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Growth Response of Lobster *Panulirus homarus* Reared in Tanks and in Floating Net Cages Fed with Compound and Fresh Feed

(Tindak Balas Pertumbuhan Udang Galah *Panulirus homarus* Diternak dalam Tangki dan Sangkar Jaring Terapung Diberi Makanan Sebatian dan Segar)

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ABSTRACT

Feeding efficiency is an important factor that supports the success of lobster grow-out culture. This study aimed to investigate growth of different sex of lobsters after being fed with combination of compound and fresh feed in indoor tanks (experiment-1) and growth of male lobster fed with different co-feeding diets in net floating cages (experiment-2). In experiment-1, five replicates of ten lobsters (191.1 ± 18.1 g) for each different sex (i.e., all males, males and females, and all females) were reared in 2 × 2 × 1 m^3 tanks with flow through water system and fed with combination of compound (75%) and fresh feed (25%) for 120 days. In experiment-2, four replicates of 20 all male lobsters (183.6 ± 17.6 g) were reared in 12 net cages of 2 × 2 × 2.5 m^3 and were fed with compound feed only, compound feed (75%) and fresh feed (25%), and compound feed (75%) with mussel meat (25%) for 120 days. Experiment-1 showed that growth (quantified as weight gain) of all male lobsters was the highest (59.4 ± 10.0 g). Experiment-2 showed that compound feed combined with fresh feed resulted in higher growth of lobster (0.73 ± 0.17 g) than 100% compound feed. There was no difference in survival rate of lobster among the treatment in both experiments with 94 – 100% in experiment-1 and 75.2 to 85.0% in experiment-2. The mortality of lobster caused by Milky Hemolymph Disease (MHD) in floating cage was found higher than in indoor tanks.

Keywords: Compound feed; fresh feed; growth; *Panulirus homarus*

ABSTRAK

Kecekapan memberi makan adalah faktor penting yang menyokong kejayaan pembiakan kultur udang galah. Penyelidikan ini bertujuan untuk mengkaji tumbesaran udang galah berbeza jantina selepas diberi makan dengan gabungan makanan sebatian dan segar dalam tangki dalaman (uji kaji-1) dan tumbesaran udang galah jantan yang diberi makan dengan diet makanan berbeza dalam sangkar jaring terapung (uji kaji-2). Dalam uji kaji-1, lima replikasi sepuluh udang galah (191.1 ± 18.1 g) untuk setiap jantina berbeza (semua jantan, jantan dan betina serta semua betina) telah diternak dalam tangki 2 × 2 × 1 m^3 dengan aliran melalui sistem air dan diberi makan dengan gabungan sebatian (75%) dan makanan segar (25%) untuk 120 hari. Dalam uji kaji-2, empat replikasi 20 udang galah semua jantan (183.6 ± 17.6 g) diternak dalam 12 sangkar jaring 2 × 2 × 2.5 m^3 dan diberi makan dengan makanan sebatian sahaja, makanan sebatian (75%) dan makanan segar (25%) dan makanan sebatian (75%) dengan daging kerang (25%) untuk 120 hari. Uji kaji-1 menunjukkan bahawa tumbesaran (dihitung sebagai penambahan berat badan) udang galah semua jantan adalah yang tertinggi (59.4 ± 10.0 g). Uji kaji-2 menunjukkan bahawa makanan sebatian digabungkan dengan makanan segar menghasilkan tumbesaran udang galah yang lebih tinggi (0.73 ± 0.17 g) daripada makanan sebatian 100%. Tiada perbezaan dalam kadar kemandirian udang galah antara rawatan dalam kedua-dua uji kaji dengan 94 - 100% dalam uji kaji-1 dan 75.2 hingga 85.0% dalam uji kaji-2. Kematian udang galah disebabkan oleh *Milky Hemolymph Disease* (MHD) dalam sangkar terapung didapati lebih tinggi daripada dalam tangki dalaman.

Kata kunci: Makanan sebatian; makanan segar; *Panulirus homarus*; pertumbuhan

Introduction

Spiny lobster is one of the most valuable marine products which were produced mainly from capture fisheries. Strong market demand had been reported primarily on live lobsters in Asia particularly derived from China and Taiwan (Priyambodo & Jones 2015), and this market demand for spiny lobsters continues to grow. Global production of spiny lobster in 2014 was 306 thousand tons which valued at more than US $2.7 billion (FAO 2016). On the other hand, lobster production from the fishery tends to be static. Increasing lobster production from aquaculture is the only way to meet that market demand. However, for the development of lobster farming, there are several obstacles that must be considered e.g., the availability of feed and disease attacks. The main disease in lobster farming that can cause 70-100% mortality has been reported to be triggered by milky haemolymph diseases (MHD) (Jones 2015; Koesharyani, Lasmika & Sugama 2021; Sudewi et al. 2020). Therefore, one of the mitigation techniques to minimize this disease is by providing good quality of feed and maintaining good water quality.

Indonesia has a significant number of lobster seeds from the wild-caught which can be utilized for grow-out culture. Annual lobster seed caught in Lombok area reached 3 million in 2013 (Bahrawi, Priyambodo & Jones 2015) which significantly supports the lobster grow-out industry. However, grow-out technology for lobster in Indonesia is not yet developed well, although some fish farmers have tried to culture lobster since 2004 in addition to seaweed and fish culture (Jones 2018). The success of Vietnam in developing lobster grow-out industry could be a model to develop lobster culture in Indonesia (Petersen, Jones & Priyambodo 2015). Lobster grow-out in Vietnam mainly uses trash fish, a combination of low-value fish, mollusks, and crustaceans as feed, with feed conversion ratios reaching 20, even higher (Hoang et al. 2009). Trash fish could be a problem for the sustainability of lobster culture, most importantly in its availability and its impact on the environment. An attempt to reduce the reliance on trash fish has been done by substituting the trash fish with formulated diets. Formulated diet has been reported to provide nutritionally completed diet and could increase lobster immunity (Mai & Tran 2022). Therefore, compound feed for lobster culture should be developed.

The advancement and long-term sustainability of lobster aquaculture are considerably affected by the development of manufactured pellet feeds (Irvin & Shanks 2015). Pellet diets are believed to be more sustainable because they have less environmental impact compared to trash fish diets (Huong et al. 2015). In addition, trash fish is intensely varied in nutritional profile and its availability is seasonal dependent (Irvin & Williams 2009). Some research on nutritional requirements and feed development for lobster has been done (Irvin & Shanks 2015; Williams 2007). Recently, Nankervis and Jones (2022) reviewed more detail on nutrient requirements and practical diet formulation for tropical Palinurid lobster aquaculture. Rathinam et al. (2009) reported that the pellet prepared with clam meal as a protein source gave better growth for lobster *P. homarus* compared to squid meal or fish meal. Smith et al. (2003) found that the optimal dietary protein and lipid content of the diet for lobster *P. ornatus* was 53 and 10%, respectively. Furthermore, Alexander Chong et al. (2017) conducted the study on the effect of different dietary lipid sources on tissue lipid profiles and found the fatty acids composition in the hepatopancreas and muscle tissues was positively correlated with the fatty acids composition of the respective diet.

Although lobster was observed to accept pellet feed, its growth and feed consumption were still lower compared to that of lobster fed fresh fishery products (fish, crab, mussel, and clam). For example, Marchese et al. (2019) reported that the best growth and survival rate of spiny lobster were still achieved by feeding with blue mussel. The same study also showed that good growth were also obtained when lobster fed formulated feed with inclusion of 10% krill meal. This suggests that feed formulation, feeding strategy and culture methods of lobster should be improved to support better growth of lobster.

A Benchmark feed formulation for grow-out of lobster *P. homarus* had been developed by ACIAR Project FIS/2014/059. Based on experimental results, formulated pellet feeds provided good growth of lobster. In 2019, a preliminary study through a series of laboratory-scale research had been conducted to increase the feed consumption of lobster by several feeding regimes including the use of feed additives and co-feeding with fresh fishery diet (fish, mussels, and crabs). Results showed that fresh food inclusions in the co-fed strategy had remarkably increased the feed consumption of lobster.

The present study aimed to investigate growth performance of different sex of lobster after being fed with combination of compound and fresh feed in indoor tanks and to investigate the effectiveness of co-feeding strategy on the growth of single sex (male) lobster in net floating cage.

Materials and Methods

Experimental feed

There were two experiments in this study to evaluate the growth of lobsters fed with a combination of compound diet and fresh feed. Compound feed was prepared as moist pellets in the Laboratory of the Institute for Mariculture Research and Fisheries Extension (IMRAFE), Gondol. The ingredients of the compound feed are presented in Table 1. Probiotic specific for lobster has been reported to increase lobster immunity (Haryanti et al. 2017) was added in feed formulation. This probiotic was prepared in the Laboratory of IMRAFE. Fresh ingredients, such as fish flesh, mussel flesh, and squid were extruded through a 3 mm die plate of meat grinder to form a homogenous mince. The mixture of dry ingredients and minced fresh ingredients were thoroughly mixed and then extruded through a 3 mm die plate of the pellet machine. The produced pellet was air dried at room temperature for 2 hours to decrease the moisture content of the feed. The feed was then packed in plastic boxes and stored at -20 °C until used for the feeding experiment.

The fresh feed consists of fisheries products, i.e., fish flesh, crab, and mussel meat. Fish and crab were chopped to the appropriate size for lobster feeding. All fresh feeds were washed with fresh water and stored in a freezer at -20 °C before feeding. Proximate analyses of all feeds were carried out according to AOAC (1990) methods. Moisture was analyzed by drying samples of feed at 110 °C in an oven (Memmert 854, Germany) until a constant weight was obtained. Crude protein was determined according to the Kjeldahl procedure (using KjeltecTM 8100, Foss). Total lipid was extracted using chloroform and methanol. Ash was analyzed using a muffle furnace (Carbolite ESF S20, England) at 550 °C. The proximate compositions of all feeds used in this experiment are presented in Table 2.

TABLE 1. Composition of the compound feed (g/100 g)

Ingredients	Feed
Fish meal	65.3
Cholesterol	0.5
Wheat flour	6
Wheat gluten	6
MOS	0.5
Fish (fresh)	6
Mussel (fresh)	6
Squid (fresh)	1
Fish Oil	2.6
Astaxanthin	1
Lecithin	1.7
Mineral premix	0.6
Vitamin premix	1.1
Stay C	0.4
Binder (CMC)	1.3
Probiotic	2

TABLE 2. Proximate compositions and energy contents of the experimental feeds

	Compound feed	Fish flesh	Mussel meat	Crab
Moisture (%)	24.9	75.3	88.3	83.6
Crude protein (% DM)	55.6	75.1	61.0	44.8
Lipid (% DM)	16.8	9.1	9.6	5.1
Ash (% DM)	15.9	10.3	20.2	30.5
Fiber (% DM)	2.2	2.0	3.3	16.5
NFE (% DM)[1)]	9.5	3.5	5.9	3.1
Gross Energy (MJ/kg)[2)]	21.4	21.9	19.2	13.1

[1)]NFE: Nitrogen-free extract

[2)]Gross energy of the experimental diets was calculated according to the gross energy values 23.61 kJ/g crude protein, 17.21 kJ/g carbohydrate, and 39.52 kJ/g crude fat, respectively (NRC 1993)

PROBIOTIC CULTURE

Four probiotic bacterial strains isolated from digestive system of wild lobster (*Photobacterium damsela* N-5, *Bacillus subtilis* C-1, *Bacillus oceanisediminis*-H-3 and *Bacillus amyloliquefaciens* I-5) have the ability to inhibit the growth of pathogenic bacteria. Those bacteria strains could produce extra cellular enzymes to hydrolyze proteins, lipid and carbohydrates. Probiotics bacteria were cultured with media of Marine Broth (Himedia) to reach densities of $10^{10\text{-}11}$ CFU/mL. Culture media must be sterilized at 121 °C for 15 min. In probiotics culture, it is equipped with aeration through 0.22 µm filtration to stimulate cell growth. Incubation time was 48 h. Culture volume of probiotics were 2.5 liters using glass flask.

EXPERIMENT SET-UP

EXPERIMENT-1. GROWTH OF LOBSTER, *Panulirus homarus* REARED IN INDOOR TANKS WITH DIFFERENT SEX PROPORTIONS AND FED A COMBINATION OF COMPOUND AND FRESH FEED

The experiment was conducted using 15 fiberglass tanks, 2 × 2 × 1 m^3 that were installed in an indoor hatchery. The experimental tanks were equipped with a flow-through seawater system and aeration. Water from the sea was pumped directly into the tanks without filtration. Lobsters for the experiment were collected from the Pekutatan Village of Jembrana Regency, Bali province through middlemen. A total of 150 good-quality lobsters with mean initial weight of 191.1 ± 18.1 g; and mean initial total length of 19.3 ± 0.7 cm were selected and used in this experiment. Ten lobsters were allocated for each tank with sex differentiation, i.e., (a) All males, b) Mixed of five males and five females, and (c) All females.

Each group of treatments had five replications. Lobster fed the mixture of compound feed (75%) and fresh feed (25%). The fresh feed consists of fish, crab, and mussel meat, each with the same proportion. Lobster fed twice every day in the morning and afternoon for 120 days feeding experiment. Uneaten feed and the tank bottom were cleaned every day before feeding. Dead lobsters and molt exuviate were removed from the tanks. The carapace of the dead and molted lobsters was photographed for individual identification. Water quality parameters of temperature, salinity and dissolve oxygen (DO) were measured during the experiment. The values of each parameter were 27.3 – 29.4 °C, 32 – 33 ppt and 5.0 – 6.2 ppm for temperature, salinity and DO, respectively.

At the initial of the experiment, a hemolymph sample of 100 µL was taken from each lobster and directly put onto an automatic temperature-compensated digital refractometer to measure the Brix index (%) (Hanna HI96801, Hanna Instruments, Australia) (Simon et al. 2015). The carapace of each lobster was photographed and used for individual identification of lobster (Figure 1). Body weight, carapace length, and total length was recorded for each lobster every four weeks.

At the same time, the hemolymph of one lobster from each tank was taken for Milky Hemolymph Disease (MHD) detection. The detection of MHD from the hemolymph sampling in this study was supporting

parameters to identify the possibility of disease presented during experiment as previously reported by Koesharyani, Lasmika and Sugama (2021). MHD was measured using PCR method. Haemolymph were collected from each lobster from ventral-sinus cavity using nidle 25 gauge and syringe 1 mL. DNA extraction was conducted using chelex 10% in TE buffer (pH 8.0) for each haemolymph sample. Extracted genomic DNA was purified using QIAcolumn purification kit based on manufacturer protocol. This is essential to prevent the presence of inhibitors.

Gene segment was amplified using PCR (Polymerase Chain Reaction). Amplification used Qiagen Fast Cycling PCR Kit (Lot 154036091). Primer for MHD detection was 254 F : 5' CGA GGA CCA GAG ATG GAC CTT 3' and 254 R : 5' GCT CAT TGT CAC CGC CAT TGT 3' with targeted region of 254 bp. Amplification was conducted under pre-denaturation 95 °C for 5 min, followed by 35 cycles consisting of denaturation 95 °C for 30 s; annealing 65 °C for the 30 s; and extension 72 °C for 30 s and a final extension of 72 °C for 2 min. The amplicons were tested electrophoresis using 1.5% agarose gel in 1X TAE (Tris-Acid-EDTA) buffer.

EXPERIMENT-2. THE STRATEGY OF COMPOUND FEED APPLICATION AND CO-FEEDING WITH FRESH FEED ON GROW-OUT OF SPINY LOBSTER, *Panulirus homarus* IN FLOATING SEA CAGES

Twelve net cages of 2 × 2 × 2.5 m^3 were used in this experiment. The experiment was conducted in the Net Cage Experimental Station of IMRAFE, Gondol at Pegametan Bay, Buleleng, Bali. A total of 240 lobsters with initial weight of 183.6 ± 17.6 g and initial total length of 18.9 ± 0.6 cm were selected and used in this experiment. Only male and good-quality lobsters were used in the experiment. Twenty lobsters were allocated for each cage. Three different feeding schemes were applied in this experiment, i.e., (a) Pellet, (b) A combination of pellet (75%) and fresh feed (25%) which consists of fish, crab, and mussel meat in the same proportion, and (c) A combination of pellet (75%) and mussel meat (25%).

Each treatment group had four replications. Lobster fed twice every day in the morning and afternoon for 120 days feeding experiment. Water quality parameters of temperature, salinity and dissolve oxygen (DO) were measured during the experiment. The values of each parameter were 27.4 - 29.2 °C, 32 - 33 ppt and 4.9 - 6.1 ppm for temperature, salinity and DO, respectively.

Brix index measurement and individual identification of lobster were done according to the methods mentioned above in experiment-1. Measurement of body weight, carapace length, and total length of each lobster was done every four weeks. MHD detection by PCR method was also done every four weeks from the hemolymph of one lobster of each cage. At the same time, all net cages were changed with clean ones for better water circulation and waste removal. The dead lobster and carapace molting found in the cage were removed. The carapace of the dead and molted lobsters was photographed to identify which lobster was dead or molted.

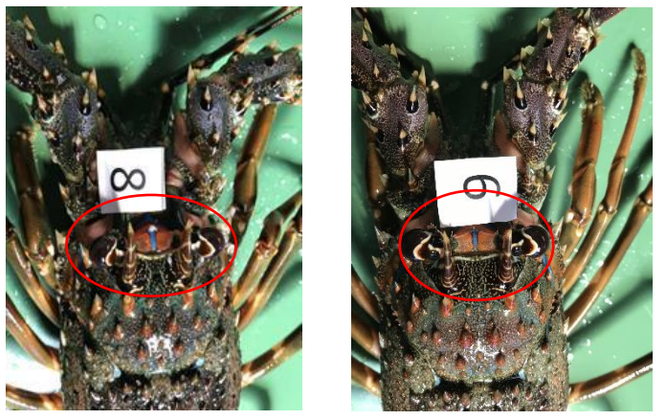

FIGURE 1. Photograph of carapace: a unique pattern of body markings on the carapace, rostrum, and eyes as well as the existence of small spines on each carapace which is shown in a red circle was used for individual lobster identification

DATA ANALYSIS

One-way ANOVA was used to compare the mean value of all parameter observed i.e., final weight, weight gain, and survival rate of lobster (dependent variable) among different sex group of lobster (independent variable) in experiment-1 and among different feed treatments (independent variable) in experiment-2. Normality and homogeneity of variances of the data was confirmed prior to ANOVA. A non-parametric Kruskal-Wallis test was applied when the assumption of normality and homogeneity of variances could not be met. A Tukey's post-hoc test for parametric analysis and Dunn's test for non-parametric analysis at a significance level of $\alpha = 0.05$ was used where the analysis indicated overall significant difference among treatments.

RESULTS

The growth pattern of lobster reared in tanks during 120 days experiment is presented in Figure 2. The average body weight of lobsters in all groups increased consistently during the experiment. Better growth was observed in all male group lobster. Analysis of variance and Tukey's test showed that the weight gain of the all-male group lobster was significantly higher than the other groups ($F_{(2,14)} = 7.91$, $p<0.05$) as shown in Table 3. Although the weight gain of all female group lobsters was the lowest, it was not significantly different from the group of mixed-sex ($p>0.05$). At the end of the experiment, some female lobsters were found bearing eggs, i.e., 8 and 7 lobsters in the mixed-sex group and in the female group, respectively.

Survival of lobsters during the 120 days rearing period in indoor tanks was high, 94.0 - 100%. A non-parametric test, Kruskal-Wallis test showed that the survival of lobster was not significantly different among group of sex ($\chi^2_{(2)} = 4.67$, $p>0.05$). This high survival was likely supported by appropriate water management to maintain the good quality of water during the experiment. Two lobsters were dead due to cannibalism in the first month of rearing and 4 lobsters were dead due to MHD. Results of MHD analysis from the hemolymph of one lobster from each tank every four weeks showed lobsters in 13 tanks (of 15 experimental tanks) were infected by MHD (Table 5).

The growth of lobster reared in floating net cages with different feed compositions for 120 days is presented in Table 4 and Figure 3. The weight of lobsters in all treatment groups increased during the feeding experiment. Lobsters fed with the combination of pellet and mixed fresh feed had the best growth. A non-parametric Kruskal-Wallis analysis showed that the weight gain of lobster fed a combination of pellet (75%) and mixed fresh feed (25%) was significantly higher than that of lobster fed only pellet ($\chi^2_{(2)} = 8.34$, $p<0.05$). Lobsters fed a combination of pellet (75%) and mussel meat (25%) showed better growth compared to lobster fed only pellet, but it was not significantly different (Table 4).

Survival of lobsters reared in floating net cages was 72.5 - 85.0% during the 120 days of the feeding experiment. Survival of lobster increased when fed with additional fresh feed instead of fed with only pellet.

TABLE 3. Weight, carapace length (CL), and survival of lobster, *Panulirus homarus* reared in indoor tanks with different sex proportions and fed a combination of compound and fresh feeds

Parameters	Treatment		
	All male (10)	Male (5)+Female (5)	All female (10)
Initial weight (g)	184.2 ± 10.1	198.3 ± 5.1	190.8 ± 3.0
Final weight (g)	243.5 ± 13.2	243.3 ± 13.5	230.5 ± 5.6
Weight gain (g)	59.4 ± 10.0^a	44.9 ± 8.7^b	39.7 ± 5.0^b
Weight gain (g/day)	0.49 ± 0.08^a	0.37 ± 0.07^b	0.33 ± 0.04^b
Weight gain (%)	32.3 ± 5.8^a	22.6 ± 3.8^b	20.9 ± 2.7^b
Initial CL (cm)	8.45 ± 0.14	8.52 ± 0.08	8.43 ± 0.13
Final CL (cm)	8.87 ± 0.27	8.68 ± 0.15	8.54 ± 0.10
Survival (%)	94.0 ± 5.5^a	94.0 ± 5.5^a	100.0 ± 0.0^a

Values with the same superscript letter in the same row shows no significant difference at 95% confidence level

Even though the overall survival rate was not statistically different among treatment groups ($F_{(2,11)}$ = 0.57, p>0.05),the highest value of survival was obtained in lobster fed a combination of pellet with a mixture of fish flesh, crab, and mussel meat (Table 4). Compared to lobsters reared in indoor tanks (Table 3), the survival of lobsters reared in floating net cages was much lower. It was difficult to control and maintained good water quality and prevents the spread of diseases in the floating net cages during the experiment. A total of fifty-two lobsters were dead during the 120 days of feeding experiment in net cages. Among them, 11 dead lobsters could not be identified because no dead body or carapace was found. Significant mortality (27 lobsters) occurred during the 100 -120 days rearing period. Results of a periodic MHD detection from the hemolymph of one lobster from each cage every four weeks showed that lobster in 8 cages (of 12 experimental cages) were infected by MHD (Table 5) and some lobster dead due to this disease. Although MHD infection was found lower compared to that of lobster reared in the tanks (Table 5), dead lobster due to MHD was found higher in the floating net cages experiment.

Table 4. Weight, carapace length (CL), and survival of lobster, *Panulirus homarus* reared in net cages and fed a combination of compound and fresh feeds

Parameters	Treatment		
	Pellet (100 %)	Pellet (75%) + fresh food (25%)	Pellet (75%) + mussel meat (25%)
Initial weight (g)	180.1 ± 2.5	186.1 ± 2.6	184.7 ± 3.6
Final weight (g)	223.5 ± 13.4	274.1 ± 20.4	254.3 ± 13.2
Weight gain (g)	41.5 ± 16.2^{a}	88.0 ± 20.9^{b}	69.6 ± 11.8^{ab}
Weight gain (g/day)	0.35 ± 0.13^{a}	0.73 ± 0.17^{b}	0.58 ± 0.10^{ab}
Weight gain (%)	23.0 ± 9.0^{a}	47.4 ± 11.6^{b}	37.7 ± 6.1^{ab}
Initial CL (cm)	8.50 ± 0.09	8.56 ± 0.03	8.50 ± 0.10
Final CL (cm)	8.60 ± 0.20	9.15 ± 0.21	8.95 ± 0.10
Survival (%)	72.5 ± 15.5^{a}	85.0 ± 16.8a	77.5 ± 25.3a

Values with the same superscript letter in the same row shows no significant difference at 95% confidence level

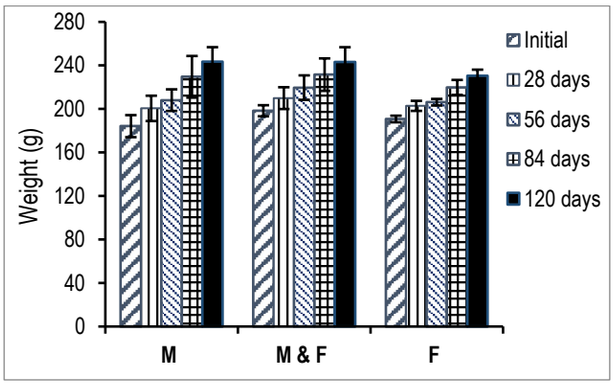

FIGURE 2. Changes in body weight of lobster reared in indoor tanks with different proportions of male and female and fed experimental feed for 120 days (M = male, F = female)

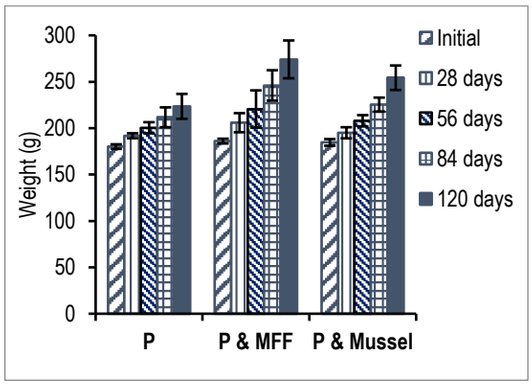

FIGURE 3. Changes in body weight of lobster reared in floating net cages with different feed compositions for 120 days (P = pellet, MFF = mixed fresh feed)

During the 120 days feeding experiment conducted in indoor tanks, the average number of molting was 19.4, 16.8, and 17.2 for each group of all males, mixed males and females, and all females, respectively. Individual identification showed that lobsters experienced one to three times molting during the experiment. Furthermore, the maximum and minimum weight increment per molting was 93.0 g and 14.3 g, respectively, for the all-male group. For the all-female group, the maximum and minimum weight increment per molting was only 71.0 g and 4.7 g, respectively, much lower compared to the male group (Figure 4). Meanwhile, it was difficult to obtain accurate individual molting data from the experiment conducted in floating net cages. Some carapaces of the molted lobsters could not be obtained. The available molting data showed that some lobsters also had two to three times molting during the experiment with the average number of molting 0.75, 3.25, and 4.0 for the feeding treatment of pellet, pellet mixed fresh feed, and pellet and mussel meat, respectively. The average weight gain of lobster reared in floating net cages was 41.5 to 88.0 g (Table 4) was higher than the weight gain of lobster reared in the tanks (Table 3). Based on these growth data, it could be assumed that lobsters reared in floating net cages also had high numbers of molting, as a consequence, the body weight of the lobster increased during the experiment.

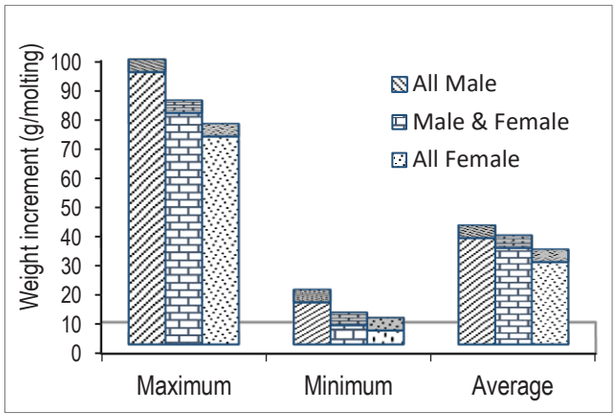

FIGURE 4. Maximum, minimum, and average weight increment per molting of lobsters reared in indoor tanks with different proportions of male and female

TABLE 5. Data of MHD detection from the hemolymph of one lobster from each tank

Treatments (Sex)	Rearing period in indoor tanks				
	Initial	28 days	56 days	84 days	120 days
M	-	+	-	-	-
M	-	-	+	-	-
M	+	+	-	-	-
M	-	+	+	-	-
M	-	+	-	-	-
M&F	-	+	-	-	+
M&F	-	-	+	-	+
M&F	-	+	-	-	+
M&F	-	+	-	+	+
M&F	-	-	-	-	-
F	+	-	-	+	-
F	-	+	-	-	-
F	-	+	-	+	+
F	-	+	-	-	+
F	-	-	-	-	-

Note: M = male, F = female

TABLE 6. Data of MHD detection from the hemolymph of one lobster from each cage

Treatments (Feed)	Rearing period in net cages				
	Initial	28 days	56 days	84 days	120 days
P	-	+	+	-	-
P	-	-	-	-	-
P	-	+	-	+	-
P	-	-	+	+	-
P & MFF	-	-	+	-	-
P & MFF	-	+	-	-	-
P & MFF	-	-	-	-	+
P & MFF	-	-	-	-	-
P & Mussel	-	-	-	-	-
P & Mussel	-	-	+	-	-
P & Mussel	+	-	-	+	-
P & Mussel	-	-	-	-	-

Note: P = pellet; MFF = mixed fresh feed (fish, crab, mussel)

DISCUSSIONS

Growth of lobster occurs through the process of molting (Wijaya, Aslamyah & Usman 2011), and the body weight of lobster increases after the molting process succeeds. Data in the present study showed maximum weight increment per molting of male lobsters was much higher (93.0 g) than female ones (71.0 g). In addition to the factor of feed consumed, this biological behavior may contribute to the lower weight gain of female lobster *P. homarus* compared to male ones in the present study. The lower growth of female lobsters in the present study agrees with the results of the research reported by Balkhair, Al-Mashiki and Chesalin (2012). In their research, lobsters with an average weight of 305 g for males and 290 g for females were reared in tanks for 187 days and fed with trash fish (sardine). Lobsters grew to 403.5 g and 367.3 g with a growth rate of 0.52 g/day and 0.45 g/day for males and females, respectively. The growth rate of lobster in the present study was 0.49 g/day and 0.33 g/day for the male and female groups, respectively (Table 3). This growth rate was comparable with the result of the experiment reported by Subhan et al. (2018) which was 0.50 – 0.53 g/day with an initial weight of 130.39 g. Vijayakumaran, Anbarasu and Kumar (2010) reported that lobster *P. homarus* had a weight increment of 23.1 - 36.3 g after the molting process succeed by communal rearing method in the tank. On the other hand, they had a much lower weight increment of 11.8 -16.6 g after the molting process succeeded by individual rearing method. Rathinam et al. (2009) reported that lobster *P. homarus* with 165.8 g initial weight and fed dry pellet for 45 days had a weight gain of 0.25 - 0.38 g/day, and that dry pellet prepared using clam meat as protein sources resulted in the best growth of lobster. *Panulirus ornatus*-fed dry pellet also had lower growth compared to that fed fresh mussel as reported by Marchese et al. (2019). Growth performance of lobsters is controlled by the efficiency to digest and then absorb nutrients from food (Simon & Jeffs 2008). Gora et al. (2018) reported lobsters fed with formulated feed demonstrated a general difficulty in the absorption and mobilization of nutrients from the feed and resulted in lower growth.

Vijayakumaran et al. (2009) reported high growth of lobster *P. homarus* reared in net cages in the open sea. Lobsters with an initial weight of 111.7 - 138.1 g had growth of 0.70 - 0.97 g/day during the 91 – 225 days rearing period. In their experiment, lobsters were

fed with fresh marine clam *Donax* spp. as the main feed and supplemented with gastropods, green mussels, crab, squid, and trash fish. In the present study, it was found that the growth of lobster rearing in floating net cages for 120 days was 0.35 – 0.73 g/day, lower compared to the result reported by Vijayakumaran et al. (2009). The lower growth obtained in the present study might be due to lobsters being mainly fed with pellet (75%). Nevertheless, the results of the present study indicated that compound feed was acceptable and could support the growth of lobsters. Furthermore, the growth of lobsters increased when it was given an additional feed of fresh mussel meat or a mixture of fresh fish flesh, crab, and mussel meat. This was agreed with the results reported by Sudewi et al. (2021) that lobster *P. homarus* grew significantly higher when they were fed a combination of pellet feed with fresh food (a mixture of fish, crabs, shrimp, and small mussels) compared to those that were only fed pelleted feed. A previous study by Johnston, Melville-Smith and Hendriks (2007) also reported best growth and survival in the spiny lobster *P. cygnus* was not possible to achieve without the use of raw molluscan flesh in diet. Adiyama and Pamungkas (2017) found the lobster *P. homarus* fed fresh fish for 60 days in a circulation aquaculture system (RAS) grew to 53.6 – 73.7 g from the initial weight of 50.07 g, with the weight gain of 3.5 – 23.6 g. The proximate composition of feed used in the present study was presented in Table 2. Fish flesh and mussel meat have high protein content that could support better growth of lobster compared to that fed only pellet. Crab has a high content of ash indicating high content of minerals that was required by lobster for supporting the success of the molting process and growth.

In the present study, the survival of lobsters reared in indoor tanks was much higher (94.0-100%) than that of lobsters reared in net cages (72.5 - 85.0%). Two factors might contribute to lobster's high mortality, i.e., cannibalism and MHD in the present study, especially for lobster reared in net cages. Although large-size lobsters were used in the present study, mortality due to cannibalism still occurred. Cannibalism usually occurs if the condition of the lobster is weak, such as just after molting. Minimizing contact among lobster individuals in rearing captivity is one method to prevent cannibalism. Irvin and Williams (2009) reported individual rearing of lobster was effective to prevent cannibalism and resulted in higher survival. However, the growth of lobster *P. ornatus* in the individual-rearing method was reported much lower than in the communal-rearing method (Marchese et al. 2019; Ratunil 2017). Another method to prevent cannibalism is by using shelters for hiding (Adiyana & Pamungkas 2017; Adiyana et al. 2014). Therefore, optimum stocking density on rearing of lobsters should be considered to minimize individual contact among lobsters.

Limited information is available on the optimum stocking density for grow-out rearing of *P. homarus*. Subhan et al. (2018) reported that initial stocking density of 18 lobsters/m^3 resulted in the best for growth for lobster reared in tank, but their survival were similar (84.6-86.1 %) across all stocking density treatments. However, this study was conducted for a shorter rearing period (30 days) than the present study (120 days). For *P. polyphagus* reared in net cage, Solanki et al. (2012) reported that stocking density of 20 lobsters (90 ± 10 g body weight) per cage (2×1×1 m^3) showed a better growth compared to higher stocking density, but their survival was low (0-24.7 %) for all density treatment tested. Study on the optimum stocking density in cubic meter of water volume needs to be further investigated as lobsters do not occupy water column but on the bottom of the tanks or cages.

The data in Table 5 shows that both groups of lobsters reared in tanks and floating net cages were infected by MHD. However, only one lobster dead due to MHD for lobster reared in the indoor tank. Meanwhile, 27 lobsters died due to MHD for lobsters reared in net cages, much higher than for lobsters reared in indoor tanks. MHD in lobster farming could cause 70-100% mortality as reported by Jones (2015), Koesharyani, Lasmika and Sugama (2021) and Sudewi et al. (2020). However, data in the present study indicates that although all the group of treatments showed that some lobster was found to be positively infected with MHD, MHD will not likely infect and cause mass mortality as long as the lobster has good health and they were kept in good water quality conditions. In the present study, probiotics and MOS were added in preparing compound feed for the experiment. The immunity of lobsters could be improved by feeding them probiotic-supplemented moist pellet (Haryanti et al. 2021, 2017). Nankervis and Jones (2022) stated that the growth and health condition of spiny lobsters could be improved by manipulation of gut microflora composition. The inclusion of mannan oligosaccharides (MOS) in the diet was also reported effective in improving the growth, survival, and gastrointestinal health of the lobster, *P. homarus* (Huu & Jones 2014). The probiotic used in the present study consisting of four bacteria, i.e., *Photobacterium damsela*, *Bacillus subtilis*, *Bacillus* sp.,

and *Bacillus amyloliquefaciens*. Those bacteria were isolated from the digestive tract of lobster *P. homarus*. The four bacteria probiotics are thought to not only play a role in the mechanism of the immune system, but also in the process of protection, namely directly blocking pathogenic microbes and increasing mucus integration with epithelial cells. Haryanti et al. (2021) reported that lobsters fed diet containing probiotics (4 strains) and prebiotics and then were challenged with MHD for six gene immunity targets provided better immunity.

Blood protein (Brix) might have a relation with the growth, health condition, and intermolt status of lobster. Wang and McGaw (2014) reported that serum protein concentration changed during the molt cycle of lobster, reaching its highest levels during the premolt stage followed by a sharp decrease after the lobsters had molted. Initial Brix score of all lobsters rearing in indoor tanks ranged between 5.3 - 24.0%, and the average Brix score for all three group treatments was not so much different, i.e., 14.8, 14.5, and 15.6% for all males, mixed male and female and all-female group, respectively. Among the lobsters used in this experiment, only six lobsters have a Brix score of less than 10%. Furthermore, the Brix score of lobster reared in floating net cages was 8.3 - 21.7%, 6.1 - 22.8%, and 5.0 - 23.0% for feeding treatment of pellet, pellet and mixed fresh feed, and pellet and mussel meat, respectively, with an average of 13.3 - 14.4%. These data indicated lobsters have good health conditions at the initial of the experiment. The Brix index was strongly related to total protein in the hemolymph and was therefore considered a good indicator of the nutritional condition in lobsters (Battison 2018; Mendo et al. 2016; Simon et al. 2015). Based on these Brix score values and the weight gain of the lobster, it might be no correlation between the Brix score and the growth of the lobster.

Conclusions

The combination of compound feed and fresh feed at a proportion ratio of 75% and 25% in this study resulted in good growth performance (observed by weight gain) in both indoor rearing tanks and net floating cage. Male lobsters fed with compound and fresh feed appear to have a better growth rate than females and mixed sex group of lobster. These findings suggest that rearing single sex lobster (males only) by applying co-feeding strategy that combine compound feed and fresh fish (i.e., fish, crab and mussel meat) can potentially improve better growth and overall production. Future study will include investigating the same co-feeding application and using single sex (males only) population with higher stocking density.

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