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Research Article

GROWTH AND SURVIVAL OF SPINY LOBSTER, *Panulirus homarus* JUVENILES FED WITH DIFFERENT FORMULATED FEEDS

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ARTICLE HIGLIGHTS

- Indonesia has remarkable natural resources of spiny lobster *Panulirus homarus* and an exceptional opportunity to establish the largest lobster aquaculture industry in the world.
- However, culturing lobsters still relies on natural feeds (trash fish of fresh fishery by-catch) which are inefficient and environmentally unsustainable.
- Developing formulated feed based on locally available ingredients is invaluable for replacing natural feeds to support sustainable lobster culture.
- Formulated feed made of local fish meal provided the best growth of juvenile spiny lobster *Panulirus homarus* compared to imported fish meal.

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ABSTRACT

Several studies suggested that good growth performance and survival of lobster juveniles can be achieved by feeding the lobsters with formulated feeds. This study aimed to (1) evaluate the growth and survival of spiny lobster *Panulirus homarus* juveniles fed with different diets having profiles based on a basal diet formulation and (2) to compare the growth of lobsters fed with an identical formulated feed with variations only in sources of fish meal and dry matter content. The feeding experiment was designed following a completely randomized design (CRD) with five diet treatments and five replicates for each treatment. Five experimental diets were prepared in this experiment, i.e., Diet A (basal diet prepared as moist pellets); Diet B (moist pellets with the same formulation as Diet A, using local fish meal); Diet C (moist pellets with the same formulation as Diet A, using imported fish meal); Diet D (dry pellets with the same formulation as Diet A, using imported fish meal); and Diet E (fresh fish). Juveniles of spiny lobster with an average weight of 3.00±0.07 g were allocated in 25 net cages (60 x 60 x 60 cm) placed in a circular HDPE canvas tank (7.5 m in diameter) at a density of 15 lobsters/cage in a flow-through seawater system. The juveniles were fed with the experimental diets twice/day for 12 weeks. The experiment showed that the best growth was observed in juveniles fed with moist feed containing local fish meal (Diet B) with the final weight reaching 18.74±2.30 g. Moist feed containing imported fish meal (Diet C) resulted in a higher survival rate (46.66%), and the lowest survival rate (13.33%) was obtained from juveniles fed with fresh fish (Diet E).

Keywords:
formulated feeds, growth, Panulirus homarus, survival

INTRODUCTION

Marine lobsters are highly favorable and considered prized luxury export products (Plaganyi *et al.* 2018), mainly exported to China, the United States of America, and Hong Kong. Chinese imports of lobsters increased dramatically to 38.6% or reached 11.461 MT from January to March 2021 compared to the same period in 2020 (FAO 2021). Grow-out culture of wild-captured spiny lobster juveniles to market size has been considered an evolving aquaculture industry in Asia, most markedly in Vietnam, and emerging in Indonesia (Nankervis & Jones 2022a). Indonesia has a remarkable natural resource of pueruli, and therefore, Indonesia has an exceptional opportunity to build the largest lobster aquaculture industry in the world (Priyambodo *et al.* 2020). In Indonesia, spiny lobster seed resources are found mainly in Lombok, Java, and Sumbawa Islands.

In 2013-2014, the catch of lobster seeds was estimated to be over 3 million per year in Lombok (Bahrawi *et al.* 2015), and by 2016, more than 100 million lobster seeds were captured from Java, Lombok, and Sumbawa (Jones *et al.* 2019). Despite the growing lobster aquaculture industry, feeding in grow-out culture commonly relies only on fisheries products, such as trash fish or fresh fishery by-catch (Nankervis & Jones 2022a).

Fresh fishery products and trash fish for feeding in aquaculture have been well-identified as environmentally unsustainable and inefficient. For example, feeding with mussel flesh has a poor feed conversion ratio of up to 17.26:1 (Rivaie *et al.* 2023). Trash fish as feed lead to significant water quality deterioration, causing environmental degradation and disease introduction (Nankervis & Jones 2022a). In addition, trash fish vary in their supply and nutritional profile. Moreover, there are difficulties in storage and possible spoilage during storage (Jones *et al.* 2015). The shrinking supply of trash fish and their downstream environmental effects have strengthened the urgency to develop sustainable formulated feeds (Nankervis & Jones 2022b). The development of formulated feed would provide benefits, such as nutrition optimization, feeding methods flexibility, easiness in usage and storage, waste minimization, and environmental sustainability improvement (Perera & Simon 2014) for the development of lobster aquaculture (Saleela *et al.* 2015).

Some studies on formulated feed development and nutritional requirements for lobsters were conducted, such as studies on the appropriate level of protein for better growth and survival of spiny lobster *P. homarus* (Rathinam *et al.* 2014), and on the effects of binders on palatability and stability of formulated dry feed for *P. homarus* (Saleela *et al.* 2015). A review on the nutritional requirement and feed development for spiny lobster in post-larval stage reported that tropical lobster *P. ornatus* had the best growth when they were fed with a diet having a high digestible protein (> 56% DM) and 10-11% total lipid in dry matter (Nankervis & Jones 2022a). The use of moist pellets was also examined for the growth of lobster *P. homarus* (Ridwanudin *et al.* 2018). One of the major problems in aquaculture of spiny lobsters is an appropriate formulation of lobster feeds that are readily consumed, attractive, and stimulate optimal growth and survival (Marchese *et al.* 2019).

In a previous study, a basal diet formulation for 2-g lobsters had been developed and promoted good growth performance and survival rates of juveniles (Irvin & Shanks 2015). This formulated feed was produced using ingredients sourced from Australia. Finding local, high-quality ingredients is the first step that needs to be addressed before Indonesian lobster farmers apply formulated feed for growing lobsters. Therefore, this study assessed 3-g lobster juveniles fed with a basal diet made from ingredients sourced from Australia and locally from Indonesia. This experiment was carried out to evaluate the growth and survival of 3-g lobster juveniles fed with different formulated feeds having a profile based on the basal diet, as well as to compare the growth of the lobsters fed with an identical formulated feed with variations only in fish meal sources and dry matter content.

MATERIALS AND METHODS

Study Location

The study was undertaken at the Institute for Mariculture Research and Fisheries Extension (IMRAFE), Gondol, Bali, Indonesia.

Rearing of *Panulirus homarus* Post-larvae to 3-g Juveniles

Feeding experiment was conducted by using *P. homarus* 3-g juveniles. To obtain the 3-g juveniles, pigmented *P. homarus* post-larvae were obtained from Lombok, West Nusa Tenggara Province, from which healthy post-larvae were selected to be reared up to juveniles weighing 3 g. The initial weight of the healthy lobster post-larvae was 0.2 g on average. Post-larvae rearing was conducted in floating net cages, concrete tanks, and an HDPE canvas tank with a flow-through seawater system. For the first 2 weeks, the post-larvae were fed with mysids and dry pellets and continued with dry pellets only until reaching 3-g juveniles. Grading of the post-larvae was carried out every 2 weeks to prevent cannibalism. Post-larvae rearing was conducted until the juveniles reached 3 g in weight. The 3-g juveniles were then used for the feeding experiment.

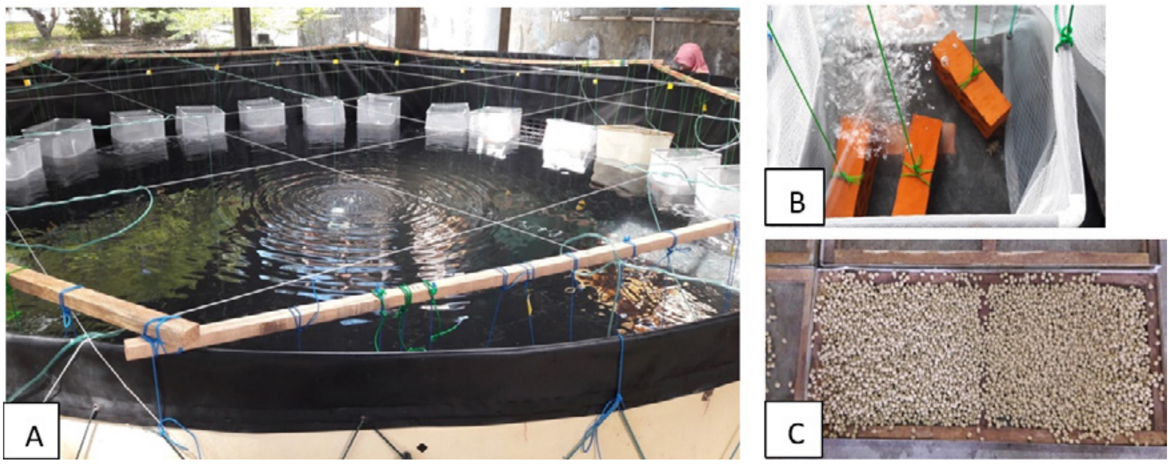

Figure 1 Feeding experiment using 3-g spiny lobster *Panulirus homarus* juveniles in 25 net cages placed in an HDPE canvas tank
Notes: A = Net cages placed in an HDPE canvas tank; B = shelters and an aeration stone inside a net-cage; C = experimental feed).

Feeding Experiment

The feeding experiment was designed following a completely randomized design (CRD) with five diet treatments and five replicates for each treatment. The experiment was carried out in 25 net cages (60 x 60 x 60 cm) that were placed in an HDPE canvas tank (Ø 7.5 m) with a flow-through seawater system, and equipped with aeration as oxygen supply (Fig. 1).

Lobster juveniles used in this experiment had an average weight of 3.00±0.07 g. The juveniles were stocked at a density of 15 ind./cage and fed with experimental diets according to the treatments, twice every day, at 08.30 a.m. and 3.30 p.m.

Waste materials, including uneaten feed and feces, exuviae of the molted juveniles, and dead juveniles, were removed from the net cages each time before feeding the juveniles. The feeding experiment was carried out for 12 weeks.

Data on weight and survival rate were collected every four weeks to calculate the percentage of weight gain (WG), specific growth rate (SGR), and survival rate (SR) as follows:

$$\text{Percentage of weight gain (\%)} = 100 \times (Wt - Wo)/Wo$$

$$\text{Specific growth rate (\%/day)} = 100 \times (\ln Wt - \ln Wo)/t$$

$$\text{Survival rate (\%)} = 100 \times (Nt/No)$$

where:

Wo = weight of lobster juveniles at the initial stage of feeding experiment (g)

Wt = weight of lobster juveniles at the final stage of feeding experiment (g)

t = duration of the feeding experiment (days)

No = number of lobster juveniles at the initial stage of feeding experiment (ind.)

Nt = number of lobster juveniles at the final stage of feeding experiment (ind.)

Experimental Diets Preparation

Four formulated diets were prepared based on the basal diet, i.e., Diet A (basal diet prepared as moist pellets), Diet B (moist pellets with the same formulation as Diet A, using local fish meal), Diet C (moist pellets with the same formulation as Diet A, using imported fish meal), and Diet D (dry pellets with the same formulation as Diet A, using imported fish meal). The fifth diet was fresh fish (Diet E). Diet A was prepared at CSIRO's Aquaculture Feed Technology Laboratory in Bribie Island, Australia. Diets B, C, and D were prepared in the Feed and Nutrition Laboratory of IMRAFE, Gondol, Bali, Indonesia. The formulation and nutrient composition of the experimental diets are shown in Table 1.

Table 1 Formulation (% of ingredients) and nutrient composition of experimental diets for the feeding experiment of spiny lobster *Panulirus homarus* 3-g juveniles

Ingredients	**Diet A** 83% dry matter (basal diet)	**Diet B** 83% dry matter (local fish meal)	**Diet C** 83% dry matter (imported fish meal)	**Diet D** 92% dry matter (imported fish meal)	**Diet E** (fresh fish)
Fish meal	65.30				Fresh fish
Fish meal B[1)]		65.30			
Fish meal A[2)]			65.30	65.30	
Wheat flour	6.00	6.00	6.00	6.00	
Wheat gluten	6.00	6.00	6.00	6.00	
MOS[3)]	0.50	0.50	0.50	0.50	
Fish (fresh)	6.00	6.00	6.00	6.00	
Mussel (fresh)	6.00	6.00	6.00	6.00	
Squid (fresh)	1.00	1.00	1.00	1.00	
Fish oil	2.60	2.60	2.60	2.60	
Astaxanthin	1.00	1.00	1.00	1.00	
Cholesterol	0.50	0.50	0.50	0.50	
Lecithin	1.70	1.70	1.70	1.70	
Mineral premix	0.60	0.60	0.60	0.60	
Vitamin premix	1.10	1.10	1.10	1.10	
Stay C	0.40	0.40	0.40	0.40	
Binder (CMC)	1.30	1.30	1.30	1.30	
Nutrient composition by proximate analysis (% of diet)					
Dry matter	83.8	83.3	83.2	91.8	
Crude protein	47.1	52.0	51.1	51.3	
Lipid	13.4	14.7	15.1	13.1	
Ash	5.9	5.4	5.6	7.9	
Fiber	10.3	8.5	8.7	10.1	

Notes: [1)] = local; [2)] = imported; [3)] = mannan oligosaccharides; CMC = carboxymethyl cellulose.

Diet B and C were prepared as moist pellets with a dry matter content of about 83%. On the other hand, Diet D was prepared as dry pellets with a dry matter content of approximately 92%. The four experimental diets were made into a 2-mm diameter pellet. The diets were then stored in a freezer before and during the feeding experiment.

Proximate Analysis

Proximate analysis of the experimental diets was carried out in the Chemistry Laboratory of IMRAFE according to the procedure of the Association of Official Analytical Chemist (AOAC, 2000) for crude protein, lipid, fiber, water, and ash contents. Crude protein was determined using Kjeldahl method (Kjeltec 8100, Foss), while lipid content was measured by chloroform-methanol extraction (v/v) method. Crude fiber was determined by loss on combustion of dried residue following consecutive digestion of the samples using H_2SO_4 and NaOH (Fibertec, Foss). Water content was defined by oven-drying samples at 105 °C to a constant weight using Memmert, Germany oven. Crude ash was analyzed by calculating the residue after heating the samples at 550 °C in a Carbolite, England furnace.

Data Analysis

Data were analyzed descriptively and inferentially using the R commander package in the R statistical program. Data obtained from the present study had equal variances; however, those data were not normally distributed which means that the assumption of Analysis of Variance (ANOVA) was violated. Therefore, a non-parametric method, i.e., the Kruskal-Wallis rank sum test, was used to determine the effect of different formulated feeds on the growth and survival of lobster juveniles. Differences in growth and survival between treatments were analyzed using the Wilcoxon rank sum test.

RESULTS AND DISCUSSION

Growth of Lobster

Different characteristics of formulated feeds significantly affected the weight of lobster juveniles (P < 0.05). The present study showed that higher growth of lobster juveniles was obtained from the feed containing local fish meal (Diet B) and the basal diet (Diet A) which resulted in a final weight of 18.74±2.30 g and 17.74±2.54 g, respectively (Fig. 2) with daily growth rate of 6.37±0.91%/day and 5.81±0.89%/day, respectively (Table 2). Feed formulated with local fish meal showed significantly better growth than feed formulated with imported fish meal (Diet C) (P < 0.05). No differences in the growth of juveniles were observed as a result of feed having 83% dry matter (moist pellets) and 92% dry matter (dry pellets) (Diets C and D) (P > 0.05). Growth of lobster juveniles fed with fresh fish (Diet E) was slightly higher (12.34±1.63 g with a daily growth rate of 3.51±0.54%/day) than those fed with imported fish meal (Diet C and D), however, the growth was significantly lower than those fed with the basal feed (Diet A) (P < 0.05) and those fed with feed containing local fish meal (Diet B).

This study found that higher growth rates were achieved by feeding lobster juveniles with feed containing local fish meal and that containing basal diet compared to the other treatments, most importantly to the fresh fish diet. The results suggested that formulated feeds can be used to reduce or replace the use of fresh feed which is unsustainable in lobster aquaculture. The good growth performance of lobster juveniles fed with formulated feeds in this study was supported by the nutrient contents of the experimental feeds. The diet containing local fish meal (Diet B) had crude protein levels of 52.0% and lipid content of 14.7% (Table 1). A review study stated that juvenile and adult lobsters require diets with high protein, low lipid, and moderate to high carbohydrate contents. Formulated pellet feed with 56% digestible proteins (61% crude protein) increased the growth of lobsters (Nankervis & Jones 2022a).

Daily observation indicated that the formulated feeds were well consumed by the juveniles and supported their growth. The feeds used in this study were formulated with the addition of fresh fish, mussels, and squid. This experiment verified a hypothesis that fresh ingredients included in feed formulation promote good growth performance in spiny lobster juveniles. Fresh ingredients contain high levels of chemo-attractants to promote feeding response in crustaceans (Irvin & Shanks 2015).

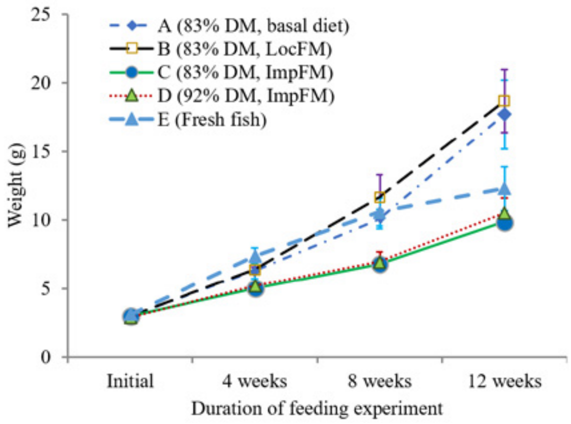

Figure 2 Weight (g) of lobster *Panulirus homarus* juveniles fed with different diets for 12 weeks
Notes: DM = dry matter; LocFM = local fish meal; ImpFM = imported fish meal.

Table 2 Growth and survival of spiny lobster *Panulirus homarus* juveniles fed with different diets for 12 weeks

	Diet A 83% dry matter (basal diet)	Diet B 83% dry matter (local fish meal)	Diet C 83% dry matter (imported fish meal)	Diet D 92% dry matter (imported fish meal)	Diet E (Fresh fish)
Initial weight (g)	3.01±0.12	2.95±0.09	2.98±0.19	2.94±0.10	3.12±0.08
Final weight (g)	17.74±2.54b	18.74±2.30b	9.94±0.44a	10.54±1.14a	12.34±1.63a
Weight gain (g)	14.73±2.47b	15.79±2.27b	6.95±0.50a	7.60±1.10a	9.22±1.58a
Weight gain (%)	488.41±75.58b	535.15±76.78b	234.03±28.69a	258.66±37.08a	294.91±45.61a
Daily growth rate (g/day)	0.17±0.02b	0.18±0.02b	0.08±0.00a	0.09±0.01a	0.10±0.01a
Daily growth rate (%/day)	5.81±0.89b	6.37±0.91b	2.78±0.34a	3.08±0.44a	3.51±0.54a
Specific growth rate (%/day)	2.10±0.15b	2.19±0.14b	1.43±0.10a	1.51±0.12a	1.62±0.14a
Survival (%)	24.00±5.96b	36.00±12.99b	46.66±8.16b	45.33±8.69b	13.33±6.66a

Note: Different letters in the same row indicate significant differences (P < 0.05).

In terms of different sources of fish meal, the present experiment showed that lobster juveniles fed with the diet containing local fish meal had significantly higher growth than those fed with the diet containing imported fish meal. This result contradicts a previous study which reported that lobsters fed with a diet containing imported fish meal (from Peru) had a significantly higher weight gain than those fed with a diet containing Indonesian fish meal (Irvin & Shanks 2015). These two experiments indicated that the ingredients of the feed vary in quality, and as a consequence, they have different effects on the growth of lobster. The quality of fish meal and levels of protein vary because of differences in the species of fish used, sources of origin, as well as fish meal processing and storage (Irvin & Shanks 2015). In the present study, Diet B which was formulated using local fish meal had the highest crude protein content (Table 1) and may support the highest growth of lobster compared to those fed with the other diets. An increase in growth was also found in lobster *P. ornatus* fed with a diet containing increased protein content (Smith *et al*. 2003).

This study found that there was no difference in the growth of lobster fed with moist (83% DM) and dry (92% DM) pellets. Feed intake of lobsters fed with these types of feed was probably the same and had similar effects on the growth of lobster. However, an earlier study reported that growth rates of lobster juveniles fed with dry pellets (90% DM) were significantly lower than those fed with moist pellets having dry matter content ranging from 65% to 82%. This study suggested that moist pellets improved the growth of the juveniles. The soft texture of moist pellets may be suitable for the infantile mouthparts of juveniles and therefore, increased feed intake. Improved feed intake was arguably a result of increased palatability provided by high moisture content (Irvin & Shanks 2015). A 30-day feeding experiment in juveniles of *P. versicolor* using a semi-moist artificial diet (83% DM) resulted in a high specific growth rate (SGR) of 1.81-2.28%/day (Syafrizal *et al.* 2018).

In the present study, the specific growth rate of *P. homarus* juveniles fed with moist pellets reached 1.42-2.19%/day. The SGR of *P. homarus* juvenile in the present study was lower than that of *P. versicolor* which may suggest that juvenile of *P. versicolor* has a relatively faster growth rate (Syafrizal *et al.* 2018).

Feeding with fresh fish resulted in similar growth performance to those fed with feed containing imported fish meal (diet C and D), but significantly lower when compared to those fed with feed containing local fish meal (diet B) and the basal diet (diet A). These results could be due to the high mortalities of lobster juveniles fed with fresh fish which affected the average weight of juveniles in each replicate cage. Feeding with fresh fish in the present study resulted in a specific growth rate of 1.62%/day. This growth performance was similar to that of *P. homarus* juveniles with an initial weight of 1.58 g fed with marine clam, *Donax* spp. which exhibited a specific growth rate of 1.64%/day (Vijayakumaran *et al*. 2009). Another study in *P. homarus* juveniles indicated that moist diet resulted in a higher specific growth rate of lobsters than those fed with trash fish (Ridwanudin *et al*. 2018). A study on *P. ornatus* juvenile fed with commercial shrimp pellets and fresh mussels had SGR values ranging from 0.49%/day to 1.35%/day (Jones 2007).

Juveniles fed with fresh fish exhibited pale coloration, in contrast to those fed with formulated feed which showed normal body color (Fig. 3). Similarly, juvenile of *P. ornatus* fed with frozen mussel *Mytilus edulis* had poorer body color than juveniles fed with formulated diets (Barclay *et al*. 2006). The normal body color of the juveniles fed with formulated diet obtained in the present study was likely due to the pigmentation role of astaxanthin which was included in the diet (1% inclusion level). The lack of a particular nutrient in the fish used in this study may have influenced the lobster's body color. It has been recommended to add carotenoids to the diet of lobster to obtain a similar color to the wild-caught lobsters (Crear *et al*. 2002; Williams 2007).

Figure 3 Pale coloration of juveniles of lobster *Panulirus homarus* fed with fresh fish (black arrow)

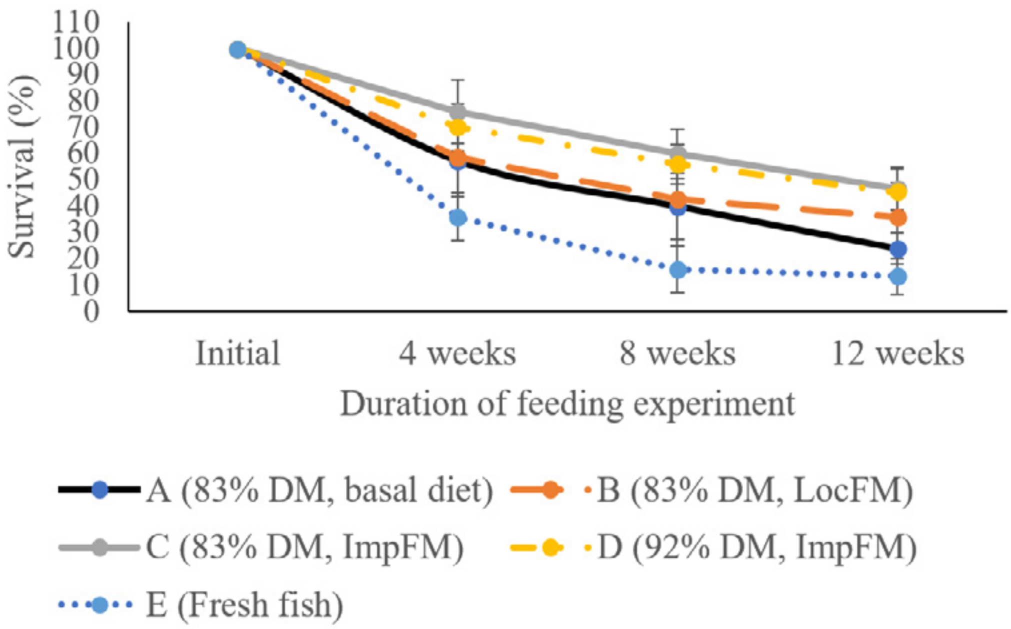

Figure 4 Survival (%) of juveniles of spiny lobsters *Panulirus homarus* fed with different diets for 12 weeks
Notes: DM = dry matter; LocFM = local fish meal; ImpFM = imported fish meal.

Survival

The present study revealed that survival of the lobster juveniles for all treatments decreased significantly during the 12 weeks of the experiment (Fig. 4). Mortalities and cannibalism caused low survival in the present study. High mortalities occurred during the first 4 weeks of the experiment (Fig. 4). This study showed that different characteristics of feeds in this study significantly affected the survival of lobster juveniles (P < 0.05).

Higher survival was observed in juveniles fed with feed containing imported fish meal (diet C and D, 46.66% and 45.3%, respectively), followed by those fed with moist pellets containing local fish meal (diet B, 36%) and the basal diet (diet A, 24%). There were no differences in survival of juveniles fed with moist pellets (83% DM) and those fed with dry pellets (92% DM), i.e., diets C and D (P > 0.05). Fresh fish diet showed the lowest survival (13.33%) (P < 0.05) (Fig. 4).

Based on daily observation, the low survival in diet E (fresh fish) was mainly due to cannibalism and unsuccessful molting. The unsuccessful molting may have been caused by the lack of nutrient content in the diet. In *P. ornatus*, low survival was reported when nutrients in the diet were inadequate (Smith *et al.* 2003). High mortality in juveniles of *P. ornatus* was also found to be mainly caused by cannibalism of newly molted lobsters (Jones *et al.* 2001).

It has been suggested that inappropriate feeding strategy and shelter may contributed to high cannibalism in juveniles of *P. ornatus* (Jones *et al.* 2001).

Results of this study was in line with the survival rates of *P. homarus* juveniles which were significantly higher when they were fed with moist feed compared to those fed with trash fish (Ridwanudin *et al.* 2018). Feeding *P. ornatus* with green mussels (*Perna viridis*) resulted in considerably low survival (6%) after 4 months of rearing (Junio-Menez & Ruinata 1996). Similarly, survival of *P. ornatus* juveniles fed with blue (*Mytilus edulis*) or green-lipped (*P. canaliculus*) mussels declined after 4 weeks of the experiment, with high mortalities occurring at molting (Smith *et al.* 2005).

Juveniles fed with moist (83% DM) and dry (92% DM) pellets containing imported fish meal (Diets C and D) resulted in higher survival, however, it indicated lower growth of juveniles. It was likely that the density of juveniles in the cage affected the growth performance of the juveniles. A study in juveniles of *P. ornatus* showed that the highest survival and weight of lobsters were resulted from the low-density treatment (Jones *et al.* 2001). In the present study, the higher the density, the lower the growth performance of the lobster juveniles. Cannibalism in the present study caused the low survival of the juveniles, however, cannibalism provided nutrition for the predatory lobsters. This incident resulted in an inverse relationship between the growth and survival of lobsters. A previous study also reported that lobsters fed with trash fish had superior growth compared to those fed with commercial lobster pellets. However, an inverse survival relationship had been found, with superior survival achieved by feeding lobsters with commercial pellets (Irvin & Shanks 2015).

CONCLUSION

The best growth performance was shown by juveniles fed with moist pellets containing local fish meal (Diet B) with the final weight reaching 18.74±2.30 g. Moist pellets containing imported fish meal (Diet C) resulted in higher survival (46.66%), while the lowest survival (13.33%) obtained by juveniles fed with fresh fish (Diet E).

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