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Effects of different dietary lipid sources on growth performance and tissue fatty acid composition of juvenile swimming crab *Portunus trituberculatus**

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Abstract This study was conducted to evaluate the effects of dietary lipid sources on the growth performance and fatty acid composition of the swimming crab, *Portunus trituberculatus*. Four isonitrogenous and isoenergetic experimental diets were formulated to contain four separate lipid sources, including fish, soybean, rapeseed, and linseed oils (FO, SO, RO, and LO, respectively). With three replicates of 18 crabs each for each diet, crabs (initial body weight, 17.00 ±0.09 g) were fed twice daily for 8 weeks. There were no significant differences among these groups in terms of weight gain, specific growth rate, and hepatosomatic index. However, the RO groups' survival rate was significantly lower than FO groups. The feed conversion and protein efficiency ratios of RO groups were poorer than other groups. The proximate compositions of whole body and hepatopancreas were significantly affected by these dietary treatments. Tissue fatty acid composition mainly reflected dietary fatty acid compositions. Crabs fed FO diets exhibited significantly higher arachidonic, eicosapentaenoic, and docosahexaenoic acid contents in muscle and hepatopancreas compared with VO crabs. Linoleic, oleic, and linolenic acids in muscle and hepatopancreas were the highest in the SO, RO, and LO groups, respectively. The present study suggested that SO and LO could substitute for FO in fishmeal-based diets for swimming crabs, without affecting growth performance and survival.

Keyword: swimming crab; *Portunus trituberculatus*; lipid source; growth performance; fatty acid composition

1 INTRODUCTION

The swimming crab, *Portunus trituberculatus*, is distributed along the coastal waters of Korea, Japan, China, and Southeast Asian countries (Dai et al., 1986; Meng et al., 2009), and is also one of the most important species contributing to the overall world of portunid fisheries production (Secor et al., 2002). However, overfishing and natural waters pollution have caused a significant decline in their stock in the East China Sea since the 1990s (Yu et al., 2003). In the past decade, swimming crab has become an important aquaculture species in China (Chen et al., 2006). However, limited studies have been conducted regarding this species' nutritional requirements that focus on lipids (Han et al., 2013a), cholesterol (Han et al., 2013b), phospholipids (Li et al., 2014a, b), and protein (Jin et al., 2013).

Fish oil (FO), rich in eicosapentaenoic acid (EPA, *n*-3 20:5) and docosahexaenoic acid (DHA, *n*-3 22:6), is widely used in commercial diets for aquaculture. Currently, the global aquafeed industry is the largest consumer of FO, using about 75% of the global supply (Thompson et al., 2010). However, FO production has been decreasing, in line with declining global fishmeal production, and prices increasing

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Table 1 Ingredients and proximate composition of the experimental diets

Ingredient (g/100 g)	Experimental diets			
	FO	SO	RO	LO
White fishmeal	40.61	40.61	40.61	40.61
Casein	13.54	13.54	13.54	13.54
Pollack fish oil	6.00	-	-	-
Soybean oil	-	6.00	-	-
Rapeseed oil	-	-	6.00	-
Linseed oil	-	-	-	6.00
Corn starch	22.00	22.00	22.00	22.00
Taurine	1.00	1.00	1.00	1.00
Cholesterol	0.60	0.60	0.60	0.60
Choline	1.00	1.00	1.00	1.00
Sodium alginate	3.00	3.00	3.00	3.00
Mineral mixture[1]	3.00	3.00	3.00	3.00
Vitamin mixture[2]	4.00	4.00	4.00	4.00
Calcium biphosphate	0.50	0.50	0.50	0.50
Cellulose	4.75	4.75	4.75	4.75
Proximate composition (% dry weight)				
Moisture	4.64	5.00	4.83	4.88
Crude protein	46.85	47.87	46.37	45.73
Crude fat	8.91	8.81	8.95	8.77
Ash	10.34	10.10	10.11	10.16
Energy (kJ/g)	19.67	19.66	19.64	19.69

[1] vitamin mixture (% mixture): thiamine B1: 0.5; riboflavin: 0.8; nicotinamide: 2.6; biotin: 0.1; calcium pantothenate: 1.5; vitamin B6: 0.3; folic acid: vitamin B: 0.5; vitamin C: 12.1; vitamin K 0.202; p-aminobenzoic acid: 3; vitamin B12: 0.1; cellulose: 50.4; vitamin A: 2.5; vitamin D3: 2.5; vitamin E: 5; inositol: 18.1; [2] mineral mixture consisted of (g/kg mixture): calcium dihydrogen phosphate: 122.87; lactate: 474.22; sodium dihydrogen phosphate: 42.03; potassium persulfate: 163.83; ferrous sulfate: 10.78; iron citrate: 38.26; magnesium sulfate: 44.19; zinc sulfate: 4.74; manganese sulfate: 0.33; copper sulfate: 0.22; cobalt chloride: 0.43; iodate: 0.02; sodium chloride: 32.33; potassium chloride: 65.75.

(Globefish, 2009). Thus, it is essential to find new lipid sources to replace FO for long-term sustainability of the aquaculture industry. Vegetable oils (VO) are abundant and their prices are relatively stable and cheap. They are suitable alternatives to FO when the essential fatty acid requirements are satisfied in the diet (Sargent et al., 1999; Turchini et al., 2003; Xue et al., 2006). Some VOs are more cost-effective than FO in the aquafeed industry and have been used in previous research with no deleterious effects on growth. Previous research has used VO (Drew et al., 2007; Shapawi et al., 2008; Wang et al., 2012), such as soybean oil (SO, rich in linoleic acid, *n*-6 18:2), linseed oil (LO, rich in linolenic acid, *n*-3 18:3), and rapeseed oil (RO, rich in oleic acid, *n*-9 18:1).

Highly unsaturated fatty acids (HUFA) have been reported to be necessary for maintaining normal cell membrane structure and function (Sargent et al., 1999; Lee, 2001). The main C_{18} fatty acids have been suggested to be less efficiently digested than EPA and DHA in marine crustaceans and fish (composed in descending proportions of EPA, DHA, *n*-3 18:3, *n*-6 18:2, and *n*-9 18:1; Kanazawa et al., 1979a; Turchini et al., 2009). Marine crustaceans have a limited ability to synthesize *n*-3 HUFA to meet metabolic requirements (Kanazawa and Teshima, 1977; Kanazawa et al., 1979b; Xu et al., 1994; Lim et al., 1997; Suprayudi et al., 2004). Moreover, different lipid sources, with different fatty acid profiles, might affect the physiological mechanisms that control energy metabolism and lipid homeostasis in aquaculture species (Wang et al., 2012).

The main purpose of this study was to investigate the effects of different dietary lipid sources on growth performance, body tissue proximate composition, and fatty acid composition of juvenile *P. trituberculatus*. The results could provide preliminary information on the practical application of lipid sources in their diets.

2 MATERIAL AND METHOD

2.1 Experimental diets

The ingredients and proximate composition of the experimental diets are presented in Table 1. Four isonitrogenous (45.73%–47.87%) and isoenergetic (19.64–19.69 kJ/g) experimental diets were formulated based on our previous studies (Han et al., 2013a, b; Li et al., 2014a, b). These diets contained four different lipid sources, including FO, SO, RO, and LO. White fishmeal and casein were used as protein sources and corn starch as a carbohydrate source. The ingredients were finely ground, sieved through a 250-μm mesh, blended in a dough mixer, and oil and sufficient water were added to form a soft dough. The mixture was then extruded through a 2.5-mm die using a double screw extruder (Institute of Chemical Engineering, South China University of Technology, Guangzhou, China). The resulting formed diets were dried at 45°C overnight and stored at -20°C until use.

The fatty acid composition of experimental diets is shown in Table 2. FO diets contained the highest HUFA content, such as arachidonic acid (ARA, *n*-6 20:4), EPA, and DHA. SO, RO, and LO diets were

highest in *n*-6 18:2, *n*-9 18:1, and *n*-3 18:3, respectively.

2.2 Feeding experiment

Juvenile swimming crabs were obtained from Zhejiang Province Key Lab of Mariculture and Enhancement (Zhoushan, China). Prior to experiments, crabs were maintained on a temporary rearing diet for 7 d. At the end of the acclimation period, 216 healthy crabs with similar body weight (17.00±0.09 g) and carapace width (6.04±0.79 cm) were randomly held individually in containers. There were three replicates (18 crabs per replicate) for each diet.

The crabs were fed to apparent satiation twice daily (at 07:30 and 16:30) for 8 wk. Wet weights of surviving individuals were determined every 2 wk and daily rations adjusted accordingly. Water quality parameters were monitored every 2 d, with the water temperature at 27.4±1.9°C and dissolved oxygen above 6 mg/L. Lighting was provided by overhead fluorescent ceiling lights on a 12-h:12-h light:dark cycle.

2.3 Sample collection

At the end of the 8-wk feeding trial, crabs were individually weighed and sampled for tissue analysis after they were starved for 24 h. Four crabs were randomly captured from each replicate for whole body proximate compositional analyses and the muscle and hepatopancreas collected from another six crabs. Tissues were then frozen in liquid nitrogen and stored at -80°C until use.

2.4 Proximate and fatty acid compositional analyses

Proximate compositional analysis was based on the AOAC (1995) method. Moisture contents of experimental diets and whole body were determined in an oven at 105°C for 24 h, while muscle and hepatopancreas samples were freeze-dried (LL1500: Thermo Fisher scientific Inc., Pittsburgh, PA, USA). Crude protein (nitrogen×6.25) was determined by the Kjeldahl method (K355 and K358, Buchi Labortechnik AG, Flawil, Switzerland). Crude lipid was determined by Soxhlet extraction using petroleum ether (E-816, Buchi Labortechnik AG). Ash was determined by incineration in a muffle furnace at 550°C for 12 h. Finally, dietary gross energy was measured using an adiabatic bomb calorimeter (HWR-15E fast calorimeter, Shanghai, China).

The fatty acid composition was analyzed by extracting the total lipid from diets, muscle, and hepatopancreas with chloroform/methanol (2/1, v/v; Folch et al., 1957). Fatty acid methyl esters were prepared by acid-catalyzed transmethylation using boron trifluoride/methanol, as described by Shantha and Ackman (1990), and analyzed in a gas chromatograph (GCMS-QP2010 Plus, Shimadzu Corp., Kyoto, Japan) equipped with a 0.25 mm×30 m capillary column (SPB-50, Supelco Inc., Bellefonte, PA, USA) and flame ionization detector. The programmed temperature regime was 150°C for 3.5 min, 150–200°C at 20°C/min, 200°C for 5 min, 200–280°C at 5°C/min, and held at 280°C for 20 min. The carrier gas was helium at 44.1 mL/min and injector temperature at 250°C.

Table 2 Fatty acid composition (% total fatty acids) of the diets with different lipid sources

Fatty acid	FO	SO	RO	LO
<14:0	5.76	1.08	1.28	1.11
15:0	1.1	-	0.14	0.11
16:0	19.48	13.95	11.13	9.92
17:0	0.37	-	0.07	-
18:0	3.86	4.37	3.21	5.03
20:0	-	-	0.05	0.17
∑SFA[1]	30.57	19.4	15.88	16.34
16:1n-7	8.17	1.76	2.13	1.81
18:1n-9	17.38	22.15	35.42	23.45
20:1n-9	6.97	3.52	6.94	4.54
22:1n-9	5.74	1.5	7.17	4.05
24:1n-9	0.73	-	0.69	0.54
∑MUFA[2]	38.99	28.93	52.35	34.39
16:2n-4	0.57	-	-	-
18:2n-6	1.92	35.76	12.39	10.46
18:3n-3	1.27	4.78	4.76	28.21
20:4n-6	0.99	0.36	0.68	0.47
20:5n-3	12.2	4.45	5.86	4.35
22:6n-3	13.49	6.32	8.08	5.78
∑PUFA[3]	30.44	51.67	31.77	49.27
∑n-3[4]	26.96	15.55	18.7	38.34
∑n-6[5]	2.91	36.12	13.07	10.93
n3/n6	9.26	0.43	1.43	3.51
n-3 HUFA[6]	25.69	10.77	13.94	10.13
DHA/EPA	1.11	1.42	1.38	1.33

1∑SFA: saturated fatty acid; 2∑MUFA: monounsaturated fatty acids; 3∑PUFA: polyunsaturated fatty acid; 4∑n-3:18:3n-3: 20:3n-3: 20:5n-3: 22:6n-3; 5∑n-6:18:2n-6: 20:4n-6; 6∑n-3 HUFA: 20:5n-3: 22:6n-3.

Table 3 Survival, growth performance, feed utilization and body indices of swimming crab fed diets with different lipid sources*

	FO	SO	RO	LO
Survival rate (%)[1]	81.25±6.25[b]	68.75±10.83[ab]	60.42±9.55[a]	72.92±3.61[ab]
IBW (g)[2]	17.02±0.06	16.95±0.10	17.03±0.09	17.01±0.09
FBW (g)[3]	67.60±4.06	62.22±5.22	59.34±16.58	61.07±6.77
WG (%)[4]	297.24±24.69	266.95±29.16	248.26±95.66	259.15±41.15
SGR (%/day)[5]	2.19±0.10	2.06±0.13	1.90±0.35	2.02±0.19
FCR[6]	1.56±0.20[a]	1.68±0.08[a]	2.39±0.18[b]	1.47±0.11[a]
PER[7]	1.38±0.18[b]	1.25±0.06[b]	0.91±0.07[a]	1.50±0.12[c]
HSI[8]	6.84±0.41	7.96±0.52	7.44±1.44	6.49±0.34

* data are mean±SD. (n=3), means in same column with different superscripts are significantly different (P<0.05). [1] survival rate=100×(initial crab number-dead crab number)/(initial crab number); [2] IBW: initial body weight; [3] FBW: final body weight: [4] WG: weight gain=100×(FBW–IBW)/IBW; [5] SGR: specific growth rate=100×[ln(FBW)–ln(IBW)]/feeding days; [6] FCR: feed conversion ratio=dry feed fed/(final weight–intial weight); [7] PER: protein efficiency ratio=(final weight–intial weight)/mass of protein fed; [8] HSI: hepatosomatic index=100×wet hepatosomatic weight/body weight.

Table 4 Proximate composition (% wet weight) of swimming crabs fed diets with different lipid sources*

	Initial	FO	SO	RO	LO
Whole body					
Moisture	72.06±0.67	73.89±2.49[a]	77.87±0.86[b]	73.95±1.48[a]	73.41±0.66[a]
Crude protein	10.85±0.24	11.69±0.88[b]	9.71±0.41[a]	11.59±0.45[b]	11.94±0.55[b]
Crude lipid	1.13±0.05	1.69±0.16[b]	1.18±0.08[a]	1.71±0.21[b]	2.26±0.11[c]
Ash	11.10±2.40	7.37±0.27	7.10±0.64	8.42±1.23	7.75±0.52
Muscle					
Moisture		81.02±1.11	79.74±0.87	78.54±2.16	80.89±1.45
Crude protein		14.97±0.99	16.23±0.71	16.23±1.58	15.15±1.17
Crude lipid		0.75±0.11	0.79±0.04	0.77±0.19	0.76±0.09
Hepatopancreas					
Moisture		66.44±2.34[c]	61.42±2.40[b]	58.00±0.91[a]	63.59±0.59[bc]
Crude protein		11.75±0.72[ab]	11.58±0.44[ab]	12.97±1.19[b]	10.86±0.42[a]
Crude lipid		17.21±1.03[a]	22.40±2.09[bc]	23.85±0.31[c]	20.33±0.67[b]

* data are mean±SD (n=3), means in same column with different superscripts are significantly different (P<0.05).

2.5 Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to confirm the effects of the four experimental diets and expressed as the mean ±SD. Significance level was set at P<0.05. All statistical analyses were performed using SPSS 17.0 for Windows 7.

3 RESULT

3.1 Growth performance and survival

In this study, experimental diets were accepted well by these swimming crabs. Their growth performance and feed utilization are presented in Table 3. The survival rate ranged from 60.42 to 81.25%, and RO groups were significantly lower than FO groups (P<0.05). No significant differences were observed in weight gain (WG, 248.26%–297.24%), specific growth rate (1.70%–1.91%/d), or hepatosomatic index (6.49%–7.96%) among all groups (P>0.05). However, the food conversion ratio (FCR) in RO groups was significantly higher than in other groups (P<0.05), and the protein efficiency ratio (PER) in RO groups was significantly lower than in other groups (P<0.05).

3.2 Proximate composition

The proximate compositions of whole body, muscle, and hepatopancreas are shown in Table 4. At

Table 5 Fatty acid composition (percentage of total fatty acids) in hepatopancreas of swimming crab fed diets with different lipid sources*

Fatty acid	FO	SO	RO	LO
<14:0	3.42±0.24^{b}	1.04±0.07^{a}	1.04±0.12^{a}	1.02±0.14^{a}
15:0	0.67±0.02^{b}	0.13±0.02^{a}	0.13±0.04^{a}	0.14±0.02^{a}
16:0	19.68±0.96^{c}	15.18±0.61^{b}	13.35±1.22^{a}	13.18±0.53^{a}
17:0	0.57±0.32^{b}	0.12±0.10^{a}	0.05±0.01^{a}	0.16±0.02^{a}
18:0	6.39±0.85^{b}	7.15±0.48^{b}	5.17±0.33^{a}	6.42±0.59^{b}
24:0	-	0.10±0.01	0.08±0.02	0.10±0.00
∑SFA[1]	30.74±0.02^{c}	23.72±0.72^{b}	19.82±1.14^{a}	21.03±0.02^{a}
16:1n-7	5.00±0.21^{b}	1.89±0.14^{a}	1.99±0.28^{a}	1.94±0.29^{a}
18:1n-9	23.06±1.43^{a}	23.02±0.80^{a}	36.13±0.65^{c}	27.99±0.63^{b}
20:1n-9	7.28±0.37^{c}	4.33±0.27^{a}	6.85±0.39^{c}	5.72±0.06^{b}
22:1n-9	4.41±0.06^{b}	1.93±0.07^{a}	5.75±0.35^{c}	4.50±0.12^{b}
24:1n-9	0.77±0.07^{c}	0.48±0.02^{a}	0.58±0.07^{b}	0.61±0.04^{b}
∑MUFA[2]	40.52±1.15^{b}	31.66±0.66^{a}	51.31±0.56^{c}	40.77±0.74^{b}
18:2n-6	2.50±0.13^{a}	25.10±0.46^{c}	10.22±0.64^{b}	9.55±0.13^{b}
18:3n-3	0.68±0.10^{a}	2.12±0.38^{b}	1.66±0.98^{ab}	12.23±0.51^{c}
20:2n-7	0.37±0.16^{a}	1.77±0.25^{c}	0.91±0.15^{b}	0.88±0.17^{b}
20:4n-6	2.04±0.17^{b}	1.18±0.09^{a}	1.37±0.28^{a}	1.20±0.16^{a}
20:3n-3	-	-	0.78±1.10	0.95±0.14
20:5n-3	8.90±0.19^{b}	5.91±0.24^{a}	5.68±0.58^{a}	5.68±0.10^{a}
22:6n-3	14.26±0.65^{b}	8.55±0.28^{a}	8.25±0.55^{a}	7.69±0.20^{a}
∑PUFA[3]	28.74±1.14^{a}	44.63±1.05^{c}	28.87±1.65^{a}	38.19±0.75^{b}
∑n3[4]	23.83±0.94^{b}	16.58±0.31^{a}	16.37±1.22^{a}	26.55±0.56^{c}
∑n6[5]	4.54±0.15^{a}	26.28±0.54^{d}	11.59±0.56^{c}	10.76±0.28^{b}
n3/n6	5.25±0.22^{d}	0.63±0.01^{a}	1.41±0.04^{b}	2.47±0.08^{c}
n-3 HUFA[6]	23.15±0.84^{b}	14.46±0.13^{a}	13.93±1.12^{a}	13.37±0.10^{a}
DHA/EPA	1.60±0.04^{b}	1.45±0.10^{a}	1.46±0.05^{a}	1.36±0.06^{a}

* data are mean±SD (n=3), means in same column with different superscripts are significantly different (P<0.05). 1∑SFA: saturated fatty acid; 2∑MUFA: monounsaturated fatty acids; 3∑PUFA: polyunsaturated fatty acid; 4∑n-3:18:3n-3: 20:3n-3: 20:5n-3: 22:6n-3; 5∑n-6:18:2n-6: 20:4n-6; 6∑n-3 HUFA: 20:5n-3: 22:6n-3.

Table 6 Fatty acid composition (percentage of total fatty acids) in muscle of swimming crab fed diets with different lipid sources*

Fatty acid	FO	SO	RO	LO
<14:0	1.31±0.11^{b}	0.99±0.18^{a}	0.98±0.06^{a}	1.06±0.13^{ab}
15:0	0.38±0.01	-	-	-
16:0	17.34±0.06^{c}	16.23±0.06^{b}	15.81±0.50^{b}	14.82±0.21^{a}
17:0	0.95±0.12	-	-	-
18:0	8.55±0.55^{b}	8.49±0.19^{b}	6.59±0.11^{a}	8.26±0.06^{b}
∑SFA[1]	28.53±0.63^{c}	25.71±0.41^{b}	23.38±0.56^{a}	24.13±0.34^{a}
16:1n-7	2.15±0.25	1.07±0.04	6.44±8.80	1.21±0.11
18:1n-9	22.25±0.36 ^{b}	19.84±0.89^{a}	30.16±1.12^{d}	24.19±1.02^{c}
18:1n-7	0.44±0.09	-	-	-
20:1n-9	3.01±0.23^{b}	2.23±0.16^{a}	3.46±0.25^{c}	2.92±0.18^{b}
22:1n-9	1.01±0.38^{a}	1.00±0.22^{a}	1.99±0.13^{b}	1.64±0.22^{b}
∑MUFA[2]	28.86±1.06^{a}	24.14±1.19^{a}	42.05±9.71^{b}	29.96±1.20^{a}
18:2n-6	4.18±0.75^{a}	19.59±0.74^{d}	11.67±2.19^{c}	9.06±0.16^{b}
18:3n-3	0.71±0.03^{a}	1.45±0.39^{ab}	1.71±0.51^{b}	8.38±0.58^{c}
20:2n-9	-	-	-	0.86±0.06
20:2n-7	-	1.59±0.11	0.83±0.03	-
20:2n-6	0.47±0.06	-	-	-
20:3n-3	-	-	-	0.96±0.08
20:4n-6	2.20±0.07^{b}	1.20±0.05^{a}	1.27±0.30^{a}	1.31±0.10^{a}
20:5n-3	18.69±0.03^{b}	13.41±1.01^{a}	12.49±1.61^{a}	13.14±0.52^{a}
22:6n-3	16.37±0.60^{b}	12.91±0.67^{a}	11.69±0.95^{a}	12.19±0.91^{a}
∑PUFA[3]	42.61±0.81^{b}	50.15±0.81^{d}	39.65±0.88^{a}	45.90±0.87^{c}
∑n-3[4]	35.77±0.62^{b}	27.77±1.30^{a}	25.89±2.50^{a}	34.68±0.82^{b}
∑n-6[5]	6.84±0.80^{a}	20.79±0.70^{d}	12.94±1.89^{c}	10.36±0.20^{b}
n-3/n-6	5.27±0.61^{c}	1.34±0.10^{a}	2.04±0.45^{a}	3.35±0.08^{b}
n-3 HUFA[6]	35.06±0.63^{b}	26.32±1.68^{a}	24.18±2.52^{a}	25.33±1.40^{a}
DHA/EPA	0.88±0.03^{a}	0.96±0.02^{b}	0.94±0.05^{ab}	0.93±0.04^{ab}

* data are mean±SD (n=3), means in same column with different superscripts are significantly different (P<0.05); 1∑SFA: saturated fatty acid; 2∑MUFA: monounsaturated fatty acids; 3∑PUFA: polyunsaturated fatty acid; 4∑n-3:18:3n-3: 20:3n-3: 20:5n-3: 22:6n-3; 5∑n-6:18:2n-6: 20:4n-6; 6∑n-3 HUFA: 20:5n-3: 22:6n-3.

the end of the growth trial, proximate compositions of whole body and hepatopancreas were found to be significantly affected by these dietary treatments. The highest whole body moisture content appeared in SO groups. However, whole body protein and lipid contents were significantly lower in SO groups compared with other groups (P<0.05). The hepatopancreas lipid contents in VO groups were significantly higher than FO groups (P<0.05). Muscle proximate compositions did not show significant differences among these groups (P>0.05).

3.3 Fatty acid composition

Swimming crab muscle and hepatopancreas fatty acid compositions were clearly influenced by dietary treatments (Tables 5 and 6). Crabs fed FO diets were significantly higher in proportions of ARA, EPA, and DHA in muscle and hepatopancreas compared with crabs fed VO diets. The n-6 18:2, n-9 18:1, and n-3 18:3 in muscle and hepatopancreas were highest in the SO, RO, and LO groups, respectively. Although

saturated fatty acid (SFA) and *n*-3 HUFA content in muscle and hepatopancreas were higher than in the diets, the *n*-6 18:2 and *n*-3 18:3 content in muscle and hepatopancreas were lower than in the diets.

4 DISCUSSION

To date, no research has examined the utilization of VOs to replace FO in formula feed for juvenile swimming crabs. In this study, WGs ranged from 248.26%±95.66% to 297.24%±24.69%, which was similar to results from a previous study of this species with similar initial weights (WGs ranged from 213.43%±5.48% to 275.28%±21.71%; Li et al., 2014a). Moreover, the present data showed that the growth performance of swimming crabs was not significantly different between VO and FO groups. These results also showed that good growth in these crabs could be obtained with VO diets. This observation was in agreement with results reported for juvenile red claw crayfish, which used similar VOs (corn, linseed, and canola oils are rich in *n*-6 18:2, *n*-9 18:1, and *n*-3 18:3, respectively) to replace menhaden oil in crab diets (Thompson et al., 2010). In general, the essential fatty acid (EFA) requirement of crustaceans is not greater than 1% of the diet (D'Abramo, 1997). Sheen and Wu (1999) have also found that the concentrations of 0.5%–1.3% EPA and DHA together in the diet supported good growth in mud crabs. In the present work, VO diets contained available concentrations of *n*-3 HUFA at 0.88%–1.25%, which suggested that the inherent lipid content from fishmeal satisfied the *n*-3 HUFA requirements for growth in crabs fed VO diets.

This study showed that crabs fed an RO diet obtained relatively poorer FCR and PER values than the results from previous studies (based on SO and FO diets; Han et al., 2013a, b). Moreover, the FCR and PER values of crabs fed the RO diet were significantly poorer than other groups in this study, which indicated that RO group crabs showed poor feed utilization, despite the use of the same protein source. In agreement with Turchini et al. (2009), these results also suggested that the substitution of FO with alternative lipid sources influenced protein, dry matter, and other micronutrient digestibility. In addition, the present data showed that crabs fed the RO diet had the lowest survival rate (at 60.42% RO), which was significantly lower than crabs fed FO diets (at 81.25% FO). It has also been reported that high monounsaturated fatty acid (MUFA) content in diets disturbs normal EFA metabolism and high concentrations of *n*-9 18:1 in tissues indicate EFA deficiency to some extent (Takeuchi et al., 1990; Halver and Hardy, 2002; Xue et al., 2006). A previous study has also reported that prawn growth performance improves with EFA (DHA, EPA, *n*-6 18:2 and *n*-3 18:3) supplementation compared with a diet with only *n*-9 18:1 (Kanazawa et al., 1979a). Moreover, Zhou et al. (2007) have suggested that EFA is important for maximal survival of some crustaceans, such as *P. indicus* (Read, 1981) and *P. vannamei* (Lim et al., 1997). In the present study, *n*-9 18:1 was rich in muscle and hepatopancreas from crabs fed RO diets (rich in MUFA). This phenomenon might explain, to some extent, why crabs fed RO diets showed low survival rates and poor feed utilization.

Hepatopancreas lipid contents in VO groups were significantly higher than FO groups. The reason for lipid accumulation appeared to be related to a decrease in dietary *n*-3 HUFA content. This decrease would result in impaired lipoprotein synthesis (Watanabe, 1982; Kanazawa, 1985; Olsen et al., 1999; Caballero et al., 2003), thereby enhancing lipid accumulation.

In animals capable of converting *n*-6 18:2 and *n*-3 18:3 to longer chain HUFA, Δ6-desaturase acts at the first step, which converts *n*-6 18:2 to *n*-6 18:3 and *n*-3 18:3 to *n*-3 18:4 (Castell et al., 2004). Although diets of SO and LO groups were rich in *n*-6 18:2 and *n*-3 18:3, respectively, *n*-6 18:3 and *n*-3 18:4 were not detected in crab muscle or hepatopancreas from these groups. Moreover, ARA, EPA, and DHA concentrations in muscle and hepatopancreas of SO and LO groups were similar to RO groups. These results appeared to agree with the hypothesis that marine crustaceans generally lack the necessary enzymes to convert *n*-6 18:2 and *n*-3 18:3 to longer chain FAs, and therefore their ability for de novo essential HUFA synthesis is limited (Kanazawa et al., 1979b; Bottino et al., 1980; Merican and Shim, 1996; Unnikrishnan et al., 2010).

The present results showed that tissue fatty acid composition reflected the crabs' dietary fatty acid composition. Similar results have also been observed in other studies (Greene and Selivonchick, 1990; Xu et al., 1993; Lim et al., 1997; Izquierdo et al., 2003; Regost et al., 2003; Lin et al., 2007; Zhou et al., 2007; Unnikrishnan et al., 2010; Li et al., 2011). However, some peculiar fatty acids are selectively used or retained (Mourente et al., 2005). Here, SFA and *n*-3 HUFA concentrations in muscle and hepatopancreas were higher than in the diets, indicating that SFA and *n*-3 HUFA were preferably deposited. In contrast, *n*-6

18:2 and *n*-3 18:3 concentrations in muscle and hepatopancreas were lower than the diets, and especially lower in *n*-3 18:3. These observations follow a similar trend to that observed in Atlantic salmon with regard to utilization of easily metabolized dietary FA for energy production via ß-oxidation (Menoyo et al., 2007). This might indicate a preferential order of utilization for energy production of *n*-3 18:3 > *n*-6 18:2 (Trushenski et al., 2006; Asdari et al., 2011). Moreover, here, crab muscle displayed higher *n*-3 HUFA concentrations than that in hepatopancreas and the diets. Similar results have also been observed in *Litopenaeus vannamei* (González-Félix et al., 2002; Hu et al., 2011; Sánchez et al., 2014).

In general, muscle and hepatopancreas are important edible parts of swimming crabs. Thus, an increase in *n*-3 HUFA content of muscle and hepatopancreas will provide consumers better access to these health-promoting fatty acids (Calder, 2001; Valfré et al., 2003; Naylor et al., 2009). FO possesses higher nutritional value than VOs because of their higher *n*-3 HUFA concentrations (Catacutan, 1991; Lim et al., 1997; Ponat and Adelung, 1980; Zhou et al., 2007). In agreement with this observation, the present study suggested that crabs fed an FO diet contained larger amounts of EPA and DHA in both muscle and hepatopancreas than those fed other diets.

In conclusion, SO and LO could be substituted for FO in fishmeal-based diets for swimming crab without affecting growth performance and survival. These alternative lipid sources are more cost-effective and marine resource-friendly. However, the nutritional values of swimming crabs were affected when FO was completely replaced in their diets. Further studies involving partial VO supplementation to meet the requirements of this species are warranted.

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