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Evaluation of dietary vitamin C supplementation on growth performance and oxidative responses of pacific whiteleg shrimp juvenile *Litopenaeus vannamei* Boone

Evaluasi suplementasi vitamin C dalam pakan terhadap kinerja pertumbuhan dan respons oksidatif juvenil udang vaname *Litopenaeus vannamei* Boone

Ratnawati Rifai[1*], Asrianti Sani[1], Fauziah Nurdin[1], Bustamin[1], Rahmadina Rahmadina[2]

[1]Aquaculture Study Program, Pangkep Agricultural State Polytechnique, South Sulawesi, Indonesia
[2]Student Aquaculture Study Program, Pangkep State Polytechnic Agriculture
*Corresponding author: rifaiharianto5@gmail.com

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ABSTRACT

Vitamin C cannot be synthesized by shrimp and must be obtained from dietary sources; however, its supply through feed is often insufficient. This study aimed to evaluate the growth performance and oxidative response of juvenile whiteleg shrimp (*Litopenaeus vannamei*) supplemented with dietary vitamin C. Juvenile shrimp at the PL-25 stage, acclimated to a salinity of 20 ppt, were stocked into 16 rearing containers, each filled with 40 L of seawater from a total 250 L fiberglass tank. Each container contained 20 juveniles with an initial average body weight of 0.001 g per individual. The rearing period lasted 60 days, during which shrimp were fed three times daily. The feeding rate (FR) was initially set at 11% of body weight for the first 30 days and then reduced to 7% thereafter. The experimental design followed a completely randomized design (CRD) consisting of four treatments and four replicates: A (0 mg/kg), B (150 mg/kg), C (300 mg/kg), and D (450 mg/kg) of dietary vitamin C supplementation. The results showed that supplementation of vitamin C at 300 mg/kg diet produced the best growth performance (RGR, PER, FCR) and survival rate, which were positively correlated with SOD enzyme activity. The oxidative response parameters (HSI and MDA) showed the lowest values in this optimal treatment. The highest growth performance was obtained from the treatment with 300 mg vitamin C/kg diet, achieving a relative growth rate of 4,358.6%, the lowest MDA concentration of 3.58 ± 0.37 nmol/L, and the highest SOD activity of 88.35 ± 2.22 U/mL enzyme.

Keywords: growth, oxidative responses, shrimp, vitamin C

ABSTRAK

Vitamin C tidak dapat disintesis oleh udang, zat ini dapat diperoleh dari pakan namun pasokannya masih belum mencukupi. Tujuan dari penelitian ini untuk menguji kinerja pertumbuhan dan respon oksidatif juvenil udang vaname (*Litopenaeus vannamei*) dengan suplementasi vitamin C dalam pakan. Juvenil udang vaname PL 25, yang telah diadaptasikan di salinitas 20 ppt ditebar pada 16 wadah pemeliharaan yang diisi dengan air laut volume 40 L dari volume total bak fiber 250 L dengan jumlah total juvenil 20 ekor dan rata-rata berat awal juvenile 0,001 g/ekor; waktu pemeliharaan udang;selama 60 hari dengan pemberian pakan tiga kali perhari dan jumlah pakan ditentukan dengan FR 11% dan setelah pemeliharaan 30 hari FR diturunkan menjadi 7%. Rancangan penelitian menggunakan rancangan acak lengkap (RAL) dengan empat taraf perlakuan dan empat ulangan dengan komposisi perlakuan; A (dosis 0 mg/kg), B (dosis 150 mg/kg). C (dosis 300 mg/kg) D (dosis 450 mg/kg). Hasil penelitian menunjukkan bahwa suplementasi vitamin C dosis 300 mg/kg pakan menghasilkan kinerja pertumbuhan (LPR, REP, RKP) dan TKH terbaik dan berkorelasi dengan aktivitas enzim SOD. Hasil uji respons oksidatif (IHS dan MDA) menunjukan hasil terendah pada perlakuan terbaik di penelitian ini. Kinerja pertumbuhan optimal diperoleh dari trreatment 300 mg vitamin C/kg sebesar 4.358.6% dengan nilai MDA terendah 3,58 ± 0,37 nmol/L dan aktivitas SOD tertinggi 88,35 ± 2,22 Unit/mL enzim.

Kata kunci: pertumbuhan, respons oksidatif, udang vaname, vitamin C

INTRODUCTION

In Indonesia, the Pacific *whiteleg* shrimp (*Litopenaeus vannamei* Boone) is a major aquaculture commodity with steadily increasing production (Roshith *et al.,* 2018; Asmild *et al.,* 2024). This species dominates national shrimp production, positioning Indonesia as the world's fourth-largest shrimp exporter, following Ecuador, India, and Thailand (Yuliadi & Sani, 2023; Wati, 2023). According to Alsy *et al.* (2023), shrimp production in 2021 accounted for 20.52% of national fishery export volume, while contributing 39.98% to the total export value. Aquaculture systems exert considerable pressure on water quality and can generate secondary pollution that damages aquatic resources (Ariadi *et al.,* 2019). Increasing water recycling efficiency and reducing wastewater discharge are therefore essential for sustainable aquaculture (Melaku *et al.,* 2024; Liu *et al.,* 2024; Tom *et al.,* 2021). However, the adoption of supra-intensive farming technologies introduces environmental challenges, primarily linked to higher feed inputs (Lal *et al.,* 2024; Ahmad *et al.,* 2022).

Imbalanced nutrient input is a key constraint: insufficient nutrient supply limits shrimp growth (Chaikaew *et al.,* 2019), whereas excessive nutrient input accelerates environmental degradation and increases production costs (Yadav, 2024; Zhang *et al.,* 2019). Sustainable shrimp production thus requires effective waste management, including identification of sources, components, and mitigation strategies (Dauda *et al.,* 2019). Accumulation of poorly degradable waste can otherwise disrupt ecosystems and harm aquatic organisms (Mustafa *et al.,* 2023). Feed quality represents the most significant determinant of shrimp farming success and constitutes the largest proportion of operational costs (Borges *et al.,* 2024). Proper feed management enhances growth and productivity (Liang *et al.,* 2025).

Shrimp generally require diets containing 25–40% protein to achieve optimal growth (Nunes *et al.,* 2022). In tiger shrimp (*Penaeus monodon*), a 40% protein diet resulted in a protein efficiency ratio (PER) of 0.50 and a survival rate of 49% (Susanto *et al.,* 2019). Diets with 60% protein yielded a feed conversion ratio (FCR) of 0.31 and a PER of 3.225, both favorable for the growth of Pacific *whiteleg* shrimp (Elsayed *et al.,* 2022). Several studies have demonstrated the benefits of vitamin C supplementation in aquaculture. Kaur *et al.* (2022) reported that 100 ppm vitamin C improved growth performance and hematobiochemical parameters in Amur carp (*Cyprinus carpio*) exposed to sodium fluoride (NaF).

Khalil *et al.* (2023) found that 1.6 g/kg vitamin C improved antioxidant status in red swamp crayfish (*Procambarus clarkii*). Similarly, Luc *et al.* (2021) demonstrated that 0.4 g/kg ascorbic acid enhanced relative growth rate, PER, and hematological profiles in juvenile Waigieu seaperch (*Psammoperca waigiensis*). At the same dosage, vitamin C also enhanced immune responses in tilapia challenged with *Aeromonas sobria*, reducing mortality to 20.83% and increasing lysozyme activity (Ibrahim *et al.,* 2020). Xu *et al.* (2022) further showed that dietary vitamin C levels of 0.09–0.2 g/kg reduced aspartate transaminase (AST) and alanine transaminase (ALT) activities while enhancing superoxide dismutase (SOD) activity in Coho salmon (*Oncorhynchus kisutch*).

The Pacific *whiteleg* shrimp is characterized by high protein content (Li *et al.,* 2021), unsaturated fatty acids (Jannathulla *et al.,* 2022), vitamins, and minerals, supporting rapid growth, uniform body size, adaptability to environmental variation, survival rates of 50–60%, and high productivity (Naser *et al.,* 2022). This species can be cultured in intensive systems at high stocking densities (Kannadasan *et al.,* 2024; Inayah *et al.,*2023; Tantu *et al.,* 2020), requiring less dietary protein and fewer chemical inputs than *P. monodon* (Sutradhar *et al.,* 2021; Jeyasanta & Patterson, 2017). Nevertheless, high stocking densities can increase susceptibility to oxidative stress. For example, Ardiansyah *et al.* (2020) reported that shrimp affected by white feces syndrome (WFS) exhibited impaired endogenous antioxidant defenses, resulting in free radical accumulation and enzyme inactivation within 72 h post-infection.

Molting failures associated with lunar cycle fluctuations also weaken immune function (Covarrubias *et al.,* 2020). In addition, low water temperatures have been shown to disrupt antioxidant defenses and induce oxidative stress in Chinese shrimp (*Fenneropenaeus chinensis*) (Xu *et al.,* 2017). One of the major challenges in shrimp aquaculture is the presence of unutilized feed (Eggink *et al.,* 2024), which contributes to ammonia accumulation (nitrogen waste) and induces oxidative stress (Iber & Kasan, 2021). Oxidative stress has profound effects on shrimp health (Zhao *et al.,* 2025), weakening the immune system (Veryer, 2023), increasing susceptibility to pathogens, and reducing growth performance (Gambini & Stromsnes, 2022), thereby lowering overall productivity. To mitigate these effects, various antioxidants have been applied in aquaculture, including alpha-lipoic acid (ALA). Feeding trials have demonstrated its ability to improve growth performance, with optimal doses reported at 0.8 g/kg feed in striped catfish (Rifai *et al.,* 2022a; Rifai *et al.,* 2022b), 1.2 g/kg feed in catfish (Siagian *et al.,* 2021), and 0.6 g/kg feed in gourami (*Osphronemus goramy*) (Samuki *et al.,* 2020).

Alpha-lipoic acid is considered a universal antioxidant capable of restoring the antioxidant properties of vitamin C when depleted (Superti & Rosso, 2024; Serhiyenko *et al.,* 2018). Despite its strong and rapid antioxidant activity (Shi *et al.,* 2018), its use in aquaculture is limited by high cost, import dependence, and long procurement times (Choudhary *et al.,* 2023; Tibullo *et al.,* 2017). In contrast, vitamin C is more affordable, readily available, and cost-effective. Its supplementation in aquafeeds provides a practical strategy for mitigating oxidative stress and nitrogen waste while simultaneously enhancing shrimp health and productivity (Lykkesfeldt, 2020).

Meanwhile the shrimp cannot synthesize the vitamin C endogenously, it must be supplied through the diet in small quantities (as a micronutrient) (Dawood & Koshio, 2016), unlike macronutrients such as proteins, lipids, and carbohydrates (Pathinathan & Roshan, 2024). However, dietary supplementation in practical aquaculture often remains insufficient. Vitamin C plays a pivotal role in strengthening the immune system and enhancing feed palatability (Khadim & Al-Fartusie, 2021). It supports growth performance, restores immune functions against pathogenic infections, and improves both survival rate and overall disease resistance in shrimp (Khalil *et al.,* 2023).

As a water-soluble compound, vitamin C also facilitates mineralization during carapace formation, particularly during molting (Lykkesfeldt & Carr, 2024). In addition, it acts as an antioxidant by preventing the peroxidation of fatty acid chains into toxic by-products (Lu *et al.,* 2021). In its ascorbic acid form, vitamin C is essential for cellular processes, including metabolism, growth, and defense against environmental stressors (Zhitkovich, 2022). Given these diverse biological functions, the present study was conducted to evaluate the effects of dietary vitamin C supplementation on growth performance and oxidative responses in Pacific *whiteleg* shrimp.

MATERIAL AND METHODS

Experimental diets

A crumbled artificial feed containing 30% protein was used as the basal diet in this study, with different levels of vitamin C supplementation. Prior to supplementation, vitamin C was dissolved in 100 mL of distilled water and blended with 30 g of egg whites and 0.9 g of egg yolk to form a suspension. This suspension was then uniformly coated onto the diet. The supplemented diets were air-dried at room temperature, stored in airtight containers, and subsequently used for shrimp feeding. The proximate composition of the experimental diets is presented in Table 1.

Experimental design

This study applied a completely randomized design with four treatments and four replications (16 experimental units). The vitamin C dosage was composed of 0 mg/kg diet (A), 150 mg/kg diet (B), 300 mg/kg diet (C), and 450 mg/kg diet (D).

Table 1. Nutrient contents (proximate analysis results) of test diets.

Nutrient	Vitamin C (mg/kg diet)			
	0	150	300	450
Ash	6.33	6.5	5.86	7.60
Moisture	6.79	6.83	7.19	6.62
Protein	30.02	30.18	30.36	30.58
Lipid	6.92	6.67	6.28	5.86
Carbohydrate	46.54	48.41	52.31	51.31
GE	423.974	418.259	426.719	414.303

Note: GE = gross energy; 1 g protein = 5.6 kcal; 1 g lipid = 9.4 kcal; 1 g carbohydrate = 4.1 kcal (Watanabe, 1988).

Shrimp rearing

Juvenile Pacific *whiteleg* shrimp (*Litopenaeus vannamei*, PL25) were acclimated at a salinity of 20 ppt and stocked into 16 containers at a density of 20 individuals per container. Each container was filled with 40 L of seawater, and shrimp had an average initial body weight of 0.001 g. For initial weight measurement, shrimp were gently collected using a plastic spoon and placed on cotton tissue prior to weighing.

Shrimp were fed three times daily at 08:00, 13:00, and 18:00 local time (GMT+7). Diets were initially provided at 11% of shrimp biomass. After 15 days of rearing, biomass sampling was conducted. As shrimp growth increased, the feeding rate was reduced to 7% of biomass and maintained until the end of the experimental period. To maintain water quality, a 15–20% daily water exchange was performed. Wastewater was siphoned from the rearing containers using a small hose fitted with a filtration net, after which fresh seawater was added. The rearing trial lasted 45 days. At the end of the experiment, shrimp were anesthetized with clove oil (0.02 mL/L) before final weight measurements were taken.

Growth performance was assessed by calculating the relative growth rate (RGR) based on the mean weight per container, while survival rate (SR) was determined from the final count of live shrimp. Water quality parameters, including temperature, dissolved oxygen (DO), pH, and salinity, were measured twice daily at 07:00 and 16:00 (GMT+7).

Growth performance and survival rate

At the final rearing period, all shrimp biomasswere measured, and the survival rate was calculated. Before final sampling, shrimps were fasted for 24 hours and anesthetized with 0.025 ml/l of clover oil (Rifai *et al.,* 2022a). The growth performance parameters were composed of relative growth rate (RPR), protein efficiency ratio (PER), and feed conversion ratio (FCR).

Relative growth rate (RGR)

$$RGR(\%) = \frac{(Wt - W0)}{W0} \times 100$$

Note:
RGR = Relative growth rate (%)
Wt = Average Initial shrimp weight (g)
W0 = Average final shrimp weight (g)

Protein efficiency ratio (PER)

PER was applied to determine the association of weight gain and the total consumed protein, as calculated using the formula Tacon (1987):

$$PER(\%) = \frac{Wt - W0}{KPP \times FI}$$

Note:
PER = Protein efficiency ratio
Wt = Total shrimp biomass at the final rearing period (g)
W0 = Total shrimp biomass at the initial rearing period (g)
KPP = Diet protein content (%)
FI = Feed intake (g)

Feed conversion ratio (FCR)

The feed intake was recorded during rearing. The feed intake was determined by measuring the difference of total feed applied to the shrimp and the total unconsumed feed. Feed conversion ratio was calculated based on Tacon (1987) formula:

$$FCR = \frac{F}{(Wt + D) - W0}$$

Note:
FCR = Feed conversion ratio
F = Total feed intake (g)
Wt = The total shrimp biomass weight at final rearing (g)
Wd = The dead shrimp total weight (g)
W0 = The total shrimp biomass weight at initial rearing (g)

Oxidative responses

Oxidative responses were evaluated from hepatosomatic index (HSI), superoxide dismutase enzyme activity (SOD), and malondialdehyde content (MDA). After recording their final weight, shrimps were reared to gain liver weight at least 0.5 g/individual.

Hepatosomatic index (HSI)

Hepatosomatic index (HSI) was calculated based on the measurement results of total shrimp weight and total liver weight of eight shrimps from each replication. The hepatosomatic index value was calculated using the following:

$$HSI\ (\%) = \frac{\text{Liver weight (g)}}{\text{Total shrimp weight (g)}}$$

Superoxide dismutase (SOD)

The SOD was analyzed following the Misra and Fridovich (1972) method was determined by adding 100 µL of epinephrin (0.05 mg/10 mL HCl 0.01 N) to the sample. Inhibitory level was read from sample absorbance and blank absorbance, following the formula:

$$\text{SOD (unit/mL of enzyme)} = \frac{\text{\% inhibition} \times 10 \text{ (dilution factor)}}{0.5 \times 0.1}$$

Superoxide dismutase (SOD) activity was measured spectrophotometrically at a wavelength of 480 nm. Absorbance was recorded at 0, 1, 2, 3, and 4 minutes, and the change in absorbance at each interval was used for calculations. The blank consisted of 100 µL of distilled water mixed with 3 mL of carbonate buffer and 100 µL of epinephrine. SOD activity was expressed as the percentage of inhibition of the sample absorbance relative to the blank, calculated according to the following formula:

$$\text{SOD (unit/Ml enzyme)} = \frac{\text{\% inhibition} \times 10 \text{ (dilution)}}{0.5 \times 0.1}$$

Malondialdehyde (MDA)

The malondialdehyde (MDA) content was determined following the thiobarbituric acid (TBA) method described by Conti *et al.* (1991). Briefly, 0.5 g of fresh catfish liver tissue was finely minced on a 5 cm² marble surface placed over ice to maintain low temperature. The minced tissue was homogenized in 2.5 mL phosphate-buffered saline (PBS; pH 7.4) containing 11.5 g/L KCl until smooth. The homogenate was centrifuged at 10,000 rpm for 20 minutes, and 0.5 mL of the resulting supernatant was collected.

The supernatant was mixed with 2 mL of a reagent solution containing 2.23 mL concentrated HCl, 10 g trichloroacetic acid (TCA), 0.57 g butylated hydroxytoluene (BHT), and 0.75 g thiobarbituric acid (TBA), dissolved in 100 mL distilled water. The mixture was incubated at 80°C for 1 hour and then cooled to room temperature. After cooling, the samples were centrifuged at 3000 rpm for 5 minutes. A 200 µL aliquot of the clear supernatant was transferred to a microplate, and absorbance was measured at 532 nm using a spectrophotometer. MDA concentration was determined using a standard curve prepared with MDA solutions at 0, 1, 2, 3, 4, 5, 10, 20, 40, and 80 µmol/L, and calculated using the following formula:

$$\text{MDA (nmol/g protein)} = \frac{\text{A (nmol/g)} \times 5 \text{ mL}}{500 \text{ mg}}$$

Data analysis

Prior to statistical evaluation, data were tested for normality. A one-way analysis of variance (ANOVA) was conducted to assess the effects of treatments on each parameter. When significant differences were detected, Tukey's post hoc test was applied to identify pairwise differences among treatment groups. All statistical analyses were performed using SPSS version 25.0.

RESULTS AND DISCUSSION

Result

Whiteleg shrimp growth performance

Growth performance, survival rate, feed nutrient utilization, and oxidative responses were evaluated to determine the effectiveness of shrimp culture productivity following a 60-day rearing period. The growth performance and survival rate of Pacific whiteleg shrimp fed diets supplemented with varying levels of vitamin C are presented in Table 2. The ANOVA results revealed that dietary vitamin C supplementation significantly affected the relative percentage growth rate (RPR) ($P<0.001$), confirming its role in enhancing shrimp growth performance. According to Tukey's test, treatment C (300 mg/kg diet) provided the optimal dosage, resulting in the highest RPR. Shrimp in treatment C achieved the greatest weight gain (22.90 g), whereas the lowest weight gain was recorded in treatment A (0 mg/kg diet). Similarly, treatment C yielded the highest relative growth rate (RGR) of 4,358.6 ± 1,181.9%.

Responses oxidative status and hepatosomatic index

The results of the oxidative response data analysis showed that the treatment supplemented with vitamin C at a dose of 300 mg/kg of feed produced the lowest MDA levels of 3.58 ± 0.37 nmol/L with the highest SOD enzyme levels of 88.35 ± 2.22 units/ml Enzyme. The results of the analysis correlated with the lowest hepatosomatic index analysis in the same treatment with an average of 2.869 ± 0.42.

Discussion

Mechanistically, vitamin C plays an essential role in carbohydrate metabolism by accelerating glycolysis (Gupta *et al.,* 2022), allowing glycogen to function as an energy reserve to support growth. In this study, vitamin C also exhibited a protein-sparing effect, whereby its contribution to energy metabolism enabled dietary protein to be utilized more efficiently for growth. Similar findings have been reported in other aquatic species (Ibrahim *et al.,* 2020; Dhewantara *et al.,* 2023). In crustaceans, vitamin C is predominantly stored in the hepatopancreas and digestive tract (Vogt, 2021). Dietary supplementation of 171.16 mg/kg vitamin C improved growth performance and feed utilization in juvenile chu's croaker (*Nibea coibor*) (Zou *et al.,* 2019). Comparable results were obtained by Carvalho *et al.* (2021), who reported improved growth at dosages of 188.7 mg/kg and 897.4 mg/kg in juvenile zebrafish (*Danio rerio*). Likewise, Liu *et al.* (2018) found that supplementation at 40 mg/kg and 80 mg/kg diet enhanced growth performance in juvenile discus fish (*Symphysodon haraldi*).

As an antioxidant, vitamin C reduces oxidative stress by minimizing free radical damage, thereby improving growth performance. However, excessive dietary vitamin C is not fully absorbed by the digestive tract and is excreted in urine (Bhoot *et al.,* 2023), highlighting the importance of consistent daily supplementation at appropriate levels (Ali *et al.,* 2024). Furthermore, vitamin C functions as an enzyme cofactor, supports nonspecific immunity, and enhances antioxidant capacity (Li *et al.,*2024). Acting as a reducing agent and free radical scavenger, it protects against oxidative damage from hydroxyl radicals and hydrogen peroxide (H_2O_2) (Pehlivan, 2017).

Shrimp reared without dietary vitamin C supplementation displayed reduced appetite, indicated by partially filled stomachs, infrequent molting, and weak swimming activity during feeding. These observations align with previous reports that vitamin C deficiency in shrimp leads to poor feed intake, growth retardation, reduced molting frequency, increased disease susceptibility, tail rot, broken antennae, and mortality (Omoniy & Ovie, 2018). Similarly, Shefat and Karim (2018) reported that vitamin C deficiency impairs collagen synthesis, reduces growth, disrupts equilibrium, and significantly increases mortality rates. Pathinathan and Roshan

Table 2. Growth performance and oxidative responses of shrimps.

Parameters	Vitamin C (mg/kg diet)			
	A (0)	B (150)	C (300)	D (450)
IIW I W (g)	0.001 ± 0.00^{a}	0.001 ± 0.00^{a}	0.001 ± 0.00^{a}	0.001 ± 0.00^{a}
FIW (g)	11.28 ± 2.89^{b}	18.48 ± 3.65^{ab}	22.90 ± 6.21^{a}	22.08 ± 5.70^{a}
ITW(g)	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}
FTW (g)	124.08 ± 4.53^{d}	277.20 ± 8.29^{c}	412.20 ± 7.69^{a}	309.12 ± 8.11^{b}
RGR (%)	2.147.6 ± 549.95^{b}	3.262.7 ± 695.93^{ab}	4.358.6 ± 1181.98^{a}	4.201.4 ± 1085.59^{a}
PER	0.37 ± 0.10^{b}	0.64 ± 0.13^{ab}	0.84± 0.23^{a}	0.83 ± 0.21^{a}
FCR	0.74 ± 0.15^{b}	0.47 ± 0.08^{a}	0.39 ± 0.09^{a}	0.43 ± 0.09^{a}
SR (%)	95.2 ± 4.1	91.2 ± 7.6	94.0 ± 2.8	98.0 ± 5.7
HSI)	7.039 ± 0.95^{c}	5.704 ± 0.36^{b}	2.869 ± 0.42^{a}	5.784 ± 0.58^{bc}
MDA (nmol/L)	6.31 ± 0.49^{c}	4.43 ± 0.37^{ab}	3.58 ± 0.37^{a}	4.82 ± 0.86^{b}
SOD (unit/ml enzyme)	27.11 ± 6.94^{d}	38.59 ± 1.65^{bc}	88.35 ± 2.22^{a}	41.55 ± 13.04^{b}

Note: Different superscript letters on the same line indicate a significant difference (P<0.05). All values are presented in average± standard deviation. IIW = initial individu weight, FIW = final individual weight, ITW=initial total weight, FTW= final ttotal weight, RGR= relative growth rate, PER= protein efficiency ratio, FCR= food convertion ratio, SR= survival rate, HSI= hepatosomatic index, MDA = malondialdehyde SOD = superoxide dismutase.

(2024) recommended a dietary requirement of 150 mg/kg vitamin C for *Litopenaeus vannamei*. Overall, nutritional imbalances or deficiencies, particularly in essential micronutrients such as vitamin C, can result in impaired growth, weakened immunity, and reduced survival in shrimp populations.

Asaikkutti *et al.* (2016) emphasized that vitamin C supplementation enhances growth performance, antioxidant status, digestive enzyme activity, and survival in *Macrobrachium malcolmsonii*. Consistent with this, dietary vitamin C supplementation at 300 mg/kg feed in the present study resulted in a markedly higher appetite compared to the other treatments (150 mg/kg, 450 mg/kg, and 0 mg/kg). The highest protein efficiency ratio (PER) was recorded in treatment C (300 mg/kg diet), with a value of 0.84 ± 0.23 g, whereas the lowest PER occurred in treatment A (0 mg/kg diet), at 0.37 ± 0.10 g. ANOVA results confirmed that vitamin C supplementation significantly influenced PER ($P<0.05$). Optimal dietary protein levels are essential to support maximum growth (Alberts *et al.,* 2025), as also demonstrated by Rahman *et al.* (2023).

PER, which is commonly used to evaluate protein quality in diets, is closely associated with digestibility (Adhikari *et al.,* 2024). Highly digestible protein improves amino acid absorption, which directly contributes to enhanced growth performance. Increased feed consumption was observed in shrimp supplemented with vitamin C, as reflected in the higher appetite scores (Table 2). This increase can be attributed to nutraceutical compounds, including vitamin C, that activate adenosine monophosphate-activated protein kinase (AMPK). AMPK acts as an energy sensor, signaling cells to utilize energy and regulating hunger and satiety (Aguilar *et al.,* 2017; Zhitkovich, 2022; Przibytho & Langner, 2020). Enhanced feed consumption improved nutrient intake and utilization, resulting in more efficient feed conversion and ultimately greater growth performance.

Shrimp in treatment C (300 mg/kg diet) demonstrated a higher PER and superior growth compared to the other treatments, indicating that supplementation at this dosage reduced the dietary requirements for both feed energy and protein relative to treatments B and D. These findings are consistent with studies in tilapia (Ibrahim *et al.,* 2020) and black tetra (*Gymnocorymbus ternetzi*) (Dhewantara *et al.,* 2023), where vitamin C supplementation was shown to activate phosphorylation via the AKT/mTOR/4EBP pathway in muscle tissue, thereby enhancing PER through improved non-protein energy utilization. Dietary vitamin C supplementation also significantly affected the feed conversion ratio (FCR). Tukey's test revealed that treatment A (0 mg/kg diet) differed significantly from treatments B, C, and D, whereas no significant differences were observed among the supplemented groups. The lowest FCR was achieved in treatment C (300 mg/kg diet), with a value of 0.39 ± 0.09, indicating optimal feed utilization at this dosage.

According to Zainuddin *et al.* (2019), juvenile shrimp exhibit feed conversion ratio (FCR) values ranging from 1.02 to 4.45. Pacific whiteleg shrimp are particularly efficient in feed utilization, with crude protein levels of 30–35% being optimal for pond-based production (Strebel *et al.,* 2023). In controlled environments, dietary vitamin C supplementation further enhances physiological processes, improves immune responses, and positively influences feed intake. Rifai *et al.* (2022a) reported that antioxidants in aquatic organisms improve appetite, while Rifai *et al.* (2022b) demonstrated that antioxidant-rich diets allow shrimp to utilize the water column more efficiently, thereby minimizing feed waste and nitrogen discharge. This improved nutrient utilization not only enhances growth performance but also stabilizes water quality parameters, such as dissolved oxygen, ultimately reducing oxidative stress.

The survival rate of *L. vannamei* over the 60-day culture period differed notably between treatments. Shrimp deprived of dietary vitamin C exhibited weakness, as evidenced by reduced movement during siphoning and increased mortality toward the end of the experiment. In contrast, shrimp supplemented with vitamin C displayed vigorous swimming activity, strong responses to siphoning, and lower mortality rates. Survival rate is strongly influenced by the adequacy of dietary nutrients, with vitamin C functioning as a key immunostimulant that enhances immune resistance, thereby enabling shrimp to better tolerate stress and pathogen exposure (Khalil *et al.,* 2023). These results emphasize the critical role of vitamin C as an essential micronutrient in shrimp aquaculture.

Vitamin C also plays a fundamental role in maintaining liver and blood biochemistry. Acting as an antioxidant, it protects the liver a major detoxification organ from oxidative damage. Hepatosomatic index (HSI) values can serve as indicators of liver function, where enlargement is often linked to excessive lipogenesis, impaired fatty acid oxidation, and disrupted triglyceride export (Heeren & Scheja, 2021). In the present study, dietary vitamin C supplementation at 300 mg/kg feed enhanced superoxide dismutase (SOD) activity while reducing malondialdehyde (MDA) levels, consistent with findings by Zhang *et al.* (2022) in *Pampus argenteus* and Cheng *et al.* (2017) in juvenile pufferfish (*Takifugu obscurus*). These results demonstrate that vitamin C enhances antioxidant capacity, mitigates oxidative stress, and supports faster growth rates in shrimp (Ibrahim *et al.,* 2020).

Beyond HSI, oxidative capacity was further assessed using stress biomarkers, particularly MDA levels. MDA is a reliable indicator of lipid peroxidation and oxidative stress (Asfandyar *et al.,* 2024), reflecting the accumulation of reactive oxygen species (ROS) that may cause hepatic cell damage (Rifai *et al.,* 2022b; Yusuf *et al.,* 2020). Elevated SOD activity within the effective supplementation range of vitamin C enhanced free radical scavenging and increased SOD enzyme activity in the hepatopancreas (Asaikkutti *et al.,* 2016; Pathinathan & Roshan, 2024). Evidence from Yin *et al.* (2020) further supports the antioxidative role of vitamin C in aquatic species, demonstrating its ability to alleviate cadmium (Cd) and lead (Pb) toxicity in grass carp kidney cells. Mechanistically, vitamin C increases the reduced-to-oxidized glutathione (GSH/GSSG) ratio (Biswas *et al.,* 2020), which activates the Nrf2 pathway and induces SOD enzyme production in the liver. This antioxidative mechanism strengthens the cellular defense system against ROS, thereby optimizing shrimp health, survival, and growth performance (Baranska *et al.,*2020; Barreiro *et al.,* 2022; Njus *et al.,* 2020).

CONCLUSION

Dietary supplementation of vitamin C at 300 mg/kg diet is recommended for optimal growth performance in Pacific whiteleg shrimp. At this dosage, shrimp exhibited the highest relative growth rate (4,358.6%), along with enhanced oxidative defense, as indicated by the lowest malondialdehyde (MDA) level (3.58 ± 0.37 nmol/L) and the highest superoxide dismutase (SOD) activity (88.35 ± 2.22 U/mL). These findings highlight the critical role of vitamin C in promoting growth, improving antioxidant capacity, and supporting the overall health of *Litopenaeus vannamei*.

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