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Original article

Evaluation of paralytic shellfish poisoning of fresh green mussels (*Perna viridis*) affected by the Lampung Coast red tide phenomenon

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Abstract

The Lampung Coast red tide phenomenon caused not only ecological disaster but also financial loss. This was the second case reported from Lampung. Toxin residues have been found in shellfish as planktonic feeder organisms, particularly in green mussel (*Perna viridis*) meat. This study aimed to investigate the effect of green mussel administration from various locations along the Lampung Coast that were exposed to red tide (Ringgung and Mutun beaches) and from unexposed locations (Klara and Kota Agung beaches). The green mussel powder was administered to mice to evaluate its toxicity. Morphological and proximate examinations were also performed to compare the abnormalities in both samples. This study found that green mussels from the red tide locations caused diarrhea and death in mice despite no differences in both samples from morphologic and proximate analyses. Our findings could be significant in providing early information on green mussel intake. The location source may be important information for consumers to be aware of the consequences of consuming green mussels.

Keywords: green mussel, Lampung coast, red tide, saxitoxin

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Introduction

Green mussels are a popular food commodity due to their high economic value and nutritional content (Saritha et al., 2015). The nutritional value of green mussels is 21.9% protein, 14.5% fat, and 18.5% carbohydrates (Basri & Rizki, 2023). Also, green mussels contain essential and non-essential amino acids, vitamins, minerals, and low cholesterol, making them widely traded and consumed (Chakraborty et al., 2016). Green mussels are widely distributed along the coastal areas of Indonesia and have a main role as bioindicators of marine pollution due to their ability to accumulate various contaminants, including heavy metals, microplastics, and polycyclic aromatic hydrocarbons (PAHs) (Rahim & Yaqin, 2022; Soegianto et al., 2021; Yogaswara et al., 2021).

Interestingly, harmful algal bloom, including red tides, could also affect green mussels, which have severe ecological and economic impacts. These algal blooms can produce toxins that accumulate in green mussels and threaten human health through seafood consumption (Blake et al., 2022; Luang-on et al., 2023; Mahdy et al., 2022). Due to the occurrence of a red tide or algal bloom in 2020, the consumption of green mussels needs to be cautious, especially for the people around Lampung Coast. This is the second time this phenomenon has been reported on Lampung Coast. Similar cases have been seen in subtropical regions such as Europe, Australia, the United States, Canada, and Japan. Red tides are possible in Lampung Coast due to excess nutrients carried by the water and trapped in the bay. The main cause of red tides is the presence of eutrophic plankton blooms in the waters, which occur when the plankton density exceeds 1000–10,000 cells/mL and might threaten the fish survivability (Sidabutar et al., 2021).

Since red tides can last for six weeks, more research is needed to determine their impact on consumption commodities because they contain toxins such as brevetoxin, domoic acid, and okadaic acid that are harmful to fish and humans (Pouil et al., 2018; Vilariño et al., 2018). Toxins like these typically accumulate in the flesh of planktonic feeder organisms (shellfish) like green mussels (Hendrickson et al., 2022; Pouil et al., 2018). One syndrome caused by shellfish toxins is paralytic shellfish poisoning caused by saxitoxin (Andayani & Sumartono, 2012; Finch et al., 2023; Peteva et al., 2019). Shellfish poisoning symptoms include dizziness, joint pain, cramps on the lips and tongue, difficulty breathing, convulsions, and even death (Cord, 2009).

However, there is still limited research investigating the residual toxin content of green mussel meat, particularly from Lampung Coast. Our current study aimed to distinguish the different morphology, yield, proximate analysis, and response of mice after being treated with green mussels collected from four sites that might be red tide-affected and non-affected in Lampung Coastal areas.

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Methods

Research design

This study uses a survey method with samples from several places. The independent research variable used was the difference in sampling locations for green mussels exposed to red tides and those that were not. The morphology of green mussels was smooth, elongated, and oval-shaped in the ventral area, narrowing to a triangular shape in the dorsal half, with a downward-pointing beak. The shell is enveloped by a thin outer cover, dark green in juveniles, but fades to brown with green borders in adults. It is 100 mm in size or above. A total of 20 green mussels were collected and compared for each place they were taken. While the parameters observed were morphological characteristics related to comparing differences in abnormalities in samples from red tide-exposed and non-exposed locations, the presence of toxins in green mussel meat was determined qualitatively. The data obtained were then analyzed and compared using the maximum toxin standard (AOAC).

Green mussel sampling

Green mussel samples were collected from various locations on the Lampung Coast. The locations that have been determined for sampling are Ringgung Beach and Mutun Beach, which are exposed to red tides, while Klara Beach and Kota Agung Beach are not. The determination of whether the location was exposed to red tides was based on the previous study and direct visits. The samples were then washed to remove adhering sand before being placed in a cool box filled with curdled ice and in the laboratory. The sample was prepared by separating the outer shells with its meat. The meats were cut into small pieces, then crushed using a blender, and sifted to form flour.

Green mussel morphology observation

Morphological observations were made to compare the body parts of green mussels from exposed and non-exposed areas. This observation includes determining whether there are any visual anomalies in green mussel meat , such as color, odor, or other specific characteristics.

Green mussel yield

Yield is the percentage of meat weight compared to total weight (meat and shell). The green mussel weight was measured before and after the shells were removed for the test.

$$\text{Yield} = \frac{\text{final weight}}{\text{initial weight}} \text{ X } 100\%$$

Mice acclimatization

Twenty-five male mice *Balb/C*, 5-6 weeks with weight around 20-25 g, were randomly divided into five groups. The mice were acclimatized for three weeks and given deworming, antibiotics, and antiprotozoal on the seventh day (Lea et al., 2004). The rearing cages were made of plastic containers and covered with wire mesh at room temperature, 25–30°C. Feeding and drinking were provided ad libitum until the mice were ready for the experiment.

Paralytic shellfish poisoning (PSP) analysis

For PSP analysis, the AOAC method (1999) was used, which involved extracting the sample with 0.1 N HCl and then correcting the extract with 0.003 N HCl. The filtrate was then injected in a volume of 1 mL into three male mice (weighing 18-20 g) and given orally. Three mice were injected with 1 mL of acetic acid as controls (pH adjusted to 4). Furthermore, the consistency of feces in mice was observed for 72 hours to determine the mortality rate and the level of diarrhea. The protocol of animal experiments was registered and approved by the Ethical Committee of Brawijaya University with reg. no. 210-KEP-UB-2023, in compliance with ARRIVE guidelines and associated guidelines, EU Directive 2010/63/EU for animal experiments.

Results

Yield

The green mussels used in this study were collected from four locations on the Lampung Coast: Mutun Beach, Ringgung Beach, Klara Beach, and Kota Agung Beach (Figure 1). The size of the green mussels is selected so that they are uniformly around 70-100 mm in length or have an average age of 7-10 months.

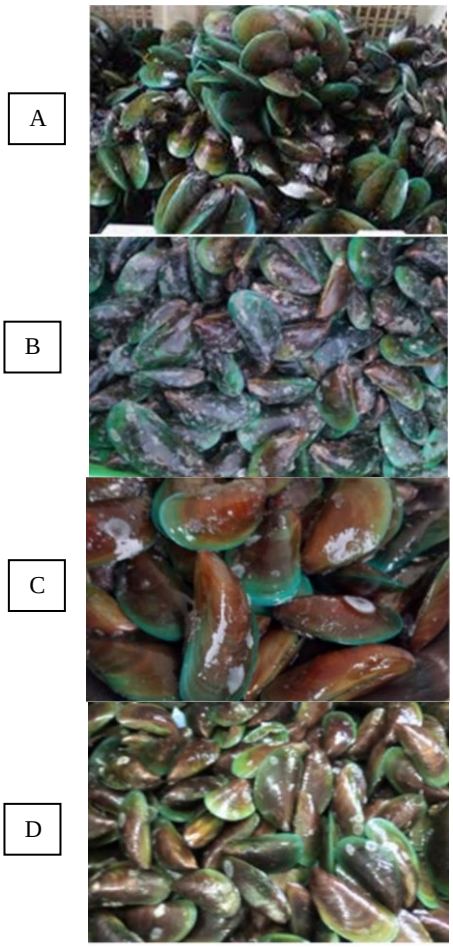

Figure 1. The morphology of green mussels collected from (A) Ringgung Beach, (B) Mutun Beach, (C) Klara Beach, and (D) Kota Agung Beach.

The yield value of green mussel meat did not differ at each sampling station (Table 1).

Table 1. Green mussel yield

Sample	Yield (%)
Ringgung beach	34.2
Mutun beach	33.5
Klara beach	34.1
Kota Agung beach	33.4

Proximate analysis

A proximate analysis was performed to determine the nutritional content of green mussels from various locations. Table 2 shows no significant difference in protein, water, carbohydrate, and ash content among Ringgung, Mutun, Klara, and Kota Agung beaches. The water content was higher in Mutun and Kota Agung Beach green mussels than Ringgung and Klara Beach. The ash content was higher in green mussels collected at Kota Agung beach (Table 2).

Table 2. Proximate Analysis of Green Mussels in Various Location

Sample	Proximate content (%)				
	Protein	Fat	Water	Carbohydrate	Ash
Ringgung beach	18.84	2.69	78.14	3.61	3.04
Mutun beach	18.27	2.29	85.24	3.45	3.13
Klara beach	18.23	2.46	78.43	3.27	2.88
Kota Agung beach	18.85	2.75	85.15	2.43	4.00

Organoleptic Analysis

Organoleptic analyses were performed to determine the quality of green mussels produced at each sampling location and any morphological differences caused by red tide exposure. The sensory test of a food's quality is typically determined by taste, texture, color, and aroma factors, but taste observations were not conducted in this study due to concerns about the panelists' health and safety. Sensory tests revealed that the texture of the obtained green mussel meat was relatively consistent, with no significant differences between locations. Color testing revealed the exact color of fresh clams. The only difference is the aroma of the green mussel from Kota Agung Beach, due to the long distance traveled by the sample before preparation.

Paralytic shellfish poisoning (PSP) analysis

According to the mortality analysis, mice treated with green mussel extract from Mutun Beach experienced up to 50% mortality during the three days of observation, compared to 16.7% for the Ringgung Beach sample and 16.7% for the Klara Beach sample. The mortality rate of mice treated with samples from red tide-affected locations was high, whereas mice treated with samples from non-affected areas had a low mortality rate, and even all of them lived.

The consistency of the feces excreted by the mice after treatment revealed that the feces tended to be mushy, which was possible because the mice's intestines had not been able to adapt to the green mussel extract (Table 3). On the second day of observation, the feces consistency of the samples from Mutun and Ringgung beaches remained mushy, whereas the other two samples were normal and solid. This indicated that the mice given green mussel extract from a location exposed to red tides had diarrhea, whereas their stool controls were relatively normal.

Table 3. Observation of Feces Consistency

Sample	Feces Weight and Consistency					
	24 h		48 h		72 h	
	Weight (g)	Consistency	Weight (g)	Consistency	Weight (g)	Consistency
Ringgung beach	0.57	Mushy	0.82	Mushy	0.74	Normal
Mutun beach	1.49	Mushy	1.44	Mushy	1.25	Mushy
Klara beach	0.24	Mushy	0.22	Normal	0.22	Mushy
Kota Agung beach	1.17	Mushy	1.21	Normal	1.24	Normal
Control	1.14	Mushy	1.29	Normal	1.17	Normal

The feces from the Mutun Beach sample returned to normal on the third day of treatment, possibly because the mice had adapted to the green mussel extract. However, this did not occur in the Ringgung Beach and Klara Beach samples; the feces of the Klara Beach samples became slightly mushy, indicating that the toxin contained in the mussel meat only influenced the third day.

Discussion

Green mussels are a frequent biota in Indonesian waters. Green mussels are popular among the public for their great and savory taste. Our study demonstrated that green mussel meat samples from various collection locations were around 33.8%. This value is consistent with the previous finding that green mussel meat represents about 30% of the total weight (Swastawati et al., 2021). The yield value of green mussel meat did not differ significantly at each sampling station, as determined by the results of statistical tests, because the samples used were selected to be uniform in size and weight.

There is no difference in yield values between green mussels exposed to red tide and those from unexposed waters, indicating that red tide does not affect yield. Exposure to red tides in Lampung Coast lasted approximately 6 weeks and killed farmed fish such as grouper and barramundi, mostly cultivated in floating net cage areas around Lampung Coast.

The chemical composition of green mussels varies greatly depending on species, sex, age, and habitat (Zakharikhina et al., 2022). Green mussels meat also contains important minerals such as calcium, phosphate, iron, iodine, copper, and low amounts of thiamin, riboflavin, and niacin (Saritha et al., 2015). Green mussels, as a food source, have a fairly high nutritional content, as shown in Table 2, and they nutritionally can be comparable to beef, chicken, and eggs (Swastawati et al.,

2021). Unlike beef, which has been associated with higher saturated fat content, green mussels are rich in polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) and essential minerals such as zinc, copper, and iron (Jachimowicz et al., 2022; Soegianto et al., 2021). In contrast, the nutritional of green mussels might vary depending on their environment and cultivation methods (Rejeki et al., 2021; Supono et al., 2023).

However, a previous study reported that green mussels on the East Java Coast accumulated Cd, Pb, and Cr levels above minimum consumption recommendations. Further, the cancer risk values for Cr and Cd in some sites were higher than acceptable levels, suggesting possible carcinogenic effects from long-term exposure to green mussels (Soegianto et al., 2021). Paralytic shellfish poisoning (saxitoxin/STX) is typically produced by Dinoflagellate phytoplankton, which includes the *Vibrio* spp. and blue-green algae (Andayani & Sumartono, 2012; Raposo et al., 2020) as well as the algae *Alexandrium* spp., *Gymnodinium* spp., and *Pyrodinium* spp (Al-Has et al., 2023). PSP in humans is acute, and there is no way to remove poison (an antidote) or provide effective treatment when poison has entered a patient's body (Andayani & Sumartono, 2012). As a result, PSP poisoning is a serious health issue. The first symptom of PSP poisoning is tingling in the lips, followed by numbness in the oral cavity, which spreads throughout the body.Furthermore, the muscles of the body, including the lung muscles, will be paralyzed, causing the patient to stop breathing.

The limitation of the study is that the PSP effect is only observed morphologically. The detailed analysis through blood and intestine change might benefit future research to elucidate the effect of green mussels consumption. In summary, the current findings suggested no difference macronutrient based on proximate analysis of green mussels collected from Ringgung, Mutun, Klara, and Kota Agung beaches. The animal testing demonstrated that only the consumption of green mussels from Mutun beach remained mushy feces during 72 h observations. Our result might be important to provide early information about green mussels' consumption. The location source might be key information for consumers to be aware of the implications that might arise after the consumption of green mussels.

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