Pakistan J. Zool., pp 1-9, 2021. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20200524130518

Effects of Different Dietary Lipid Levels on the Growth Performance, Body Composition and Digestive Enzymes of the Dog Conch, *Laevistrombus canarium*

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Article Information
Received 24 May 2020
Revised 20 June 2020
Accepted 27 June 2020
Available online 02 October 2020

Authors' Contribution
JHC supervised the research and prepared manuscript. YWL conducted the feeding trial. SSS provided all materials for the research. AC helped in statistical analysis and helped in manuscript revision.

Key words
***Laevistrombus canarium*, Lipid, Fatty acids, Weight gain, Dog conch**

ABSTRACT

A feeding experiment investigated the effects of dietary lipid levels on the growth, muscle composition and digestive enzyme of 0.28 g *Laevistrombus canarium*. Five semi-purified diets containing 0% (L0), 2% (L2), 4% (L4), 6% (L6), and 12% (L12) lipids were formulated. Each diet was randomly assigned to three replicate groups of *L. canarium* larvae. The final weight, weight gain percentage and specific growth rate of *L. canarium* larvae fed with diets L6 and L12 were significantly higher than those of larvae fed with diets L0, L2 and L4. The feed conversion ratio (FCR) of *L. canarium* larvae fed with treatment diets was significantly lower than that of conch fed with control diet. The minimum dietary n-3 highly unsaturated fatty acid (HUFA) (C20:5n-3 + C22:6n-3) requirement for *L. canarium* was 19.59% of total lipid. Lipase activity in soft body increased with an increasing dietary lipid level, but amylase activity was not significantly affected by the dietary lipid level. Based on a broken-line of the final weight of *L. canarium* larvae, the optimum dietary lipid levels were 5.6%.

INTRODUCTION

Laevistrombus canarium, commonly known as the dog conch, is a marine gastropod mollusc in the family Strombidae. *L. canarium* is a highly valued animal species in Asian countries, including Taiwan. It is an Indo-Pacific species naturally distributed from India and Sri Lanka to Melanesia, Australia, and southern Japan (Man *et al*., 1998; Poutiers, 1998). In many parts of Southeast Asia, the flesh of *L. canarium* is a staple food for human consumption (Cob *et al*., 2008). The shell can be ornamental value (Purchon and Purchon, 1981) and used as sinkers for nets by fisheries (Poutiers, 1998). Due to its high economic value, *L. canarium* has been overexploited and overfished in many areas. Therefore, several ecologists have recommended reducing exploitation rates to maintain the sustainability of this natural resource (Cob *et al*., 2009a). In Taiwan, the consumption of *L. canarium* has increased year by year; therefore, the Marine Life Propagation Station in Penghu County, Taiwan released 48,000 *L. canarium* larvae into the wild in 2018. Unfortunately, catch of large *L. canarium* individuals has become an increasingly difficult task in the waters around Taiwan where this species inhabits. The mass production technology of *L. canarium* larvae has been established since 2009 (Cob *et al*., 2009b), but seed production is still inconsistent and of insufficient quantity to meet the high demands of grow-out fish. *L. canarium* larval development and survival are most affected by several environmental conditions, such as temperature and food sources, particularly, food quality and availability (Cob *et al*., 2009c). *L. canarium* lives on muddy and sandy bottoms in its wild habitat, where most food source are algae and detritus. To develop this species on an economic scale, background study of nutritional requirements should be established, such as the amounts of protein (Chu *et al*., 2018), lipids, carbohydrates, vitamins, and minerals.

Lipids are one of the essential nutritional elements required for animal development and growth. Lipids play several roles, such as providing energy, phospholipids, sterols, fat-soluble vitamins, and essential fatty acids (Watanabe, 1982). Fatty acids are important constituents of cell membranes in the brain and retina, particularly in the larval and juvenile stages (Chu and Sheen, 2016).

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0030-9923/2021/0001-0001 $ 9.00/0

There are protein-sparing effects of non-protein nutrients; for example, when dietary lipids are in an inadequate supply, proteins might be used as an energy source and thus would reduce the utilization of protein for animal growth. Therefore, the dietary energy supply form lipid for animal growth not only improves protein utilization but also reduces feed costs and limits ammonia production (Vergara *et al*., 1999).

The lipid requirements of mollusk species may differ from different species and in different life stages of mollusks (Gallager *et al*., 1986). *Haliotis discus hannai* weighing 4.36 and 0.39 g fed with diets containing 5% and 3.11%~7.09% lipids, respectively, achieved optimal growth performance (Uki *et al*., 1985; Mai *et al*., 1995). *Babylonia areolata* weighing 0.1 and 0.16 g fed diets containing 10% and 6.54% lipids, respectively, achieved optimal growth performance (Chaitanawisuti *et al*., 2011; Zhou *et al*., 2007). For 0.59 g abalone *Haliotis tuberculata*, the optimal dietary lipid level was 3.11% (Mai *et al*., 1995). The size of mollusks mentioned above was large enough to digest the artificial diets.

Lipid, which play important roles in the physiological and nutritional status provide fatty acids for the successful metamorphosis of mollusk larval stages (Gallager *et al*., 1986). However, most studies relevant to the quantitative lipid requirements and their utilization is available for abalone, with little information on conch. Therefore, it is necessary to develop a dietary lipid requirement for 0.28 g *L. canarium* larvae.

MATERIALS AND METHODS

Five isonitrogenous (46.4%) and isoenergetic (321 kcal/100 g) experimental diets were formulated to contain 0% (L0), 2% (L2), 4% (L4), 6% (L6), and 12% (L12) lipids. Casein and fish meal were served as protein sources. Before formulation of the diets, the endogenous lipid in fish meal was extracted and removed using hot ethanol (1:1, w/v) in five successive treatments. The lipid source was a mixture of fish oil and corn oil (2:1, w/w), α-starch was added as a carbohydrate source and binder, and dextrin was used to adjust the energy level. Cellulose was included in the diets to balance the dietary compositions. A hammer mill was used to grind all ingredients into small particles that could pass through a 150-μm mesh sieve. Water was added approximately 20% of the mash dry weight to form a moist dough. The dough was then cold-extruded through a chopper (3.0-mm die diameter) to produce pellets. The pellets were dried at 60 °C for 12 h to approximately 10% moisture content. The experimental diets were stored at 4°C in a refrigerator until use.

The proximate compositions of the experimental diets and muscle of *L. canarium* were analyzed based on AOAC (1984) 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 (Folch *et al*., 1957). Moisture and ash were determined by conventional methods using a 200 °C oven and a muffle furnace. The crude protein of the experimental diets ranged from 46.24% to 46.26% (dry weight). Crude lipid analyses indicated that the diets formulated to contain 0%, 2%, 4%, 6%, and 12% total lipids actually contained 0.16%, 2.19%, 4.18%, 6.15%, and 12.19%, respectively (Table I).

Table I. Ingredient composition of dietary treatments for *Laevistrombus canarium*.

Ingredient (%)	Dietary level of lipid (% dry weight)				
	0%	**2%**	**4%**	**6%**	**12%**
Casein	30	30	30	30	30
Fish meal	23.73	23.73	23.73	23.73	23.73
Oil[1]	0	2	4	6	12
Lecithin	1	1	1	1	1
Yeast	1	1	1	1	1
α-Starch	2	2	2	2	2
Dextrin	32.0	27.5	23	18.5	5
Vitamin[2]	2	2	2	2	2
Vitamin C	0.03	0.03	0.03	0.03	0.03
Minerals[3]	1	1	1	1	1
Cellulose	7.24	9.74	12.24	14.74	22.24
Proximate analysis					
Moisture	10.60	10.57	10.51	10.1	10.12
Crude protein[4]	46.25	46.24	46.26	46.24	46.24
Crude lipids[4]	0.16	2.19	4.18	6.15	12.19
Ash[4]	6.15	6.45	6.35	6.24	6.28
Crude fiber[4]	9.12	11.05	14.06	16.45	24.21
NFE[5]	29.72	23.5	18.64	14.82	0.96
Dietary energy (kcal/100 g)[6]	305.32	298.67	297.22	299.59	298.51

[1]Fish oil: corn oil, (2:1 v/v); [2]Thiamin HCl 0.5%, riboflavin 0.8%, niacinamide 2.6%, D-biotin 0.1%, Ca-pantothenate 1.5%, pyridoxine HCl 0.3%, folic acid 0.5%, inositol 18.1%, ascorbic acid 12.1%, cyanobalamin 0.1%, para-aminobenzoic acid 3%, BHT 0.1%, and cellulose 60.3%; [3]Bernhart-Tomarell, modified (Bernhart and Tomarell, 1966); [4]Expressed as a percent of dry weight; [5]Nitrogen free extract (NFE): [100 - (crude protein + crude lipids + crude fiber + ash)] (%); [6]Calculated digestible energy = [4 (% crude protein) + 9 (% crude lipid) + 4 (% NFE)].

In total, 35 days after hatch *L. canarium* larvae were transported from the Marine Life Propagation Station, Penghu County to National Pingtung University of Science and Technology, Pingtung, Taiwan by air. The larvae were transported in tightly sealed bags, quarter-filled with seawater, inflated with oxygen, and enclosed in an insulated container. After 1-week of acclimation in the laboratory, *L. canarium* individuals weighing 0.28 g were randomly distributed into 15 aquarium (45 × 30 × 30 cm) containing 50 L of seawater with 10 *L. canarium* in each aquarium. Five experimental diets were randomly assigned to three replicate aquaria.

Table II. Major fatty acid composition (% of total fatty acids) of the experimental diets.

Fatty acids	Dietary level of lipid (% dry weight)				
	0%	2%	4%	6%	12%
14:0	6.68	0.03	0.06	0.08	0.11
14:1	8.38	0.02	0.12	0.1	0.06
16:0	14.99	13.56	12.03	13.12	14.62
16:1	2.84	0.35	0.63	0.45	0.46
18:0	46.86	44.79	30.97	35.19	32.24
18:1	1.11	1.06	0.96	1.03	0.99
18:2 n-6	1.68	1.95	1.52	1.56	1.48
18:3 n-3	1.14	2.14	2.01	2.01	2.36
20:0	n.d.	0.14	0.12	0.15	0.15
20:1	n.d.	0.16	0.21	0.16	0.17
20:2	8.01	0.41	0.29	0.64	0.65
20:3 n-6	0.69	1.63	1.23	1.62	1.36
20:3 n-3	2.61	1.62	16.5	2.06	2.31
20:4 n-6	n.d.	13.21	13.21	15.01	15.21
20:5 n-3	n.d.	0.83	1.36	1.03	1.67
22:0	4.22	0.21	0.14	0.21	0.25
22:1	n.d.	1.03	1.01	0.63	0.39
22:5 n-3	n.d.	5.63	4.66	6.39	6.78
22:6 n-3	4.47	11.23	13.57	18.56	19.12
SFAs	72.75	58.73	43.32	48.75	47.37
MUFAs	12.33	2.62	2.93	2.37	2.07
PUFAs	14.13	7.75	21.55	7.89	8.16
n-3 HUFAs	4.47	17.69	19.59	25.98	27.57

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids; n.d., not detectable.

Triplicate groups of *L. canarium* larvae were fed with the experimental diets (at a rate of approximately 5% of their body weight) twice (08:00 and 18:00) a day by hand for 56 days. During the feeding period, the water temperature, dissolved oxygen, and salinity ranged from 27 to 28°C, 5.0 to 5.1 ppm, and 34‰ to35‰, respectively. Experiment was maintained on a 12-h dark: 12-h light photoperiod. Uneaten feed and feces were siphoned out before the next feeding. At the beginning and termination of the experiment, all *L. canarium* were starved for 24 h before being weighed and the wet weights of survival individuals in each aquarium were determined. The following growth factors were calculated as described: the weight gain percentage = 100 × (W_t – W_0) / W_0; survival = 100 x (F_i – F_d) / F_i); feed conversion ratio (FCR) = total feed intake (g) / (W_t - W_0) (g); specific growth rate (SGR) = 100 (ln W_t - ln W_0) / feeding days; soft muscle/shell weight ratio (SB/S) = 100 × (W_s / W_t); shell length increase = 100 (S_f - S_i) / S_i; mean protein gain (MPG) = SB_f × (1 - M_f) × P_f - SB_i × (1 - M_i) × P_i; where W_0 is the initial mean body weight (g), W_t is the final mean body weight (g), W_s is the final soft-body weight (g), F_i is the initial *L. canarium* number, and F_d is the number of dead *L. canarium*, S_i is the initial *L. canarium* length (mm), S_f is the final *L. canarium* length (mm), $SB_{f,i}$, is the final or initial *L. canarium* soft-body weight (g), $M_{f,i}$ is the final or initial moisture level in the soft body (%), and $P_{f,i}$ is the final or initial protein level in the soft body (%).

At the termination of the experiment, all survival *L. canarium* were sacrificed by immersing them in ice water, and the muscle was carefully dissected. Some of the muscle was analyzed for amylase, lipase and fatty acid profile, while others were dried in an oven at 60 °C, crushed using a homogenizer, and then stored in a refrigerator at -20 °C until being analyzed. The muscle and liver were weighed to calculate the soft-body weight to shell weight ratio (SB/S), and analyzed for the proximate composition.

Diets and the muscle of survival *L. canarium* from each treatment were separately homogenized in chloroform/methanol (2:1, v/v) for 5 min to extract total lipids (Folch *et al*., 1957) and refluxed in 50% KOH for 40 min. The saponified lipids were then methylated by refluxing for 20 min in 2 ml 14% boron-trifluoride in methanol (BF_3-MeOH) as described by Metcalfe and Schmitz (1961) and then extracted with 50 ml ether and 20 ml distilled water in a separatory funnel. Fatty acid methyl esters (FAMEs) 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, ST, USA) isothermally at 208 °C. The injection and detector temperatures were maintained at 250 and 200 °C, respectively. Nitrogen was used as the carrier gas.

Table III. Initial weight, final weight, weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and survival of *L. canarium*.

Dietary level of lipid	Initial weight(g)	Final weight(g)	Weight gain (%)	FCR	SGR (%)	Survival (%)
0%	0.28±0.00	0.59±0.08^{d}	125.86±30.66^{d}	5.79±1.53^{a}	1.44±0.24^{d}	100
2%	0.28±0.00	1.09±0.09^{c}	317.31±34.21^{c}	3.46±0.34^{b}	2.55±0.15^{c}	100
4%	0.28±0.00	1.98±0.10^{b}	660.20±33.93^{b}	3.55±0.21^{b}	3.62±0.08^{b}	100
6%	0.28±0.00	2.42±0.20^{a}	830.25±74.09^{a}	2.46±0.10^{b}	3.98±0.14^{a}	100
12%	0.28±0.00	2.44±0.10^{a}	838.17±34.04^{a}	2.48±0.09^{b}	4.00±0.07^{a}	100

^{a,b,c,d} Means(mean ± s.d.) in the same column with different letters significantly differ at $p<0.05$.

Fatty acids were identified by comparison with retention times of reference standards (GLC-68A, Nu-Check-Prep) consisting of a mixture of saturated (SFAs) and polyunsaturated fatty acids (PUFAs).

The muscle was homogenized (1:9 w/v) in ice-cold double distilled buffer. The homogenates were centrifuged at 30,000g for 30 min at 4°C and collected the resultant supernatants stored at -80°C until analysis digestive enzyme. The digestive enzyme activities were determined with a commercial kit (Spinreact, Girona, Spain, ref. 41201 for amylase, and ref.1001275 for lipase).

Data are presented as the mean ± standard deviation. A one-way analysis of variance (ANOVA) was performed to examine differences in weight gain percentages, SGR, FCR, and data of shell growth and survival among treatments. When a significant difference was observed, Tukey's test was used to compare differences among treatment means. The significance level was set to 0.05, and all statistical analyses were conducted using the SAS software program for Windows (V9.4, SAS Institute, Cary, NC, USA).

RESULTS

Fatty acid compositions of the diets containing different levels of lipid are presented in Table II. The diet without lipid addition contained higher proportions of SFAs and lower proportions of highly unsaturated fatty acids (HUFAs). Dietary levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increased with increasing dietary lipid levels.

The final weight, weight gain percentage, FCR, SGR, and survival of *L. canarium* are presented in Table III. The *L. canarium* fed with diets containing 6% and 12% lipid showed significantly higher final weight, weight gain percentage and SGR than those fed with diet containing 0, 2 and 4% lipid. *L. canarium* fed with the diet without lipid supplementation showed the lowest growth performance. There is no mortality occurred during the 56-day feeding trial and survival was not affected by the different dietary treatments.

The proximate compositions of dog conch muscle are presented in Table IV. Moisture and crude protein contents of muscles ranged from 76.03 to 76.41 % and 45.28 to 45.35 %, respectively. In contrast, the lipid contents of dog conch muscle increased with increasing dietary lipid ranging from 2.24 to 5.00%.

Table IV. Proximate composition of muscle of *L. canarium* fed diets containing different levels of lipid.

Dietary level of lipid	Muscle composition (% dry weight)			
	Moisture	Ash	Crude protein	Crude lipids
0%	76.03±0.12^{c}	1.12±0.03	45.35±0.05	2.24±0.07^{e}
2%	76.41±0.02^{a}	1.09±0.03	45.33±0.02	3.12±0.03^{d}
4%	76.33±0.04^{ab}	1.11±0.04	45.28±0.07	4.01±0.04^{c}
6%	76.14±0.18^{bc}	1.10±0.04	45.33±0.03	4.15±0.03^{b}
12%	76.30±0.08^{ab}	1.10±0.03	45.34±0.02	5.00±0.02^{a}

$^{a, b, c, d, e}$ Means (mean ± s.d.) in the same column with different letter significantly differ at $p<0.05$.

Fatty acid profiles of dog conch muscle are presented in Table V. Percentages of C14:0, C14:1, C18:2n-6 and C20:0 in the muscle of *L. canarium* fed with the diet without lipid supplementation were significantly higher than those fed with the other treatment diets. Percentages of C20:5n-3, C22:5n-3 and C22:6n-3 of the muscle of *L. canarium* fed with the diets containing 4, 6 and 12% lipid were significantly higher than those fed the other treatment diets. The percentage of C20:4n-6 of the muscle of *L. canarium* fed with the diet containing 12 % lipid was significantly lower than those of dog conch fed with the other diets.

Table V. Major fatty acid composition (% of total fatty acids) of muscle of *L. canarium*.

Fatty acid	Dietary level of lipid (dry weight %)				
	0%	2%	4%	6%	12%
14:0	12.36±0.03^{a}	12.12±0.16^{b}	11.41±0.06^{c}	5.41±0.05^{d}	5.04±0.09^{e}
14:1	0.14±0.01^{a}	0.10±0.02^{b}	0.06±0.01^{c}	0.07±0.01^{c}	0.07±0.01^{c}
16:0	10.81±0.20^{d}	23.55±0.06^{a}	3.73±0.09^{e}	19.55±0.26^{c}	21.09±0.23^{b}
16:1	6.81±0.07^{d}	8.67±0.03^{c}	14.21±0.40^{a}	12.78±0.20^{b}	13.87±0.20^{a}
18:0	24.20±0.06^{a}	16.16±0.17^{b}	10.83±0.67^{c}	5.54±0.17^{e}	6.88±0.18^{d}
18:1	9.87±0.16^{e}	14.17±0.14^{c}	22.78±0.81^{a}	12.55±1.02^{d}	19.87±0.35^{b}
18:2 n-6	0.93±0.06^{a}	0.25±0.02^{c}	0.35±0.03^{c}	0.47±0.13^{b}	0.28±0.02^{c}
18:3 n-3	0.37±0.02^{c}	0.09±0.01^{d}	0.16±0.02^{cd}	2.17±0.20^{a}	1.70±0.24^{b}
20:0	21.07±0.14^{a}	0.37±0.02^{d}	0.79±0.02^{c}	4.56±0.35^{b}	0.98±0.02^{c}
20:1	0.37±0.06^{d}	1.53±0.04^{a}	1.07±0.02^{b}	0.36±0.01^{d}	0.55±0.04^{c}
20:2 n-6	0.11±0.02^{d}	0.56±0.10^{b}	0.31±0.05^{c}	3.37±0.17^{a}	0.36±0.01^{c}
20:3 n-6	n.d.	0.15±0.03^{c}	0.19±0.02^{b}	0.26±0.01^{a}	0.14±0.01^{c}
20:3 n-3	n.d.	0.12±0.03^{b}	0.05±0.01^{b}	0.43±0.04^{a}	0.48±0.08^{a}
20:4 n-6	0.69±0.02^{a}	0.68±0.04^{a}	0.60±0.04^{b}	0.70±0.01^{a}	0.36±0.05^{c}
20:5n-3	0.19±0.01^{c}	0.24±0.02^{b}	0.66±0.02^{a}	0.67±0.02^{a}	0.68±0.01^{a}
22:0	9.92±0.12^{c}	13.37±0.60^{ab}	14.46±0.96^{a}	12.53±0.24^{b}	8.00±0.94^{d}
22:1	1.31±0.30b^{c}	1.33±0.06^{bc}	1.49±0.23^{b}	1.01±0.06^{c}	2.66±0.26^{a}
22:5 n-3	0.15±0.03^{c}	4.65±0.35^{b}	12.70±0.26^{a}	13.30±0.58^{a}	12.96±0.02^{a}
22:6 n-3	0.70±0.17^{c}	1.62±0.14^{b}	4.14±0.15^{a}	4.26±0.08^{a}	4.03±0.03^{a}
SFAs	78.35±0.08^{a}	65.57±0.60^{b}	41.21±1.36^{d}	47.59±0.23^{c}	41.98±0.79^{d}
MUFAs	18.49±0.10^{d}	25.80±0.10^{c}	39.62±1.29^{a}	26.77±0.92^{c}	37.03±0.60^{b}
PUFAs	1.42±0.07^{c}	1.16±0.13^{d}	1.07±0.05^{d}	6.70±0.19^{a}	2.95±0.18^{b}
n-3 HUFAs	1.05±0.19^{d}	6.52±0.47^{c}	17.5±0.16^{b}	18.24±0.62^{a}	17.68±0.02^{ab}

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; HUFAs, highly unsaturated fatty acids; n.d., not detectable.

Table VI. Effects of dietary lipid levels on the shell length increase, soft-body weight/shell weight (SB/S) ratio, and mean protein gain (MPG) of *L. canarium* fed diets containing different levels of lipid.

Dietary level of lipid	Shell length increase (mm)	SB/S (%)	MPG (g/ shell)
0%	19.79±0.22^{e}	0.23±0.03^{b}	0.02±0.00^{d}
2%	57.22±1.65^{d}	0.27±0.04^{b}	0.07±0.00^{c}
4%	71.18±0.67^{c}	0.38±0.02^{a}	0.12±0.00^{b}
6%	88.43±0.55^{a}	0.39±0.04^{a}	0.13±0.00^{a}
12%	83.04±1.87^{b}	0.35±0.02^{a}	0.13±0.00^{a}

a, b, c, d Means (mean ± s.d.) in the same column with different letters significantly differ at $p<0.05$.

The shell length increase, SB/S and MPG of *L. canarium* are shown in Table VI. The shell length increase of *L. canarium* fed with the diet containing 6% lipid was significantly higher than that of conch fed with the other diets, whereas the shell length increase of dog conch fed with the control diet was significantly lower than that of conch fed the other diets. The SB/S of *L. canarium* fed with the diets containing 4, 6 and 12 % lipid was significantly higher than that of conch fed with diets containing 0 and 2 % lipid. The conch fed with diets containing 6 and 12 % lipid had significantly higher MPG than those fed with other treatment diets.

The lipase and amylase of conch muscle are presented in Table VII. Lipase activity of muscle of *L. canarium* fed diets containing 4, 6 and 12% lipid was significantly

higher than that of conch fed with diets containing 0 and 2% lipid. However, the amylase activity was not affected by the dietary lipid levels.

Table VII. Effect of dietary lipid level on the activities of digestive enzymes of *L. canarium* fed with diets containing different levels of lipid.

Dietary level of lipid	Lipase (mU mg^{-1} protein)	Amylase (mU mg^{-1} protein)
0%	0.16±0.01^{d}	2.05±0.08
2%	0.21±0.02^{c}	2.07±0.07
4%	0.29±0.02^{b}	2.01±0.09
6%	0.31±0.02^{b}	2.05±0.05
12%	0.35±0.02^{a}	2.03±0.17

^{a,b,c,d} Means (mean ± s.d.) in the same column with different letters significantly differ at $p<0.05$.

The broken-line analysis based on the final weight for estimating the optimal dietary lipid requirement for *L. canarium* is shown in Figure 1. The regression equations are $Y = 0.35X + 0.47 (r^2 = 0.97)$ and $Y_{max} = 2.43$. The broken point occurred at 5.6 g lipid/100 g diet was estimated to provide the adequate level of dietary lipid for *L. canarium*.

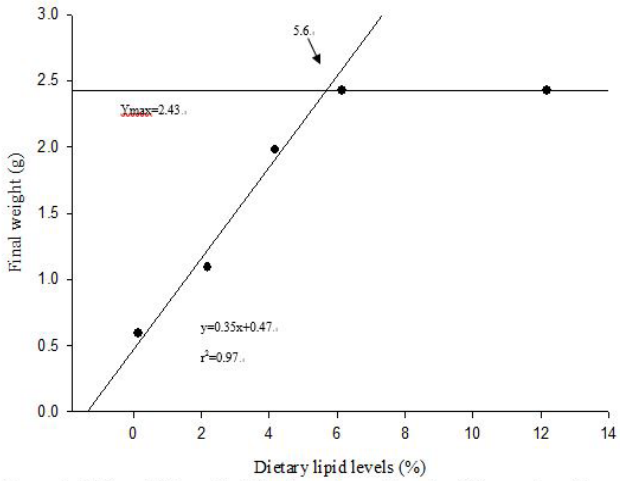

Fig. 1. Effect of dietary lipid levels on the final weight of *L. canarium*. The regression line that fits the dietary lipid requirement has a breakpoint at 5.6g lipid/100 g diet.

DISCUSSION

Our study indicated that a dietary lipid level ranging from 6 to 12 % is satisfactory for dog conch. This study supports the observed relationship of comparatively low dietary lipid levels and reduced growth performance in certain aquatic animals (NRC, 2011). The reduced growth response to low levels of dietary lipid is probably due to insufficient lipid utilization, particularly when other energy sources are available (Sheen and D'Abramo, 1991). Under these conditions, protein may serve as an energy source. Although carbohydrate can be converted into fatty acids, the quantity of carbohydrate converted into fatty acids might be insufficient for animal growth. Therefore, the growth performance of dog conch fed with the control diet without lipid addition was lower than those of dog conch fed with the diets containing lipid, although the diets were isoenergetic. The significantly lower weight gain of dog conch fed with the diet without oil probably reflects a response to insufficient levels of dietary essential fatty acids rather than the ratio itself. These results were similar to the results of other mollusk species, such as abalone, *H. tuberculate* and *H. discus* hannai (Mai *et al*., 1995).

Ivory shell larvae, *B. areolate*, fed with diets containing above 7.8% lipid (Zhou *et al*., 2007), *H. asinine* fed with diets containing above 6.1% lipid (Bautista-Teruel *et al*., 2011) and *H. tuberculate* and *H. discuss* fed with diets containing above 5 % lipid (Mai *et al*., 1995), showed a decrease in growth performance. Diets containing high levels of lipid have the adverse effect on the weight gain of ivory shell and abalone. However, dog conch fed with the diets containing higher than 5.6 % lipids showed no adverse effect on the growth performance. The magnitude of a weight gain response to different levels of lipid appears to be partially dependent on the amount of available dietary energy. Differences in response of lipid may be most pronounced at higher levels of total available energy. If a diet contains a sufficient level of non-protein energy, little protein will be used as an energy source (Huo *et al*., 2014). We suggest that in diets which do not satisfy the protein requirement the relative proportion of energy sources can exert a significant effect on growth.

This study tried to maintain the same dietary protein to energy ratio while changing the total levels of a dietary oil mixture and to satisfy the protein requirement by supplying the proper quality and quantity of protein. Under these conditions, the relative proportions of lipid and carbohydrate differed and the carbohydrate:lipid ratio ranged from 0.08 to 10.7. Comparable weight gain responses of juvenile dog conch to lipid levels were between 6 to 12 % and all essential fatty acid requirements were satisfied at the 6% level of the oil mixture. The significant weight gain of dog conch was observed in response to the 1.21 % dietary EPA and DHA when expressed as a percentage of the dry diet in this study. A level of dietary lipid as low as 6% can be sufficient if appropriate levels of protein, energy and essential fatty acids are provided.

There was a positive correlation between muscle

lipid contents and dietary lipid levels in this study. Muscle lipid contents increased as dietary lipid supplementation increased. It was indicated that when dietary lipids were supplied in excess, a proportion of these lipids is deposited as body fat. This observation was similar to those in *H. tuberculata*, *H. discus hannai* (Mai *et al*., 1995), *B. areolata* (Zhou *et al*., 2007), and some marine fishes, *Epinephelus bruneus* (Yoshii *et al*., 2010), Atlantic cod (Morais *et al*., 2001), *Epinephelus coioides,* and *Epinephelus lanceolatus* (Chu and Sheen, 2016). Protein contents in muscle tissues of *L. canarium* were not correlated with dietary lipid levels, even though these experimental diets were isonitrogenous; this result was similar to those of abalone (Mai *et al*., 1995) and ivory shell (Zhou *et al*., 2007). The SB/S and MPG of *L. canarium* increased as the dietary lipid level rose to 6%, but then decreased when the dietary level exceeded 6%, the finding that agrees with previous results reported for ivory shell (Zhou *et al*., 2007). Those results also suggest mollusks seem to be able to digest and utilize dietary lipids or carbohydrates to achieve good growth parameters.

The fatty acid profiles in muscle of *L. canarium* fed diets supplemented with lipids generally reflect those of the dietary source. This is especially obvious when the dietary lipid content exceeded 4%. This result was similar to several reports on abalone (Bautista-Teruel *et al*., 2011; Thongrod *et al*., 2003), hybrid tilapia (Huang *et al*., 1998), freshwater prawn (D'Abramo and Sheen, 1993), grouper (Chu and Sheen, 2016), Chinook salmon (Silver *et al*., 1993), and a snail (Lee and Lim, 2005). The increase of tissue EPA, C22:5n-3 and DHA as the level of dietary lipid increased was of particular importance. The dietary n-3 fatty acids (EPA + C22:5n-3 + DHA) ranged from 0.007 to 3.36% when expressed as a percentage of the dry diet in this study. However, there have been no studies on the essential fatty acid requirement for dog conch. The levels of EPA + C22:5n-3 + DHA (1.60 to 3.36%) found in the diets supplemented with 6 to 12% lipid in the present study supported good growth of dog conch. Sargent *et al*. (1997) and Sargent *et al*. (1999) indicated that marine aquatic animals has a limited capacity to desaturate C18:3n-3 to EPA and DHA because marine aquatic animals lack Δ-5 desaturase. Hence, DHA and EPA are essential dietary constituents for marine aquatic animals. Bautista-Teruel *et al*. (2011) documented that n-3 HUFAs such as DHA and EPA can potentially improve abalone growth. Dog conch requires higher 1.60% n-3 HUFA for superior growth performance.

CONCLUSIONS

In conclusion, based on the broken line analysis of final weight of *L. canarium*, the optimal dietary lipid requirement was found to be 5.6 g lipid /100 g diet. The dietary lipids could supply essential fatty acid for *L. canarium* in this study. The growth of *L. canarium* was influenced by n-3 HUFA levels in the diets.

Statement of conflict of interest

The authors have declared no conflict of interest.

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