

Physiological responses of scalloped spiny lobster *Panulirus homarus pueruli* raised at different calcium doses

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Abstract. In the lobster aquaculture systems, several challenges commonly arise, including poor water quality (low pH and alkalinity), molting failure, delayed development, and high mortality due to deficiencies in essential macrominerals such as calcium. To address this issue, calcium supplementation is necessary to the maintenance medium. This study aims to evaluate the effect of different doses of calcite added to the maintenance medium on the physiological responses of scalloped lobster pueruli. The study demonstrates that the appropriate amount of calcium in the maintenance medium can physiologically address these problems of environmental change, molting, and growth. Several calcium doses, including 0, 20, 40, and 60 mg L⁻¹ CaCO₃, were used in this thoroughly randomized investigation. The concentration or body calcium content in the lobster's meat, exoskeleton, hepatopancreas, hemolymph, molting lobster body, dead lobster body, water content, protein, and body lipid, as well as its total hemocyte count, glucose, alkaline phosphatase, total protein, glucose, malondialdehyde, lysozyme activity, and alanine aminotransaminase, was significantly altered ($P < 0.05$). Supplementation with 40 mg L⁻¹ calcium carbonate can be considered an effective strategy to improve mineral balance, physiological performance, molting success, and overall health condition in lobsters.

Keywords: absorption, environmental change, growth, macrominerals, molting.

Introduction. Spiny lobster (*Panulirus homarus*) is one of the most widely consumed seafood products worldwide with substantial commercial value. Lobster is expensive in both home and international markets. In 2025, Indonesia announced a shift in focus from lobster seed exports toward domestic aquaculture development (FAO 2026). In Indonesia, a nation with significant lobster potential, spiny lobster has the third-largest natural availability, after ornate and rock lobster (Wahyudin 2018). Excessive natural lobster catching operations will affect the availability of lobster, hence culture activities are necessary.

Although lobster farming has been developed in Indonesia, not much progress has been accomplished. One of the problems in lobster farming is a lack of macrominerals like calcium, which impacts molting failure, declining water quality (low alkalinity and pH), delayed growth, and death. Marine species must primarily obtain calcium from the water since they are unable to store much of it (Greenaway 1985). Ca²⁺ is recognized to be involved in almost every biological function, including growth and development, inflammation, immunological response, and cell death (Hogan et al 2003). Thus, its availability will determine the lobster's ability to survive. Crustaceans have a far more

active calcium metabolism than other invertebrates because they need to gather large amounts of calcium from their surroundings in order to form shells (Greenaway 1985). Shi et al (2013) discovered that there is a reduction in the availability of macro-minerals such as calcium because it is extensively absorbed and used during molting, an essential process for crustacean growth.

Lobster growth and molting may also be hindered by low alkalinity. The intermoult period increases and the calcium absorption process is hindered at pH values below 5.75 (Adhikari et al 2007). Effectively controlling environmental elements like optimum alkalinity and mineral availability is essential in aquaculture systems because they can affect water chemistry, physiological processes, and the growth of farmed organisms (Bhatnagar & Devi 2013). Both diet and the environment can provide calcium, but environmental calcium is far more important for the hardening of crustacean shells (Greenaway 1985). In addition to calcium, bicarbonate and pH can affect the mineralization of the environment (Greenaway 1974; Kurihara 2008). The solution can be made more alkaline by adding calcium carbonate, which acts as a pH buffer (Boyd 2016). Low pH levels might cause stress, which could impede the growth and survival of crustaceans (Wickins 1984).

According to a study on the effects of calcium addition and alkalinity variations, lobsters with an initial weight of 58.08 g that were reared at an alkalinity range of 200 mg L⁻¹ CaCO₃ were able to increase by 86.67% (Supriyono et al 2022). The lobsters were raised to an alkalinity range of 32.1 to 241.3 mg L⁻¹ CaCO₃ with the individual compartment method (Pratiwi 2016). An alkalinity of 160 mg L⁻¹ CaCO₃ was found to extend the survival rate of lobsters by 96.67% (weighing 51.22 g) (Aji et al 2019). The effects of adding calcite (CaCO₃) as a mineral source to enhance pueruli development and improve water quality in the lobster pueruli rearing water have not yet been studied. Therefore, the goal of this study was to ascertain how the physiological responses, water quality, and growth performance of scalloped lobster pueruli are impacted by varying doses of calcite added to rearing water.

Material and Method

Containers and media research. Four treatments were employed: calcium delivery at 20, 40, and 60 mg L⁻¹ CaCO₃, as well as control (no calcium administration). Each treatment had four replicates. The average weight of the pueruli employed in this investigation was ±0.22 g. They were from fishermen in the districts of Sukabumi and Lampung. Before receiving the diet treatment, *P. homarus* was acclimated for a week in an aquarium. After that, they were raised for 40 days in sixteen 60 x 40 x 40 cm³ (96 L) units with a protein skimmer and a stocking density of 15 lobsters per aquarium. We employed four administrations at 0–100 mg L⁻¹ to determine the initial doses of treatments, and we observed pH and alkalinity for 48 hours. This procedure is in accordance with that carried out by Lesmana et al (2024) with different test parameters. In this study, the test carried out was on the physiological aspect, not the growth performance.

Body calcium and chemistry profile. Observation of calcium concentration in *P. homarus* body included calcium content in hepatopancreas, hemolymph, dead lobster body, meat, molting lobster body and exoskeleton. The method of determining calcium in samples in lobster body parts using atomic absorption spectrophotometry (AAS). The method for determining calcium levels using Atomic Absorption Spectrophotometry (AAS) is based on the ability of free calcium atoms to absorb light energy at specific wavelengths. The lobster body sample is first crushed and dissolved through a digestion process, converting the calcium minerals into an ionic form that can be analyzed. The solution is then aspirated into an AAS flame, causing the calcium atoms to absorb light from a hollow cathode lamp (HCL) at a specific wavelength of calcium, approximately 422.7 nm. The resulting absorbance is proportional to the calcium concentration in the sample. The method of determining the chemical composition of the lobster body is using the proximate test to determine the main nutritional content in the lobster body, including water content, ash content, protein content, fat content, and carbohydrate content.

Blood biochemistry analysis. The analysis was carried out on glucose of hemolymph (GH), glycogen (Gly), total protein (TP), total triglycerides (Trig), aspartate transaminase (AST), Alanine Aminotransaminase (ALT), and alkaline phosphatase (ALP). Blood samples from the EDTA tube were taken with a 1 mL syringe, then transferred into the analysis tube and put into the ARKRAY blood chemical analyzer machine (SPOTCHEMEZ sp 4430). Paper test indicators were prepared and arranged on a panel inside the tool. The blood chemical analyzer machine was set and coded according to the treatment, the analysis on the machine lasted 10 minutes, the results automatically came out in the form of a print out. The blood chemical analyzer machine was prepared and calibrated prior to analysis to ensure accurate and reliable measurement results. Before sample examination, the instrument was connected to a stable power source and allowed to warm up according to the manufacturer's operational instructions. Calibration and quality control procedures were performed using standard control solutions to verify that the analyzer was functioning within the acceptable analytical range. All reagent cartridges, diluents, and test strips required for biochemical analysis were checked for expiration date, cleanliness, and proper installation in the analyzer system.

Antioxidant status analysis. Analysis of antioxidant status, including levels of malondialdehyde (MDA) and superoxide dismutase (SOD) enzymes, were carried out at the end of the maintenance period using five *P. homarus* per replicate. Measurement of liver and blood plasma malondialdehyde (MDA) levels and superoxide dismutase (SOD) enzyme activity was conducted 24 hours after the final feeding treatment in order to evaluate oxidative stress status and antioxidant defense responses in the experimental lobster samples. The analysis was performed to determine the physiological effects of the dietary treatments on lipid peroxidation and antioxidant enzyme activity. All sampling and analytical procedures were carried out under controlled laboratory conditions to minimize oxidation and degradation of biological samples. MDA levels and SOD enzyme activity were measured using a spectrophotometer (HITACHI U-2001).

Data analysis. This study used a completely randomized design (CRD). Data of total haemocyte count (THC), GH, Gly, TP, Trig, AST, ALT, and ALP, malondialdehyde (MDA) and superoxide dismutase (SOD) were statistically analyzed using one-way ANOVA (F-test) with 95% confidence interval, with Microsoft Excel 2013 and Minitab 19.0. When significant differences were detected ($P < 0.05$), Tukey's post-hoc test was applied. Water quality data were analyzed descriptively.

Results and Discussions

Body calcium profile. The results demonstrated that varied calcium administration significantly changed the concentration or content of body calcium in the meat, exoskeleton, hepatopancreas, hemolymph, molting lobster body, and dead lobster body ($P < 0.05$) (Table 1).

Table 1

The calcium composition of the lobster body in the meat, exoskeleton, hepatopancreas, hemolymph, molting and dead body of *Panulirus homarus*

Parameter	<i>CaCO₃</i> doses (mg L^{-1})			
	0	20	40	60
Hepatopancreatic	28,667±10.08 ^c	105,200±31.70 ^a	71,333±7.51 ^b	81,200±0.70 ^{ab}
Hemolymph	148,667±3.17 ^a	32,000±3.35 ^c	67,467±2.35 ^b	70,667±2.64 ^b
Exoskeleton	13,242.91±0.14 ^c	14,091.19±0.06 ^b	9,855.12±0.23 ^d	14,923.37±0.35 ^a
Meat	1,150.17±0.15 ^c	1,197.93±0.46 ^c	1,564.75±0.13 ^a	1,502.70±0.23 ^b
Moulting body	1,160.65±4.36 ^b	1,512.33±2.67 ^{ab}	1,786.40±1.12 ^a	1,310.22±4.15 ^{ab}
Dead body	1,092.70±0.05 ^c	871.82±0.03 ^d	1,657.73±0.16 ^a	1,616.09±0.28 ^b

Values with different superscripts are significantly different ($P < 0.05$)

In comparison to the control, the addition of 40 mg L⁻¹ calcium carbonate had an impact on the high calcium content of the meat, molting lobster body, and dead lobster body. Compared to the control, the treatment that included the addition of 60 mg L⁻¹ calcium carbonate had an impact on the exoskeleton's high calcium content. In comparison to controls, treatment involving the addition of 20 mg L⁻¹ calcium carbonate had an impact on the calcium concentration of the hepatopancreas. The control treatment (without the addition of calcium carbonate) had the highest calcium level in hemolymph, though (P<0.05). The calcium in the hemolymph has not been optimally utilized for molting activities, so the calcium in the hemolymph is still high.

Body chemistry profile. The findings of the analysis of variance (ANOVA) revealed that the water content, protein content, and body lipid of the pueruli were significantly affected by the various calcium doses (Table 2). The obtained lipid content varied from 1.52-3.23% while the protein level varied from 5.49-12.01%. The amount of protein in lobster pueruli at dosage 0 (without the addition of calcium) varied significantly from the amount at dose 60 mg L⁻¹ of calcium. At a calcium dose of 60 mg L⁻¹, lobster pueruli had a lower body lipid content than at a dose of 20 mg L⁻¹, which was statistically significant. Although lobster at a dose of 60 mg L⁻¹ had much less water than lobster at a level of 0 (without calcium).

Table 2

Chemical composition of body of *Panulirus homarus* at different doses of CaCO₃

Wet weight (%)	CaCO ₃ doses (mg L ⁻¹)			
	0	20	40	60
Water	84.34±2.34 ^a	80.58±1.16 ^{ab}	81.65±3.66 ^{ab}	77.04±3.22 ^b
Ash	5.55±1.46	5.74±1.91	7.55±3.17	6.80±1.69
Protein	5.49±1.69 ^b	7.86±1.16 ^{ab}	7.13±2.34 ^b	12.01±1.82 ^a
Lipid	3.23±0.58 ^{ab}	4.52±1.03 ^a	1.92±1.09 ^b	1.52±0.41 ^b
Carbohydrate				
Coarse fiber	0.93±0.02	0.64±0.52	0.91±0.19	1.47±0.32
Nitrogen-free extract	0.47±0.16	0.66±0.73	0.85±0.69	1.17±0.34

Values with different superscripts are significantly different (P<0.05).

The calcium concentration in the meat, molting lobster body, and dead lobster body was higher with the addition of 40 mg L⁻¹ CaCO₃ dosage than in other treatments. It is believed that the addition of a dosage of CaCO₃ 40 mg L⁻¹ resulted in good calcium absorption by the pueruli. The amount of calcium in the body that is high or low depends on how much calcium is taken in by the body from the environment (Greenaway 1985). Chitin, protein, and calcium carbonate are the three main constituents of the cuticle. Other minor constituents include proteoglycans, lipids, and other organic materials (Nagasawa 2012). The lipid value in the treatment with the addition of a dose of 40 mg L⁻¹, which tends to be lower, can be used to determine how lipids were used by the lobster's pueruli in the process of hardening the cuticle after molting. In order to cause the breakdown of animal protein, calcium activates neutral proteases, also known as calcium activated neutral protease (CANP). The amount of protein breakdown is influenced by the CANP activity linked to ionic Ca. In this study, it was discovered that lobster meat's calcium and protein contents tended to be lower in the control group than the treatment group. Calcium is an essential mineral that plays a crucial role in various physiological processes in lobsters, particularly in exoskeleton formation, mineralization, osmoregulation, muscle contraction, and the molting cycle (Chang & Mykles 2011). In particular, Ca²⁺ is crucial for the transmission of nerve impulses, osmoregulation, muscle contraction, and as a cofactor in a number of enzyme reactions. The proper amount of calcium is hypothesized to stimulate immunological and enzymatic responses to environmental stressors and pathogenic bacterial assaults, allowing the lobster pueruli to develop more rapidly.

Immune response. The findings of the analysis of variance (ANOVA) revealed that the value of THC was significantly impacted by the various calcium doses ($P < 0.05$). At a calcium dose of 40 and 60 mg L^{-1} , lobster pueruli had a greater THC and lysozyme activity (ASZ) values than control. Figures 1 and 2 below show the immunological response of the lobster pueruli along the study.

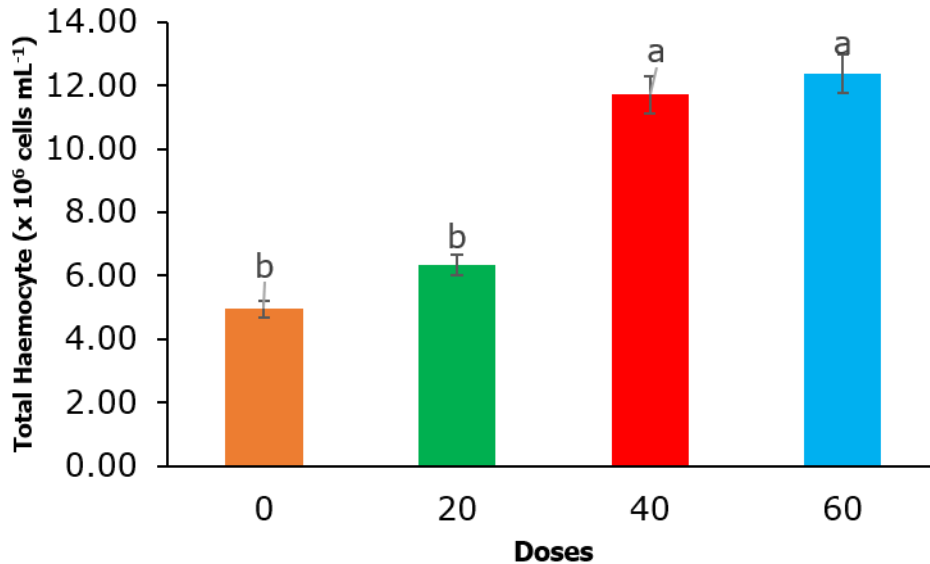


Figure 1. Total hemocyte count with different doses of calcium.

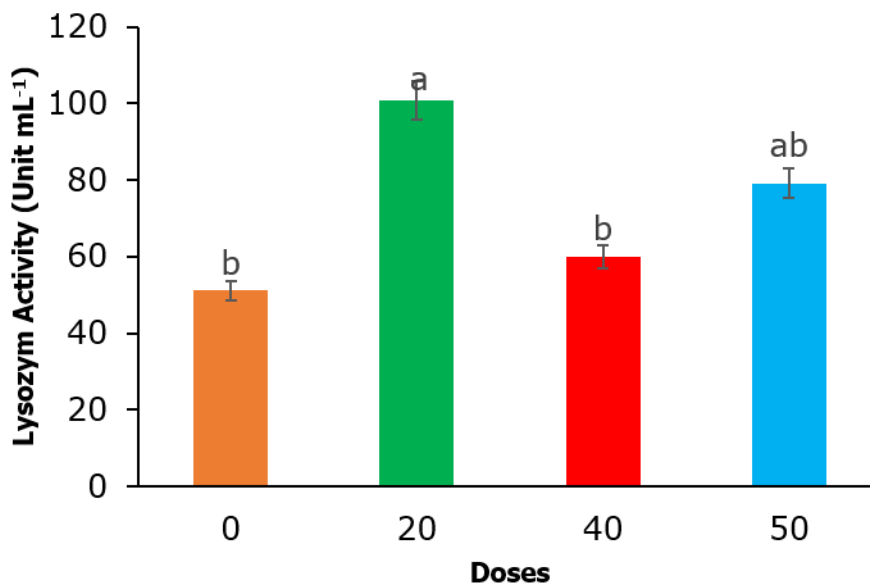


Figure 2. Lysozyme activity with different doses of calcium.

Since lobsters lack a distinct immune response, they rely on a range of nonspecific immunological responses. Cellular and humoral immune responses make up the nonspecific immune response. A crucial part of the defense of the lobster body and an unfavorable environment is played by hemocytes, which are a type of cellular immune response. In general, lobster pueruli treated with 40 mg L^{-1} of calcium showed a greater THC value than the control, indicating that the immune system was successful in fending off pathogenic infections and levels of environmental stress. One physiological indicator that can be used to assess the degree of stress in lobsters is THC. The THC of the treated sand lobster generally had greater values than the control, indicating that the immune system was successful in fending off pathogenic infections and levels of environmental stress. In comparison to the control, the ASZ value in the calcium addition therapy often exhibited a

greater value ($P < 0.05$). As a result, it is believed that the addition of calcium will increase the lobster pueruli' resistance to sickness by causing enzyme activity in its body. Although both THC and ASZ were still within normal ranges, lobsters raised without the addition of calcium tended to have a higher hemolymph glucose content than the therapy. According to this, lobsters in the control treatment are more vulnerable to stress. Hyperglycemia or elevated glucose levels are signs that the lobster is under stress. Stress causes the glucose levels in lobsters to rise or perhaps reach hyperglycemia. A significant indication of adaptive stress in crustaceans is hyperglycemia, which prompts CHH to release glucose from glycogen stores and utilize it as a substrate for anaerobic respiration (Powell et al 2017). In order to provide energy sources and boost homeostasis during stress, the processes of glycogenolysis and gluconeogenesis produce glucose (Ocampo et al 2003). It has been shown that a variety of proteins have the capacity to bind chitin and form chitin/protein complexes. The basic components of chitin, according to Zaidy (2007), are complex proteins and glycogen. The addition of 40 mg L^{-1} calcium tends to result in lower glycogen levels than other treatments ($P < 0.1$). This is due to the fact that during post-molting, lobsters utilize glycogen to help produce chitin and harden their cuticles. Environmental changes have an impact on changes in crab hemolymph protein levels. Additionally, hemolymph proteins are involved in a variety of physiological processes in crustaceans, including oxygen delivery and stress response (Lorenzon et al 2011). The amount of protein in hemolymph is also thought to be a measure of lobster strength (Bolton et al 2009). According to Lorenzo et al (2011), each species of crustacean hemolymph included a significantly different proportion of total protein, with Penaeid shrimp having the greatest concentration. According to Mercier et al (2006), crustacean physiological changes can be observed using metabolic measures such total protein, glucose, triglycerides, and hemocyanin. In this investigation, the treatment that included an extra dose of 60 mg L^{-1} CaCO_3 had the greatest total protein value. While there was no discernible difference in the triglyceride levels across the regimens.

Blood chemistry. According to the analysis of variance (ANOVA) results, the treatment with various calcium doses had a significant impact on the levels of GH, ALP, TP, ALT, and AST ($P < 0.05$). AST and ALT enzymes are often used as biomarkers of health and stress in crustaceans. AST values in the control treatment were higher ($1,066.22 \text{ U L}^{-1}$) than in other treatments. Lobsters reared in the control treatment tend to experience metabolic stress. ALT values in the 20 mg L^{-1} of calcium treatment were higher than in other treatments. Stressed lobster conditions can increase ALT values. The Gly value was considerably impacted by calcium treatment at various doses ($P < 0.01$). Table 3 shows the blood biochemistry of the lobster pueruli that was retained throughout the study.

Table 3

Blood biochemistry of *Panulirus homarus* pueruli reared with different doses of calcium

Parameter	CaCO_3 doses (mg L^{-1})			
	0	20	40	60
GH ($\text{mg } 100 \text{ mL}^{-1}$)	48.51 ± 0.29^{ab}	46.75 ± 0.28^{ab}	37.80 ± 0.49^b	51.15 ± 0.20^a
Gly ($\text{mg } 100 \text{ mL}^{-1}$)*	4.38 ± 0.23^{ab}	4.46 ± 0.38^a	3.89 ± 0.17^b	4.13 ± 0.04^{ab}
TP (g L^{-1})	30.07 ± 1.64^b	33.27 ± 4.95^{ab}	37.80 ± 3.14^{ab}	39.32 ± 1.78^a
Trig (mg dL^{-1})	94.20 ± 1.36	61.86 ± 0.52	74.84 ± 0.38	93.97 ± 0.95
ALP (U L^{-1})	47.67 ± 0.74^b	246.40 ± 1.30^a	79.69 ± 3.18^b	60.00 ± 1.19^b
AST (U L^{-1})	$1,066.22 \pm 0.25^a$	683.30 ± 1.07^c	927.10 ± 0.32^b	699.00 ± 0.44^c
ALT (U L^{-1})	839.80 ± 2.40^a	356.20 ± 2.42^{bc}	196.72 ± 0.16^c	472.0 ± 0.54^b

Values with different superscripts are significantly different ($P < 0.05$). *($P < 0.01$)

Antioxidant status. The results of analysis of variance (ANOVA) showed that different doses of calcium had a significant effect on the value of malondialdehyde (MDA) and not significant effect on the value of superoxide dismutase (SOD). Compared to other treatments, the MDA value in the 40 treatment is lower. Table 4 shows the antioxidant status of the lobster pueruli that was kept throughout the investigation.

Table 4

Antioxidant status of *Panulirus homarus pueruli* reared with different doses of calcium

<i>Dosis CaCO₃</i> (mg L ⁻¹)	<i>Parameter</i>	
	<i>SOD (U μg⁻¹ protein)</i>	<i>MDA (μmol L⁻¹)</i>
0	7.29±1.07	1.51±0.49 ^{bc}
20	6.45±1.39	4.03±0.74 ^a
40	7.67±0.90	0.68±0.02 ^c
60	8.47±1.23	2.12±0.12 ^b

Values with different superscripts are significantly different (P<0.05).

The AST, ALT, and ALP enzyme groups were also examined in this investigation as additional blood biochemical markers. Researchers frequently utilize this metric as a measure of kidney and liver health. Under normal circumstances, this enzyme's activity is at low, steady levels, but when liver injury and environmental factors are present, the concentration rises (Luo et al 2013). The AST and ALT readings in this study were greater in the control group than in the treatment group. The ALP value was higher than the control and other treatments at a dose of 20 mg L⁻¹. In earlier research, an increase in alkaline phosphatase (ALP) activity was closely related to how aquatic animals responded to stressful situations (Molina et al 2005). As a biomarker for cell membrane damage, MDA is frequently employed (Marciniak et al 2009). It was discovered in this study that when lobster pueruli were raised with a calcium dose of 40 mg L⁻¹, the MDA value was comparatively low in comparison to other treatments. This indicates that lobsters reared with doses <40 mg L⁻¹ and >40 mg L⁻¹ are relatively experiencing oxidative stress. Stress conditions in lobsters can result in disruption of metabolic processes and growth. The growth of absolute weight of lobster reared with calcium dose of 40 mg L⁻¹ tended to be higher than control (P<0.05). One of the endogenous enzymatic antioxidants that combat free radicals in the body that can lessen oxidative stress is superoxide dismutase (SOD). However, additional antioxidants are required to neutralize these free radicals if the body's free radical molecules surpass the capacity of endogenous antioxidant protection (Umeno et al 2017). In the study, it was discovered that the lobster pueruli in each treatment adapted to unfavorable environmental conditions (the presence of free radicals) by turning on the SOD enzyme's role in scavenging free radicals. The reason lobsters do this is because free radicals can result in tissue death and damage.

Conclusions. In general, calcium carbonate supplementation improved the physiological responses of lobsters. The addition of 40 mg L⁻¹ calcium carbonate resulted in higher calcium content in lobster meat and body tissues during the molting process, which contributed to better exoskeleton formation and mineralization. Furthermore, the treatment reduced lobster mortality, indicating improved physiological stability and adaptation during molting. The enhanced calcium availability also appeared to support the immune system, making lobsters more resistant to pathogenic infections and environmental stress. Therefore, supplementation with 40 mg L⁻¹ calcium carbonate can be considered an effective strategy to improve mineral balance, physiological performance, molting success, and overall health condition in lobsters.

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Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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