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The Effect of Liquid Organic Fertilizer Derived from Leucaena (*Leucaena leucocephala*) Leaves as a Substitute for Urea on the Proximate Composition of Pacific White Shrimp (*Litopenaeus vannamei*) in Traditional Aquaculture

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Abstract

The cultivation of Pacific white shrimp (*Litopenaeus vannamei)* commonly relies on inorganic fertilizers, particularly urea, to stimulate primary productivity in aquaculture ponds. However, the increasing scarcity and cost of urea have encouraged the exploration of environmentally friendly alternatives. This study evaluated the effectiveness of liquid organic fertilizer (LOF) derived from *Leucaena leucocephala* leaves as a substitute for urea in improving proximate composition of Pacific white shrimp cultured under traditional pond systems. Five treatments were applied, consisting of a negative control, a positive control using urea fertilizer, and LOF concentrations of 10%, 20%, and 30%. Water quality parameters (temperature, pH, and salinity), Compound of LOF Leucaena leaf and proximate composition (moisture, ash, lipid, protein, and carbohydrate contents) were evaluated. Data were analyzed using one-way ANOVA followed by Duncan's Multiple Range Test at α 95% confidence level. The results indicated that LOF application had no significant effect on moisture, ash, and lipid contents ($p>0.05$), whereas protein and carbohydrate contents were significantly affected ($p<0.05$). The highest protein content (19.84%) were obtained from the 30% LOF treatment. Water quality remained within the optimal range throughout the culture period. These findings demonstrate that *Leucaena leucocephala* leaf liquid organic fertilizer has considerable potential as an environmentally friendly substitute for urea fertilizer in traditional Pacific white shrimp farming.

Keywords: liquid organic fertilizer; *Leucaena leucocephala*; proximate composition; survival rate; Pacific white shrimp.

Abbreviations: Liquid Organic Fertilizer (LOF)

INTRODUCTION

Pacific white shrimp (*Litopenaeus vannamei)* is one of the most economically valuable aquaculture commodities and contributes significantly to Indonesia's fisheries exports (Ministry of Marine Affairs and Fisheries [KKP], 2023). The increasing market demand has driven efforts to enhance aquaculture productivity through various technological innovations, including improved pond environmental management. One of the common practices during pond preparation is fertilization to stimulate primary productivity by promoting phytoplankton growth, which serves as a natural food source for shrimp (FAO, 2024). Urea has long been the most widely used inorganic fertilizer in shrimp farming due to its high nitrogen content and its ability to stimulate phytoplankton growth (Nasuki, 2022). However, the continuous application of inorganic fertilizers may deteriorate aquatic environmental quality through the accumulation of chemical residues and increase production costs due to fertilizer shortages and price fluctuations in the market (Hijrah et al., 2017). These challenges highlight the need for alternative nutrient sources that are environmentally friendly, readily available, and sustainable.

One potential organic material is leucaena *(Leucaena leucocephala)* leaves. Leucaena leaves contain essential nutrients such as nitrogen, phosphorus, and potassium, which play important roles in enhancing phytoplankton productivity in aquaculture ponds (Handayani et al., 2017). Moreover, the abundant availability of leucaena throughout many regions of Indonesia makes it an economical raw material for producing liquid organic fertilizer (LOF) (Khoirul et al., 2020). The application of LOF is expected not only to supply essential nutrients to the pond ecosystem but also to improve environmental

quality compared with conventional inorganic fertilizers. Enhanced phytoplankton productivity increases the availability of natural food, thereby supporting shrimp growth, improving feed utilization efficiency, and enhancing shrimp nutritional quality (Ratnawati et al., 2023). The nutritional quality of shrimp can be evaluated through proximate analysis, which includes the determination of moisture, ash, lipid, protein, and carbohydrate contents. In addition to reflecting nutritional value, these parameters can also serve as indicators of aquaculture system performance and the effectiveness of the applied treatments. Previous studies have demonstrated that organic fertilizers can improve the productivity of aquaculture waters. However, information regarding the use of liquid organic fertilizer derived from leucaena leaves as a substitute for urea fertilizer and its effects on the nutritional quality of Pacific white shrimp remains limited. Most previous research has focused primarily on shrimp growth performance and water quality, whereas the effects on shrimp proximate composition have received little attention. This knowledge gap highlights the need for studies that integrate environmental quality, shrimp survival, and nutritional composition within a single aquaculture system.

Therefore, this study aimed to evaluate the effects of liquid organic fertilizer derived from *Leucaena leucocephala* leaves as a substitute for urea fertilizer on water quality, fertilizer composition and the proximate composition of Pacific white shrimp (*Litopenaeus vannamei)* cultured under a traditional farming system. The findings are expected to provide a scientific basis for the development of more environmentally sustainable aquaculture technologies while promoting the utilization of locally available biological resources as alternatives to inorganic fertilizers.

MATERIALS AND METHODS

Time and Study Location

The study was conducted over a two-month period, from September to November, at a traditional Pacific white shrimp *(Litopenaeus vannamei*) farming pond. The fertilizer content analyzed is N, P2O5, and K2O content. Proximate analyses, including the determination of moisture, ash, lipid, protein, and carbohydrate contents, were carried out in the laboratory using standard proximate analysis methods. Shrimp farming was conducted at shrimp ponds in Ujung Pangkah Village, Gresik, while laboratory testing was carried out at the Integrated Laboratory of Walisongo State Islamic University and the UNS Agricultural Laboratory.

Experimental Design

This study employed an experimental method using a Completely Randomized Design (CRD) consisting of five treatments:KN: No fertilizer application (negative control); KP: Urea fertilizer application (positive control); A: Application of 10% Leucaena leucocephala leaf liquid organic fertilizer (LOF);B: Application of 20% Leucaena leucocephala leaf liquid organic fertilizer (LOF);C: Application of 30% Leucaena leucocephala leaf liquid organic fertilizer (LOF). Each treatment was repeated 3 times. Each treatment was applied to culture units maintained under relatively homogeneous environmental conditions to ensure that the observed differences in response were primarily attributable to the treatments administered.

Preparation of Liquid Organic Fertilizer

The liquid organic fertilizer (LOF) was prepared using *Leucaena leucocephala* leaves as the primary raw material through a fermentation process until a fertilizer solution suitable for application in the culture medium was obtained. The fertilizer was prepared at concentrations of 10%, 20%, and 30%, corresponding to the treatment groups, while the positive control treatment received urea fertilizer according to standard shrimp farming practices. The composition of the Leucaena leaf extract liquid organic fertilizer consists of Leucaena leaves, EM4, sugar solution, distilled water, rice-washing water, and palm sugar. Lamtoro leaves intended for use as raw material in fertilizer production are first sorted. They are then thoroughly washed to remove dirt or other impurities. The washed leaves are cut into small pieces to reduce their size and facilitate decomposition. EM4, a sugar solution, and distilled water are added to the leaves, using a ratio of 10 kg of leaves to 20 L of water, 4 L of rice-washing water, 1 L of palm sugar solution, and 1 L of EM4 (Monica, 2015). The organic fertilizer undergoes fermentation for 14 days. Once fermentation is complete, the mixture is filtered to extract the liquid for use as fertilizer. Prior to application, the liquid is diluted according to the specific concentration treatment until the total volume reaches 1,000 ml.

Pond Preparation and Shrimp Rearing

Prior to stocking, the culture ponds were prepared by cleaning the pond bottom, drying, liming, and filling with water to the operational depth. Fertilization was then carried out according to the respective treatments to stimulate phytoplankton growth as a natural food source. Pacific white shrimp (*Litopenaeus vannamei)* post-larvae were stocked after the water quality met the recommended standards for shrimp culture. During the rearing period, the shrimp were fed a commercial diet according to their nutritional requirements. Routine pond management included regular monitoring of water quality, fertilizer application according to the assigned treatments, and health observation of the cultured shrimp. The shrimp are cultivated in ponds measuring 1.5 m x 1 m and depth of 80 cm. Each pond contains 50 Pacific white shrimp juveniles. Feeding is carried out in stages, three times daily: 30% of the ration is given at 07:00 and

16:00, and 40% at 22:00; this schedule accounts for the nocturnal foraging behavior of Pacific white shrimp. The feed used is TONGWEI 8830, which has a nutritional content of at least 40% protein and 6% fat.

Research Parameters
The parameters evaluated in this study included water quality, Analysis of fertilizer content *(N, P2O5, and K2O)*, and the proximate composition of Pacific white shrimp (*Litopenaeus vannamei*).

Water Quality
The water quality parameters monitored were temperature, pH, and salinity. Measurements were taken periodically throughout the culture period to ensure that environmental conditions remained within the optimal range for the growth of Pacific white shrimp.

Analysis of fertilizer content (N, P2O5, and K2O)
The nutrient composition of the liquid organic fertilizer (LOF), including total nitrogen (N), available phosphorus (expressed as P_2O_5), and soluble potassium (expressed as K_2O), was analyzed prior to its application in the culture ponds. All analyses were performed in accordance with the Indonesian National Standard (SNI) for organic fertilizers and the AOAC Official Methods of Analysis. The total nitrogen content was determined using the Kjeldahl method. The total nitrogen content was calculated and expressed as a percentage (%). The available phosphorus content was determined using the molybdenum blue spectrophotometric method. The phosphorus concentration was calculated from a calibration curve prepared using standard phosphate solutions, and the results were expressed as P_2O_5 concentration (ppm or mg L^{-1}). The potassium content was determined using Atomic Absorption Spectrophotometry (AAS). The potassium concentration was calculated using a standard calibration curve and converted to potassium oxide (K_2O), expressed as a percentage (%).

Proximate Analysis
Shrimp samples were collected at harvest for proximate composition analysis, including moisture, ash, lipid, protein, and carbohydrate contents. The analytical methods used were as follows: Moisture content was determined using the thermogravimetric method (SNI 01-2891-1992), Ash content was determined using the gravimetric method (SNI 01-2354.1-2010), Lipid content was determined using the Soxhlet extraction method, Protein content was determined using the Kjeldahl method, Carbohydrate content was calculated using the by-difference method.

Data analysis
The data obtained were analyzed statistically using one-way Analysis of Variance (ANOVA) at a 95% confidence level ($\alpha = 0.05$). Prior to the analysis, the data were tested for the assumptions of normality and homogeneity of variance. When the ANOVA indicated significant differences among treatments ($p < 0.05$), the analysis was followed by Duncan's Multiple Range Test (DMRT) to identify significant differences between treatment means.

Water quality parameters and proximate composition data are presented as mean ± standard deviation (SD). The results of the statistical analyses were used as the basis for interpreting the effects of liquid organic fertilizer derived from *Leucaena leucocephala* leaves on the nutritional quality and survival of Pacific white shrimp *(Litopenaeus vannamei).*

RESULTS AND DISCUSSION

Effect of *Leucaena leucocephala* Leaf Liquid Organic Fertilizer on Water Quality

Temperature
The first environmental parameter used was temperature. Temperature is a measure indicating the degree of heat or coldness of an object or environment (Nakkir et al., 2023). The unit used in this study to express temperature is degrees Celsius (°C). The average temperature values were derived from five temperature readings taken weekly for each treatment pond.

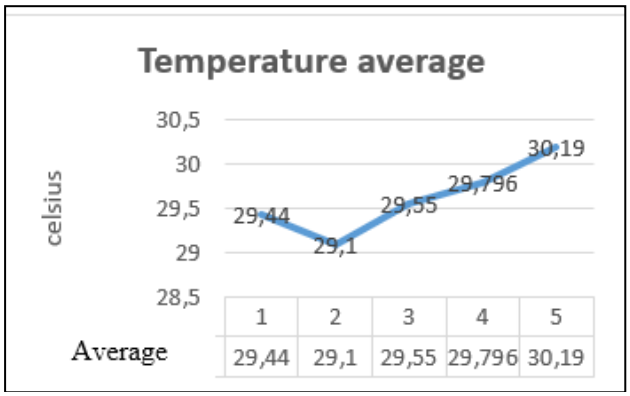

Figure 1. Graph of Average Temperatures for Each Treatment.
Note: negative control (1), positive control (2), liquid organic fertilizer (POC) at 10% concentration (3), fertilizer at 20% concentration (4), and fertilizer at 30% concentration (5).

According to Regulation of the Minister of Marine Affairs and Fisheries of Indonesia No. 75/2016 (PERMEN-KP-75, 2016), the optimum water temperature for shrimp pond culture ranges from 28 to 32°C. As shown in Figure 1, the water temperature recorded during the study ranged from 29.10°C to 30.19°C. This temperature range is considered normal and suitable for the cultivation of *Litopenaeus vannamei.* Therefore, the observed temperatures were within the optimal range for the growth and survival of Pacific white shrimp. The treatment with a 30% fertilizer concentration exhibited the highest average water temperature (30.19°C). Elevated water temperatures can

increase the metabolic and respiratory rates of aquatic organisms, resulting in greater oxygen consumption. This finding is consistent with Putra et al. (2024), who reported that increased metabolic activity and respiration elevate oxygen demand. Moreover, higher daytime temperatures may stimulate shrimp feeding activity but can also contribute to increased pH and ammonia concentrations due to the accumulation of feces and uneaten feed. However, sudden fluctuations in water temperature should be avoided, as rapid temperature changes can induce physiological stress in shrimp. Excessively high temperatures may cause muscle spasms or convulsions, whereas low temperatures reduce shrimp activity and feeding behavior, potentially leading to Early Mortality Syndrome (EMS) (Pambudi, 2018). Similarly, Nurhasanah et al. (2021) reported that low water temperatures decrease feed consumption rates, while excessively high temperatures may completely suppress feeding activity.Water temperature is influenced by various environmental factors, including seasonal variation, latitude, altitude, time of day, air circulation, cloud cover, water flow, and water depth (Saputra, 2022). Temperature plays a crucial role in regulating aquatic ecosystem processes, including photosynthetic activity, the solubility of dissolved substances, and the physical, chemical, and biological characteristics of pond water. These factors directly affect the physiological performance of cultured organisms. Within the optimal range, increasing water temperature enhances metabolic activity and promotes shrimp growth. Nevertheless, temperatures exceeding the tolerance threshold may negatively affect shrimp health and overall aquaculture productivity.

pH

The second environmental parameter used was pH. pH is a physicochemical parameter used to indicate the acidity or alkalinity of a solution (Mufida et al., 2020). The pH for each treatment was measured once a week. The average pH values were derived from five weekly measurements taken for each treatment pond (Appendix 6). The average pH results are presented in the graph below. The following are the average results obtained from the pH measurements for each treatment:

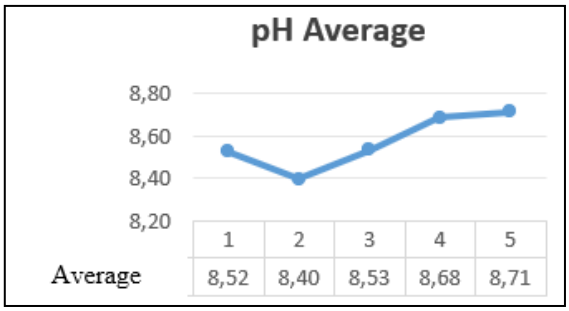

Figure 2. Graph of Average pH for Each Treatment.
Note: negative control (1), positive control (2), liquid organic fertilizer (POC) at 10% concentration (3), fertilizer at 20% concentration (4), and fertilizer at 30% concentration (5).

Based on Figure 2, the average water pH ranged from 8.40 to 9.71. The optimal pH for shrimp culture is generally reported to range from 7.5 to 8.5 (Rafiqie, 2021), while Ibrahim (2023) suggested that the ideal pH for shrimp growth lies between 7.4 and 8.9. Water pH plays a critical role in regulating shrimp appetite and growth performance. Furthermore, when the pH falls below the tolerance threshold, the molting process may be disrupted, resulting in a softened exoskeleton and reduced shrimp survival (Aris et al., 2022). Although several treatments in the present study exhibited pH values slightly above the recommended range, the observed pH conditions generally remained within the tolerance limits for *Litopenaeus vannamei* culture and were able to support normal growth and survival. Water pH is one of the most important environmental parameters influencing the physiological performance of Pacific white shrimp. Both excessively acidic and highly alkaline conditions can induce physiological stress, impair growth, and increase mortality. In addition, inappropriate pH levels may weaken the shrimp exoskeleton, making it more susceptible to physical damage and disease (Yew-Hu Chien, 1992). Variations in pH also affect shrimp metabolism and feeding behavior. Highly acidic conditions can reduce feed intake and ultimately cause mortality, whereas excessively alkaline conditions may inhibit growth and negatively affect shrimp health (Jelinda et al., 2024).The results of the present study are consistent with these findings. The 30% fertilizer treatment, which exhibited the highest pH value, also produced the highest feed efficiency. This observation suggests that water pH plays an important role in stimulating shrimp feeding activity and improving feed utilization efficiency, provided that the pH remains within the shrimp's physiological tolerance. Improved feed efficiency under this treatment may be attributed to enhanced metabolic activity and more favorable environmental conditions that support nutrient assimilation.

Previous studies have shown that water with a pH below 7.0 may damage shrimp gills and impair ion regulation, thereby reducing the efficiency of feed utilization (Yuniartik, 2013). Conversely, pH values exceeding 9.0 may accelerate shrimp metabolism and increase oxygen demand. High pH also promotes the conversion of ammonium (NH_4^+) to the more toxic un-ionized ammonia (NH_3), thereby increasing ammonia toxicity and posing a significant risk to shrimp health and survival. Consequently, maintaining water pH within the optimal range is essential not only for maximizing feed efficiency and growth but also for minimizing physiological stress and preventing adverse effects associated with elevated ammonia concentrations.

Salinity

The third environmental parameter is salinity. The quantity of dissolved ions in the water determines the

salinity level (Nugroho & Koprawi, 2024). Pacific white shrimp are capable of surviving across a wide range of salinity levels, a characteristic known as euryhalinity (Nurhasanah et al., 2021). Nevertheless, salinity levels must align with the shrimp's requirements, as salinity affects their osmotic systems. Salinity is measured in parts per million (ppm). The average salinity values were derived from five measurements taken weekly in each treatment pond. The following are the average results obtained from the salinity measurements for each treatment:

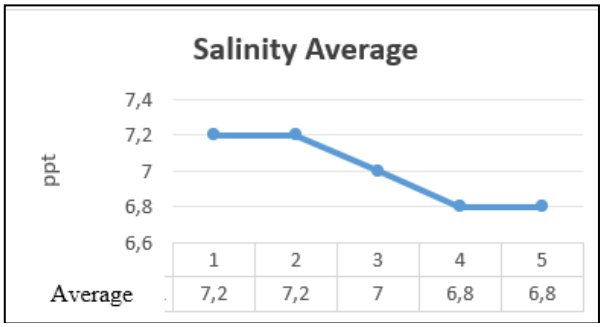

Figure 3. Graph of Average Salinity for Each Treatment.
Note: negative control (1), positive control (2), liquid organic fertilizer (POC) at 10% concentration (3), fertilizer at 20% concentration (4), and fertilizer at 30% concentration (5).

Based on Figure 3, the highest average salinity was observed in the negative control (Treatment 1) and positive control (Treatment 2), with a value of 7.2 ppt. In contrast, the lowest average salinity was recorded in the 20% POC treatment (Treatment 4) and the 30% POC treatment (Treatment 5), both measuring 6.8 ppt. Overall, the measured salinity values were relatively low compared with the optimal salinity recommended for *Litopenaeus vannamei* culture. Nevertheless, the shrimp survived well throughout the experiment because L. vannamei is a euryhaline species, capable of tolerating a wide range of salinity conditions (Scabra et al., 2023). This finding is consistent with Jayanti et al. (2022), who reported that *L. vannamei* can survive in salinity ranging from 5 to 30 ppt, although the optimum salinity for growth and culture performance is 15–23 ppt (Putra et al., 2024). The relatively low salinity observed in this study was primarily attributed to the location of the experimental ponds, which were situated far from the coastline. The primary water source for the ponds was groundwater supplied through bore wells rather than seawater. Groundwater obtained from inland bore wells generally exhibits low salinity, typically ranging from 0 to 10 ppt (Cahyono, 2024). Furthermore, the limited availability of seawater in the ponds resulted from the considerable distance between the ponds and the seawater intake point. This condition has been exacerbated by coastal accretion caused by sediment deposition, which has created new land and reduced the accessibility of seawater to the aquaculture area (Utama et al., 2023). Despite the suboptimal salinity conditions, no substantial adverse effects on shrimp performance were observed during the study. This result demonstrates the remarkable osmoregulatory capacity of *L. vannamei*, enabling the species to maintain physiological homeostasis under low-salinity conditions. The ability to regulate internal osmotic balance allows Pacific white shrimp to adapt to fluctuations in environmental salinity without experiencing severe physiological stress, thereby sustaining normal growth and survival. To minimize the effects of low salinity, the researchers periodically increased pond salinity by pumping brackish groundwater into the culture ponds using a diesel-powered water pump. This management practice helped maintain salinity within the minimum tolerance range required by the shrimp and contributed to the stable environmental conditions observed throughout the experimental period. Although the recorded salinity remained below the optimum range for commercial culture, the adaptive capacity of *L. vannamei* combined with appropriate water management enabled satisfactory shrimp performance under the prevailing environmental conditions.

Nutrient content of *Leucaena leucocephala* Leaf Liquid Organic Fertilizer (N, P2O5, and K2O)

Among the various forms of nitrogen, nitrate (NO_3^-) is the primary form readily utilized by aquatic organisms. Nitrate is highly soluble in water and serves as an essential nutrient for the growth of algae and phytoplankton (Nurjumanis et al., 2024). It plays a crucial role in phytoplankton photosynthesis and is often considered one of the limiting nutrients controlling primary productivity in aquatic ecosystems. Adequate nitrate availability promotes phytoplankton growth, whereas nitrate deficiency can restrict photosynthetic activity and reduce primary production. In addition to nitrate, phosphate (PO_4^{3-}) is another essential macronutrient required for phytoplankton growth. Nitrate and phosphate are utilized by phytoplankton during both photoautotrophic and chemoautotrophic metabolic processes, ultimately producing carbohydrates and oxygen through photosynthesis (Mishbach et al., 2021). These nutrients are indispensable for cellular metabolism because they are involved in the synthesis of fundamental biomolecules, including nucleic acids, proteins, adenosine triphosphate (ATP), and phospholipids. During photoautotrophic photosynthesis, light energy is converted into chemical energy, enabling the transformation of carbon dioxide and water into carbohydrates and oxygen, while nitrate and phosphate support the biochemical pathways required for biomass production.

Tabel 1. Nutrient Content of Leucaena Leaf Fertilizer.

Concentration	Total N	P_2O_5	K_2O
10%	0,22%	151,33 ppm	0,05%
20%	0,31%	168,23 ppm	0,07%
30%	0,45%	231, 59 ppm	0,12%

The availability of nitrate and phosphate directly influences the primary productivity of aquaculture ponds. Sufficient concentrations of these nutrients stimulate phytoplankton growth, leading to higher natural productivity and increased dissolved oxygen production during daylight hours. Conversely, nutrient limitation may suppress phytoplankton abundance, thereby reducing the availability of natural food and affecting the ecological stability of the culture system. Therefore, maintaining adequate nitrate and phosphate concentrations is essential to support phytoplankton development, which serves as an important natural food source for *Litopenaeus vannamei,* particularly during the early stages of cultivation. A stable and productive phytoplankton community also contributes to improved water quality, nutrient cycling, and overall pond ecosystem health, thereby supporting optimal shrimp growth and culture performance.

Effect of *Leucaena leucocephala* Leaf Liquid Organic Fertilizer on Proximate Composition

Effect of Leucaena leucocephala Leaf Liquid Organic Fertilizer on Moisture Content

The proximate analysis showed that the moisture content of Pacific white shrimp ranged from 74.19% to 76.72%, with no significant differences among treatments ($p > 0.05$). These results indicate that the application of *Leucaena leucocephala* leaf liquid organic fertilizer (LOF) did not affect the ability of shrimp muscle tissue to maintain its water content.The observed moisture values fall within the range reported by Li et al. (2025) (75.63–79.22%), indicating that the shrimp produced in this study were of acceptable quality. High moisture content is a normal physiological characteristic of shrimp because muscle tissue is composed primarily of water, which is maintained through the water-holding capacity (WHC) of the tissue. The absence of significant differences among treatments may be attributed to the relatively stable water quality maintained throughout the experiment, allowing normal osmoregulatory processes to occur. According to Khutni et al. (2024), extreme changes in salinity can disrupt osmoregulation and alter the water content of shrimp muscle tissue. In the present study, salinity remained within the tolerance range for Pacific white shrimp, thereby preventing significant changes in tissue moisture content.

Tabel 2. Moisture Content Results for Litopenaeus vannamei.

Component	Moisture Content Results for *Litopenaeus vannamei*				
	NC	PC	10%	20%	30%
Moist Content (%)	74.195	76.719	75.364	75.567	75.705

The moisture content of *Litopenaeus vannamei* muscle is influenced by both intrinsic factors, such as age, body size, and physiological condition, and extrinsic factors, including culture methods, post-harvest handling, and microbial activity. Among these factors, the aquaculture system plays an important role in determining the chemical composition of shrimp muscle. Ramli et al. (2025) reported that the moisture content of vannamei shrimp cultured under intensive, traditional, and floating net cage systems was 77.26%, 76.70%, and 72.64%, respectively. Although the differences were relatively small, these findings indicate that culture practices can influence the proximate composition of shrimp, including moisture content. Moisture content is widely recognized as an important indicator of shrimp freshness and product quality. Fresh shrimp generally possess high moisture content, whereas a reduction in water content may indicate dehydration and quality deterioration during storage or handling. Hafiludin and Najah (2023) investigated the effect of different thawing methods on the quality of frozen L. vannamei and reported moisture contents ranging from 72.64% to 73.43%, depending on the thawing technique employed. These findings demonstrate that post-harvest processing methods can alter the water-holding capacity of shrimp muscle and consequently affect product quality.

In addition to thawing techniques, storage conditions are critical in maintaining shrimp quality after harvest. Nuryanti et al. (2020) reported that storing seafood products under refrigerated conditions effectively suppresses microbial proliferation and enzymatic activity, thereby preserving product quality and extending shelf life. Appropriate cold storage minimizes moisture loss and delays quality deterioration, allowing seafood products to be stored for extended periods without significant changes in their physicochemical characteristics. Therefore, post-harvest handling practices play an essential role in preserving the moisture content of shrimp muscle. The influence of post-harvest handling on moisture content is particularly important because shrimp are classified as highly perishable food products. Their high water activity and nutrient content make them highly susceptible to microbial contamination, enzymatic degradation, and

physicochemical changes during storage (Herawati et al., 2020). Consequently, selecting appropriate post-harvest handling and storage methods is essential for maintaining moisture content, preserving freshness, and ensuring the overall quality and market value of shrimp. Proper handling not only reduces water loss but also minimizes quality deterioration, thereby improving the shelf life and consumer acceptability of *Litopenaeus vannamei.*

Effect of Leucaena leucocephala Leaf Liquid Organic Fertilizer on Ash Content

The ash content ranged from 0.060% to 0.098% and did not differ significantly among treatments ($p > 0.05$). This finding suggests that the application of Leucaena leaf LOF did not significantly influence mineral accumulation in shrimp tissues. According to Smith et al. (2023), ash content reflects the total mineral composition of aquatic organisms and is influenced by feed composition, water quality, and the organism's capacity to absorb minerals. In the present study, all treatment groups received the same commercial feed, resulting in a relatively uniform mineral supply. The ash content obtained in this study was lower than that reported by Ramli et al. (2025), who found an ash content of approximately 2.42% in shrimp cultured under traditional farming systems. This discrepancy may be attributed to differences in culture systems, salinity, feed type, harvest age, and laboratory analytical methods.

Tabel 3. Ash Content Results for Litopenaeus vannamei.

Component	Ash Content Results for *Litopenaeus vannamei*				
	NC	**PC**	**10%**	**20%**	**30%**
Ash Content (%)	0.098	0.070	0.060	0.079	0.076

The highest ash content observed in this study was recorded in the negative control (KN) treatment, where no fertilizer was applied. The elevated ash content in this treatment may indicate that the shrimp were unable to utilize and digest feed efficiently, resulting in greater mineral residue accumulation within the muscle tissue. This interpretation is supported by the high feed conversion ratio (FCR) recorded in the KN treatment (18.75). Feed conversion ratio is inversely related to feed efficiency; therefore, a higher FCR indicates lower feed utilization efficiency (Setyono et al., 2020). Consequently, the shrimp in the negative control treatment likely exhibited less efficient nutrient utilization, which may have influenced the mineral composition of the harvested shrimp. Ash content represents the total mineral content of biological tissues. In crustaceans, the highest ash concentration is generally found in the exoskeleton because it contains large amounts of chitin and mineral deposits, particularly calcium. This observation is consistent with the findings of Syed et al. (2017), who reported that the inedible portions of *Litopenaeus vannamei,* primarily the shell, contained significantly higher ash content than the edible muscle tissue. Samples containing the exoskeleton exhibited ash contents ranging from 6.66% to 9.03% (wet basis). The high ash content is mainly attributed to the abundance of chitin reinforced by calcium-rich minerals that provide structural rigidity to the exoskeleton. Chitin is a linear polysaccharide composed of N-acetyl-D-glucosamine units and possesses structural properties similar to those of cellulose.

Mineral accumulation in crustaceans is closely associated with their developmental stage and molting cycle. Truong et al. (2023) reported that in slipper lobster, ash content decreased from the first larval stage (39.1%) to the post-larval stage (15.3%). However, following the first molt during the juvenile stage, the exoskeleton underwent extensive mineralization, resulting in a substantial increase in ash content to 50.8%, primarily due to increased calcium deposition. These findings suggest that mineral requirements and deposition patterns vary considerably throughout crustacean development and are strongly influenced by the process of exoskeleton formation. Minerals perform numerous physiological functions in shrimp, including supporting tissue development, regulating metabolic processes, maintaining osmotic balance, and contributing to body pigmentation (Luthfiani, 2016). Therefore, variations in ash content may reflect differences in mineral metabolism, nutritional status, and exoskeleton mineralization rather than merely differences in mineral intake. Maintaining appropriate mineral availability and improving feed utilization efficiency are therefore essential for supporting normal physiological functions, growth, and overall shrimp quality during culture.

Effect of Leucaena leucocephala Leaf Liquid Organic Fertilizer on Lipid Content

The lipid content ranged from 0.53% to 1.36%, with the highest value observed in the 10% LOF treatment. However, statistical analysis indicated that these differences were not significant ($p > 0.05$). These findings suggest that the increased nutrient availability resulting from LOF application was insufficient to promote significant lipid accumulation in shrimp tissues. In shrimp, lipids are primarily utilized as an energy source during metabolic activities rather than being stored in body tissues. These results are consistent with the findings of Pires et al. (2018), who reported that shrimp generally contain relatively low lipid levels

despite being rich in nutritionally valuable polyunsaturated fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Similarly, Bastian et al. (2024) reported that shrimp reared in traditional culture systems generally exhibit low lipid content because much of the available energy is allocated to growth and metabolic processes.

Tabel 4. Results of Fat Content in Litopenaeus vannamei.

Component	Results of Fat Content in *Litopenaeus vannamei*				
	NC	PC	10%	20%	30%
Fat Content (%)	0.525	0.619	1.36	0.747	0.596

The liquid organic fertilizer (LOF) derived from *Leucaena leucocephala* leaf extract at a concentration of 10% contained 0.22% nitrogen. Nitrogen in the form of nitrate plays an important role in promoting the growth of algae and phytoplankton (Nurjumanis et al., 2024). This nutrient is essential for supporting the growth of phytoplankton and zooplankton, which serve as natural food sources for shrimp and may influence meat quality, including lipid content. Phytoplankton are known to contain relatively high lipid levels, ranging from 2.2–10% (Erlina et al., 2004). The increased availability of lipid-rich natural feed can contribute to higher lipid accumulation in shrimp tissue. Therefore, it is assumed that the application of 10% LOF treatment can optimize lipid content in shrimp by enhancing the availability of natural feed resources in the culture environment.

Effect of Leucaena leucocephala Leaf Liquid Organic Fertilizer on Protein Content

Protein content differed significantly among treatments ($p < 0.05$), with the highest value (19.84%) recorded in the 30% LOF treatment. The increase in protein content was likely associated with enhanced phytoplankton productivity resulting from the increased availability of nitrogen supplied by the Leucaena leaf LOF. Nitrogen is an essential nutrient for protein synthesis and phytoplankton growth. According to Nurjumanis et al. (2024), increased nitrogen availability in aquatic environments enhances phytoplankton biomass, thereby enriching the natural food supply for cultured organisms. The present findings are consistent with those of Bastian and Hudi (2024), who reported a protein content of 15.12% in traditionally cultured shrimp. The higher protein content observed in the 30% LOF treatment suggests that Leucaena leaf LOF has the potential to improve the nutritional quality of Pacific white shrimp. Furthermore, Li et al. (2021) reported that the protein content of Pacific white shrimp ranges from 21.1% to 23.3%. Although the protein content obtained in the present study was slightly lower, this difference may be attributed to variations in culture systems, feed type, harvest age, and environmental conditions. Nevertheless, the results clearly demonstrate that increasing the LOF concentration to 30% positively influenced body protein deposition.

Tabel 5. Protein Content Results for Litopenaeus vannamei.

Component	Protein Content Results for *Litopenaeus vannamei*				
	NC	PC	10%	20%	30%
Protein (%)	10.50	19.125	14.29	17.53	19.84

The highest protein content in *Litopenaeus vannamei* was observed in the 30% concentration of liquid organic fertilizer (LOF) treatment (C) and urea fertilizer treatment (KP). The increased protein content is presumed to be associated with the higher availability of natural feed in the culture environment. *Leucaena leucocephala* leaves contain essential nutrients, including nitrogen (N), phosphorus (P), and potassium (K), which support primary productivity in aquatic ecosystems. The nitrogen content in the 30% LOF treatment derived from *L. leucocephala* leaf extract was 0.45%, while urea fertilizer contained approximately 46% nitrogen per 50 kg. The availability of nitrogen may enhance the growth of phytoplankton and other natural food organisms, thereby increasing the nutritional input available for shrimp. The highest crude protein content in shrimp is found in the muscle tissue, ranging from 12.33–15.09%. Shrimp protein consists of approximately 70–80% myofibrillar proteins and 20–30% sarcoplasmic proteins, indicating that shrimp is a high-quality seafood product (Liu et al., 2021). Protein plays an essential role in shrimp physiology, particularly in supporting growth and development. It is the primary component of body structures, including the exoskeleton, muscle tissue, and other tissues. Furthermore, protein contributes to various metabolic processes, such as enzyme and hormone synthesis, and can also serve as an energy source when required (Subki, 2024).

Effect of Leucaena leucocephala Leaf Liquid Organic Fertilizer on Carbohydrate Content

The analysis revealed significant differences in carbohydrate content among treatments ($p < 0.05$), with the highest value (14.27%) observed in the unfertilized control and the lowest value (4.38%) in the 30% LOF treatment. The reduction in carbohydrate content in the LOF treatments was likely associated with increased protein synthesis, resulting in a lower carbohydrate value when calculated using the by-difference method. Because carbohydrate content is estimated by subtracting the measured proximate components from 100%, an increase in protein content directly reduces the calculated carbohydrate proportion. According to Yenrina (2015), carbohydrate values obtained using the by-difference method are strongly influenced by changes in moisture, protein, lipid, and ash contents. Therefore, the higher protein content observed in the 30% LOF treatment mathematically resulted in a lower calculated carbohydrate content.

Tabel 6. Carbohydrate Content Results for Litopenaeus vannamei.

Component	Carbohydrate Content Results for Litopenaeus vannamei				
	NC	PC	10%	20%	30%
Carbohydrate (%)	14.27	4.80	8.42	6.17	4.38

Leucaena leucocephala leaves contain essential nutrients, including nitrogen (N), phosphorus (P), and potassium (K). The nitrogen content in the 30% concentration of liquid organic fertilizer (LOF) derived from L. leucocephala leaf extract (treatment C) was 0.45%, whereas urea fertilizer contained 46% nitrogen per 50 kg. Nitrogen in the form of nitrate plays an important role in supporting the growth of algae and phytoplankton (Nurjumanis et al., 2024). This nutrient is essential for enhancing the productivity of phytoplankton and zooplankton, which serve as natural food sources for shrimp. Therefore, the higher nitrogen availability in the urea fertilizer treatment (KP) and 30% LOF treatment (C) was assumed to promote greater natural feed availability, while the absence of fertilization in the control treatment (KN) resulted in limited natural food resources. This finding is supported by Arfah et al. (2019), who reported that the lowest phytoplankton growth occurred in treatments without fertilizer application, whereas the highest phytoplankton abundance was observed in urea application at a concentration of 45%. The highest carbohydrate content observed in the KN treatment (14.27%) was presumed to be related to the limited availability of natural food, which may reduce shrimp feeding activity and movement. Consequently, less energy was expended for foraging, resulting in greater carbohydrate storage in the form of glycogen. In contrast, shrimp in the KP and C treatments, which had higher nitrogen availability, were assumed to have access to more abundant natural food resources. The increased availability of food may stimulate higher shrimp activity, resulting in greater energy expenditure and lower carbohydrate accumulation. Reduced shrimp movement under limited food availability may occur because shrimp have difficulty detecting the presence of feed sources (Nadlifah, 2019).

Bastian and Hudi (2024) reported that the carbohydrate content of L. vannamei cultured under conventional farming systems ranged from 4.11–6.16%. In the present study, which also applied a conventional culture system, carbohydrate levels ranged from 4.38–14.37%. The differences in carbohydrate content may be attributed to variations in feed quality and environmental conditions in shrimp ponds. In crustacean nutrition, carbohydrates are utilized as an energy source through the protein-sparing effect mechanism, which allows dietary protein to be used more efficiently for growth and is considered economically beneficial (Zainuddin et al., 2014). The carbohydrate content showed an inverse relationship with protein content. In this study, the highest carbohydrate level was recorded in the KN treatment, whereas the lowest levels were observed in the KP and C treatments (Figure 4.59). Conversely, the highest protein content was found in the KP and C treatments, while the lowest protein content occurred in the KN treatment (Figure 4.56). The inverse relationship between protein and carbohydrate content in proximate analysis occurs because carbohydrate content is commonly calculated by difference, representing the remaining fraction after subtracting moisture, ash, protein, and lipid components from the total composition. Shrimp are characterized by high protein content, while their carbohydrate requirements are relatively low. In shrimp metabolism, carbohydrates primarily function as glycogen reserves in muscle tissue and participate in energy production through the Krebs cycle (Luthfiani, 2016).

CONCLUSIONS

The application of liquid organic fertilizer (LOF) derived from *Leucaena leucocephala* leaves as a substitute for urea fertilizer influenced the culture performance of Pacific white shrimp *(Litopenaeus vannamei)* reared under a traditional aquaculture system. The 30% LOF

treatment produced the highest result, indicating that the application of Leucaena leaf LOF improved the culture environment by enhancing the availability of natural food through increased primary productivity. The proximate analysis showed that the application of Leucaena leaf LOF had no significant effect on moisture, ash, or lipid contents ($p > 0.05$), but significantly affected protein and carbohydrate contents ($p < 0.05$). The highest protein content (19.84%) was obtained in the 30% LOF treatment, whereas the lowest carbohydrate content (4.38%) was observed in the same treatment. The increase in protein content was likely associated with greater nitrogen availability, which stimulated phytoplankton growth and increased the supply of natural food for shrimp. Overall, the findings demonstrate that the 30% *Leucaena leucocephala* leaf liquid organic fertilizer treatment was the most effective in improving both shrimp survival and nutritional quality, particularly protein content, without adversely affecting other proximate composition parameters. These results suggest that Leucaena leaf LOF has considerable potential as an environmentally friendly alternative to urea fertilizer, contributing to more sustainable traditional culture of Pacific white shrimp (*Litopenaeus vannamei*).

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