Aceh Journal of Animal Science (2024) 9 (1): 10 - 17

Aceh Journal of Animal Science

Journal homepage: www.jurnal.unsyiah.ac.id/AJAS

Amino acid profiles and growth performance of spiny lobster *Panulirus ornatus* fed on combination of three mollusk meals

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ARTICEL INFO

Keywords:
amino acid,
weight gain,
mollusk meal,
spiny lobster

Received: 04 December 2023
Accepted: 24 February 2024
Available online: 28 February 2024

DOI: 10.13170/ajas.9.1.35793

ABSTRACT

Spiny lobster is an economically important marine fish commodity that has a high price and one of the important protein source for people worldwide. The quality of the protein is strongly depending on the amino acid compositions and concentrations. This study aimed to determine of amino acid profiles and growth of spiny lobster (*Panulirus ornatus*) fed with the diet contained combination of three mollusk meal. Four experimental diets were prepared to contain of 40% fish meal,FM (Diet A), 15% telescopium meal, + 15% gold snail meal + 10% scallops meal (Diet B), 15% telescopium meal + 10% gold snail meal + 15% scallops meal (Diet C), 10% telescopium meal + 15% gold snail meal + 15% scallops meal (Diet D) in the test diet. A total of 36 spiny lobster juvenile (initial weight : 120± 15,38 g) were distributed into twelve plastic tanks (three lobster/ tank) with sized of 60×50 ×50 cm. Feeding the lobster two times a day (08.00 a.m and 05.00 p.m) in dosage of 8% total biomass weight for 60 days of rearing period. The experiment was designed by using completely randomized design with four treatments and three replications. Collecting data included of weight gain, survival rate, and amino acid content in the fish body. The results showed that feeding the lobster with different feed test was significantly different in weight gain of spiny lobster. The weight gain of spiny lobster fed with combination of mollusk meal had higher than the feed contain fish meal. The highest amount level of amino acid in the lobster body was leucine , while the lowest one was tryptophan. The types and total number of some amino acid profiles in the fish body was similarly with amino acid profile in the diets which contained mollusk meal. This study concluded that the amino acid profiles in the diet contained mollusk meal were suitable for requirement to improve the growth of spiny lobster.

Introduction

Spiny lobster is an economically important marine fish commodity that has a high price and it is included in Indonesia's fisheries export commodity. Generally, there are 6 species of lobster has been cultivated in Indonesia, namely spiny lobster (*Panulirus ornatus*), bamboo lobster (*Panulirus versicolor*), painted lobster (*Panulirus longiceps*), sand lobster (*Panulirus homarus*), rock lobster (*Panulirus penicillatus*), and slipper lobster (*Thenus orientalist*). The advantages of rock ornate lobsters compared to other types of lobsters are that they are more expensive and grow faster.

Apart from genetic factors, the growth of lobsters, including ornate rock lobsters, is also determined by the availability of quality feed, especially protein. The quality of a protein is related to the amino acid profile it contains. Classification of amino acids is based on the body's ability to synthesize them and their metabolic needs. This classification is known as essential and non-essential amino acids (Nina and Wayan, 2018). Most animals, including fish, require 10 essential amino acids, namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenyl alanine, threonine, tryptophan and valine (NRC, 1993).

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Printed ISSN 2502-9568; Electronic ISSN 2622-8734

Several studies related to the optimum protein requirements of nursery size (juvenile) spiny lobsters required 55 % protein and 9% lipid in feed, spiny lobster (*Sagmariasus verreauxi*) required 50% protein in the diet (Wang *et al*., 2021) , *Panulirus ornatus* juveniles required 59.6% of protein in the feed formulation (Arumugam *et al*., 2020; Saputra and Fotedar, 2022). The compatibility of amino acids between the feed provided and the amino acids in the rock ornate lobster's body is a determining factor for increasing growth. Fish including lobster could grow optimally when the feed can be used efficiently and has a complete amino acid profile and it is suitable or similar to amino acid in the body of the fish being farmed (Maryam *et al*., 2019).

In the natural habitat, lobsters consume feed from the best protein sources, with mollusks dominated by bivalves, gastropods, and chitons being the preferred type in lobster feed. Additionally, crustaceans (predominantly barnacles, crabs, and other decapods), polychaete worms, echinoderms, and a small amount of macroalgae are also part of their diet (Williams, 2007; Astuti *et al*., 2023). Fresh green mussel and pellet feed containing green mussel showed higher lobster growth compared to those fed with dry feed containing green mussel (Landman *et al*., 2021; Simon and Jeffs, 2008). Feed ingredients from mollusks, such as telescopium meal, have the potential to enhance the growth of black tiger shrimp (Kurnia *et al*., 2016). Other mollusk-based feed ingredients with potential include golden snail meal and mud scallops meal. The protein content of golden snail meal and mud scallops meal were 44% and 46.75%, respectively (Nurjannah *et al*., 2017; Kelana *et al*., 2015).

The utilization of fresh golden snail as feed has been shown to enhance the growth performance of mud crabs (Rahmawati, 2022). The inclusion of golden snail meal in feed has demonstrated growth improvement in vannamei shrimp (Agustono *et al*., 2016), mud crabs (Raznawati *et al*., 2020), and Lanchester's freshwater prawns (Tongmee *et al*., 2021). Meanwhile, the use of the mollusk species mud clam has been found to enhance growth in mud lobsters (Kizakhudan and Pitel, 2011). The provision of fresh mollusk shells results in the highest growth rate in sand lobsters compared to feeding with white fish meat, beef, or their mixture (Kotiya and Vadher, 2020). However, information regarding the combination of these three mollusk meals (mangrove snail meal, golden snail meal, and giant clam meal) in feed to enhance the growth of rock ornate lobsters is still limited.

Therefore, the purpose of this study was to observe and analyze the growth and amino acid profile of lobster fed on combination of three species of mollusk meals: telescopium meal (*Telescopium telescopium*), gold apple snail meal (*Pomacea canaliculata*), and mud scallops meal (*Polymesoda erosa*).

Materials and Methods

Research time and location

This study was conducted over a period of three months (August – October 2023) at the Fish Breeding Laboratory, Faculty of Fisheries and Marine Sciences, Halu Oleo University, Kendari. Proximate analysis and amino acid content of the test feed were conducted at the PT Saraswanti Laboratory in Bogor, West Java.

Test feeds

The test feed in this study took the form of pellet formulations with a protein content of 36%. Four types of test feeds with different composition of protein sources consisted of : 20% sardine fish meal (SFM) + 20% mackerel fish meal (MFM) (Diet A), 10% telescopium meal (TM) + 15% gold snail meal (GSM) + 15% mud scallops meal (MSM) (Diet B), 15% TM + 10% GSM + 15% MSM (Diet C), and 15% TM + 15% GSM + 10% MSM (Diet D) in the test diet. The other feed ingredients composing the lobster test feed in the study were include shrimp head meal, soybean meal, corn meal, fine bran meal, sago meal, corn oil, fish oil, squid oil, and minerals and vitamins mixture. The detailed formulation of the test feed and proximate analysis results are presented in Table 1.

Table 1. Feed formulation and results of proximate analysis of rock ornate lobster Test Feed

Feed Ingredients	Test Feed (g/100 g feed)			
	Feed A	Feed B	Feed C	Feed D
Sardine fish meal	20	0	0	0
Mackerel fish meal	20	0	0	0
Telescopium meal	0	10	15	15
Gold snail meal	0	15	10	15
Mud scallops meal	0	15	15	10
Shrimp head meal	15	15	15	15

Soybean meal	25	25	25	25
Corn meal	9	9	9	9
Fine bran meal	5	5	5	5
Sago meal	4	4	4	4
Fish oil	0.5	0.5	0.5	0.5
Squid meal	0.5	0.5	0.5	0.5
Corn oil	0.5	0.5	0.5	0.5
Vitamins and minerals mix.	0.5	0.5	0.5	0.5
Total	100	100	100	100
Results of proximate analysis (%)				
Moisture	9.93	9.36	9.15	9.71
Crude Protein	38.52	33.76	35.52	35.56
Crude Lipid	2.96	1.74	1.99	1.11
Crude Ash	13.17	11.34	10.39	10.9
Fiber	9.66	10.39	11.36	11.12
Carbohydrate	35.42	43.80	42.95	42.72
Lipid Energy (Kcal/100 g)	26.64	15.66	17.91	9.99
Total Energy (Kcal/100 g)	322.4	325.9	331.79	323.11

Lobster rearing

A total of 36 rock ornate lobsters (average initial weight: 126.7±9.38 g) were placed into 12 plastic tanks (3 lobsters/tank) measuring 60×50×40 cm. Prior to the experiment, all rock ornate lobsters were acclimated to the research conditions for one week with the test feed provided once a day (4:00 p.m.). One day before start the experiment, the test lobsters were fasted to obtain the initial weight of all test lobsters, ensuring that only the pure body weight was measured without the weight of any feed in their bodies.

Fish maintenance and feeding were conducted for 50 days with daily feeding at a dosage of 3% of the total body weight. Weighing of rock ornate lobsters was performed three times on day 0, day 25, and day 50. The maintenance system for the test lobsters utilized a recirculating system with a weekly water exchange of 50% of the total water volume. Siphoning was conducted every morning before feeding the lobster. Observations of temperature, salinity, dissolved oxygen (DO), and pH were carried out daily.

Data Processing

Some of the data measured in this research include Weight gain (WG)

The WG value of lobster was determined by comparing the initial and end weights. Weight gain of lobster can be calculated using the formula: WG = Wt – Wo (Peterson *et al.*, 2012)

Note: WG is weight gain (g), Wt is final weight (g), W0 is initial weight (g).

Daily growth rate (DGR)

DGR is the average daily weight gain of shrimp and is calculated using the formula:

DGR=$[(Wt-Wo)/t]$ (Muchlisin *et al.*, 2016a)

Note: DGR is daily growth rate (g), Wt stands for final weight (g), W0 stands for initial weight (g), and t stands for research time (day).

Specific growth rates (SGR)

SGR were a parameter that used to overcome problems associated with exponential growth rates (Crear *et al.*, 2020).

SGR (% BW day− 1) = (ln final mean lobster weight − ln initial mean lobster weight) * 100/number of days

Feed conversion ratio (FCR)

FCR was calculated by devided the amount of feed consumption by the total weight growth of lobster: FCR =$[F/Wt\text{-}Wo]$ (Muchlisin *et al.*, 2016b).

Where, FCR is feed conversion ratio, F stands for weight of the feed consumed during rearing (g), Wt is for lobster weight at the end of rearing (g), W0 is lobster biomass at the beginning of rearing (g).

Feed efficiency (FE)

FE is defined as total amount of feed that can be digested by lobster. Good feed quality will show a high efficiency value as well. Formula of Feed efficiency calculation is opposite with FCR: FE = $1/FCR \times 100$ (Peterson *et al.*, 2012) Where: FE is feed efficiency (%), FCR is feed conversion ratio.

Proximate and Amino Acid Analysis of Feed

Proximate analysis of the feed includes determining the moisture content using the drying method in an oven at 105°C, protein content analysis using the Kjeldahl method, fat content analysis using the Soxhlet method, ash content analysis by burning the sample in a furnace at 600 °C, and crude fiber analysis using a vacuum pump (Takeuci, 1988). Meanwhile, the analysis of feed amino acids was conducted at the Saraswanti

Indogenetech Laboratory in Bogor, West Java.

Data Analysis

Research data, including absolute growth, specific growth rate, daily growth rate, feed consumption, feed efficiency, feed conversion ratio, survival rate, and moulting frequency, were analyzed using analysis of variance (ANOVA). If there were significant effects, the Duncan test was then applied to determine the best treatment and significant differences between treatments. The data from amino acid analysis of the feed and water quality were analyzed descriptively.

Results

The results of proximate and amino acid analysis of protein test feed consisting of telescopium meal, golden snail meal, and mud scallops meal are presented in Tables 2 and 3. Meanwhile, the results of weight gain (WG), specific growth rate (SGR), daily growth rate (DGR), feed consumption (FC), feed efficiency (FE), feed conversion ratio (FCR), and survival rate (SR) of rock ornate lobsters after 50 days of maintenance are presented in Table 5.

In Table 2 showed that the highest of crude protein content was found in telescopium meal (67.6%) and followed by mud scallops meal (54.66%) and gold snail meal (35.2%). Also telescopium meal contained crude lipid (6.35%) was more higher compared to gold snail meal (3.58%) and mud scallops meal (4.26%). However, gold snail meal and mud scallops meal contained higher crud ash (35.94% and 25.57%, respectively) than telescopium meal (772%).

Results of amino acid profiles (Table 3), showed that commonly mud scallops meal contained more higher amino acid content than amino acid content in telescopium meal and gold snail meal. The highest of some amino acids in mud scallops meal were aspartate acid, glutamic acid, serine, glycine, arginine, threonine, alanine, tyrosine, valine, isoleucine, leucine, phenylalanine, and lysine. Telescopium meal had higher content of amino acid only in histidine (13500 mg/kg) and proline (28500 mg/kg) compared to histidine and proline in gold snail meal and mud scallops meal. The lowest of amino acid content between three mollusk meal was gold snail meal.

Table 2. Results of proximate analysis of Telescopium meals, Golden snail meals, and Mud scallops meals

Parameters	Telescopium meal	Gold snail meal	Mud scallops meal
Moisture (%)	9.64	10.76	5.92
Crude Protein (%)	67.6	35.2	54.66
Crude Lipid (%)	6.35	3.58	4.26
Crude Ash (%)	7.72	35.94	25.57
Fiber (%)	2.54	2.59	2.10

Table 3. Results of amino acid analysis of telescopium meal, gold snail mail, and mud scallops meal

Amino Acids	Telescopium meal (mg/kg)	Gold snail meal (mg/kg)	Mud scallops meal (mg/kg)
Aspartat acid	26900	22926	43753
Glutamic acid	39700	32207	67277
Serine	9000	14528	28283
Glycine	20100	16027	30265
Histidine	13500	5435	11780
Arginine	15300	20537	48370
Threonine	8200	15837	28461
Alanine	7900	18035	31192
Proline	28500	10989	21716
Tyrosin	11000	10158	18873
Valine	7200	16771	27011
Iso-leucine	14200	14098	25393
Leucine	17300	24421	43806
Phenylalanine	8400	15580	24111
Lysine	21200	15199	35837

Table 4. Results of amino acid in the test diet for rock ornate lobster

Amino acids	Test Diet (%)			
	A	**B**	**C**	**D**
Alanine	1.81	1.55	1.68	1.65
Arginine	2.05	2.66	2.84	2.77
Aspartat acid	2.38	2.09	2.25	2.19
Glycine	2.01	1.96	2.1	2.07
Glutamic acid	3.79	3.45	3.71	3.67
Histidine	1.16	1.05	1.07	1.12
Iso-leucine	1.43	1.2	1.27	1.25
Leucine	2.51	2.28	2.44	2.4
Lycine	1.91	1.33	1.44	1.3
Valine	1.71	1.45	1.55	1.52
Phenylalanin	1.83	1.77	1.86	1.89
Proline	1.51	1.37	1.49	1.47
Serine	1.68	1.79	1.88	1.83
Threonin	1.8	1.75	1.81	1.8
Tyrosin	1.16	1.23	1.29	1.31

Results of amino acids in the test diet showed that the highest of amino acid content was glutamic acid (3.45 – 3.79%) and the lowest of amino acid in the diet was histidine (1.05 – 1.16%). The diet that contained fish meal only (Diet A: without mollusk meal) had more higher in some amino acids content (such as : alanine, aspartate acid, glutamic acid, histidine, iso-leucine, leucine, lysine, valine, and proline) than diet contained mollusk meal (Diet B, Diet C and Diet D). Between test diets that contained of combination of mollusk meal showed that Diet C had more higher content of alanine (1.68%), arginine (2.84%),aspartate acid (2.25%), some amino acids . In table 5 showed that the growth performance (WG, DGR, SGR, FC, FE, FCR and SR) of rock ornate lobster fed with Diet B had more higher values than the other treatments. The results of WG, GDR and SGR of the lobster fed with test diet B were 6.84±3.13 g, 0.137±0.04% and 0.084±0.05%, respectively. In addition values of FC, FE and FCR of the lobster in treatment B were 348.84±9.97g, 1.97± 0.97% and 56.18±26.22, respectively. During the experiment, all water quality parameters were optimum. The water temperature and water salinity were ranged between 28-31 ^{0}C and 25-30 ppt. While the pH and ammonia value were ranged between 5-7 and 0.0031-0.0037 mg/l (Table 6).

Table 5. Weight gain, specific growth rate, daily growth rate, feed consumption rate, feed efficiency, feed conversion ratio, and survival rate of rock ornate lobsters

Parameters	Treatment			
	A	**B**	**C**	**D**
WG (g)	2.45±1.42^{a}	6.84±3.13^{b}	5.025±0.67^{ab}	3.19 ± 1.26^{a}
DGR (g/day)	0.049±0.02^{a}	0.137±0.04^{b}	0.100±0.01^{ab}	0.064±0.02^{a}
SGR (%)	0.039±0.02^{a}	0.084±0.05^{a}	0.056±0.03^{a}	0.051±0.02^{a}
FC (g)	240.77±4.63^{d}	348.84±9.97^{b}	273.67±8.41^{c}	294.65±0.58^{b}
FE (%)	1.01 ± 0.57	1.97± 0.97	1.83±0.19	1.28±0.35
FCR	117.83 ± 66.60	56.18±26.22	54.84±5.65	81.11±22.18
SR (%)	100	100	100	100

Table 6. Results of water quality parameters of the experiment

Parameter	Results	Optimum values
Temperature	28-31 ^{0}C	24-31 ^{o}C (Bunga, 2008)
Salinity	25-30 ppt	28-33 ppt (Subiyakto and Cahyaningsih, 2003)
pH	5-7	8.0 – 8.2 (Suprakto ane Fahlevi, 2007)
Ammonia	0,0031-0,0037 mg/l	< 0.00 (Bunga, 2008)
Dissolved oxygen	4.5-5.7 mg/l	3.95 – 4.28 (Subiyakto and Cahyaningsih, 2003)

Discussions

The results of water quality analysis (temperature, salinity, pH, ammonia content, and dissolved oxygen) during the study indicated optimal values to support the growth and survival of rock ornate lobsters. Meanwhile, the analysis results of the nutritional quality of the test feeds (Feed A, Feed B, Feed C and Feed D) in terms of proximate analysis values (protein, fat, moisture, ash, and crude fiber) remained within the optimum limits for the nutritional needs of rock ornate lobsters. The protein content of the test feed for rock ornate lobsters ranged from 33.76% to 38.52%. The protein content of the test feed containing fish meal was slightly higher than the protein content of the test feed containing a combination of mollusk meal. This is because the protein content of fish meal consists of mackerel fish meal at 74.6% (Cahyono and Mardani, 2020) and sardine fish meal protein at 66.67%, which is higher than the protein content of golden snail meal and mud scallops meal, which were 35.2% and 54.7%, respectively. Except for telescopium meal, which has a protein content similar to that of fish meal at 67.6% (Kurnia *et al*., 2016). However, the protein content of the test feed is still lower compared to the protein content of test feeds in previous lobster studies (Kurnia *et al*., 2016; Barclay *et al*., 2006; Landman *et al*., 2021).

The highest amino acid content in telescopium meal compared to golden snail meal and mud scallops meal was proline and histidine at 28,500 mg/kg and 13,500 mg/kg, respectively. Meanwhile, the amino acids contained in golden snail meal, which lives in freshwater in rice fields, generally have lower amounts content of amino acid compared to both mollusk types living in seawater (telescopium meal and mud scallops meal). The highest amino acid in golden snail meal was glutamic acid, and the lowest amino acid is valine. Meanwhile, the highest amino acid in mud scallops meal was glutamic acid at 67.277 mg/kg, and the lowest amino acid in giant clam meal was histidine at 11.780 mg/kg. Overall, the amino acid content in mud scallops meal was higher than the amino acid content in telescopium meal and golden snail meal.

The test feeds were formulated based on the differences in feed ingredients by comparing the growth of lobsters fed a combination of fish meal (mackerel fish meal and sardine fish meal) and a combination of mollusk meal (telescopium meal, golden snail meal, and mud scallops meal). The amino acid analysis results of the all test feed showed that the highest amino acid content was glutamic acid (3.79 mg/kg), and the lowest amino acid content in the test feed was histidine (1.05 mg/kg). Glutamic acid plays a crucial role in amino acid metabolism due to its involvement in transamination reactions and is required for the synthesis of key molecules, such as glutathione, needed to eliminate highly toxic peroxides and polyglutamate folate cofactors. Dietary supplementation with glutamic acid to farmed fish can improve their growth performance, intestinal development, innate and adaptive immune responses, skeletal muscle development and fillet quality, ammonia removal, and the endocrine status. Similar glutamic acid values have been reported in other fish species such as mackerel and red salmon and in beef as well (Li *et al*., 2020).

Statistical analysis using analysis of variance (ANOVA) showed that the test feed significantly affected lobster growth. This is because of the protein content of the test feed relatively has the same protein content except for Feed A, which has a slightly higher protein content (38.52%) compared to the other test feeds that contained combination of mollusk meal , Feed B (33.76%) Feed C (35.52%) and Feed D(35.56%). However, the protein content of the test feed is still lower than the protein requirement of rock ornate lobsters in general, which requires a protein content of 47.8-60.0% (Kurnia *et al*., 2016; Landman *et al*., 2021).

At the end of the study, it was observed that the absolute growth and feed efficiency of lobsters fed a test feed containing a combination of mollusk meal were higher than those of lobsters fed a feed containing only fish meal. This study's results align

with the findings of Landman *et al.* (2021), Marchese *et al.* (2018), Kurnia *et al.* (2016), and Murugam *et al.* (2020). The absolute growth of rock ornate lobsters in this study was lower than in previous studies (Smith et al., 2005; Landman et al., 2021). This is due to the difference in the test animal species, which is the slipper lobster. One factor influencing growth is species differences (Dangeubun *et al.*, 2019). The rock ornate lobster fed with combination of 10% telescopium meal,15% gold snail meal and 15% mud scallops meal in the diet (Diet B) had highest in WG, DGR, FC, FE, and FCR compared to other treatments. Meanwhile, the results of feed protein analysis showed that protein content in diet B was the lowest compared to the protein content of other treated feeds.. These results were different with the results of previously experiments that stated that optimum protein to rearing the lobster which ranged between 45% - 60% (Kurnia *et al.*, 2016; Barclay *et al.*, 2006; Landman *et al.*, 2021). Total of feed consumption of the ornate spiny lobster in this experiment was lower. It might be caused the test diet didn't have any contain attractant compounds (Rivaie *et al.*, 2023).

Furthermore, the survival data of female rock ornate lobsters until the end of the study ranged from 77.5% to 80%. The analysis of variance results showed that the differences in the test feed provided did not significantly affect the survival rate of rock ornate lobsters (P>0.05). The survival rate of rock ornate lobsters in this study is similar to the survival rate of rock ornate lobsters in the study by Syafrizal *et al.* (2018). This value is also lower than the results of the study by Rahmawati (2013), which reported a survival rate of rock ornate lobsters in her research at 95-98%. According to Harahap *et al.* (2023) , the survival of cultured fish is influenced by internal and external factors.

Conclusion

Based on the above description, it can be concluded that, the combination of mollusk meal, consisting of 10% mangrove snail meal, 15% golden snail meal, and 15% giant clam meal, results in the highest growth of marine lobsters.

Acknowledgment

Thank you very much to Ministry of Education, Culture, Research and Technology of Indonesia for funding this research through scheme of Fundamental Research in 2023.

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