



EFFECT OF DIETARY PROTEIN TO ENERGY RATIOS ON GROWTH, DIGESTIVE ENZYME ACTIVITY AND BODY COMPOSITION OF CAPTIVE JUVENILE SPINY LOBSTERS, *PANULIRUS ORNATUS* (FABRICIUS, 1798)

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Abstract

An evaluation of six dietary protein-to-energy (P/E) ratios was conducted to the early juvenile lobsters (*Panulirus ornatus*) by investigating the ratio effects on physiology, digestive enzyme activities, body composition, and amino acid profiles. Juvenile lobsters (0.25 ± 0.01 g) were individually kept in aquaria and treated with six isoenergetic formulated diets of P/E ratios of 21.8, 22.5, 23.5, 24.6, 25.3 and 26.6 mg KJ⁻¹. After 8 weeks of feeding experiment, the differentiation on dietary P/E ratios was found to have affected the specific growth rates, but not the survival, moult increment, moulting rate, moult interval and average daily moulting rate of juvenile lobsters. The dietary P/E ratio also influenced the protease, lipase, superoxidase dismutase activities, and body composition of juvenile lobster. The increase in dietary protein levels significantly affected the increase of the specific growth rate, while increased dietary lipid levels only affected the length increments. Both protein and lipid content were found in the whole body of the juveniles which reflected the treated dietary composition. Results indicated that a P/E ratio of 26.6 mg KJ⁻¹ (55% dietary protein and 9% dietary lipids) can yield the maximum growth and digestive enzyme activities from among the studied diets.

Key words: *Panulirus ornatus*, P/E ratio, protein, lipid

Tropical ornate spiny lobster, *Panulirus ornatus* is one of the commercially exploited spiny lobster species of the *Panulirus* genera and it spreads in the Indo-Pacific region (Holthuis, 1991). The commercial fisheries of the species reached 26.999 tons in 2022 (FAO, 2023). The demands for *P. ornatus* have followed an upward trend and, together with other spiny lobster species, *P. ornatus* has become a valuable commodity. Instead of relying on the wild-caught *P. ornatus*, some countries started to use the aquaculture-based ones by growing out the non-feeding stage of the spiny lobsters as an initial stock (Jones et al., 2019). This method is recognized as the most efficient one to grow out the spiny lobster. Success stories on this method implementation have been reported in Vietnam and Indonesia (Jones, 2015), with a respective total production of 2,271 tons and 700 tons in 2022 (FAO, 2023). Since the commercially formulated feed for spiny lobster is not yet available, bivalves are widely used as the natural diet for spiny lobster aquaculture, which leads to an inconsistent production due to inadequate dietary nutrition (Nankervis and Jones, 2022). Therefore, the development of formulated or complete feed for ornate spiny lobsters becomes vital and demands further research (Francis et al., 2014; Nankervis and Jones,

2022; Perera and Simon, 2014; Phillips and Matsuda, 2011).

The protein requirement to optimize the growth of *P. ornatus* has been researched with mixed results (Irvin and Shanks, 2015; Johnston et al., 2008; Smith et al., 2003, 2005). Some findings report that the increased growth of *P. ornatus* juvenile is strongly associated with the increase in dietary protein content, and the recommended high dietary protein for juvenile lobster is 530 g.kg⁻¹ and 610 g.kg⁻¹ (Smith et al., 2003, 2005). In contrast, Irvin and Shanks (2015) found that low protein levels in a formulated diet can result in a comparable growth response, indicating that high dietary protein is not the only factor to maximize the growth in juvenile lobsters. A sufficient amount of lipid as the main energy source in a formulated diet can play a crucial role in the growth of juvenile lobsters (Smith et al., 2003). Previous studies have reported that the optimum dietary lipid for spiny juvenile lobsters ranges between 10% and 16% (Johnston et al., 2003; Smith et al., 2003, 2005). In a recent study by Marchese et al. (2018), the formulated diet for spiny lobster contains 12.2 g/100 g⁻¹ lipid, which is higher than the established lipid requirements for most crustaceans (D'Abramo et al., 1984; Nankervis and Jones, 2022). In

contrast, low lipid portions (8–9%) in a formulated diet were found to be optimal for spiny lobster growth under cage culture (Irvin and Shanks, 2015). High lipids in a diet are expected to reduce the protein requirement or result in better growth compared to the low ones through protein sparing (Capuzzo and Lancaster, 1979; Wang et al., 2021 a, b). However, reports have indicated that excessive lipid can lead to fatty acid deposition in the tissues of shrimp (*Penaeus vannamei*), thus resulting in a reduced growth (Braga et al., 2023; Xu et al., 2018).

Protein has a significant role in the process of tissue synthesis, with the possibility of occurrence of the synthesis when energy needs are properly met only. Otherwise, protein will be utilized as an energy source (Cuzon and Guillaume, 1997; Wang et al., 2021 c). This makes investigations on the optimum P/E ratios of a formulated diet essential, whereas only a few studies have been conducted on the P/E ratio requirements for ornate spiny juvenile lobsters (Smith et al., 2003, 2005). The early nutritional study on *P. ornatus* juvenile indicated that a high dietary P/E ratio ranging between 28 and 29 mg KJ⁻¹ is the required ratio for maximum growth and survival (Smith et al., 2003, 2005). Similar P/E ratio was recommended for some crustaceans, including *Jasus edwardsii* (Simon and Jeffs, 2013), *Penaeus monodon* (Chuntapa et al., 1999), and *Litopenaeus setiferus* (Guzman et al., 2001). However, findings of *P. ornatus* studies have not reflected the protein and lipid combinations in the protein-energy sparing effect mechanism. Smith et al. (2003) reported that the high amount of lipid content in the formulated diet failed to reduce the protein requirement, and vice versa. In some other crustaceans, like Chinese mitten crab (*Eriocheir sinensis*), white leg shrimp (*Litopenaeus vannamei*), and oriental river prawn (*Macrobrachium nipponense*), the proper formulated dietary protein to energy ratio was proven to maximize the growth and immune performance (Cui et al., 2017; Wang et al., 2021 a; Zhang et al., 2017), while for ornate spiny juvenile lobsters, the optimum ratio remained unclear. In addition, such high P/E ratio requires high amount of diet protein, mostly sourced from fishmeal. Since feed takes up the most production cost in aquaculture, lowering the P/E ratio by reducing fishmeal may improve the efficiency of aquaculture. Therefore, the present experiment aimed to re-evaluate the effects of optimum protein in combination with six P/E ratios on the growth, survival, and digestive enzyme activities of *P. ornatus* juveniles.

Material and methods

Animals

A total of 200 heads of early-stage juvenile lobsters or puerulii (0.25±0.01 g) were purchased from a local supplier in the southern coast area of Banten Province, Indonesia, and transported by land to the Mariculture Facility Station of Jakarta Technical University of Fisheries. The puerulii were treated in fully equipped rectangular

fibreglass aquaria with corals, plastic net cuts, sufficient aeration, and a water filtration system for one week to let them grow into pigmented juveniles. During acclimation, the feeding was intermittently stopped, and a visual observation was conducted to check their general health condition. Juvenile lobsters that were unhealthy or less attractive or losing body appendages were excluded.

Experiment system and design

A total of 180 heads of ornate spiny juvenile lobsters were selected and distributed to 18 rectangular, 60 L acrylic fish tanks (60 cm × 29.5 cm × 35 cm) with densities of 10 heads in each tank. All tanks were equipped with an individual water filtration and an aeration system. The ornate spiny juvenile lobsters were individually caged in a 1.5 L plastic basket with sufficient holes. Net cut (4 × 4 cm) and pipe cut (Ø 0.5", 4 cm long) were provided in each basket. The ornate spiny juvenile lobsters were cultured under the experimental laboratory condition which was maintained at the natural photoperiod (12-h light/12-h dark) for 8 weeks. There were six experimental diet treatments and triplication. The feeding rate was maintained at approximately 15% of wet body weight and the diets were given as moist diets using stainless steel pincers three times per day until satiation. The uneaten diet was removed after 1 h of feeding. During the feeding trial, the water temperature was 29.4±0.03°C and the salinity was 30.9±0.18 ppt, the pH level was 8.4±0.11, and the dissolved oxygen was 5.08±0.26 mg L⁻¹.

Experimental diet

The composition and proximate analysis of the tested diets in this study are presented in Table 1. A total of six experimental diets (F1 to F6) with various protein contents (45% to 55%), lipid levels (7% to 13%) and P/E ratios from 21.8 to 26.6 mg KJ⁻¹ were formulated. The experimental diets used fishmeal as the main protein source, and fish oil as the lipid source. The fishmeal was originated from bycatch fishes (*Eleotris fusca* and *Nemipterus virgatus*) with minimum protein of 56%. The other ingredients are the common ingredients in fish feed manufacturers (Fish Nutrition Department, IPB University, Bogor, Indonesia), namely sodium caseinate (Friesland Campina, The Netherlands), and soy lecithin concentrate (PT. Cheil Jedang, Indonesia). All feed ingredients, except fish oil and soy lecithin, were mixed and sieved manually using a 1.0 mm stainless steel sieve (Retch Test Sieve, Germany), then combined thoroughly with fish oil and soy lecithin using a milling machine (Kawano 250G, Korea). The fine-formulated mixture was then stored in a sealed plastic bag and kept in a dry place until used. The amino acid profiles from the formulated diets were analysed, and the result is presented in Table 2.

Proximate and biochemical analysis

The crude protein content was determined using the Kjeldahl method by calculating the nitrogen percentage × 6.25 (AOAC, 2005). Furnace heating was applied to

calculate the moisture content ($110^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 2 hours) and the ash content (600°C for 4 hours) of 1–2 g of samples. The percentage of ash was calculated by weighing the remaining samples after heating. The crude fibre was calculated as the sample residue using H_2SO_4 1.25% and NaOH 1.25% solution. The total lipid content was extracted through the mixing of chloroform and methanol (2:1), then the mixing solution was filtrated and evaporated using a vacuum as described in the established protocol (AOAC, 2005). The amino acid composition in the experimental diets and whole-body lobster was measured following AOAC (2006). Digestive amylase enzyme activity was determined by Bergmeyer's method (Mulyasari et al., 2018) using soluble starch in citrate buffer (pH 5.7), vortexed and incubated at 32°C for 30 min before heating at 100°C for 10 min. The amylase enzyme activity was calculated on the absorbance basis at 578 nm. For protease activity analysis, samples were mixed with boric acid buffer (0.01 M, pH 8.0), casein substrate (20 mg mL^{-1} pH 8.0), HCl (0.05 mg mL^{-1}), and standard tyrosine (5 mmol L^{-1}), then subsequently incubated in a water bath

at 37°C for 10 min. Following the process, 0.1 M TCA was added to each sample and stored at 37°C for 10 min continued with centrifugation at 3,500 rpm for 10 min. The mixture was then stored at 37°C for 20 min before the measurement of absorbance at 578 nm. Meanwhile, the lipase activity was observed following an established protocol (Tietz and Fiereck, 1966).

The superoxidase dismutase (SOD) analysis was performed according to Wang et al. (2018) by using the SOD test kit (Abcan, UK). Tissue from each sample (0.3 g) was diluted into 10 mL sulphuric acid 10 M, then centrifuged. The supernatant was transferred into a new sterile tube and mixed with 20 μL working solution (20 μL SOD assay buffer and 50 μL SOD enzyme solution in 100 mL cool PBS solution), following 20 min of incubation. The reaction in the incubation process was stopped by incorporating 50 μL of butylated hydroxytoluene, followed by an absorbance measurement at 550 μm . The SOD was calculated by an equation as follows:

$$\text{SOD} = \text{absorbance} \times 70.16 / \text{total protein}$$

Table 1. Formulated diet ingredients (dry weight basis in g) and proximate composition

Ingredients	Dietary treatments (Protein/lipid)					
	F1 (50/9)	F2 (50/13)	F3 (50/7)	F4 (55/9)	F5 (45/9)	F6 (45/13)
Fishmeal	578	582	540	648	530	530
Astaxanthin (8%)	2	2	2	2	2	2
Binder (inert)	22	22	22	22	22	22
Casein	170	173	191	191	135	147
Wheat (10 CP)	154	107	191	70	233	185
Corn/wheat starch	25	25	25	25	25	25
Cholesterol	4	4	4	4	4	4
Fish oil	20	60	0	13	24	60
Vitamin premix ¹	10	10	10	10	10	10
Vitamin C	5	5	5	5	5	5
Soy lecithin	5	5	5	5	5	5
Trace mineral premix ²	5	5	5	5	5	5
Total	1000	1000	1000	1000	1000	1000
DM (%)	91.33	91.79	91.06	91.5	91.15	91.58
Ash (%)	7.94	7.97	7.6	8.8	7.3	7.31
GE (MJ/kg)	20.4	21.35	19.86	20.73	20.04	20.93
CP (%)	50.22	50.24	50.19	55.28	45.18	45.74
Lipid (%)	9.87	13.84	7.48	9.87	9.82	13.34
Fibre (%)	0.61	0.58	0.65	0.59	0.62	0.59
*P/E ratio (mg KJ^{-1})	24.62	23.53	25.28	26.67	22.54	21.85

*P/E ratio= protein/gross energy

¹Vitamin mix (mg/kg ingredient): retinol, 0.9 mg; ascorbic acid, 200 mg; cholecalciferol, 0.2 mg; menadione, 10 mg; α -tocopherol, 100 mg; choline, 1000 mg; inositol, 100 mg; thiamine/ B_1 , 15 mg; riboflavin/ B_2 , 20 mg; pyridoxine/ B_6 , 15 mg; d-pantothenic acid/ B_5 , 50 mg; nicotinic acid, 75 mg; biotin, 0.5 mg; cyanocobalamin/ B_{12} , 0.05 mg, and folic acid, 5 mg.

²Mineral mix (mg/kg ingredient): $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.5 mg; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 5 mg; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 50 mg; KI, 4 mg; $\text{CrCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 mg; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 150 mg; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 25; NaSeO_3 , 0.1 mg; and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 100 mg.

Table 2. Amino acid analysis profile of tested diets (%)

Amino acids	Dietary treatments (Protein/lipid)					
	F1 (50/9)	F2 (50/13)	F3 (50/7)	F4 (55/9)	F5 (45/9)	F6 (45/13)
Non-essential amino acids						
Alanine	1.66	1.57	1.57	1.67	1.50	1.47
Aspartate	1.81	1.76	1.76	1.79	1.68	1.71
Glutamine	3.47	3.67	3.21	3.51	3.18	3.24
Serine	0.69	0.72	0.65	0.71	0.70	0.67
Glycine	1.42	1.35	1.34	1.38	1.37	1.39
Tyrosine	0.79	0.69	0.78	0.69	0.79	0.86
Proline	0.50	0.53	0.47	0.56	0.49	0.51
Total	10.37	10.31	9.80	10.3	9.73	9.89
Essential amino acids						
Histidine	0.68	0.79	0.67	0.79	0.70	0.68
Arginine	0.57	0.51	0.57	0.58	0.59	0.60
Threonine	1.12	1.15	0.98	1.12	1.14	1.01
Valine	1.42	1.32	1.27	1.27	1.24	1.17
Methionine	0.45	0.50	0.48	0.56	0.47	0.50
Cysteine	0.39	0.39	0.49	0.46	0.46	0.49
Isoleucine	0.50	0.50	0.59	0.61	0.61	0.65
Leucine	0.89	0.89	0.89	0.96	0.98	0.95
Phenylalanine	0.58	0.57	0.62	0.60	0.57	0.54
Lysine	0.88	0.84	0.81	0.89	0.86	0.82
Total	7.52	7.50	7.41	7.88	7.66	7.45

Table caption (P/E ratio): F1 (24.6 mg KJ⁻¹), F2 (23.5 mg KJ⁻¹), F3 (25.3 mg K⁻¹), F4 (26.6 mg KJ⁻¹), F5 (22.5 mg KJ⁻¹) and F6 (21.8 mg KJ⁻¹).

Data collection

At the end of the feeding trial, the survived juvenile lobsters were collected and counted. The excess water from their body was removed by using a paper towel before the weighing process. Total length was measured by using a calibrated digital vernier calliper (Insize Digital Calliper 1108-200, China) from the base of the uropod to the end of the rostrum.

The surviving number of juvenile lobsters and the weight of each juvenile lobster in each tank were recorded to count the survival rate (SR), specific growth rate (SGR), total length increment (TLi), moult interval (MI), moulting rate (MR) and average daily moulting (ADM), by using the following:

- (1) SR (%) = (Final number of survived juvenile lobsters)/(Initial number of juvenile lobsters) × 100,
- (2) SGR (% BW.day⁻¹) = 100 × [ln final weight (g)–ln initial weight (g)]/days × 100,
- (3) TLi (%) = (Final total length – initial total length)/(initial total length) × 100,
- (4) MI (days) = duration between two consecutive moults,
- (5) MR (%) = (Number of moulted juvenile lobsters)/(Number of total juvenile lobsters) × 100,
- (6) ADM (Number of moults lobster⁻¹ day⁻¹).

$$ADM = \frac{X_{R1} + X_{R2} + \dots + X_{RN}}{R_N}$$

$$X_R = \frac{\left(\frac{N_R}{S_R}\right)_{Day1} + \left(\frac{N_R}{S_R}\right)_{Day2} + \dots + \left(\frac{N_R}{S_R}\right)_{Day(t)}}{t}$$

Where: X_r is the average number of juvenile lobster moults, S_r is the number of moults in replicate, N_r is the number of animals in replicate, t is the number of replicates, and is the duration of the feeding trial.

Data analysis

All data were analysed using SPSS for Windows version 25.0 (IBM, New York, USA). The effects of the P/E ratio on all test parameters of the juvenile lobster were analysed by using a one-way ANOVA analysis, and the significant differences at $P \leq 0.05$ were located using the post hoc Tukey's HSD multiple comparison tests. A quadratic regression analysis was used to analyse the trend of the results.

Results

Growth performance

The physiological responses of juvenile lobsters fed with different dietary P/E ratios are presented in Table 3. Different dietary P/E ratio treatments significantly affected the specific growth rate but did not affect the survival rate, length increment, moulting rate, moult interval, and average daily moulting rate. Lobsters offered with a dietary P/E ratio of 26.6 mg KJ⁻¹ containing 55% protein and 9% lipid experienced the highest growth rate ($P \leq 0.05$). Lobsters offered with dietary P/E ratio of 23.5 mg KJ⁻¹ containing 50% protein and 13% lipid showed the highest survival rate and the shortest moult interval, although not significantly different from other treatments. The length increment ranged from 1.69% to 13.20% with no significant differences among the treatments ($P \geq 0.05$). The increase of protein content from 45% (P/E ratio of 22.5 mg KJ⁻¹) to 55% (P/E ratio of 26.6 mg KJ⁻¹) resulted in a significant growth (Table 3), but the growth was not affected by the increase of lipid level in the same dietary

protein level (P/E ratio of 23.5, 24.6, and 25.2 mg KJ⁻¹). Polynomial regression analysis indicated a relationship between P/E ratio and specific growth rate as $Y = 0.0375x^2 - 1.7347x + 20.439$ (Y = specific growth rate; x = P/E ratio). The minimum P/E ratio for the specific growth rate of juvenile lobster was 23.12 mg KJ⁻¹, with $R^2 = 0.8036$ (Figure 1).

Digestive enzymes

The results on the digestive enzyme activity and superoxidase dismutase are presented in Table 4. The protease, lipase enzyme activities, and superoxidase dismutase of juvenile lobsters were significantly affected by the dietary P/E ratio in feed with a high P/E ratio ($P \leq 0.05$). Lobsters receiving dietary P/E ratio of 26.6 mg KJ⁻¹ had the highest protease and lipase activities ($P \leq 0.05$). The highest value of SOD was obtained by lobsters fed with dietary P/E ratio of 21.8 mg KJ⁻¹. The amylase activity ranged from 1.43 to 2.52 U/mg protein⁻¹ with no significant differences among the P/E ratio treatments.

Table 3. The physiological response of juvenile lobsters fed with different dietary P/E ratio and interaction between dietary protein and dietary lipid inclusion level, specific growth rate, survival rate, length increment, moulting rate and moult interval. Initial weight (W_0) = 0.25 ± 0.01 g and initial total length (mm) = 22.93 ± 0.29

Dietary treatments (Protein/lipid)	SGR (% g.day ⁻¹)	Survival rate (%)	Length increment (%)	Moulting rate (%)	Moult interval (days)	ADM rate (%.day ⁻¹)
F1 (50/9)	0.39 ± 0.06 b	58.33 ± 11.0	7.56 ± 1.34	62.50 ± 19.09	20.00 ± 1.0	0.01 ± 0.00
F2 (50/13)	0.47 ± 0.07 ab	70.83 ± 11.0	5.95 ± 1.64	45.83 ± 8.33	14.50 ± 7.8	0.02 ± 0.00
F3 (50/7)	0.54 ± 0.08 ab	58.33 ± 8.33	12.13 ± 2.79	83.33 ± 11.02	23.80 ± 6.1	0.03 ± 0.03
F4 (55/9)	0.69 ± 0.05 a	58.33 ± 11.0	13.20 ± 3.84	83.33 ± 11.02	25.20 ± 5.3	0.03 ± 0.01
F5 (45/9)	0.36 ± 0.10 b	54.17 ± 11.0	1.69 ± 2.89	83.33 ± 16.67	28.33 ± 10.5	0.03 ± 0.01
F6 (45/13)	0.58 ± 0.04 ab	58.33 ± 4.43	7.49 ± 1.95	62.50 ± 14.43	18.33 ± 3.5	0.02 ± 0.00

Values are presented as means $\pm$ SE from three replicates. Data in the similar column with different letters (a, b) indicated a significant difference in means value at $P \leq 0.05$. Table caption (P/E ratio): F1 (24.6 mg KJ⁻¹), F2 (23.5 mg KJ⁻¹), F3 (25.3 mg KJ⁻¹), F4 (26.6 mg KJ⁻¹), F5 (22.5 mg KJ⁻¹) and F6 (21.8 mg KJ⁻¹).

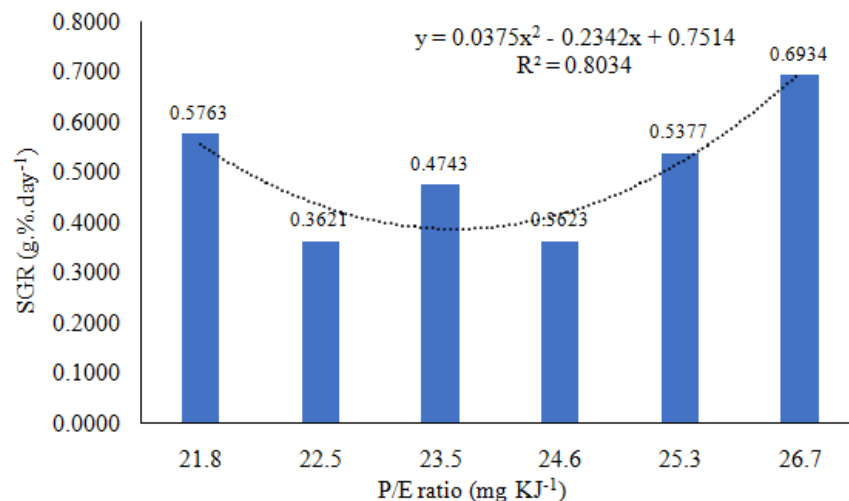


Figure 1. Polynomial regression of specific growth rate of *P. ornatus* juvenile against different P/E ratios in an 8-week feeding trial

Table 4. Digestive enzyme activity and superoxidase dismutase of juvenile lobsters fed the experimental diets. Initial weight (W_0) = 0.25±0.01 g and initial total length (mm) = 22.93±0.29

Dietary treatments (Protein/lipid)	Protease (U ml ⁻¹)	Lipase (m.U L ⁻¹)	Amylase (U mg protein ⁻¹)	SOD (U ml ⁻¹)
F1 (50/9)	0.19±1.4 b	11.63±1.16 b	1.78±0.06	8.59±0.32 b
F2 (50/13)	0.18±0.6 a	22.09±1.16 c	1.43±0.02	10.29±0.12 c
F3 (50/7)	0.19±1.7 bc	5.81±0.58 a	2.02±0.08	6.08±0.19 a
F4 (55/9)	0.21±0.3 d	28.48±0.58 d	1.51±0.02	7.39±0.51 ab
F5 (45/9)	0.19±0.4 ab	18.60±1.16 c	1.81±0.03	7.89±0.20 b
F6 (45/13)	0.19±0.1 c	4.65±1.16 a	2.51±0.38	10.89±0.17 c

Values are presented as means ± SE from three replicates. Data in the similar column with different letters (a, b) indicated a significant difference in means value at $P \leq 0.05$. SOD: superoxidase dismutase. Table caption (P/E ratio): F1 (24.6 mg KJ⁻¹), F2 (23.5 mg KJ⁻¹), F3 (25.3 mg KJ⁻¹), F4 (26.6 mg KJ⁻¹), F5 (22.5 mg KJ⁻¹) and F6 (21.8 mg KJ⁻¹).

Table 5. The whole-body proximate composition of early juvenile lobsters fed with experimental diets. Initial weight (W_0) = 0.25±0.01 g and initial total length (mm) = 22.93±0.29

Dietary treatments (Protein/lipid)	Proximate composition (%)				
	Protein	Lipid	Ash	Crude fibre	NFE
F1 (50/9)	20.99±1.19 a	6.07±0.12 cd	58.39±0.67 ab	6.24±0.28 b	9.74±0.77 c
F2 (50/13)	30.27±0.38 b	7.19±0.24 ef	62.39±1.45 bc	4.89±0.12 ab	1.17±0.18 a
F3 (50/7)	23.05±0.15 a	5.76±0.19 bc	56.95±0.25 a	4.81±0.27 ab	10.47±0.88 c
F4 (55/9)	33.87±0.21 c	4.83±0.29 ab	63.34±0.47 c	4.27±0.04 ab	6.49±0.24 b
F5 (45/9)	19.90±0.79 a	3.99±0.12 a	58.59±0.62 ab	4.65±0.29 ab	10.11±0.35 c
F6 (45/13)	28.29±0.13 b	7.05±0.13 de	56.60±0.21 a	3.07±0.31 a	1.72±0.11 a

Values are presented as means ± SE from three replicates. Data in the similar column with different letters (a, b) indicated a significant difference in means value at $P \leq 0.05$. Table caption (P/E ratio): F1 (24.6 mg KJ⁻¹), F2 (23.5 mg KJ⁻¹), F3 (25.3 mg KJ⁻¹), F4 (26.6 mg KJ⁻¹), F5 (22.5 mg KJ⁻¹) and F6 (21.8 mg KJ⁻¹).

Table 6. Amino acid analysis profile of whole-body juvenile lobster (%). Initial weight (W_0) = 0.25±0.01 g and initial total length (mm) = 22.93±0.29

Amino acids	Dietary treatments (Protein/lipid)					
	F1 (50/9)	F2 (50/13)	F3 (50/7)	F4 (55/9)	F5 (45/9)	F6 (45/13)
Non-essential amino acids						
Alanine	1.51	1.49	1.42	1.57	1.36	1.43
Aspartate	1.62	1.69	1.57	1.76	1.59	1.65
Glutamine	3.01	3.19	2.96	3.31	2.89	2.98
Serine	0.64	0.67	0.59	0.65	0.62	0.57
Glycine	1.19	1.28	1.21	1.38	1.17	1.20
Tyrosine	0.57	0.63	0.65	0.69	0.61	0.63
Proline	0.43	0.45	0.41	0.42	0.39	0.40
Total	9.00	9.43	8.82	9.80	8.65	8.90
Essential amino acids						
Histidine	0.60	0.67	0.58	0.63	0.59	0.55
Arginine	0.47	0.46	0.47	0.50	0.47	0.43
Threonine	0.91	0.94	0.89	0.98	0.93	0.92
Valine	1.12	1.16	1.07	1.11	1.04	0.98
Methionine	0.40	0.41	0.36	0.39	0.36	0.38
Cysteine	0.32	0.31	0.34	0.32	0.31	0.31
Isoleucine	0.47	0.42	0.45	0.41	0.42	0.46
Leucine	0.79	0.79	0.80	0.82	0.812	0.78
Phenylalanine	0.46	0.45	0.43	0.48	0.46	0.42
Lysine	0.73	0.75	0.72	0.78	0.76	0.71
Total	6.30	6.39	6.15	6.46	6.18	5.97

Table caption (P/E ratio): F1 (24.6 mg KJ⁻¹), F2 (23.5 mg KJ⁻¹), F3 (25.3 mg KJ⁻¹), F4 (26.6 mg KJ⁻¹), F5 (22.5 mg KJ⁻¹) and F6 (21.8 mg KJ⁻¹).

Tissue proximate composition

Different P/E ratio dietary treatments significantly affected the crude protein, lipid, ash, crude fibre and NFE composition in juvenile lobster tissue. The body composition of lobsters offered with dietary P/E ratio of 26.6 mg KJ⁻¹ containing 55% protein showed the highest protein content ($P \leq 0.05$). The highest lipid content was obtained by lobsters fed with dietary P/E ratio of 23.5 mg KJ⁻¹ containing 13% of lipid. The lowest ash, crude fibre, and NFE were observed in the lobster receiving dietary P/E ratio of 21.8 mg KJ⁻¹. While it was not conclusive that the body composition trends increased with the dietary P/E ratio, this study indicated a general trend that the higher the protein or lipid content in the diets, the higher their contents in the lobster body composition (Table 5).

Amino acids

The present study revealed that different dietary P/E ratios containing different levels of protein and lipid affected the amino acids in the lobster's body composition. The results indicated a general trend in the amino acids composition, where high amino acids content in the diets translated into higher amino acids in the lobster's whole body (Table 6). More specifically, the non-essential amino acids (NEAA) were higher than the essential amino acids (EAA) in both experimental diets and the whole body of juvenile lobsters. In comparison, a higher protein content in lobster's body was observed in lobsters receiving dietary P/E ratio of 26.6 mg KJ⁻¹ containing 55% protein than those with 21.8 mg KJ⁻¹ P/E ratio containing 45% of protein (16.3 % vs 14.87%, respectively).

Discussion

In this study, the dietary P/E ratio influenced the specific growth rate. The dietary P/E ratio of 26.6 mg KJ⁻¹ of the experimental diet F4 produces the maximum growth at 0.69 g day⁻¹ of juvenile lobsters. This growth rate is low compared to that of Smith et al. (2003) who reported a growth rate of 1.1 g day⁻¹ when *P. ornatus* (1.7 g) were fed a formulated feed with a P/E ratio of 27.3 mg KJ⁻¹. Additionally, Smith et al. (2005) reported that an optimum growth of 1.18% day⁻¹ can be achieved whenever *P. ornatus* are fed with a P/E ratio of 26.2 mg KJ⁻¹ at a protein level of 540 g kg⁻¹ formulated diet. Several studies indicate that the optimum P/E ratio for juvenile lobster ranges from 27.9 to 30.39 mg KJ⁻¹ (Irvin and Williams, 2009 b; Johnston et al., 2008; Marchese et al., 2018; Smith et al., 2003, 2005; Ward et al., 2003). On the contrary, several other studies on *P. ornatus* showed that the P/E ratio could be lower than 27.9–30.39 mg KJ⁻¹ without compromising any growth rates (Rathinam et al., 2009; Simon and Jeffs, 2013). In this study, the stress level of juvenile lobsters might increase following the individual rearing system which ultimately led to a suboptimal growth. This result is in agreement with a previous report on different growth response of lobsters under solitary

or separated system versus communal system (Kropielnicka-Kruk et al., 2019).

An analysis using a quadratic equation calculation is commonly used to predict the trend of the P/E ratio against the growth rate. In this study, the increase of the P/E ratio in the experimental diet did not show a linear increase in the growth rate of juvenile lobsters. The result showed that juvenile lobsters have the lowest extreme point of growth rate when fed dietary F2 at a P/E ratio of 23.12 mg KJ⁻¹ as shown in the negative value which points out towards the lowest value of the data set. Smith et al. (2003), using a similar method of statistical analysis, reported that the increase in P/E ratio did not always improve the growth rate. The growth rate of the juvenile lobsters peaked at the P/E ratio of 29.1 mg KJ⁻¹ and decreased beyond this level.

Different levels of dietary protein formulated in this study (45%, 50%, and 55%) have improved the specific growth rate and the length increments. A previous study reported that the growth of *P. ornatus* juvenile increased with the quantity of protein in the diet (Smith et al., 2005). As a carnivorous species, lobster requires a sizeable amount of protein, and therefore, low dietary protein content will affect its growth (Marchese et al., 2018; Smith et al., 2003; Smith et al., 2005; Wang et al., 2019). Juvenile lobsters fed on dietary F4 with P/E ratio of 26.6 mg KJ⁻¹ containing 55% protein and 9% lipid had the most significant growth rate within this study, thus demonstrating that F4 was suitable for growth and survival of juvenile lobsters, which aligned with Smith et al. (2003). Results have shown that compared to increased lipid, increased protein in the tested diets was more effective in improving lobster growth. The dietary treatments with high vs. low lipid in the diet containing 50% protein resulted in similar growth rate. In contrast, at similar lipid level (9%), the growth of juvenile lobster was improved when the protein level increased. In addition, the dietary lipids improved the growth only when the protein level was sufficient. It demonstrates that sufficient protein within the diets is important because, according to Mente (2010), protein plays a critical role in providing amino acids for the growth of decapod crustaceans. Although only little attention has been addressed to lipid requirements for lobster (Nankervis and Jones, 2022), some researches have shown that the lipid requirement for lobster ranges between 8% and 16 % of the diets (Irvin and Shanks, 2015; Johnston et al., 2008; Smith et al., 2003, 2005). In the present study, the optimum growth was achieved at the dietary P/E ratio of 26.6 mg KJ⁻¹ with 9% lipid content, thus within the accepted range (Irvin and Shanks, 2015). At some point, excessive lipid can have a detrimental effect on the growth of crustaceans (Chuntapa et al., 1999; Méndez-Martínez et al., 2018; Zhang et al., 2017).

The energy-sparing effect was shown on juvenile lobsters fed with formulated diet and assigned low protein (45% protein, 9% lipid) and low protein–high oil (45% protein, 13% lipid) experimental diets. The energy-spar-

ing effect is a condition where lipids as an energy source can replace protein functions that are not available in sufficient quantities for growth. Therefore, a certain amount of lipids in the feed will have a positive growth effect despite the low protein (Cui et al., 2017; Wang et al., 2021a). In those experimental diets, the increased lipid levels in the diets have promoted the growth rate of juvenile lobsters. This energy-sparing effect mechanism has also been reported in other decapods (Méndez-Martínez et al., 2018; Zhang et al., 2017).

The survival of juvenile lobsters in this study remained constant, which confirmed Smith et al. (2003), but higher than that reported by Haryanti et al. (2017). The individual rearing system used in this study successfully protected the juvenile lobsters from cannibalism and simplified the assessment of moult rates, moult intervals, and ADM of the juvenile lobsters. Most of the juvenile lobsters died in their moulting stage due to failure to complete the moulting process which might have been affected by insufficient dietary nutrients warranted in the ecdysis process (Irvin and Williams, 2009a; Smith et al., 2005).

Significant variations of moult frequencies were observed on the juvenile lobsters which were able to moult two to four times during the feeding trial, but not on others which died immediately after their first moult. The moult frequency which is reflected by the average daily moulting rate of juvenile lobster in this study was, as predicted, lower than that of Kropielnicka-Kruk et al. (2019), and it occurred due to the culture system where juvenile lobsters were housed individually. The average daily moult was higher on juvenile lobsters in the communal rearing system fed with multi-diet sources compared to those in the individual system (Kropielnicka-Kruk et al., 2019). In this study, the moult frequencies, intervals, and increments were not affected by the dietary P/E ratio of experimental diets. The increase of dietary lipids in the experimental diets was found to shorten the moult interval. This study had difficulty in directly associating both moulting rates and moult intervals with the dietary P/E ratios due to the value of both parameters which indicated non-significant statistical difference. However, juvenile lobsters fed with 13% dietary lipid showed a shorter moult interval than the ones fed with less than 13% dietary lipid. Phospholipids and sterols are part of the lipids and have vital roles during the moulting process of crustaceans (Teshima, 1998). An increase in dietary lipid levels may translate into a higher availability of phospholipids and sterols which lead to a shorter interval in the moulting process.

In this study, the protease and lipase activities as part of digestive enzymes in juvenile lobsters were affected by the dietary P/E ratio and significantly influenced by the levels of dietary protein and dietary lipids. These findings confirm other studies on crustacean species (Cui et al., 2017; Méndez-Martínez et al., 2018; Sudtongkong et al., 2020). Digestive enzyme activities play some important roles in the process of food digestion and signifi-

cantly impact moulting and growth (Méndez-Martínez et al., 2018; Rodriguez-Viera et al., 2016, 2017). Juvenile lobsters fed on a diet containing P/E ratio of 26.6 mg KJ⁻¹ were able to produce the highest protease and lipase, thus reflecting a faster capability to process protein and lipid which leads to an improved growth rate. This result showed that dietary P/E ratio had positive effects on protease and lipase activities which led to better growth rate and total length increment of *P. ornatus* juvenile. The positive correlation between the increase of protein content and the protease activity was reported in Chinese mitten crab and did not affect the growth performance (Cui et al., 2017). The optimum nutrient absorption was influenced by a higher availability of digestive enzymes which led to a higher growth rate.

Studies conducted in 2018 stated that the increase in the dietary protein level improved the amylase activity in crustaceans (Hamidoghli et al., 2018; Méndez-Martínez et al., 2018). The present study has discovered that the amylase enzyme activity of juvenile lobsters is not significantly affected by changes in dietary protein between 45% and 55%. The high amylase enzyme activity was observed in juvenile lobsters fed with low dietary protein and low P/E ratio. Similar findings on amylase activity were also reported by a study in *Episesarma singaporense* (Sudtongkong et al., 2020). In the present study, the increase of dietary protein and lipid in the diets leads to a higher availability of both nutrients in the juvenile lobster body, which aligns with Cui et al. (2017). Proximate analysis of amino acids also reflected the amino acid retention in the experimental diets and juvenile lobster whole-body. In addition, the total amino acids of the experimental diet were linear with the dietary protein in the diet. The increased dietary protein in the experimental diets resulted in an increased amino acid level of the experimental diet. These findings illustrate the importance of a proper dietary protein in diet formulation to achieve a suitable feed. A similar result was reported by Moreno-Arias et al. (2018) that the higher protein content in the formulated diet for shrimp, the higher the amino acid content in the diets. Apart from nutrition retention, it was shown that the total quantity of non-essential amino acids was higher than essential amino acids in experimental diets as well as the juvenile lobster whole-body, which is in agreement with studies on *E. singaporense* crabs (Sudtongkong et al., 2020) and *L. vannamei* (Moreno-Arias et al., 2018). Although no conclusions can be drawn regarding the relationship between amino acids and juvenile lobster growth in this study, the amino acid profile of the diets in the feeding trial can be used as a reference for further research on lobster nutrition, particularly on their amino acids requirements.

Conclusion

The present study has demonstrated that the increased dietary P/E ratio improves the growth performance and digestive enzyme activities of juvenile lobsters. Based on the growth rate, total length increment, and protease and

lipase enzyme activities, the dietary P/E ratio of 26.6 mg KJ⁻¹ (55% protein and 9% lipid) is optimum for *P. ornatus* juvenile. Further research is required to determine the possibility of using alternatives to fishmeal protein sources for juvenile lobsters.

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