Hindawi
Aquaculture Research
Volume 2023, Article ID 5336474, 12 pages
https://doi.org/10.1155/2023/5336474

Research Article

Effects of Various Lipid Sources and L-Carnitine Supplementation on the Growth Performance, Body Composition, and Antioxidants Enzyme Ability of Dog Conch Larvae, *Laevistrombus canarium*

Jen-Hong Chu

Department of Aquatic Biosciences, National Chiayi University, Chiayi 600, Taiwan

Correspondence should be addressed to Jen-Hong Chu; jhchu@mail.ncyu.edu.tw

Received 15 September 2022; Revised 29 November 2022; Accepted 7 December 2022; Published 7 February 2023

Academic Editor: Femi Fawole

A feeding experiment was conducted to study the effects of dietary lipid sources and L-carnitine on the growth performance, body component, muscle fatty acid composition, and antioxidant enzyme ability of average weight of 0.28 g dog conch larvae, *Laevistrombus canarium*. Three sources of lipid: fish oil, soybean oil, and beef tallow were tested in combination with two levels (0 and 0.5%) of L-carnitine. The dog conch larvae fed diets containing fish oil with L-carnitine supplementation exhibited the highest weight gain. After air exposure challenge, the liver tissue of dog conch fed the diets containing soybean oil and beef tallow without supplemented L-carnitine had lower levels of superoxide dismutase (SOD, U min^{-1}), phenoloxidase (PO, U min^{-1}), and glutathione peroxidase (GPx, mU/mL) activity. The activity of SOD, PO, and GPx of dog conch larvae increased with increasing levels of dietary L-carnitine. In particular, dog conch larvae fed the diet containing fish oil supplemented with L-carnitine exhibited significantly ($P < 0.05$) enhanced antioxidant responses. The thiobarbituric acid reactive substances (TBARS) in the muscle tissue of dog conch larvae fed the diet containing fish oil without L-carnitine supplementation showed significantly ($P < 0.05$) higher activity. It was concluded that dietary administration of L-carnitine can enhance resistance against beta-oxidation, and the administration of fish oil in the diet was the best strategy to promote growth due to high nutritional availability.

1. Introduction

Laevistrombus canarium (Linnaeus, 1758), known as dog conch, is widely distributed in India, including Andhra Pradesh, Tamil Nadu, and the Andamans [1], and Asian countries including Thailand, Indonesia, Philippines, Vietnam, and Taiwan [2, 3]. Dog conch is an economically important species of marine gastropod mollusc in many coastal areas of Southeast Asia [4]. Currently, the supply of *L. canarium* for human consumption is obtained by fishing in the wild, without aquaculture. Gong et al. [5] indicated that *L. canarium* has been successfully reproduced and released to the wild to help offset overfishing and environmental damage. Nutritional quantity and quality of artificial diet are crucial factors for the successful commercial aquaculture of many aquatic animals because these food resources provide balanced energy and essential nutrients [6]. Boonyaratpalin [7] indicated the importance of artificial diets to successful aquatic larval cultivation. However, food resources consumed by dog conch larvae in their natural habitat include living organisms such as seagrass, macroalgae, and zooplankton [8]. Insufficient food supply and environmental changes in natural habitat can render inadequate conditions for successful larval cultivation.

In order to improve normal growth of aquatic larvae, it is necessary to evaluate nutritional requirements, including lipids, proteins, carbohydrates, minerals, and vitamins [9, 10]. In the aquaculture industry there is a need for diets containing high lipid levels that elevate dietary energy concentration and improve the growth efficiency and protein utilization of aquatic animals [11–13]. Fish oil is a brown liquid obtained by cooking, pressing, and separation. Most

of the water and dry matter from fish or fish waste are removed. Generally, fish oil is sold in liquid form and is used mostly in compound foods for poultry, pigs, and farmed fish. The countries with major industrial fisheries are Norway, Iceland, Chile, and Denmark. Fish oil will continue to be an important ingredient in many aquafeeds. The careful management of the fishery stocks will include short-term periods of unsustainable production, and during periods of shortage, the future growth of aquaculture will be limited by the short supply of this important dietary ingredient. Therefore, increasing the capability of aquatic biota utilizing various sources of lipid in diets has become a focus of study in the lipid nutrition of aquafeeds [14]. The important component, L-carnitine, has been given attention due to its potential for having a positive effect on the growth and lipid metabolism of aquatic animals [15, 16].

Carnitine is present in both L- and D-forms, with L-carnitine being the biologically active form. L-carnitine, also known as vitamin B_T, is a small molecular weight (161.2), water soluble, quaternary nitrogen-containing compound. L-carnitine serves to promote growth and the synthesis of a derivative of lysine in the liver of animals [17]. Carnitine was used to improve growth factors in the mealworm (*Tenebrio molitor*) in the early 1950s [18]. L-carnitine has several roles and essential functions in mammals, including mitochondrial long-chain fatty acid oxidation, buffering of the mitochondrial acyl-CoA/CoA ratio, removal of potentially toxic acyl-groups, and fatty acid oxidation in peroxisomes [17]. L-carnitine supplementation has been administered in aquafeeds to promote growth, reduce adverse effects of toxic levels of ammonia, alleviate stress related to water temperature extremes, and facilitate acclimation to changes in water temperature [17].

L-carnitine is not only a derivative from lysine and methionine but also a growth promoter synthesized in the liver. It is transported to skeletal and cardiac muscle tissues that utilize fatty acids as primary fuel through beta-oxidation in mitochondria [17]. Growth-promoting effects of dietary L-carnitine have been found in European sea bass (*Dicentrarchus labrax* L.) [19], African catfish (*Clarias gariepinus*) [20], Red Sea bream (*Pagrus major*) [21, 22], tilapia (*Oreochromis mossambicus*) [23–25], hybrid striped bass (*Morone saxatilis* male × *M. chrysops* female) [26], freshwater prawn (*Macrobrachium rosenbergii*) [27], grouper (*Epinephelus lanceolatus*) [28], and narrow clawed crayfish (*Astacus leptodactylus*) [16]. However, several negative growth effects have been found in channel catfish (*Ictalurus punctatus*) [29], rainbow trout (*Oncorhynchus mykiss*) [15, 30], ornamental cichlid (*Pelvicachromis pulcher*) [31], European sea bass (*Dicentrarchus labrax*) [32], hybrid striped bass (*Morone chrysops* × *M. saxatilis*) [12, 33], tilapia [34], and Atlantic salmon (*Salmo salar*) [35]. These variations in results suggest that the effects of L-carnitine on the growth of aquatic animals may be attributed to interactions among several factors, such as dietary components, aquatic animal species, initial aquatic animal size, and aquatic animal life stage [17, 36].

The dog conch, originally an important seawater shellfish in the Indo-Pacific region, is one of the major wild-caught seawater shellfish in Malaysia and Taiwan [8, 37]. Previous studies indicated that the complete replacement of feed components from wild, such as diatoms, detritus, foraminifera, seagrass and macroalgal fragments, sand particles, and shell fragments with the administration of artificial feed has no significant effects on growth performance [38]. Wild feed may carry virus and bacteria which can destroy larval cultivation [37, 39]; therefore, the development of artificial feeds for shellfish can be helpful in preventing larvae from encountering pathogenic infections [40]. Lipids are essential nutrients, which play several roles, such as improving feed protein utilization and providing energy, phospholipids, sterols, fat-soluble vitamins, and essential fatty acids [41]. Proteins can be used as an energy source when supply of dietary lipids is limited and this metabolic pathway can reduce animal growth. Lipid nutrients have protein-sparing effects; therefore, the dietary energy supply from lipids for animal growth not only improves protein utilization, but also reduces feed costs and limits ammonia production [42].

Aquaculture industry provides half of the amount of seafood required for human consumption [43]. Fish oil is still widely used as the main lipid source in commercial diets for the cultured aquatic biota all over the world due to its high nutritional value, high level of docosahexaenoic acid and eicosapentaenoic acid, and good palatability [6]. However, due to overfishing and global climate change, the price of fish oil has increased from $314 USD per tonne in year 1999 to $2000 USD per tonne in year 2020; therefore, fish oil has become an uneconomical ingredient used in fish diet (FAO, 2020) [44, 45]. Thus, there is an urgent need to find suitable alternatives to fish oil, such as beef tallow and soybean oil. The beef tallow and soybean oil have advantages over fish oil in both price and yield (FAO, 2020) [46]. Therefore, the aim of the present study is to investigate the effects of diets supplemented with various sources of lipid, with or without 0.5% L-carnitine on the growth performance, lipid, protein, and fatty acid composition of the muscle of 0.28 g dog conch larvae.

2. Materials and Methods

2.1. Experimental Diets. In preparation of this experiment, seven experimental diets (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 g·L-carnitine/100 g diet) and a control diet (0 g·L-carnitine/100 g diet) were fed to dog conch larvae. Based on lipid and protein requirements of *L. canarium* recommended by Chu et al. [39], 6 isolipidic (5.6%) and isonitrogenous (46%) diets were formulated to contain three different sources of lipid (fish oil, soybean oil, and beef tallow) and one of the two levels of L-carnitine (Table 1) (0% and 0.5%) (*A*: fish oil + 0% L-carnitine, *B*: soybean oil + 0% L-carnitine, *C*: beef tallow + 0% L-carnitine, *D*: fish oil + 0.5% L-carnitine, and *E*: soybean oil + 0.5% L-carnitine, *F*: beef tallow + 0.5% L-carnitine). Before formulation of the diets, lipids were extracted from fish meal and shrimp meal using hot ethanol (1 : 1, w/v) in three successive treatments to minimize the contribution from dietary lipid. Mixed ingredients were cold-extruded through a chopper (0.1 cm die diameter) and were dried at 30°C to approximate 8% moisture content. The diets were

Table 1: Feed formula and proximate analysis of experimental diets for larval dog conch, *Laevistrombus canarium*

Ingredients	Treatments					
	A	*B*	*C*	*D*	*E*	*F*
Basal mix[a]	93.9	93.9	93.9	93.9	93.9	93.9
Fish oil	5.6	0	0	5.6	0	0
Soybean oil	0	5.6	0	0	5.6	0
Beef tallow	0	0	5.6	0	0	5.6
α-cellulose	0.5	0.5	0.5	0	0	0
L-carnitine	0	0	0	0.5	0.5	0.5
Analyzed composition (as fed)						
Moisture	8.68	8.03	9.22	8.41	8.02	8.92
Crude protein[b]	46.29	46.16	46.18	46.23	46.28	46.26
Crude fat[b]	5.68	5.68	5.67	5.67	5.65	5.66
Ash[b]	10.43	9.59	10.42	11.01	9.54	10.62
Crude fiber[b]	1.56	1.52	1.54	1.48	1.49	1.46
L-carnitine[c]	0.056	0.056	0.056	5.046	5.046	5.045

[a]lipid-extracted fish meal (Liandong Industry Co., Ltd., Taiwan) 59%, lipid-extracted shrimp meal (Jian-Bao Foods Co., Ltd., Taiwan) 6%, β-glucan (Toong Yeuan Enterprise Co., Ltd., Taiwan) 1%, yeast (Sun Right Foods Co., Ltd., Taiwan) 4%, and mineral mix [47] modified (calcium phosphate 0.0735%, potassium phosphate 0.0081%, potassium sulfate 0.0068%, sodium chloride 0.0031%, calcium carbonate 0.0021%, sodium phosphate 0.0022%, magnesium oxide 0.0025%, and trace element mix 0.0018% (trace element mix: ferric citrate 0.0031%, zinc carbonate 0.0005%, manganese carbonate 0.0024%, copper carbonate 0.0002%, potassium iodide 0.0005%, and citric acid 0.0039%)) 2%, vitamin mix [48] modified (vitamin D3 0.001%, vitamin A 0.06%, α-tocopheryl acetate 0.45%, vitamin K3 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%, B12 0.001%, inositol 20%, ascorbic-monophosphate-Mg 2.5%, choline chloride 40%, and α-cellulose 31.978%) 1%, α-Starch (Ping Tung Foods Co., Ltd., Taiwan) 20.9%, and L-carnitine (Tokyo Chemical Industry Co., Ltd). [b]Expressed as percent dry weight. [c]L-carnitine (g/kg).

then ground by Mortar Grinder RM 100 (Retsch, Germany) into 0.2 mm particles.

2.2. Experimental Dog Conch. Fertilized eggs of dog conch obtained from spontaneous spawning by cultured broodstock were hatched and reared for 80 days at a hatchery farm in the Aquatic Conservation Center, National Chiayi Country. Two thousand 80-day-post-hatch (DAH) dog conch larvae were transported to the laboratory. After one week of acclimation in the laboratory, a total of 720 0.28 ± 0.01 g dog conch larvae were randomly distributed into 18 fiber reinforced plastic (FRP) aquaria ($45 \times 30 \times 30$ cm) containing 50 L seawater, with 40 dog conch larvae in each aquarium.

All animal experimentation conformed to the principles for the use and care of laboratory animals in accordance with the Institutional Animal Care and Use Committee (IACUC) of National Chiayi University (NCYU) (approval no. NCYU-11101). Dog conch larvae were hand-fed to excess once daily at 09 : 30 AM. A continuous aeration was provided to each aquarium through an air stone connected to a central air compressor. Approximately 50% of the water volume was exchanged every day in the morning to remove uneaten food and fecal material. The following water quality parameters and ranges were recorded during each feeding period: water temperature (27 to 28°C), dissolved oxygen (5 to 6 ppm), ammonia nitrogen (0.7 to 0.8 ppm), and salinity (32 to 33‰). Dog conch were fasted for 24 h before weighing. Wet weights (surface water removed) of surviving individuals in each aquarium were measured at the initiation and termination of the experiment. The duration of the experiment was 120 days. The shell length, shell width, and shell height were measured with electronic digital calipers according to the method of Guzmán and Viana [49]. Growth parameters including weight gain, survival, specific growth rate (SGR), feed conversion ratio (FCR), and soft muscle/final mean body weight (SB) ratio were calculated according to the following formulae:

$$\text{Weight gain}(\%) = 100 \times (Wt - W0)/W0;\quad \text{mortality}(\%) = 100 \times (Fi - Fd)/Fi;\quad \text{SGR}(\%\,\text{day}^{-1}) = 100 \times (\ln Wt - \ln W0)/\text{feeding days};\quad \text{FCR} = \text{total feed intake}(g)/(Wt - W0)\,(g);\quad \text{shell length, shell width, shell height increase}(\%) = 100 \times (Sf - Si)/Si;\quad \text{and}\quad SB(\%) = 100 \times (Ws/Wt);$$

where $W0$ is the initial mean body weight (g); Wt is the final mean body weight (g); Ws is the final soft-body weight (g); Fi is the initial dog conch number; Fd is the number of dead dog conch; Si is the initial dog conch shell length, shell width, and shell height (mm); Sf is the final dog conch shell length, shell width, and shell height (mm).

2.3. Sample Collection and Chemical Analysis. The experimental diets and muscle of dog conch were analyzed for proximate composition based on AOAC [50] methods. Crude protein was determined with a Kjeltec semi-autoanalyzer model 1007 (Tecator, Sweden). Crude lipid was determined by the chloroform-methanol (2 : 1, v/v) extraction method [51]. Crude fiber was determined by acid and alkaline digestion using Fibertec system M1020 (Foss Tecator, Sweden). Ash and moisture were determined by conventional methods using a muffle furnace and an oven, respectively. Dietary L-carnitine was extracted and the assay method was analyzed by high performance liquid chromatography (HPLC) equipped with a Hitachi L-6200 intelligent pump and a Hitachi L-4200 UV-VIS detector in accordance with procedures described by Cao et al. [52]. The mobile phase was prepared using 700 ml 0.1 M ammonium acetate mixed with 300 ml acetonitrile. The samples were separated on a C18 column (Supelco Ascentis TM), and the detector wavelength was set at 248 nm. The crude protein of the experimental diets ranged from 46.16 to 46.29% (dry weight). Crude lipid of the experimental diets ranged from 5.65 to 5.68% (dry weight). The experimental diet without L-carnitine supplement contained 0.056 $g \cdot kg^{-1}$ L-carnitine and diets supplemented with 0.5% L-carnitine contained 5.05 $g \cdot kg^{-1}$, respectively (Table 1).

At the termination of the experiment, the fatty acid profile of the muscle of dog conch and diets were analyzed. The muscle of dog conch and diets from each aquarium were homogenized and separated in chloroform/methanol (2 : 1, v/v) for 5 min to extract total lipid [51], and refluxed in 50% KOH for 40 min. The saponified lipids were then methylated by refluxing for 20 min in 14% boron trifluoride in methanol (BF_3MeOH) as described by Metcalfe and Schmitz [53] in

the preparation for fatty acid analysis by gas chromatography. 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), and temperature was set at 208°C. Injection 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.

2.4. Air Exposure Challenge. In preparation for this experiment, four temperature (25°C, 30°C, 35°C, and 40°C) were used for air exposure challenge trial. After an 8-hour challenge trial, the survival of dog conch larvae in 25°C, 30°C, and 35°C were significantly higher ($P < 0.05$) than 40°C treatment (data are not shown). The air exposure challenge followed modified procedures described by Stoner [54]. Ten dog conch from each aquarium were exposed to warm air (35°C) for 8 hours on the first day after the feeding trial. At the termination of the air exposure challenge, the ten dog conch from each aquarium were analyzed for activities of phenoloxidase activity (PO), superoxide dismutase (SOD), glutathione peroxidase (GPx), thiobarbituric acid-reactive substance (TBARS), and superoxide anion (O_2^-) production ratio in the liver tissue. These results were used to evaluate biological responses of the dog conch to the air exposure challenge, with respect to the various experimental diets.

Phenoloxidase activity (PO) was assayed spectrophotometrically by recording from Coomassie blue staining using a method modified by Wang et al. [55]. Coelomic fluid samples from experimental dog conch were homogenized using a homogenizer (PREMA, IADI-HG-300D). The coelomic fluid samples were placed in a 5 ml Eppendorf tube and centrifuged at 2500 × g for 15 min at 4°C, then the isolated samples were harvested at the surface layer. For measurement of the PO activity, a 100 μl coelomic fluid sample was placed in 96-well microtiter plates and incubated for 30 min with 50 μl of L-dopa (3 mg/mL) (L-3,4 dihydroxy phenyl alanine). Optical density was measured at a final reading of 492 nm using a microplate reader. Enzyme activity was expressed as the change in absorbance min^{-1} $100\,\mu\text{l}^{-1}$ of coelomic fluid.

The SOD assay was conducted using the Ransod kit (Randox Laboratories, Crumlin, UK) following the manufacturer's instructions. The 0.2 g liver samples were homogenized with 2.7 ml of phosphate buffer containing 1% Trition X-100 (0.05 M, pH 7.8) for 5 min. The cell suspension was transferred to the tubes and centrifuged at 13000 × g for 30 min at 4°C. A 25 μL volume of the muscle tissue solution or HBSS (as a control) were added to 850 μL of the reaction substrate containing a solution of xanthine and INT (2-(4-iodophenyl)-3-(4-nutrophenol 3-5-phenltetrazolium), followed by the addition of 125 μL of xanthine oxidase (XOD), while the decrease in absorbance at 505 nm was recorded for 30 and 210 seconds. The specific activity was defined as a unit of SOD that could cause a 50% reduction in the rate of formazan dye formation.

The glutathione peroxidase (GPx) assay was conducted using the assay kit (colorimetric) (ab102530) following the manufacturer's instructions. The 0.2 g of the liver were placed in liquid nitrogen upon extraction and the samples were stored immediately at −80°C. The 100 mg tissue sample was washed with cold PBS, and resuspended in 200 μL of cold assay buffer, then transferred to the tubes and centrifuged at 10,000 × g for 15 min at 4°C. A 50 μL sample of the liver tissue solution or a 100 μL standard dilution (as a control) was added to 40 μL of the reaction mix, and placed in positive control(s) and reagent control wells. Samples were mixed well and incubated at room temperature for 15 minutes to deplete all GSSG, then 10 μL of cumene hydroperoxide solution were added, while the decrease in absorbance at 340 nm was recorded on a microplate reader, and incubated at 25°C for 5 min. The output was measured on a microplate reader at OD340 nm. Samples producing signals greater than that of the highest standard were further diluted in appropriate buffer and reanalyzed, then the concentration was adjusted by multiplying the appropriate dilution factor.

Thiobarbituric acid-reactive substances (TBARS) in the liver were measured using a method described by Chu et al. [56]. In brief, tissues were homogenized with a 20% trichloroacetic acid extracting solution containing 1% butylated hydroxytoluene (BHT) and incubated with 50 mM thiobarbituric acid (TBA). The samples were then placed in a boiling water bath for 10 min and centrifuged. The optical density of the solution was then measured at 532 nm. TBARS is expressed as micromole of malondialdehyde (MDA) per gram of tissue using a molar extinction coefficient of $1.56 \times 10^5\ \text{M}^{-1}\cdot\text{cm}^{-1}$ [57].

Superoxide anion production ratio in the liver were measured using a method described by Cheng et al. [58]. The liver samples were dispersed in AIM medium and L-15 medium (Leibovitz, GIBCO, Rockville, Maryland, U.S.A.) in a plastic petri plate. The cell mixture was filtered through 100 μm nylon net filter. The 2 ml 30% Percoll (Sigma) was added and centrifuged at 400 × g for 40 min (4°C) to separate cells. The leukocyte layers were obtained from the interface, and then cultured in an incubator at 25°C. The leukocyte was placed in an incubator and incubated for 1 hour at 30°C. Nitro blue tetrazolium solution (Sigma) was added after incubation. The leukocytes were treated in sequence with absolute alcohol, 120 μl of 2 M potassium hydroxide, and 140 μl of dimethyl sulfoxide for formazan formation. Superoxide anion production was quantified by measuring OD 620 nm.

2.5. Statistical Analysis. Data were analyzed in a completely randomized design using each aquarium as an experimental unit. Data analysis was completed by performing one-way analysis of variance (ANOVA) using Statistic Analysis

System [59]. 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 [60].

3. Results

Fatty acid compositions of the diets containing different sources of lipid are presented in Table 2. The diet supplemented by soybean oil and beef tallow contained higher proportions of PUFA and SFA, respectively. However, the $n3$ highly unsaturated fatty acid ($n-3$ HUFA) of the diets containing soybean oil and beef tallow was not detectable. Dietary levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increased with the diet supplemented by fish oil. Survival for all treatment groups was 100%.

The muscle protein, muscle lipid, liver lipid levels, and SB of dog conch larvae fed with the treatment diets are shown in Table 3. The dog conch larvae fed with the diet containing fish oil without L-carnitine supplement showed the highest muscle lipid (3.97%) and liver lipid (5.60%) levels ($P < 0.05$). Whether the lipid was fish oil, soybean oil, or beef tallow, the lipid levels of dog conch fed with the diets containing L-carnitine were significantly lower than those of dog conch fed diets without L-carnitine supplementation. The muscle protein levels of dog conch fed with the diets containing fish oil supplemented with L-carnitine was significantly higher than those of dog conch fed with the other treatment diets ($P < 0.05$). Both dietary lipid and L-carnitine contributed significant effects on muscle lipid, liver lipid, and muscle protein levels. The SB (1.87%) of dog conch fed with the diet containing fish oil supplemented with L-carnitine was significantly higher than that of dog conch fed the other treatment diets ($P < 0.05$).

Weight gain, FCR, and SGR of dog conch larvae fed the experimental diets for 120 days are shown in Table 4. There was a significant dose-dependent growth-promoting effect when the supplemental L-carnitine level was 0.5%. The dog conch larvae fed diets supplemented with L-carnitine had significantly higher weight gain (984.47%) and SGR (1.99% day^{-1}) than those fed diet without L-carnitine supplementation ($P < 0.05$). Also, dog conch larvae fed diet supplemented with fish oil had significantly higher weight gain than those fed diet containing soybean oil and beef tallow ($P < 0.05$). ANOVA results indicated that the two factors (L-carnitine and lipid sources) on the growth parameters of dog conch larvae were significant.

The increases in shell length, shell width, and shell height of dog conch larvae fed with experimental diets for 120 days are shown in Table 5. Shell length increase (134.01 mm), shell width increase (124.09 mm), and shell height increase (130.05 mm) of dog conch larvae fed with the diet containing fish oil supplemented with L-carnitine was significantly higher than those of dog conch larvae fed with the other diets ($P < 0.05$).

Fatty acid compositions of the muscle of dog conch larvae are shown in Table 6. The percentages of 18 : 0 (2.62%) and 18 : 1 (4.15%) level of the muscle of dog conch larvae fed diet containing fish oil without L-carnitine supplementation was significantly lower than those of dog conch larvae fed diets containing soybean oil and beef tallow ($P < 0.05$). The percentages of $20:5n-3$ (1.23%), $22:5n-3$ (2.29%), and $22:6n-3$ (13.92%) in the muscle of dog conch larvae fed diet supplemented with fish oil without L-carnitine supplementation were significantly higher than those of dog conch fed diets supplemented with soybean oil and beef tallow ($P < 0.05$). The percentages of $20:5n-3$, $22:5n-3$, and $22:6n-3$ in the muscle of larval dog conch fed diets supplemented with L-carnitine were significantly lower than those of dog conch larvae fed diets containing the three lipid sources without L-carnitine supplementation.

The SOD, PO, and GPx activities of dog conch larvae fed with the experimental diets for 120 days are shown in Table 7. The SOD (6.04 $U \cdot min^{-1}$), PO (7.76 $U \cdot min^{-1}$), and GPx (5.73 $mU \cdot mL^{-1}$) activities of dog conch larvae fed diet supplemented with fish oil with L-carnitine supplementation were significantly higher than those of dog conch fed other diets ($P < 0.05$). The dog conch larvae fed diets supplemented with L-carnitine had significantly higher antioxidant enzyme ability than those fed diets without L-carnitine supplementation.

The superoxide anion production in dog conch liver macrophages is shown in Figure 1. The superoxide anion activities (1.51) of dog conch larvae fed diet supplemented with fish oil without L-carnitine supplementation was significantly higher than that of dog conch fed other diets ($P < 0.05$). The dog conch larvae fed diets supplemented with L-carnitine had significantly lower superoxide anion ability than those fed diet without L-carnitine supplementation.

Dietary lipid sources and L-carnitine levels also influenced lipid peroxidation in muscle tissues of the dog conch (Figure 2). TBARS of dog conch fed diet containing fish oil was significantly higher than those fed soybean oil and beef tallow ($P < 0.05$). The dog conch larvae fed diets supplemented with L-carnitine had significantly lower TBARS than those fed diet without L-carnitine supplementation.

4. Discussion

There was no mortality in the dog conch fed experimental diets. Dog conch larvae fed the fish oil diet containing 0.5% L-carnitine exhibited the highest weight gain (984.47%) and SGR (1.99% day^{-1}), and the lowest FCR (1.76) in this study. The same increasing trends were observed for all measured shell dimensions, including shell length (134 mm), shell width (124 mm), and shell height (130 mm) for dog conch larvae fed the diets containing fish oil supplemented with 0.5% L-carnitine, indicating a higher utilization of the dietary lipid for metabolic energy and protein for growth; whereas those values were slightly lower when the dietary lipid sources were soybean oil and beef tallow. Feeding a diet supplemented with L-carnitine enhanced growth performance in giant grouper (*Epinephelus lanceolatus* larvae) [28], juvenile narrow clawed crayfish (*Astacus leptodactylus leptodactylus*) [16], and juvenile largemouth bass (*Micropterus salmoides*) [61]. The L-carnitine has been demonstrated by Jalai et al., 2010, to enhance the oxidation of triglycerides, protein-sparing effect, and aquabiota growth

Table 2: Major fatty acid composition (% of total fatty acid) of diets for larval dog conch, *L. canarium*.

Fatty acid	Treatments					
	A	*B*	*C*	*D*	*E*	*F*
14 : 0	0.89	0.3	7.68	0.78	0.29	6.99
14 : 1	0.05	n.d.	0.66	0.06	n.d.	0.56
16 : 0	14.42	13.27	50.38	13.95	15.65	48.77
16 : 1	0.18	0.14	7.11	0.21	0.21	7.35
18 : 0	45.36	3.44	34.12	44.52	3.56	36.25
18 : 1	0.28	19.44	n.d.	0.2	18.69	n.d.
18 : 2	0.51	57.07	0.05	0.48	54.2	0.08
18 : 3	1.46	5.81	n.d.	1.59	5.93	n.d.
20 : 0	0.98	0.21	n.d.	0.82	0.19	n.d.
20 : 1	0.48	0.32	n.d.	0.36	1.28	n.d.
20 : 2	0.34	n.d.	n.d.	0.37	n.d.	n.d.
20 : 3$n-6$	1.12	n.d.	n.d.	0.99	n.d.	n.d.
20 : 3$n-3$	1.86	n.d.	n.d.	1.89	n.d.	n.d.
20 : 4	12.12	n.d.	n.d.	11.14	n.d.	n.d.
20 : 5$n-3$	1.34	n.d.	n.d.	1.35	n.d.	n.d.
22 : 0	0.37	n.d.	n.d.	0.48	n.d.	n.d.
22 : 1	1.1	n.d.	n.d.	1.54	n.d.	n.d.
22 : 5$n-3$	4.14	n.d.	n.d.	4.89	n.d.	n.d.
22 : 6$n-3$	13	n.d.	n.d.	14.38	n.d.	n.d.
SFA	62.02	17.22	92.18	60.55	19.69	92.01
MUFA	2.09	19.9	7.77	2.37	20.18	7.91
PUFA	5.29	62.88	0.05	5.32	60.13	0.08
$n-3$ HUFA	18.48	0	0	20.62	0	0

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, HUFA: highly unsaturated fatty acids, n.d. = not detectable, *A*: fish oil + 0% L-carnitine, *B*: soybean oil + 0% L-carnitine, *C*: beef tallow + 0% L-carnitine, *D*: fish oil + 0.5% L-carnitine, *E*: soybean oil + 0.5% L-carnitine, and *F*: beef tallow + 0.5% L-carnitine.

Table 3: The muscle protein and lipid content, liver lipid content, and soft muscle/final mean body weight ratio (SB) of larval dog conch, *L. canarium* fed with experimental diets for 120 days.

Treatment	Muscle protein (%, dry matter)	Muscle lipid (%, dry matter)	Liver lipid (%, dry matter)	SB (%)
A	77.96 ± 1.67^{b}	3.97 ± 0.77^{a}	5.60 ± 1.15^{a}	1.62 ± 0.04^{b}
B	76.98 ± 0.43^{b}	3.68 ± 0.28^{ab}	4.79 ± 0.36^{b}	1.39 ± 0.02^{c}
C	69.32 ± 1.01^{d}	2.15 ± 0.17^{cd}	2.69 ± 0.21^{cd}	0.95 ± 0.05^{e}
D	82.89 ± 2.32^{a}	3.19 ± 0.61^{bc}	4.69 ± 0.89^{b}	1.87 ± 0.05^{a}
E	79.65 ± 1.86^{b}	2.89 ± 0.31^{bc}	3.73 ± 0.40^{bc}	1.57 ± 0.02^{b}
F	73.35 ± 0.86^{c}	1.96 ± 0.06^{d}	2.15 ± 0.07^{d}	1.29 ± 0.09^{d}

^{a,b,c,d,e}means in the same column with different letters are significantly different ($P < 0.05$). Data are expressed as mean values ± S.E. (30 dog conch per tank; $n = 3$). *A*: fish oil + 0% L-carnitine, *B*: soybean oil + 0% L-carnitine, *C*: beef tallow + 0% L-carnitine, *D*: fish oil + 0.5% L-carnitine, *E*: soybean oil + 0.5% L-carnitine, and *F*: beef tallow + 0.5% L-carnitine.

Table 4: Final weight (g), weight gain (%), feed conversion ratio (FCR), and specific growth rate (SGR, % day^{-1}) of larval dog conch, *L. canarium* fed with experimental diets for 120 days.

Treatment	Initial weight (g)	Final weight (g)	Weight gain (%)	FCR	SGR (% day^{-1})
A	0.28 ± 0.01	2.28 ± 0.04^{c}	713.26 ± 21.71^{c}	2.48 ± 0.27^{c}	1.75 ± 0.02^{c}
B	0.28 ± 0.00	1.96 ± 0.06^{d}	599.89 ± 14.12^{d}	2.92 ± 0.15^{c}	1.62 ± 0.02^{d}
C	0.28 ± 0.01	1.22 ± 0.10^{f}	335.64 ± 32.53^{f}	6.91 ± 1.26^{a}	1.22 ± 0.06^{f}
D	0.28 ± 0.00	3.04 ± 0.07^{a}	984.47 ± 22.65^{a}	1.76 ± 0.08^{c}	1.99 ± 0.02^{a}
E	0.28 ± 0.01	2.71 ± 0.13^{b}	866.49 ± 41.48^{b}	2.15 ± 0.10^{c}	1.89 ± 0.04^{b}
F	0.28 ± 0.00	1.59 ± 0.05^{e}	467.80 ± 15.12^{e}	4.65 ± 1.02^{b}	1.45 ± 0.02^{e}

^{a,b,c,d,e,f}means in the same column with different letters are significantly different ($P < 0.05$). Data are expressed as mean values ± S.E. (30 dog conch per aquarium; $n = 3$). *A*: fish oil + 0% L-carnitine, *B*: soybean oil + 0% L-carnitine, *C*: beef tallow + 0% L-carnitine, *D*: fish oil + 0.5% L-carnitine, *E*: soybean oil + 0.5% L-carnitine, and *F*: beef tallow + 0.5% L-carnitine.

Table 5: Increase in shell length, width, and height of larval dog conch, *L. canarium* fed experimental diets for 120 days.

Treatment	Shell length increase (mm)	Shell width increase (mm)	Shell height increase (mm)
A	103.09 ± 4.22^{c}	94.16 ± 4.01^{bc}	99.90 ± 4.85^{c}
B	97.63 ± 3.37^{c}	88.80 ± 2.95^{c}	95.04 ± 3.33^{c}
C	87.78 ± 2.79^{d}	85.80 ± 13.65^{c}	85.14 ± 5.91^{d}
D	134.01 ± 2.86^{a}	124.09 ± 2.44^{a}	130.05 ± 1.97^{a}
E	112.51 ± 6.74^{b}	104.03 ± 6.64^{b}	108.97 ± 7.02^{b}
F	104.40 ± 6.74^{c}	97.49 ± 6.36^{bc}	98.39 ± 2.92^{c}

^{a,b,c,d}means in the same column with different letters are significantly different ($P < 0.05$). Data are expressed as mean values ± S.E. (30 dog conch per aquarium; $n = 3$). *A*: fish oil + 0% L-carnitine, *B*: soybean oil + 0% L-carnitine, *C*: beef tallow + 0% L-carnitine, *D*: fish oil + 0.5% L-carnitine, E: soybean oil + 0.5% L-carnitine, and F: beef tallow + 0.5% L-carnitine.

Table 6: Fatty acid composition (% of total fatty acid) of the muscle of larval dog conch, *L. canarium* fed with experimental diets for 120 days.

Fatty acid	Treatments					
	A	*B*	*C*	*D*	*E*	*F*
14 : 0	9.45 ± 0.12^{a}	2.27 ± 0.10^{d}	7.26 ± 0.12^{b}	5.31 ± 0.96^{c}	2.47 ± 0.12^{d}	7.99 ± 0.86^{b}
14 : 1	0.03 ± 0.00^{b}	10.17 ± 1.05^{a}	0.24 ± 0.02^{b}	0.14 ± 0.02^{b}	10.18 ± 0.19^{b}	0.27 ± 0.03^{b}
16 : 0	21.56 ± 0.26^{c}	23.84 ± 0.27^{b}	26.76 ± 0.48^{a}	24.12 ± 0.66^{b}	23.27 ± 0.91^{b}	25.58 ± 1.34^{a}
16 : 1	12.39 ± 0.20^{b}	4.69 ± 0.45^{c}	14.61 ± 0.52^{a}	15.37 ± 0.21^{a}	4.65 ± 0.38^{c}	15.38 ± 0.80^{a}
18 : 0	2.62 ± 0.05^{d}	8.90 ± 0.12^{a}	7.35 ± 0.06^{b}	3.57 ± 0.12^{c}	8.64 ± 0.88^{a}	7.12 ± 0.15^{b}
18 : 1	4.15 ± 0.16^{c}	10.08 ± 1.49^{b}	16.20 ± 0.15^{a}	4.65 ± 0.38^{c}	10.34 ± 0.30^{b}	15.95 ± 0.68^{a}
18 : 2 $n-6$	1.48 ± 0.03^{c}	2.72 ± 0.06^{b}	7.61 ± 0.64^{a}	1.65 ± 0.10^{c}	3.00 ± 0.69^{b}	8.20 ± 0.06^{a}
18 : 3 $n-3$	0.35 ± 0.02^{b}	0.63 ± 0.08^{b}	4.13 ± 0.42^{a}	0.41 ± 0.07^{b}	0.68 ± 0.04^{b}	4.14 ± 0.18^{a}
20 : 0	4.46 ± 0.22^{b}	0.14 ± 0.03^{e}	1.63 ± 0.22^{c}	4.98 ± 0.03^{a}	0.15 ± 0.01^{e}	0.65 ± 0.09^{d}
20 : 1	0.84 ± 0.04^{c}	15.09 ± 1.08^{b}	0.20 ± 0.02^{c}	1.07 ± 0.08^{c}	16.12 ± 0.84^{a}	1.18 ± 0.20^{c}
20 : 2 $n-6$	0.30 ± 0.03^{c}	4.36 ± 0.36^{a}	2.06 ± 0.05^{b}	0.29 ± 0.02^{c}	4.24 ± 0.59^{a}	2.26 ± 0.21^{b}
20 : 3 $n-6$	0.07 ± 0.01^{f}	0.32 ± 0.05^{d}	1.10 ± 0.01^{a}	0.17 ± 0.02^{e}	0.41 ± 0.02^{c}	0.99 ± 0.02^{b}
20 : 3 $n-3$	0.34 ± 0.02^{d}	2.29 ± 0.38^{b}	1.81 ± 0.10^{c}	0.27 ± 0.02^{d}	3.19 ± 0.36^{a}	1.45 ± 0.05^{c}
20 : 4 $n-6$	1.14 ± 0.01^{b}	0.35 ± 0.03^{c}	2.36 ± 0.29^{a}	1.17 ± 0.03^{b}	0.42 ± 0.03^{c}	2.29 ± 0.06^{a}
20 : 5 $n-3$	1.23 ± 0.02^{a}	0.49 ± 0.07^{c}	0.16 ± 0.06^{e}	1.06 ± 0.11^{b}	0.38 ± 0.04^{d}	0.09 ± 0.02^{e}
22 : 0	21.99 ± 0.21^{a}	3.92 ± 0.21^{c}	0.74 ± 0.05^{e}	21.21 ± 0.96^{b}	4.21 ± 0.12^{c}	1.53 ± 0.14^{d}
22 : 1	1.38 ± 0.02^{a}	0.56 ± 0.05^{c}	0.55 ± 0.20^{c}	1.48 ± 0.07^{a}	0.78 ± 0.12^{b}	0.65 ± 0.07^{bc}
22 : 5 $n-3$	2.29 ± 0.11^{a}	1.34 ± 0.19^{c}	0.32 ± 0.07^{e}	1.93 ± 0.13^{b}	0.99 ± 0.11^{d}	0.22 ± 0.04^{e}
22 : 6 $n-3$	13.92 ± 0.07^{a}	7.83 ± 0.66^{c}	4.92 ± 0.01^{e}	11.13 ± 0.88^{b}	5.89 ± 0.47^{d}	4.04 ± 0.13^{f}
SFA	60.08 ± 0.30^{a}	39.07 ± 0.50^{c}	43.74 ± 0.44^{b}	59.20 ± 0.93^{a}	38.74 ± 1.29^{c}	42.87 ± 0.77^{b}
MUFA	18.79 ± 0.30^{f}	40.59 ± 0.30^{b}	31.80 ± 0.21^{d}	22.72 ± 0.10^{e}	42.07 ± 0.95^{a}	33.45 ± 0.55^{c}
HUFA	18.58 ± 0.08^{a}	8.99 ± 0.60^{c}	8.78 ± 0.35^{c}	15.29 ± 0.82^{b}	6.90 ± 0.44^{d}	7.42 ± 0.11^{d}
$n-3$ HUFA	17.44 ± 0.09^{a}	8.64 ± 0.59^{c}	6.42 ± 0.25^{d}	14.12 ± 0.79^{b}	6.48 ± 0.46^{d}	5.13 ± 0.07^{e}

^{a,b,c,d,e,f}means in the same column with different letters are significantly different ($P < 0.05$). Data are expressed as mean values ± S.E. (30 dog conch per aquarium; $n = 3$). MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, HUFA: highly unsaturated fatty acids, SFA: saturated fatty acids, *A*: fish oil + 0% L-carnitine, *B*: soybean oil + 0% L-carnitine, *C*: beef tallow + 0% L-carnitine, *D*: fish oil + 0.5% L-carnitine, *E*: soybean oil + 0.5% L-carnitine, and *F*: beef tallow + 0.5% L-carnitine.

[20, 62]. In this study, dog conch may need more energy for growth during the larval stage, thus the L-carnitine supplementation enhanced the available energy produced through beta-oxidation. The lipid energy sources supplemented with L-carnitine are not appropriate for some species, such as rainbow trout (*Oncorhynchus mykiss*) [36], juvenile hybrid striped bass (*Morone chrysops* female × *M. saxatilis* male) [12], and hybrid tilapia (*O. niloticus* × *O. aureus*) [14]. The lower weight gain associated with L-carnitine could be attributed to L-carnitine supplementation levels, dietary composition, animal species, duration and fluctuation of the rearing water temperature, fish size, and stability of diets [17, 63]. The reduction of muscle lipid and liver lipid content with an incremental increase in the dietary L-carnitine level confirmed that lipids supplemented with L-carnitine are beneficial as energy sources. In the present study, the synchronous improvement of FCR and SGR in dog conch fed L-carnitine-supplemented diets was accompanied by enhanced weight gain. Therefore, less protein is used for metabolic energy requirements and less feed is needed to obtain the same growth parameters as the nonsupplemented group [17, 64].

The dog conch larvae fed with diets supplemented with L-carnitine exhibited higher weight gain and lower lipid levels in the muscle tissue than those fed with diets without L-carnitine supplementation. The fatty acid metabolism occurs in the mitochondrial matrix and L-carnitine is essential towards the transport of long-chain fatty acids into

Table 7: Superoxide dismutase (SOD, U·min^{-1}), phenoloxidase (PO, U·min^{-1}), and glutathione peroxidase (GPx, mU·mL^{-1}) in the liver tissue of larval dog conch fed experimental diets after 8 hours of warm-air (35°C) exposure challenge.

Treatment	SOD*	SOD	PO	GPx
A	1.22 ± 0.01	3.96 ± 0.10^{b}	4.44 ± 0.11^{b}	3.77 ± 0.10^{b}
B	1.21 ± 0.02	2.01 ± 0.03^{e}	2.25 ± 0.03^{cd}	1.91 ± 0.03^{e}
C	1.21 ± 0.01	1.23 ± 0.08^{f}	1.38 ± 0.09^{d}	1.17 ± 0.08^{f}
D	1.23 ± 0.02	6.04 ± 0.07^{a}	7.76 ± 1.81^{a}	5.73 ± 0.07^{a}
E	1.21 ± 0.01	3.01 ± 0.04^{c}	3.38 ± 0.04^{bc}	2.86 ± 0.04^{c}
F	1.22 ± 0.01	2.76 ± 0.08^{d}	3.09 ± 0.09^{bc}	2.63 ± 0.08^{d}

^{a,b,c,d,e,f}means in the same column with different letters are significantly different ($P < 0.05$). Data are expressed as mean values ± S.E. (10 dog conch per aquarium; $n = 3$). *before warm-air (35°C) exposure challenge. *A*: fish oil + 0% L-carnitine, *B*: soybean oil + 0% L-carnitine, *C*: beef tallow + 0% L-carnitine, *D*: fish oil + 0.5% L-carnitine, *E*: soybean oil + 0.5% L-carnitine, and *F*: beef tallow + 0.5% L-carnitine.

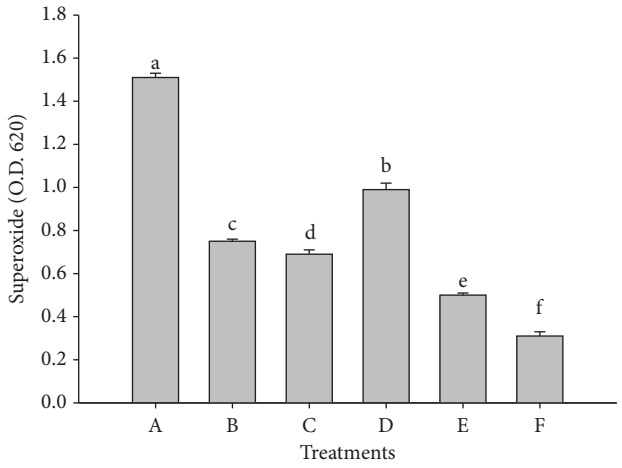

Figure 1: Superoxide anion (O_2^-) production ratio in the leukocyte isolated from the liver of larval dog conch fed with experimental diets after 8 hours of warm-air (35°C) exposure challenge (10 dog conch per plastic aquarium; $n = 3$). ^{a,b,c,d,e,f}means in the same column with different letters are significantly different ($P < 0.05$). Different superscripts at the end of each bar chart indicate significant differences ($P < 0.05$) among treatments. *A*: fish oil + 0% L-carnitine, *B*: soybean oil + 0% L-carnitine, *C*: beef tallow + 0% L-carnitine, *D*: fish oil + 0.5% L-carnitine, *E*: soybean oil + 0.5% L-carnitine, and *F*: beef tallow + 0.5% L-carnitine.

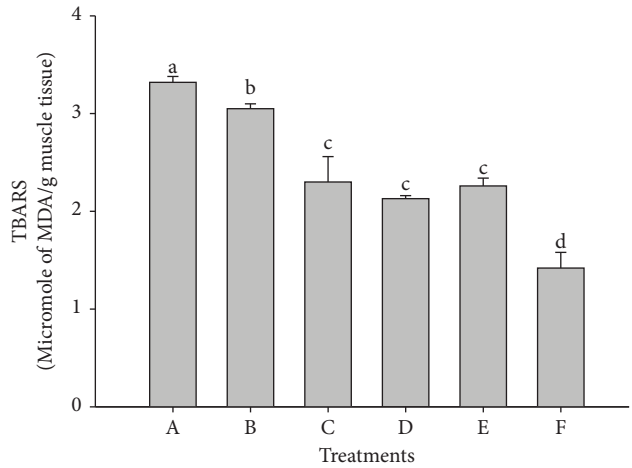

Figure 2: Thiobarbituric acid reactive substances (TBARS) in the muscle tissue content of larval dog conch, *Laevistrombus canarium*, fed various experimental diets after 8 hours warm-air (35°C) exposure challenge (10 dog conch per plastic aquarium; $n = 3$). ^{a,b,c,d}means in the same column with different letters are significantly different ($P < 0.05$). *A*: fish oil + 0% L-carnitine, *B*: soybean oil + 0% L-carnitine, *C*: beef tallow + 0% L-carnitine, *D*: fish oil + 0.5% L-carnitine, *E*: soybean oil + 0.5% L-carnitine, and *F*: beef tallow + 0.5% L-carnitine.

the mitochondrial matrix, producing extra energy from beta-oxidation [32]. Similarly, results of the current study show positive linear relationships between growth performance and dietary L-carnitine levels. Similar results have been reported for Atlantic salmon (*Salmo salar*) [35], carp (*Cyprinus carpio* L.) [65], and rainbow trout (*Oncorhynchus mykiss*) [62]. Chu et al. [48] indicated that giant grouper larvae, *Epinephelus lanceolatus*, need more energy for growth, and L-carnitine supplementation might increase energy availability due to an increase in triglycerides oxidation. In the current study, dog conch larvae fed diet supplemented with 0.5% L-carnitine treatments showed significantly higher growth parameters than those fed diet without L-carnitine supplementation, perhaps because larvae can use more dietary lipid for energy consumption and growth when receiving a diet supplemented with L-carnitine. Thus, dog conch larvae consumed energy in lipid form and saved the protein for growth, as observed by Torrelee et al. [20] and Jalali et al. [66].

Long-chain (Carbon >22) fatty acids are degraded to shortened fatty acids, which then need to be transported into mitochondria for complete beta-oxidation [67–69]. Dog conch larvae fed diets supplemented with L-carnitine had significantly less $n-3$ HUFA in the muscle tissue than those fed diets without L-carnitine supplements in this study. In contrast, dog conch larvae fed diets containing soybean oil and beef tallow supplemented with L-carnitine had higher levels of 18 : 0, 18 : 1, and 18 : 2 $n-6$ in the muscle than those fed fish oil treatment diet without L-carnitine supplementation. Dikel et al. [62] indicated that rainbow trout fed diets supplemented with L-carnitine showed increasing C14–18 and decreasing C20–22 fatty acid profiles in the muscle tissue, and similar results have been observed in Red Sea bream (*Pagrus major*) [22] and African catfish (*Clarias gariepinus*) [70]. Chatzifotis and Takeuchi [15] indicated that mitochondria require carnitine for the oxidation of long-chain fatty acids. Therefore, L-carnitine causes an increase in the oxidation rate of highly polyunsaturated fatty acids and is assumed to

promote fatty acid disappearance. Chatzifotis and Takeuchi [15] indicated that carnitine is required for the oxidation of long-chain fatty acids by mitochondria. In this study, the fatty acid profile of dog conch larvae showed that dietary L-carnitine can decrease the $n-3$ HUFA content of the muscle and it can be inferred that L-carnitine may increase the oxidation rate of highly polyunsaturated fatty acids in muscle tissues. Similar results have also found in juvenile European sea bass (*Dicentrarchus labrax* L.) [19, 71], channel catfish (*Ictalurus punctatus*) [29], Atlantic salmon (*Salmo salar*) [35], and grouper (*Epinephelus lanceolatus*) [28, 48].

The use of L-carnitine in dog conch diets also affects the function of the antioxidant system. Safari et al. [16] found that the use of L-carnitine in narrow clawed crayfish, *Astacus leptodactylus*, diets can influence increase the production of haemolymph as well as enhance antioxidant enzymes. The successful use of L-carnitine as a feed additive to improve antioxidant response has also been confirmed in broiler chickens [72], black carp (*Mylopharyngodon piceus*) [73], juvenile black sea bream (*Sparus macrocephalus*) [74] and rainbow trout (*Oncorhynchus mykiss*) [75]. In this study, the dog conch fed the diets supplemented with L-carnitine showed enhanced status of antioxidants and immunological enzyme activity. Safari et al. [16] indicated that malondialdehyde and glutathione peroxidase activity may be used to determine whether the body is under oxidative stress. These processes may explain why dog conch receiving L-carnitine dietary supplement had lower malondialdehyde and higher glutathione peroxidase activities than dog conch fed the diets without L-carnitine supplementation. The results showed that antioxidant enzyme activities of superoxide dismutase and phenoloxidase were significantly enhanced by the 0.5% L-carnitine dietary supplement. Antioxidant enzymes, such as SOD, PO, and GPx, constitute a natural defense system against the activity of oxidants. The growth and survival of dog conch may depend on the ability of the organism to overcome the toxic effects of reactive oxygen species. The ability of L-carnitine dietary supplementation to reduce lipid peroxide content and to enhance the activities of enzymatic antioxidants has also been confirmed by Kalaiselvi and Panneerselvam [76], Ma et al. [74], and Mohseni and Ozório [64]. Thus, L-carnitine can reduce lipid peroxidation and mitigate injury from oxidative stress in dog conch.

In conclusion, the present study indicated that growth parameters of dog conch larvae fed with a diet containing fish oil supplemented with 0.5% L-carnitine was significantly ($P < 0.05$) higher than those fed other diets, L-carnitine supplementation did impact muscle fatty acid profiles and enhanced antioxidant, fatty acids beta-oxidation, and antioxidant ability in dog conch larvae. Furthermore, our findings revealed that dietary L-carnitine supplementation has an important role in helping dog conch larvae recover from the stress induced by warm-air exposure.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] N. V. Subba Rao, "Indian seashells (Part 1): polyplacophora and gastropoda," *Records of the Zoological Survey of India Occasional Paper*, pp. 145-146, Zoological Survey of India, Kolkata, India, 2003.

[2] L. A. Man In 'T Veld and K. De Turck, "Contributions to the knowledge of Strombacea. 6. A revision of the subgenus Laevistrombus Kira, 1955 including the description of a new species from the New Hebrides," *Gloria Maris*, vol. 36, pp. 73–107, 1998.

[3] J. M. Poutiers and K. E. Carpenter, "Gastropods," in *The Living Marine Resources of the Western Central Pacific*p. 471, Food and Agriculture Organization of the United Nations (FAO), Rome, 1998.

[4] R. Ramses, F. Syamsi, and N. Notowinarto, "Length-weight relationship, growth patterns and sex ratio of dog conch Strombus canarium linnaeus, 1758 in the waters of kota batam," *Omni-Akuatika*, vol. 15, no. 1, pp. 19–29, 2019.

[5] S. R. Gong, M. R. Wang, W. Y. Zheng, H. Y. Xie, and D. S. Huang, "Artificial feeding experiment of dog conch, Laevistrombus canarium," *FRI Newsletter*, vol. 65, pp. 15–19, 2019.

[6] E. Prabu, S. Felix, N. Felix, B. Ahilan, and P. Ruby, "An overview on significance of fish nutrition in aquaculture industry," *International Journal of Fisheries and Aquatic Studies*, vol. 5, pp. 349–355, 2017.

[7] M. Boonyaratpalin, "Nutrient requirements of marine food fish cultured in Southeast Asia," *Aquaculture*, vol. 151, no. 1-4, pp. 283–313, 1997.

[8] W. N. W. H. Husna, A. G. Mazlan, and Z. C. Cob, "Ontogenetic changes in feeding and food preferences of the dog conch Laevistrombus canarium Linnaeus 1758 (Mollusca: gastropoda) from Merambong shoal, Malaysia," *Chinese Journal of Oceanology and Limnology*, vol. 35, no. 5, pp. 1230–1238, 2016.

[9] M. J. Leaver, J. M. Bautista, B. T. Björnsson et al., "Towards fish lipid nutrigenomics: current state and prospects for fin-fish aquaculture," *Reviews in Fisheries Science*, vol. 16, no. 1, pp. 73–94, 2008.

[10] Z. Luo, Y. J. Liu, K. S. Mai, and L. X. Tian, "Advances in the study on nutrient requirements of grouper (Epinephelus sp.): a review," *Journal of Ocean University of China*, vol. 4, no. 2, pp. 93–98, 2005.

[11] J. Dias, M. J. Alvarez, A. Diez et al., "Regulation of hepatic lipogenesis by dietary protein/energy ratio in juvenile European seabass (*Dicentrarchus labrax*)," *Aquaculture*, vol. 161, no. 1-4, pp. 169–186, 1998.

[12] T. G. Gaylord and D. M. Gatlin, "Effects of dietary carnitine and lipid on growth and body composition of hybrid striped bass (Morone chrysops × *M. saxatilis*)," *Fish Physiology and Biochemistry*, vol. 22, pp. 297–302, 2000.

[13] M. Naz, E. Yilmaz, and M. Türkmen, "A preliminary study on African catfish (*Clarias gariepinus*) larvae fed with diets containing different E/P rations and L-carnitine

supplementation," *Jouranl of Animal and Veterinary Advances*, vol. 4, pp. 871–875, 2005.

[14] S. D. Yang, Y. C. Wen, C. H. Liou, and F. G. Liu, "Influence of dietary L-carnitine on growth, biological traits and meat quality in tilapia," *Aquaculture Research*, vol. 40, no. 12, pp. 1374–1382, 2009.

[15] S. Chatzifotis and T. Takeuchi, "Effect of supplemental carnitine on body weight loss, proximate and lipid compositions and carnitine content of red sea bream (*Pagrus major*) during starvation," *Aquaculture*, vol. 158, no. 1-2, pp. 129–140, 1997.

[16] O. Safari, M. M. S. Atash, and M. Paolucci, "Effects of dietary L-carnitine level on growth performance, immune responses and stress resistance of juvenile narrow clawed crayfish, *Astacus leptodactylus* leptodactylus Eschscholtz," *Aquaculture*, vol. 439, pp. 20–28, 2015.

[17] S. Harpaz, "L-Carnitine and its attributed functions in fish culture and nutrition-a review," *Aquaculture*, vol. 249, no. 1-4, pp. 3–21, 2005.

[18] H. E. Carter, P. K. Bhattacharyya, K. R. Weidman, and G. Fraenkel, "Chemical studies on vitamin B_T isolation and characterization as carnitine," *Archives of Biochemistry and Biophysics*, vol. 38, no. 1, pp. 405–416, 1952.

[19] A. Santulli and V. D'Amelio, "Effect of supplemental dietary carnitine on the growth and lipid metabolism of hatchery-reared sea bass (*Dicentrarchus labrax* L)," *Aquaculture*, vol. 59, no. 3-4, pp. 177–186, 1986.

[20] E. Torreele, A. V. D. Sluiszen, and J. Verreth, "The effect of dietary L-carnitine on the growth performance in fingerlings of the African catfish (*Clarias gariepinus*) in relation to dietary lipid," *British Journal of Nutrition*, vol. 69, no. 1, pp. 289–299, 1993.

[21] S. Chatzifotis, T. Takeuchi, and T. Seikai, "The effcet of dietary L-carnitine on growth performance and lipid composition in red sea bream fingerlings," *Fisheries Science*, vol. 61, no. 6, pp. 1004–1008, 1995.

[22] S. Chatzifotis, T. Takeuchi, and T. Seikai, "The effect of dietary carnitine supplementation on growth of red sea bream (*Pagrus major*) fingerlings at two levels of dietary lysine," *Aquaculture*, vol. 147, no. 3-4, pp. 235–248, 1996.

[23] K. Becker, S. Schreiber, C. Angoni, and R. Blum, "Growth performance and feed utilization response of *Oreochromis niloticus* × *Oreochromis aureus* hybrids to L-carnitine measured over a full fattening cycle under commercial conditions," *Aquaculture*, vol. 174, no. 3-4, pp. 313–322, 1999.

[24] A. F. M. El-Sayed, N. F. Abdel-Hakim, H. A. Abo-State, K. F. El- Kholy, and D. A. Al-Azab, "Effects of L-carnitine on growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings fed basal diet or diets containing decreasing protein levels," *Journal of Aminal Science*, vol. 6, pp. 165–172, 2010.

[25] V. Jayaprakas, C. Sambhu, and S. Sunil Kumar, "Effect of dietary L-carnitine on growth and reproductive performance of male (*Oreochromis mossambicus*)," *Fishery Technology*, vol. 33, pp. 84–90, 1996.

[26] R. G. Twibell and P. B. Brown, "Effects of dietary carnitine on growth rates and body composition of hybrid striped bass (*Morone saxatilis* male × *M. chrysops* female)," *Aquaculture*, vol. 187, no. 1-2, pp. 153–161, 2000.

[27] R. K. Singh, A. S. Desai, S. L. Chavan, and P. A. Khandagale, "Growth performance, survival and body composition of post larvae of the freshwater prawn (*Macrobrachium rosenbergii*) fed graded L-carnitine diets," *Israeli Journal of Aquaculture - Bamidgeh*, vol. 60, pp. 161–167, 2008.

[28] J. H. Chu and S. S. Sheen, "Effects of dietary L-carnitine and lipid supplementation on growth performance, survival and body composition of grouper larvae, *Epinephelus lanceolatus*," *Journal of the Fisheries Society of Taiwan*, vol. 41, pp. 189–204, 2014.

[29] G. J. Burtle and Q. Liu, "Dietary carnitine and lysine affect channel catfish lipid and protein composition," *Journal of the World Aquaculture Society*, vol. 25, no. 2, pp. 169–174, 1994.

[30] M. Rodehutscord, "Effects of supplemental dietary L-carnitine on the growth and body composition of rainbow trout (*Oncorhynchus mykiss*) fed high-fat diets," *Journal of Animal Physiology and Animal Nutrition*, vol. 73, no. 1-5, pp. 276–279, 1995.

[31] S. Harpaz, K. Becker, and R. Blum, "The effect of dietary L-carnitine supplementation on cold tolerance and growth of ornamental cichlid fish (Pelvicachromis pulcher) – preliminary results," *Journal of Thermal Biology*, vol. 24, no. 1, pp. 57–62, 1999.

[32] J. Dias, J. Arzel, G. Corraze, and J. Kaushik, "Effect of dietary L-carnitine supplementation on growth and lipid metabolism in European sea bass (*Dicentrarchus labrax*)," *Aquaculture Research*, vol. 32, no. 1, pp. 206–215, 2001.

[33] T. G. Gaylord and D. M. Gatlin, "Dietary lipid level but no L-carnitine affects growth performance of hybrid striped bass (Morone chrysops × *M. saxatilis*)," *Aquaculture*, vol. 190, no. 3-4, pp. 237–246, 2000.

[34] C. Schlechtriem, V. Bresler, L. Fishelson, M. Rosenfeld, and K. Becker, "Protective effects of dietary L-carnitine on tilapia hybrids (*Oreochromisnil oticus* × *Oreochromis aureus*) reared under intensive pond-culture conditions," *Aquaculture Nutrition*, vol. 10, no. 1, pp. 55–63, 2004.

[35] H. Ji, T. M. Bradley, and G. C. Tremblay, "Atlantic salmon (*Salmo salar*) fed L-carnitine exhibit altered intermediary metabolism and reduced tissue lipid, but no change in growth rate," *The Journal of Nutrition*, vol. 126, no. 8, pp. 1937–1950, 1996.

[36] Z. Selcuk, S. U. Tiril, F. Alagil et al., "Effects of dietary L-carnitine and chromium picolinate supplementations on performance and some serum parameters in rainbow trout (*Oncorhynchus mykiss*)," *Aquaculture International*, vol. 18, no. 2, pp. 213–221, 2010.

[37] J. H. Chu, Y. W. Lan, and S. S. Sheen, "The protein requirement for the juvenile dog conch, Laevistrombus canarium, in a recirculating aquaculture system," *Journal of the Fisheries Society of Taiwan*, vol. 45, pp. 261–269, 2018.

[38] Z. C. Cob, A. Arshad, B. Japar Sidik, W. H. W. Nurulhusan, and A. G. Mazlan, "Feeding behaviour and stomach content analysis of Laevistrombus canarium (Linnaeus, 1758) from Merambong shoal, Johor," *Malayan Nature Journal*, vol. 66, pp. 159–170, 2014.

[39] J. H. Chu, Y. W. Lan, S. S. Sheen, and A. Chien, "Effects of different dietary lipid levels on the growth performance, body composition and digestive enzymes of the dog conch, Laevistrombus canarium," *Pakistan Journal of Zoology*, vol. 53, no. 5, pp. 1–9, 2021.

[40] P. J. Britz, T. Hecht, J. Kanuer, and M. G. Dixon, "The development of an artificial feed for abalone farming," *South African Journal of Science*, vol. 90, pp. 7-8, 1994.

[41] T. Watanabe, "Lipid nutrition in fish," *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, vol. 73, no. 1, pp. 3–15, 1982.

[42] J. M. Vergara, G. Lopez-Calero, L. Robaina et al., "Growth, feed utilization and body lipid content of gilthead seabream (*Sparus aurata*) fed increasing lipid levels and fish meals of

different quality," *Aquaculture*, vol. 179, no. 1-4, pp. 35–44, 1999.

[43] FAO, *The state of world fisheries and aquaculture*, FAO Fisheries and Aquaculture Department Food and Agriculture Organization of the United Nations, Rome, 2009.

[44] A. G. J. Tacon and M. Metian, "Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects," *Aquaculture*, vol. 285, no. 1-4, pp. 146–158, 2008.

[45] S. Barlow, "Fishmeal and fish oil: sustainable feed ingredients for aqua feeds," *Global Aquaculture Advocate*, vol. 4, pp. 85–88, 2000.

[46] G. M. Turchini, B. E. Torstensen, and W. K. Ng, "Fish oil replacement in finfish nutrition," *Reviews in Aquaculture*, vol. 1, pp. 10–57, 2009.

[47] F. W. Bernhart and R. M. Tomarelli, "A salt mixture supplying the national research council estimates of the mineral requirements of the rat," *The Journal of Nutrition*, vol. 89, no. 4, pp. 495–500, 1966.

[48] J. H. Chu, Y. C. Hong, A. Chien, and S. S. Sheen, "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, pp. 1–9, 2022.

[49] J. M. Guzmán and M. T. Viana, "Growth of abalone *Haliotis fulgens* fed diets with and without fish meal, compared to a commercial diet," *Aquaculture*, vol. 165, no. 3-4, pp. 321–331, 1998.

[50] AOAC (Association of Analytical Chemists), *Official Methods of Analysis of the Association of Official Analytical Chemists*, AOAC. Inc, Arlington, VA, 14th edition, 1984.

[51] J. Folch, M. Lees, and C. H. Sloane-Stanley, "A simple method for the isolation and purification of total lipids from animal tissue," *Jouranl of Biology Chemistry*, vol. 22, pp. 497–509, 1957.

[52] Q. R. Cao, S. Ren, M. J. Park, Y. J. Choi, and B. J. Lee, "Determination of highly soluble L-carnitine in biological samples by reverse phase high performance liquid chromatography with fluorescent derivatization," *Archives of Pharmacal Research*, vol. 30, no. 8, pp. 1041–1046, 2007.

[53] L. D. Metcalfe and A. A. Schmitz, "The rapid preparation of fatty acid esters for gas chromatographic analysis," *Analytical Chemistry*, vol. 33, no. 3, pp. 363-364, 1961.

[54] A. W. Stoner, "Assessing stress and predicting mortality in economically significant crustaceans," *Reviews in Fisheries Science*, vol. 20, no. 3, pp. 111–135, 2012.

[55] Y. N. Wang, X. H. Mu, N. S. Feng, J. Song, and Y. Q. Chang, "Analysis of phenoloxidase activity in the coelomic fluid of sea cucumber Apostichopus japonicus," *Journal of Dalian Ocean University*, vol. 28, pp. 319–322, 2013.

[56] J. H. Chu, S. M. Chen, and C. H. Huang, "Growth, haematological parameters and tissue lipid peroxidation of soft-shelled turtles, Pelodiscus sinensis, fed diets supplemented with different levels of ferrous sulphate," *Aquaculture Nutrition*, vol. 15, no. 1, pp. 54–59, 2009.

[57] J. A. Buege and S. D. Aust, "Microsomal lipid peroxidation," *Methods in Enzymology*, vol. 52, pp. 302–310, 1978.

[58] A. C. Cheng, C. Y. Chen, C. H. Liou, and C. F. Chang, "Effects of dietary protein and lipids on blood parameters and superoxide anion production in the grouper, *Epinephelus coioides* (serranidae: epinephelinae)," *Zoological Studies*, vol. 45, pp. 492–502, 2006.

[59] S. P. Joyne, *SAS/STAT Guide for Personal Computers*, SAS Institute, Cary North Carolina, USA, version 6 edition, 1985.

[60] R. G. D. Steel and J. H. Torrie, *Principles and Procedures of Statistics. A Biometrical Approach*, McGraw-Hill Book Co, New York, 2nd Ed edition, 1980.

[61] Y. Chen, Z. Suna, Z. Liang et al., "Effects of dietary fish oil replacement by soybean oil and L-carnitine supplementation on growth performance, fatty acid composition, lipid metabolism and liver health of juvenile largemouth bass," *Micropterus salmoides. Aquaculture*, vol. 516, pp. 1–10, 2020.

[62] S. Dikel, B. O. Ünalan, T. Eroldoğan, and H. A. Özlüer, "Effects of dietary L-carnitine supplementation on growth, muscle fatty acid composition and economic profit of rainbow trout (*Oncorhynchus mykiss*)," *Turkish Jouranl of Fisheries and Aquatic Science*, vol. 10, pp. 173–180, 2010.

[63] R. O. A. Ozório, "Dietary L-carnitine supplementation to cultivated fish: a mini-review," *Current Nutrition & Food Science*, vol. 5, no. 1, pp. 40–48, 2009.

[64] M. Mohseni and R. O. A. Ozório, "Effects of dietary L-carnitine level on growth performance, body composition and antioxidant status in beluga (*Huso* L. 1758)," *Aquacult. Nutr.*vol. 20, no. 5, pp. 477–485, 2014.

[65] U. Focken, K. Becker, and P. Lawrence, "A note on the effects of L-carnitine on the energy metabolism of individually reared carp, *Cyprinus carpio* L," *Aquaculture Nutrition*, vol. 3, no. 4, pp. 261–264, 1997.

[66] H. S. M. A. Jalali, S. N. Mahboobi, A. A. Sadeghi, M. Chamani, and G. H. Riazi, "Effects of supplemental dietary L-carnitine and ractopamine on the performance of juvenile rainbow trout, *Oncorhynchus mykiss*," *Aquaculture Research*, vol. 41, no. 11, pp. 1582–1591, 2010.

[67] H. Ishii, S. Horie, and T. Suga, "Physiological role of peroxisomal beta-oxidation in liver of fasted rats," *The Journal of Biochemistry*, vol. 87, no. 6, pp. 1855–1858, 1980.

[68] H. Osmundsen, J. Bremer, and J. I. Pedersen, "Metabolic aspects of peroxisomal β-oxidation," *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, vol. 1085, no. 2, pp. 141–158, 1991.

[69] N. M. Verhoeven, D. S. Roe, R. M. Kok, R. J. Wanders, C. Jakobs, and C. R. Roe, "Phytanic acid and pristanic acid are oxidized by sequential peroxisomal and mitochondrial reactions in cultured fibroblasts," *Journal of Lipid Research*, vol. 39, no. 1, pp. 66–74, 1998.

[70] R. O. A. Ozório, *Dietary L-Carnitine and Energy and Lipid Metabolism in African Catfish (Clarias gariepinus) Juveniles PhD Dissertation*, Wageningen University, The Netherlands, 2001.

[71] A. Santulli and V. DAmelio, "The effects of carnitine on the growth of sea bass (*Dicentrarchus labrax*) fry," *J Fish Biol*, vol. 28, pp. 81–86, 1986.

[72] M. Azizi-chekosari, M. Bouye, and A. R. Seidavi, "Effects of L-carnitine supplementation in diets of broiler chickens," *Journal of the Hellenic Veterinary Medical Society*, vol. 72, no. 1, pp. 2611–2628, 2021.

[73] J. Ming, J. Ye, Y. Zhang et al., "The influence of maggot meal and l-carnitine on growth immunity antioxidant indices and disease resistance of black carp (*Mylopharyngodon piceus*)," *Journal of the Chinese Cereals and Oils Association*, vol. 28, pp. 80–86, 2013.

[74] J. J. Ma, Z. R. Xu, Q. J. Shao et al., "Effect of dietary supplemental L-carnitine on growth performance, body composition and antioxidant status in juvenile black sea bream, Sparus macrocephalus," *Aquaculture Nutrition*, vol. 14, no. 5, pp. 464–471, 2008.

[75] M. N. Farsani, S. M. A. Jalali, and M. J. Dehkordi, "Evaluation the meat composition and immunity parameters of rainbow trout (*Oncorhynchus mykiss*) fed by dietary different oil sources, L-carnitine and ractopamine supplement," *ARPN Journal of Agricultural and Biological Science*, vol. 10, pp. 108–115, 2015.

[76] T. Kalaiselvi and C. Panneerselvam, "Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats," *The Journal of Nutritional Biochemistry*, vol. 9, no. 10, pp. 575–581, 1998.

[77] Z. R. Xu, M. Q. Wang, H. X. Mao, X. A. Zhan, and C. H. Hu, "Effects of L-carnitine on growth performance, carcass composition, and metabolism of lipids in male broilers," *Poultry Science*, vol. 82, no. 3, pp. 408–413, 2003.