Effect of Restricted Feeding to Nutrients Utilization of Spiny Lobster (Panulirus homarus)

Pranawengrum Sekartadji
Post Graduate Program, Faculty of Fisheries and Marine Science, Brawijaya University, Malang, East Java, Indonesia, pranawengrums@gmail.com

Arning W. Ekawati
Department of Aquaculture, Faculty of Fisheries and Marine Science, Brawijaya University, Malang, East Java, Indonesia

Mohamad Fadjar
Department of Aquaculture, Faculty of Fisheries and Marine Science, Brawijaya University, Malang, East Java, Indonesia

Abstract

Restricted feeding has been known to have an impact on reducing feed usage without disrupting the growth of spiny lobster (P. homarus). As growth was affected by nutrients i.e protein, lipid, and carbohydrate from feed, informations about the ability of spiny lobster to utilize and store nutrients during feed restricted condition is important. Nutrient utilizations of spiny lobster can be described by digestive enzymes activities and nutrient retentions. Spiny lobster (P. homarus) measuring 50.0 ± 10.0 g was reared for 32 days in a restricted feeding regime by fasting. Lobsters were fasted at intervals of 1 fasting day/1 day of feeding, 1 fasting day/2 days of feeding, and fed daily. At the end of cultivation, spiny lobster (P. homarus) that fed daily showed the highest protease (0.019 IU/ml), lipase (0.02 IU/ml), and amylase (0.66 IU/ml). High protein retention (25.1%) and fat retention (36.5%) were found in spiny lobster (P. homarus) reared with restricted feeding in 1 day feeding/1 day fasting cycle. Feeding management with a restricted feeding system allowed the spiny lobsters (P. homarus) to utilize nutrients from the feed more optimally.

Keywords: *Energy Reserve, Feed, Growth, Lobster, Panulirus.*

INTRODUCTION

Feed is one factor that determines the success of spiny lobster cultivation. Feeding management is related to the growth rate, which if the nutrients in the feed can be utilized optimally it will result in an increase in body weight (Setiawati et al., 2013). Meanwhile, the cost that needed for feed in spiny lobsters (P. homarus) culture can reach 60% of the total operational costs (Petersen and Phuong, 2011), that is partly due to the slow growth and the long rearing time of spiny lobster (Mulyani et al., 2014).

One of the strategy that can be implemented to increase feed efficiency in crustacean culture. Feed restriction is carried out by dividing the feeding time into 2 periods, the period when biota are not fed/starved and the period when biota are fed again (refeeding). This feeding regime has been known to result in compensatory growth, i.e. body weight gain after experiencing fasting (Stumpf et al., 2020). Restricted feeding also has an impact on reducing feed usage without disrupting the growth of spiny lobster (P. homarus) (Karimah et al., 2018).

Spiny lobsters can survive in conditions of limited feed availability by utilizing energy reserves in the form of lipids and proteins to meet specific energy needs without reducing life and growth performance when feed availability is limited and reducing energy use when feed is available. When lobsters are starving, there are changes in the activity of protease, lipase, and amylase enzymes that indicates a critical state of utilization of energy and nutrients which may have undergone metabolism or been utilized at the beginning of the period of food shortage (Johnston et al., 2004).

The activity of digestive enzymes indicates the ability of spiny lobsters to hydrolyze proteins and carbohydrates from feed (Perera and Simon, 2015). According to Haryasakti et al. (2010), digestive enzymes will help in the process of hydration of nutrients present in the feed, such as proteins, fats and carbohydrates into simple molecular forms so as to facilitate the process of digestion and absorption in the digestive tract. Protease activity indicates protein catabolism, lipase activity indicates lipid utilization, and amylase activity indicates carbohydrate catabolism, including starch and glycogen (Johnston et al., 2004). Protease enzyme activity indicates protein catabolism, lipase enzyme activity indicates lipid utilization, and amylase enzyme activity indicates carbohydrate catabolism such as starch and glycogen (Johnston et al., 2004). The lipase enzyme hydrolyzes fat into fatty acids to facilitate fat absorption by the body. Fat from feed is used as a source of energy and maximizes protein for the growth process (Kurniawan et al., 2017).

The ability of organisms to utilize nutrients from feed can be determined by calculating nutrient retention. Protein retention describes the amount of protein that can be utilized to build and repair damaged body cells and be used in metabolism (Sukmaningrum et al., 2014). Fat retention describes the ability of lobsters to absorb and utilize fat from feed to be stored in the body (Ekawati et al., 2019). Information regarding the ability of spiny lobsters (P. homarus) to utilize nutrients under limited feed conditions can be useful in determining the appropriate feeding strategy for aquaculture.

Material and Method

Research method. The research conducted was an experimental study with a completely randomized design consisting of 3 treatments with 3 replications each. Treatments in the study were (A) Fasting for 1 day after 1 day of being fed, (B) Fasting for 1 day after 2 days of being fed, and (C) Feeding every day.

Spiny lobster cultivation. Spiny lobsters (P. homarus) maintenance was carried out for 6 weeks. Average weight of reared spiny lobster was50.0 ± 0.40 g/ind with a stocking density of 7 lobsters/m2. The feed given was fresh mangrove snail meat (Telescopium telescopium) with feeding rate of 20% of the total lobster biomass in each container (Arumugam et al., 2020). Feed were given 2 times a day.

Feed nutrition. Nutrition content of feed were measured by using proximate analysis (AOAC, 2005). Proximate composition of feed that used during spiny lobster culture were showed in Table 1.

Table 1. Nutrition content of mangrove snail (Telescopium telescopium)

Parameters	Amount
Moisture (%)	12,52
Ash (%)	22,60
Protein (%)	34,20
Lipid (%)	1,51
Crude fiber (%)	7,21
Carbohydrate (%)	22,03
Total	100

Sample preparation. Spiny lobsters (P. homarus) was dissected by splitting the shell from the cephalothorax to the tail to take the intestines and meat. The sample organs that

have been taken were put into a plastic clip then stored in a flask. The flask were stored in the freezer to prevent enzyme damage due to changes in temperature above 5oC.

Protease activity. Protease activity was measured based on the method of Bergmeyer and Grassi (1983). Test tubes for blanks, standards and samples were prepared. A total of 1 ml of 0.05 M phosphate buffer pH 7 was put into all test tubes. Furthermore, 1 mL of casein substrate solution of 20 mg/mL pH 7 was added to all test tubes. 0.2 mL of sample was put into a test tube containing the sample. A total of 0.2 mL of 5 mmol/L Tyrosine standard solution was added to the test tube for the standard, while 0.2 ml of distilled water was added to the blank test tube and incubated at 37oC for 10 minutes. Furthermore, as much as 2 ml of 0.1 M TCA solution as much as 2 ml was added to all tubes. 0.2 mL of 2 mmol/L CaCl2 solution was added to the blank and standard tubes, while 0.2 mL of aquadest was added to the sample tube. All mixtures were then allowed to stand at 37oC for 10 minutes, then centrifuged for 10 minutes at 3,500 rpm. The filtrate from each tube was taken as much as 1.5 mL, then 5 mL of 0.4 M Na2CO3 and 1 ml of Folin Ciaocalteau's solution were added to all the tubes. The mixture was allowed to stand for 20 minutes at 37oC and the absorbance was measured with a spectrophotometer at a wavelength of 578 nm.

Lipase activity. Lipase activity was measured using olive oil emulsion as a substrate of Tris-HCl as a buffer according to Borlongan (1990). A total of 1 ml of enzyme crude extract sample was added to 1 ml of stable lipase enzyme substrate in 1.5 ml of buffer containing 0.1 M Tris-HCl at pH 8.0. The mixture was incubated for 6 hours at 37oC, then the hydrolysis was stopped by adding 3 ml of 95% ethyl alcohol. The mixture was then titrated with 0.01 N NaOH using 0.9% thymolphthalein in ethanol as indicator. The blank treatment was carried out in the same way, except that the enzyme crude extract sample was put into the test system after incubation for 6 hours and immediately before the titration. One unit of lipase activity was defined as the volume of 0.01 N NaOH required to neutralize the fatty acids released from the substrate during 6 hours of incubation and after correcting with the appropriate blank.

Amylase activity. Amylase activity was measured using 1% starch solution as a substrate in sodium phosphate buffer pH 6.9, and containing 6.0 mM NaCl following the method of Worthington (1993). 0.5 ml of substrate solution was added to 0.5 ml of crude enzyme extract sample, then incubated for 3 minutes at 95oC. Dinitrosalicylic acid (DNS) was added as much as 0.5 ml, then the sample was incubated again in a boiling water bath for 5 minutes. The absorbance value of the mixture was measured using a spectrophotometer at a wavelength of 540 nm. The amount of maltose released in the test is determined from the standard curve. One unit of enzyme activity is defined as the amount of amylase required to hydrolyze 1 μg maltose per minute.

Nutrient retention. Protein and lipid of muscle and hepatopancreas spiny lobster (P. homarus) were measured by proximate analysis (AOAC, 2005) at the start and the end of rearing period. Protein and lipid retention were calculated by the formula:

Protein retention (%) = ((final body protein content (g) – initial body protein content (g))/feed protein (g)) x 100%.

Lipid retention (%) = ((final body lipid content (g) – initial body lipid content (g))/feed lipid (g)) x 100%.

Statistical analytics. Mean of obtained data were compared using One-Way ANOVA and post-hoc Least Significant Difference (LSD) test at 95% confidence interval were done.

Statistical analytics were done by using IBM SPSS Statistic 22 software for Windows.

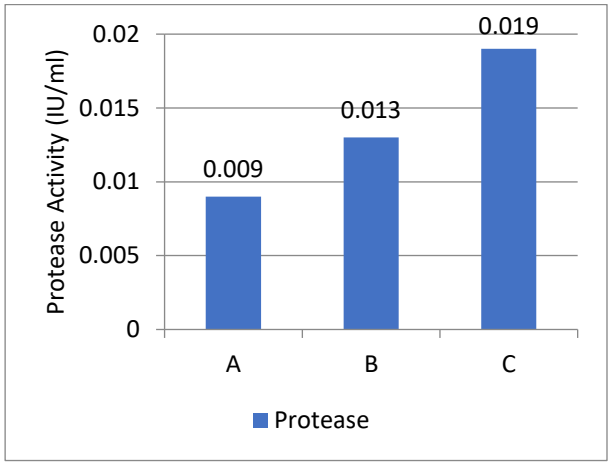
Results and Discussion

Digestive Enzymes Activities. Digestive enzymes activities of spiny lobsters

(P. homarus) were displayed in Fig. 1., Fig. 2., and Fig 3. The lowest enzymes activity was found in lobsters reared with 1 day fasting/1 day feeding i.e. treatment A. The result was assumpted due to the shorter feeding time compared to other treatments. As the lobsters were fasted, less food was digested that impacted to low production of digestive enzymes. According to Dai et al. (2018), crustaceans adapted to starvation by reducing the activity of digestive enzymes. Growth, feed composition, feeding habits, and genetics are known to affect digestive enzyme activities (Shen et al., 2022). The activity of digestive enzymes indicates the ability of spiny lobsters to hydrolyze proteins and carbohydrates from feed (Perera and Simon, 2015). Digestive enzymes helps the process of nutrients hydration such as proteins, fats and carbohydrates into simple molecular forms so as to facilitate the process of digestion and absorption in the digestive tract (Haryasakti et al., 2010).

Highest protease activity were found in The increase in protease activity indicates that protein catabolism occurs, which is an important energy source for lobsters. In contrast, lipase activity decreased indicating that lipids were probably stored as a reserve energy source for molting (Johnston et al., 2004).

Figure 1. Protease Activity of Spiny Lobster. A is 1 day feeding/1 day fasting, B is 2 days feeding/1 day fasting, and C is daily feeding



Low lipase activity in fasted biota indicates that lipids may be stored in the body and used to meet energy needs during molting (Johnston et al., 2004). However, the results of the proximate test on fat in meat and hepatopancreas were not significantly different. The low lipase activity in digestion is thought to be due to the less feed consumed. The lipase enzyme in the digestive tract is known to be unregulated when the organism is in a fasted state, but the intracellular lipase enzyme which is found to be expressed in a number of body tissues (walking legs, swimming legs, gills, hemocytes, muscles, and gonads) is regulated when the organism is starving which shows its role in lipid mobilization (Sacristan et al., 2014).

Figure 2. Digestive Lipase Activity of Spiny Lobster. A is 1 day feeding/1 day fasting, B is 2 days feeding/1 day fasting, and C is daily feeding

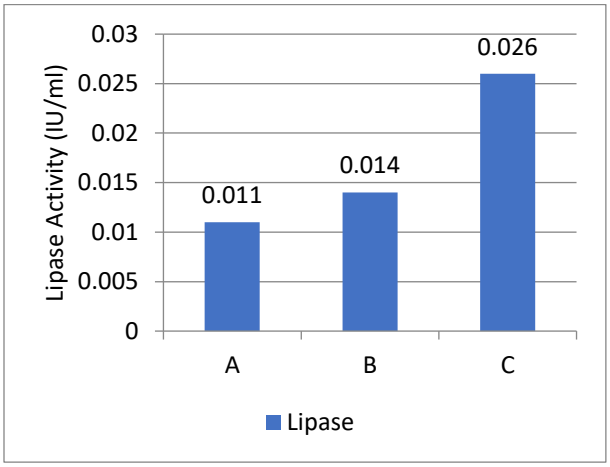
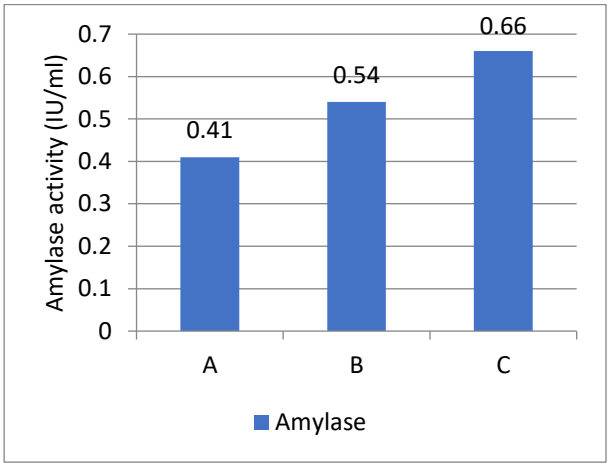


Figure 3. Digestive Amylase Activity of Spiny Lobster. A is 1 day feeding/1 day fasting, B is 2 days feeding/1 day fasting, and C is daily feeding



The low amylase activity in the fasted spiny lobsters (P. homarus) in treatment A and B was assumpted to be the effect of the ability of the spiny lobsters (P. homarus) to accumulate carbohydrates to be used as an energy source. The relationship between decreased amylase activity and feed shortages may illustrate the implementation of energy optimization strategies when available feed is limited (Charron et al., 2014). Hungry lobsters showed lower amylase activity, indicating that carbohydrate accumulation from feed had not occurred and could not be used as an energy reserve (Johnston et al., 2004).

Protein and lipid retention. Table 2. showed the protein and lipid retention of spiny lobster (P. homarus) reared with restriced feeding regime. There were significant difference in both protein retention and lipid retention ($p > 0.05$).

Table 2. Protein retention and lipid retention (mean ± SD) of spiny lobster (P. homarus)

Parameters	Treatments		
	A	B	C
Protein retention (%)	$25,1 \pm 0,80^{a}$	$16,0 \pm 0,52^{b}$	$9,1 \pm 0,62^{c}$
Lipid retention (%)	$36,5 \pm 2,10^{a}$	$12,8 \pm 4,80^{b}$	$11,2 \pm 1,85^{b}$

Different superscripts in the same column shows that there are significant differences ($p <0.05$).

High protein retention indicates the ability of biota to absorb and utilize protein from feed for growth (Ekawati et al., 2019). In line with this statement, spiny lobsters (P. homarus) reared with treatment A produced the highest protein retention and weight gain, which correlated with their growth rate. According to Tantri et al. (2019), the growth rate is

determined by protein which can be absorbed and utilized by the body as a building material. Body tissues bind amino acids and are stored intracellularly to be formed into proteins or body cells. Furthermore, the high growth rate may be due to the more optimal utilization of protein for growth rather than for body energy.

The average muscle protein content at the end of the study was 60.18% (treatment A), 59.44% (treatment B), and 59.01% (treatment C). Even though the feed given to treatments A and B was less than treatment C, the protein levels of lobster muscles at the end of culture were not significantly different. This might be related to the activity of protease enzyme which plays a role in the absorption of protein from digested feed. In this study, fasted spiny lobsters (P. homarus) i.e. lobsters in treatment A and B, showed low protease activity that might be increase when the lobsters consumed feed. Furthermore, fasting was known to have an impact on hyperphagia or an increase in appetite after biota are fasted, which will lead to a higher level of feed consumption (Mulyani et al, 2014). The amount of feed consumed is thought to result in a higher expression of the protease enzyme resulting in more optimal absorption and utilization of protein. According to Cellis-Guerrero et al. (2004), protease enzymes are expressed when organisms consume feed and work to hydrolyze proteins into amino acids that can be absorbed by the body.

The value of lipid retention was related to the level of lipid in the body. Starved lobsters were known to store lipd reserves in body tissues, especially muscles and hepatopancreas that will be used when feed was not available (Gora et al., 2018). According to Kurniawan et al. (2017), absorption of lipid by the body is related to lipase activity which plays a role in hydrolyzing fat into fatty acids. Fasted spiny lobsters (P. homarus) in this study might be able to digest lipid from the feed when the feed is available. Digested fat is then stored in body tissues, mainly in hepatopancreas. Lobsters that reared with more frequent feeding frequency show lower lipid retention presumably because they did not need to adapt to conditions of limited feed so that the lipid from feed tends to be used for metabolism instead of being stored in body tissues. This was in line with the statement of Johnson et al. (2004) that the low activity of the lipase enzyme in starved organisms was probably due to the lipid in the feed being stored in the body's tissues. According to Priya et al. (2013), lipid was utilized by organisms as a source of energy and stored for transportation to organs and body tissues. Utilization of fat as an energy source allows organisms to utilize protein more optimally for growth.

Conclusions

The activity of lobster digestive enzymes decreased during food deprivation. High protein retention, and fat retention were found in spiny lobster (P. homarus) reared with restricted feeding, 25.1% and 36.5% respectively. Feeding management with a restricted feeding system allows the spiny lobsters (P. homarus) to utilize nutrients from the feed more optimally.

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Reference

Arumugam, A., Dineshkumar, R., Rasheeq, A.A., Gowrishankar, M.P., Murugan, S. & Sampathkumar, P. (2020) Growth performance of spiny lobster, Panulirus homarus (Linnaeus, 1758). Indian Journal of Geo Marine Sciences, 49(05): 812-819

Celis-Guerrero, L. E., García-Carreño, F. L., & del Toro, M. A. N. (2004). Characterization of proteases in the digestive system of spiny lobster (Panulirus interruptus). Marine Biotechnology, 6, 262-269.

Charron, L., Geffard, O., Chaumot, A., Coulaud, R., Jaffal, A., Gaillet, V., ... & Geffard, A. (2014). Influence of Molting and Starvation on Digestive Enzyme Activities and Energy Storage in Gammarus fossarum. Plos one, 9(4), e96393.

Dai, W. F., Zhang, J. J., Qiu, Q. F., Chen, J., Yang, W., Ni, S., & Xiong, J. B. (2018). Starvation Stress Affects the Interplay Among Shrimp Gut Microbiota, Digestion and Immune Activities. Fish & shellfish immunology, 80, 191-199.

Ekawati, A. W., Fakhri, M., Abdillah, J., & Indahsari, W. N. (2019). Limbah Bandeng (Chanos Chanos Forsskal) Sebagai Sumber Protein Pengganti Tepung Ikan Dalam Pakan Udang Galah (Macrobracium Rosenbergii De Man). Journal of Aquaculture and Fish Health, 8(3), 149-158.

Haryasakti, A., Imanuddin, I., & Wahyudi, M. (2019). Pengaruh Pemberian Probiotik Terhadap Kandungan Protein Pada Pakan Komersial. Jurnal Pertanian Terpadu, 7(2), 183-189. https://doi.org/10.36084/jpt.v7i2.198

Johnston, D. J., Ritar, A. J., dan Thomas, C. W. (2004). Digestive enzyme profiles reveal digestive capacity and potential energy sources in fed and starved Spiny Lobster (Jasus edwardsii) phyllosoma larvae. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 138(2), 137–144. https://doi.org/10.1016/j.cbpc.2004.02.013

Karimah, U., Samidjan, I., dan Pinandoyo, --. (2018). Performa pertumbuhan dan kelulushidupan Ikan Nila Gift (Oreochromis niloticus) yang diberi jumlah pakan yang berbeda. Journal of Aquaculture Management and Technology, 7(1), 128–135.

Kurniawan, L. A., Manan, A., Arief, M., & Nindarwi, D. D. (2017). Pengaruh Pemberian Probiotik Berbeda Pada Pakan Terhadap Retensi Protein Dan Retensi Lemak Udang Vaname (Litopenaeus vannamei). Journal of Aquaculture and Fish Health. https://www.e-journal.unair.ac.id/JAFH/article/view/11272

Mulyani, Y. S., Yulisman, dan Fitrani, M. (2014). Pertumbuhan dan efisiensi pakan Ikan Nila (Oreochromis niloticus) yang dipuasakan secara periodik. Jurnal Akuakultur Rawa Indonesia, 2(1), 1–12. https://doi.org/10.36706/jari.v2i1.1958

Perera, E., dan Simon, C. (2015). Digestive physiology of spiny lobsters: Implications for formulated diet development. Reviews in Aquaculture, 7(4), 243–261. https://doi.org/10.1111/raq.12066

Petersen, E. H., dan Phuong, T. H. (2011). Bioeconomic analysis of improved diets for lobster, Panulirus ornatus, culture in Vietnam. Journal of the World Aquaculture Society, 42(1), 1–11.

Sacristán, H. J., Nolasco-Soria, H., & Greco, L. S. L. (2014). Effect of attractant Stimuli, Starvation Period and Food Availability on digestive Enzymes in the Redclaw Crayfish Cherax quadricarinatus (Parastacidae). Aquatic Biology, 23(1), 87-99.

Setiawati, J. E., Adiputra, Y. T., & Hudaidah, S. (2013). Pengaruh penambahan probiotik pada pakan dengan dosis berbeda terhadap pertumbuhan,

kelulushidupan, efisiensi pakan dan retensi protein ikan patin (Pangasius hypophthalmus). E-Jurnal Rekayasa Dan Teknologi Budidaya Perairan, 1(2), 151–162.

Shen, W., Qu, Y., Zhang, H., Zhang, X., Masell, F. B., & Ren, G. (2022). Response of Gut Microbiota, Digestive Enzyme Ability, and Immune Function to Starvation in the Oriental River Prawn Macrobrachium nipponense. Aquaculture Nutrition, 2022.

Stumpf, L., Timpanaro, S., Battista, A., dan López Greco, L. (2020). Effects of intermittent starvation on the survival, growth, and nutritional status of the Freshwater Prawn Macrobrachium borellii Nobili, 1896 (Decapoda: Caridea: Palaemonidae). Journal of Crustacean Biology, 40(5), 489–497. https://doi.org/10.1093/jcbiol/ruaa051

Sukmaningrum, S., Setyaningrum, N., & Pulungsari, A. E. (2014). Retensi protein dan retensi energi ikan cupang plakat yang mengalami pemuasaan. Omni-Akuatika, 10(1).

Tantri, A.F., Rahardja, B.S. dan Agustono, A.,)2019). Penambahan lisin pada pakan komersial terhadap retensi protein dan retensi energi udang galah (Macrobrachium rosenbergii). Journal of Aquaculture and Fish Health, 5(2), pp.78-84