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Fatty Acid Profiles and Digestive Enzyme Responses of *Birgus latro* at Developmental Stages Fed Artificial Diets with Varying Coconut Meal Levels in Controlled Environments

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ABSTRACT

This study investigated the impact of different percentages of coconut meal in pellet diets on the fatty acid composition and lipase enzyme activity of coconut crabs (*Birgus latro*). Five experimental diets containing 30, 35, 40%, fresh coconut meal, and shrimp pellet were fed to juvenile coconut crabs. The fatty acid profiles were analyzed, and the response to different coconut meal inclusions was evaluated. Results showed significant differences in the concentrations of caprylic, capric, lauric, myristic, palmitic, stearic, arachidonic, oleic, linoleic, and linolenic acids, with the highest concentrations observed in diets with 40% coconut meal. Lipase enzyme activity recorded the highest values in the 40% coconut meal treatment, suggesting a positive correlation between dietary fat content and enzyme activity. These findings indicate that coconut meal can enhance both the fatty acid composition and digestive enzyme activity in coconut crabs, supporting their growth and molting processes. The results offer insights into optimizing coconut meal inclusion in aquaculture diets for improved crustacean health and performance.

INTRODUCTION

The coconut crab (*Birgus latro*, Linnaeus, 1767) is recognized as an economically important species in several Pacific Island nations, including Jungafsa (Papua New Guinea) and the Vanuatu Islands **(Lindner, 2004)**, and in Indonesia, particularly in the Maluku and Papua regions. *B. latro* is utilized and sold in high-end restaurants at premium prices. Consequently, the population of coconut crabs in their natural habitats has been steadily declining, including in Liwo Island, Central Halmahera **(Widiyanti *et al.*, 2015)**.

Efforts to cultivate coconut crabs during larval, juvenile, and adult phases have been carried out but have not shown significant progress. These cultivation efforts remain below the expected production targets, as growth patterns largely mimic those observed in the wild (constant growth rates), and mortality rates remain high **(Sulistiono *et al.*, 2009; Hamasaki *et al.*, 2014; Murhum *et al.*, 2019)**.

Studies have shown that coconut crabs prefer diets rich in lipids, with lesser interest in protein-based feeds **(Fletcher *et al.*, 1991; Wilde *et al.*, 2004; Stensmyr *et al.*, 2005; Buden, 2012)**. In their natural habitats, coconut crabs consume diverse food sources, including plant materials (seeds and fruits such as coconuts), animal matter (wild crabs), and organic materials. Meanwhile, in captivity, coconut meat is primarily provided as feed.

Attempts to increase molting rates and growth include enhancing the nutritional value of feeds to align with the physiological and biochemical conditions of *B. latro*. Physiological factors, such as the activity of specific enzymes, are known to play critical roles in digestion **(Zonneveld *et al.*, 1991)** and molting processes **(Vega-Villasante *et al.*, 1999)**, particularly enzymes like protease, lipase, and amylase. By understanding enzyme activity, fundamental information can be provided to develop engineered artificial feed formulations and optimize feeding schedules.

Coconut meat has been identified as a nutrient-rich feed ingredient, with lipids being one of its key components **(Warisno, 2003; USDA, 2004)**. Lipids are an essential dietary component, serving as an energy source, providing essential fatty acids, forming the primary structural components of biomembranes, acting as carriers for fat-soluble vitamins, and serving as precursors for eicosanoids, hormones, and enzyme cofactors in crustaceans (**Watanabe, 1982; Sargent *et al.*, 1989; Martinez *et al.*, 1999; Higgs & Dong, 2000; Chen *et al.*, 2006; Hamza *et al.*, 2007)**. However, excessive dietary lipids can negatively impact growth performance, reduce feed intake, impair nutrient utilization, and lead to lipid deposition in the hepatopancreas and other tissues **(Shiau & Huang, 1990)**. The dietary lipid requirements vary significantly among species, life stages, and environmental conditions **(D'Abramo, 1997)**. This study was conducted to investigate the fatty acid responses and lipase enzyme activity at the developmental stages of coconut crabs when fed pelleted diets with varying percentages of coconut meal.

MATERIALS AND METHODS

Experimental design

The study employed a completely randomized design (CRD) with five treatments: three with experimental diets and two controls. Tank assignments were randomized following published methods (**Gomez & Gomez, 2007)**.

Study duration and location

The test animal samples were collected from Liwo Island. After collection, the samples were transported to the Field Laboratory of the Faculty of Fisheries and Marine

Sciences, Universitas Khairun, Kastela Village, Ternate City. Proximate analysis of feed ingredients, pelleted feeds, fatty acid characterization, and digestive enzyme assessments were conducted at the Fish Nutrition Laboratory, Faculty of Fisheries and Marine Sciences, IPB University, Bogor. The study was carried out over five months, from May to September 2024, starting with preparation and feed trials and concluding with data analysis.

Fatty acid analysis and lipase enzyme activity

The fatty acid profiling of coconut meal was conducted using the methods of **AOAC (2005)**. Lipase enzyme activity was measured following the procedure according to the method of **Borlongan (1990)**. For this analysis, 1.5mL of pure lipase substrate (olive oil) was pipetted into a 100–125mL Erlenmeyer flask. Subsequently, 1mL of 0.1 M Tris-HCl buffer (pH 8.0) and 1mL of the sample were added to the flask. The mixture was homogenized and incubated at 37°C for 6 hours. To halt the hydrolysis process, 3mL of 95% ethanol was added. The solution was then titrated using 0.01 N NaOH with 0.9% thymolphthalein as an indicator. Lipase activity was calculated using the formula: Lipase (Unit/mg protein) = (Volume of titration – Blank) × mg Protein.

Cultivation tank design and construction

The cultivation tanks were designed and constructed to accommodate the biological and ecological characteristics of *Birgus latro*. Each tank was made from a plastic container with dimensions of 30cm in height, 20cm in top width, and 15cm in bottom width. The tank bases were filled with a substrate comprising an 80:20 mixture of sand and soil, over which coconut husks were placed to mimic the natural habitat. To prevent escape, the tanks were secured with double-layered wire mesh, with a hole diameter of 2cm and wire thickness of 0.2mm. Reused plastic mineral bottles were modified and attached to the tank walls to serve as feed containers, while water containers were partially embedded in the substrate to ensure stability. Behavioral monitoring, particularly during nocturnal periods, was facilitated by installing a CCTV camera above the tanks. This design aimed to replicate natural conditions and support effective observation and management of the test organisms.

Pelleted feed composition

The cultivation tanks were designed and constructed to align with the biological and ecological characteristics of *Birgus latro*. Plastic containers, measuring 30cm in height, 20cm in top width, and 15cm in bottom width, were utilized as tanks. The base of each tank was filled with a substrate composed of a sand-soil mixture at an 80:20 ratio, with coconut husks placed on the surface to simulate the natural habitat. Escape prevention was ensured by covering the tanks with double-layered wire mesh, featuring a hole diameter of 2cm and a wire thickness of 0.2mm. Feed containers were created from

repurposed plastic mineral bottles, which were cut and securely attached to the tank walls, while water containers were partially embedded in the substrate to maintain stability. Behavioral activity, including nocturnal patterns, was monitored using a CCTV camera installed above the tanks. This setup was designed to facilitate the effective observation and maintenance of the test organisms under controlled conditions.

Table 1. Feed composition (%)

Ingredient	**Treatment (%)**
Fish meal	20
Fresh coconut meat	Varied
Shrimp grower feed	-
Fish oil	5
Crab shell meal	16
Rice bran	10
Coconut meal	30-40
Tapioca flour	15
Vitamins	2
Minerals	2
Total	100

Table 2. Feed nutritional composition

Nutrient	**A**	**B**	**C**	**D**	**E**
Moisture (%)	15.90	5.89	6.17	7.33	11.23
Ash (%)	15.23	17.14	14.29	7.86	11.79
Protein (%)	33.28	31.40	28.04	7.36	32.16
Lipid (%)	15.23	19.76	23.03	56.94	6.92
Crude Fiber (%)	9.84	12.22	14.58	19.52	3.08
Nitrogen-free extract (NFE) (%)	2.72	13.59	21.69	6.54	34.82
Gross energy (GE, kcal/kg)**	434,998.8	914,167	1,259,637.4	843,480.4	1,670,135.6

Note:

GE Calculation: Based on the **NRC (1988)** energy equation:

- 1 g carbohydrate = 2.5 kcal
- 1 g protein = 3.5 kcal
- 1 g lipid = 8.1 kcal

Feed trial protocol

A feed trial was conducted following the collection and preparation of juvenile coconut crabs (*Birgus latro*). Twenty juvenile crabs were collected from Liwo Island and

transported to the Field Laboratory of Universitas Khairun. The crabs were acclimatized for one month under controlled conditions, during which they were fed fresh coconut meat and provided with fresh water. Prior to the feed trial, the crabs underwent a fasting period of three days to ensure digestive system clearance.

Initial measurements of each crab, including body weight and carapace width, were recorded using an electronic scale and calipers. Feed was administered at a rate of 10% of body weight, with fresh water provided in a volume of 300mL per individual. Water and feed were replenished every three days. Feeding sessions were conducted daily between 17:00 and 18:00 WIT. This regimen was designed to standardize the feeding conditions and to optimize the evaluation of the trial outcomes.

Statistical analysis

Data were analyzed using ANOVA (Analysis of variance) with a 95% confidence interval using MS Excel 2013. Significant differences ($P< 0.05$) were further analyzed using Duncan's test via SPSS version 16. Environmental parameters were tabulated and compared against relevant references.

RESULTS AND DISCUSSION

Response of fatty acids in coconut crabs fed with pellet diet containing different coconut meal percentages

The fatty acid composition in coconut crabs fed pellet diets with varying coconut meal percentages is presented in Table (3). The highest percentage of caprylic acid (C8:0) was observed in treatment A, significantly different ($P < 0.05$) from treatments B and E but not from treatments C and D. The highest percentage of capric acid (C10:0) was recorded in treatment C, significantly different ($P< 0.05$) from treatments A, B, and E, but not from treatment D. Lauric acid (C12:0) was highest in treatment A, differing significantly ($P< 0.05$) from treatment B but not from treatments C, D, and E. Palmitic acid (C16:0) was at its highest value in treatment B, showing no significant difference ($P> 0.05$) from treatment D but significantly different ($P< 0.05$) compared to all other treatments. Stearic acid (C18:0) was the highest in treatment A, significantly differing ($P< 0.05$) from treatments B and C, but not from treatments D and E.

Table 3. Results of fatty acid tests on coconut crabs fed with different percentages of coconut flour

Fatty acid	Treatments				
	A	B	C	D	E
Caprylate (%)	$0,17^{c}$	$0,05^{a}$	$0,16^{bc}$	$0,13^{bc}$	$0,10^{ab}$
Caprate (%)	$0,11^{ab}$	$0,09^{a}$	$0,22^{c}$	$0,19^{bc}$	$0,11^{ab}$
Laurate (%)	$16,42^{b}$	$13,38^{a}$	$14,78^{ab}$	$14,77^{ab}$	$14,76^{ab}$

Myristate (%)	10,34[a]	9,86[a]	7,85[a]	12,33[a]	12,29[a]
Palmitate (%)	29,92[c]	32,52[d]	21,61[a]	31,19[cd]	25,20[b]
Stearate (%)	15,57[b]	5,94[a]	6,49[a]	12,57[ab]	12,98[ab]
Arachidonate (%)	0,02[a]	0,02[a]	0,02[a]	0,02[a]	0,02[a]
Oleate (%)	27,13[b]	27,54[b]	21,30[a]	26,67[b]	22,04[a]
Linoleate (%)	13,30[a]	9,90[a]	11,26[a]	14,01[a]	13,83[a]
Linolenate (%)	1,21[a]	0,89[a]	1,05[a]	1,22[a]	1,11[a]

Description: A: Coconut flour 30%, B: Coconut flour 35%, C: Coconut flour 40%, D: Fresh coconut meat and D: Shrimp pellets. The numbers in the same row followed by the same letter indicate no significant difference ($P > 0.05$).

A significant variation in the fatty acid composition indicates that different percentages of coconut meal in the diet influence the lipid profile of coconut crabs. Oleic acid (C18:1) did not show significant differences ($P > 0.05$) in treatments A, B, and D, but all these treatments differed significantly ($P < 0.05$) from treatments C and E. The essential fatty acid, linoleic acid (C18:2), did not differ significantly ($P > 0.05$) across treatments. These findings are consistent with the literature, as linoleic acid plays an important role in the integrity of cellular membranes in crustaceans **(Kanazawa *et al.*, 1978; Sheen & Wu, 1999)**.

The present results suggest that the formulated pellet diets can support the lipid requirements of coconut crabs, marked by the increase in ten fatty acids, including caprylic, capric, lauric, myristic, palmitic, stearic, arachidonic, oleic, linoleic, and linolenic acids. These findings contrast with earlier studies in the first phase of this research, which identified only eight fatty acids in the crabs. The balance of fatty acids in the diet is crucial for supporting the growth and molting processes in aquatic animals **(Warisno, 2003)**. The formulation of fish diets in the future should be guided by the consideration of additional nutrients derived from native and non-native plant resources **(Pratama *et al.*, 2020; Islamy *et al.*, 2024a; 2024b; Serdiati *et al.*, 2024)**. These nutritional components are increasingly being identified and analyzed to enhance the sustainability and efficiency of aquaculture feed **(Kilawati & Islamy, 2019; Islamy *et al.*, 2024c; 2024d; Kilawati *et al.*, 2024)**. The potential of such resources is being evaluated through systematic studies, aiming to integrate them into diet formulations. By focusing on these alternative ingredients, the reliance on conventional feed sources is expected to be reduced, contributing to more sustainable aquaculture practices.

The use of native and non-native plants in fish feed formulation can be controlled to manage their populations effectively **(Islamy *et al.*, 2024e)**. By incorporating these plants into aquaculture practices, their growth and spread are regulated, preventing potential ecological imbalances. This approach is being developed to ensure that their utilization contributes positively to both environmental sustainability and the optimization of fish nutrition.

Furthermore, the coconut oil content in coconut meal comprises mainly saturated fatty acids (SFA) like caproic, caprylic, capric, lauric, myristic, palmitic, and stearic acids, contributing approximately 91%, with unsaturated fatty acids (MUFA) like oleic and linoleic acids comprising about 9%. The highest concentrations of lauric acid (44.3–52.1%) are particularly important for the development of coconut crabs.

These results are supported by published research, which found that palmitic and stearic acids were dominant SFAs in coconut crabs, while oleic acid and palmitoleic acid were the main MUFAs **(Sato *et al.*, 2015)**. No significant differences in SFA and MUFA percentages were found between male and female crabs, suggesting that fatty acid composition may be relatively consistent regardless of sex.

Lipase enzyme activity in coconut crabs fed pellet diets with different coconut meal percentages

The lipase enzyme activity in coconut crabs fed with different coconut meal percentages is shown in Fig. (1). The highest enzyme activity was observed in treatment C (40% coconut meal), significantly different ($P< 0.05$) from treatments A, B, and D, but not from treatment E. Treatments A and B exhibited no significant differences ($P> 0.05$), but both were significantly different ($P< 0.05$) from the other treatments.

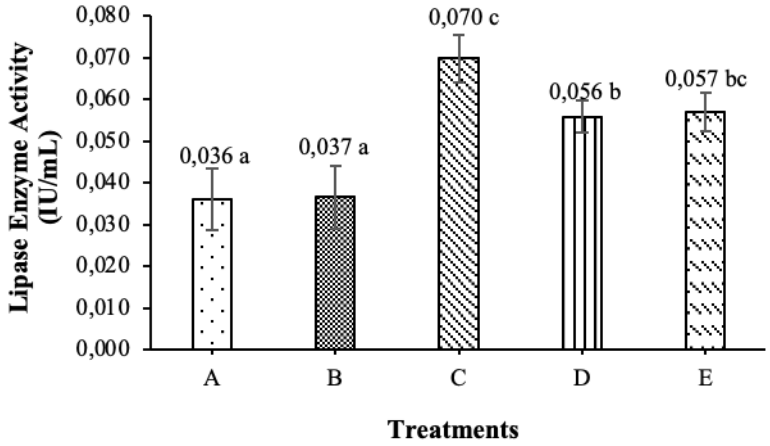

Fig. 1. Lipase enzyme activity in coconut crab fed with different coconut flour contents. Percentage of coconut flour 30% (A), 35% (B), 40% (C), positive control/fresh coconut flesh (D), negative control/shrimp pellet feed (E). Different letters indicate significance.

The increase in lipase activity is linked to the dietary fat content, as higher fat levels in the diet tend to enhance digestive enzyme activities, facilitating the breakdown and absorption of fats. Published article reported that enzyme activity is influenced by the development of the digestive system and the quantity and quality of dietary components (**Bakkara *et al.*, 2015)**. Additionally, a research found that higher lipase activity

increases energy availability, thereby promoting growth and molting in crustaceans **(Zhao *et al.*, 2014)**.

Diets with higher fat content enhance lipase activity, contributing to improved growth and molting **(Huo *et al.*, 2014)**. In this study, the highest lipase activity in treatment C (40% coconut meal) suggests that higher lipid content in the diet is more effective in stimulating enzyme activity compared to lower lipid diets (treatments A and B). This correlation between dietary fat and lipase activity is crucial for understanding how different diets influence digestive efficiency and the overall health of coconut crabs.

CONCLUSION

The provision of pellet diets with varying coconut meal percentages significantly increased the response of fatty acids, with ten types observed in the coconut crabs. These included caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidonic (C20:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids. The highest lipase enzyme activity was observed in treatment C, significantly different ($P< 0.05$) from treatments A, B, and D, but not from treatment E. Treatments A and B did not differ significantly ($P> 0.05$), but were significantly different from the other treatments.

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