatty acid (MUFA) and n-3 HUFA of the muscle of grouper larvae fed with a diet containing 14% lipid, 0% L-carnitine and 0% L-lysine were significantly higher than that of fish fed with the other diets. The levels of n-3 PUFA of the muscle of grouper larvae fed with diets containing 5% lipid and

Table 3. Weight gain (%), HSI and survival of E. lanceolatus fed with experimental diets for 42 days

Dietary treatments			Weight gain (%)	HSI	Survival
Lipid (%)	L-carnitine (%)	L-lysine (%)			
5	0	0	372 ± 95^{d}	3.37 ± 0.53^{b}	96.7 ± 5.8
5	0.5	0	383 ± 61^{d}	2.49 ± 0.03^{bc}	96.7 ± 5.8
5	0	2.83	357 ± 106^{d}	2.93 ± 0.24^{bc}	96.7 ± 5.8
5	0.5	2.83	328 ± 22^{d}	2.37 ± 0.12^{c}	90.0 ± 0
14	0	0	758 ± 25^{bc}	4.97 ± 0.78^{a}	100.0 ± 0
14	0.5	0	1085 ± 298^{a}	2.86 ± 0.16^{bc}	86.7 ± 11.5
14	0	2.83	606 ± 107^{c}	4.80 ± 0.57^{a}	96.7 ± 5.8
14	0.5	2.83	921 ± 25^{ab}	3.03 ± 0.81^{bc}	90.0 ± 10
Main effects					
Lipid (%)					
5			360^{b}	2.79^{b}	
14			843^{a}	3.91^{a}	
L-carnitine (%)					
0			524^{b}	4.02^{a}	
0.5			679^{a}	2.69^{b}	
L-lysine (%)					
0			650	3.42	
2.83			553	3.28	
Interaction (ANOVA, Pr > F)					
Lipid			< 0.0001	< 0.0001	
L-carnitine			0.0078	< 0.0001	
L-lysine			0.0786	0.4960	
Lipid × L-carnitine			0.0054	0.0082	
Lipid × L-lysine			0.2479	0.5038	
L-carnitine × L-lysine			0.8012	0.4268	
Lipid × L-carnitine × L-lysine			0.8931	0.9755	

[a,b,c,d] Means in the same column with different letters are significant differences (P < 0.05). Data are expressed as mean values ± SD (n = 3).

Table 4. The lipid levels of muscle and liver and muscle protein of E.lanceolatus fed with experimental diets for 42 days

Dietary treatment			Muscle lipid	Liver lipid	Muscle protein
Lipid (%)	L-carnitine (%)	L-lysine (%)			
5	0	0	3.37 ± 0.13^{bc}	2.74 ± 0.53^{c}	14.00 ± 0.08^{c}
5	0.5	0	2.61 ± 0.09^{cd}	1.76 ± 0.16^{d}	15.07 ± 0.42^{b}
5	0	2.83	2.87 ± 0.22^{bcd}	2.71 ± 0.50^{c}	15.59 ± 0.33^{b}
5	0.5	2.83	2.15 ± 0.31^{d}	1.19 ± 0.08^{d}	15.66 ± 0.69^{b}
14	0	0	4.42 ± 0.28^{a}	5.60 ± 0.19^{a}	15.00 ± 0.60^{b}
14	0.5	0	3.57 ± 0.05^{b}	3.59 ± 0.46^{b}	20.29 ± 0.37^{a}
14	0	2.83	3.68 ± 1.11^{ab}	5.27 ± 0.54^{a}	15.59 ± 0.26^{b}
14	0.5	2.83	2.55 ± 0.24^{cd}	3.57 ± 0.59^{b}	21.25 ± 1.14^{a}
Main effects					
Lipid (%)					
5			2.75^{b}	2.10^{b}	15.08^{b}
14			3.56^{a}	4.51^{a}	18.03^{a}
L-carnitine (%)					
0			3.59^{a}	4.08^{a}	15.04^{b}
0.5			2.72^{b}	2.53^{b}	18.07^{a}
L-lysine (%)					
0			3.49^{a}	3.42	16.09^{b}
2.83			2.81^{b}	3.18	17.02^{a}
Interaction (ANOVA, Pr > F)					
Lipid			0.0004	< 0.0001	< 0.0001
L-carnitine			0.0002	< 0.0001	< 0.0001
L-lysine			0.0016	0.1856	0.0010
Lipid × L-carnitine			0.4892	0.1021	< 0.0001
Lipid × L-lysine			0.2868	0.7186	0.5054
L-carnitine × L-lysine			0.7430	0.7376	0.5087
Lipid × L-carnitine × L-lysine			0.6715	0.2427	0.1620

[a,b] Means in the same column with different letters are significantly different (P < 0.05).
Data are expressed as mean values ± SD (n = 3).

Table 5. Fatty acid composition (% of total fatty acid) of muscle of E. lanceolatus fed with experiment diets for 42 days.

Fatty acid	Treatments							
	A	B	C	D	E	F	G	H
14:0	5.27 ± 0.12^{b}	4.39 ± 0.20^{c}	2.18 ± 0.34^{d}	3.91 ± 0.50^{c}	5.56 ± 0.08^{b}	6.35 ± 0.26^{a}	3.92 ± 0.13^{c}	4.36 ± 0.78^{c}
14:1	0.69 ± 0.40^{b}	0.56 ± 0.33^{b}	3.14 ± 1.95^{a}	0.98 ± 0.47^{b}	0.39 ± 0.01^{b}	0.50 ± 0.35^{b}	1.50 ± 0.67^{b}	0.52 ± 0.06^{b}
16:0	21.91 ± 0.14^{c}	23.82 ± 0.19^{b}	24.37 ± 0.62^{b}	24.04 ± 0.16^{b}	20.66 ± 0.12^{d}	19.44 ± 0.97^{e}	27.82 ± 0.53^{a}	22.54 ± 0.03^{c}
16:1	8.87 ± 0.50^{bc}	7.39 ± 0.10^{de}	8.54 ± 0.33^{cd}	8.11 ± 0.04^{cd}	15.05 ± 0.17^{a}	9.97 ± 0.90^{b}	6.38 ± 0.15^{e}	7.29 ± 1.74^{de}
18:0	3.58 ± 0.15^{c}	4.80 ± 0.06^{b}	5.55 ± 0.06^{a}	4.81 ± 0.23^{b}	3.26 ± 0.03^{c}	4.45 ± 0.31^{b}	5.41 ± 0.62^{a}	5.54 ± 0.18^{a}
18:1	15.60 ± 0.49^{b}	17.41 ± 0.11^{a}	10.86 ± 1.82^{c}	16.00 ± 0.01^{b}	15.76 ± 0.15^{b}	15.54 ± 0.92^{b}	11.34 ± 0.19^{c}	17.78 ± 0.22^{a}
18:2 n -6	18.36 ± 0.44^{b}	15.76 ± 0.08^{c}	12.89 ± 0.39^{d}	14.69 ± 0.21^{cd}	13.85 ± 0.02^{cd}	22.94 ± 3.52^{a}	18.29 ± 0.46^{b}	20.04 ± 0.69^{b}
18:3 n-3	9.38 ± 0.15^{c}	11.57 ± 0.22^{b}	13.29 ± 0.04^{a}	13.10 ± 0.04^{a}	1.55 ± 0.02^{g}	5.14 ± 0.22^{f}	6.63 ± 0.09^{e}	7.71 ± 1.58^{d}
20:0	0.17 ± 0.05^{bc}	0.18 ± 0.04^{bc}	0.63 ± 0.10^{a}	0.12 ± 0.01^{cd}	0.18 ± 0.01^{bc}	0.07 ± 0.02^{d}	0.23 ± 0.01^{b}	0.11 ± 0.01^{cd}
20:1	0.30 ± 0.12^{bc}	0.27 ± 0.05^{bc}	0.61 ± 0.34^{a}	0.23 ± 0.06^{c}	0.53 ± 0.11^{ab}	0.22 ± 0.02^{c}	0.40 ± 0.12^{abc}	0.30 ± 0.04^{bc}
20:2 n-6	0.29 ± 0.09^{bc}	0.50 ± 0.18^{ab}	0.73 ± 0.33^{a}	0.48 ± 0.03^{ab}	0.15 ± 0.02^{c}	0.44 ± 0.18^{ab}	0.60 ± 0.06^{a}	0.62 ± 0.02^{a}
20:3 n-6	2.78 ± 0.06^{b}	2.72 ± 0.02^{b}	4.00 ± 0.53^{a}	1.88 ± 0.06^{c}	0.05 ± 0.01^{e}	0.99 ± 0.08^{d}	1.84 ± 0.21^{c}	1.76 ± 0.03^{c}
20:3 n-3	0.79 ± 0.05^{de}	1.16 ± 0.02^{bc}	1.02 ± 0.13^{c}	1.20 ± 0.10^{b}	0.66 ± 0.01^{e}	0.85 ± 0.06^{d}	1.36 ± 0.15^{a}	1.35 ± 0.08^{a}
20:4 n-6	0.48 ± 0.11^{b}	0.53 ± 0.08^{b}	0.93 ± 0.21^{a}	0.56 ± 0.01^{b}	0.10 ± 0.01^{c}	0.28 ± 0.03^{c}	0.83 ± 0.22^{a}	0.52 ± 0.04^{b}
20:5n-3	0.47 ± 0.08^{d}	0.51 ± 0.11^{d}	0.57 ± 0.04^{bcd}	0.54 ± 0.05^{cd}	0.68 ± 0.01^{b}	0.67 ± 0.09^{bc}	0.97 ± 0.04^{a}	0.89 ± 0.10^{a}
22:0	5.36 ± 0.27^{c}	4.27 ± 0.11^{f}	4.61 ± 0.26^{ef}	4.89 ± 0.25^{de}	9.52 ± 0.09^{a}	6.01 ± 0.39^{b}	5.30 ± 0.31^{cd}	4.74 ± 0.13^{e}
22:1	0.48 ± 0.11^{c}	0.63 ± 0.06^{b}	0.97 ± 0.17^{a}	0.42 ± 0.09^{c}	0.12 ± 0.01^{e}	0.22 ± 0.02^{de}	0.77 ± 0.08^{b}	0.36 ± 0.02^{cd}
22:5 n-3	1.39 ± 0.44^{bc}	0.97 ± 0.06^{d}	1.31 ± 0.28^{bcd}	1.23 ± 0.04^{cd}	3.01 ± 0.10^{a}	1.51 ± 0.24^{bc}	1.70 ± 0.11^{b}	1.14 ± 0.09^{cd}
22:6 n-3	3.85 ± 0.80^{c}	2.57 ± 0.28^{d}	3.81 ± 0.29^{c}	2.81 ± 0.13^{d}	8.90 ± 0.45^{a}	4.41 ± 0.46^{bc}	4.71 ± 0.18^{b}	2.44 ± 0.24^{d}
SFA	36.29 ± 0.19^{c}	37.46 ± 0.30^{c}	37.34 ± 0.76^{c}	37.77 ± 0.23^{c}	39.18 ± 0.14^{b}	36.32 ± 1.94^{c}	42.68 ± 0.21^{a}	37.29 ± 0.71^{c}
MUFA	25.93 ± 0.38^{b}	26.25 ± 0.32^{b}	24.12 ± 0.06^{c}	25.74 ± 0.42^{b}	31.86 ± 0.33^{a}	26.44 ± 1.66^{b}	20.39 ± 0.64^{d}	26.24 ± 1.50^{b}
HUFA	6.20 ± 0.47^{c}	4.58 ± 0.15^{d}	6.61 ± 0.66^{c}	5.13 ± 0.13^{d}	12.69 ± 0.47^{a}	6.87 ± 0.36^{c}	8.20 ± 0.35^{b}	4.99 ± 0.25^{d}
n-3 PUFA	10.17 ± 0.15^{c}	12.72 ± 0.15^{b}	14.31 ± 0.15^{a}	14.29 ± 0.13^{a}	2.21 ± 0.03^{g}	5.99 ± 0.25^{f}	8.00 ± 0.20^{e}	9.06 ± 1.6^{d}
n-6 PUFA	21.42 ± 0.43^{bc}	18.98 ± 0.17^{cd}	17.62 ± 0.47^{d}	17.05 ± 0.15^{d}	14.05 ± 0.02^{e}	24.37 ± 3.68^{a}	20.73 ± 0.30^{bc}	22.42 ± 0.67^{ab}
n-3 HUFA	5.72 ± 0.42^{d}	4.06 ± 0.21^{e}	5.68 ± 0.53^{d}	4.58 ± 0.13^{e}	12.59 ± 0.46^{a}	6.60 ± 0.35^{c}	7.37 ± 0.15^{b}	4.47 ± 0.22^{e}

^{a,b,c,d,e,f,g} Mean in the same column with the different letter are significantly different ($P < 0.05$). Data are expressed as mean values ± SD ($n = 3$).
SFA: saturated fatty acids.
MUFA: monounsaturated fatty acids.
PUFA: polyunsaturated fatty acids (18:2 + 18:3 + 20:2 + 20:3n-6 + 20:3n-3).
HUFA: highly unsaturated fatty acids (20:5n-3 +22:5n-3 + 22:6n-3).

2.83% L-lysien supplemented with or without L-carnitine and L-lysine were significantly higher than that of fish fed with the other diets. The level of n-6 PUFA of the muscle of grouper larvae fed with a diet containing 14% lipid, L-carnitine and 0% L-lysine was significantly higher than that of fish fed with the other diets.

Fatty acid compositions of liver of grouper larvae fed with treatment diets for 42 days are shown in Table 6. The levels of SFA and MUFA of the liver of grouper larvae fed with a diet containing 5% lipid, L-carnitine and 0% L-lysine were significantly higher than those of fish fed the other diets. The level of HUFA of the liver of grouper larvae fed with a diet containing 14% lipid without L-carnitine and L-lysine was significantly higher than that of fish fed with the other diets. The level of n-3 PUFA of liver of grouper larvae fed with a diet containing 5% lipid without L-carnitine and L-lysine was significantly higher than that of grouper larvae fed with the other diets. The level of n-6 PUFA of liver of grouper larvae fed a diet containing 5% lipid, L-carnitine and L-lysine supplementation and a diet containing 14% lipid, 2.83% L-lysine and 0% L-carnitine were significantly higher than that of fish fed the other diets.

4. Discussion

The grouper larvae fed a diet containing 0.5% L-carnitine and 14% lipid had the best growth performance in this study. The addition of dietary L-lysine, however, would not be able to promote the growth performance of grouper larvae even though the diet has contained 14% lipid and 0.5% L-carnitine. The weight gain of Indian major carp, *Cirrhinus mrigala* larvae fed the diets containing 6% lipid and 0.25−1% L-carnitine is significantly higher than that of fish fed the diet containing 6% lipid without added L-carnitine [51]. Moreover, the indian major carp fed the diet containing 0.25% L-carnitine and 6% lipid showed the best growth performance [51]. With a high level of dietary lipid, positive effects of dietary L-carnitine are also observed on the growth performance of fish, such as the Beluga sturgeon, *Huso huso* fed the diet consisting of 0.03% L-carnitine and 16% lipid [40], the black sea bream, *Sparus*

Table 6. Fatty acid composition (% of total fatty acid) of liver of E. lanceolatus fed with experiment diets for 42 days

Fatty acid	Treatments							
	A	B	C	D	E	F	G	H
14:0	11.26 ± 0.79^{b}	12.41 ± 0.73^{a}	8.88 ± 0.92^{de}	9.74 ± 0.67^{cd}	10.25 ± 0.09^{bc}	9.54 ± 0.07^{cd}	6.82 ± 0.58^{f}	8.29 ± 0.04^{e}
16:0	27.42 ± 0.43^{b}	29.62 ± 0.43^{a}	26.55 ± 0.58^{b}	27.43 ± 1.71^{b}	21.33 ± 0.27^{d}	24.35 ± 0.06^{c}	23.80 ± 0.26^{c}	24.13 ± 0.12^{c}
16:1	14.71 ± 0.36^{b}	18.50 ± 0.50^{a}	13.98 ± 0.15^{c}	12.76 ± 0.45^{d}	12.67 ± 0.21^{d}	14.98 ± 0.11^{b}	8.78 ± 0.17^{f}	11.95 ± 0.09^{e}
18:0	2.74 ± 0.08^{c}	2.64 ± 0.09^{c}	2.06 ± 0.09^{d}	2.64 ± 0.28^{c}	4.58 ± 0.19^{a}	3.74 ± 0.02^{b}	3.76 ± 0.07^{b}	3.84 ± 0.01^{b}
18:1	11.57 ± 0.25^{c}	15.29 ± 0.24^{b}	15.37 ± 2.24^{b}	12.51 ± 2.02^{c}	8.79 ± 0.05^{d}	14.57 ± 0.02^{b}	15.48 ± 0.23^{b}	18.58 ± 0.02^{a}
18:2 n-6	10.98 ± 0.25^{f}	8.57 ± 0.18^{g}	16.31 ± 0.60^{c}	17.91 ± 0.75^{b}	14.56 ± 0.44^{d}	11.74 ± 0.08^{e}	19.50 ± 0.19^{a}	18.48 ± 0.06^{b}
18:3 n-3	3.33 ± 0.05^{c}	3.03 ± 0.27^{d}	4.44 ± 0.12^{a}	3.77 ± 0.20^{b}	2.51 ± 0.05^{e}	2.86 ± 0.01^{d}	3.30 ± 0.07^{c}	2.85 ± 0.03^{d}
20:2 n-6	0.37 ± 0.14^{b}	0.61 ± 0.23^{a}	0.40 ± 0.12^{b}	0.73 ± 0.06^{a}	0.35 ± 0.06^{b}	0.37 ± 0.01^{b}	0.43 ± 0.03^{b}	0.71 ± 0.02^{a}
20:3 n-6	3.44 ± 0.19^{a}	1.15 ± 0.10^{d}	2.38 ± 0.41^{b}	2.30 ± 0.26^{b}	0.95 ± 0.05^{d}	1.59 ± 0.05^{c}	1.53 ± 0.10^{c}	0.82 ± 0.01^{d}
20:3 n-3	3.73 ± 0.01^{a}	1.56 ± 0.06^{de}	2.40 ± 0.11^{c}	2.73 ± 0.29^{b}	1.35 ± 0.14^{e}	1.64 ± 0.01^{d}	1.66 ± 0.05^{d}	0.52 ± 0.02^{f}
20:4 n-6	0.31 ± 0.06^{b}	0.26 ± 0.08^{b}	0.29 ± 0.08^{b}	0.67 ± 0.16^{a}	0.19 ± 0.03^{b}	0.19 ± 0.02^{b}	0.17 ± 0.02^{b}	0.18 ± 0.01^{b}
20:5n-3	0.56 ± 0.06^{e}	0.54 ± 0.11^{e}	0.36 ± 0.05^{e}	0.42 ± 0.09^{e}	4.00 ± 0.20^{b}	2.89 ± 0.41^{c}	5.00 ± 0.36^{a}	2.25 ± 0.05^{d}
22:5 n-3	0.85 ± 0.07^{c}	0.48 ± 0.06^{de}	0.57 ± 0.06^{d}	0.43 ± 0.05^{e}	2.21 ± 0.08^{a}	1.50 ± 0.02^{b}	0.87 ± 0.09^{c}	0.82 ± 0.01^{c}
22:6 n-3	3.42 ± 0.67^{b}	1.50 ± 0.15^{d}	2.28 ± 0.36^{c}	0.79 ± 0.08^{e}	6.57 ± 0.16^{a}	3.74 ± 0.05^{b}	3.34 ± 0.19^{b}	2.53 ± 0.26^{c}
SFA	45.48 ± 0.27^{b}	46.97 ± 0.43^{a}	38.26 ± 1.12^{d}	42.43 ± 1.82^{c}	45.38 ± 0.06^{b}	43.07 ± 0.10^{c}	38.91 ± 0.24^{d}	39.31 ± 0.10^{d}
MUFA	27.52 ± 0.59^{d}	35.35 ± 0.35^{a}	32.37 ± 2.04^{b}	27.80 ± 1.67^{d}	22.00 ± 0.11^{f}	30.43 ± 0.23^{c}	25.28 ± 0.40^{e}	31.52 ± 0.08^{bc}
HUFA	5.15 ± 0.72^{d}	2.77 ± 0.25^{f}	3.51 ± 0.38^{e}	2.31 ± 0.23^{f}	12.96 ± 0.18^{a}	8.31 ± 0.41^{c}	9.38 ± 0.50^{b}	5.78 ± 0.25^{d}
n-3 PUFA	7.06 ± 0.12^{a}	4.59 ± 0.28^{cd}	6.84 ± 0.19^{ab}	6.50 ± 0.46^{b}	3.87 ± 0.19^{e}	4.50 ± 0.02^{d}	4.96 ± 0.10^{c}	3.37 ± 0.01^{f}
n-6 PUFA	14.79 ± 0.32^{e}	10.33 ± 0.06^{g}	19.10 ± 0.92^{c}	20.94 ± 0.47^{a}	15.86 ± 0.33^{d}	13.69 ± 0.13^{f}	21.46 ± 0.14^{a}	20.01 ± 0.08^{b}
n-3 HUFA	4.84 ± 0.67^{e}	2.52 ± 0.18^{g}	3.21 ± 0.45^{f}	1.64 ± 0.22^{h}	12.77 ± 0.19^{a}	8.12 ± 0.42^{c}	9.21 ± 0.50^{b}	5.60 ± 0.26^{d}

^{a,b,c,d,e,f,g,h} Mean in the same column with the different letter are significantly different($P < 0.05$). Data are expressed as mean values ± SD ($n = 3$).
SFA: saturated fatty acids.
MUFA: monounsaturated fatty acids.
PUFA: polyunsaturated fatty acids.
HUFA: highly unsaturated fatty acids.

macrocephalus fed with diets containing 0.01−0.024% L-carnitine and 12.6% lipid [37], the African catfish, *Clarias gariepinus* and grouper larvae, *E. lanceolatus* fed with the diets containing 0.05% L-carnitine and 10% lipid [13,54]. Nevertheless, no improvement was found on the growth performance of fish fed with the diet containing lower than 0.1% L-carnitine, such as the rainbow trout, *Oncorhynchus mykiss* [50], juvenile hybrid striped bass, *Morone chrysops* female × *Morone saxatilis* male [22], and hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus* [57]. Such different effects on the growth performance of fish might be due to the level of L-carnitine supplementation, dietary composition, fish species, fish size, and the stability of diets [24,44].

The grouper larvae fed with diets supplemented with L-carnitine had lower lipid levels in muscle and liver than those fed with diets without L-carnitine supplementation. The acceleration of fatty acid oxidation and nitrogen retention were observed in Atlantic salmon fed with diets supplemented with L-carnitine which regulates the transport of triglycerides across the inner mitochondrial membrane [17,29]. Despite L-carnitine not being an essential nutrient [24], the dietary L-carnitine supplementation has been demonstrated to enhance the oxidation of triglycerides, the protein-sparing effect and the fish growth [18,27,54]. In this study, grouper larvae may need more energy for growth and thus the L-carnitine supplementation increase the available energy produced through β-oxidation. Furthermore, the HSI of grouper larvae fed with the diets containing a high lipid level without L-carnitine supplementation was high, while that of grouper fed the diets containing a high level of lipid supplemented with dietary L-carnitine was comparatively low. Similarly, the HSI of black sea bream, *Sparus microcephalus*, decreased as the fish were fed with diets containing 0.01−0.11% L-carnitine with the dietary lipid being 12.6% [37]. The HSI of rohu, *Labeo rohita,* fed with the diets containing 6.28% lipid supplemented with 0.05 and 0.1% L-carnitine was lower than that fed with the diet containing 6.28% lipid without added L-carnitine [31]. Given that fish would accumulate lipid in liver and subcutaneous tissues [15], the reduced HSI, muscle and liver lipid levels of grouper larvae is likely to be related to lipid metabolism by supplementation of L-carnitine.

Compared with that of grouper larvae fed diets containing 14% lipid without supplementation of L-carnitine and L-lysine, the liver of grouper larvae fed diets containing 14% lipid supplemented with L-carnitine had higher levels of mono-unsaturated fatty

acid (MUFA) and lower levels of n-3HUFA in this study. An increase in MUFA and a decrease in n-3 HUFA levels were also detected from *E. lanceolatus* liver as the dietary L-carnitine was supplemented [13]. The reduced amount of C20-22 fatty acids in the liver of red sea bream, *Pagrus major* was observed as the concentration of dietary L-carnitine increase to 0.21% [11]. The tissue of African catfish fed with diets containing L-carnitine showed reduced levels of eicosapentaenoic acid and docosahexaenoic acid [43]. The rainbow trout fed with diets supplemented with L-carnitine also showed an increase in C14-18 and a decrease in C20-22 fatty acid [18]. [9] indicated that carnitine is required for the oxidation of long-chain fatty acids by mitochondria. Therefore, it can be inferred that L-carnitine may increase the oxidation rate of highly polyunsaturated fatty acids in muscle and liver tissues.

A decrease in lipid and an increase in protein levels were detected in the muscle of grouper larvae fed with the diets supplemented with either L-carnitine or L-lysine in this study and in [13]. Despite the supplementation of both L-lysine and L-carnitine, no additive effect was observed in terms of lipid and protein deposition of grouper larvae in this study. Similarly, the diet supplemented with lysine was found to increase the protein and decrease the lipid levels in channel catfish [34,41]. However, the lipid and protein levels in the muscle of red sea bream, *P. major* fed the diet containing 1% lysine supplemented with 0.2% carnitine were not significantly different from those fed the diet containing 1% lysine without carnitine [11]. The effects of dietary carnitine or lysine on the protein and lipid levels in muscle tissues seem to be dependent on fish species. Even though lysine serves as a precursor to synthesize carnitine [26,56], the supply of lysine to a diet containing carnitine cannot yield an additive effect on nutrition deposition in fish.

In conclusion, a diet containing 14% lipid and 0.5% L-carnitine has been able to enhance the growth performance of grouper larvae, while the addition of 2.83% L-lysine to a diet containing 14% lipid and 0.5% L-carnitine fails to have any additive effect on the growth performance. With the dietary lipid being 14%, not only dietary L-carnitine but also L-lysine is able to decrease lipid and increase protein levels in grouper muscle. The supplementation of dietary L-carnitine with a high level of lipid is also able to reduce liver lipid level of grouper.

Declaration of interest

The authors declare that there are no conflicts of interest.

Acknowledgement

This work was partially supported by grants from 101AS to 6.1.1-ST.a8, 102AS-6.2.1-ST-a9 and 103AS-14.3.3-ST-a6 of the Council of Agriculture, Executive Yuan, Taiwan, R.O.C. The authors also thank funds from Guangdon Provincial Key Laboratory of Aquatic Larvae Feed, Guangdon, 515500, China.

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