Aquaculture Research

Aquaculture Research, 2017, **48**, 2243–2252 doi: 10.1111/are.13061

Effects of dietary protein to energy ratios on growth, body composition and digestive enzyme activities in Chinese mitten-handed crab, *Eriocheir sinensis*

Yanyan Cui[1], Qianqian Ma[1], Samwel Mchele Limbu[1,2], Zhenyu Du[1], Nannan Zhang[1], Erchao Li[1] & Liqiao Chen[1]

[1]Laboratory of Aquaculture Nutrition and Seafood Research (LANEH), School of Life Sciences, East China Normal University, Shanghai, China

[2]Department of Aquatic Sciences and Fisheries Technology, University of Dar es Salaam, Dar es Salaam, Tanzania

Correspondence: L Chen, Laboratory of Aquaculture Nutrition and Environmental Health (LANEH), School of Life Sciences, East China Normal University, Shanghai 200241, China. E-mail: lqchen@bio.ecnu.edu.cn

Abstract

This study aimed to determine the optimal protein to energy ratio (P/E ratio) and evaluate the effect of dietary protein and lipid levels on growth performance, body composition and digestive enzymes activities in Chinese mitten-handed crab, *Eriocheir sinensis*. Nine practical diets containing three levels both for protein (DP 30%, 35% and 40%) and lipid (DL 2%, 7% and 12%) with P/E ratios ranging from 13.69 to 19.79 mg KJ^{-1} were fed to four replicates of crabs (3.39 ± 0.10 g) for 10 weeks. Weight gain increased significantly with the increase in DP level at each DL level. Moreover, weight gain increased in crabs fed with diets containing DL level from 2% to 12% and DP level from 30% to 35%. However, the diet containing 40% DP and 12% DL levels significantly decreased the growth performance and protein efficiency of the crabs. The whole crab and hepatopancreas lipid contents also increased as dietary lipid increased, but not dietary protein. The total protease activity increased significantly with the increase in dietary protein at each lipid level. The lipase activity was statistically comparable among different DL levels at each DP level. Taken together, the crab fed the diet containing 35% protein and 12% lipid levels with P/E 15.77 mg KJ^{-1} revealed optimal growth, feed utilization efficiency and digestive enzymes activities. Moreover, our study indicated that the higher dietary lipid level at a relatively lower dietary protein level could provide protein sparing effect in *Eriocheir sinensis*.

Keywords: *Eriocheir sinensis*, dietary protein to energy ratio, lipid, growth, body composition digestive enzyme

Introduction

Chinese mitten-handed crab, *Eriocheir sinensis* is an important crustacean species for aquaculture in many Asian countries, especially in China due to its desirable taste and nutrition contents. It has become one of the most important economic species in freshwater aquaculture with a production of over 700 000 tons in 2012 in China (FAO 2014). The recent expansion of crab farming in China necessitates reduction in feed cost to make the farming more sustainable. In artificial diets formulation, protein content is the main constituent affecting animal growth performance and feed cost. However, excessive protein in diets can result in feed wastage, environmental pollution and poor growth performance (Kim & Lee 2005). Previous studies reported the optimal protein level for *E. sinensis* to range from 35% to 46% (Chen, Du & Lai 1994; Mu, Lam, Guo & Shim 2000; Pan, Xiao, Zhang & Luan 2005). In addition to protein, aquatic animals require considerable levels of dietary lipid in their diet for various functions. Compared

© 2016 John Wiley & Sons Ltd

with carbohydrates, lipid provides twice as much energy density and plays an important role in metabolism of aquatic animals (Watanabe 1982; Sargent, Henderson & Tocher 1989; Higgs & Dong 2000). Previous studies suggested that the optimal dietary lipid for *E. sinensis* varies from 3% to 9% (Qian & Zhu 1999; Qi, Wang & Ha 2002; Zhou, Liu, Wang & Zhang 2009).

There is a strong argument that protein to energy (P/E) ratio is a more rational method for determining the optimum protein requirement of cultured aquatic animals than the sole use of dietary crude protein (NRC 2011). The reason is that dietary lipid and carbohydrate are considered as energy sources to reduce the utilization of dietary protein for energy production, a concept known as protein sparing effect. Thus, proper ratios among protein, carbohydrate and lipid would result in optimum growth and reduce feeding costs for aquatic animals (Cho & Kaushik 1990; Nankervis, Matthews & Appleford 2000; Morais, Bell, Robertson, Roy & Morris 2001; Ai, Mai, Li, Zhang, Zhang, Duan, Tan, Xu, Ma, Zhang & Liufu 2004). However, imbalanced P/E ratio in feeds has been shown to induce adverse effects on growth and nutrient utilization efficiency for channel catfish fingerlings (*Ictalurus punctatus*) and juvenile Japanese seabass (*Lateolabrax japonicus*) (Garling & Wilson 1976; Ai *et al.* 2004).

In some crustacean species, the optimal P/E ratio has been reported as 26.89 mg KJ^{-1} for swimming crab, *Portunus trituberculatus* (Huo, Jin, Zhou, Li, Mai & Zhou 2014), 26.65 mg KJ^{-1} and 34.91 mg KJ^{-1} for Tiger prawn, *Penaeus monodon* (Hajra, Ghosh & Mandal 1988 and Chuntapa, Piyatiratitivorakul, Nitithamyong, Viyakarn & Menasveta 1999), respectively, 21.1 mg KJ^{-1} for White shrimp, *Litopenaeus vannamei* (Hu, Tan, Mai, Ai, Zheng & Cheng 2008) and 32.52 mg KJ^{-1} for Chinese white shrimp, *Penaeus chinensis* (Xue, Li, Dong & Zhang 1997). In Chinese mitten-handed crab, some previous studies have indicated that the recommended optimum dietary P/E ratio range from 22.2 to 28.93 mg KJ^{-1}, based on growth or protein conversion efficiency (Mu, Shim & Guo 1998; Lin, Luo, Ye, Zhou, Xue & Yang 2000; Zhu & Qian 2000). However, these studies had some limitations, such as short duration (less than 35 days) and existence of limited dietary groups raising questions on the results obtained. A 76 day study performed by Li, Li, Liu and Murphy (2012) to assess the optimum P/E ratio in *E. sinensis*, demonstrated that 18.13 to 19.20 mg KJ^{-1} was the optimum level, based on growth performance, rate of precocity and digestive enzymes activities. However, in the study by Li *et al.* (2012), carbohydrate and lipid were simultaneously changed to make only six different P/E ratios, in which the contribution of individual nutrient was difficult to evaluate. Moreover, a study conducted by Du, Tian, Liang and Liu (2009) in Grass carp, *Ctenopharyngodon idella* indicated that there was no linear relationship between dietary P/E ratio and the growth performance. In their study, Du *et al.* (2009) revealed that, the absolute amount of individual or several nutrients would affect growth and feed utilization rather than a sole P/E ratio. Therefore, based on this paradox, the optimum P/E ratio in *E. sinensis* need to be re-evaluated, together with the absolute amount of energy-producing nutrients using a standard experimental protocol and appreciable time.

In the present study, the P/E ratios were adjusted using three levels both for lipid (2%, 7% and 12%) and protein (30%, 35% and 40%). The study aimed to determine the optimal protein to energy ratio and evaluate the effect of dietary protein and lipid on growth performance, feed utilization, body composition and digestive enzymes activities in *E. sinensis*.

Materials and methods

Diet preparation

The composition and proximate analysis of the diets used in the present study are given in Table 1. A total of nine different experimental diets (D1 to D9) containing three levels both for protein (DP 30%, 35% and 40%) and lipid (DL 2%, 7% and 12%) with P/E ratios ranging from 13.69 to 19.79 mg KJ^{-1} were prepared (Table 1). The diets gross energy (ranging from 19.78 to 22.30 KJ) was determined by using an adiabatic bomb calorimeter (WELL 9000; Tangshan Shenke Equipment Co., Ltd., Tangshan, Hebei, China). Casein (Sigma-Aldrich, Shanghai, China) and gelatin (Sangon Biotech, Shanghai, China) were used as the protein sources, whereas fish oil (Xiamen Xinsha Pharmaceutical, Xiamen, China), soybean oil (National Golden Dragon Fish, Shanghai, China) and soy lecithin (Sangon Biotech, Shanghai,

 © 2016 John Wiley & Sons Ltd,

Table 1 Formulation and proximate composition of the experimental diets

Diets (P/L) Ingredients	D1 (30/2)	D2 (30/7)	D3 (30/12)	D4 (35/2)	D5 (35/7)	D6 (35/12)	D7 (40/2)	D8 (40/7)	D9 (40/12)
Casein	268	268	268	312.5	312.5	312.5	357.2	357.2	357.2
Gelatin	53.6	53.6	53.6	62.5	62.5	62.5	71.4	71.4	71.4
Fish oil	5	17.5	30	5	17.5	30	5	17.5	30
Soybean oil	15	52.5	90	15	52.5	90	15	52.5	90
Lecithin	5	5	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5	5	5
Corn starch	270	270	270	270	270	270	270	270	270
Vitamin mixture*	40	40	40	40	40	40	40	40	40
Mineral mixture†	30	30	30	30	30	30	30	30	30
Attractant‡	30	30	30	30	30	30	30	30	30
Carboxyl methyl cellulose	20	20	20	20	20	20	20	20	20
Choline Chloride	5	5	5	5	5	5	5	5	5
Cellulose	253.4	203.4	153.4	200	150	100	146.4	96.4	46.4
Proximate composition (%)									
Crude protein	31.92	32.70	32.47	36.69	36.32	36.12	41.15	40.70	40.62
Crude lipid	3.32	7.08	12.14	2.95	8.08	12.66	2.98	8.17	13.12
Gross energy (KJ g^{-1})	19.78	20.60	21.92	20.17	20.96	22.20	20.21	21.34	22.58
P/E (mg KJ^{-1})	15.17	14.56	13.69	17.35	16.70	15.77	19.79	18.74	17.71

*Vitamin premix (mg g^{-1} premix): vitamin A, 1.0; vitamin D_3, 0.63; vitamin E, 10; vitamin K_3, 1.8; niacin, 5; riboflavin, 2.63; pyridoxine, 1; aminobenzoic acid, 5; thiamin, 0.75; D-calcium pantothenate, 5; biotin, 0.75; folic acid, 0.19; vitamin B12, 0.15; inositol, 60; a-cellulose, 905.75.

†Mineral mixture (per 100 g mixture): KH_2PO_4, 21.5 g; NaH_2PO_4, 10.0 g; $Ca(H_2PO_4)_2$, 26.5 g; $CaCO_3$, 10.5 g; KCl, 2.8 g; $MgSO_4{\cdot}7H_2O$, 10.0 g; $AlCl_3{\cdot}6H_2O$, 0.024 g; $ZnSO_4{\cdot}7H_2O$, 0.476 g; $MnSO_4{\cdot}4–6H_2O$, 0.143 g; KI, 0.023 g; $CuCl_2{\cdot}2H_2O$, 0.015 g; $CoCl_2{\cdot}6H_2O$, 0.14 g; Ca-lactate, 16.50 g; Fe-citrate, 1 g. All ingredients are diluted with a-Cellulose to 100 g.

‡Attractant: glycine, 0.6; L-alanine, 0.6; L-glutamic acid, 0.6; betaine, 1.2.

China) formed the lipid sources. The carbohydrate source used was corn starch (Sigma, China National Medicine Corporation, Beijing, China). Dry feed raw materials were sieved through an 80 mesh sieve, weighed according to formulations and mixed with distilled water. After uniform mixing, two millimeter diameter pellets were wet-extruded, followed by air-drying to about 10% moisture. The formed pellets were finally stored at −20°C until needed for use.

Experimental crabs and feeding management

Experimental Chinese mitten juvenile crabs were purchased from the Chongming Fisheries, Shanghai, China and then acclimated in indoor concrete ponds for 2 weeks in the Caojing Mingyou aquaculture base (Jinshan, Shanghai, China). During this period, juvenile crabs were fed with a commercial diet (9812; Shanghai Harmony Feed Co., Ltd., Jinshan, Shanghai, China). Thirty (30) healthy crabs per tank (3.39 ± 0.10 g) were randomly distributed into 36 plastic tanks (500 L) in four replicates. A PVC pipe (12 cm long and 50 mm diameter), corrugated plastic pipe (12 cm long and 25 mm diameter) and an arched tile were placed in experimental tanks as shelters to avoid crabs fighting. During the trial, all the crabs were hand-fed to apparent visual satiation twice daily at 09:00 hours and 17:00 hours for 10 weeks. Seventy percentage (70%) of the daily diet was fed at nightfall (around 17:00 hours) and 30% in the morning (around 09:00 hours) because *E. sinensis* has higher food intake in a weak-light environment (Jiang, Chen & Li 2012; Jiang, Chen, Sun & Li 2013; Wei, Yu & Tian 2014). Two hours after feeding, uneaten feed from each tank was recovered and dried before weighing. In each tank, one-third to half of the water was exchanged on daily basis. During water exchange, faeces and other debris were siphoned. Dead crabs were removed and their weight recorded. The environmental conditions in each experimental tank were kept within optimum levels required for *E. sinensis* growth and survival. Temperature ranged from 20.0 to 24.8°C, pH was

7.6 to 8.4, dissolved oxygen was >7.6 mg L^{-1} and ammonia nitrogen was <0.01 mg L^{-1}.

Sample collection and chemical analyses

At the end of the feeding trial, total weight was determined and the number of crabs in each tank was counted to calculate growth performance and survival rate respectively. Four crabs from each tank were collected for analysis of whole body composition. The collected samples were stored at −20°C until needed for analysis. The remaining crabs were dissected. Their hepatopancreas were collected and stored at −80°C for further analysis.

The whole crab body and diet proximate compositions were analyzed according to the standard methods (AOAC 1990). Moisture was determined by drying the crabs to a constant weight in an oven at 105°C for 24 h. Ash was determined by burning the samples to constant weights using a muffle furnace at 550°C for 12 h. Crude protein was determined by using the Kjeldahl method. Crude lipid was determined by the ether extraction method using the Soxhlet system (2055 Soxhlet Avanti; FossTecator, Hoganas Sweden). Hepatopancreas total lipid content was determined by using the chloroform/methanol method according to Folch and Sloane-Stanley (1957).

To measure the digestive enzymes activities, the hepatopancreas samples were weighed and homogenized in 10 volumes (v/w) of pre-chilled phosphate buffer solution (pH 7.3). The homogenates were then centrifuged at 2500 r min^{-1} for 30 min and the supernatants were collected. Amylase activity was determined by using the iodine-starch colorimetric method by utilizing assay kits from Nanjing Jiancheng Bioengineering Institute (Cat. No. C016, Nanjing, China). Total protease and lipase activities were measured by using enzyme-linked immune sorbent assay (ELISA) kit purchased from Shanghai Hengyuan Biotechnology. One unit of amylase activity (U mg^{-1} protein) was defined as one mg substrate hydrolyzing 10 mg starch in 30 min at 37°C. The protein concentration of supernatants was measured as described by Bradford (1976).

Evaluation of growth performance, survival, feed intake and utilization efficiency

Survival, weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio and feed intake were evaluated using the following formulae:

$$\text{Survival}\,(\%) = 100 \times \left(\frac{\text{Final number of crabs}}{\text{Initial number of crabs}}\right)$$

$$\text{Weight gain}\,(\text{WG}, \%) = 100 \times \left(\frac{W_t - W_i(g)}{\text{Wi (g)}}\right)$$

$$\text{Specific growth rate}\,(\text{SGR}, \%\ \text{day}^{-1}) = 100 \times \left(\frac{\ln W_t - \ln W_i}{t}\right)$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed consumed (g)}}{\text{Weight gained (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gained (g)}}{\text{Protein intake (g)}}$$

$$\text{Feed intake}\,(\text{FI}, \text{g crab}^{-1}) = \frac{\text{Feed consumed (g)}}{\text{Final number of crabs}}$$

where, W_t is the mean final body weight (g), W_i is the mean initial body weight (g) and t is the experimental duration (days).

Statistical analyses

The results are presented as means ± standard deviation (SD). Means of each variable were analyzed using two-way analysis of variance (ANOVA) to determine the effect of dietary lipid, protein and any possible interactions among their levels. Significant differences ($P < 0.05$) for each variable were detected by the one-way ANOVA test, followed by Duncan's multiple range test to rank the groups. All statistical analyses were performed using the SPSS 19.0 software (SPSS, Chicago, IL, USA).

Results

Growth performance, survival, feed intake and utilization efficiency

The results on survival, growth performance, feed intake and utilization efficiency of juvenile crabs

 © 2016 John Wiley & Sons Ltd,

are shown in Table 2. The survival rate ranged from 79.17% to 89.16% with no significant differences among all experimental groups ($P > 0.05$). The feed intake was also not affected by the treatments ($P > 0.05$). Both DP and DL levels significantly affected the growth performance and feed utilization efficiency of crabs ($P < 0.05$). The increase in DP level from 30% to 40% increased significantly the WG and SGR at each lipid level (2%, 7%, 12%). Nevertheless, the WG and SGR for the crabs fed the 40% protein and 12% DL levels were less than those fed the same lipid level at 35% protein level (D9 versus D6). Similarly, at each protein level (30%, 35%, 40%), WG and SGR increased with the increasing DL level from 2% to 12%, except for the diet containing 40% protein and 12% lipid levels, where the WG and SGR were significantly less than those at 7% lipid level (D9 versus D8). The best values of WG and SGR were obtained in crabs fed the diet (D8) containing 40% protein and 7% lipid with a P/E ratio of 18.74 mg KJ^{-1}, followed by the diet (D6) containing 35% protein and 12% lipid with a P/E ratio of 15.77 mg KJ^{-1}. There were no significant differences in WG and SGR between crabs fed on D6 and D8 ($P > 0.05$). A similar trend obtained in WG and SGR was also found in FCR, where the lowest values were recorded for crabs fed D8 and D6. However, the highest PER value was obtained in crabs fed with D6. Nonetheless, the interaction among the different protein and lipid levels did not affect significantly the growth performance survival, feed intake and utilization efficiency of crabs ($P > 0.05$).

Body composition and hepatopancreas lipid content

The results of whole body composition and hepatopancreas lipid content are shown in Table 3. There were no significant differences in percentage moisture and ash contents among the different experimental groups ($P > 0.05$). The whole crab body protein content was significantly affected by the dietary protein and lipid levels ($P < 0.05$). Nevertheless, the interactions of dietary protein and lipid levels did not affect significantly the whole crab body protein content ($P > 0.05$). There was a general trend indicating that, the higher dietary protein and/or dietary lipid levels caused higher protein content in the whole crab body composition. The lipid content in the whole crab body composition was also significantly affected by the dietary protein and lipid levels ($P < 0.05$). Furthermore, the lipid content showed an increasing trend with the increase in dietary lipid level at each protein level. However, the lipid content in whole crab body composition showed no clear trend with the increase in dietary protein level at each lipid level. A similar trend obtained in whole crab body lipid composition was also observed in hepatopancreas lipid content.

Table 2 Effects of different dietary protein and lipid levels on growth performance, survival, feed intake and utilization of Chinese mitten crab, *Eriocheir sinensis*

Diets (P/L)	WG (%)	SGR (% day^{-1})	FCR	PER	Survival (%)	FI (%)
D1 (30/2)	89.09 ± 2.97^{a}	0.91 ± 0.02^{a}	2.02 ± 0.09^{d}	1.65 ± 0.07^{c}	79.17 ± 9.57	7.32 ± 0.61
D2 (30/7)	95.25 ± 5.50^{ab}	0.96 ± 0.04^{ab}	1.86 ± 0.26^{cd}	1.80 ± 0.15^{c}	86.67 ± 8.60	6.58 ± 0.30
D3 (30/12)	95.50 ± 6.69^{ab}	0.96 ± 0.05^{ab}	1.85 ± 0.09^{cd}	1.79 ± 0.09^{c}	83.33 ± 8.81	6.61 ± 0.55
D4 (35/2)	99.25 ± 4.94^{abc}	1.00 ± 0.03^{abc}	1.75 ± 0.22^{abc}	1.66 ± 0.24^{c}	88.33 ± 4.30	6.60 ± 0.44
D5 (35/7)	106.93 ± 5.57^{bc}	1.04 ± 0.04^{bc}	1.63 ± 0.08^{abc}	1.76 ± 0.09^{c}	89.16 ± 1.66	6.65 ± 0.45
D6 (35/12)	111.76 ± 9.11^{cd}	1.07 ± 0.06^{cd}	1.59 ± 0.09^{ab}	1.81 ± 0.10^{c}	85.56 ± 1.92	6.37 ± 0.57
D7 (40/2)	105.16 ± 13.4^{bc}	1.02 ± 0.09^{bc}	1.84 ± 0.28^{bcd}	1.38 ± 0.22^{a}	84.17 ± 8.34	7.06 ± 1.12
D8 (40/7)	121.77 ± 12.7^{d}	1.14 ± 0.08^{d}	1.55 ± 0.13^{a}	1.62 ± 0.13^{bc}	88.00 ± 2.31	7.14 ± 0.55
D9 (40/12)	102.01 ± 14.9^{abc}	1.00 ± 0.11^{abc}	1.80 ± 0.23^{abcd}	1.40 ± 0.16^{b}	83.33 ± 3.84	6.58 ± 0.46
Two-way ANOVA						
Protein	0.001	0.001	0.002	0.001	0.236	0.302
Lipid	0.044	0.044	0.027	0.040	0.230	0.203
Interaction	0.139	0.138	0.487	0.512	0.805	0.626

Values are means ± standard deviation (SD) of four replicates of crabs ($n = 4$) with 30 crabs each group; values in the same column with different superscripts are significantly different ($P < 0.05$).

WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; FI, feed intake.

Table 3 Effects of different dietary protein and lipid levels on body composition and hepatopancreas lipid content of Chinese mitten crab, *Eriocheir sinensis*

Diets (P/L)	Crude lipid (%)	Crude protein (%)	Moisture (%)	Ash (%)	Hepatic lipid (%)
D1 (30/2)	4.17 ± 0.13^{a}	12.20 ± 0.43^{a}	63.81 ± 1.36	13.28 ± 0.83	31.15 ± 4.02^{a}
D2 (30/7)	5.30 ± 0.64^{bc}	12.91 ± 0.20^{abc}	62.66 ± 0.74	13.07 ± 0.26	37.59 ± 5.10^{ab}
D3 (30/12)	8.09 ± 0.83^{e}	13.02 ± 0.45^{abc}	62.32 ± 2.64	14.09 ± 0.69	44.69 ± 5.06^{b}
D4 (35/2)	6.26 ± 0.85^{d}	12.80 ± 0.84^{ab}	62.60 ± 1.77	13.68 ± 0.45	38.29 ± 3.78^{ab}
D5 (35/7)	5.48 ± 0.43^{bcd}	14.23 ± 0.53^{d}	61.45 ± 1.90	13.78 ± 0.66	40.36 ± 6.24^{b}
D6 (35/12)	6.10 ± 0.41^{cd}	12.80 ± 0.99^{ab}	63.74 ± 2.73	13.73 ± 0.56	40.95 ± 7.94^{b}
D7 (40/2)	4.10 ± 0.22^{a}	13.52 ± 0.41^{bcd}	63.69 ± 2.30	13.49 ± 0.37	37.80 ± 2.24^{ab}
D8 (40/7)	5.14 ± 0.21^{b}	14.48 ± 0.20^{d}	61.37 ± 0.83	14.32 ± 0.18	41.25 ± 2.12^{b}
D9 (40/12)	5.71 ± 0.69^{bcd}	13.91 ± 0.25^{cd}	64.55 ± 3.42	13.20 ± 1.52	41.16 ± 3.63^{b}
Two-way ANOVA					
Protein	0.001	0.001	0.792	0.746	0.552
Lipid	0.001	0.003	0.117	0.770	0.031
Interaction	0.001	0.241	0.500	0.202	0.322

Values are means ± standard deviation (SD) for four replicates of crabs ($n = 4$) with 30 crabs each group; values in the same column with different superscripts are significantly different ($P < 0.05$).

Digestive enzymes activities

The results of the different digestive enzymes activities studied in juvenile crabs fed different diets are shown in Table 4. The total protease activity in hepatopancreas was significantly affected by the DP level ($P < 0.05$). Moreover, the total protease activity in hepatopancreas increased with the increasing DP level at each lipid level. In one hand, at each protein level, the higher dietary lipid caused higher total protease activity except in crabs fed the 40% protein diet. On the other hand, lipase activity for crabs fed the 30% DP was significant different among the three dietary lipid levels ($P < 0.05$). However, those fed the 35% and 40% DP at each lipid level had statistically similar lipase activities ($P > 0.05$). The amylase activity was generally higher at higher dietary lipid levels, but only crabs fed the 30% DP depicted significant differences in amylase activity among the three dietary lipid levels ($P < 0.05$).

Discussion

The main purpose of the present study was to investigate the ability of *E. sinensis* to utilize various dietary protein and lipids levels at different P/E ratios. The results indicated that increased DP

Table 4 Effects of different dietary protein and lipid levels on digestive enzyme activities of Chinese mitten crab, *Eriocheir sinensis*

Diets (P/L)	Protease (IU L^{-1})	Lipase (mU L^{-1})	Amylase (U mg protein^{-1})
D1 (30/2)	427.23 ± 17.65^{a}	13.70 ± 3.08^{c}	5.63 ± 1.37^{a}
D2 (30/7)	443.65 ± 7.80^{ab}	10.59 ± 2.28^{ab}	6.49 ± 0.68^{ab}
D3 (30/12)	459.78 ± 32.71^{ab}	8.50 ± 1.35^{a}	9.84 ± 1.57^{b}
D4 (35/2)	462.33 ± 17.64^{ab}	12.97 ± 1.77^{bc}	8.96 ± 2.55^{ab}
D5 (35/7)	471.34 ± 37.35^{ab}	13.54 ± 1.00^{c}	9.10 ± 2.06^{ab}
D6 (35/12)	485.78 ± 30.22^{bc}	11.40 ± 0.55^{bc}	7.18 ± 1.66^{ab}
D7 (40/2)	529.74 ± 44.72^{c}	10.84 ± 2.19^{abc}	6.25 ± 0.75^{ab}
D8 (40/7)	492.90 ± 20.83^{bc}	13.60 ± 1.68^{c}	9.54 ± 3.75^{b}
D9 (40/12)	487.92 ± 49.58^{bc}	11.70 ± 0.21^{bc}	9.01 ± 3.07^{ab}
Two-way ANOVA			
Protein	0.001	0.075	0.418
Lipid	0.804	0.014	0.132
Interaction	0.168	0.054	0.045

Values are means ± standard deviation (SD) for four replicates of crabs ($n = 4$) with 30 crabs each group; values in the same column with different superscripts are significantly different ($P < 0.05$).

 © 2016 John Wiley & Sons Ltd,

level from 30% to 40% tended to increase WG and SGR. However, there were no significant differences in WG and SGR between 35% and 40% protein levels, indicating that 35% protein level was sufficient to satisfy protein requirement for *E. sinensis*. A number of studies conducted in crustaceans have indicated that surplus protein is metabolized for energy rather than used for growth, and dietary protein at a low level can be efficiently used for protein synthesis (Hajra *et al.* 1988; Shiau & Peng 1992; Hu *et al.* 2008). As an evidence in the present study, although the increased DP level from 30% to 40% tended to increase the percentage protein in the whole crab body composition (Table 3) and the activity of protease enzyme (Table 4), the PER at the 40% DP level was lower than those at 30% and 35% DP levels. These results imply that at 40% DP level, more protein was metabolized for energy than at 30% and 35% DP levels. Thus, the diet containing 35% protein level is sufficient to meet the optimum protein required for growth in *E. sinensis*.

Dietary lipid is another important source of energy for aquatic animals with positive effects on improving growth in fish species (De Silva, Gunasekera & Shim 1991; Company, Calduch-Giner, Kaushik & Pérez-Sánchez 1999; Lee, Jeon & Lee 2002) and in crustacean species, such as American lobster (Capuzzo & Lancaster 1979) and Juvenile mud crab (Catacutan 2002). One of the positive effects of dietary lipid on aquatic organisms is known as protein sparing effect. Protein sparing effect literally means that other nutrients such as lipid and carbohydrate are efficiently metabolized for energy production and save protein for growth. However, excess lipid can also cause negative effects on growth and physiological characteristics (Du, Clouet, Huang, Degrace, Zheng, He, Tian & Liu 2008). In the present study, diets containing 30% and 35% DP level and increased DL level had positive effects in promoting WG, SGR and PER, consequently reducing FCR. On the other hand, increased DL did not affect feed intake and did not induce severe lipid accumulation in the whole crab body and hepatopancreas compositions. These results suggest that the DL level up to 12% at 30% and 35% DP levels does not cause adverse effects in *E. sinensis* based on the present study experimental conditions.

As a typical protein sparing effect of lipid, the diet (D6) containing 35% DP and 12% DL revealed a comparable growth performance with the diet (D8) containing 40% DP and 7% DL, indicating that at relatively lower DP level, relatively higher DL might save protein from energy metabolism and enhance protein utilization efficiency. As a proof, the PER value in the crabs fed D6 was the best of all the groups used in the present study.

However, it is worthwhile to note that at 40% DP, increasing DL from 7% to 12% significantly impaired growth performance of crabs. Since the diet containing 35% DP level had satisfied the protein requirement of the crabs for growth, the higher protein and lipid in the diet containing 40% DP/12% DL produced excessive total energy that induced negative impacts on the growth and protein utilization efficiency. Zhou *et al.* (2009) reported that, diets with more than 9% lipid level resulted in lower energy budget and growth for *E. sinensis*. A number of studies in crustaceans have also shown that additional dietary energy provided by lipid can have a limited effect on protein efficiency (Huo *et al.* 2014), survival (Chuntapa *et al.* 1999) and growth performance (Zhao, Wen, Li, Zhu & Li 2015). In crustaceans species, some previous studies reported that the dietary lipid requirements range from 3% to 9% for *E. sinensis* (Qian & Zhu 1999; Qi *et al.* 2002), 7% for red swamp crayfish (*Procambarus clarkii*) (Xu, Liu, Shen, Li, Wang & Zhang 2013) and 6% dietary lipid level for mud crab (*Scylla paramamosain*) (Catacutan 2002). Therefore, from the previous studies, it is clear that crustacean species do not need higher dietary lipid levels (Sheen & Wu 1999; Huo *et al.* 2014). However, it must be pointed out that the requirement for lipid or energy in a species is normally affected by the contents of other nutrients, such as protein and carbohydrate. In the present study, 12% DL promoted the growth of crabs when DP level was 30% or 35%, but it impaired the growth when DP level was increased to 40%. Thus, a comprehensive evaluation of the composition of the energy-producing nutrients and P/E ratio are important considerations in feed formulation for aquatic species.

The different growth performance with the increasing lipid level responding to the different protein level reflected that the dietary P/E ratio has a great influence on growth and nutrient utilization (Lee & Kim 2001). In our experiment, the P/E ratios ranged from 13.69 mg KJ^{-1} to 19.79 mg KJ^{-1} and the growth performance of

juvenile crabs was significantly influenced by the dietary P/E ratios. In general, based on the growth performance, protein efficiency and digestive enzymes activities, the diet containing 35% protein and 12% lipid with P/E of 15.77 mg KJ^{-1} was optimum for *E. sinensis* in the present study. Previous studies conducted by Lin *et al.* (2000) and Zhu and Qian (2000) in *E. sinensis* reported that the optimum dietary P/E ratio were 28.93 mg KJ^{-1} with 38.46% protein level and 22.22 mg KJ^{-1} with 44% protein level respectively. However, the durations of both studies were lower than 35 days and the values of weight gain obtained were not correct. A more time-extensive study (76 days) conducted by Li *et al.* (2012) reported that 18.13 to 19.20 mg KJ^{-1} (DP 35%) was the optimum dietary P/E ratio for *E. sinensis*, based on growth performance, rate of precocity and digestive enzymes activities. The optimum P/E ratio for *E. sinensis* set in the present study when compared with that obtained by Lin *et al.* (2000), Zhu and Qian (2000) and Li *et al.* (2012) is relatively lower. The disparity in the P/E ratio values of *E. sinensis* between our study and the previous studies is related to experimental set up. In the previous studies (Lin *et al.* 2000; Zhu & Qian 2000; Li *et al.* 2012), dietary carbohydrate and lipid were both changed to obtain the designed energy levels. In fact, the estimation of nutritional requirements and optimum P/E ratio in aquatic animals vary depending on different experimental dietary P/E levels designed in a particular study (Mercer 1982; Ai *et al.* 2004). Since dietary protein is the main contributor of feed costs, the relatively lower optimum P/E ratio obtained in the present study may be due to the dose-response by the lower protein level and the higher energy level (dietary lipid level). These results imply that the lower optimum P/E ratio in the present study could provide lower economic costs compared with higher P/E ratios obtained from the previous studies. Nevertheless, future studies should investigate the dependence of dietary P/E ratio on an individual or several nutrients.

In the present study, the results showed that differences in protein and lipid levels of diets fed to juvenile crabs affected digestive enzymes activities. The total protease activity in hepatopancreas was greatly enhanced with the increasing DP level. Similar results have been obtained in marine shrimp (*Penaeus vannamei*) and red swamp crayfish (*Procambarus clarkii*) (Lee, Smith & Lawrence 1984; Xu, Liu, Shen, Wang, Zhu, Xu & Cheng 2011). The increase in protease activity as DP level increased may be due to the higher availability of dietary protein as substrate for protease activity (Giri, Sahoo, Sahu & Meher 2003). Despite this, there were no significant differences in growth performance between 35% and 40% protein levels, indicating that the total protease activity did not have an absolute positive effect on the growth performance as indicated in diet D7 when compared with diet D4 or D5. The lipase activity for crabs fed the 30% DP decreased with the increasing DL level. However, the diets containing 30% and 35% DP levels had positive effects in promoting WG at each DL level. These results show that lipase activity did not directly influence the growth rate of *E. sinensis*. Previous studies have shown that digestive enzymes activities can directly reflect the capability of nutrient absorption and utilization, but not growth performance (Galgani & Ceccaldi 1988). Moreover, Lin *et al.* (2000) reported that higher digestive enzyme activity in *E. sinensis* mean higher digestive ability, which should translate into availability of more nutrients, subsequently resulting in higher growth rate. However, the present study did not provide adequate evidence to support this observation. Further studies are required to explore the relationship between digestive enzymes activities and growth performance in *E. sinensis*.

In conclusion, the present study demonstrated that the growth performance of juvenile crabs was significantly influenced by the different dietary P/E ratios. The diet containing 35% protein and 12% lipid with P/E ratio of 15.77 mg KJ^{-1} is optimum for *E. sinensis*, based on the growth rate, feed utilization and digestive enzymes activities.

Acknowledgments

This research was supported by grants from the Special Fund for Agro-scientific Research in the Public Interest (No. 201203065), National 'Twelfth Five-Year' Plan for Science & Technology Support (2012BAD25B03), the National Natural Science Foundation of China (No. 31572629, 31172422), Shanghai Industrial Technology System for Crab, and partly by the E-Institute of Shanghai Municipal Education Commission (No. E03009).

 © 2016 John Wiley & Sons Ltd,

References

Ai Q.H., Mai K.S., Li H.T., Zhang C.X., Zhang L., Duan Q.Y., Tan B.P., Xu W., Ma H.M., Zhang W.B. & Liufu Z.G. (2004) Effects of dietary protein to energy ratios on growth and body composition of juvenile Japanese seabass, *Lateolabrax japonicus*. *Aquaculture* **230**, 507–516.

AOAC (1990) Official Methods of Analysis (15th edn). Association of Official Analytical Chemists. Washington, DC, USA. 1094 pp.

Bradford M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry* **72**, 248–254.

Capuzzo J.M. & Lancaster B.A. (1979) The effects of diet on the growth energetics of postlarval lobsters (*Homarus americanus*). *Woods Hole Oceanographic Institution* **10**, 689–700.

Catacutan M.R. (2002) Growth and body composition of juvenile mud crab, *Scylla serrata*, fed different dietary protein and lipid levels and protein to energy ratios. *Aquaculture* **208**, 113–123.

Chen L.Q., Du N.S. & Lai W. (1994) Evaluation of soybean cake as a substitute for partial fish meal in formulated diets for juvenile Chinese mitten-handed crab. *Journal of Fisheries of China* **18**, 24–31 (in Chinese with English abstract).

Cho C. & Kaushik S. (1990) Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). *World Review of Nutrition and Dietetics* **61**, 132–172.

Chuntapa B., Piyatiratitivorakul S., Nitithamyong C., Viyakarn V. & Menasveta P. (1999) Optimal lipid: carbohydrate and protein: energy ratios in semi-purified diets for juvenile black tiger shrimp *Penaeus monodon* Fabricius. *Aquaculture Research* **30**, 825–830.

Company R., Calduch-Giner J., Kaushik S. & Pérez-Sánchez J. (1999) Growth performance and adiposity in gilthead sea bream (*Sparus aurata*): risks and benefits of high energy diets. *Aquaculture* **171**, 279–292.

De Silva S.S., Gunasekera R.M. & Shim K. (1991) Interactions of varying dietary protein and lipid levels in young red tilapia: evidence of protein sparing. *Aquaculture* **95**, 305–318.

Du Z.Y., Clouet P., Huang L., Degrace P., Zheng W.H., He J.G., Tian L.X. & Liu Y.J. (2008) Utilization of different dietary lipid sources at high level in herbivorous grass carp (*Ctenopharyngodon idella*): mechanism related to hepatic fatty acid oxidation. *Aquaculture Nutrition* **14**, 77–92.

Du Z.Y., Tian L.X., Liang G.Y. & Liu Y.J. (2009) Effect of dietary energy to protein ratios on growth performance and feed efficiency of juvenile Grass carp (*Ctenopharyngodon idella*). *Open Fish Science Journal* **2**, 25–31.

FAO [Food Agriculture Organization of the United States] (2014) *Yearbooks of Fisheries Statistics*. Food Agriculture Organization of the United Nations, Rome.

Folch J.L.M. & Sloane-Stanley G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry* **226**, 497–509.

Galgani F. & Ceccaldi H.J. (1988) The effect of the incorporation of soya bean and fish meals in the diet on the growth and digestive enzymes of *Penaeus vannamei*. *Aquatic Living Resources* **1**, 181–187.

Garling D.L. & Wilson R.P. (1976) Optimum dietary protein to energy ratio for channel catfish fingerlings, *Ictalurus punctatus*. *The Journal of Nutrition* **106**, 1368–1375.

Giri S.S., Sahoo S.K., Sahu A.K. & Meher P.K. (2003) Effect of dietary protein level on growth, survival, feed utilisation and body composition of hybrid *Clarias* catfish (*Clarias batrachus* × *Clarias gariepinus*). *Animal Feed Science and Technology* **104**, 1–4.

Hajra A., Ghosh A. & Mandal S.K. (1988) Biochemical studies on the determination of optimum dietary protein to energy ratio for juveniles tiger prawn, *Penaeus monodon*. *Aquaculture* **71**, 71–79.

Higgs D.A. & Dong F.M. (2000) Lipids and fatty acids. In: *Encyclopedia of Aquaculture* (ed. by R.R. Stickney), pp. 476–496. John Wiley & Sons, New York, NY, USA.

Hu Y., Tan B., Mai K., Ai Q., Zheng S. & Cheng K. (2008) Growth and body composition of juvenile white shrimp, *Litopenaeus vannamei*, fed different ratios of dietary protein to energy. *Aquaculture Nutrition* **14**, 499–506.

Huo Y.W., Jin M., Zhou P., Li M., Mai K.S. & Zhou Q.C. (2014) Effects of dietary protein and lipid levels on growth, feed utilization and body composition of juvenile swimming crab, *Portunus trituberculatus*. *Aquaculture* **434**, 151–158.

Jiang H.B., Chen L.Q. & Li E.C. (2012) Partial or total replacement of soybean meal by cottonseed meal in practical diets for Chinese Mitten Crab, *Eriocheir sinensis*: effects on oxygen consumption, ammonia excretion, O: N ratio and amino transferases activities. *Turkish Journal of Fisheries and Aquatic Sciences* **12**, 547–554.

Jiang X., Chen L.Q., Sun S.S. & Li E.C. (2013) Apparennt digestibility of 10 feed ingredients for Chinese mitten crab, *Eriocheir sinensis*. *Marine Fisheries of China* **35**, 209–216 (in Chinese with English abstract).

Kim L.O. & Lee S.M. (2005) Effects of the dietary protein and lipid levels on growth and body composition of bagrid catfish, *Pseudobagrus fulvidraco*. *Aquaculture* **243**, 323–329.

Lee S.M. & Kim K.D. (2001) Effects of dietary protein and energy levels on the growth, protein utilization and body composition of juvenile masu salmon (*Oncorhynchus masou* Brevoort). *Aquaculture Research* **32**, 39–45.

Lee P.G., Smith L.L. & Lawrence A.L. (1984) Digestive proteases of *Penaeus vannamei* Boone: relationship between enzyme activity, size and diet. *Aquaculture* **42**, 3–4.

Lee S.M., Jeon I.G. & Lee J.Y. (2002) Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish (*Sebastes schlegeli*). *Aquaculture* **211**, 227–239.

Li X., Li Z., Liu J. & Murphy B.R. (2012) Growth, precocity, enzyme activity and chemical composition of juvenile Chinese mitten crab, *Eriocheir sinensis*, fed different dietary protein-to-energy ratio diets. *Aquaculture Research* **43**, 1719–1728.

Lin S.M., Luo L., Ye Y.T., Zhou J.S., Xue M. & Yang J.K. (2000) Effects of protein to energy ration on protease activities and apparent digestibility for *Eriocheir sinensis*. *Journal of Fishery Science of China* **7**, 33–36 (in Chinese with English abstract).

Mercer L.P. (1982) The quantitative nutrient–response relationship. *The Journal of nutrition* **112**, 560–566.

Morais S., Bell J.G., Robertson D.A., Roy W.J. & Morris P.C. (2001) Protein/lipid ratios in extruded diets for Atlantic cod (*Gadus morhua* L.): effects on growth, feed utilization, muscle composition and liver histology. *Aquaculture* **203**, 101–119.

Mu Y., Shim K.F. & Guo J.Y. (1998) Effects of protein level in isocaloric diets on growth performance of the juvenile Chinese hairy crab, *Eriocheir sinensis*. *Aquaculture* **165**, 139–148.

Mu Y., Lam T.J., Guo J.Y. & Shim K.F. (2000) Protein digestibility and amino acid availability of several protein sources for juvenile Chinese hairy crab *Eriocheir sinensis* H. Milne-Edwards (*Decapoda, Grapsidae*). *Aquaculture Research* **31**, 757–765.

Nankervis L., Matthews S.J. & Appleford P. (2000) Effect of dietary non-protein energy source on growth, nutrient retention and circulating insulin-like growth factor I and triiodothyronine levels in juvenile barramundi, *Lates calcarifer*. *Aquaculture* **191**, 323–335.

NRC [National Research Council] (2011) *Nutrient Requirements of Fish and Shrimp*. National Academies Press, Washington, DC, USA 114pp.

Pan L.Q., Xiao G.Q., Zhang H.X. & Luan Z.H. (2005) Effects of different dietary protein content on growth and protease activity *Eriocheir sinensis* larvae. *Aquaculture* **246**, 313–319.

Qi H.Y., Wang L.Q. & Ha Y. (2002) The progress about lipid nutritional needs and Metabolism of Chinese mitten. *Reservoir Fisheries* **22**, 34–35 (in Chinese with English abstract).

Qian G.Y. & Zhu Q.H. (1999) Optimal levels of protein, lipid and fiber for *Eriocheir sinensis*. *Journal of Fishery Science of China* **6**, 61–65 (in Chinese with English abstract).

Sargent J., Henderson R.J. & Tocher D.R. (1989) The lipids. In: *Fish Nutrition* (ed. by J.E. Halver), pp. 154–209. Academic Press, New York, NY, USA.

Sheen S.S. & Wu S.W. (1999) The effects of dietary lipid levels on the growth response of juvenile mud crab *Scylla serrata*. *Aquaculture* **175**, 143–153.

Shiau S.Y. & Peng C.Y. (1992) Utilization of different carbohydrates at different dietary protein levels in grass prawn, *Penaeus monodon*, reared in seawater. *Aquaculture* **101**, 241–250.

Watanabe T. (1982) Lipid nutrition in fish. *Comparative Biochemistry* **73B**, 3–15.

Wei J.J., Yu N. & Tian W.J. (2014) Dietary vitamin B_{12} requirement and its effect on non-specific immunity and disease resistance in juvenile Chinese mitten crab *Eriocheir sinensis*. *Aquaculture* **434**, 179–183.

Xu W.N., Liu W.B., Shen M.F., Wang Y., Zhu J., Xu G. & Cheng L. (2011) Effect of different dietary protein and lipid level on growth performance, body composition and digestive enzymes activities of red swamp crayfish *Procambarus clarkii*. *Oceanologia et Limnologia Sinica* **42**, 521–529.

Xu W.N., Liu W.B., Shen M.F., Li G.F., Wang Y. & Zhang W.W. (2013) Effect of different dietary protein and lipid levels on growth performance, body composition of juvenile red swamp crayfish (*Procambarus clarkii*). *Aquaculture International* **21**, 687–697.

Xue M., Li A., Dong S. & Zhang X. (1997) Studies on the optimum DP/DE for different sizes of *Penaeus chinensis*. *Journal of Ocean University of Qingdao* **28**, 245–252.

Zhao J., Wen X., Li S., Zhu D. & Li Y. (2015) Effects of dietary lipid levels on growth, feed utilization, body composition and antioxidants of juvenile mud crab *Scylla paramamosain* (*Estampador*). *Aquaculture* **435**, 200–206.

Zhou H.X., Liu C.Q., Wang J.X. & Zhang Y.J. (2009) Effects of dietary fat on the energy budget and growth of *Eriocheir sinensis*. *Hebei Fishery* **9**, 1–3 (in Chinese with English abstract).

Zhu Q.H. & Qian G.Y. (2000) Study on optimal ratio of dietary protein to energy in the growing Chinese mitten crab, *Eriocheir sinensis*. *Journal of Zhengjiang Ocean University (Nature Science)* **19**, 134–138 (in Chinese with English abstract).

 © 2016 John Wiley & Sons Ltd,