

Original Article

Growth, molting, and tissue responses of Pacific whiteleg shrimp (*Litopenaeus vannamei* Boone, 1931) to garlic (*Allium sativum*) skin and guava (*Psidium guajava*) leaf extracts as dietary phytochemicals

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Abstract: The use of phytochemical feed additives has gained increasing attention in shrimp aquaculture as alternatives to synthetic growth promoters. This study evaluated the zootechnical, morphometric, and histological responses of *Litopenaeus vannamei* fed diets supplemented with garlic skin extract (GSE) and guava leaf extract (GLE). A 60-day feeding trial was conducted with four dietary treatments: Control, GSE, GLE, and a combined complex (GSE + GLE) diet. Growth parameters did not differ significantly among treatments, but numerical improvements in final weight and condition factor were observed in GLE-fed shrimp (9.02 ± 1.76 g; 1.30 ± 0.56). GSE-fed shrimp exhibited the highest biomass and survival, while the complex diet resulted in lower growth and survival relative to individual additives. Feed utilization outcomes were consistent with these trends, and strong positive correlations were recorded between survival and biomass ($r=0.985$) and between final weight and condition factor ($r=0.932$). Molting frequency increased significantly in GSE-fed shrimp ($11.62 \pm 1.02\%$). Histological examinations showed intact hepatopancreatic tubules and muscle fiber integrity in GSE and GLE groups, whereas mild epithelial thinning and fiber disruption were observed in shrimp fed the complex diet. Overall, supplementation with GSE or GLE individually improved shrimp performance and tissue condition, indicating their potential as sustainable phytochemical additives for aquaculture.

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Introduction

The Pacific whiteleg shrimp (*Litopenaeus vannamei* Boone, 1931) remains the cornerstone of global aquaculture, contributing approximately 6.3 million tons annually and ranking as the most cultured aquatic species worldwide (Romero and Autric, 2022; FAO, 2023). In the Philippines, *L. vannamei* farming plays a vital role in rural livelihoods and export earnings, with annual production reaching about 66,000 metric tons valued at USD 38 million (Bureau of Fisheries and Aquatic Resources, 2020; Philippine Statistics Authority, 2020). Despite its biological advantages, rapid growth, efficient feed conversion, and broad salinity tolerance, production performance is still constrained by inconsistent growth rates, molting irregularities, and feed conversion inefficiencies, highlighting the need for functional feed strategies to

enhance physiological performance (Cho and Lee, 2012).

Molting and growth in crustaceans are tightly coupled processes regulated by nutritional status, hormonal signaling, and environmental factors (Gao et al., 2021). Disturbances in molting cycles can impair exoskeleton development, reduce nutrient allocation efficiency, and increase vulnerability to stress (Sheikh et al., 2022). Nutritional bioactives have been shown to modulate molting and metabolic pathways by influencing endocrine regulation, antioxidant capacity, and digestive physiology (Jory, 2016; Barreto et al., 2023), supporting the growing interest in phytochemical feed additives as sustainable alternatives to synthetic growth promoters. Phytochemicals, derived from herbs, spices, and plant byproducts, can enhance feed palatability, stimulate

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Table 1. Experimental dietary treatments and inclusion levels used in the feeding trial.

Treatment	Diet Description	Inclusion Level
T ₀	Basal diet (Control)	–
T ₁	Garlic skin extract (GSE)	10 g kg ⁻¹ feed
T ₂	Guava leaf extract (GLE)	10 g kg ⁻¹ feed
T ₃	GSE + GLE (Complex)	5 g kg ⁻¹ + 5 g kg ⁻¹ feed

Table 2. Mean water quality parameters during the experimental period.

Parameter	Control	GSE	GLE	Complex
Temperature (°C)	27.65±0.96	27.93±0.92	27.93±0.92	27.97±0.86
Salinity (ppt)	15.03±0.31	15.04±0.65	15.04±0.65	15.04±0.45
pH	8.62±0.23	8.62±0.24	8.26±0.24	8.66±0.22
Ammonia (mg/L)	2.63±2.28	2.58±2.24	2.54±1.32	2.21±2.02

digestive enzyme activity, improve intestinal barrier function, and reinforce immune responses (Firmino et al., 2021; Alem, 2024; Mobashar, 2025).

Guava (*Psidium guajava*) leaf extract (GLE) contains tannins, flavonoids, and quercetin associated with antioxidant defense, gut epithelial protection, and improved nutrient assimilation (Yin et al., 2014; Giri et al., 2015). Garlic (*Allium sativum*) skin extract (GSE), an underutilized agro-industrial byproduct, is rich in phenolic and sulfur-containing compounds that support oxidative balance and feed utilization efficiency (Bayan et al., 2014; Leonardo et al., 2023). Their incorporation into aquafeeds also aligns with circular bioeconomy objectives by valorizing agricultural waste streams. However, no studies have directly compared the individual versus combined effects of GSE and GLE on simultaneous growth, molting, and tissue integrity in *L. vannamei*, creating a gap in understanding optimal phytogenic inclusion strategies. Most studies have evaluated these extracts independently, providing limited insight into potential synergistic or antagonistic interactions when combined. Additionally, investigations that integrate zootechnical, morphometric, and histological endpoints remain scarce (Cervellione et al., 2017; Sá et al., 2024), leaving unresolved questions about their coordinated physiological effects.

Therefore, this study integrates zootechnical, morphometric, and histological analyses to evaluate the individual and combined effects of GSE and GLE. We hypothesize that individual supplementation

enhances growth performance, molting frequency, and tissue integrity, while the combined supplementation may exhibit additive or antagonistic effects. This integrated assessment contributes to refining phytogenic feed strategies for sustainable shrimp aquaculture.

Materials and Methods

Experimental design and animal husbandry: A total of 240 juvenile *L. vannamei* (≈ 2.5 cm) were obtained from CPF Shrimp Hatchery, Maasim, Sarangani Province, Philippines. Shrimp were acclimated for two weeks and fed a commercial diet (40% crude protein). A completely randomized design was applied with four dietary treatments and three replicates (Table 1), with 20 shrimp stocked per 40-L tank. The overall layout of the experimental setup is illustrated in Figure 1. Shrimp were fed four times daily at 5% biomass for 60 days. Uneaten feed was removed before the first feeding. Growth was measured biweekly, and molting was recorded daily following Bardera et al. (2019).

Water quality: Temperature, salinity, pH, and dissolved oxygen were measured daily; ammonia was measured weekly. All parameters remained within recommended ranges for *L. vannamei* culture (Table 2).

Preparation of extracts: Fresh guava leaves and garlic skins were washed, air-dried (3-5 days), and processed at DOST–Caraga using ethanolic extraction (Aguinaldo et al., 2005). Filtrates were concentrated

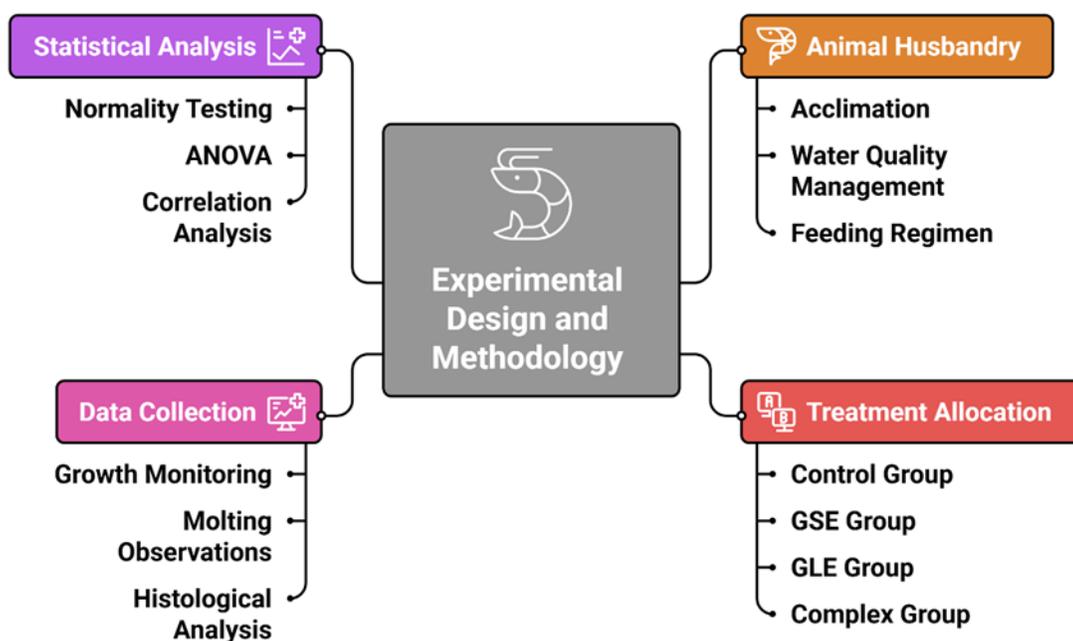


Figure 1. Schematic diagram of the experimental design.

under reduced pressure (<math> < 50^{\circ}\text{C}</math>) and stored at $0\text{--}5^{\circ}\text{C}$. Qualitative phytochemical screening for alkaloids, flavonoids, tannins, saponins, and steroids was conducted using standard procedures, including the Dragendorff, Keller–Killiani, Froth, and ferric chloride reactions, following the protocols described by Harborne (1973) and Trease and Evans (2002).

Feed preparation: Experimental diets were prepared by spraying extract solutions onto commercial feed pellets (Król and Zakeś, 2015). Extracts were diluted in 80% ethanol (100 mL kg^{-1} feed), applied uniformly, and oven-dried at 37°C for 1 h. Control feed was treated with ethanol only to maintain solvent uniformity (Kumar et al., 2023). Basal diet proximate composition was as follows: crude protein 31.11%, moisture 13.04%, crude fat 17.61%, ash 7.81%, crude fiber 8.65%.

Growth and feed utilization: The following growth indices were calculated after 60 days (Hwihy et al., 2021): total weight gain (TWG), average daily weight gain (ADWG), weight-specific growth rate (WSGR, %), feed conversion ratio (FCR), and condition factor (K), where FW and IW = final and initial weights, respectively, days = the experimental duration, W = body weight (g), and L = body length (cm); Total Weight Gain (TWG) = FW – IW, Average Daily

Weight Gain (ADWG) = TWG / days, Weight-Specific Growth Rate (WSGR%) = $[(\ln \text{FW} - \ln \text{IW}) / \text{days}] \times 100$, Feed Conversion Ratio (FCR) = feed intake / biomass gain, and Condition Factor (K) = $100 \times W / L^3$.

Morphometric measurements and molting: Total length (TL), body length (BL), carapace length (CL), carapace width (CW), and abdominal width (AW) were measured biweekly (Mohamed et al., 2013). Molting indices followed Kibria (1993): Molts per day (Md) = M / d, Molt rate (%) = $(M / N) \times 100$, and Molt cycle (days) = N / Md , where M = number of molts, N = number of live shrimps, and d = duration (days).

Histology: Three shrimp per replicate were fixed in Davidson’s AFA (Bell and Lightner, 1988), paraffin-embedded, sectioned, and stained with H&E (Sheehan and Hrapchak, 1980). Hepatopancreas and muscle were scored for histological alterations on a 0–3 scale, adapted from Baums et al. (2013).

Statistical analysis: Data normality was assessed using the Shapiro-Wilk test. One-way ANOVA with Tukey’s HSD was used when assumptions were met; otherwise, Kruskal–Wallis and Dunn’s test (Bonferroni correction) were applied. Pearson correlation was used to evaluate relationships among growth and feed utilization metrics. Analyses were

Table 3. Phytochemical composition of guava leaf and garlic skin extracts.

Phytochemical	Guava Leaf	Garlic Skin	Method Used
Alkaloids	+	+	Dragendorff, Mayer, and Wagner
Steroids	+	+	Keller-Killiani and Leibermann
Flavonoids	+	+	Bate-Smith & Metcalf
Saponins	+	+	Froth Test
Tannins	+	+	Ferric Chloride Test

Table 4. Growth performance indices of *Litopenaeus vannamei* under different dietary treatments (mean \pm SD).

Parameter	Control	GSE	GLE	Complex
FL (cm)	10.77 \pm 0.23	10.68 \pm 0.29	10.23 \pm 0.51	3.48 \pm 6.02*
FW (g)	8.31 \pm 0.22	8.41 \pm 1.15	9.02 \pm 1.76	2.43 \pm 4.22*
TLG (cm)	9.47 \pm 0.26	9.07 \pm 0.43	8.94 \pm 0.61	3.10 \pm 5.37
TWG (g)	8.24 \pm 0.21	8.25 \pm 1.08	8.89 \pm 1.81	2.40 \pm 4.16*
ADWG (g/day)	0.14 \pm 0.00	0.14 \pm 0.02	0.15 \pm 0.03	0.04 \pm 0.07*
SR (%)	58.33 \pm 15.28	60.00 \pm 8.66	43.33 \pm 29.30	20.00 \pm 34.64
WSGR (%/day)	3.43 \pm 0.14	2.95 \pm 0.35	3.13 \pm 0.56	1.06 \pm 1.84
LSGR (%/day)	1.53 \pm 0.03	1.37 \pm 0.09	1.50 \pm 0.09	0.54 \pm 0.93

Values marked with (*) indicate significant differences ($P \leq 0.05$)

performed in JASP v0.18 ($\alpha = 0.05$).

Results

Phytochemical composition of plant extracts:

Qualitative phytochemical screening (Table 3) confirmed the presence of alkaloids, steroids, flavonoids, saponins, and tannins in both *P. guajava* (guava leaf) and *A. sativum* (garlic skin) ethanolic extracts. These compounds were consistently detected using standard phytochemical tests, indicating a shared functional profile between the two plant-derived additives. No qualitative differences in phytochemical composition were observed among the extracts.

Growth performance: Significant differences were observed in final length, final weight, and average daily weight gain among treatments ($P \leq 0.05$) (Table 4). Shrimp fed the guava leaf extract (GLE) diet exhibited the highest final weight (9.02 \pm 1.76 g) and condition factor (1.30 \pm 0.56), while the garlic skin extract (GSE) group also showed enhanced growth compared to the control group. In contrast, shrimp fed the combined GSE+GLE (complex) diet exhibited markedly reduced growth performance and survival, indicating a negative interaction between the combined extracts.

Feed utilization: Feed conversion ratio (FCR) and biomass did not differ significantly between treatments ($P > 0.05$); however, GSE-fed shrimp demonstrated the highest biomass accumulation (97.67 \pm 18.18 g), while GLE-fed shrimp recorded the highest condition factor (1.30 \pm 0.56) (Table 5). The complex diet resulted in lower biomass and efficiency indices. Correlation analysis (Fig. 2) indicated strong positive relationships between final weight and condition factor ($r = 0.932$) and between survival rate and biomass ($r = 0.985$), confirming that improved condition and survivability contribute directly to production efficiency.

Morphometric characteristics: Morphometric traits (Table 6; Fig. 3) indicated that shrimp in the GSE and GLE treatments had more proportionally sized body dimensions and greater structural uniformity than those receiving the complex diet. Strong positive correlations were observed between total weight and carapace width ($r = 0.914$) and between total length and body length ($r = 0.996$), indicating synchronized somatic development.

Molting frequency: Molting frequency differed significantly among treatments ($P \leq 0.05$) (Table 7). Shrimp fed GSE exhibited the highest molt rate (11.62 \pm 1.02%) and shortest molt cycle (10.62 \pm 3.17

Table 5. Feed utilization indices of *Litopenaeus vannamei* (mean ± SD).

Parameter	Control	GSE	GLE	Complex
FCR	2.58±0.88	2.26±0.30	5.10±5.05	0.68±1.18
Biomass	95.83±27.32	97.67±18.18	65.30±47.55	28.60±49.54
Condition Factor	0.97±0.06	1.10±0.06	1.30±0.56	0.30±0.52

Table 6. Morphometric parameters of *Litopenaeus vannamei* (mean ± SD).

Parameter	Control	GSE	GLE	Complex
TW (g)	8.31±0.22 ^a	8.41±1.15 ^a	9.02±1.76 ^a	7.21±1.73 ^b
TL (cm)	10.77±0.23 ^a	10.68±0.29 ^a	10.23±0.51 ^a	9.43±0.98 ^b
BL (cm)	5.53±0.023 ^a	5.42±0.12 ^a	5.09±0.36 ^b	4.31±1.09 ^b
CW (cm)	1.26±0.011 ^a	1.16±0.032 ^a	1.09±0.37 ^b	1.18±0.20 ^a
CL (cm)	3.62±0.008 ^a	3.64±0.065 ^a	3.37±0.34 ^b	3.32±0.0019 ^b
AW (cm)	1.05±0.07 ^a	1.07±0.084 ^a	1.04±0.08 ^a	1.00±0.14 ^b

Different superscripts denote significant differences ($P < 0.05$)

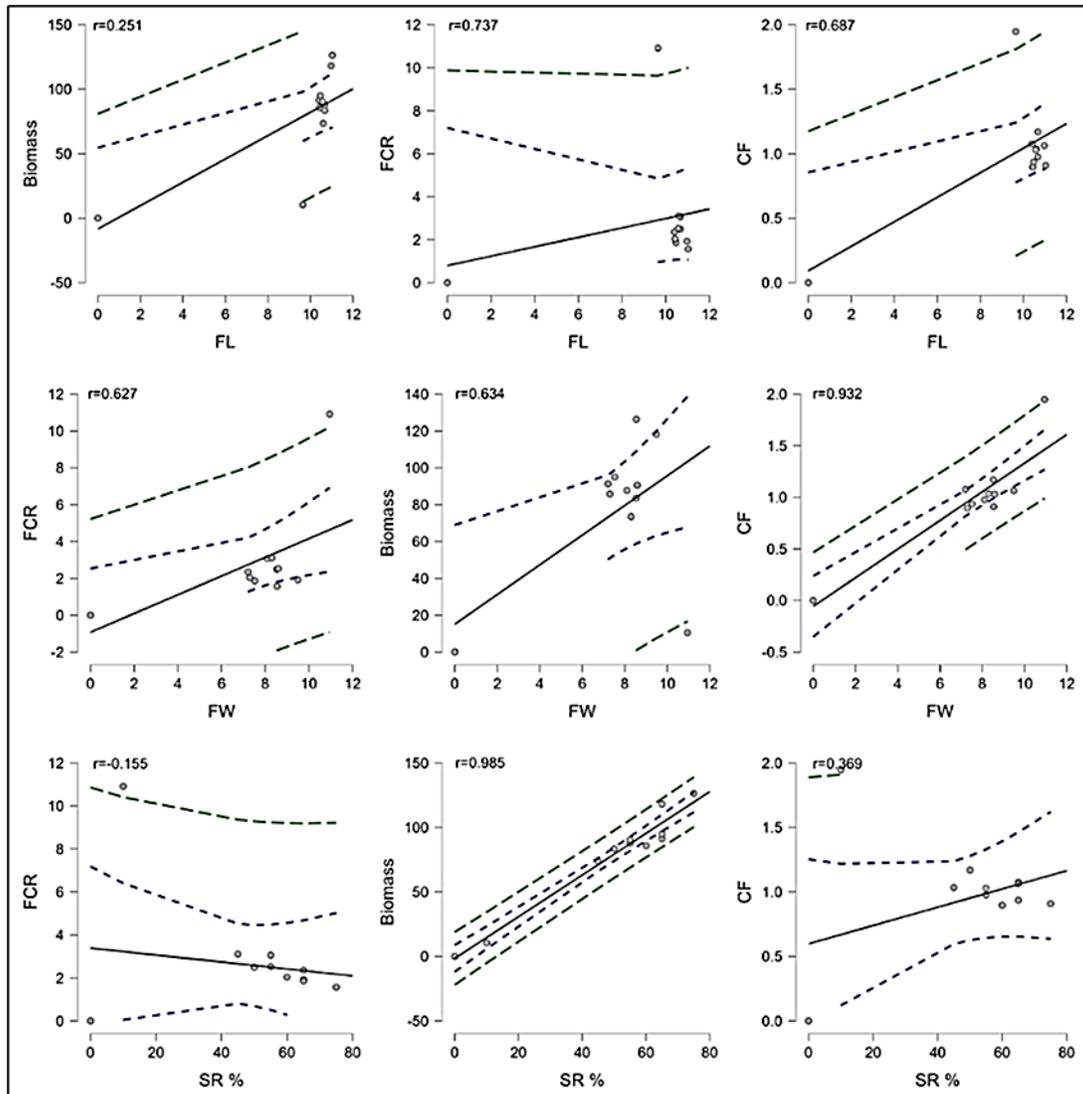


Figure 2. Correlation between growth, survival, and production performance indicators of *Litopenaeus vannamei* fed different dietary treatments.

days), followed by GLE. Both extract-fed groups performed better than the control. The complex group

showed molting activity comparable to that of GSE and GLE.

Table 7. Molting frequency of *Litopenaeus vannamei* (mean ± SD).

Treatment	Molts/day	Molt Rate (%)	Molt Cycle (days)
Control	1.87±1.40 ^b	9.42±1.53 ^b	11.42±3.89 ^b
GSE	2.29±2.08 ^a	11.62±1.02 ^a	10.62±3.17 ^a
GLE	2.22±1.95 ^a	11.17±1.87 ^a	9.48±2.80 ^a
Complex	2.20±1.90 ^a	11.00±1.48 ^a	9.16±3.92 ^a

Different superscripts denote significant differences ($P \leq 0.05$)

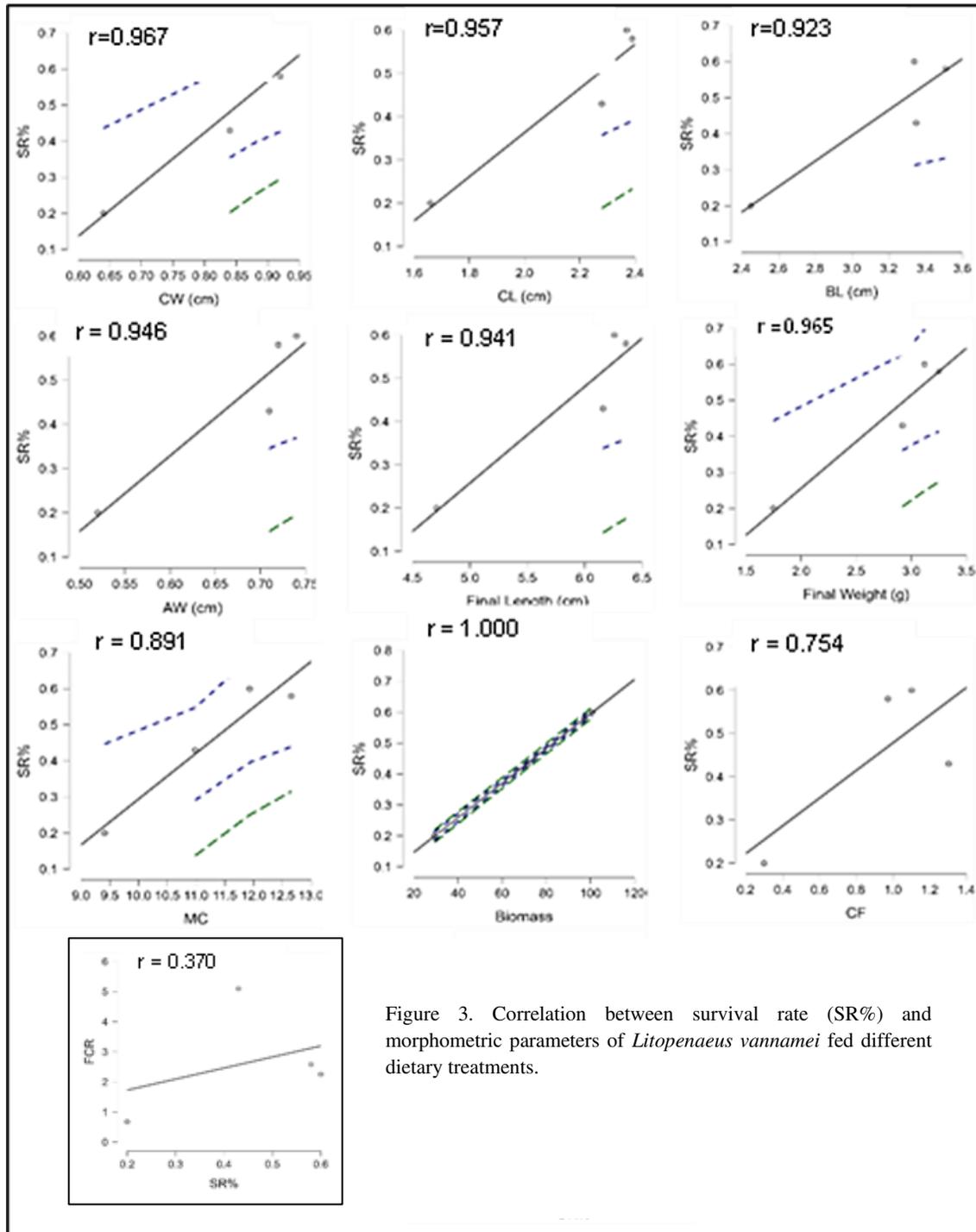


Figure 3. Correlation between survival rate (SR%) and morphometric parameters of *Litopenaeus vannamei* fed different dietary treatments.

Histological observations: Histological examination (Fig. 4) revealed intact hepatopancreatic tubules and

narrow lumens in shrimp fed GSE- and GLE-based diets, consistent with stable digestive and metabolic

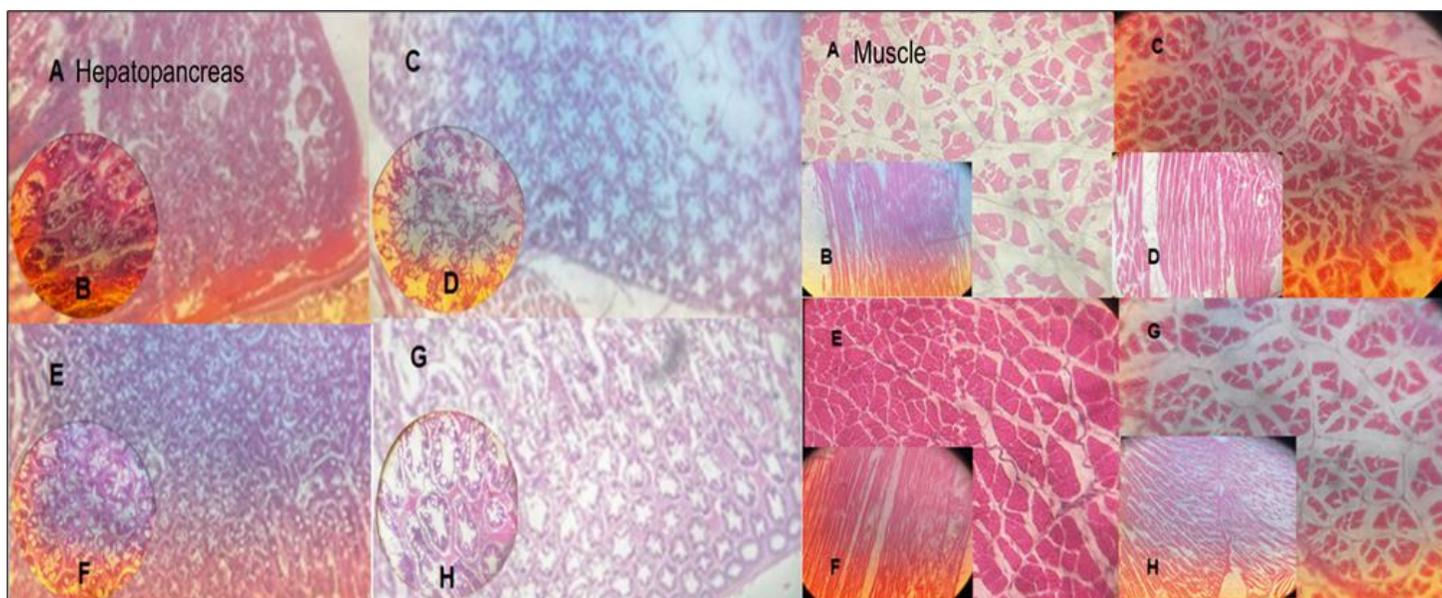


Figure 4. The histological structure of *Litopenaeus vannamei* hepatopancreas in control, GSE, and GLE groups (100x, 400x) showed normal tissues with narrow lumens, intact tubules, and thick epithelium, while the complex group showed minimal damage with wider lumens and thinner epithelium. In muscle tissue, Control and Complex groups (400x, and 100x) showed high damage, GSE showed moderate damage, and GLE showed normal structure.

function. In contrast, shrimp fed the complex diet exhibited mild epithelial thinning and lumen dilation. Muscle tissue in the GLE-treated group showed well-organized fibers, whereas control and complex groups exhibited fiber separation and fragmentation.

Discussions

The phytochemical profiles of guava leaf and garlic skin extracts provide a strong biochemical foundation for their functional roles in enhancing shrimp physiology. The detected flavonoids, phenolics, tannins, and organosulfur compounds are well-established modulators of antioxidant defense, digestive enzyme secretion, antimicrobial resistance, and immune signaling (Chakraborty et al., 2014; Giri et al., 2015; Bayan et al., 2014; Kumar et al., 2021; Naseer et al., 2018; Shang et al., 2019; Phan et al., 2023). These phytochemicals likely contributed to the improved growth performance, condition factor, and morphometric integrity observed in shrimp fed GSE and GLE.

The superior performance of the GLE and GSE treatments is consistent with reports that phytochemical feed additives enhance nutrient utilization efficiency, antioxidant status, and immune resilience in

L. vannamei and other cultured species (Chakraborty et al., 2014; Jana et al., 2018; Reverter et al., 2020; Li et al., 2022). Meta-analytic evidence further confirms that ethanol-extracted herbal supplements enhance growth and survival more effectively than water extracts (Saleh et al., 2024). The decline in growth and survival in the complex group suggests phytochemical antagonism, where overlapping biochemical pathways may interfere with nutrient absorption or metabolic stability (Francis et al., 2002).

Molting enhancements in GSE- and GLE-fed shrimp suggest improved hormonal regulation and metabolic coordination. Flavonoids, phenolics, and sulfur compounds are known to modulate ecdysteroid synthesis and stabilize molt-inhibiting hormone (MIH) signaling (Chang and Mykles, 2011; Hosamani et al., 2017; Naiel et al., 2023; Barua et al., 2025). Faster and more synchronized molting leads to faster size-class progression and improved harvest turnover, key economic advantages in intensive shrimp culture.

Histological results confirm tissue-level resilience, with phytochemical supplementation supporting hepatopancreatic stability, reduced inflammatory stress, and improved muscle integrity (Ng and Koh, 2017; Abdel-Latif et al., 2020; Goh et al., 2022; De

Marco et al., 2023). These cellular effects validate the observed improvements in growth and feed efficiency.

Overall, GSE and GLE function as effective, sustainable, and non-antibiotic phytogetic feed additives that enhance physiological resilience, growth performance, and molting efficiency in *L. vannamei*. However, their combination in a single feed formulation requires careful dose optimization to avoid antagonistic interactions.

Conclusion

This study demonstrated that individual supplementation with ethanolic extracts of *Allium sativum* (garlic skin) and *Psidium guajava* (guava leaf) can improve key physiological and production traits in *Litopenaeus vannamei*. Diets containing either GSE or GLE supported enhanced growth performance, improved molting frequency, and maintained hepatopancreatic and muscle tissue integrity, reflecting better metabolic stability and structural resilience. Although not all parameters showed significant differences, consistent directional trends and strong trait correlations indicate meaningful biological responses. GSE supplementation was particularly associated with improved survival and molting efficiency, while GLE enhanced somatic growth and muscle fiber organization. In contrast, the combined supplementation resulted in reduced growth and tissue condition, suggesting potential phytochemical antagonism when both extracts are used concurrently. Overall, GSE and GLE represent promising, sustainable phytogetic additives that can support health and performance in *L. vannamei* culture. However, further work is needed to determine optimal inclusion levels, evaluate long-term effects, and elucidate mechanisms of interaction when extracts are combined. Future studies should also incorporate dosage, response modeling, and immune and oxidative stress biomarkers to strengthen mechanistic interpretation.

Ethical statement: Procedures complied with Republic Act No. 8485 and were approved by the NEMSU Institutional Animal Care and Use

Committee.

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