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The Effect of Various Levels of Protein and Carbohydrate in the Feed on the Carcass, Chemical Composition, and RNA/DNA Ratio of the Mud Crab (*Scylla olivacea)* in an Apartment System

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ABSTRACT

Protein, despite its high cost, is crucial for the growth of mud crabs. To optimize protein efficacy, carbohydrates are used combined. In an attempt to minimize the expensive protein- rich feed and reduce the production costs of crab cultivation, an innovative method has been used, namely the apartment system. Thus, the current study aimed to investigate the effects of various levels of protein and carbohydrate in the feed on the carcass, chemical composition, and RNA/DNA ratio of mud crabs undergoing fattening in an apartment system. This experiment used a completely randomized design (CRD) with five treatments and three replicates consisting of different protein and carbohydrate levels: A (P 60%-C 20%), B (P 50%-C 30%), C (P 40%-C 40%), D (P 30%-C 50%), and E (P 20%-C 60%). The study utilized monosex mud crabs (*S. olivacea*) with an average weight of 129.59± 0.98 grams. A total of 150 crabs were placed individually in crab boxes measuring 40 x 36 x 16cm^3 at a feeding rate of 5% body weight, and the feeding frequency was two times/day over a period of 60 days. The results showed that the levels of protein and carbohydrate feed had a significant effect ($P< 0.05$) on the protein content, meat carcass, and RNA/DNA ratio of mud crabs, but had no significant effect ($P> 0.05$) on the levels of fat, crude fiber, and nitrogen free extract (NFE) mud crab. Protein and carbohydrates levels of 40% produced the highest values for protein content (68.25%), meat carcass (51.25%), and RNA/DNA ratio (1.52ng/nμL) of the mud crabs. Other ranges of body chemical composition values for mud crabs include fat content (14.10-15.92%), crude fiber (1.16- 1.54%), and NFE (2.11- 2.44%). Thus, protein and carbohydrates levels of 40% in feed can improve the quality of the mud crab meat. Addintional research is required to further explore this topic comprehensively, ideally using a more economical protein source and a more improved filter.

INTRODUCTION

The cultivation of the mud crab (*Scylla olivacea*) continues to expand in line with the high consumer demand for mangrove crabs, both for domestic consumption and as an export commodity **(Rinaldy *et al*., 2023)**. One of the cultivation systems that has begun

to develop by the community is mud crab fattening, which is considered more profitable **(Islam *et al*., 2021)**. The mud crab fattening is typically conducted in mangrove areas or ponds. However, there are shortcomings to be considered when applying this method **(Yulianto *et al*., 2019: Putra *et al*., 2023)**. These include the requirement of a large area of land, high cannibalism, and low safety. Furthermore, monitoring and harvesting are difficult, which results in a decrease in productivity **(Islam *et al*., 2021)**. Consequently, it is imperative to implement measures to facilitate the fattening of the mud crabs in a controlled manner. One such measure is the utilization of vertical crab houses.

The vertical crab house, a novel approach to mangrove crab cultivation utilizing PVC or plastic boxes arranged vertically in a controlled environment with a recirculation system, offers various benefits such as space efficiency, prevention of cannibalism, improved feeding efficiency, water conservation, and enhanced monitoring capabilities **(Aqza *et al*., 2023)**. The recirculation system, comprising multiple filters, positively impacts the maintenance media quality by optimizing nitrogen pollutant decomposition in the water **(Lu *et al*., 2020)**. An essential factor influencing the success of fattening mud crabs in an apartment system is the quality of the feed, necessitating a feeding regimen that meets the crabs' nutritional requirements in terms of both quality and quantity **(Wahida *et al*., 2022)**. The increasing use of artificial feed in crab cultivation is gaining popularity due to its perceived profitability, continuous availability, ease of preparation, and the ability to tailor the nutritional content to the cultivar's needs **(Haryati & Trijuno, 2021)**.

The nutrient balance of aquaculture feed is a critical determinant of the success of aquaculture operations. Protein, a crucial component of aquaculture feed, is also the most expensive ingredient, which can significantly impact production costs. Therefore, it is essential to maintain the percentage of protein within an optimal range to ensure efficient feed utilization and minimize costs **(Olakiya & Kotiya, 2022)**. The protein requirement of the mud crabs has been reported to fall within a range of 30 to 47%, emphasizing the importance of balancing protein levels in the diet **(Nguyen *et al*., 2022)**. Carbohydrates, on the other hand, represent a cost-effective source of non-protein energy and can be used to reduce the percentage of feed protein **(Zhang *et al*., 2022)**. By acting as a protein-sparing effect, carbohydrates can serve as a substitute energy source for protein, allowing for the full utilization of energy derived from protein for protein synthesis and meat production **(Wen *et al*., 2022)**. Additionally, low levels of feed protein can result in reduced nitrogen waste discharge in water, which is a significant environmental benefit **(Hasnidar *et al*., 2024)**. Nevertheless, elevated carbohydrate levels can impede growth and energy metabolism in the body. As reported by **Zhan *et al*. (2020)**, the carbohydrate requirements for the crabs range from 25.64 to 32.50%. A 49% carbohydrate level in the feed results in a lower nitrogen discharge in the water compared to a 30% level **(Hasnidar *et al*., 2024)**. The optimal balance of protein and carbohydrates in the diet is

crucial for enhancing the efficiency of feed protein utilization, thereby improving the quality of meat and rearing media for the mud crabs.

The current understanding of the optimal balance of protein and carbohydrate levels in the diet for fattening the mud crabs reared in apartment systems is still limited. Given the significant impact of feed composition on growth, survival, and meat quality, it is crucial to investigate the effects of varying protein and carbohydrate levels on the chemical composition of the body, carcass-to-body weight ratio, RNA-to-DNA ratio, and rearing media quality. This study aimed to investigate the effects of different protein and carbohydrate levels on these parameters to optimize the nutritional support for the mud crabs in apartment systems, ultimately enhancing their overall health and meat quality.

MATERIALS AND METHODS

Description of the study sites

This research was conducted at the CV. Kreatif Laut Indonesia, Barru Regency, South Sulawesi, Indonesia. Proximate analysis of the mud crab and experimental feed was conducted at the Chemistry of Feed Labrotary, Faculty of Animal Science, Hasanuddin University. RNA/DNA ratio analysis was conducted at the brackish water aquaculture center, Takalar Regency, South Sulawesi, Indonesia.

Experimental animals and procedure

The experimental design was a completely randomized design (CRD) with five treatments and triplicate. A total of 150 mud crabs (129.57± 0.98) were obtained from the crab collectors in Mangempang Village, Barru District, Barru Regency, South Sulawesi, Indonesia. The crabs were adapted for one week (they were fed two times daily with their respective control feed) and then divided randomly into five experimental groups with three replicates at rate of 30 crabs/ treatment in boxes with size of 40 x 36 x 16cm^3. All boxes were arranged vertically and equipped with a recirculation system (Fig. 1). Feeding frequencies were two times/day (06:00 AM and17:00 PM).

Fig. 1. Experimental design

Experimental feed

The preparation of artificial feed for this study involved a multi-step process that began with the selection and processing of various ingredients. The ingriedents used in this study include fish, corn, and soybeans, as well as fish silage and fresh fish. The dry ingredients were initially pulverized into flour. To produce fish silage, the fish were first scaled and washed before being grounded into a smooth consistency. The grounded fish were then placed in a bucket covered with a plastic bag and mixed with 1.5 liters of formic acid per 50 kilograms of fish. The mixture was stirred four times a day for five days, after which it was left to stand for two days. The water on the surface was removed, and the mixture was then inoculated with microorganisms at a dose of 10 milliliters per 100 grams. Moreover, the mixture was incubated for seven days. The fish were then scaled and washed thoroughly before being drained and mashed. All ingredients were mixed, and five experimental feeds were formulated. The pellets were made and dried for about 72 hours at 32^0C. The particle size of the diets was 1cm. The feed was then placed in plastic bag and stored in a dry location until the further test **(Aslamyah *et al*., 2022)**. The result of the proximate analysis of the experimental feed are presented in Table (1).

Table 1. Composition (% dry matter) of feed ingredients and results of feed proximate analysis

Composition	Treatment of protein and carbohydrate ratio of feed (%)				
	(P 60-C 20)	(P 50-C 30)	(P 40-C 40)	(P 30-C 50)	(P 20-C 60)
Water (%)	8.43	8.17	8.35	9.17	9.32
Ash (% wt)	8.42	7.53	6.09	6.03	5.5
Protein (% wt)	59.62	49.50	39.65	29.54	19.44
Fat (% wt)	8.03	7.81	7.78	6.87	6.79
Crude fiber (% wt)	4.07	5.82	6.8	7.91	8.87
BETN (% wt)	19.85	29.34	39.67	49.65	59.40
DE (kcal/kg.)[1]	3,233.77	3,098.43	3,010.11	2,831.64	2,715.58
C/P (DE/g Protein)	5.42	6.26	7.59	9.59	13.97

[1]Calculation results based on the energy equation (NRC, 1988): 1g carbohydrate = 2.5 kcal DE; 1g protein = 3.5 kcal DE; 1 g fat =8.1 kcal DE

Measurement parameters

Chemical composition of the crabs and feed

The proximate analysis was carried out according to the standard methods by the Association of Official Analytical Chemists **(AOAC, 2012)**. A total of three mud crabs from each treatments were randomly selected for analysis. The following nutrients were analyzed: moisture, crude protein (CP), crude fat (CF), crude fiber, ash, and nitrogen-free extract (NFE). For protein content, the Kjeldahl method was used, where nitrogen content was converted to protein percentage using a conversion factor of 6.25. Fat content was determined through the Soxhlet method, and ash content was obtained by heating samples in a muffle furnace until a constant weight was achieved. NFE was calculated as the

difference between 100% of the dry matter and the sum of ether extract %, crude protein%, crude fiber%, and ash%.

Carcass comparison

The mud crab carcass weight was calculated by separating meat from non-carcass consisting of body, shell, claws, walking legs, swimming legs and viscera. Calculation of carcass weight was carried out at the beginning and end of the study. A total of three mud crabs form each treatments were randomly selected to calculate the carcass weight. The crab was anesthetized using ice cubes, and was steamed for 15- 20 minutes, then the crab meat was separated from the body, claws, walking legs and swimming legs. Next, the meat was weighed using an electric scale with an accuracy of 0.01g. The percentage of meat was determined by comparing the weight of the meat with the total weight of the crab multiplied by 100. The percentage of non-carcass consisting of carapace, claws, walking legs, swimming legs, walking legs and other organs was calculated by comparing the weight of non-carcass organs with the total crab multiplied by 100 **(Sari *et al*., 2022)**.

RNA/DNA ratio

The analysis of the mud crab RNA/DNA ratio was carried out at the beginning and end of the study. A total of three mud crabs form each treatments were randomly selected for analysis RNA/DNA ratio using the NanoDrop Spectrophotometer method, with the following stages **(Gallagher, 1994; Alegria *et al*., 2020)**. This process begins with the aseptic transfer of 0.050µL of mud crab meat into a 1.5mL microtube, followed by the addition of 500µL of lysis buffer and grinding until the tissue was thoroughly destroyed. The sample was then incubated on a hot plate at 90°C for 10 minutes, after which it was centrifuged at 12,000rpm for 10 minutes to separate the components. Next, 200µL of the supernatant was transferred to a new microtube, and 400µL of 95% ethanol was added. The mixture was vortexed and centrifuged at 12,000rpm for 10 minutes to further separate the components. The supernatant and sediment were then discarded, and the sediment was allowed to dry. Subsequently, 25µL of RNase was added to the mixture, which was then left to stand at room temperature for 15 to 30 minutes. This step is crucial in degrading the RNA, allowing for the isolation of high-quality genomic DNA. Following this, 500µL of phenol was added, and the sample was allowed to stand at room temperature for 10 minutes. The mixture was then centrifuged at 13,000rpm for 4 minutes to separate the components. Afterward, the supernatant was then transferred to a new microtube, and an additional 500µL of phenol was added. The sample was centrifuged at 13,000rpm for 4 minutes, and the supernatant was added to a volume equal to that of the original sample. Chloroform was then added in one portion, and the mixture was centrifuged at 13,000rpm for 2 minutes to separate the components. The supernatant was then precipitated using cold absolute ethanol by inverting the microtube, followed by centrifugation at 6,000rpm for 30 minutes. The pellet was washed with 1mL of 70% ethanol and centrifuged at 6,000rpm for 15 minutes. The DNA pellet was then air-dried

for approximately 20 minutes, after which 50µL of TE buffer was added to facilitate complete dissolution. The pellet was subsequently stored in a freezer at a temperature of -20°C until required for the subsequent process.

Total RNA extraction

To extract and purify the RNA from the mud crab meat, a precise protocol was followed. First, 0.050mg of muscle per gram of the marine crab meat was carefully placed in a 1.5mL microtube. The sample was then dissolved in 200µL of isogen, a specialized solution designed for RNA extraction, in a container filled with ice to prevent degradation. Next, 800µL of isogen was added to the crushed sample in the microtube, and the mixture was incubated at room temperature for 4 minutes to facilitate the extraction process. Subsequently, 200µL of chloroform was added to the mixture, which was then vortexed and left at room temperature for 2- 3 minutes to allow for the separation of the RNA and other components. The sample was then centrifuged at 12,000rpm for 10 minutes to separate the phases. After allowing the sample to stand at room temperature for 5 minutes, the supernatant formed was carefully transferred into a new microtube containing 400µL of isopropanol. The sample was homogenized and allowed to stand at room temperature for a period of 5- 10 minutes to facilitate the precipitation of the RNA. A second centrifugation at 12,000rpm at 40°C for 15 minutes was performed to further purify the RNA. The pellet formed was then dissolved in 1mL of cold 70% ethanol and centrifuged at 12,000rpm at 4°C for 15 minutes. The supernatant was discarded, and the pellet was dried in a microtube under a stream of dry air to remove any remaining ethanol. Finally, the RNA pellet was dissolved in 50µL of TE buffer, a solution specifically designed for storing and handling RNA.

The concentration of DNA and RNA was quantified using the GeneQuant instrument. A total of 7µL of DNA or RNA was placed in a 5mm cuvette using TE buffer as the standard solution. The absorbance measurements were conducted at the wavelengths of 260 and 280nm. RNA concentration (µg/mL) was calculated as A260 x 50 x dilution factor, while RNA concentration (µg/mL) was calculated as A260 x 40 x dilution factor. The ratio of RNA to DNA was calculated by dividing the total RNA concentration by the genomic DNA concentration.

Water quality

The water quality parameters that were measured during the treatments period included temperature, salinity, pH, dissolved oxygen, nitrite, and ammonia. The temperature was measured using a thermometer, salinity with a hand refractometer, pH with a pH meter, and dissolved oxygen with a DO meter. Temperature, salinity, pH, and dissolved oxygen were measured twice daily during the study, at 06:00 PM in the morning and at 17:00 AM in the afternoon. Ammonia and nitrite measurements were taken three times during the study.

Statistical analysis

The data were all subjected to one-way analysis of variance (ANOVA) to determine if significant differences occur among the dietary treatments. The data were statistically analyzed with one-way ANOVA, and W-Tuckey's was used to compare differences between means at a significance level of 95% ($P< 0.05$). The water quality parameters were analyzed descriptively. Statistical analyses were performed using SPSS (Statistical Package for Social Sciences, Version 21, IBM Corporation, New York, USA).

RESULTS

Chemical body composition

The results of the initial and final mean body composition of the mud crab fed with various levels of protein and carbohydrate are presented in Table (2). At the end of the experimental period, a significant effect ($P< 0.05$) was observed on the protein content of the mud crabs. Nevertheless, no significant effect ($P> 0.05$) was observed in terms of fat, crude fiber, and NFE levels of the mud crabs. The higher value of protein content of the mud crap was observed in treatment C (68.25± 0.71) which wasn't significantly different from treatment B, but significantly different from other treatments.

Table 2. Mean body chemical composition of mud crabs treated with various levels of dietary protein and carbohydrate

Treatment	Protein (P) carbohydrate (C) content of feed (%)	Chemical body composition of the mud crab (%) ± Stdv			
		Protein	Fat	Crude fiber	NFE
	Initial	56.36 ± 2.53	13.52 ± 0.99	1.17 ± 0.14	2.16 ± 0.31
A	(P 60 – C 20)	63.58 ± 1.14^{a}	14.10 ± 0.28^{a}	1.16 ± 0.15^{a}	2.36 ± 0.05^{a}
B	(P 50 – C 30)	68.02 ± 0.37^{b}	15.92 ± 0.86^{a}	1.53 ± 0.24^{a}	2.41 ± 0.18^{a}
C	(P 40 – C 40)	68.25 ± 0.71^{b}	15.82 ± 2.17^{a}	1.44 ± 0.20^{a}	2.44 ± 0.27^{a}
D	(P 30 – C 50)	63.12 ± 2.48^{a}	15.63 ± 1.05^{a}	1.54 ± 0.06^{a}	2.27 ± 0.31^{a}
E	(P 20 – C 60)	60.43 ± 0.75^{a}	15.34 ± 0.84^{a}	1.42 ± 0.14^{a}	2.11 ± 0.32^{a}

Notes: Different letters in the same column indicate significant effect ($P< 0.05$).

Carcass comparison

The results of the carcass measurements, which comprise the percentage of the meat and non-meat mud crabs treated with varying levels of protein and carbohydrate feed are presented in Table (3). The results demonstrated that the inclusion of varying levels of protein and carbohydrates in the diet of the mud crabs had a statistically significant impact ($P< 0.05$) on the proportion of the meat and non-meat in the crabs. It was also indicated that the highest percentage of the mud crab meat was observed in treatment C (51.25± 0.17) which wasn't significantly different from treatment B but was significantly different from other treatments. In contrast, the highest percentage of the

non-meat mud crab was observed in treatment E (62.78± 0.80), which was significantly different from all other treatments.

Table 3. Avarage percentage of meat and non-meat of mud crab treated with various levels of protein and carbohydrate feeds

Treatment	Protein (P) carbohydrate (C) content of feed (%)	Avarage meat percentage (%) ± Stdv	Avarage non-meat percentage (%) ± Stdv
	Initial	30.72 ± 3.51	69.28 ± 3.51
A	(P 60 – C 20)	41.57 ± 0.98^{c}	58.43 ± 0.98^{b}
B	(P 50 – C 30)	51.22 ± 0.16^{d}	48.78 ± 0.16^{a}
C	(P 40 – C 40)	51.25 ± 0.17^{d}	48.75 ± 0.17^{a}
D	(P 30 – C 50)	39.41 ± 0.07^{b}	60.59 ± 0.07^{c}
E	(P 20 – C 60)	37.22 ± 0.80^{a}	62.78 ± 0.80^{d}

Notes: Different letters in the same column indicate significant effect (P< 0.05).

RNA/DNA ratio

The results of RNA/DNA ratio of the mud crabs fed diets with varying levels of protein and carbohydrates are presented in Table (4). There was a highly significant impact of the dietary composition on the RNA/DNA ratio of the mud crabs. The highest ratio of RNA/DNA was observed in treatment C (1.52± 0.14), which was significantly different from all other treatments, indicating that the optimal combination of protein and carbohydrates in the diet of the mud crabs was crucial for achieving the highest RNA/DNA ratio.

Tabel 4. Mean RNA/DNA ratio of mud crabs treated with various levels of protein and carbohydrate feeds

Treatment	Protein (P) and carbohydrate (C) content of feed (%)	Mean RNA/DNA ratio of the mud crab (ng/µL) ± Stdv
	Initial	0.51 ± 0.12
A	(P 60 – C 20)	0.80 ± 0.06^{a}
B	(P 50 – C 30)	0.64 ± 0.06^{a}
C	(P 40 – C 40)	1.52 ± 0.14^{b}
D	(P 30 – C 50)	0.63 ± 0.03^{a}
E	(P 20 – C 60)	0.70 ± 0.08^{a}

Notes: Different letters in the same column indicate significant effect (P< 0.05).

Water Quality

The physico-chemical parameters of the rearing media measured during the study were within the tolerance range of mud crabs and are presented in Table (5).

Table 5. The physico-chemical parameters of the mud crabs (*S.olivacea*) during the rearing period

Physico-chemical variable	Measurement result	Optimal level	Reference
Temperature (^{0}C)	25-29	25-35	Hastuti *et al*., (2019)
Salinity (ppt)	10-35	15-35	Eddwiwan *et al*., (2021)
pH	7.1-7.9	7.9-9.0	Hastuti *et al.*, (2019)
DO (ppm)	3.6-7.1	>3	Karim *et al*., (2019)
Carbondioxyde (ppm)	2.1-3.7	<5	Agus, (2015)
Ammonia (NH_3) (ppm)	0.065-0.145	<0.1	Karim *et al*., (2019)
Nitrite (NO_2) (ppm)	0.059-0.387	<0.5	Karim *et al*., (2019)

DISCUSSION

Chemical body composition

The chemical composition of the body analyzed includes, protein, fat, crude fiber, and nitrogen-free extract (NFE) (Table 2). The highest protein content for the mud crabs was observed in feeds containing 40% protein and 40% carbohydrates. This is likely due to the nutritional content of the feed aligning with the specific needs of the mud crabs. Protein is the primary source of energy for growth and tissue repair, and the protein requirements of the mud crabs fall within the range of 30- 47% **(Nguyen *et al*., 2022)**. Carbohydrates, being the most economical non-protein energy source, are essential for the mud crabs, with requirements ranging from 25.64 to 32.50% **(Zhan *et al*., 2020)**. The balance of protein and energy in the feed significantly influences the protein content of the mud crabs. A balanced feed with a suitable ratio of protein and carbohydrates optimizes energy utilization, ensuring that energy from protein is fully utilized for protein synthesis and meat production, while energy from carbohydrates supports other bodily activities **(Hasnidar *et al*., 2024)**. According to **Zhang *et al*. (2022)**, the balance of feed protein and carbohydrate is crucial for achieving an optimal growth. This aligns with the protein-sparing effect, where the majority of energy derived from protein is utilized for tissue growth, while non-protein energy is allocated for metabolic processes and other activities. However, an excessive feed energy can lead to a reduction in feed consumption, resulting in a decline in the nutrients received by the body, including protein. Low levels of feed protein can result in a deficiency of essential amino acids that can be absorbed by crabs, thereby reducing the protein produced **(Prakoso *et al*., 2020)**. Conversely, elevated protein levels can lead to increased ammonia accumulation due to excretion, which can subsequently impact metabolic processes. As observed by **Sudtongkong *et al*. (2020)**, a high intake of protein can lead to increased ammonia production in the hemolymph, which has a negative impact on the balance of osmotic pressure and oxygen transport and increases toxicity due to the accumulation of free

amino acids. Furthermore, a reduction in the protein content of the diet can result in reduction in excretion of nitrogenous waste products in water **(Hasnidar *et al*., 2024)**.

The fat content of the mud crabs obtained in this study was not significantly different ($P > 0.05$), indicating that a diet comprising varying levels of protein and carbohydrate is consistent with the nutritional requirements of the mud crabs, both in terms of quantity and nutritional quality. The fat content of mud crabs is influenced by the fat content of their diet, which ranged from 6.79 to 8.03% in this study. This range is within the optimal feed fat content for the growth of the mud crabs, which is between 2 and 10%, as suggested by **Zhao *et al*. (2014)**. A high percentage of feed fat can inhibit growth, reduce feed utilization, lipolysis, and fat deposition in the hepatopancreas, as reported by **Sun *et al*. (2019)**. Furthermore, the fat content of mud crabs is influenced by the carbohydrate content of the feed. Carbohydrates serve as a source of energy in the body and are also converted into fat, resulting in a higher fat content than carbohydrates. **Aslamyah and Karim (2013)** indicated that the availability of sufficient nutrients, including protein, fat, and carbohydrates, in the diet is utilized as body fat stores during the process of lipogenesis. This process involves the conversion of carbohydrates into triglyceride fatty acids, which occur in the liver and fat tissue, as described by **Bou *et al*. (2016)**.

The crude fiber content of the mud crabs obtained in this study was relatively low, indicating that the feed provided had a low crude fiber content. This low crude fiber content allows for proper consumption and digestion, ensuring optimal utilization by the mud crabs. The crude fiber content of the mud crabs is influenced by the crude fiber content of the feed, which is affected by the utilization of fish silage in the diet. The presence of lactic acid bacteria in fish silage facilitates the breakdown of cellulose into simpler molecules through the production of cellulase enzymes. As a result, the greater the proportion of silage in the diet, the lower the crude fiber content. This is consistent with the findings of **Herdiyanti *et al*. (2018)**, who noted that crude fiber is a component of carbohydrates that comprises a mixture of hemicellulose, cellulose, and lignin, which cannot be digested by organisms. An increase in crude fiber content is accompanied by a reduction in digestibility. The reduction in crude fiber content in the feed can be achieved by incorporating fish silage into the diet, which contains lactic acid bacteria such as *Lactobacillus* sp. These bacteria produce enzymes that degrade crude fiber, specifically cellulose and hemicellulose, into simple carbohydrates, thereby facilitating digestion. This is supported by the study of **Pratama *et al*. (2023)**, which demonstrated that the utilization of fish silage in the diet can reduce the crude fiber content and improve digestibility.

Nitrogen-free extract (NFE) is a crucial carbohydrate component that exhibits high digestibility, which is essential for an optimal absorption and utilization by the mud crabs. The NFE content in the feed does not vary significantly across different treatments, indicating that the feed has good digestibility, thereby supporting growth and meat

production. The NFE percentage is influenced by other nutrient components, particularly crude fiber. Research has shown that NFE content is inversely proportional to crude fiber content, as reported by **Annamalai *et al*. (2021)**. This inverse relationship highlights the importance of balancing NFE and crude fiber levels in the feed to achieve an optimal digestibility. NFE is a component of carbohydrates that are soluble in acid-base solutions, including monosaccharides, polysaccharides, and disaccharides, as stated by **Amrullah *et al*. (2015)**. Its high digestibility makes it a valuable component in the mud crab feed. The percentage of NFE is contingent upon other biochemical compositions, including crude protein, crude fiber, and crude fat. The chemical composition analysis of the mud crab body reveals the following values: protein content (60.43- 68.25%), fat content (15.34- 15.92%), crude fiber content (1.16- 1.54%), and NFE content (2.11- 2.44%). These values demonstrate the importance of maintaining a balanced nutrient profile in the feed to support an optimal growth and a meat production for the mud crabs.

Carcass comparison

According to this study findings, the mud crabs fed a diet consisting of 40% protein and 40% carbohydrates generated the greatest amount of flesh (Table 3). The development of flesh is positively impacted by the ideal protein to carbohydrate ratio in the mud crab diets. The percentage of meat in the mud crabs is a key metric for evaluating their quality, as it directly affects their economic value. The greater the percentage of meat, the higher the quality of the crab, and the more valuable it is for consumption. Conversely, the percentage of non-meat components such as shells exhibits an inverse proportionality with the percentage of meat, indicating that these components have no significant economic value. The composition of meat carcasses is influenced by various factors, including the type, weight, and size of the carcass, as well as the growth rate of the animal from which it originates. For instance, **Rizal *et al*. (2014)** emphasized the importance of carcass composition in assessing aquatic productivity, as it is a key component that has a selling price and can be consumed.

The nutritional content of crab feed significantly influences the increase in body weight, with protein being a crucial component. Proper energy allocation in the crab body facilitates the formation of meat. When crab nutritional requirements are met, they can maintain their survival and undergo energy transformation for growth and meat production **(Karim *et al*., 2017)**. This process is closely linked to protein synthesis, which is essential for growth and meat production. As **Carter and Mente (2014**) noted, the growth process is based on protein synthesis. Growth depends on the occurrence of protein synthesis on a significant scale. Protein synthesis can only occur if the body's energy is sufficient for metabolism and other activities. To fulfill the energy requirements of the mangrove crabs in an optimal manner, non-protein energy sources like carbohydrates are employed. Optimal carbohydrate levels provide the necessary energy for the mud crabs, thereby conserving protein. This approach is supported by studies that have shown that the crabs are opportunistic feeders and can digest plant-

based feed efficiently, with digestibility ranging from 94.4 to 96.1%. The percentage of the mud crab meat is also influenced by the cooling and steaming process. Steaming foodstuffs can result in weight reduction and the production of a specific quantity and weight of meat yield, as **Suharto *et al*. (2016)** found. The percentage of the mud crab meat obtained in this study ranged from 37.22 to 51.25%, with the percentage of the non-meat carcasses ranging from 48.75 to 62.78%. Overall, the nutritional content of crab feed and the energy allocation in the crab body play critical roles in the formation and production of crab meat. Meeting crab nutritional requirements and optimizing energy sources can facilitate growth and meat production, ultimately influencing the body weight of the crab.

RNA/DNA ratio

The RNA/DNA ratio of the mud crabs is a crucial parameter used to assess their quality and growth performance. This ratio is closely linked to protein synthesis, which is influenced by the size and number of body cells. Higher RNA/DNA concentrations indicate superior growth and quality of the mud crab meat. In this study, the RNA/DNA ratio of the mud crabs fed a diet consisting of 40% protein and 40% carbohydrate was found to be significantly higher (Table 4), indicating that this feed composition is suitable for the nutritional needs of the mud crabs. The incorporation of high levels of protein into the diet up to the optimal limit has a positive effect on protein synthesis, which is further enhanced by the presence of carbohydrates, leading to an increase in the RNA/DNA ratio. Optimal protein levels in the diet significantly boost protein synthesis, contributing to a higher RNA/DNA ratio. Carbohydrates, too, play a critical role by providing a protein-sparing effect, further enhancing the RNA/DNA ratio in the mud crabs. According to **Wu *et al*. (2022)**, the RNA/DNA ratio is extensively used as a growth parameter because it provides a comprehensive measure of protein synthesis activity, directly correlating with body weight gain. While DNA content remains constant, RNA levels fluctuate based on protein synthesis activity. Therefore, increased protein synthesis leads to a higher RNA concentration and, consequently, a higher RNA/DNA ratio, indicating faster growth. Several factors influence the RNA/DNA ratio, including environmental conditions, food intake, and internal biosynthesis processes. Research by **Foley *et al*. (2011)** has shown that, well-nourished and metabolically active crabs exhibit higher RNA/DNA ratios compared to underfed and metabolically inactive crabs. The study's findings, showing RNA/DNA ratios ranging from 0.63 to 1.52ng/ µL, underscore the positive impact of high protein content and favorable carcass percentages on the mud crab meat quality. These results convincingly demonstrate that a diet rich in protein and carbohydrates significantly enhances growth and quality in the mud crabs, as reflected by the elevated RNA/DNA ratios.

Additionally, alterations in the chemical composition of the mud crab's body, as well as changes in the ratio of RNA/DNA in the mud crab's carcass meat, are also influenced by the composition of the rearing media. The fattening of the mud crabs in this

study employs an apartment system utilizing medium-sized containers, thereby limiting the available space for the crabs to move. The limited space for movement, coupled with the availability of an optimal feed in terms of both quality and quantity, results in the crabs expending minimal energy on activities such as searching for food, defending themselves, and so on. This energy efficiency is beneficial for the crabs.

CONCLUSION

The feed composition with 40% protein and 40% carbohydrate was found to be superior to other treatments in terms of its impact on the mud crab's nutritional parameters. Addintional research is required to further explore this topic comprehensively, ideally using a more economical protein source and a more improved filter.

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