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Squid Ink Extract as Immunostimulant on Blood Glucose and THC of Sand Lobster (*Panulirus homarus*)

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ABSTRACT

Sand lobster (*Panulirus homarus*) is a high-value aquaculture commodity but is vulnerable to physiological stress and Vibrio sp. infection. This study aimed to analyze the effect of squid ink extract (Uroteuthis duvaucelii) as a feed immunostimulant on blood glucose level, blood profile (Total Hemocyte Count/THC and Differential Hemocyte Count/DHC), and Total Vibrio Count (TVC) of sand lobster at different doses and rearing durations. A factorial completely randomized design was used with four doses (0, 400, 500, and 600 ppm) and five observation times (days 0, 7, 14, 21, and 28) with five replications. Data were analyzed using ANOVA followed by Duncan's test (α=0.05). The results showed that dose, rearing duration, and their interaction had a significant effect ($p<0.05$) on all parameters. Squid ink extract lowered blood glucose and TVC, increased THC and the proportion of granular and semi-granular cells, while the proportion of hyaline cells decreased. The best response was achieved at a dose of 600 ppm on day 21, namely the lowest glucose level (30.20 mg/dL), the highest THC (7.24×10^4 cells/mL), and the lowest TVC (3.12 CFU/g). Squid ink extract has potential to be developed as a natural immunostimulant in sand lobster aquaculture.

INTRODUCTION

Sand lobster (*Panulirus homarus*) is one of the fishery commodities of high economic value, with continuously increasing demand in both domestic and global markets (Suryanto & Wahjuningrum, 2019; KKP, 2023). However, lobster aquaculture still faces serious obstacles in the form of high mortality due to declining environmental quality and infectious disease outbreaks (Putra *et al*., 2020; Sudewi *et al*., 2018).

One of the main threats in lobster aquaculture is infection by *Vibrio* sp., an opportunistic bacterium that often emerges when water quality declines. This infection causes systemic disorders, reduced appetite, body lesions, and even mass mortality that harms farmers (Fitrani & Purnama, 2020). Disease control efforts have so far been dominated by antibiotic use, even though long-term use causes bacterial resistance, tissue residues, and negative environmental impacts (Pasaribu, 2021). Therefore, a safer and more sustainable lobster health management strategy is needed.

The use of natural immunostimulants is a promising alternative because it can enhance the non-specific immune response of organisms without causing toxic effects (Bricknell & Dalmo, 2005; Austin & Austin, 2016). Squid ink (*Uroteuthis duvaucelii*) has potential as a source of natural immunostimulant because it contains melanin, peptides, polysaccharides, and

phenolic compounds that possess antibacterial, antioxidant, and immunomodulatory activities (Derby, 2014; Liu *et al*., 2023). Previous studies have shown that squid ink supplementation improves the immune response and resistance to *Vibrio* infection in white shrimp (*Litopenaeus vannamei*) (Zhao *et al*., 2019), and exhibits antibacterial activity against aquatic pathogens (Fitrial & Khotimah, 2017).

Most studies on the use of squid ink are still limited to fish and shrimp, while its application to sand lobster is relatively rare, even though this commodity is highly susceptible to *Vibrio* sp. and environmental stress. The physiological response of lobster can be monitored through the blood glucose level, while the immunological response can be observed through the blood profile (Witeska *et al*., 2023). This study aimed to analyze the effect of squid ink extract as a feed immunostimulant on blood glucose level, blood profile (THC and DHC), and TVC of sand lobster at different doses and rearing durations, as well as their interaction.

METHOD

Time, Place, and Materials

The research was conducted from August to December 2025. Lobster rearing as well as the measurement of blood glucose level and blood profile were carried out at the Laboratory of Anatomy and Aquaculture, while TVC examination was conducted at the Laboratory of Microbiology. The test animals were sand lobsters (Panulirus homarus) weighing 45–55 g, totaling 200 individuals, obtained from Banyuwangi, East Java. Squid (Uroteuthis duvaucelii) as the extract material was obtained from markets in Sidoarjo and Surabaya. The feed used was trash fish.

Research Design

The study used a factorial completely randomized design (CRD) with two factors and five replications (Kusriningrum, 2012). The first factor was the squid ink extract dose, namely A0 (0 ppm/control), A1 (400 ppm), A2 (500 ppm), and A3 (600 ppm). The second factor was the observation time, namely days 0, 7, 14, 21, and 28. Lobsters were reared at a stocking density of 10 individuals per aquarium (20 L) for 28 days. The dose determination referred to Fadjar et al. (2022).

Extract and Test Feed Preparation

Squid ink was extracted by dissolution using Phosphate Buffer Saline (PBS), precipitation with ethanol, centrifugation, and freeze drying to obtain a dry ink powder. The test feed was prepared by mixing the ink extract according to the dose (400, 500, and 600 ppm) into trash fish using a binder (progol), referring to Setiyowati et al. (2022). Feeding followed a feeding rate of 4% of lobster biomass per day, while the control feed (A0) had no extract added.

Research Parameters

Blood glucose level was measured from hemolymph taken from the ventral sinus between the fourth and fifth walking legs using a digital glucose meter (Mahasri et al., 2020). The blood profile included the Total Hemocyte Count (THC), counted using a haemocytometer under a microscope at 100× magnification (Jussila et al., 1997), and the Differential Hemocyte Count (DHC), namely the percentage of hyaline, granular, and semi-granular cells. The Total Vibrio Count (TVC) was counted using the plate count method on TCBS medium and expressed in CFU/g (Buller, 2004; Idami & Nasution, 2020). Supporting parameters in the form of water quality (temperature, salinity, pH, and dissolved oxygen) were measured during rearing.

Data Analysis

Data were analyzed using Analysis of Variance (ANOVA). If a significant effect was found, it was followed by Duncan's Multiple Range Test (DMRT) at a significance level of $\alpha = 0.05$ to determine differences among treatments (Blanca et al., 2023; Deepthy & Kalesh, 2025).

RESULTS AND DISCUSSION

Table 1. Average blood glucose level (mg/dL) of sand lobster during rearing

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
K (0 ppm)	49.80±0.83^{c}	51.80±0.83^{b}	52.40±0.54^{ab}	53.40±0.54^{a}	52.40±0.54^{ab}
A1 (400 ppm)	49.80±0.83^{c}	46.80±0.83^{d}	42.80±0.83^{f}	39.80±0.83^{h}	37.80±0.83^{i}
A2 (500 ppm)	50.20±0.83^{c}	46.20±0.83^{de}	41.20±0.83^{g}	37.20±0.83^{ij}	36.20±0.83^{j}
A3 (600 ppm)	50.20±0.83^{c}	45.20±0.83^{e}	38.20±0.83^{i}	30.20±0.83^{l}	33.20±0.83^{k}

Note: values are mean ± standard deviation. Different superscripts in the same column indicate significant differences among treatments ($p \leq 0.05$).

Table 1 shows that on day 0 all treatments were not significantly different (49.80–50.20 mg/dL) because the treatments had not yet been applied. From day 7, the glucose level of the treatment groups decreased and differed significantly from the control. On day 14, the control glucose level reached 52.40 mg/dL, while A1, A2, and A3 were 42.80, 41.20, and 38.20 mg/dL, respectively. The sharpest decrease occurred in A3 (600 ppm), reaching 30.20 mg/dL on day 21, whereas the control actually increased to 53.40 mg/dL. This pattern indicates that the higher the squid ink extract dose, the lower the lobster blood glucose level.

Blood glucose level is a sensitive indicator of physiological stress in crustaceans. The high glucose level in the control group indicates that the lobsters were under stress. Under stressful conditions, lobsters require additional energy to survive, so the body triggers the release of *Crustacean Hyperglycemic Hormone* (CHH). This hormone activates glycogenolysis and gluconeogenesis in the hepatopancreas, namely the breakdown of glycogen reserves into glucose that is subsequently mobilized into the hemolymph (Sukmawati *et al*., 2023). As a result, glycogen reserves decrease while blood glucose increases as a rapid energy source to respond to the pressure. The high glucose in the control therefore reflects a heavy stress load, whereas the decrease in glucose in the treatment groups shows that the bioactive content of squid ink is able to suppress the intensity of that stress response so that the need for emergency energy mobilization is reduced.

This decrease is related to the activity of melanin and polysaccharides in squid ink extract. Melanin has an antioxidant capability that neutralizes free radicals during oxidative stress so that physiological pressure is reduced (Liu *et al*., 2023). Jiang *et al*. (2020) reported similar findings in *Litopenaeus vannamei* given squid ink melanin, where hemolymph glucose levels were lower and more stable than in the control. Hoseinifar *et al*. (2021) stated that immunostimulants based on natural compounds suppress the oxidative stress burden so that glucose homeostasis can be maintained. The increasingly consistent decreasing pattern up to days 21 and 28 confirms that the glucose-stabilizing effect is cumulative (Mulia, 2022; Fitrani & Purnama, 2020).

Table 2. Average Total Hemocyte Count (THC) ($\times 10^4$ cells/mL) of sand lobster during rearing

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
K (0 ppm)	5.28±0.83^{j}	5.34±0.54^{ij}	5.44±0.54^{hi}	5.54±0.54$_{gh}$	5.44±0.54^{hi}
A1 (400 ppm)	5.28±0.83^{j}	5.58±0.83^{g}	5.88±0.83^{f}	6.08±0.83^{e}	6.28±0.83^{d}
A2 (500 ppm)	5.32±0.83^{j}	5.82±0.83^{f}	6.22±0.83^{d}	6.62±0.83^{c}	6.74±0.54^{b}
A3 (600 ppm)	5.32±0.83^{j}	6.02±0.83^{e}	6.62±0.83^{c}	7.24±0.11^{a}	6.84±0.11^{b}

Note: values are mean ± standard deviation. Different superscripts in the same column indicate significant differences among treatments ($p \leq 0.05$).

Table 2 shows that the THC values of the treatment groups increased over time, with the highest values consistently in A3 (600 ppm), namely 6.02, 6.62, and 7.24×10^4 cells/mL on

days 7, 14, and 21. In contrast, the control only increased slightly from 5.28 to 5.54×10^4 cells/mL. The higher the squid ink dose, the higher the lobster THC value.

THC reflects the cellular defense capacity of lobster because hemocytes play a role in phagocytosis, encapsulation, and the production of immune effector molecules, analogous to leukocytes in vertebrates (Liu *et al*., 2020). The increase in THC in the treatment groups indicates that the bioactive compounds of squid ink stimulate the proliferation and mobilization of hemocytes from the hematopoietic tissue (Siregar *et al*., 2024). Jiang *et al*. (2020) reported that squid ink melanin increased THC and resistance to infection in *Litopenaeus vannamei*, while Bouallegui (2021) stated that marine bioactive materials rich in polysaccharides have the potential to activate hematopoiesis. The low THC in the control indicates the consumption of hemocytes to fight infection without adequate regeneration (Arifah & Khatimah, 2024), and the progressive increase in the treatments confirms an effect that is cumulative and dose-dependent (Idami & Nasution, 2020).

The relationship between blood glucose level and THC can be explained through the same stress-response framework. When lobsters experience stress, as seen in the control group, blood glucose increases as a form of energy mobilization for survival. At the same time, lobsters attempt to defend themselves through the cellular immune system; however, under prolonged stress and *Vibrio* sp. infection, hemocytes are continuously consumed for phagocytosis and encapsulation faster than their regeneration rate, so the hemocyte count (THC) in the control group remains low (Arifah & Khatimah, 2024). Conversely, the administration of squid ink extract reduces the stress burden—indicated by the decreasing blood glucose level—while also supplying bioactive compounds that stimulate hemocyte proliferation so that THC increases. Thus, when glucose decreases, signifying that the lobster is no longer under stress, the immune capacity reflected in the THC value recovers and increases. This opposing pattern underlies the formation of a very close negative relationship between the two parameters.

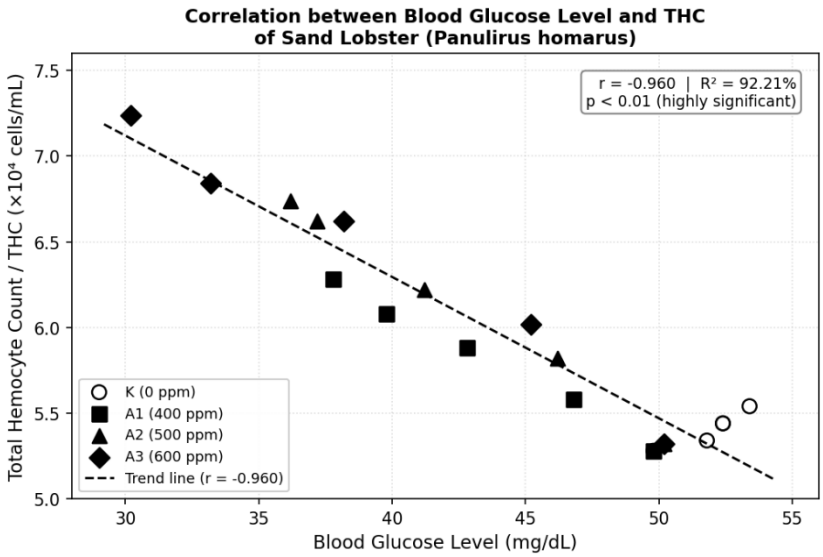


Figure 1. Correlation graph between blood glucose level (mg/dL) and Total Hemocyte Count/THC (×10^4 cells/mL) of sand lobster during rearing with squid ink extract administration at various doses (r = -0.960; p ≤ 0.01)

Figure 1 shows the scatter pattern of the data points between blood glucose level and THC, forming a downward trend line from the upper left to the lower right, reflecting a consistent negative relationship across all treatment groups. The control group (K, 0 ppm)

occupies the lower right of the graph with the highest glucose level (49.80–53.40 mg/dL) and the lowest THC value (5.28–5.54×10^4 cells/mL), showing that without an immunostimulant the lobsters were in a physiological stress condition that inhibited the immunological response. Conversely, group A3 (600 ppm) is located in the upper left with the lowest glucose level (30.20–50.20 mg/dL) and the highest THC (5.32–7.24×10^4 cells/mL), indicating that the highest dose of squid ink extract was the most effective in suppressing stress while increasing immunity. Groups A1 (400 ppm) and A2 (500 ppm) lie gradually between the two extremes, reinforcing the dose-dependent response pattern.

The relationship between the two parameters was measured using the Pearson correlation test and produced a correlation coefficient of r = -0.960 with a significance of $p < 0.01$. The negative sign of r indicates an inverse direction; that is, the lower the blood glucose level, the higher the THC value, and vice versa. The magnitude of r approaching -1 indicates that the strength of the relationship is very strong, while the value of $p < 0.01$ confirms that the relationship is statistically significant and did not occur by chance. The coefficient of determination (R^2) of 92.21% means that 92.21% of the variation in THC can be explained by changes in blood glucose level, while the remaining 7.79% is influenced by other factors outside the model. This close correlation occurs because both variables lie on the same physiological pathway: blood glucose level represents the stress burden, while THC represents the immune defense capacity, so that every reduction in stress pressure marked by a decreasing blood glucose is directly followed by the recovery and increase of the hemocyte count. Thus, the decrease in blood glucose level correlates very strongly with the increase in the immunological capacity of sand lobster.

The water quality parameters during the study (temperature, salinity, pH, and dissolved oxygen) were within the optimal range for sand lobster, with a temperature of 26–30°C (Louhenapessy *et al*., 2023). The stability of water quality ensured that the observed differences in response were treatment effects, not the result of environmental variation, while also helping to suppress the proliferation of *Vibrio* sp. (Norfolk *et al*., 2023). Overall, the best physiological and immunological responses were consistently found at a dose of 600 ppm (A3) with a rearing time of day 21. Administration through feed is a practical and stress-friendly method for lobster (Fadjar *et al*., 2022; Setiyowati *et al*., 2022), so squid ink extract has the potential to be applied as a functional feed supplement in healthier and more sustainable lobster aquaculture (Pasaribu, 2021; KKP, 2023).

CONCLUSION

Based on the research conducted, it was found that herbal therapy using young teak leaf solution was effective in treating catfish infected with A. hydrophila bacteria with an optimal dose in treatment B (10 g /L), with a decrease in the number of bacteria by 0.06 x 10^7 in the kidneys and 0.02 x 10^7 in the liver, with a survival rate and cure rate of 100%. This result is influenced by the concentration in each treatment, the highest concentration has the lowest survival rate and the lowest concentration has the highest survival rate, this is because bioactive compounds always become toxic when given at high or excessive doses such as in treatment D (20 gr/L) namely stress or shock in fish where the body that was initially infected with bacteria meets a high dose of treatment that triggers stress in fish and high doses can make fish gasp due to tannins that accumulate in the gills, causing hypoxia or lack of oxygen and eventually death. The results of this study are expected to be easy to apply and useful for farmers.

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