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Evaluation of different lipid sources in diet of pacific white shrimp *Litopenaeus vannamei* at low salinity

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ABSTRACT

Litopenaeus vannamei (1.98 ± 0.28 g) were fed diets containing soybean oil (SBO), beef tallow (BFT), fish oil (FIO), linseed oil (LNO), and an equal combination of SBO + BFT + FIO (SBF) or SBO + BFT + LNO (SBL) as dietary lipid source respectively for 8 weeks at low salinity of 3‰. The shrimp fed the SBL diet had the highest weight gain and survival rate. The whole body fatty acid composition including the EPA and DHA of *L. vannamei* generally reflected the composition of dietary fatty acids with the highest DHA and EPA found in *L. vannamei* fed FIO. The activities of fatty acid synthetase, acyl-CoA, diacylgycerol acyltransferase 2, elongase of long-chain fatty acids family member 6, Δ5 and Δ6 fatty acid desaturases of shrimp fed SBL were significantly lower than those fed BFT. The results indicated that fish oil could not be the only lipid source for *L. vannamei* cultured at low salinity, and the shrimp fed non-fish oil diet with a suitable proportion of PUFAs could obtain the same growth and survival rate as those fed diets with fish oil.

1. Introduction

The Pacific white shrimp *Litopenaeus vannamei* is one of the most important shrimp species cultured worldwide (Cheng et al., 2006; Hu et al., 2004). Nowadays, the rearing of L. vannamei in inland saline water has become an emerging industry due to its wide range of salinity tolerance from 0.5 to 50‰ (Saoud et al., 2003). Although relatively high economic profit can be obtained from the culture of *L. vannamei* in inland low salinity water, there are some problems and potential risks of failure in commercial production, such as slow growth and low survival (Li et al., 2007), low immune ability (Ponce-Palafox et al., 1997), and low stress resistance to some water born toxicants (Li et al., 2007, 2008). Low salinity can reduce the salt diffusion from the blood to the body tissues or ambient environment and water will be absorbed from the environment, leading to swollen cells (Davis et al., 2002). At salinity stress, aquatic animals are forced to adapt to the fluctuant water environment by osmoregulation via the change of various enzymes and transporters, and the process has been proved a high energy cost process (Tseng and Hwang, 2008). Therefore, it would be of potency to develop a suitable and practical method from the aspect of dietary nutrition modulation to solve the problems in shrimp production at low salinity as osmoregulation is a high energy-cost process in many decapod species (Tseng and Hwang, 2008).

Previous studies have showed that dietary lipids have positive influence to reduce osmotic shock in aquatic animals (Sui et al., 2007; Chen et al., 2014). When marine species are transferred into a low salinity environment, more energy is needed to maintain the humoral environment balance and intracellular and extracellular osmotic equilibrium (Tseng and Hwang, 2008). Supplementation of dietary long-chain polyunsaturated fatty acids (lc-PUFAs) can improve productivity of aquatic animals at low salinities (Romano et al., 2012). Although dietary lc-PUFAs are important for osmoregulation when the ambient salinity changes (Hurtado et al., 2007), the optimal requirement of lc-PUFAs in the diet is usually species-specific in marine crustaceans (Deering et al., 1997; Lim et al., 1997). The requirement of lc-PUFAs of *L. vannamei* is 5–10 g/kg in 25‰ salinity (Gonzalez-Felix et al., 2002), but the optimal amount of lc-PUFAs in diet of *L. vannamei* is still unknown at low salinity. Although some studies have explored fatty acid requirements (Gonzalez-Felix et al., 2002), lipid source (Ju et al., 2012; Lim et al., 1997), and the replacement of fish oil by plant oils (Gonzalez-Felix et al., 2010) in *L. vannamei*. However, few studies focused on the

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type of lipid source in the diet formulation for the culture of *L. vannamei* at low salinity.

In aquaculture feed, dietary lipids from different sources vary in fatty acid profiles and contents, and may affect the growth performance and health of aquatic animals. Therefore, this study aimed to evaluate the growth performance, whole proximate body composition, muscle fatty acids profile and activities of enzymes in white shrimp fed various lipid sources. Furthermore, we also measured biochemical parameters relevant to lipid synthesis, catabolism and transport in *L. vannamei* in an attempt to understand the shrimp response to diets formulated with different lipid sources.

2. Materials and methods

2.1. Experimental diets, animals, design and facilities

Six semi-purified diets were formulated to contain equal targeted levels of crude protein (352 g/kg), crude lipid (72.0 g/kg), ash (118.8 g/kg), moisture (98.9 g/kg) and 16.7 MJ of digestible energy (DE/kg) (Table 1). Different lipid sources including soybean oil (SBO), beef tallow (BFT), fish oil (FIO), linseed oil (LNO), a combination of equal levels of SBO + BFT + FIO (SBF) and another similar combination of SBO + BFT + LNO (SBL) were added to the basal diet at a level of 70 g/kg. Before oil addition, the dry ingredients of each diet were mixed thoroughly in a mixer. Then 250 ml of deionized water per kg of diet was added into the diet. Ethoxyquin was blended in the oil and added to each diet at a level of 200 mg/kg diet as an antioxidant agent. A meat grinder was used to extrude the mixture into 2-mm diameter pellets. The pellets were air-dried at room temperature to the moisture content less than 10%. Pellets were then ground into small pieces, sieved to obtain appropriate sizes and stored at −20 °C until use.

Juvenile white shrimp (1.98 ± 0.28 g) were obtained from the Shenzhen base of South China Sea Fisheries Research Institute (CAFS), Shenzhen, China, and were cultured in 18 tanks at a density of 40 shrimp per tank (500 L). The salinity of original habitat of shrimp was 17psu, and the obtained shrimps were acclimated to 3 psu through daily increments of 2 psu at acclimation period before the start of the 8-week experiment. During the periods of acclimation, shrimp were fed thrice daily at 0800, 1600 and 2200 h with a commercial diet containing 10% moisture, 40% crude protein, 8% crude lipid, 12% ash, 30% carbohydrates, digestible energy (16.7 kJ/g) and known fatty acid compositions (Table 2). During the periods of acclimation and experiment, shrimps were fed thrice daily at 0800, 1600 and 2200 h. Based on the amount of feed remaining on the following day, daily rations were adjusted to approximate a feed input slightly above satiation. The unfed feed was daily removed with a siphon tube. The photoperiod was 12 h light and 12 h dark. Sea water was pumped from the Dayawan Coast (Shenzhen, China), and filtered through an activated carbon cartridge for at least 3 d before entering the culture system. The tap water was aerated thoroughly before being added to the tank to adjust the salinity by 2–3‰ per day and the salinity was determined by a salinity meter. During the experiment, water was adjusted the salinity and deposited in the storage tank, the daily exchanged water was 1/4–1/3 of the tank volume. Water quality parameters were maintained at pH 7.5–7.9, temperature 26.7–27.8 °C, dissolved oxygen 5.0–6.1 mg/L, and total ammonia nitrogen ≤0.05 mg/L. The pH, temperature and dissolved oxygen were tested by YSI 550A Dissolved Oxygen Instrument (YSI Company Ltd, USA) according to the operation manual every three days, the ammonia nitrogen was tested by ammonia nitrogen test kit (Lohand biological) once a week.

At the end of the feeding trial, all shrimp were deprived of feed for 24 h before weighing. Five shrimp at intermold stage C in each tank were used for the body composition analysis. Another 10 shrimp at intermold stage C in each tank were dissected to obtain the tissue of muscle and hepatopancreas, and all samples were stored at −80 °C for further biochemical analysis and enzyme assay. Weight gain and survival were calculated to assess the growth performance of shrimp, which were calculated as follows: weight gain (%) = 100 × (final weight-initial weight)/initial weight; survival rate (%) = 100 × (final shrimp number)/(initial shrimp number).

Table 1
Formulation and proximate composition of experimental diets (g/kg diet).

Ingredients	SBO	BFT	FIO	SBF	LNO	SBL
Casein	320	320	320	320	320	320
Gelatin	80	80	80	80	80	80
Corn starch	330	330	330	330	330	330
Lipid[a]	70	70	70	70	70	70
Cholesterol	5	5	5	5	5	5
Lecithin	10	10	10	10	10	10
vitamin premix[b]	20	20	20	20	20	20
Stay-C	1	1	1	1	1	1
Amino acid mixture[c]	30	30	30	30	30	30
mineral premix[d]	5	5	5	5	5	5
CMC	30	30	30	30	30	30
$Ca(H_2PO4)_2$	5.3	5.3	5.3	5.3	5.3	5.3
$CaCO_3$	2.2	2.2	2.2	2.2	2.2	2.2
Calcium lactate	3.3	3.3	3.3	3.3	3.3	3.3
NaH_2PO_4	4.2	4.2	4.2	4.2	4.2	4.2
α-cellulose	84	84	84	84	84	84

[a] Lipid: lipid resources including soybean oil (SBO), beef tallow (BFT), fish oil (FIO), linseed oil (LNO), and equal combinations of SBO + BFT + FIO (SBF) or SBO + BFT + LNO (SBL).

[b] Vitamin premix (g/kg premix): thiamin HCl, 0.5; riboflavin, 3.0; DL Ca-pantothenate, 5.0; nicotinic acid, 5.0; biotin, 0.05; folic acid, 0.18; B12, 0.002; choline chloride, 100.0; inositol, 5.0; menadione, 12.0; A acetate (20,000 IU/g), 5.0; D3 (400,000 IU/g), 0.002; DL-alpha-tocopheryl acetate (250 IU/g), 8.0; alpha-cellulose, 866.266.

[c] Amino acid mixture contained the following (g/300 g diet): glycine, 0.6 g; L-aLanine, 0.6 g; L-gLutamic acid, 0.6 g; and betaine, 1.2 g.

[d] Mineral premix (g/100 g premix): sodium dihydrogen phosphate, 21.5; calcium dihydrogen phosphate, 26.5; calcium carbonate, 10.5; Ca-lactate, 16.5; cobalt chloride, 0.001; cupric sulfate pentahydrate, 0.0625; ferrous sulfate, 1.0; magnesium sulfate heptahydrate, 7.0995; manganous sulfate monohydrate, 0.1625; potassium iodide, 0.0167; sodium selenite, 0.0025; zinc sulfate heptahydrate, 3.298.

Table 2
Fatty acids composition of experimental diets (percent by weight of total fatty acids).

Fatty acid	SBO	BFT	FIO	SBF	LNO	SBL	Commercial diet
16:0	14.37	23.05	21.47	18.05	9.99	13.67	16.63
18:0	6.40	28.53	5.41	13.51	8.68	13.38	4.36
$\sum$SFA	22.85	53.86	32.66	34.26	19.60	28.23	24.34
18:1(*n*-9)	24.25	34.83	23.87	26.08	21.01	24.43	26.21
$\sum$MUFA	27.00	36.93	32.49	34.44	22.84	27.30	30.60
18:2(*n*-6)	39.72	8.15	10.06	23.14	16.13	23.22	28.16
18:3(*n*-3)	5.59	0.42	3.92	0.21	40.80	20.96	2.72
20:5(*n*-3)	1.41	0.12	6.63	2.11	0.16	0.10	4.16
22:6(*n*-3)	1.91	0.15	8.96	2.86	0.21	0.12	7.23
$\sum$PUFA	50.15	9.21	34.85	31.30	57.56	44.47	45.07
$\sum$*n*-3	9.24	0.69	20.77	5.64	41.17	21.17	15.31
$\sum$*n*-6	40.39	8.52	14.08	25.66	16.30	23.30	29.75
$\sum$(*n*-3)/$\sum$(*n*-6)	0.23	0.08	1.48	0.22	2.53	0.91	0.51

Not all analyzed fatty acids fractions were included in this table.
Total saturated fatty acid ($\sum$SFA): 14:0, 16:0, 18:0, 20:0, 22:0.
Total monounsaturated fatty acid ($\sum$MUFA): 16:1, 18:1(*n*-9), 20:1, 22:1.
Total polyunsaturated fatty acid ($\sum$PUFA): 18:2(*n*-6), 18:3(*n*-3), 20:2, 20:3(*n*-6), 20:4(*n*-6), 20:3(*n*-3), 20:5(*n*-3), 22:2, 22:3, 22:4, 22:5(*n*-3), 22:6(*n*-3).
Total *n*-3 polyunsaturated fatty acid ($\sum$*n*-3): 18:3(*n*-3), 20:3(*n*-3), 20:5(*n*-3), 22:5(*n*-3), 22:6(*n*-3).
Total *n*-3 polyunsaturated fatty acid ($\sum$*n*-6): 18:2(*n*-6), 20:3(*n*-6), 20:4(*n*-6).

2.2. Whole-body proximate composition

All experimental samples and diets were analyzed in triplicate for proximate composition following the standard methods (AOAC 2000). Moisture was determined by oven drying at 105 °C to a constant weight. Samples for dry matter determination were digested with nitric acid and incinerated in a muffle furnace at 600 °C overnight for ash determination. Protein was measured by the combustion method using an FP-528 nitrogen analyzer (Leco, USA). Lipid was determined by the ether extraction method using the 2055 Soxtec system (Foss, Sweden).

2.3. Fatty acids analysis

Total lipids of muscle were extracted in triplicate using chloroform: methanol (2:1, v/v) according to Folch et al. (1957). The saponifiable lipids were converted to their methyl esters by using the standard boron tri-fluoride–methanol method (Morrison and Smith 1964). Fatty acids methyl esters (FAME) were analyzed on an Agilent 6890 gas chromatograph (Agilent Technologies, USA), equipped with a flame ionization detector (FID) and a SP-2560 fused silica capillary column (100 m, 0.25 mm i.d. and 0.20 μm film thickness). Injector and detector temperatures were 270 and 280 °C, respectively. Column temperature was held at 120 °C for 5 min then programmed to increase at 3 °C/min up to 240 °C where it was maintained for 20 min. Carrier gas was helium (2 ml/min), and the split ratio was 30:1. Identification of fatty acids was carried out by comparing the sample FAME peak relative retention times with those obtained for the Sigma–Aldrich standards (St. Louis, MO, USA). The concentration of individual fatty acids was calculated and expressed as the mass percentages of total identified fatty acids.

2.4. Enzyme activity assay

Activity assays of all enzymes tested in this study were determined by the enzyme-linked immunosorbent assay (ELISA) kit (Xinyu, ShangHai) following the protocol with specific antibody corresponding to the parameter. The enzyme activities of fatty acid synthetase (FAS), hormone sensitive lipase (HSL), lipoprotein lipase (LPL), adipose triacylglycerol lipase (ATGL), acyl-CoA, diacylgycerol acyltransferase 2 (DGAT2), elongase of very long chain fatty acids 6 (ELOVL6), Δ5 and Δ6 fatty acid desaturases (Δ5FAD and Δ6FAD) were expressed as U/g protein and detected in hepatopancreas. The protein content of each sample was determined by the method of UV–vis spectrophotometer (Thermo Scientific, NanoDrop, 2000).

2.5. Statistical analysis

Data are expressed as mean ± standard errors (Mean ± S.E.), and were subjected to one-way analysis of variance (SPSS for Windows, version 11.5) to determine significant differences between treatments. Fatty acid composition data and survival were transform into arcsine, and then calculated for one-way analysis. If a significant difference was identified, differences between means were compared by Duncan's multiple range tests. The level of significant difference was set at $p < 0.05$.

3. Results

3.1. Growth and whole-body proximate composition

Diet lipid sources significantly affected shrimp growth performance including survival and weight gain (Table 3). Survival rates of shrimp fed SBL, SBF, FIO and LNO were higher than shrimp fed BFT, but there were no significant differences between the first four groups. Shrimp survival was similar between the SBO, LNO, FIO and SBF groups or between SBO and BFT groups. Weight gain in the shrimp fed SBL or SBF was higher than those fed SBO or BFT, but there was no significant difference of weight gain between the shrimp fed SBO, BFT, LNO and FIO, or between LNO, FIO and SBL, or between LNO, SBL and SBF. The whole body proximate protein, lipid, ash and moisture were not affected by dietary lipid source (Table 4).

Table 3
Body composition (percent live weight) of white shrimp grown in diluted sea water 3‰ (percent).

	Protein	Lipid	Ash	Moisture
SBO	16.94 ± 0.74	0.88 ± 0.04	2.72 ± 0.08	77.01 ± 0.71
BFT	17.01 ± 0.14	1.07 ± 0.10	2.57 ± 0.24	76.18 ± 0.64
FIO	15.86 ± 0.83	1.36 ± 0.22	2.42 ± 0.11	77.34 ± 1.19
SBF	16.45 ± 0.61	1.25 ± 0.16	2.69 ± 0.11	77.20 ± 0.93
LNO	17.71 ± 0.11	0.85 ± 0.09	2.91 ± 0.30	75.15 ± 1.86
SBL	16.67 ± 0.21	1.46 ± 0.20	2.76 ± 0.06	76.54 ± 0.39
p value	0.56	0.06	0.50	0.70

Different letters in the same column represent significant difference ($P < 0.05$).

3.2. Muscle fatty acid composition

Fatty acid compositions in the muscle of shrimp were significantly affected by dietary treatments (Table 5). Shrimp fed the SBO diet had the highest linoleic acid (18:2[*n*-6], LOA) level among all group, and the SBL and SBF groups had significantly higher LOA than shrimp fed BFT, LNO and FIO diets. Shrimp fed SBL diet had significantly higher linolenic acid (18:3[*n*-3], LNA) than those fed the SBO, BFT, FIO and SBF, but significantly lower than those in the LNO treatment. The content of eisosapentaenoic acid (EPA, C20:5[*n*-3]) and docosahexaenoic acid (DHA, C22:6[*n*-3]) in the muscle of shrimp fed the FIO diet were highest among all treatments. The content of EPA of shrimp fed FIO diet had significantly higher than those fed the SBO, BFT, LNO and SBL. DHA content was similar between the SBO and BFT, BFT and SBF, or LNO and SBL. Shrimp fed BFT diet had the highest total monounsaturated fatty acids ($\sum$MUFAs) and the lowest total polyunsaturated fatty acids ($\sum$PUFAs) in all treatments, but there were no differences between the other five groups. Shrimp fed the LNO or FIO diet had significantly higher $\sum n$-3 fatty acids than shrimp fed other diets. The highest *n*-6 fatty acids ($\sum n$-6) value was found in shrimp fed the SBO diet followed by those fed the SBL and SBF diets, which were all significantly higher than those fed BFT, LNO and FIO diets. Total saturated fatty acids did not differ in shrimp muscle between various treatments.

3.3. Activities of enzymes related to lipid metabolism

The activities of DGAT2 and Δ5FAD were higher in shrimp fed the BFT diet than in other five treatments ($p = 0.006$), and there was no difference between those five treatments. FAS was higher in shrimp fed the BFT diet than that fed FIO, SBL and SBF ($P = 0.042$), but FAS was similar between the SBO, BFT and LNO, or between the FIO, SBL and SBF. ELOVL6 was higher in shrimp fed the BFT and SBL

Table 4
Growth, survival of white shrimp grown in diluted sea water 3‰ (percent).

	Survival	Initial weight	Final weight	Weight gain
SBO	52.50 ± 8.04^{ab}	2.13 ± 0.01	8.96 ± 0.37	321.12 ± 17.88^{a}
BFT	43.33 ± 4.64^{a}	2.11 ± 0.02	9.17 ± 0.28	335.13 ± 9.80^{a}
FIO	66.96 ± 7.77^{bc}	2.11 ± 0.01	9.60 ± 0.27	343.28 ± 8.06^{ab}
SBF	68.62 ± 7.65^{bc}	2.11 ± 0.02	9.34 ± 0.23	377.24 ± 17.27^{bc}
LNO	70.32 ± 4.28^{bc}	2.12 ± 0.01	10.46 ± 0.27	355.40 ± 9.60^{abc}
SBL	86.54 ± 7.54^{c}	2.11 ± 0.01	10.07 ± 0.42	394.36 ± 6.56^{c}
p value	0.01			0.01

Different letters in the same column represent significant difference ($P < 0.05$).

Table 5
Fatty acid composition (percent by weight of total fatty acids) in muscle of white shrimp grown in diluted sea water 3‰ (percent).

Fatty acid	SBO	BFT	FIO	SBF	LNO	SBL	*p* value
∑SFA	31.23 ± 0.27	30.82 ± 0.75	32.05 ± 0.84	32.33 ± 0.45	31.11 ± 0.33	33.41 ± 2.09	0.50
∑MUFA	24.97 ± 1.12[a]	39.34 ± 1.46[b]	28.38 ± 0.43[a]	28.55 ± 0.74[a]	27.46 ± 2.16[a]	27.71 ± 0.61[a]	0.01
18:2*n*-6	25.31 ± 0.77[c]	12.36 ± 0.40[a]	9.56 ± 2.68[a]	17.15 ± 0.57[b]	13.04 ± 0.89[a]	18.86 ± 1.11[b]	0.01
18:3*n*-3	2.01 ± 0.14[a]	1.36 ± 0.20[a]	1.20 ± 0.55[a]	1.54 ± 0.57[a]	16.53 ± 1.33[c]	9.32 ± 0.79[b]	0.01
EPA	5.56 ± 0.78[a]	5.65 ± 0.76[a]	13.78 ± 1.43[c]	8.57 ± 0.59[b]	4.21 ± 0.30[a]	3.88 ± 0.41[a]	0.01
DHA	6.09 ± 0.34[b]	6.71 ± 0.43[bc]	11.01 ± 0.90[d]	8.20 ± 0.35[c]	4.42 ± 0.36[a]	4.23 ± 0.26[a]	0.01
∑PUFA	43.80 ± 1.31[b]	29.84 ± 0.71[a]	39.57 ± 0.56[b]	39.12 ± 0.58[b]	41.42 ± 2.07[b]	38.88 ± 2.51[b]	0.01
∑(*n*-3)	16.60 ± 1.31[a]	16.73 ± 1.14[a]	28.93 ± 2.13[b]	20.84 ± 0.46[a]	27.29 ± 1.18[b]	19.13 ± 1.40[a]	0.01
∑(*n*-6)	25.43 ± 0.86[c]	12.53 ± 0.43[a]	9.77 ± 2.71[a]	17.23 ± 0.57[b]	13.15 ± 0.84[a]	18.89 ± 1.09[b]	0.01
∑*n*-3/∑*n*-6	0.63	1.34	2.96	1.21	2.12	1.01	

Different letters in the same row represent significant difference ($P < 0.05$).
Not all analyzed fatty acids fractions were included in this table.
∑SFA: 14:0, 16:0, 18:0, 20:0, 22:0.
∑MUFA: 16:1, 18:1(*n*-9), 20:1, 22:1.
∑PUFA: 18:2(*n*-6), 18:3(*n*-3), 20:2, 20:3(*n*-6), 20:4(*n*-6), 20:3(*n*-3), 20:5(*n*-3), 22:2, 22:3, 22:4, 22:5(*n*-3), 22:6(*n*-3).
∑*n*-3: 18:3(*n*-3), 20:3(*n*-3), 20:5(*n*-3), 22:5(*n*-3), 22:6(*n*-3).
∑*n*-6: 18:2(*n*-6), 20:3(*n*-6), 20:4(*n*-6).

diet than those fed SBO and FIO ($p = 0.032$), but ELOVL6 was similar between the SBO, FIO, SBF and LNO, or between the BFT, LNO, SBL and SBF treatments. Δ6FAD was higher in shrimp fed the BFT diet than that fed SBO, SBL and FIO ($p = 0.024$), but ELOVL6 was similar between the SBO, FIO, SBF, SBL and LNO, or between the BFT, LNO and SBF. No significant differences were found in activities of HSL, ATGL and LPL among all groups (Table 6).

4. Discussion

At low salinity, low survival rate (~70%) at low salinity of 3‰ contrast to higher survival rate (~100%) at higher salinity as 17 or 30‰ has been observed in various studies in *L. vannamei* (Laramore et al., 2001; Li et al., 2007, 2009, 2010; McGraw et al., 2002; Wang et al., 2014), which can be explained the hyposaline stress faced by *L. vannamei* at low salinity. In this study, similar survival rates of shrimp feed diets other than diets of BTO and SBO were obtained. In our studies, shrimp fed LNO, FIO, SBL and SBF showed better growth performance and survival rate, which could be due to the function of dietary polyunsaturated fatty acids (PUFAs) for improving growth and osmoregulation capacity in aquatic animals (Hurtado et al., 2007; Martins et al., 2006; Palacios et al., 2004b). The routine metabolic functions of most organisms require the participation of fatty acids, especially *n*-3 PUFAs as they are an integral component of cell membranes and precursors of eicosanoids (Hurtado et al., 2007; Palacios et al., 2004b). Higher *n*-3 PUFA levels can result in a larger gill area by modifying the fatty acid composition of the gills to improve the osmoregulatory capacity and survival rate of shrimp under low salinity stress (Palacios et al., 2004a). However, SBO contained a level of high PUFA (50.15%), but still showed a poor growth performance, possibly due to the inhibition effect on the mRNA of fatty acid synthase (FAS) by excess PUFA content in experiment diets (Blake and Clarke, 1990; Clarke et al., 1990). Similarly, the FAS activities of shrimp fed LNO, FIO and SBL were significantly lower than those fed the BFT diet in this study. These previous findings are consistent with the results of this study, and similar findings were also reported on other crustacean species, including *Fenneropenaeus indicus*, *Litopenaeus stylirostris* larvae (Léger et al., 1985), *Penaeus monodon* and *Penaeus chinensis* (Xu et al., 1993).

The requirements of essential fatty acid vary among penaeids species. The dietary *n*-3 PUFA requirement was 0.5% in *L. vannamei* (Gonzalez-Felix et al., 2002), 0.5–1% in *P. monodon* (Chen and Tsai, 1986) and 1% in post-larval penaeids (Kanazawa et al., 1979a). The optimal LNA and DHA requirements were 2.50% and 1.44% for *L. vannamei*, respectively (Merican and Shim, 1997). The optimum EPA and DHA levels were both at 1% in the diet of juvenile *Marsupenaeus japonicus* (Kanazawa et al., 1979b). Similarly, the optimal DHA was about 1% in the diet for *Fraxinus chinensis* (Xu et al., 1993). Besides, excessive essential fatty acid (>3%) in the diet can lead to detrimental effects on both growth and survival in *L. vannamei* (Glencross and Smith, 2001). In this study, the shrimp fed the BFT diet had the lowest survival and weight gain possibly due to the low (*n*-3) content (0.048%). In contrast, the SBO diet containing a high amount of PUFA (3.5%) but a very low level of EPA and DHA (<0.13%) did not produce better growth and survival of *L. vannamei*. Since the *n*-3 PUFA level ranged from 0.39% to 2.8% in diets of LNO, FIO, SBL and SBF, which all yielded relative higher growth performance, the precise requirement of *n*-3 PUFA in diet of *L. vannamei* should be further studied by feeding shrimp diets with graded levels of PUFAs at low salinity less than 5‰, even previous study has

Table 6
The lipid metabolism related enzyme activities (in units per gram protein) in hepatopancreas of white shrimp raised in diluted sea water 3‰.

	SBO	BFT	FIO	SBF	LNO	SBL	*p* value
ATGL	2.47 ± 0.39	2.88 ± 0.30	2.11 ± 0.26	2.22 ± 0.12	2.68 ± 0.16	2.27 ± 0.03	0.20
HSL	1.08 ± 0.11	1.49 ± 0.08	1.19 ± 0.24	1.27 ± 0.08	1.35 ± 0.11	1.11 ± 0.07	0.30
LPL	0.37 ± 0.06	0.51 ± 0.07	0.29 ± 0.04	0.35 ± 0.01	0.37 ± 0.02	0.41 ± 0.02	0.06
FAS	1.70 ± 0.28[ab]	2.11 ± 0.13[b]	1.27 ± 0.09[a]	1.58 ± 0.07[a]	1.65 ± 0.08[ab]	1.58 ± 0.14[a]	0.04
DGAT2	2.94 ± 0.41[a]	4.29 ± 0.42[b]	2.36 ± 0.28[a]	2.96 ± 0.12[a]	3.22 ± 0.11[a]	2.63 ± 0.12[a]	0.01
ELOVL6	0.22 ± 0.03[a]	0.30 ± 0.03[b]	0.18 ± 0.02[a]	0.25 ± 0.03[ab]	0.25 ± 0.01[ab]	0.21 ± 0.01[a]	0.03
Δ6 FAD	0.96 ± 0.13[a]	1.37 ± 0.09[b]	0.78 ± 0.08[a]	1.12 ± 0.16[ab]	1.10 ± 0.08[ab]	0.87 ± 0.06[a]	0.02
Δ5 FAD	0.76 ± 0.19[a]	1.18 ± 0.11[b]	0.68 ± 0.03[a]	0.55 ± 0.01[a]	0.76 ± 0.04[a]	0.61 ± 0.04[a]	0.01

Different letters in the same row represent significant difference ($p < 0.05$).
Where ATGL: triacylglycerol lipase, HSL: hormone sensitive lipase, LPL: lipoprotein lipase, FAS: fatty acid synthetase, DGAT2: acyl CoA: diacylgycerol acyltransferase 2, Δ6FAD and Δ5FAD were Δ6 and Δ5 fatty acid desaturases respectively.

shown that the dietary *n*-3 PUFA requirement was 0.5% in *L. vannamei* at 23.8–25.8‰ (Gonzalez-Felix et al., 2002). Although, fish oil has been traditionally regarded as the best oil for *L. vannamei* (Lim et al., 1997), our result in this study still can suggest that fish oil should not be the only lipid source for *L. vannamei* at low salinity.

Although *n*-3 PUFAs are vital for aquatic animals, the requirements for marine crustaceans are often species-specific (Deering et al., 1997; Lim et al., 1997), because there is wide variation among aquatic animals in their ability to synthesize *n*-3 PUFAs (Sargent et al., 1999; Sargent et al., 1995). Marine shrimp are believed to have a limited ability to synthesize *n*-3 PUFAs (Suprayudi et al., 2004), and EPA and DHA are essential for these animals including *L. vannamei* (Gonzalez-Felix et al., 2002). In this study, shrimp fed FIO diet had the highest DHA and EPA contents, because fish oil contains higher levels DHA and EPA, since fatty acid composition of tissues of aquatic animals is generally a reflection of dietary fatty acid. Feeding unsaturated oils has been shown to increase the body and tissue unsaturated fatty acid content in a number of species (Kalogeropoulos et al., 1992; Yildirim-Aksoy et al., 2007; Takeuchi et al., 1991). However, in this study, despite low levels of EPA and DHA (<0.16% and 0.21%, respectively) in the BFT, SBL and LNO diets, shrimp fed these diets showed a certain level of accumulation of EPA and DHA in the tissue of muscle. Although DHA and EPA content were low in the LNO and SBL diets, shrimp fed these diets still achieved the similar growth performance as those fed fish oil diet (FIO and SBF). The highest activities of ELOVL6, Δ6FAD and Δ5FAD were detected in shrimp fed BFT diet with lowest *n*-3 PUFA content, and their activities decreased as dietary *n*-3 PUFA increased, might showing a feed-back regulation. In our previous study, *L. vannamei* fed commercial diet cultured in water with low salinity as 3‰ accumulated higher DHA and EPA in hepatopancreas than those at 17 and 30‰ (Chen et al., 2014). On the other hand, shrimp fed BFT with low dietary *n*-3 PUFA had high *n*-3 PUFA in the muscle (16.73%), which is not different from muscle *n*-3 PUFA content in the fast growing shrimp fed SBL (19.13%) or SBF (20.84%). Therefore, the accumulation of DHA and EPA in tissue of *L. vannamei* not only can be effected by dietary fatty acid composition, but also has a relationship with the ability to convert LNA to *n*-3 PUFA at low salinity of *L. vannamei*. However, the hypothesis on this conversion ability still needs further studies.

Previous studies have shown that shrimp can selectively use certain fatty acids for energy by oxidation (Deering et al. 1997; Palacios et al. 2004a,b). *P. monodon* prefer to use shorter-chain fatty acids in energy metabolism, and can selectively retain longer-chain unsaturated fatty acids (Deering et al. 1997). It has been found that *L. vannamei* exposed to low salinity contained low levels of several SFAs and MUFAs but high PUFAs, indicating that a selective pathway may exist for fatty acid oxidation to obtain energy (Palacios et al. 2004a,b). In this study, the accumulation of saturated fatty acids (SFAs) in muscle at a level near 31% in shrimp was similar among all treatments except for the shrimp fed BFT diet with 54% dietary SFAs or LNO diet with a low SFAs level (19.60%). This finding may suggest that *L. vannamei* prefer using SFA to obtain energy when fed an excess SFA diet, and prefer using MUFA to obtain energy when fed a diet with SFA deficiency. The oleic acid accumulation in fish liver might be related to the sufficient energy supply of other tissues to guarantee the normal physiological status. However, since there is no direct evidence showing the relationship between fatty acid metabolism and fatty acid composition in the diet, and the fatty acid profile and composition in *L. vannamei* hepatopancreas is not determined, further study is suggested to test this energy-fatty acid pathway.

In conclusion, *L. vannamei* fed diets with mixed oil with a suitable proportion of PUFAs can achieve desirable growth performance. Fish oil possess higher content of highly unsaturated fatty acids, and has been traditionally regarded as the best oil incorporated into aquafed to improve growth or survival of aquatic animals. However, fish oil could not be the only lipid source in *L. vannamei* diet at low salinity as shrimp fed non-fish oil diet could grow and survive equally well as those fed diet with fish oil. Diets with linseed oil, equal combinations of soybean oil, beef tallow and fish oil or, or equal combinations of soybean oil, beef tallow and linseed oil as dietary lipid sources, should be considered for inland low salinity cutlure of *L. vannamei*. The potential ability to synthesize DHA and EPA from LNA at low salinity of *L. vannamei* should be conducted in the future.

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