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Effects of Tryptophan Addition to Green Mussel (*Perna viridis*) on Cannibalism and Physiological Responses in Lobster (*Panulirus homarus*)

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ABSTRACT

The increasing demand for sand lobsters requires higher aquaculture productivity. However, sand lobster production is still constrained by high mortality due to cannibalism, a behavior in which individuals attack and consume conspecifics. One approach to suppress cannibalism is through supplementation of the amino acid tryptophan as a precursor to serotonin, which plays a role in regulating stress and aggressiveness. The present study aimed to determine the effectiveness and optimal formulation of tryptophan in green mussel feed on cannibalism, survival, and growth rate in sand lobsters. The study used a completely randomized design with four treatments and five replications. The treatments consisted of adding tryptophan to green mussels used as feed, including a control without tryptophan (K0), and supplementation at doses of 5 g/kg (T1), 10 g/kg (T2), and 15 g/kg (T3). Observed parameters included physiological responses, cannibalism levels, and growth. The results revealed that T3 treatment was most effective in increasing serotonin levels (89.45 ± 3.069 ng/mL) and survival rate (55.29 ± 5.26 %), and reducing cannibalism (7.06 ± 2.63 %), which caused lower glucose (14.19 ± 3.206 mg/dL) and total hemocyte count (81.00 ± 4.243 cell/mL) levels. However, the highest dose (T3; 15 g/kg) was less effective in enhancing growth performance, indicating that this level of supplementation was not optimal compared to the lower doses. In contrast, T1 (5 g/kg) resulted in relatively higher growth performance compared to the other treatments, as indicated by moulting frequency (1.40 ± 0.23%), carapace length (1.25 ± 0.15 cm), length increase (1.70 ± 0.12 cm), absolute weight (1.14 ± 0.35 g), specific growth rate (3.13 ± 0.46 %/day), feed conversion ratio (5.04 ± 0.93), and protein efficiency ratio (1.42 ± 0.18). However, in T1 (5 g/kg), serotonin (70.66 ± 4.165 ng/mL), survival rate (42.35 ± 4.92%), cannibalism (38.82 ± 3.22%), glucose (16.26 ± 3.352 mg/dL), and total hemocyte count (110.50 ± 9.192 cell/mL) were not as optimal as those observed in T3. Tryptophan supplementation revealed potential to enhance physiological condition, survival, and growth under the experimental conditions.

Keywords: Cannibalism, Growth, Lobster, Physiological, Tryptophan

INTRODUCTION

The sand lobster (*Panulirus homarus*) is a high-value food commodity whose global demand increases annually (Efrizal et al., 2025). Sand lobster is a leading fishery product that contributes significantly to national exports and the income of farmers and coastal communities in Indonesia (Zuhriyah et al., 2025). Currently, sand lobster is one of the most widely cultivated lobster species (Petersen et al., 2013; Kintani et al., 2020), with national production reaching 8,279 tons and a production value of IDR 1.94 trillion (PDSPKP, 2023). The application of sustainable cultivation technology supported by optimal feed management has the potential to increase sand lobster cultivation productivity in coastal areas (Effendi et al., 2025). However, lobster cultivation still faces different complex challenges, particularly related to physiological, behavioral, and environmental aspects, which directly impact lobster health and productivity (Sarkar et al., 2026). One of the main problems in lobster cultivation is the high mortality of seeds in the early stages of life, which has been reported to range from 96.0 to 99.4% (Subagio et al., 2021).

The high mortality of lobster larvae in the early stages of life is closely related to several management issues, including limited food availability, which triggers cannibalism among individuals (Kelly et al., 2023). Cannibalism is a crucial issue in lobster cultivation, particularly during the moulting phase, which leaves lobsters physiologically highly vulnerable to attack (Codabaccus et al., 2025). Furthermore, nutritional deficiencies, high population densities, and limited shelter also contribute to cannibalism (Ma et al., 2021; Kelly et al., 2023). Physiological stress can also result from degraded water quality, leading to higher mortality and slower growth (Spencer et al., 2025; Sarkar et al., 2026).

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Therefore, the development of effective technology and managerial approaches to suppress cannibalism and stress is needed, one of which is through the use of the amino acid tryptophan in feed formulations, which has the potential to play a role in suppressing stress responses and aggressive behavior in lobsters (Feng et al., 2024; Debnath et al., 2024).

Tryptophan is an essential amino acid that acts as the main precursor of serotonin (5-hydroxytryptamine, 5-HT), a neurotransmitter that has an important function in modulating physiological responses, stress, and aggressive behavior in crustaceans, including lobsters (Rathore and Sarkar, 2021; Dinu et al., 2022). Through the neuroendocrine signaling pathway, tryptophan influences the synthesis and secretion of serotonin, which is regulated by neuroendocrine cells sensitive to environmental signals, thereby regulating the function of the central and peripheral nervous systems and maintaining organism homeostasis (Liu et al., 2020). Enrichment of tryptophan in the feed enables lobsters to adapt better to stressful conditions, especially during critical phases such as moulting, which is often followed by increased aggression and cannibalism (Leopoldo et al., 2010). Additionally, tryptophan enrichment in the feed can suppress cannibalism and promote growth in freshwater lobsters (Trisnasari et al., 2020), vannamei shrimp (Rachmawati et al., 2021), and mangrove crabs (Suharyanto and Yudhistira, 2012). These findings highlight the need for further investigation into the effectiveness of dietary tryptophan supplementation in sand lobsters for enhancing growth and reducing cannibalism.

Green mussels (*Perna viridis*) are the primary natural feed commonly used in lobster cultivation due to their high availability and their rich in nutritional and bioactive compounds (Jusadi et al., 2020; Rejeki et al., 2021). Green mussels contain high levels of protein, lipids, and essential fatty acids, thus playing a vital role in supporting lobster growth and health (Chakraborty et al., 2016). Different studies have reported that lobsters fed green mussels exhibited better growth performance, indicated by increased carapace length, body length, and total weight compared to those fed trash fish or pellets (Arumugam et al., 2020; Rivaie et al., 2023). The addition of the amino acid tryptophan to green mussels is expected to address the mortality due to stress and cannibalism, especially during the critical phase of lobster cultivation. The present study aimed to determine the effects and optimal formulation of tryptophan in green mussels on cannibalism, survival rate, and growth parameters in sand lobsters.

MATERIALS AND METHODS

Ethical approval

The present study was approved by the Health Research Ethics Committee, Faculty of Medicine, Diponegoro University, Indonesia (No. 297/EC/KEPK/FK-UNDIP/X/2025). All procedures were conducted in accordance with established laboratory and field guidelines. Experimental animals were observed daily, and feeding was administered according to the respective treatment groups.

Study place and time

The study was conducted from October to November 2025 at the Fish Health and Environment Laboratory, Lampung Marine Aquaculture Center, and Central Research and Clinical Diagnostic Laboratory, Healthy Animals, Malang, East Java, Indonesia.

Study design

The study used an experimental method to evaluate the effectiveness of tryptophan-enriched green mussel feed at different doses over a 60-day period. The experiment was designed using a completely randomized design consisting of four treatments with five replications, including a control without tryptophan supplementation (K0) and tryptophan supplementation in green mussels at doses of 5 g/kg (T1), 10 g/kg (T2), and 15 g/kg (T3) of feed.

Sand lobster seed sample

The sand lobsters used in the present study were in the puerulus stage, with an average total length of 2.2-2.4 cm and an average weight of 0.2-0.4 g. The pueruli were obtained from fishermen or collectors in the Pesisir Barat Regency, Lampung Province, Indonesia, and a total of 340 individuals were selected, all healthy and unblemished. The animals were distributed into experimental units at a density of 17 individuals per container. For transportation, the pueruli were packed in plastic bags filled with seawater, equipped with two sheets of black netting (0.5 cm mesh size), supplemented with pure oxygen, and tightly sealed. The bags were then placed in Styrofoam boxes containing ice wrapped in paper to maintain temperature stability, then closed, wrapped in plastic, and labeled according to lobster seed packaging standards (BSNI, 2024).

Preparation of the study container

The present study used 20 square plastic containers measuring 70 cm × 45 cm × 35 cm, operated under a flow-through water system. Each container was filled to an operational water depth of approximately 25 cm, resulting in a total water volume of about 70-80 L per container. Each unit was equipped with two aeration points for oxygen supply and six outlet holes (1 cm in diameter), with three outlets on each side, which served as overflow drainage to maintain a stable water level and continuous water exchange. The inflow water rate was maintained at 3 L min^{-1}. Additionally, each

container was provided with two netting shelters inserted into ½-inch polyvinyl chloride (PVC) pipes to provide refuge for the pueruli.

Test feed preparation

The test feed was prepared according to the method of Suharyanto and Yudhistira (2012) using green mussel (*Perna viridis*) meat as the main ingredient. The green mussels were opened using a knife, then the meat was removed and roughly chopped using a blender. 30 g of green mussel meat was prepared for each treatment. Next, the amino acid tryptophan was added at the treatment dosages reported in previous studies, namely 5 g/kg (Leopoldo et al., 2010), 10 g/kg (Trisnasari et al., 2020), and 15 g/kg (Ardina, 2021). Progol was added at 0.15 g as an adhesive and 2 mL of distilled water as a diluent. All test ingredients were stirred until homogeneous and then mixed evenly with the green mussel meat. The prepared feed was placed in plastic bags, labeled according to the tryptophan dosage, and stored in a refrigerator at approximately 4˚C until use.

Distribution and maintenance of sand lobster seeds

Sand lobster puerulus seeds were stocked into plastic containers at a density of 55 per m², equivalent to 17 per container. Before the test feed, the lobster seeds were acclimatized for 7 days by feeding them green mussels without added tryptophan to aid environmental adaptation, reduce stress, and accustom them to the feed. After acclimatization, the pueruli were reared for 60 days and fed green mussel meat enriched with tryptophan according to the treatment dosage. The daily feeding rate was set at 30% of biomass (body weight day^{-1}; BBPBL, 2022), which was divided into three feeding times: 30% of the daily ration at 07:00, 20% at 12:00, and 50% at 17:00 Western Indonesia Time (WIB), considering the higher nocturnal feeding activity of lobsters. Uneaten feed was siphoned out prior to the next feeding to maintain water quality. During the rearing period, approximately 20% of the population (four individuals per container) was sampled every two weeks.

Physiological parameters of lobster

Total hemocyte count

Total hemocyte count (THC) is commonly used as an indicator of stress in crustaceans. In this study, THC in lobster hemolymph was measured on days 0 and 60 of the culture period. Hemolymph samples were collected using a 1 mL syringe pre-rinsed with 10% (w/v) citric acid solution, which functions as an anticoagulant to prevent hemocyte aggregation and clotting, and were drawn from the base of the walking legs near the abdomen (Domínguez-Borbor et al., 2018). A drop of hemolymph was then loaded onto a hemocytometer (Neubauer chamber; chamber depth 0.1 mm), covered with a coverslip, and observed under a light microscope at 400× magnification. Hemocyte counts were performed in five large squares of the counting chamber, corresponding to a known volume, and THC values were calculated using the following formula by Abdollahi-Arpanahi et al. (2018).

$$\text{THC} = \frac{\sum \text{Total hemocyte counted}}{\text{Volume Box}} \text{ x Dilution factor}$$

Hemolymph glucose levels

Hemolymph samples were collected with a syringe rinsed with anticoagulant, drawn from the base of the walking leg near the abdomen, and transferred to microtubes. Samples were incubated for 10-20 minutes at 20-25˚C before analysis. Hemolymph glucose levels were determined by the glucose oxidase-peroxidase (GOD-PAP) method (Barham and Trinder, 1972). Briefly, 10 µL of hemolymph was mixed with 1000 µL of reagent solution containing glucose oxidase, peroxidase, phenol, and 4-aminoantipyrine. A glucose standard (100 mg dL^{-1}) was prepared and processed identically. Reaction mixtures were incubated at 37˚C for 10 minutes, and absorbance was measured at 500 nm with a spectrophotometer (PerkinElmer, USA). Glucose concentration was calculated by the following formula through comparing the sample absorbance with that of the standard (Barham and Trinder, 1972).

KG [mg/dl] = 100×ΔASTDΔAsample

Hemolymph glucose level (KG) was calculated as the ratio of the sample absorbance (ΔA_sample) to the standard solution absorbance (ΔA_STD), multiplied by 100 to express the result in mg/dL. The absorbance values were obtained using a spectrophotometer at the specified wavelength, where ΔA_sample represents the absorbance of the hemolymph sample, and ΔA_STD represents the absorbance of the glucose standard solution.

Serotonin content

Serotonin testing was performed to evaluate lobster aggression, with higher serotonin levels associated with reduced aggression (Esmaeili et al., 2025). Observations were made by taking hemolymph samples on day 0 (1 sample before

treatment) and day 60 (4 samples after treatment). Serotonin levels were measured using an Enzyme-Linked Immunosorbent Assay (ELISA) method and expressed in ng/mL to assess the relationship between stress and changes in serotonin levels in each treatment.

Cannibalism level

Observation of cannibalism levels was conducted using Closed Circuit Television (CCTV) installed above the experimental containers, with recordings taken before and after feeding at 5-hour intervals during the first 14 days of rearing. Cannibalism events were identified based on direct visual evidence of aggressive interactions leading to the attack and consumption of conspecifics. Mortality was classified as cannibalism when individuals indicated clear physical damage, such as missing body parts (such as the abdomen and appendages) or bite marks, or when predation events were directly recorded by CCTV. In contrast, mortality without visible signs of injury or predation was classified as non-cannibalism-related death. The level of lobster cannibalism was calculated using the formula below of Hseu et al. (2003).

$$K=\frac{KA-KS-KBK}{KA}\times 100\%$$

The cannibalism rate (K) was calculated as the percentage of individuals lost due to cannibalism relative to the initial number of individuals (KA). In this formula, KA represents the initial number of lobsters at the beginning of the experiment, KS represents the number of surviving individuals at the end of the observation period, and KBK represents mortality not caused by cannibalism. Thus, the value of K reflects the proportion of mortality specifically attributed to cannibalistic behavior.

Moulting

Moulting is a physiological process in crustaceans involving the periodic shedding of the exoskeleton (exuviae) to allow growth and development. The moulting frequency of lobsters was calculated based on the following formula described by Handayani and Syahputra (2018).

$$MFq = \frac{X_{molt}}{N_{tot}}$$

Moulting frequency (MFq) was calculated as the ratio of the total number of moulting events (X_{molt}) to the total number of individuals observed during the study period (N_{tot}). X_{molt} represents the cumulative number of moulting events recorded, while N_{tot} is the total number of lobsters maintained throughout the experimental period. The result is expressed as the average number of moulting events per individual.

Lobster growth parameters

Absolute length growth

The absolute length growth of sand lobsters during the study was calculated using the formula of Effendie (1997).

$L = L_t - L_0$

Absolute length increase (L) was calculated as the difference between the final length (L_t) and the initial length (L_o) of the lobster during the culture period. The parameter L_t represents the length of the lobster at the end of the experiment, while L_o represents the length at the beginning of the experiment. The result is expressed in centimeters (cm).

Absolute weight gain

The absolute weight growth of sand lobsters during the study was calculated using the formula of Effendie (1997).

$W = W_t - W_0$

where W is the absolute weight gain (g), W_t is the average final weight (g), and W_0 is the average initial weight (g). The parameter represents the total increase in body weight during the experimental period.

Kerapas length

Carapace length was measured from the tip of the rostrum to the posterior margin of the carapace using a digital caliper, following Baskoro et al. (2019). Carapace length increment was calculated as the difference between final and initial measurements ($CL = CL_t - CL_o$). The initial carapace length of the lobsters at the beginning of the experiment ranged from 2.2 to 2.4 cm.

Specific growth rate

The specific growth rate is the daily percentage increase in lobster weight. The specific growth rate was calculated using the formula of Wu et al. (2024).

SGR (% day 1) = 100 × (ln (FBW) - ln (IBW)) / day

Specific growth rate (SGR) was calculated as the natural logarithmic difference between the final body weight (FBW) and the initial body weight (IBW) over the experimental period, expressed as a percentage per day (% day^{-1}). FBW represents the average body weight of lobsters at the end of the rearing period, while IBW represents the average body weight at the beginning of the experiment.

Survival rate

Survival rate (SR) is the ratio of the total number of lobsters that survive until the end of cultivation to the number of lobsters at the start of stocking. The survival rate was calculated using the formula of Santoso et al. (2024).

$$SR = \left[\frac{Nt}{No}\right] x\ 100\%$$

The survival rate was calculated as the percentage of individuals remaining at the end of the experiment relative to the initial number stocked. In this calculation, Nt represents the number of lobster seeds at the end of the study, while N_0 represents the number of seeds at the beginning of the study. The result is expressed as a percentage (%).

Feed conversion rate

The feed conversion ratio (FCR) of sand lobsters during maintenance was calculated using the formula of Zonneveld et al. (1991).

$$FCR = F / (Wt - Wo)$$

Feed conversion rate (FCR) was calculated as the ratio between the total feed provided (F) and the weight gain of lobsters during the study period. In this calculation, F represents the total amount of feed given (g), W_t represents the total weight of lobsters at the end of the study (g), and W_0 represents the total weight of lobsters at the beginning of the study (g).

Protein efficiency ratio

The protein efficiency ratio (PER) of sand lobsters during the rearing period was calculated based on Jafri (1999). Protein intake was determined by measuring the total feed consumption, calculated as the difference between the amount of feed offered and the uneaten feed collected after each feeding. The uneaten feed was carefully collected, dried, and weighed to obtain accurate estimates of consumption. The amount of protein intake was then calculated by multiplying the actual feed consumption by the crude protein content of the feed, as determined through proximate analysis. PER was subsequently calculated as the ratio between weight gain and total protein intake.

$$PER = Wg / P$$

Protein efficiency ratio (PER) was calculated as the ratio between total weight gain (Wg) and protein intake (P). In this calculation, Wg represents the total absolute weight gain of lobsters during the rearing period (g), while P represents the total amount of protein consumed (g), which was estimated from feed intake multiplied by the crude protein content of the feed.

Water quality

Water quality measurements were conducted throughout the maintenance period. Several parameters were measured, including temperature, pH, dissolved oxygen (DO), salinity, nitrite, and nitrate. The methods and water quality parameters measured during the study are shown in Table 1.

Table 1. Water quality parameters measured during the study

Parameter	Unit	Methods/tools	Measurement time
Temperature	˚C	Thermometer	once a week
pH	-	pH meter	once a week
Dissolved oxygen	mg/L	Dissolved oxygen meter	once a week
Salinity	Ppt	Refractometer	once a week
Nitrite	mg/L	Spectrophotometer	once a week
Ammonia	mg/L	Spectrophotometer	once a week

Data analysis

Data on total hemocyte count (THC), haemolymph glucose levels, serotonin, cannibalism levels, moulting frequency, carapace length, absolute length, absolute weight, specific growth rate, feed conversion ratio, survival rate, protein efficiency, and water quality during maintenance were tabulated using Microsoft Excel and statistically analyzed using IBM SPSS Statistics (version 25). A least significant difference (LSD) test was performed when significant differences among treatments were detected.

RESULTS

Physiological parameters

The results of the physiological response test revealed that all observed parameters had relatively similar initial values in all treatments (week 0), indicating that the lobsters' initial physiological state was homogeneous. The initial THC value was recorded at 203 ± 70.74 cells/mL, hemolymph glucose levels were 19.48 ± 1.075 mg/dL, and serotonin levels were 100.35 ± 11.667 ng/mL. The uniformity of these initial values indicated that the lobsters had not yet experienced the effects of the feed treatment and remained in a normal physiological state.

After feeding tryptophan-enriched green mussels for eight weeks of maintenance, different changes occurred in each physiological parameter. THC values experienced a significant decrease ($p < 0.05$) in all treatments compared to the initial conditions. The lowest THC values were found in the T3 and control treatments, at 81.00 ± 4.243 cells/mL and 91.50 ± 4.950 cells/mL, respectively, followed by the T1 treatment at 110.50 ± 9.192 cells/mL, while the highest value was found in T2 at 129.00 ± 7.071 cells/mL.

Contrary to THC parameters, hemolymph glucose levels did not differ significantly between treatments ($p > 0.05$), ranging from 14.05 ± 0.276 mg/dL to 20.54 ± 3.465 mg/dL. The THC results indicated that tryptophan supplementation does not directly affect energy metabolism, and lobsters are still able to maintain their physiological balance. Meanwhile, serotonin levels differed significantly among treatments ($p < 0.05$), with the lowest value observed in the control (56.37 ± 5.812 ng/mL), followed by T1 (70.66 ± 4.165 ng/mL). Treatments T2 (90.09 ± 6.463 ng/mL) and T3 (89.45 ± 3.069 ng/mL) were not significantly different from each other ($p > 0.05$), but both were significantly higher than the control and T1 treatments ($p < 0.05$). The results of the physiological parameters of the lobsters after treatment are shown in Table 2.

Table 2. Physiological parameters of lobsters before and after treatment of adding tryptophan to green mussel feed during the 60-day rearing period.

Parameter	Treatment	Observation time	
		Initial of maintenance (Week 0)	End of maintenance (Week 8)
Total hemocyt count (cell/mL)	K0	203 ± 70.74	91.50 ± 4.950[a]
	T1		110.50 ± 9.192[b]
	T2		129.00 ± 7.071[c]
	T3		81.00 ± 4.243[a]
Glucose (mg/dL)	K0	19.48 ± 1.075	14.05 ± 0.276[a]
	T1		16.26 ± 3.352[a]
	T2		20.54 ± 3.465[a]
	T3		14.19 ± 3.206[a]
Serotonin (ng/mL)	K0	100.35 ± 11.667	56.37 ± 5.812[a]
	T1		70.66 ± 4.165[b]
	T2		90.09 ± 6.463[c]
	T3		89.45 ± 3.069[c]

K0**:** Control (green mussels without tryptophan supplementation), T1**:** 5 g/kg, T2**:** 10 g/kg, T3**:** 15 g/kg

Cannibalism level

The results indicated significant differences in cannibalism rates among treatments ($p < 0.05$). The highest cannibalism rate was observed in the control treatment (47.06 ± 4.16%), followed by T1 (38.82 ± 3.22%) and T2 (34.12 ± 4.92%), which were not significantly different from each other ($p < 0.05$). The lowest cannibalism rate was recorded in T3 (7.06 ± 2.63%). A comparison of cannibalism rates among treatments is presented in Figure 1.

Moulting frequency

The moulting frequency of sand lobsters fed green mussels enriched with tryptophan revealed significant differences among treatments ($p < 0.05$). The highest moulting frequency was observed in T1 (1.40 ± 0.23 times per individual), followed by the control treatment (K0; 0.98 ± 0.30 times per individual). Lower moulting frequencies were recorded in T3 (0.64 ± 0.03 times per individual) and T2 (0.60 ± 0.06 times per individual). The moulting frequency of sand lobsters fed green mussels enriched with tryptophan is shown in Figure 2.

Carapace length

The results indicated that enrichment of green mussel feed with tryptophan had a significant effect on the carapace length of sand lobsters ($p < 0.05$). The highest carapace length values were observed in T1 (1.25 ± 0.15) and T2 (1.23 ± 0.11), which were not significantly different from each other ($p > 0.05$). Lower values were recorded in the control treatment (K0; 0.95 ± 0.13) and T3 (0.86 ± 0.15), which were also not significantly different from each other ($p > 0.05$). The results of carapace length growth measurements are presented in Figure 3.

Absolute length

The results indicated that enrichment of green mussel feed with tryptophan had a significant effect on the absolute length of sand lobsters ($p < 0.05$). The highest absolute length values were observed in T1 (1.70 ± 0.12) and T2 (1.70 ± 0.06), which were not significantly different ($p > 0.05$). Lower values were recorded in the control treatment (K0; 1.48 ± 0.13) and T3 (1.35 ± 0.17), which were also not significantly different from each other ($p > 0.05$). The results of absolute length measurements of sand lobsters are presented in Figure 4.

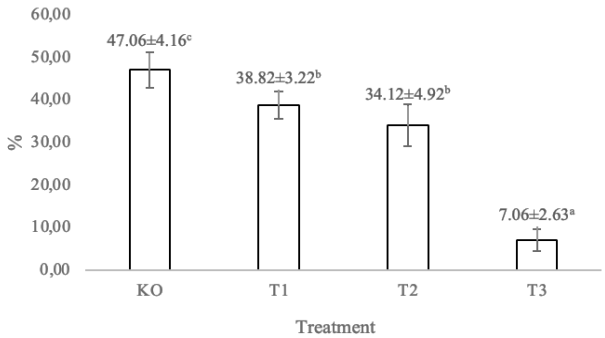

Figure 1. Cannibalism levels of sand lobsters during the 60-day rearing period fed with tryptophan-enriched green mussels at different doses. Treatments consisted of K0 (control, without tryptophan), T1 (5 g/kg), T2 (10 g/kg), and T3 (15 g/kg).

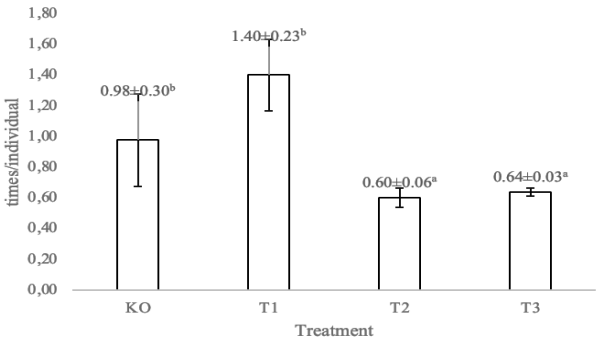

Figure 2. Moulting frequency of sand lobsters during the 60-day rearing period fed with tryptophan-enriched green mussels at different doses. Treatments consisted of K0 (control, without tryptophan), T1 (5 g/kg), T2 (10 g/kg), and T3 (15 g/kg).

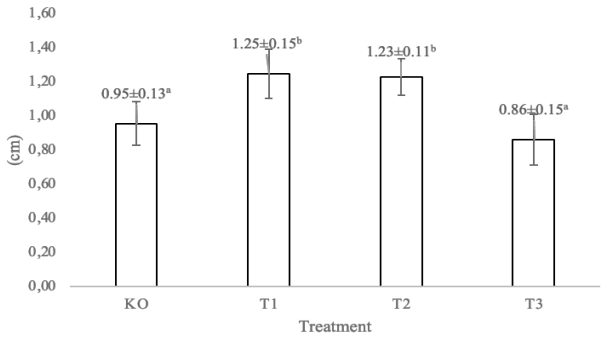

Figure 3. Carapace length of sand lobsters during the 60-day rearing period fed with tryptophan-enriched green mussels at different doses. Treatments consisted of K0 (control, without tryptophan), T1 (5 g/kg), T2 (10 g/kg), and T3 (15 g/kg).

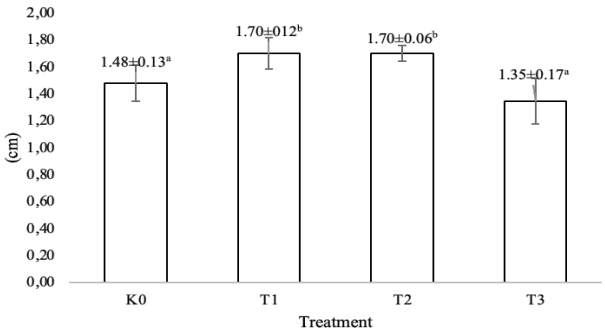

Figure 4. Absolute length of sand lobsters during the 60-day rearing period fed with tryptophan-enriched green mussels at different doses. Treatments consisted of K0 (control, without tryptophan), T1 (5 g/kg), T2 (10 g/kg), and T3 (15 g/kg).

Absolute weight

The results indicated that enrichment of green mussel feed with tryptophan had a significant effect on the absolute weight of sand lobsters ($p < 0.05$). The highest absolute weight values were observed in T1 (1.14 ± 0.35) and T2 (1.02 ± 0.15), which were not significantly different ($p > 0.05$). Lower values were recorded in the control treatment (K0; 0.68 ± 0.12) and T3 (0.60 ± 0.10), which were also not significantly different from each other ($p > 0.05$). The results of absolute weight measurements of sand lobsters are presented in Figure 5.

Specific growth rate

The results of the study revealed that enriching green mussel feed with tryptophan resulted in different specific growth rates among treatments. T1 (3.13 ± 0.46) and T2 (3.02 ± 0.22) were not significantly different from each other ($p > 0.05$), and both revealed higher values compared to the control (K0; 2.43 ± 0.26) and T3 (2.27 ± 0.23), which were also not significantly different from each other ($p > 0.05$). The results of specific growth rate are presented in Figure 6.

Feed conversion ratio

The results of the study indicated that enriching green mussel feed with tryptophan significantly improved the feed conversion ratio of sand lobsters ($p < 0.05$). The lowest feed conversion ratio values were observed in T1 (5.04 ± 0.93) and T2 (5.65 ± 0.54), which were not significantly different from each other ($p > 0.05$). The next highest values were recorded in the control treatment (K0; 6.43 ± 1.00), while the highest value was observed in T3 (7.69 ± 0.91). The feed conversion ratio values for sand lobsters are presented in Figure 7.

Protein efficiency ratio

The results indicated that enriching green mussel feed with tryptophan significantly affected the protein efficiency ratio (PER) of sand lobsters ($p < 0.05$). The highest PER value was observed in T1 (1.42 ± 0.18), which was significantly different from T3 (0.95 ± 0.08) ($p < 0.05$), but not significantly different from the control treatment (K0; 1.33 ± 0.20) ($p > 0.05$). The PER in T2 (1.17 ± 0.11) was not significantly different from K0 or T1 ($p > 0.05$), but was significantly higher than T3 ($p < 0.05$). Overall, T3 revealed the lowest PER value and differed significantly from the higher treatments ($p < 0.05$). The protein efficiency ratio values of sand lobsters are presented in Figure 8.

Survival rate

The results revealed that enriching green mussel feed with tryptophan significantly increased the survival rate of sand lobsters ($p < 0.05$). The highest survival rate values were observed in T3 (55.29 ± 5.26) and T2 (52.94 ± 7.50), which were not significantly different from each other ($p > 0.05$). These were followed by T1 (42.35 ± 4.92), while the lowest value was recorded in the control treatment (K0; 28.24 ± 7.67). The survival rate values of sand lobsters are presented in Figure 9.

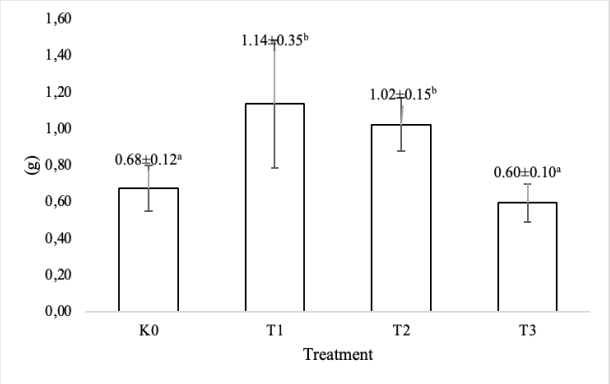

Figure 5. Absolute weight of sand lobsters during the 60-day rearing period fed with tryptophan-enriched green mussels at different doses. Treatments consisted of K0 (control, without tryptophan), T1 (5 g/kg), T2 (10 g/kg), and T3 (15 g/kg).

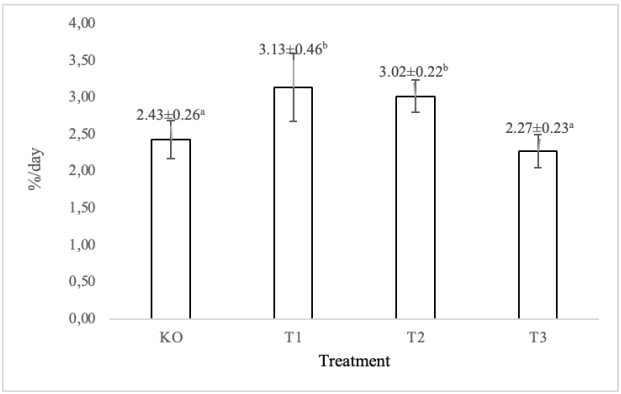

Figure 6. Specific growth rate of sand lobsters during the 60-day rearing period fed with tryptophan-enriched green mussels at different doses. Treatments consisted of K0 (control, without tryptophan), T1 (5 g/kg), T2 (10 g/kg), and T3 (15 g/kg).

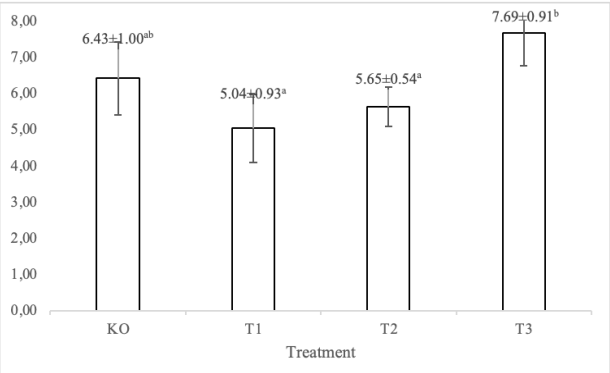

Figure 7. Feed conversion ratio values of sand lobsters during the 60-day rearing period fed with tryptophan-enriched green mussels at different doses. Treatments consisted of K0 (control, without tryptophan), T1 (5 g/kg), T2 (10 g/kg), and T3 (15 g/kg).

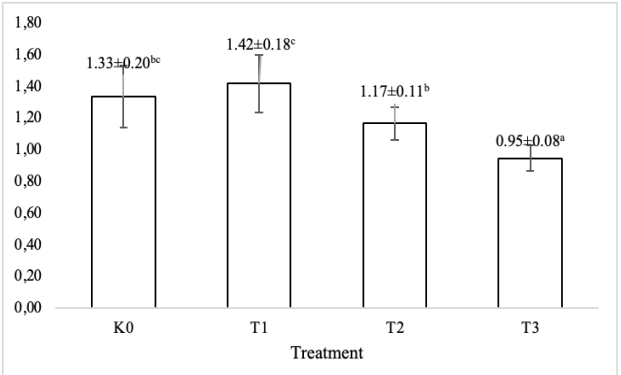

Figure 8. Protein efficiency ratio value of sand lobster during the 60-day rearing period fed with tryptophan-enriched green mussels at different doses. Treatments consisted of K0 (control, without tryptophan), T1 (5 g/kg), T2 (10 g/kg), and T3 (15 g/kg).

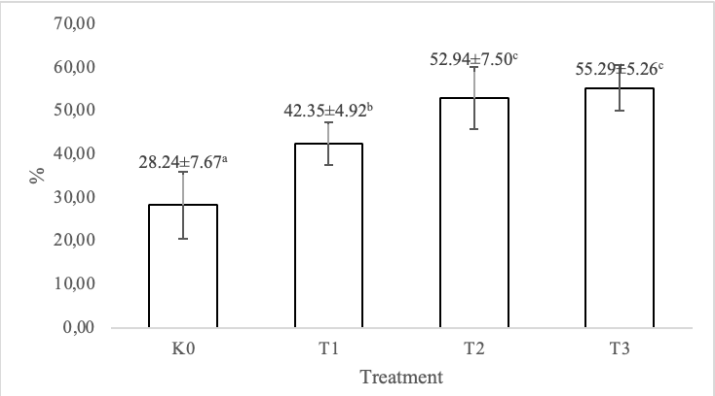

Figure 9. Survival rate of sand lobsters during the 60-day rearing period fed with tryptophan-enriched green mussels at different doses. Treatments consisted of K0 (control, without tryptophan), T1 (5 g/kg), T2 (10 g/kg), and T3 (15 g/kg).

Water quality

Water quality during the maintenance period ranged within values that remained optimal for sand lobster cultivation. The measured parameters included a temperature of 29.43-29.54˚C, pH 7.66-8.01, salinity 31.40-31.60 ppt, dissolved oxygen (DO) 5.98-6.07 mg/L, ammonia 0.11-0.12 mg/L, and nitrite 0.08 mg/L. The complete results of the water quality measurements are shown in Table 3.

Table 3. Average water quality parameters during the 60-day rearing period of sand lobsters

Parameter \ Treatment	Unit	K0	T1	T2	T3
Temperature	˚C	29.43 ± 0.315	29.45 ± 0.277	29.47 ± 0.286	29.54 ± 0.367
Dissolved Oxygen	(mg/L)	6.07 ± 0.075	6.02 ± 0.076	5.98 ± 0.087	6.02 ± 0.058
Salinity	(ppt)	31.40 ± 0.894	31.60 ± 0.894	31.40 ± 0.894	31.40 ± 0.894
pH	-	7.96 ± 0.084	7.96 ± 0.069	7.66 ± 0.708	8.01 ± 0.113
Ammonia	(mg/L)	0.12 ± 0.005	0.11 ± 0.002	0.12 ± 0.003	0.12 ± 0.003
Nitrite	(mg/L)	0.08 ± 0.001	0.08 ± 0.002	0.08 ± 0.002	0.08 ± 0.001

K0: Control (green mussels without tryptophan supplementation), T1: 5 g/kg, T2: 10 g/kg, T3: 15 g/kg

DISCUSSION

Physiological stress responses in crustaceans, particularly lobsters, are generally evaluated using biochemical and immunological parameters, such as hemolymph glucose levels and Total hemocyte count (THC) values , during culture (Djai et al., 2017). The results of the present study indicated that there was no significant increase in hemolymph glucose levels between treatments at either the beginning or end of culture, suggesting that the tryptophan content in green mussels does not directly affect hemolymph glucose metabolism. Consistent increases in glucose levels are usually indicators of environmental or physiological stress in crustaceans (Conneely and Coates, 2021; Turnbull et al., 2024), so the stability of glucose levels in the present study indicated that the lobsters were under relatively controlled environmental conditions. On the other hand, THC values at the beginning and end of culture revealed a significant decrease, which is thought to reflect the process of cellular immune system adjustment during culture in response to tryptophan supplementation (Mayall et al., 2021). THC is an important parameter in evaluating crustacean health because changes in hemocyte count are closely related to immune responses, physiological adaptation, and stress exposure (Ooi et al., 2019; Baladrat et al., 2022). Overall, the combination of stable hemolymph glucose levels and changes in THC indicates that lobsters fed a diet supplemented with the amino acid tryptophan are not under severe physiological stress, as stress generally results in an increase in L-lactate followed by an increase in glucose, and changes in THC are significant predictors of stress in crustaceans (Aji et al., 2019; Conneely and Coates, 2024). Furthermore, these results suggest that tryptophan supplementation in green mussel feed may primarily function through serotonergic pathways that regulate behavior, thereby reducing aggression and cannibalism, rather than directly affecting primary energy metabolism (Cao et al., 2025).

Serotonin levels in all tryptophan-supplemented treatments were relatively high, ranging from 70.66-90.09 ng/mL, and were higher than those observed in the control treatment. The increase in serotonin levels was accompanied by a reduction in cannibalism rates in sand lobsters during the study period, with tryptophan-supplemented treatments showing lower cannibalism rates (7.06-47.06%) than the control. According to Trisnasari et al. (2020) and Cao et al. (2025), tryptophan is an essential amino acid that has been shown to reduce cannibalism rates. Furthermore, tryptophan is considered a major precursor in serotonin synthesis, a process that occurs primarily in the nervous system, including

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the brain, through a conserved metabolic pathway across species, including crustaceans (Roth et al., 2021; Moon et al., 2022). Increased serotonin not only plays a role in neurotransmission and the control of aggressive behavior but also contributes to the regulation of animal metabolism and physiological responses, as well as to the modulation of stress responses and cannibalism in aquatic organisms (Höglund et al., 2019; Maffei, 2021). The use of tryptophan in suppressing cannibalism rates has also been reported in a study by Ardina (2021) which indicated that the addition of 1.5% tryptophan to commercial whiteleg shrimp feed was able to suppress cannibalism by up to 2.50%, and in a study by Trisnasari et al. (2020) which reported that 1% tryptophan supplementation in artificial feed can increase growth while reducing cannibalism rates in freshwater lobsters by 10.00 ± 7.07%.

The present study revealed that the addition of tryptophan to green mussels at a dose of 5 g/kg (T1) resulted in a relatively high moulting frequency, reaching 1.40 ± 0.23 times/individual, indicating an acceleration of the sand lobster growth process during the rearing period. A high moulting frequency is known to be positively correlated with crustacean growth, as the moulting process allows for an increase in body size after the formation of a new exoskeleton; the more frequent the moulting, the greater the opportunity for increased biomass (Zhang et al., 2021; Ding et al., 2024). Hormones and metabolic regulation that control moulting are also strongly influenced by nutritional status and the availability of essential amino acids, which play a role in the synthesis of structural proteins and the formation of new tissue after moulting (Shyamal et al., 2018). The results of the present study are in line with the findings reported by Shyamal et al. (2018), where the 5 g/kg (T1) dose treatment indicated higher specific growth rate, carapace length, absolute weight gain, and absolute length gain compared to other treatments. Tryptophan, as an essential amino acid, is also known to influence feeding behavior and energy utilization efficiency, thus supporting overall crustacean growth (Mohammady et al., 2025). Furthermore, the lower FCR and higher protein efficiency observed in the tryptophan-supplemented treatment (T3) indicated that tryptophan supplementation increases nutrient utilization efficiency, allowing more feed protein to be allocated to growth rather than metabolic activity or stress responses (Agustiana et al., 2022; Mohammady et al., 2025).

The survival rate values for all tryptophan-enriched green mussel feed treatments were higher than those for the control, with the highest value, 55.29%, observed in the T3 treatment. The high value is attributed to the fact that high mortality rates generally occur during the puerulus phase of lobster seed rearing, reaching 96.0-99.4% (Subagio et al., 2021). The high survival rate in the tryptophan-supplemented treatment is related to a decrease in cannibalism rates during rearing, where lobster mortality in the tryptophan treatment tended to be normal compared to the control, which was largely caused by cannibalism. These findings are supported by studies by Kelly et al. (2023) and Rivaie et al. (2023), which state that cannibalism is the main cause of low survival in the post-puerulus phase and that this behavior becomes more dominant when lobsters experience physiological stress that can weaken the immune system and trigger aggressiveness (Conneely and Coates, 2024). Tryptophan, an essential amino acid, plays a role in serotonin synthesis, which suppresses stress responses, reduces aggressive behavior and cannibalism, and maintains the physiological balance of lobsters, allowing for more efficient energy utilization for body maintenance and environmental adaptation (Esmaeili et al., 2025). Furthermore, although the survival rate and lobster growth were relatively high during the maintenance period, the measured ammonia concentrations (0.11-0.12 mg/L) were higher than the optimal level for sand lobster reported by Prama and Kurniaji (2022), which is < 0.01 mg/L. According to Prama and Kurniaji (2022), optimal water quality parameters for sand lobster cultivation include a temperature of 25-30˚C, salinity of 32-36 ppt, pH of 7.5-8.5, dissolved oxygen > 5 mg/L, ammonia < 0.01 mg/L, and nitrite < 0.1 mg/L. The water quality conditions, particularly ammonia levels, were not fully optimal and may have induced suboptimal or mild stress in the culture system. Therefore, the relatively high survival rate values observed in the present study are likely influenced not only by water quality conditions but also by other factors, including tryptophan supplementation, which may have contributed to stress mitigation and improved physiological performance of sand lobsters (Cao et al., 2025).

CONCLUSION

Based on the present results, dietary tryptophan supplementation in green mussel feed affected the physiological responses and growth performance of sand lobsters, with variations observed among the tested doses. The highest dose (15 g/kg; T3) was associated with higher serotonin levels, survival rate, and lower cannibalism, but tended to result in reduced growth performance. In contrast, the lower dose (5 g/kg; T1) indicated higher values in several growth parameters, including carapace length, absolute length, weight gain, specific growth rate, feed conversion ratio (FCR), and protein efficiency ratio (PER), while exhibiting relatively lower effects on physiological responses. These results suggest a possible trade-off between physiological responses and growth performance in relation to tryptophan dosage; however, this interpretation should be considered cautiously in relation to the statistical outcomes presented in the main text. The study is limited by the range of doses tested and the absence of long-term observations; therefore, further

research is recommended to evaluate a broader dosage range and to assess long-term effects on growth, health status, and stress physiology in sand lobsters.

DECLARATIONS

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Authors' contributions

Muhamad Sabar Syafi conducted data collection and prepared the original manuscript. Meci Desi Yulia and Yos Baragama were involved in the research implementation and data collection. Vivi Endar Herawati and Aninditia Sabdaningsih contributed to data analysis, administration, and conceptualization. Sri Hastuti, Pujiono W. Purnomo, and Arief Rahman Rivaie contributed to data interpretation, drafting, and manuscript preparation. All authors have read and approved the final version of the manuscript.

Availability of data

All relevant data generated during the study are included in this published article and are available upon reasonable request from the corresponding author.

Competing interests

The authors declare no conflict of interest.

Ethical considerations

All authors have carefully reviewed the manuscript to ensure there are no ethical concerns, including plagiarism, research misconduct, data fabrication or falsification, and redundant publication. The authors confirmed that no AI tools were used during the preparation of the present study.

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