Evaluating Fresh Earthworm as an Alternative Feed for the Spiny Lobster *Panulirus homarus*: Growth and Immune Responses

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Abstract

Limited feed availability is one of the major challenges in scaling up lobster aquaculture in Indonesia. At present, most lobster farmers rely heavily on trash fish, mainly sardines, which are nutritionally unbalanced, compete with human consumption, and are subject to seasonal fluctuations in price and availability. This study evaluated the use of earthworms as an alternative feed for juvenile scalloped spiny lobster (Panulirus homarus), assessing their nutritional value and effects on growth, survival, and immune responses. A 63-day feeding trial was conducted with four treatments and three replicates per treatment: 100% fish (Control), 70% fish + 30% earthworm (R7C3), 30% fish + 70% earthworm (R3C7), and 100% earthworm (C10). Hemolymph samples were collected to analyze total hemocyte count (THC) and phenoloxidase (PO) activity alongside growth measurements. Lobsters fed earthworm-based diets (C10, R3C7, and R7C3) exhibited significantly higher specific growth rates (0.98–1.73%/day) compared with the control (0.42–0.59%.day^{-1}). Survival followed a similar pattern, with markedly higher rates in earthworm-fed groups (60–75%) than in the control (~25%). Immune parameters, including THC and PO activity, were significantly elevated in lobsters receiving earthworm diets. These findings demonstrate that earthworms are a promising, sustainable feed alternative that improves growth, survival, and immunity in P. homarus, reducing dependence on trash fish in lobster aquaculture.

Keywords: Spiny lobster, Panulirus homarus, earthworm, sustainable, feed, growth, immune

Introduction

Sustainable aquaculture is increasingly important in securing global food supply by providing animal-based protein to meet rising demand. Spiny lobsters (*Panulirus homarus*), valued for their high market price, are a key aquaculture species in Southeast Asia, particularly in Indonesia and Vietnam (Jones, 2010). However, more than five decades after initial development efforts, feed availability remains a major obstacle, as no commercial lobster feed is currently available (Nankervis and Jones, 2022). Vietnam, the region's leading lobster producer, relies almost entirely on low-value seafood as feed, while Indonesia, despite its abundant seedstock, struggles with the scarcity of suitable feed resources (Jones *et al.*, 2019).

In Indonesia, lobster farmers typically depend on trash fish, mainly sardines, as their primary feed. This practice has drawbacks because sardines often do not meet lobsters' dietary needs, leading to slow growth and low survival (Petersen *et al.*, 2013), and they are also an important protein source for humans, creating competition with aquaculture (Warren and Steenbergen, 2021). These challenges highlight the urgent need for nutritionally balanced, sustainable,

and economically viable alternatives. According to Goncalves *et al.* (2024), live feed offers several advantages over formulated diet. For example, *Artemia* provides highly digestible, fresh nutrients in a natural form, with lower nutrient leaching and waste accumulation, helping maintain stable water quality and reduce microbial load. In contrast, artificial diets can negatively affect water quality, potentially compromising lobster health and increasing mortality (Hinchcliffe *et al.*, 2022). Hence, live feed as an alternative diet is considered more advantageous (Kalaiselvan *et al.*, 2024) as it provides higher nutritional availability, enhances feed palatability, and align with natural feeding behavior, supporting better growth performance (Martins *et al.*, 2026).

Recent efforts in lobster aquaculture have focused on developing sustainable feed alternatives to reduce reliance on trash fish while supporting both growth performance and animal health. Several potential alternatives have been explored, such as black soldier fly larvae (Agustin *et al.*, 2023), golden apple snails (Anggraini *et al.*, 2018), and chicken egg hatching waste (Okta *et al.*, 2023). Despite promising nutritional profiles, these options face challenges regarding feed acceptance, lipid balance, biosecurity, and compositional consistency.

Earthworms, although not yet tested in lobster diets, are a promising candidate: they provide high-quality protein, essential amino acids, and moderate lipid levels (Musyoka *et al.*, 2019; Parolini *et al.*, 2020), which could better align with lobsters' nutritional needs while avoiding lipid-related health issues (Pucher *et al.*, 2014). Studies in other aquaculture species demonstrate that earthworms can enhance growth, immune response, and nutrient utilization (Bhuvaneshwaran *et al.*, 2019; Genodepa and Apines-Amar, 2024). Further, early juvenile lobsters prefer feeds that are both tender and cohesive (Kropielnicka-Kruk *et al.*, 2024), and fresh earthworms meet this need while releasing chemical cues such as amino acids and small peptides (Kavle *et al.*, 2023) that stimulate lobsters' chemosensory-driven feeding, promoting rapid acceptance and efficient ingestion (Williams, 2006). Earthworms can also be grown on organic waste, turning it into nutritious feed while reducing biosecurity risks and supporting a circular economy (Pérez-Godínez *et al.*, 2017). While these attributes underscore their potential as a superior feed choice, further research is needed to confirm their effectiveness in lobster aquaculture.

This study investigates the potential of fresh earthworms as an alternative feed for juvenile spiny lobsters, focusing on growth, survival, and immune responses. The findings aim to contribute to the development of sustainable and efficient lobster aquaculture systems by reducing reliance on trash fish.

Materials and Methods

Study site and experimental setup

The experiment was conducted at the Marine Bioindustry Laboratory, Kurnaen Sumadiharga Science Complex, National Research and Innovation Agency (BRIN), located in North Lombok, West Nusa Tenggara. Wild juvenile lobsters were procured from local fishermen in the southern part of Lombok Island, Indonesia. Due to the limited availability of juvenile specimens, we utilized a total of 150 lobsters with initial body weights ranging from 4 to 39 g. After collection, the animals were transported to the laboratory and acclimated for approximately three weeks in four 500-L concrete tanks supplied with flow-through filtered seawater and continuous aeration. During this period, they were fed a mixture of sardines and small amounts of earthworms to facilitate adaptation to the experimental diets. However, stress from mishandling during transport and natural mortality during acclimation reduced the cohort to 71 surviving individuals. From these, a final group of sixty lobsters was selected for the experimental trials.

Diet preparation and proximate analysis

Sardines used as feed were purchased in bulk from local fishermen. The head and tail were removed due to their lower edible content, and the remaining portions were cut into 1.5–2 cm pieces, in line with feed size ranges recommended for small palinurid lobsters weighing 2-50 g, as described by Nankervis and Jones (2022). The prepared feed was then stored frozen in small plastic bags. Frozen stock was thawed in the refrigerator for 24 h prior to feeding to allow gradual defrosting while minimizing spoilage, and thawed fish were fed to lobsters on the same day. Earthworms were obtained from a local vermiculture facility and maintained alive in containers filled with spent mushroom substrate (SMS). They were fed organic food scraps to ensure freshness and harvested immediately before use. For feeding, the earthworms were provided to lobsters whole and live, with no further preparation. Both fish and earthworms were washed thoroughly and weighed prior to feeding.

To determine nutritional content, a proximate analysis was conducted on both feed types following standard AOAC methods (1995) with minor adjustments to suit laboratory conditions. Moisture content was measured by oven-drying 2 g of sample at 100–105°C until constant weight. Ash content was determined by burning 3–5 g of sample in a furnace at 400–550°C. Protein content was quantified by the Kjeldahl method, with nitrogen converted to protein using the factor 6.25. Lipid content was analysed using Soxhlet extraction with n-hexane, followed by evaporation and weighing. Carbohydrates were measured spectro-photometrically at 490 nm using a glucose standard curve, while crude fiber was determined by refluxing a fat-free sample with sulfuric acid and sodium hydroxide, filtering, drying, and weighing the residue. The proximate composition of sardine and earthworms used in this experiment is presented in Table 1.

Feeding trial

The feeding trial was carried out in twelve 100-L fiberglass tanks, with five lobsters per tank. Each tank underwent 100% water exchange every other day and was equipped with a pump, filtration system, shelters, and continuous aeration to maintain water quality between exchanges. Due to variation in individual size, lobsters were grouped into five weight classes (4-6 g, 6-9 g, 9-13 g, 13-20 g, and >20 g), defined based on natural weight distribution and biological relevance rather than equal intervals. Each tank contained one lobster from each class, thereby standardizing size structure and tank biomass across treatments.

Table 1. Proximate analysis of feed used in the experiment

Analysed nutrient content (% of dry matter)	Sardines	Earthworm
Dry matter (% fresh weight)	25.37	18.07
Ash	10.03	8.69
Protein	74.89	65.74
Fat	3.00	1.77
Carbohydrate	12.12	23.80
Fiber (% carbohydrate)	1.95	0.55

Lobsters were fed ad libitum once daily at 16:00, at a ration equivalent to 15% of their total biomass (fresh weight). Uneaten feed was siphoned before the next feeding. The trial lasted 63 days and consisted of four dietary treatments with three replicates each: Control (100% fish), R7C3 (70% fish + 30% earthworm), R3C7 (30% fish + 70% earthworm), and C10 (100% earthworm). Temperature, salinity, pH, and dissolved oxygen (DO) were measured once a week just before water replacement with a multiparameter water quality meter (HI98194, Hanna Instruments). Measurements of ammonia, nitrite, nitrate, and phosphate were conducted every two weeks with Sera test kits for ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), and phosphate (PO_4) (Sera GmbH, Germany). Water quality was maintained under ambient conditions, and measured parameters remained within or close to the optimum range for spiny lobster (Table 2).

Survival and growth measurement

Survival of the lobsters in each tank was monitored daily and expressed as survival rate (SR). Growth measurements, based primarily on body weight, were taken every two weeks. To ensure accuracy, lobsters were dried to minimize water droplets before being weighed individually. At the end of the feeding trial, Specific Growth Rate (SGR) was calculated following the formula below. Because of variation in lobster size within each tank, calculating an average tank weight was not appropriate; instead, SGR was measured individually by tracking the growth of each lobster, facilitated by the distinct size classes present in each tank.

$$SGR\ (\%.day^{-1}) = \left(\frac{\ln W_t - \ln W_0}{t} \right) \times 100\%$$

Where W_0 is the initial weight (g), W_t is the final weight (g), t is the duration of the trial in days.

Sample extraction and haemolymph collection

At the end of the experiment, haemolymph samples were extracted using a 1 mL syringe prefilled with 0.5 mL of precooled anticoagulant solution (0.27 M trisodium citrate, 0.385 M NaCl, 0.115 M glucose; pH 7.5) at a 1:1 ratio (Huang *et al*., 2009). The haemolymph was drawn from the coxa of the fourth walking leg, as described by Perdomo-Morales *et al*. (2007). The samples were immediately transferred into 1.5 mL microtube and kept on ice throughout the extraction process to preserve stability.

Total Haemocyte Count (THC)

The total haemocyte count (THC) was determined according to Gomez-Jimenez *et al*. (2000). After extraction, a 100 µL aliquot of haemolymph was immediately fixed in 4% formalin solution with 0.45 M NaCl at a 1:2 ratio and stored at 4°C until analysis. To count haemocytes, 2 µL of the fixed haemolymph mixture was placed on a haemocytometer and observed under a microscope at 400× magnification.

Phenoloxidase (PO) activity

Phenoloxidase activity was analysed by measuring dopachrome formation from L-DOPA using a spectrophotometric method adapted from Liu *et al*. (2004). The analysis was conducted on the same day haemolymph was collected. A 10 µL plasma sample was diluted with 90 µL of 0.1 M potassium phosphate buffer (PPB, pH 6.6). Then, 100 µL of trypsin solution (1 mg.mL^{-1} in 0.1 M PPB, pH 7.6) was added, and the mixture was incubated at 27°C for 5 min before being placed on ice to stop the reaction. Subsequently, 4 µL of the plasma–trypsin mixture was combined with 176 µL of L-DOPA solution (1.5 mg.mL^{-1} in 0.1 M PPB, pH 6.6), and PO activity was measured spectrophotometrically at 490 nm using Multimode Microplate Readers (BioTek Synergy HTX, Agilent), with absorbance recorded every min for 11 min. PO activity was calculated from the slope of the linear portion of the absorbance increase, expressed as the change in optical density per min per mL haemolymph.

Statistical analysis

All statistical analyses were performed using R software (version 2024.12.1). Prior to analysis, data were assessed for normality and homogeneity of

Table 2. Water quality measurements result

Parameter	Measured value (Average±SD; min-max)				Expected value
	Control	R7C3	R3C7	C10	
pH	7.91±0.182; 7.68-8.41	7.91±0.205; 7.55-8.41	7.92±0.182; 7.70-8.41	7.93±0.173; 7.74-8.41	7-8.5 [1]
Salinity (ppt)	35.05±0.489; 34.31-36.30	35.00±0.388; 34.34-35.76	35.02±0.469; 34.29-36.17	35.08±0.443; 34.21-36.01	33-35 [1]
Temperature (^{o}C)	26.29±0.474; 25.33-26.84	26.29±0.497; 25.34-26.95	26.28±0.490; 25.31-26.94	26.42±0.454; 25.56-26.96	24-28 [2]
Dissolved oxygen (mg.L^{-1})	5.62±0.519; 4.88-7.72	5.65±0.365; 4.96-6.30	5.64±0.362; 4.79-6.16	5.64±0.322; 4.99-6.20	>5 [1]
NH_3 (mg.L^{-1})	0.27±0.036; 0.00-0.50	0.25±0.000; 0.00-0.50	0.25±0.000; 0.00-0.50	0.25±0.000; 0.00-0.50	<0.5 (NH_3) [1]
NO_2 (mg.L^{-1})	0.05±0.000; 0.00-0.10	0.05±0.000; 0.00-0.10	0.05±0.000; 0.00-0.10	0.05±0.000; 0.00-0.10	<5.0 (NO_2) [3]
NO_3 (mg.L^{-1})	31.25±0.000; 0.00-50.00	31.25±0.000; 0.00-50.00	31.25±0.000; 0.00-50.00	31.25±0.000; 0.00-50.00	<100 (NO_3) [3]
PO_4 (mg.L^{-1})	1.38±0.000; 0.00-2.50	1.38±0.000; 0.00-2.50	1.38±0.000; 0.00-2.50	1.38±0.000; 0.00-2.50	<20 (PO_4) [4]

Notes: [1]Indonesian Government Regulation No. 22 of 2021 concerning the Implementation of Environmental Protection and Management; [2] Kemp and Britz (2008); [3] Drengstig and Bergheim (2013); [4] Martins *et al.* (2009)

variance using the Shapiro–Wilk and Levene's tests, respectively. Because of variation in initial weights, statistical models were adjusted to control for this confounding factor when evaluating treatment effects on growth.

Analysis of Covariance (ANCOVA) for spiny lobster's Specific Growth Rate (SGR)

For growth analysis, a one-way ANCOVA was used to account for differences in initial body weight among treatments, which would otherwise violate ANOVA assumptions. By including Initial Weight as a covariate, ANCOVA enhanced precision and statistical power, reducing bias and yielding more reliable results (Florien *et al.*, 2022; Rakse *et al.*, 2024). The model assumed a linear relationship between initial weight and SGR across treatments, specified as:

$$SGR = \beta_0 + \beta_1 \, (Initial\ Weight) + \beta_2 \, (Treatment) + \epsilon$$

In this model, SGR is the dependent variable. The intercept, β_0, represents the expected SGR when the initial weight is zero, and the control treatment is at the reference level. The coefficient β_1 quantifies the effect of Initial Weight as a covariate on SGR, while β_2 estimates the effect of each Treatment relative to the reference group. The residual term, ϵ, accounts for unexplained variation in SGR.

Survival rate analysis

For survival rate, complementary approaches were applied. First, repeated measures ANOVA was performed to test treatment and time effects, with sphericity verified by Mauchly's test and

Greenhouse–Geisser corrections applied when necessary. Normality was checked with the Shapiro–Wilk test; however, because of the small sample size, assumptions for ANOVA were not fully met. Therefore, a generalized linear model (GLM) was also applied.

A GLM with binomial distribution and logit link was employed to model survival data (alive= 1, dead= 0) across treatments (Bowlby and Gibson, 2015). Treatment was included as a categorical predictor (Control= reference) and Initial Weight as a continuous covariate:

$$\log \frac{p}{1 - p} = \beta_0 + \beta_1 \, (Initial\ Weight) + \beta_2 \, (Treatment) + \epsilon$$

where p is the probability of survival, β_0 is the intercept, and β_1 and β_2 represent the effects of Initial Weight and Treatment, respectively. Variability followed a binomial distribution, with significance assessed at α = 0.05.

Results and Discussion

Specific Growth Rate (SGR)

The average SGR of lobsters fed diets containing earthworms was significantly higher than that of the Control group, which received only sardines (Figure 1). Control lobsters grew at 0.42–0.59%.day^{-1}, while treatments (R7C3, R3C7, C10) achieved 0.98–1.73%.day^{-1}, nearly double the Control growth. An ANCOVA model controlling for initial weight (Figure 2), confirmed that all treatments outperformed the Control ($P< 0.05$) and revealed a strong negative relationship between initial weight and SGR ($P< 0.001$, $F_{(4,28)} = 23.09$), indicating that

larger lobsters exhibited lower growth rates. This pattern reflects allometric growth, whereby larger lobsters allocate more energy to maintenance and exhibit higher specific dynamic action (SDA), resulting in greater energy expenditure on digestion and metabolism, and thereby reducing the energy available for somatic growth (Goncalves *et al.*, 2020). Overall, the model explained 74.1% of the variation in SGR, underscoring that substituting sardines with earthworms substantially enhanced growth performance.

Proximate analysis of feeds in this experiment indicated that earthworms contained high protein (65.74%), slightly lower than sardines (74.89%). On average, earthworms contain 55–65% protein, while fishmeal contains around 60–68%, providing energy of about 1500 kJ.100 g^{-1} dry weight and 1800–2300 kJ.100 g^{-1} dry weight, respectively (Rachmawati and Nurhayati, 2022; Gbai *et al.*, 2024). Carbohydrate levels, however, were nearly double those of sardines (24% vs. 12%), enabling lobsters to utilize carbohydrates for energy while conserving protein for growth (Zhou *et al.*, 2013; Martínez-Antonio *et al.*, 2019; Xiao *et al.*, 2025). Earthworms also contain a balanced spectrum of essential amino acids, such as lysine, methionine, leucine, and isoleucine, often surpassing fishmeal in lysine and methionine, which are critical for growth and feed efficiency in crustaceans (Istiqomah *et al.*, 2009; Chiu *et al.*, 2016; Genodepa and Apines-Amar, 2024). Their extremely low fiber content (0.55%) further enhances digestibility, ensuring efficient nutrient absorption (Terrazas *et al.*, 2010; Vieira *et al.*, 2022). Studies across aquaculture species show that earthworm meal has a high protein efficiency ratio (PER) and apparent protein digestibility (ADCp), supporting growth and feed conversion comparable to or better than fishmeal in various species such as fish, shrimp, and crab (Pucher *et al.*, 2014; Rachmawati (Rachmawati and Nurhayati, 2022; Genodepa and Apines-Amar, 2024; Marjanović *et al.*, 2024). These traits explain the improved growth performance observed with earthworm substitution.
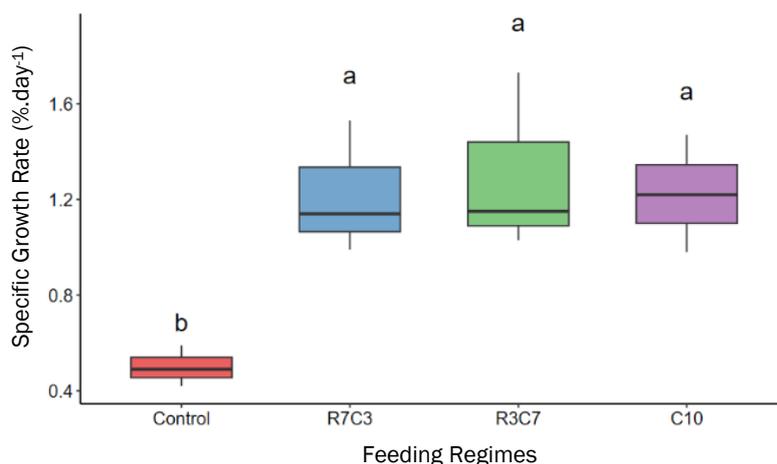

Figure 1. Specific growth rate of lobster in different feeding regimes
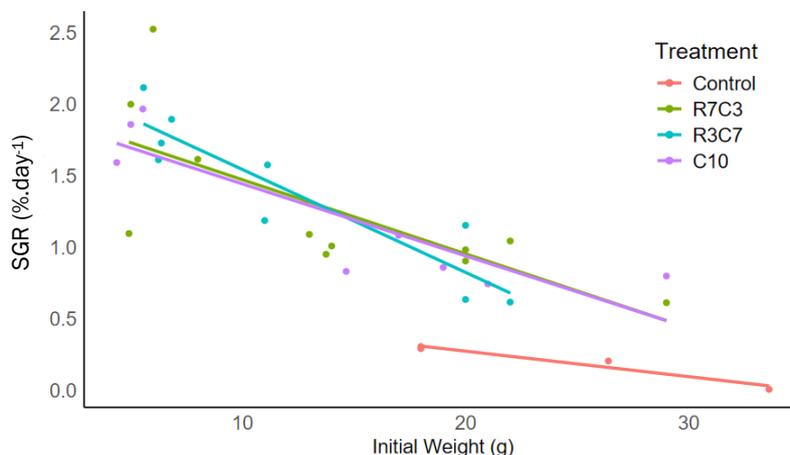

**Figure 2**. SGR ANCOVA model of lobster in different feeding regimes

In this study, lobsters fed earthworm-based diets under regular water exchange achieved SGR of $0.98\text{-}1.73\%.\text{day}^{-1}$. This exceeds the $0.95\text{-}1.05\%.\text{day}^{-1}$ reported by Ridwanudin *et al.* (2018) using moist formulated feed with 45-65% protein, and approaches the $1.89\text{-}1.98\%.\text{day}^{-1}$ reported by Slamet *et al.* (2021) using mixed feeds (pellets, trash fish, mussels) under flow-through water system. Although flow-through systems offer advantages such as continuous oxygenation, waste removal, and stable water quality (Snow *et al.*, 2012), the comparable growth achieved here highlights the superior nutritional quality of earthworms. This demonstrates that optimized diets can partly compensate for infrastructural limitations, supporting sustainable lobster culture in simpler and lower-cost systems.

Survival rate

The survival rate of lobsters appeared lower in the Control group compared to other treatments (Figure 3). However, Repeated Measures ANOVA indicated that these differences were not statistically significant. All groups experienced a gradual decline in survival over time, with the final survival rate of the Control group at approximately 25–26%, whereas treatment groups maintained 60–75%. However, a generalized linear model (Figure 4) revealed that lobster survival probability was significantly affected by dietary treatment, with the R7C3 group showing the strongest improvement ($P= 0.0092$), followed by C10 ($P= 0.0436$) and R3C7 ($P= 0.0473$). These findings suggest that fresh earthworm substitution
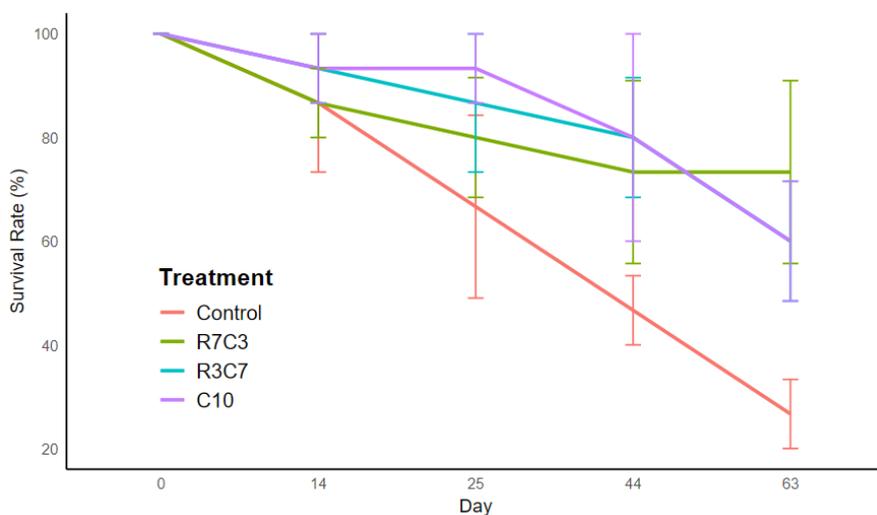
Figure 3. Survival rate of lobsters during 63 days of experiment in different feeding regimes
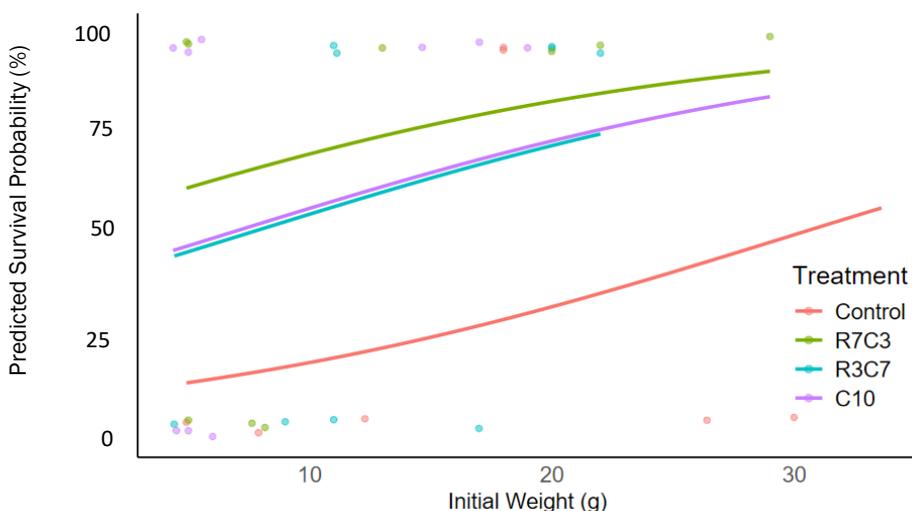
Figure 4. Generalized linear model of lobster survival probability in different feeding regimes

enhances lobster survival, particularly under the R7C3 diet.

Improved survival in groups receiving earthworm substitution may result from better digestibility and nutrient absorption (Marjanović *et al.*, 2024), particularly affected by the low fiber content that minimizes gut irritation and promotes a healthier microbiome (Duan *et al.*, 2019; Zeng *et al.*, 2024). These authors indicated that shrimp fed with lower dietary fiber develop more stable and diverse gut microbial communities by enhancing the abundance of beneficial bacteria such as *Lactobacillus* and *Bacillus*. This, in turn, results in improved digestive enzyme activity, more efficient nutrition absorption, lower gut irritation and stress, and enhanced immune response. Although no studies have directly tested earthworms in the gut microbiota of marine species, research in related aquatic species provides encouraging insight. In juvenile carp, inclusion of *Eisina fetida* significantly increased gut microbial diversity and upregulated immune-related genes without compromising performance (Yang *et al.*, 2019). Similarly, in marine finfish such as European seabass, partial replacement of fishmeal with polychaete meal (*Alitta virens*) reduced opportunistic pathogens and promoted beneficial microbes (Monteiro *et al.*, 2023). These findings suggest that worm-derived proteins not only provide high-quality nutrients but also function as microbiome modulators that improve host resilience.

Initial weight showed a positive relationship with survival, although this effect was marginally non-significant ($P= 0.0571$). This suggests a general trend that larger lobsters had greater resilience to environmental stress and nutritional variability, potentially because increased body size is associated with more developed immune systems and stronger physiological defences. In Caribbean spiny lobster *Panulirus argus*, body size positively correlated with haemocyte counts and phenoloxidase activity during viral infection (Pascual *et al.*, 2022). Additionally, genomic analysis in American lobster *Homarus americanus* reveals an extensive repertoire of detoxification and antioxidant genes, such as heat-shock proteins, cytochromes, and reactive oxygen species (ROS) defence systems, which are more robust in larger individuals (Polinski *et al.*, 2021). Cannibalism behaviour may also contribute, as smaller lobsters are more vulnerable after molting (Kelly *et al.*, 2022, 2023). This highlights the importance of size grading in lobster aquaculture, consistent with results in other crustaceans such as crayfish *Pacifastacus leniusculus* (Ahvenharju *et al.*, 2005) and mud crab *Scylla serrata* (Sanda *et al.*, 2021), where survival improved when smaller individuals were separated from larger conspecifics.

Total Haemocyte Count (THC)

The total haemocyte count (THC) differed significantly among treatments (Figure 5). Lobsters in the R3C7 group exhibited the highest median THC ($5.75\pm0.18\times 10^6$cells.ml^{-1}), followed by C10 ($4.67\pm0.59 \times 10^6$cells.ml^{-1}) and R7C3 ($4.40\pm0.71 \times 10^6$cells.ml^{-1}), while the Control group had the lowest levels ($1.71\pm0.27\times 10^6$cells.ml^{-1}). Diets containing fresh earthworm significantly increased THC compared to the control ($P<0.05$), with 70%
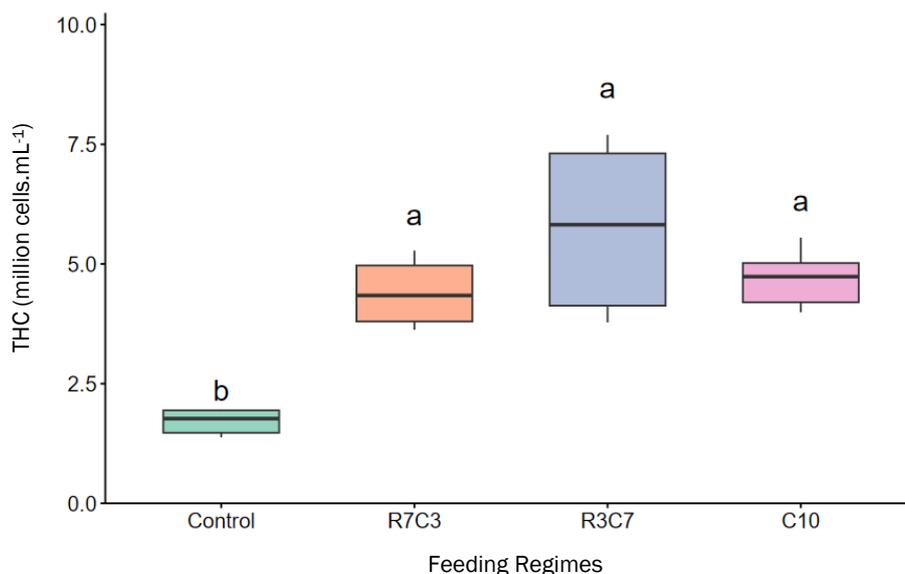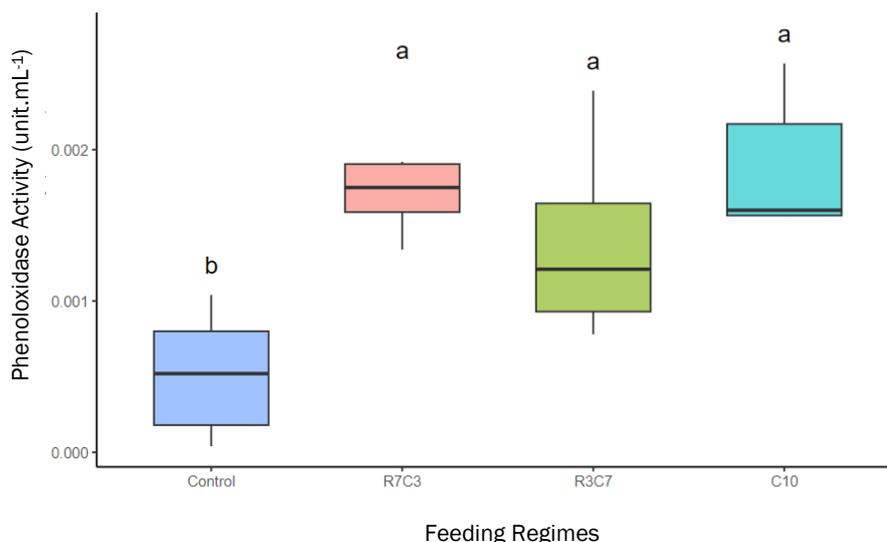

Figure 5. Total haemocyte counts of lobster in different feeding regimes

Figure 6. Phenoloxidase (PO) activity of lobster in different feeding regimes

replacement of sardines by earthworm being most effective. These results suggest that incorporating earthworm strongly stimulates haemocyte production, a key component of crustacean cellular immunity, which relies primarily on innate immune mechanisms such as cellular ingestion (phagocytosis), cluster formation (nodulation) and cellular enclosure (encapsulation) (Ren *et al*., 2021). THC is an important indicator of immune capacity, with higher values reflecting an active immune system and lower values suggesting immunosuppression or increased disease susceptibility (Gomez-Jimenez *et al*., 2000).

Normal THC levels in healthy lobsters are reported as $4.79 \pm 0.40 \times 10^6$ cells.ml^{-1} for California spiny lobster *Panulirus interruptus* (Gomez-Jimenez *et al*., 2000) and $5.6 \pm 0.7 \times 10^6$ cells.ml^{-1} for western rock lobster *Panulirus cygnus* (Jussila *et al*., 1997). In *Panulirus Homarus*, Slamet *et al*. (2021) reported THC values of 2.46–2.68×10^6 cells.ml^{-1}, slightly higher than the Control group in this study. Lobsters fed diets with fresh earthworm as substitute exhibited significantly higher THC, indicating enhanced innate immunity, which likely contributed to their improved survival. Overall, dietary supplementation with earthworms not only boosts haemocyte production but also strengthens lobster resilience and disease resistance.

Phenoloxidase activity

Phenoloxidase (PO) activity, a critical component of the humoral innate immune system, exhibited a consistent enhancement across all dietary treatments compared to the Control group (*P<*

0.05). The lowest PO activity was observed in the Control group ($5.2 \pm 3.6 \times 10^{-4}$ unit.ml^{-1}), whereas earthworm-supplemented diets resulted in markedly higher values of $13.6 \pm 6.2 \times 10^{-4}$ unit.ml^{-1}, $16.9 \pm 7.8 \times 10^{-4}$ unit.ml^{-1}, and $18.0 \pm 3.9 \times 10^{-4}$ unit.ml^{-1} for R3C7, C10, and R7C3, respectively. This enhancement may be explained by the nutritional and bioactive properties of earthworms. Phenoloxidase is a copper-dependent enzyme central to crustacean defence system, functioning in melanization, pathogen encapsulation, and wound repair through the prophenoloxidase (proPO) system (Fujieda *et al*., 2013). Activation of this pathway requires proteolytic cleavage of proPO by serine proteases (Zou *et al*., 2005), a process that may be supported by earthworm-derived proteolytic enzymes. In addition, earthworms provide antioxidant compounds that could protect PO from oxidative inactivation (Garczyńska *et al*., 2023), as well as sulphur-rich amino acids such as cysteine that contribute to the folding and stabilization of copper-binding proteins (Kavle *et al*., 2023). Collectively, these nutritional properties offer a plausible explanation for the elevated PO activity observed in lobsters fed with earthworm diets.

Conclusion

This study showed that lobsters fed diets with fresh earthworm substitute (C10, R3C7, and R7C3) exhibited significantly higher specific growth rates, survival, and immune responses compared to the control. These findings indicate that earthworms represent a promising and sustainable fresh feed alternative for *P. homarus*, with the potential to reduce dependence on trash fish in lobster

aquaculture. Based on these results, earthworms may be considered as a partial substitute for sardines, while further studies are recommended to evaluate long-term performance, optimal inclusion levels, and feasibility in formulated feeds and commercial farming systems.

Acknowledgement

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