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Enhancing the dietary value of palm oil in the presence of lysolecithin in tiger shrimp, *Penaeus monodon*

Hajah Imran Khan[1,2] · **Jagabattuala Syama Dayal**[1] · **Kondusamy Ambasankar**[1] · **Eda Purdhvi Madhubabu**[1] · **Rajabdeen Jannathulla**[1] · **Vanjiappan Rajaram**[1,3]

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Abstract The effect of four iso-nitrogenous and iso-lipidic diets containing soy lecithin and lysolecithin with fish oil (sardine) and palm oil on growth, digestibility, and fatty acid composition of tail muscle and non-muscle portions of tiger shrimp, *Penaeus monodon*, was evaluated. Shrimp fed with lysolecithin diets had significantly ($P < 0.05$) higher daily growth coefficient values (1.40–1.45% day^{-1}) than those fed with soylecithin containing diets (1.32–1.37% day^{-1}). Correspondingly, lysolecithin-supplemented diets showed significantly higher ($P < 0.05$) apparent digestibility coefficients (ADC) of fatty acids with both the oils due to higher emulsification ability of small micelle forming by lysolecithin. However, there were no significant differences in survival and FCR among all treatments. The fatty acid composition of the test diets reflected to a certain extent in the fatty acid composition of the muscle and non-muscle portions of shrimp. Arachidonic, eicosapentaenoic, and docosahexaenoic acid contents of muscle and non-muscle portions of shrimp were significantly ($P < 0.05$) higher in lysolecithin-supplemented diet compared to soylecithin-supplemented diet. The present results suggest that lysolecithin improved the fatty acid digestibility with its high emulsification properties that reflected in better performance by improving dietary value of palm oil.

Keywords Digestibility · Fatty acid · Lysolecithin · Palm oil · *Penaeus monodon* · Shrimp nutrition

✉ Hajah Imran Khan
amanimran1986@gmail.com

[1] ICAR—Central Institute of Brackishwater Aquaculture, 75, Santhome High Road, RA Puram, Chennai 600028, India

[2] ACI Godrej Agrovet Pvt. Ltd., Ja-28/8/B, Mohakhali, C/A, Dhaka 1212, Bangladesh

[3] Deepak Nexgen Foods and Feeds Pvt. Ltd, 67/2, Koyyur Road, Bommuluru, Bapulapudu (M), Krishna District, Andhra Pradesh 521105, India

Introduction

Traditionally, fish oil (FO) is used as the main lipid source in aqua feeds for marine fish and crustaceans due to its richness in highly unsaturated fatty acids (HUFA). Aqua feed industry constitutes only 4% of the animal feed industry and is using approximately 80% of world's fish oil resources (Shepherd and Jackson 2013). The present aqua feed production (35 million tons) is expected to double by 2020 to meet the aquaculture production demands. Due to economic pressures from high fish oil prices and for ensuring sustainability, evaluating alternatives to fish oil is imperative today (González-Félix et al. 2010). Research efforts were made to replace fish oil in shrimp feed with various vegetable oils (Colvin 1976; Guary et al. 1976; Kanazawa et al. 1977b; Catacutan 1991; González-Félix and Perez-Velazquez 2002; Kumaraguru Vasagam et al. 2005). Despite research efforts, the inclusion frequency of fish oil is 80% compared to the contribution of palm oil (3.1%), coconut oil (3.1%), and peanut oil (3.1%) in aqua feeds.

The strategy to include vegetable oils should not compromise the fatty acid digestibility, production performance, and also fatty acid profiles of muscle and non-muscle portion of shrimp. Palm oil (PO) is one among the major oils produced (66.83 mt; USDA 2016), but its utilization in shrimp feeds is limited due to its high content of saturated fatty acids (SFA) which are less digestible compared to the polyunsaturated fatty acids (PUFA) in fish oil (Merican and Shim 1994). Lower digestibility of SFA is due to the limitation of these fatty acid incorporation into micelles and makes them rely on efficient emulsifiers (Polin et al. 1980; Danicke et al. 2001). The effect of dietary phospholipids on digestion of olive oil in shrimp indicated that phospholipids have positive effect on solublization, emulsification, and digestion of lipids (Glencross 1998) and in other vegetable oils (Kumaraguru Vasagam et al. 2005; Yi et al. 2011).

Lysolecithin (LL) is a product of phospholipase A2 enzymatic activity of lecithin. Kontara et al. (1998) studied the efficacy of native, lyso, and hydrogenated soy lecithin in post larval stages of *Penaeus japonicus* with semi-purified diets. LL can form very small micelle because it has a critical micelle concentration (CMC) of 0.02–0.2 mM L^{-1}, which is 20–200 times more effective than bile (CMC = 4 mM L^{-1}) and soylecithin (SL) (CMC = 0.3–2 mM L^{-1}; Longmuir 2002). However, the effect of LL on digestion of fatty acids in practical diets of shrimp has not been examined so far. It is hypothesized that LL inclusion is more useful in shrimp due to its very short gut passage time by enhancing the digestibility and transportation of fatty acids in PO-based diets, enabling the total replacement of FO in shrimp feeds. Hence in the present study, the effect of replacement of FO with PO in the presence of LL on growth parameters, fatty acid digestibility, and the final product quality in terms of fatty acid profiles in tiger shrimp, *Penaeus monodon*, was evaluated.

Materials and methods

Experimental diet preparation

Four iso-nitrogenous and iso-lipidic practical diets were formulated with varying lipid sources (Table 1) viz., fish oil with soylecithin (FOSL), fish oil with lysolecithin (FOLL), palm oil with soylecithin (POSL), and palm oil with lysolecithin (POLL). The source of fish oil used in the present study is sardine and was obtained from Bismi Fisheries, Mayiladuthurai, Tamil Nadu,

India. The other oil sources viz., palm oil, soylecithin, and lysolecithin were purchased from local market (Chennai, Tamil Nadu, India), Real Soy Enterprises (Madhya Pradesh, India), and Godrej Agrovet Feed Pvt. Ltd. (Andhra Pradesh, India), respectively. The coarse ingredients were powdered in a micropulverizer and passed through 250-μm mesh screen. All the dry ingredients were weighed as per formula and were mixed manually in a domestic mixer for 10 min. Fish oil/palm oil and soylecithin/lysolecithin were gradually added to the homogenized mash. Water was then added (500 mL kg^{-1} mash) to the diet mix and manually kneaded into dough. It was steamed for 5 min and pelleted in a table top pelletizer with a 2-mm-diameter die (Dayal et al. 2003). The pellets were dried in a forced air oven at 60 °C for 12 h and stored at − 5 °C until being used.

Table 1 Ingredient and proximate composition of experimental diets

Composition	Experimental diets			
	FOSL	FOLL	POSL	POLL
Coarse ingredients[a] (g kg^{-1} as fed basis)				
Fishmeal	250	250	250	250
Acetes	120	120	120	120
Prawn head	50	50	50	50
Squid meal	50	50	50	50
Soybean meal	220	220	220	220
Wheat flour	184	184	184	184
Broken rice	50	50	50	50
Vitamin mineral mix[b]	20	20	20	20
Vitamin C	1	1	1	1
Binder	10	10	10	10
Chromic oxide	5	5	5	5
Oil sources[a] (g kg^{-1} as fed basis)				
Fish oil[c]	20	20	–	–
Palm oil[d]	–	–	20	20
Soy lecithin[e]	20	–	20	–
Lysolecithin[f]	–	20	–	20
Chemical composition (g kg^{-1} as fed basis)				
Moisture	91.84	87.45	89.24	90.64
Crude protein	392.71	393.01	394.46	393.16
Ether extract	71.86	71.27	71.44	71.71
Crude fiber	36.21	35.57	36.19	35.80
Nitrogen-free extract (NFE)	261.19	263.37	258.21	260.80
Total ash	146.19	149.33	150.46	147.89

[a] All the ingredients used are feed grade

[b] Vitamin mineral mix (mg kg^{-1} feed): vitamin A, 10,000 IU; vitamin D, 1500 IU; vitamin E, 60; vitamin K, 1.5; niacin, 200; riboflavin, 37.5; calcium pantothenate, 125; vitamin B12, 20; thiamin, 15; pyridoxine, 15; folic acid, 5.5; biotin, 750; choline chloride, 100; stable vitamin C, 1000; cobalt, 0.2; iodine, 0.6; copper, 5.0; iron, 14.0; manganese, 55.0; zinc, 24.0) and 1% dicalcium phosphate

[c] Extracted from sardine and obtained from Bismi Fisheries, Mayiladuthurai, Tamil Nadu, India

[d] Local market, Chennai, Tamil Nadu, India

[e] Real Soy Enterprises, Madhya Pradesh, India

[f] Godrej Agrovet Feed Pvt. Ltd., Andhra Pradesh, India

Growth trial

Post larvae (PL15) of *P. monodon* were procured from the private hatchery located near Chennai, India, and was reared in net cages in the Muttukadu Experimental Station, Central Institute of Brackishwater Aquaculture, Chennai, to get the juveniles for feeding trial. The shrimp were acclimatized for 48 h in 1000-L fiber glass tanks. The growth trial was conducted in a flow through seawater (1.5 mL min^{-1}) indoor wet laboratory with continuous aeration. Shrimp (mean initial body weight 5.21 ± 0.1 g) were distributed randomly into 21 oval 500-L fiber glass tanks (3 tanks/feed, 15 shrimps/tank) in a completely randomized design. The shrimp were fed ad libitum thrice daily (8:30, 12:30, and 18:00 h) at the rate of 6% to the biomass for 42 days, and the feed given was adjusted later according to growth, intake, and survival. During the feeding trial, the water temperature, salinity, and dissolved oxygen were in the range of 26–28.5 °C, 28–29 g L^{-1}, and 6.0–8.0 mg L^{-1}, respectively. Two hours after each feeding, uneaten feed was bottom-siphoned and dried to calculate the feed intake. At the end of the growth trial, weight gain (WG), daily growth coefficient (DGC), and survival for each dietary treatment were determined. DGC was used for the expression of growth pattern of *P. monodon* following the protocol of Rajaram et al. (2012). Protein efficiency ratio (PER) and apparent protein utilization (APU) were calculated to measure the protein efficiency utilization.

$$\mathrm{WG}\ (\%) = [\textit{initial weight}\ (\mathrm{g}) - \textit{final weight}\ (\mathrm{g})]/\textit{initial weight}\ (\mathrm{g}) \times 100$$

$$\mathrm{DGC} = \left[\textit{final body weight}^{1/3} - \textit{initial body weight}^{1/3}\right]/\textit{days of experiment} \times 100$$

$$\mathrm{PER} = \textit{weight gain}\ (\mathrm{g})/\textit{protein intake}\ (\mathrm{g})$$

$$\mathrm{APU} = \textit{protein gain}\ (\mathrm{g})/\textit{protein intake}\ (\mathrm{g})$$

$$\textit{Survival}\ (\%) = \textit{final number of animals}/\textit{initial number of animals} \times 100$$

Digestibility trial

The apparent digestibility coefficients (ADC) of fatty acids in feeds were measured in vivo using 5 g kg^{-1} of chromic oxide (Cr_2O_3) as an inert marker. *P. monodon* (weight 8.33 ± 0.08 g) was randomly stocked in 16 1000-L fiber glass tanks (4 tanks/feed, 15 shrimps/tank). During the feeding period, the shrimp were fed in static mode to prevent leaching of feces with 80% water exchange before the first feeding. Shrimp were fed with test diets and priorly acclimatized to the diets for 7 days before fecal collection. Fecal strand-encased membranes were siphoned out gently 2 h after first feeding by avoiding water turbulence. Feces collected were gently rinsed in distilled water, dried on filter paper, and frozen immediately at − 20 °C prior to further analysis. In order to have a representative sample, 15-day collections from all shrimps in a tank were pooled to avoid possible variation and represented one replicate. ADC of fatty acids was calculated according to Rajaram et al. (2012).

Biochemical analysis

Proximate compositions of experimental diets and muscle (edible) and non-muscle portion (head, hepatopancreas, exoskeleton, and gut) of shrimp were analyzed by standard methods of AOAC (1995). Chromium content in the diets and feces was analyzed to calculate fatty acid digestibility (Furukawa and Tsukahara 1966). The concentrations of total lipid (TL; Folch et al. 1957), total cholesterol (TC; Parekh and Jung 1970), triacylglycerides (TG; Rice 1970), and phospholipids (PL; Fiske and Subbarow 1925; Bartlette 1959) were analyzed by standard methods in experimental diets and muscle and non-muscle portions of shrimp.

Fatty acid methyl esters (FAMEs) were prepared according to Metcalfe et al. (1966). The lipids were first trans-esterified with BF -methanol and 0.5 N methanolic sodium hydroxide, and the FAMEs were extracted into petroleum ether. Routine analysis of methyl esters was performed by a gas chromatograph (GC-2014 Shimadzu) on a RTX wax capillary column (30-m length × 0.25-mm ID × 0.2-μm film thickness). Nitrogen was used as carrier gas at a linear velocity of 37.5 cm s^{-1}. The oven temperature was first increased from 100 to 140 °C at 10 °C min^{-1} and held for 5 min followed by temperature increase of 2.5 °C min^{-1} to 240 °C and held for 5 min and raised by 2.0 °C per minute to 245 °C for 5 min. Operating conditions for injection ports and flame ionization detector were 260 and 250 °C, respectively. Compounds were identified by comparisons with retention times of 37 component FAME mix (Supelco-Sigma).

Statistical analysis

Completely randomized design (CRD) was adopted for all experiments, and the data were statistically analyzed using SPSS17.0. A multiple comparison of treatments was done using Tukey's test to find a significant difference between the treatments. Prior to statistical evaluation, the data was checked for determining the homogeneity of variance after ascertaining the normal distribution. The percent survival data was arc sin transformed before testing. The means were compared at significance level of $P < 0.05$, and the results were presented as means ± SD (standard deviation of the mean).

Results

Proximate, lipid class, and fatty acid composition of test diets

Proximate, lipid class, and fatty acid composition of test diets are shown in Tables 1 and 2. All the diets contained 393.34 ± 0.94 g kg^{-1} crude protein and 71.57 ± 0.30 g kg^{-1} ether extract irrespective of the lipid sources. TG was the major lipid fraction in all the diets, and it was high in diet containing PO. PL and TC content of the test diets was relatively constant in both PO and FO-based diets (Table 2). The test diets reflected to a certain extent the fatty acid profile of the oils used in their formulation (Table 2). Higher concentrations of 16:0, 18:1n-9, and 18:2n-6 and lower levels of 14:0, 16:1, 17:1, 20:5n-3 (EPA), and 22:6n-3 (DHA) were observed in PO-based diets compared to FO-based diets. Total saturated fatty acid concentration was high in PO-based diets, whereas total of PUFA and MUFA were high in FO-based diets.

Table 2 Lipid composition and fatty acid profiles of experimental diets

Parameters	Experimental diets			
	FOSL	FOLL	POSL	POLL
Lipid composition (% total lipid)				
Phospholipids	26.14 ± 0.31	26.73 ± 0.34	25.87 ± 0.43	26.16 ± 0.36
Triglycerides	44.95 ± 0.73	44.76 ± 0.66	51.50 ± 0.48	50.38 ± 0.70
Cholesterol	5.29 ± 0.50	5.31 ± 0.51	5.18 ± 0.18	5.33 ± 0.46
Fatty acid profiles (% total fatty acid)				
14:0	4.90 ± 0.90	4.58 ± 0.47	2.29 ± 0.18	2.13 ± 0.17
15:0	0.36 ± 0.11	0.36 ± 0.12	0.25 ± 0.10	0.22 ± 0.05
16:0	19.89 ± 0.54	20.45 ± 0.58	27.64 ± 0.54	28.43 ± 0.59
17:0	0.61 ± 0.09	0.59 ± 0.09	0.43 ± 0.04	0.36 ± 0.07
18:0	4.25 ± 0.43	4.26 ± 0.15	4.46 ± 0.70	4.98 ± 0.20
20:0	0.39 ± 0.11	0.36 ± 0.08	0.36 ± 0.09	0.32 ± 0.06
22:0	0.43 ± 0.14	0.44 ± 0.03	0.44 ± 0.07	0.13 ± 0.05
24:0	0.31 ± 0.07	0.23 ± 0.05	0.31 ± 0.02	0.24 ± 0.05
16:1	5.05 ± 0.43	4.99 ± 0.21	2.48 ± 0.25	2.45 ± 0.43
17:1	0.88 ± 0.06	0.94 ± 0.26	0.30 ± 0.10	0.30 ± 0.02
18:1n-9	14.12 ± 0.30	13.29 ± 0.45	20.18 ± 0.30	19.26 ± 1.26
18:1n-7	2.26 ± 0.31	2.26 ± 0.23	1.67 ± 0.34	1.59 ± 0.29
20:1n-9	0.31 ± 0.03	0.29 ± 0.09	0.34 ± 0.07	0.32 ± 0.04
24:1	0.16 ± 0.05	0.12 ± 0.04	0.17 ± 0.06	0.16 ± 0.01
18:2n-6	16.78 ± 0.50	17.05 ± 0.73	19.71 ± 0.48	20.04 ± 0.87
γ18:3n-6	0.19 ± 0.02	0.18 ± 0.03	0.26 ± 0.08	0.26 ± 0.05
20:2n-6	0.09 ± 0.02	0.11 ± 0.02	0.05 ± 0.03	0.06 ± 0.02
20:3n-6	0.11 ± 0.04	0.11 ± 0.03	0.09 ± 0.01	0.08 ± 0.01
20:4n-6	1.08 ± 0.10	0.92 ± 0.03	0.71 ± 0.15	0.84 ± 0.10
α18:3n-3	3.40 ± 0.40	3.62 ± 0.14	2.65 ± 0.24	3.05 ± 0.18
20:5n-3	7.84 ± 0.40	7.79 ± 0.57	4.61 ± 0.32	4.73 ± 0.17
22:6n-3	8.39 ± 0.20	8.01 ± 0.75	6.39 ± 0.40	6.52 ± 0.44
Σ SFA[a]	31.14 ± 0.15	31.27 ± 0.28	36.18 ± 0.04	36.81 ± 0.56
Σ MUFA[b]	22.78 ± 0.59	21.89 ± 0.49	25.13 ± 0.64	24.08 ± 1.86
Σ PUFA and HUFA[c]	37.89 ± 1.27	37.79 ± 2.08	34.47 ± 0.43	35.57 ± 1.15
Σ n-6	18.26 ± 0.39	18.38 ± 0.73	20.82 ± 0.28	21.27 ± 0.83
Σ n-3	19.63 ± 0.93	19.48 ± 1.48	13.64 ± 0.35	14.30 ± 0.34
n-3/n-6	1.07 ± 0.03	1.06 ± 0.06	0.66 ± 0.02	0.67 ± 0.01

[a] Σ SFA—sum of saturated fatty acids: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0

[2] Σ MUFA—sum of monounsaturated fatty acid: 16:1, 17:1, 18:1n-9, 18:1n-7, 20:1n-9, 24:1

[3] Σ PUFA and HUFA—sum of poly and highly unsaturated fatty acids: 18:2n-6, γ18:3n-6, 20:2n-6, 20:3n-6, 20:4n-3, α18:3n-3, 20:5n-3, 22:6n-3

Growth performance

Data on growth performance in terms of daily growth coefficients (DGC % day^{-1}) and FCR are given in Table 3. Shrimp-fed diets containing LL (FOLL and POLL) had significantly ($P < 0.05$) higher DGC values than those fed with SL diets within the same oil source. There were no significant differences in survival among the dietary treatments. The protein efficiency ratios (1.40–1.42) and apparent protein utilization (24.99–26.28%) were essentially same for all the diets. The different dietary oils had no effect on the growth performance of *P. monodon* within the SL or LL-fed groups. Non-significantly lower FCR were observed in shrimp-fed diet containing fish oil with LL diet compared to other diets (Table 3).

Table 3 Growth performance of *Penaeus monodon*-fed experimental diets containing different lipid sources ($n = 3$; mean ± SD)

Parameters	Experimental diets			
	FOSL	FOLL	POSL	POLL
Weight gain (%)	136.4ab ± 0.91	146.36c ± 2.77	131.10a ± 1.31	141.03bc ± 5.55
DGC (% day^{-1})	1.37ab ± 0.02	1.45c ± 0.03	1.32a ± 0.02	1.40bc ± 0.04
FCR	1.96 ± 0.07	1.93 ± 0.02	1.98 ± 0.07	1.97 ± 0.08
Survival (%)	84.44 ± 10.18	88.89 ± 7.70	88.89 ± 10.18	86.67 ± 11.55
PER	1.42 ± 0.05	1.42 ± 0.02	1.40 ± 0.05	1.41 ± 0.06
APU (%)	25.94 ± 0.93	24.99 ± 0.30	25.67 ± 0.85	26.28 ± 1.07

Mean with different letters in a row is significantly different ($P < 0.05$)

DGC daily growth coefficient, *FCR* feed conversion ratio, *PER* protein efficiency ratio, *APU* apparent protein utilization

Digestibility of fatty acids in *P. monodon*

All test diets were readily accepted by the shrimp regardless of oil sources. Mean ADC of fatty acids ranged from 0.66 ± 0.011 for 24:0 (POSL) to 0.99 ± 0.007 for 20:5n-3 and 22:6n-3 (FOLL; Table 4). Digestibility values of SFA decreased with increasing chain length in all the test diets. All monoenes were well digested (> 87%). Apparent digestibilities of monoenes increased with the chain length. Most of the HUFA were well digested. Shrimp-fed LL-supplemented diets showed significantly ($P < 0.05$) higher ADC of fatty acids compared to those fed with SL-supplemented diet regardless of oil. Apparent digestibility of individual fatty

Table 4 Apparent digestibility coefficients (ADC) of fatty acids in *Penaeus monodon*-fed experimental diets containing different lipid sources

ADC (%)	Experimental diets			
	FOSL	FOLL	POSL	POLL
14:0	90.26b ± 2.69	96.22c ± 2.03	86.44a ± 1.15	92.62bc ± 1.77
15:0	87.13b ± 1.16	94.09c ± 1.07	81.75a ± 0.84	89.81b ± 2.83
16:0	85.29ab ± 2.08	93.01c ± 2.04	80.28a ± 3.09	86.97b ± 3.48
17:0	85.08b ± 1.60	90.26c ± 1.99	79.36a ± 3.93	85.35b ± 1.60
18:0	83.53b ± 1.57	88.66c ± 2.02	77.53a ± 3.12	82.35b ± 2.48
20:0	80.31b ± 1.67	85.31c ± 0.93	73.83a ± 1.26	79.19b ± 1.60
22:0	78.27b ± 1.07	84.78c ± 1.87	67.47a ± 0.58	77.95b ± 0.92
24:0	77.75b ± 2.01	79.75b ± 1.82	66.47a ± 1.12	76.90b ± 1.00
16:1	92.05b ± 0.62	97.19c ± 2.33	87.01a ± 1.54	92.75b ± 3.08
18:1n-9	92.66ab ± 2.87	96.76b ± 2.53	88.50a ± 1.11	93.10b ± 2.29
18:1n-7	91.84ab ± 1.90	95.75b ± 1.93	89.77a ± 2.36	95.24b ± 2.00
20:1	94.91ab ± 1.38	96.58b ± 1.38	92.61a ± 1.10	94.34ab ± 1.27
24:1	96.78 ± 2.22b	98.11 ± 1.63b	95.96 ± 1.4	97.78 ± 1.71b
18:2n-6	96.81b ± 2.01	98.07b ± 1.09	93.45a ± 1.44	97.20b ± 0.87
α18:3n-3	97.42a ± 0.96	97.21a ± 2.30	95.55a ± 1.08	96.87a ± 2.67
20:4n-6	95.47ab ± 0.50	98.14c ± 1.63	93.96a ± 1.09	97.27bc ± 1.48
20:5n-3	97.37ab ± 0.46	99.00c ± 0.54	96.15a ± 1.01	98.32bc ± 0.53
22:6n-3	97.11ab ± 0.23	99.15c ± 0.77	95.76a ± 0.34	98.12bc ± 1.64

Mean with different letters in a row is significantly different ($P < 0.05$)

Table 5 Proximate and fatty acid composition of the muscle portion of *Penaeus monodon*-fed experimental diets containing different lipid sources

Parameters	Experimental diets			
	FOSL	FOLL	POSL	POLL
Proximate composition[a] (g kg^{-1} wet basis)				
Moisture	776.12 ± 5.97	782.03 ± 13.13	773.44 ± 2.34	766.74 ± 11.45
Crude protein	200.34 ± 4.84	194.63 ± 14.37	202.11 ± 5.71	210.11 ± 9.43
Ether extract	5.94 ± 0.50	6.24 ± 1.26	5.61 ± 0.87	5.71 ± 0.19
Crude fiber	1.91 ± 1.11	2.03 ± 0.56	2.24 ± 0.87	1.78 ± 0.77
Nitrogen-free extract	2.32 ± 1.26	1.84 ± 0.67	3.33 ± 1.61	2.11 ± 1.44
Total ash	13.37 ± 1.05	13.23 ± 02.34	13.27 ± 0.74	13.55 ± 1.06
Fatty acid composition (% total fatty acids)				
14:0	0.47bc ± 0.07	0.54c ± 0.10	0.33a ± 0.05	0.38ab ± 0.02
15:0	0.24a ± 0.13	0.45a ± 0.34	0.38a ± 0.15	0.44a ± 0.19
16:0	17.44a ± 0.57	16.32a ± 1.96	20.56b ± 1.38	21.20b ± 1.60
17:0	0.74a ± 0.27	0.94a ± 0.40	0.77a ± 0.24	0.82a ± 0.10
18:0	8.06a ± 0.56	8.51a ± 0.54	8.15a ± 0.69	8.40a ± 0.34
22:0	0.15a ± 0.09	0.24a ± 0.13	0.26a ± 0.06	0.21a ± 0.12
24:0	0.27a ± 0.11	0.35a ± 0.27	0.29a ± 0.08	0.33a ± 0.32
16:1	0.74ab ± 0.04	0.85b ± 0.14	0.60a ± 0.05	0.55a ± 0.12
17:1	0.62a ± 0.11	0.64a ± 0.08	0.67a ± 0.12	0.72a ± 0.19
18:1n-9	10.40a ± 0.45	10.91a ± 0.81	13.53b ± 0.42	13.45b ± 0.62
18:1n-7	2.07a ± 0.98	2.26a ± 0.58	2.66a ± 1.17	2.22a ± 0.76
24:1	0.22a ± 0.07	0.27a ± 0.09	0.26a ± 0.18	0.29a ± 0.21
18:2n-6	15.27a ± 0.23	14.84a ± 1.27	17.55b ± 0.42	18.02b ± 0.75
20:2n-6	1.19a ± 0.76	1.15a ± 0.32	1.30a ± 0.26	1.18a ± 0.46
γ18:3n-6	0.20a ± 0.10	0.21a ± 0.06	0.22a ± 0.07	0.19a ± 0.07
α18:3n-3	0.50a ± 0.15	0.46a ± 0.17	0.51a ± 0.14	0.44a ± 0.05
20:4n-6	3.62b ± 0.10	4.16c ± 0.07	3.26a ± 0.08	3.57b ± 0.15
20:5n-3	12.76b ± 0.50	14.01c ± 0.52	11.60a ± 0.60	12.90b ± 0.44
22:6n-3	12.20b ± 0.14	12.92c ± 0.39	11.43a ± 0.40	12.04b ± 0.13
Σ SFA	27.37a ± 0.95	27.35a ± 1.45	30.74b ± 0.51	31.78b ± 1.68
Σ MUFA	14.05a ± 1.60	14.93a ± 0.94	17.72b ± 1.25	17.23b ± 0.73
Σ PUFA and HUFA	45.77a ± 0.32	47.75b ± 0.74	45.87a ± 0.92	48.34b ± 1.26
Σn-3	25.47b ± 0.45	27.40c ± 0.74	23.55a ± 1.13	25.38b ± 0.34
Σn-6	20.29a ± 0.69	20.37a ± 1.01	22.34b ± 0.23	22.98b ± 0.99
n-3/n-6	1.25b ± 0.06	1.34b ± 0.09	1.05a ± 0.06	1.10a ± 0.03

Mean with different letters in a row is significantly different ($P < 0.05$)

[a] No significant difference ($P > 0.05$)

acids was clearly affected by the dietary lipid source; however, no such differences were observed in shrimp fed with FOSL and POLL diets (Table 4).

Proximate, lipid, and fatty acid composition of shrimp

Proximate, lipid, and fatty acid composition of muscle and non-muscle portions of *P. monodon* is presented in Tables 5 and 6 and Fig. 1, respectively. No significant differences were observed in proximate composition of *P. monodon* among the dietary treatments. PL content significantly ($P < 0.05$) increased in LL-supplemented diet regardless of oil sources. Shrimp-fed diets with POSL and POLL had significantly ($P < 0.05$) higher level of TG in non-muscle portion, whereas in the muscle portion, no significant differences were observed. More importantly, TC content was significantly ($P < 0.05$) lower in PO-fed diets compared to FO-

Table 6 Proximate and fatty acid composition of the non-muscle portion of *Penaeus monodon*-fed experimental diets containing different lipid sources

Parameters	Experimental diets			
	FOSL	FOLL	POSL	POLL
Proximate composition[a] (g kg^{-1} wet basis)				
Moisture	715.13 ± 10.66	727.71 ± 10.72	719.11 ± 3.74	725.88 ± 10.53
Crude protein	156.24 ± 9.46	148.42 ± 7.16	153.03 ± 5.61	150.41 ± 4.11
Ether extract	13.72 ± 1.24	14.02 ± 0.89	13.47 ± 0.57	14.83 ± 0.96
Crude fiber	15.43 ± 1.36	16.67 ± 0.59	16.81 ± 1.29	14.69 ± 1.17
Nitrogen-free extract	26.12 ± 4.80	25.45 ± 4.04	24.41 ± 3.63	22.86 ± 4.71
Total ash	73.36 ± 4.31	67.73 ± 8.63	73.27 ± 6.24	71.33 ± 7.46
Fatty acid composition (% total fatty acids)				
14:0	1.41b ± 0.33	1.32b ± 0.18	0.76 a ± 0.26	0.66a ± 0.28
15:0	0.35 a ± 0.12	0.36 a ± 0.18	0.32 a ± 0.08	0.30a ± 0.19
16:0	18.38a ± 1.10	18.79a ± 1.07	21.42b ± 1.05	21.15b ± 1.41
17:0	0.74a ± 0.15	0.69a ± 0.20	0.72a ± 0.09	0.74a ± 0.15
18:0	7.04a ± 1.14	7.20a ± 0.76	6.94a ± 1.83	7.17a ± 1.97
24:0	0.38a ± 0.11	0.31a ± 0.12	0.32a ± 0.19	0.36a ± 0.07
16:1	1.74b ± 0.28	1.76b ± 0.15	1.03a ± 0.15	1.11a ± 0.22
17:1	0.36a ± 0.06	0.33a ± 0.08	0.45a ± 0.07	0.37a ± 0.14
18:1n-9	14.08a ± 0.45	15.04a ± 0.58	18.77b ± 0.69	18.03b ± 0.44
18:1n-7	2.61a ± 0.29	2.55a ± 0.36	2.42a ± 0.19	2.44a ± 0.08
24:1	0.33a ± 0.07	0.30a ± 0.12	0.31a ± 0.18	0.34a ± 0.09
18:2n-6	18.19a ± 0.39	17.98a ± 0.39	20.96b ± 0.94	21.19b ± 1.33
20:2n-6	1.15a ± 0.31	1.28a ± 0.37	1.18a ± 0.25	1.33a ± 0.08
γ18:3n-6	0.28a ± 0.13	0.30a ± 0.04	0.28a ± 0.11	0.27a ± 0.04
α18:3n-3	0.82a ± 0.11	0.76a ± 0.17	0.67a ± 0.33	0.65a ± 0.18
20:4n-6	3.32b ± 0.11	3.92c ± 0.25	2.87a ± 0.10	3.35b ± 0.14
20:5n-3	9.13b ± 0.24	10.08c ± 0.50	7.32a ± 0.78	9.09b ± 0.24
22:6n-3	8.30b ± 0.23	9.28c ± 0.15	7.37a ± 0.52	8.48b ± 0.10
Σ SFA	28.30a ± 0.39	28.67a ± 1.93	30.48a ± 2.95	30.38a ± 0.81
Σ MUFA	19.12a ± 0.28	19.98a ± 0.46	22.98b ± 1.25	22.29b ± 0.88
Σ PUFA and HUFA	41.19ab ± 0.94	43.60bc ± 0.75	40.65a ± 2.54	44.36 ± 0.98
Σn-3	18.27b ± 0.34	20.13c ± 0.52	15.37a ± 1.63	18.24b ± 0.50
Σn-6	22.95a ± 0.71	23.49a ± 0.27	25.30b ± 0.96	26.16b ± 1.19
n-3/n-6	0.79c ± 0.02	0.85c ± 0.01	0.60a ± 0.04	0.69b ± 0.04

Mean with different letters in a row is significantly different ($P < 0.05$)

[a] No significant difference ($P > 0.05$)

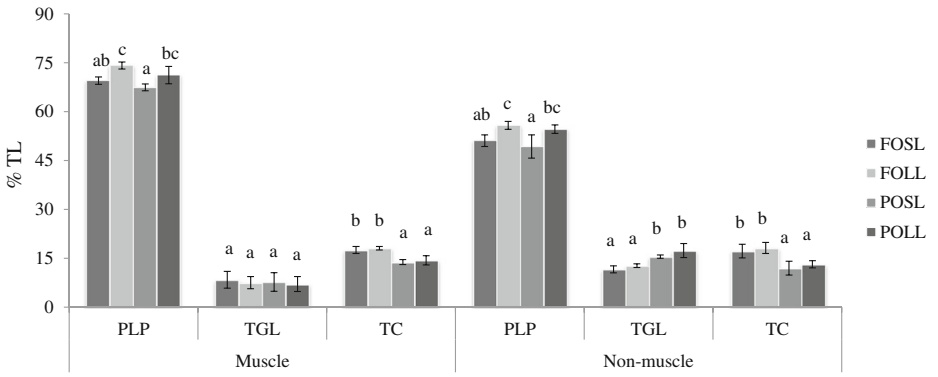

Fig. 1 Lipid composition (phospholipids (PLP), triglycerides (TGL), cholesterol (TC)) of edible and non-edible portion of *Penaeus monodon*-fed experimental diets containing different lipid sources

fed diets in both muscle and non-muscle portions. No effect of LL supplementation was observed in the TG and TC in both muscle and non-muscle portions of the shrimp. The fatty acid composition of the test diets reflected on the fatty acid composition of the muscle and non-muscle portions of shrimp (Tables 5 and 6). Fatty acids 16:0, 18:1n-9, and 18:2n-6 were significantly ($P < 0.05$) higher in muscle and non-muscle portions of shrimp-fed diets with PO. For instance, arachidonic acid (AA), EPA, and DHA contents of shrimp significantly ($P < 0.05$) increased in LL-supplemented diet compared to those not having LL regardless of oil. EPA and DHA content is significantly ($P < 0.05$) high in shrimp-fed diet FOLL compared to shrimp fed with other diets.

Discussion

Any research efforts to replace fish oil should not have any negative effect on production performance and also the final product quality. The recommended dietary lipid levels for penaeid shrimp range from 6.0 to 7.5%, and a maximum level of 10% was suggested (Akiyama et al. 1991; Hu et al. 2008). In the present study, the lipid level of experimental diets averaged 71.57 ± 0.30 g kg^{-1} (Table 1), which was within the recommended range and was contributed not only by the test oils but also by other basal ingredients (mainly by fishmeal). The lipid sources used in the formulation determined the concentration of fatty acids in test diets (Table 2). Shrimp-fed diets containing LL had significantly ($P < 0.05$) higher growth performance compared to the diets containing SL irrespective of oils (Table 3). This significant increase is due to the improvements in fatty acid digestibility (Table 4) and the overall lipid digestibility in *P. monodon* by way of enhanced emulsification of the lipid in the diet. This would have resulted in better absorption of lipid and lipid-soluble substances from shrimp gut. Many studies have demonstrated that HUFA are preferentially incorporated and conserved in the polar lipid of crustacean tissue (Clarke 1970; Kanazawa et al. 1977b; D'Abramo et al. 1980; D'Abramo and Sheen 1993). In the present study, shrimp-fed diets containing LL absorbed and provided more lipid components like HUFA and other fatty acids for the synthesis of PL that constitutes major lipid in shrimp. No significant differences were observed in growth of shrimp fed with FOSL and POSL diets similar to the earlier reported studies (Kumaraguru Vasagam et al. 2005; González-Félix et al. 2011). The present results of improved performance with LL are not in agreement with the findings of Kontara et al. (1998) in post larval stages of *Penaeus japonicus* fed with semi-purified diets. The better performance in the present study may be due to the supplementation of phospholipids from the basal feed ingredients which were absent in earlier reported study. These practical ingredients would have supplied the phospholipid with the presence of unsaturated fatty acid in sn-2 position of the PL molecule (D'Abramo and Sheen 1993; Coutteau et al. 1997; González-Félix et al. 2002) and also may be due to the juveniles having been less sensitive to dietary PL level (Kumaraguru Vasagam et al. 2005).

Chromic oxide was successfully used as an indicator for digestibility measurements of fatty acids in *P. monodon* (Merican and Shim 1994). The mean ADC of individual fatty acids in this study was in agreement with earlier measurements in *P. monodon* wherein the ADC of SFA generally decreased with increasing chain length and unsaturated fatty acids were well digested (Merican and Shim 1994). In contrast, Teshima and Kanazawa (1983) reported higher ADC value for palmitic acid than that of oleic acid in *P. japonicus*. The differences observed may be due to the differences in the form of feeding, i.e., tripalmitate or free fatty acids. Result of the

digestibility trial indicated the ADC of fatty acid increased by LL-supplemented experimental diets, suggesting higher fatty acid digestibility due to increased solubilization and emulsification. In support of this, Schwarzer and Adams (1996) reported that LL forms micelles that are smaller and more stable than those formed with other phospholipids or emulsifier such as lecithin and bile salt. LL can form very small micelle because it has a critical micelle concentration (CMC) of 0.02–0.2 mM L^{-1}, which is 20–200 times more effective than bile (CMC = 4 mM L^{-1}) and lecithin (CMC = 0.3–2 mM L^{-1}; Longmuir 2002). Reynier et al. (1985) indicated that micelle size is one of the most important factors that determine the absorption of lipid and lipophilic substances. Similarly, better digestibility was also observed with LL in broiler rations due the enhanced efficiency of micelle formation (Melegy et al. 2010; Zhang et al. 2011).

The fatty acid composition of the test diets was reflected to a certain extent in the fatty acid composition of tail muscle and non-muscle portions of shrimp. These results are in agreement with other studies reporting the fatty acid pattern in shrimp species reflecting that of dietary lipids (*Fenneropenaeus indicus*: Colvin 1976; *Marsupenaeus japonicus*: Guary et al. 1976; Kayama et al. 1980; *P. monodon*: Millamena 1989, Deering et al. 1997; *Litopenaeus vannamei*: Gonzàlez-félix et al. 2011). However, certain FA appeared to be actively synthesized and/or retained, because they were present in small amounts in PO-based diets, but relatively higher amounts in muscle portion such as in the case of AA, EPA, and DHA. In contrast, palmitic acid and oleic acid were abundant in palm oil-based diets, but their proportion was reduced in the muscle portions. Studies with *Fenneropenaeus chinensis* (Xu et al. 1994) and *P. monodon* (Deering et al. 1997) suggested relatively high levels of HUFA such as AA, EPA, and DHA. The reasons attributed for this may be due to the diversion of shorter and medium-chain FA towards energy production, whereas longer chain unsaturated fatty acids may be selectively retained by the body similar to our observations in this study. In the present study, AA, EPA, and DHA contents were significantly increased in LL-supplemented diets compared to SL-supplemented diets, suggested that lipid digestibility was high in LL-supplemented diets due to high emulsification by low CMC compared to SL-included diets. A similar result of high retention of HUFA was observed in shrimp fed with emulsifier-supplemented diet (Coutteau et al. 2000).

Concentration of PL content of muscle and non-muscle portions of shrimp was higher in LL-supplemented diets regardless of oil source, which might have been due to high emulsification by low CMC capacity of LL in *P. monodon*. Shrimp fed with the FO-based diets had higher concentration of cholesterol in muscle and non-muscle portion of shrimp than those fed the PO-based diets. Cheng and Hardy (2004), Kumaraguruvasagam et al. (2005), and Richard et al. (2006) also reported that body cholesterol lowering effect of diets containing vegetable oils in shrimp and fish. Plant oils also contain phytosterols (Phillips et al. 2002) which are known to decrease total cholesterol and LDL cholesterol in man (Moghadasian and Frohlich 1999; Matvienko et al. 2002; Vanstone et al. 2002) as well as in some teleosts (Gilman et al. 2003), by decreasing intestinal cholesterol absorption efficiency (Normen et al. 2000; Vanstone et al. 2002). Phytosterols contained in PO used in the present study may also explain the hypocholesterolemic effect of diets (POSL and POLL) in *P. monodon*. Phytosterol levels were not measured in the diets, but palm oil contains 0.5 g kg^{-1} phytosterols, mainly sitosterol, campesterol, and stigmasterol (Phillips et al. 2002). Further investigations to determine the mechanisms by which PO reduce shrimp body cholesterol are needed. Higher concentration of TG in non-muscle portion of shrimp-fed PO-included diet is the reflection of test diets.

The utilization of FO has reached its peak in aqua feeds; any further expansion of aquaculture depends on the resilience of aqua feed sector to use alternate plant oils. The results of the present study are encouraging and confirmed that LL could be supplemented in shrimp practical diets for better performance in growth and also improved final product quality through its improved fatty acid digestibility. Further research should take place in the evaluation of LL with complete replacement of fishmeal and fish oil and its effects on enzyme-substrate interactions to achieve the final goal of resilience of shrimp feed industry from marine-based ingredients.

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