

Publication status: This preprint has not been published elsewhere.

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<https://doi.org/10.1590/SciELOPreprints.17942>

Submitted on: 2026-09-03

Posted on: 2026-09-09 (version 1)

(YYYY-MM-DD)

# **Dietary vitamin C supplementation modulates gene expression and selected meat quality traits in juvenile Pacific white shrimp**

## **Vitamin C in shrimp nutrition**

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## Abstract

Vitamin C plays important roles in antioxidant defense and connective tissue metabolism in crustaceans, but its effects on molecular responses associated with shrimp meat quality remain unclear. This study evaluated dietary vitamin C supplementation on growth performance, physicochemical and sensory traits, and gene expression in juvenile *Penaeus vannamei* Boone, 1931. A total of 288 shrimp were randomly distributed among 24 experimental boxes (12 shrimp per box), with 12 experimental units per dietary treatment, for 15 days. The conventional diet contained 150 mg kg<sup>-1</sup> vitamin C according to the manufacturer, whereas the supplemented diet received 150 mg kg<sup>-1</sup> of a product containing 35% vitamin C, corresponding to a nominal addition of 52.5 mg kg<sup>-1</sup> vitamin C. Final dietary vitamin C concentrations were not analytically determined. Dietary treatment did not affect final weight, length, fillet yield, or biomass. Supplemented shrimp showed lower thawing and cooking losses and higher sensory scores for taste, consistency, and overall liking. Supplementation was also associated with higher transcript abundance of *GPx*, *SOD*, *SREBP*, *ACC*, *MYCH5*, *COL1A2*, and *RYS*. These findings indicate that vitamin C supplementation modulated selected molecular and meat-quality responses without affecting growth performance under the conditions evaluated.

**Keywords:** antioxidant defense; ascorbic acid; gene expression; meat quality; shrimp nutrition.

## Introduction

Pacific white shrimp (*Penaeus vannamei* Boone, 1931) is the most widely farmed crustacean worldwide and is valued for its productive characteristics and consumer acceptance (Li et al., 2021; Ximenes and Vidal, 2023; Kir et al., 2023). Intensification of shrimp farming, however, can increase physiological challenges and reinforce the importance of nutritional strategies that support animal health and product quality (Abdel-Rahim et al., 2021). Among these strategies, dietary vitamins have received attention because of their roles in metabolism, antioxidant defense, and tissue integrity.

Vitamin C (ascorbic acid) is essential for shrimp and contributes to disease resistance, exoskeleton maintenance, antioxidant defense, and connective tissue metabolism (Dawood and Koshio, 2018; Kong et al., 2021; Ko and Lee, 2024). Ascorbic acid is also a cofactor for enzymes involved in hydroxyproline formation and collagen biosynthesis (Hunter et al., 1979; Pinnell, 1985). These functions may be relevant to muscle characteristics because collagen organization, oxidative processes, and water retention can influence texture, juiciness, and other quality attributes of aquatic products (Hagen and Johnsen, 2016; Huang et al., 2025).

Despite evidence of the nutritional and antioxidant functions of vitamin C, information remains limited on its association with transcriptional responses related to antioxidant defense, lipid metabolism, and muscle-associated processes in *P. vannamei* and on how these responses coincide with meat-quality traits. Therefore, this study

evaluated the effects of dietary vitamin C supplementation on growth performance, physicochemical and sensory characteristics of the meat, and expression of genes associated with antioxidant defense, lipid metabolism, and muscle-related processes in juvenile *P. vannamei*.

## **Materials and Methods**

This study was conducted at the Laboratory of Nutrition of Aquatic Organisms and Beekeeping (LANOAA), Federal University of Sergipe (UFS), São Cristóvão, Sergipe, Brazil. For use in the experiments, 500 juveniles of *P. vannamei* weighing about 15 g each were purchased from a local nursery in São Cristóvão and transported in plastic bags with water to LANOAA.

At the time the experiment was conducted, ethical approval by an institutional animal care and use committee was not required for studies involving decapod crustaceans under the regulations applicable at the institution where the study was performed.

## **Acclimatization and quarantine**

The shrimp were acclimatized for 7 days in a 1,000-L polyethylene tank equipped with a forced aeration system. Partial water exchange was performed to maintain adequate water quality while minimizing animal stress. The stocking density during acclimatization was 1,000 individuals m<sup>-3</sup>. Water temperature, dissolved oxygen, salinity, and pH were maintained within the recommended ranges for the species according to Boyd (2000). Feed was provided twice daily (09:00 and 16:00 h) at a rate of 4% of total biomass.

97

98 **Animals and experimental design**

99       After the acclimatization period, 288 shrimp were randomly distributed among 24  
100 experimental boxes, with 12 shrimp per box. The experiment followed a completely  
101 randomized design with two dietary treatments and 12 experimental replicates per  
102 treatment, with each box considered an experimental unit. The 24 boxes were distributed  
103 among six experimental batteries, with four boxes per battery.

104       The dietary treatments consisted of a commercial diet containing 150 mg kg<sup>-1</sup> of  
105 vitamin C, according to the manufacturer's specification (conventional diet), and the same  
106 commercial diet supplemented with an additional vitamin C source. According to the  
107 manufacturer's specification, the commercial diet contained 150 mg kg<sup>-1</sup> of vitamin C.  
108 For the supplemented treatment, 150 mg kg<sup>-1</sup> of a vitamin C product containing 35%  
109 vitamin C was added to the feed, corresponding to an additional 52.5 mg kg<sup>-1</sup> of vitamin  
110 C. Thus, the supplemented diet had a nominal vitamin C concentration of approximately  
111 202.5 mg kg<sup>-1</sup>, based on the vitamin C content declared for the commercial diet and the  
112 added supplement. The actual vitamin C concentration in the final diets was not  
113 analytically determined.

114       The supplemented diet was prepared by spraying 150 mg of the vitamin C product  
115 (35% vitamin C) and 1% soybean oil per kg of feed, according to Okamura et al. (2007).  
116 Soybean oil was used as a vehicle to facilitate adhesion of the supplement to the feed and  
117 minimize losses during application. The conventional diet was sprayed with 1% soybean  
118 oil only. Both diets were dried in a forced-ventilation oven at 50 °C for 4 h before use.  
119 The prepared diets were provided to the shrimp shortly after preparation. During the

experimental period, feed was provided twice daily at 4% of biomass, with the amount adjusted according to the biomass of each experimental unit.

During the 15-day experimental period, water temperature, pH, salinity, total ammonia, and nitrite were monitored daily using colorimetric kits. The values were  $28.0 \pm 0.5$  °C, pH  $8.0 \pm 0.2$ , salinity  $20 \pm 1$ ‰, total ammonia  $0.3 \pm 0.1$  mg L<sup>-1</sup>, and nitrite  $0.10 \pm 0.05$  mg L<sup>-1</sup>.

At the end of the experimental period, shrimp were euthanized by immersion in ice, as recommended by Weineck et al. (2018). A transverse section was made between the posterior region of the carapace and the anterior abdominal segment. The cephalothorax was separated from the abdomen to collect hepatopancreas and muscle samples for gene expression analysis. Subsequently, the carapace, intestine, telson, and ventral nerve cord were removed from the fillet. The fillets were washed with distilled water and stored frozen until analysis.

### **Animal performance**

Initial and final biometric measurements were obtained using a digital scale and manual caliper, respectively. Final biomass was calculated as the sum of the final body weight of all shrimp in each experimental unit. Uniformity was calculated as 100 minus the coefficient of variation of body weight. Fillet yield (%) was calculated as:

$$Fillet\ yield\ (\%) = \left( \frac{fillet\ weight}{shrimp\ body\ weight} \right) \times 100.$$

No mortality was observed during the experimental period; therefore, survival was 100% in both treatments.

### **Gene expression**

Muscle and hepatopancreas samples were collected from six shrimp from each experimental box, resulting in six biological samples per experimental unit. Samples were placed in RNAlater™ (Life Technologies do Brasil, Brazil) and stored at -20 °C until RNA extraction.

Total RNA was extracted from 100 mg of tissue using 1 mL of TRIzol® reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. RNA samples were treated with DNase I Amplification Grade (Invitrogen, Carlsbad, CA, USA) to remove potential genomic DNA contamination. Complementary DNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, USA), according to the manufacturer's instructions.

Real-time quantitative PCR (RT-qPCR) was performed using SYBR™ Green PCR Master Mix (Applied Biosystems™, USA). Primers (Table 1) targeted genes associated with antioxidant defense (superoxide dismutase, *SOD*; glutathione peroxidase, *GPx*; and catalase, *CAT*), lipid metabolism (fatty acid synthase, *FAS*; acetyl-CoA carboxylase, *ACC*; and sterol regulatory element-binding protein, *SREBP*), muscle-associated processes (myosin heavy chain 1, *MYCH1*; myosin heavy chain 2, *MYCH2*; myosin heavy chain 6A, *MYCH6A*; myosin heavy chain 5, *MYCH5*; and myosin heavy chain 15, *MYCH15*), and extracellular matrix and muscle-related processes (collagen alpha-1 type I, *COL1A1*; collagen alpha-2 type I, *COL1A2*; and ryanodine receptor, *RYP*). Shrimp  $\beta$ -actin was used as the reference gene. The stability of  $\beta$ -actin expression was verified across treatments. All reactions were performed in duplicate, and relative gene expression was calculated using the  $2^{-\Delta CT}$  method.

#### **Physicochemical characteristics of shrimp meat**

Color parameters were measured using a CR-10 colorimeter (Konica Minolta Inc., Tokyo, Japan) and expressed according to the CIELab system: L\* (lightness), a\* (green–red), and b\* (blue–yellow).

Fillet texture was analyzed according to Gontijo et al. (2017), with modifications. Fillet fragments measuring  $1 \times 1 \times 2$  cm were positioned perpendicular to the probe axis. Texture analysis was performed using a CT3® digital texture analyzer (AMETEK Brookfield, Middleborough, MA, USA), equipped with a 5-mm-diameter TA41 cylindrical probe, operating at  $1 \text{ mm s}^{-1}$ , with a penetration depth of 2 mm and a force of 0.5 N in two cycles. The evaluated parameters were adhesiveness (mJ), cohesiveness, hardness (kg), elasticity (mm), chewiness (mJ), resilience, and gumminess (kg). Data were obtained using TexturePro CT software.

Water-holding capacity was determined using the filter-paper press method described by Hamm (1961). Thawing loss and cooking loss were determined according to Esteves et al. (1998). Thawing loss (TL, %) was calculated as:

$$TL (\%) = \left[ \frac{(\text{frozen weight} - \text{thawed weight})}{\text{frozen weight}} \right] \times 100$$

Cooking loss (CL, %) was calculated as:

$$CL (\%) = \left[ \frac{(\text{raw weight} - \text{cooked weight})}{\text{raw weight}} \right] \times 100$$

## Sensory analysis

Sensory analysis was conducted at the Laboratory of Quantitative Analysis of Animal Products (LAPOA), Department of Animal Science, Federal University of

Sergipe. The panel consisted of 80 untrained seafood consumers who were randomly selected based on their interest in participating in the study. The analysis followed the Guidelines for Sensory Evaluation of Fish and Shellfish in Laboratories (CAC/GL 31-1999).

A total of 160 shrimp fillet samples were evaluated, comprising 80 samples from each dietary treatment. Samples were wrapped in aluminum foil and cooked at 72 °C for 5 min. After cooking, the samples were placed in labeled, closed containers and allowed to cool to an appropriate temperature for sensory evaluation.

Each participant evaluated one sample from each dietary treatment. The order of sample presentation was randomized, and the samples were identified using three-digit random codes so that participants were unaware of the dietary treatment. A cream cracker biscuit and mineral water were provided between samples to minimize residual taste effects.

Appearance, taste, consistency, and overall liking were evaluated using a 9-point hedonic scale, ranging from 1 ("dislike extremely") to 9 ("like extremely"), according to Dutcosky (2019). Purchase intention was evaluated using a 5-point scale, ranging from 1 ("definitely would not buy") to 5 ("definitely would buy").

## **Statistical analysis**

Data were initially assessed for normality using the Shapiro–Wilk test and for homogeneity of variances using Bartlett's test. Performance and physicochemical variables were analyzed using analysis of variance, considering dietary treatment as the main fixed effect and the experimental box as the experimental unit. The significance level was set at 5%.

For sensory analysis, because each consumer evaluated samples from both dietary treatments, the paired nature of the observations was considered in the statistical analysis. Consumer identity was treated as a random effect, with dietary treatment as the fixed effect.

For gene expression, the six-shrimp sampled from each experimental box were considered subsamples of the corresponding experimental unit. Therefore, statistical inference was based on the experimental box rather than treating individual shrimp from the same box as independent experimental replicates.

All statistical analyses were performed using R software version 4.3.1 (R Core Team, 2022).

## **Results**

### **Animal performance**

Dietary treatment did not affect final body weight, final length, fillet yield, or final biomass ( $P > 0.05$ ). However, body weight uniformity was higher in shrimp fed the conventional diet than in those fed the vitamin C-supplemented diet ( $P < 0.001$ ) (Figure 1).

Figure 1

### **Physicochemical characteristics of shrimp meat**

Dietary treatment significantly affected thawing loss and cooking loss. Fillets from shrimp fed the vitamin C-supplemented diet exhibited lower thawing and cooking losses than those from shrimp fed the conventional diet ( $P < 0.05$ ) (Fig. 2). No significant

differences were observed between treatments for the texture or color parameters evaluated ( $P > 0.05$ ) (Fig. 3).

Figure 2

Figure 3

### Sensory analysis

Dietary treatment significantly affected taste, consistency, and overall liking ( $P < 0.05$ ). Shrimp fillets from the vitamin C-supplemented treatment received higher scores for these attributes than fillets from the conventional diet treatment (Fig. 4). No significant differences were observed between treatments for appearance or purchase intention ( $P > 0.05$ ).

Figure 4

### Gene expression

The effects of dietary treatment on gene expression in the hepatopancreas are presented in Fig. 5. Vitamin C supplementation was associated with higher transcript abundance of *GPx* and *SOD* compared with the conventional diet ( $P < 0.05$ ), whereas *CAT* expression did not differ between treatments ( $P > 0.05$ ). Among the genes related to lipid metabolism, *SREBP* and *ACC* expression were higher in shrimp receiving the supplemented diet ( $P < 0.05$ ), while *FAS* expression was not affected by dietary treatment ( $P > 0.05$ ).

Figure 5

In muscle tissue, dietary treatment affected the expression of *MYH5*, *COL1A2*, and *RYR*, which showed higher transcript abundance in shrimp fed the vitamin C-

supplemented diet ( $P < 0.05$ ) (Fig. 6). No significant differences were observed for *MYCH1*, *MYCH2*, *MYCH6A*, *MYCH15*, or *COL1A1* ( $P > 0.05$ ).

Figure 6

## Discussion

Previous studies have demonstrated the importance of dietary vitamin C for performance and physiological functions in aquatic animals (Chen et al., 2015; Liang et al., 2017; Dawood and Koshio, 2018). In the present study, the conventional diet contained a declared vitamin C concentration of  $150 \text{ mg kg}^{-1}$ , whereas the supplemented diet had a nominal concentration of approximately  $202.5 \text{ mg kg}^{-1}$  based on the manufacturer's declaration and the added supplement. The absence of differences in final body weight, length, fillet yield, and biomass may be related to the vitamin C already supplied by the basal diet, the 15-day experimental period, and the relatively narrow difference between treatments. Chen et al. (2017) likewise reported no performance differences among juvenile *P. vannamei* fed 30 to  $460 \text{ mg kg}^{-1}$  L-ascorbyl-2-polyphosphate, whereas Ko et al. (2026) reported improved growth after 49 days at selected vitamin C levels. Together, these findings indicate that growth responses depend on dietary concentration, experimental duration, and physiological stage.

Although growth performance was unchanged, vitamin C supplementation was associated with transcriptional and meat-quality responses. *SREBP* and *ACC* transcript abundance increased, whereas *FAS* was unaffected. *SREBP* regulates genes involved in lipid homeostasis, and *ACC* catalyzes the formation of malonyl-CoA, an important step in fatty acid biosynthesis (Eberlé et al., 2004; Satoh et al., 2020). Thus, the simultaneous response of *SREBP* and *ACC* suggests modulation of lipid-metabolism pathways.

However, these transcriptional changes do not demonstrate increased lipid synthesis or deposition because *FAS* expression, tissue lipid composition, enzyme activity, and fatty acid profiles were not measured. Consequently, possible links between lipid metabolism and texture, flavor, or moisture retention remain speculative under the conditions evaluated (Yang et al., 2022).

Vitamin C supplementation also increased *GPx* and *SOD* transcript abundance, while *CAT* was unchanged. *SOD* and *GPx* are components of the enzymatic antioxidant system involved in controlling reactive oxygen species and peroxides (Wang et al., 2012). Similar responses to dietary vitamin C have been reported in crustaceans: Asaikkutti et al. (2016) observed increased *SOD* expression, Ko and Lee (2024) reported upregulation of antioxidant-related genes in *P. vannamei*, and Ebadi et al. (2021) described changes in antioxidant enzyme activity that depended on interactions among dietary vitamin C, vitamin E, and lipid levels. These studies support a role for vitamin C in antioxidant defense, but the higher *GPx* and *SOD* transcript abundance observed here cannot be interpreted as direct evidence of reduced oxidative stress because reactive oxygen species, oxidative damage, and antioxidant enzyme activities were not measured.

Fillets from supplemented shrimp had lower thawing and cooking losses. Water retention is an important meat-quality trait and can be affected by biochemical and structural changes in muscle proteins (Huff-Lonergan and Lonergan, 2005; Pearce et al., 2011). Oxidative modification of myofibrillar proteins can alter muscle organization and contribute to water loss (Medina et al., 2012). Therefore, the antioxidant-related transcriptional response observed in supplemented shrimp is biologically consistent with the lower water losses, but causality cannot be established. Moreover, the reduction in thawing and cooking losses was not accompanied by significant changes in the texture or

color variables evaluated. Measurements of protein and lipid oxidation, antioxidant enzyme activity, and muscle microstructure would be required to clarify this response.

Sensory evaluation showed higher scores for taste, consistency, and overall liking in the supplemented treatment, whereas appearance and purchase intention were unaffected. These findings indicate that the dietary treatment influenced specific aspects of consumer perception. Nevertheless, volatile compounds, lipid oxidation, fatty acid composition, and other chemical characteristics that could explain the sensory differences were not measured. The sensory outcomes should therefore be interpreted as treatment-associated quality responses rather than consequences directly caused by the observed gene-expression changes.

In muscle, *MYCH5*, *RYS*, and *COLIA2* showed higher transcript abundance in supplemented shrimp. Myosin heavy chains are major components of the contractile apparatus (Koyama et al., 2013), while *RYS* participates in intracellular calcium release and excitation-contraction coupling (Matsukawa and Murayama, 2023). The responses of *MYCH5* and *RYS* therefore indicate transcriptional modulation of muscle-associated pathways, but they do not demonstrate increased myosin synthesis, altered channel activity, or improved muscle function because these endpoints were not measured.

The *COLIA2* response is particularly relevant because ascorbic acid is a cofactor for enzymes involved in collagen hydroxylation and normal collagen biosynthesis (Hunter et al., 1979; Pinnell, 1985; Sipilä et al., 2018). Recent evidence in juvenile *P. vannamei* also indicates that dietary vitamin C can increase muscle hydroxyproline and collagen concentrations (Ko et al., 2026). This physiological evidence is consistent with the higher *COLIA2* transcript abundance observed here. However, collagen concentration, hydroxyproline content, collagen organization, and protein abundance

were not measured; therefore, the result demonstrates transcriptional modulation of a collagen-related gene rather than increased collagen synthesis or deposition.

Overall, dietary vitamin C supplementation at the level evaluated was associated with changes in genes related to antioxidant defense, lipid metabolism, and muscle-associated processes, together with lower thawing and cooking losses and improved scores for selected sensory attributes. Because the experiment compared only two dietary conditions over 15 days and did not include biochemical or structural validation of the transcriptional responses, longer dose-response studies integrating gene expression with enzyme activity, oxidative markers, tissue composition, collagen measurements, and muscle structure are needed to clarify the mechanisms involved.

## Conclusion

Dietary vitamin C supplementation at the level evaluated in the present study was associated with increased transcript abundance of genes related to antioxidant defense, lipid metabolism, and muscle-associated processes in juvenile *Penaeus vannamei*. Supplemented shrimp also exhibited lower thawing and cooking losses and higher sensory scores for taste, consistency, and overall liking, whereas growth performance was not significantly affected. These findings suggest that vitamin C supplementation may influence physiological and quality-related responses beyond growth performance. However, the observed changes in gene expression should not be interpreted as direct evidence of reduced oxidative stress, increased lipid synthesis, collagen deposition, or improved muscle functionality, as these responses were not directly measured. Further studies using graded dietary vitamin C levels, longer feeding periods, and biochemical,

enzymatic, and structural assessments are needed to establish dose-response relationships and clarify the mechanisms underlying the observed effects.

### **Acknowledgments**

This study was supported by the Brazilian National Council for Scientific and Technological Development (CNPq; process 403205/2021-2) and the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES; Finance Code 001).

### **Conflict of interest**

The authors declare no conflict of interest.

### **Authors' Contributions**

**Jefté dos Santos Vieira:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation.

**Juliana dos Santos Conceição:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing (original draft, Writing) review & editing.

**Amanda Silva Carvalho:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing (original draft, Writing) review & editing.

**Thaís Pacheco Santana:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing (original draft, Writing) review & editing.

**Jefferson Wayne da Silva Cartaxo:** Investigation.

**Vittor Tuzzi Zancanela:** Investigation.

**Hannah Caroline Santos Araujo:** Investigation.

**Jodnes Vieira Sobreira:** Investigation.

**Maria Terezinha Santos Leite Neta:** Investigation.

**Ana Paula Del Vesco:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Writing (review & editing).

### **Ethics statement**

At the time the experiment was conducted, approval by an institutional animal ethics committee was not required for studies involving decapod crustaceans under the regulations applicable at the institution where the study was performed.

### **Declaration of use of Artificial Intelligence**

No artificial intelligence tools were used for data generation, data analysis, interpretation of results, scientific decision-making or language editing and improvement of the readability of the manuscript.

### **Data availability statement**

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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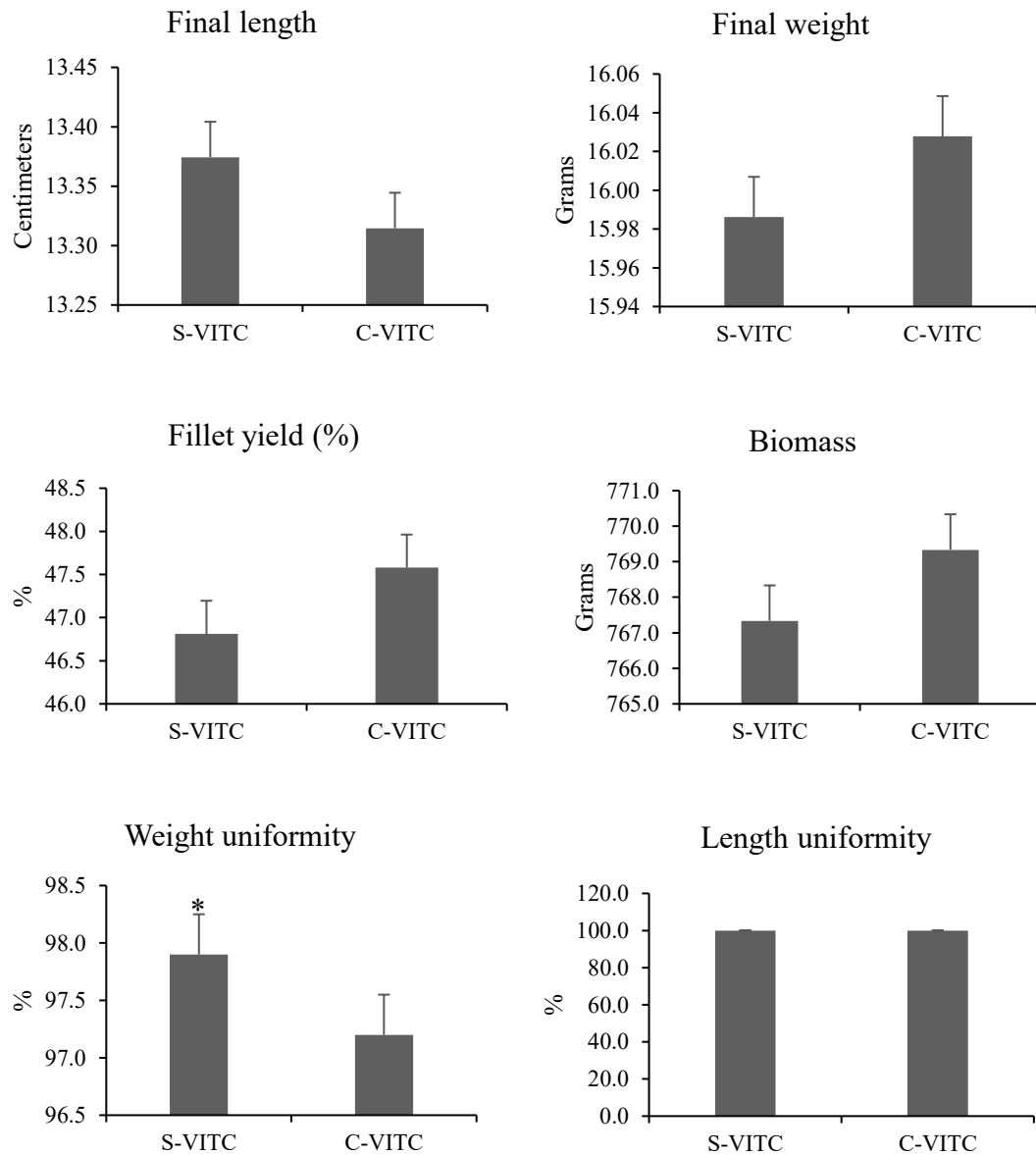
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**Table 1.** Nucleotide sequence of primers used for amplification

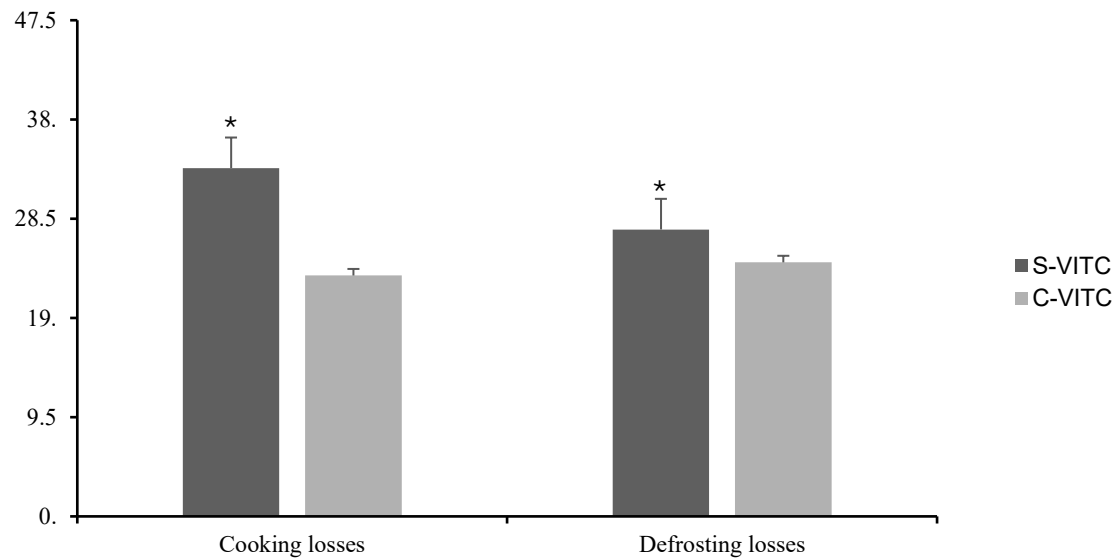
| Gene            | Sequence (5'– 3')                                          | Base pairs | GenBank                            |
|-----------------|------------------------------------------------------------|------------|------------------------------------|
| <i>FAS</i>      | F: TGCTACTGTGCCTGTTGTGTATGC<br>R: CCACCAGAACCTGCGTGAATGAG  | 47         | HM595630                           |
| <i>ACC</i>      | F: CGGCAGACAACATCCATACCACAG<br>R: GCAACCAGCGAGAGCAGTAACC   | 46         | XM_027360190                       |
| <i>SREBP</i>    | F: AGATGGCTGAGATGTTGGTAATGGC<br>R: CCCTTGTGGCTCTTCCTCTTTGC | 48         | MG770374                           |
| <i>sMYCH1</i>   | F: CGGTGCCTCTGAGAAGAAAG<br>R: AGGAGTTGTCGTTACGGGTG         | 119        | AB758443                           |
| <i>sMYCH2</i>   | F: CGATATTTACGACTACCGCTACG<br>R: CGACCCCTCTGCTTGAACCTT     | 192        | AB758444.1                         |
| <i>sMYCH6a</i>  | F: ATCCGAACCTTGCTGATGCC<br>R: TCAGCACGGAGTTCGTCAGC         | 207        | AB759104.1                         |
| <i>sMYCH5</i>   | F: ATGCTCAACGAAGCCAGACA<br>R: CCTTCATCGCATTTGTTTCG         | 193        | AB759100.1                         |
| <i>sMYCH15</i>  | F: GCAACTACGCCACCGAACAC<br>R: CCTCACCGATCTGGTCCATCAA       | 132        | LVANscaffold_903<br>446522: 459841 |
| <i>COL1A1</i>   | F: CTGGAAGCCTGTTCGGAATAA<br>R: CACATCGTTGGTGAAGAGGTAG      | 43         | XM_027350742.1                     |
| <i>COL1A2</i>   | F: GCCAGGATTCCAGGGATTAG<br>R: GCCTACCAACATCTCCCTTT         | 40         | XM_027369130.1                     |
| <i>SOD</i>      | F: ATCCACCACACAAAGCATCA<br>R: AGCTCTCGTCAATGGCTTGT         | 229        | XM_027352840.1                     |
| <i>GPx</i>      | F: TTTTCCGTGCAAAAAGGAC<br>R: TAATACGCGATGCCCTAAC           | 239        | XM_027376216.1                     |
| <i>CAT</i>      | F: CAAGTGGCGATTACCCCTCAT<br>R: CCCATGAGGCCATACTTTGGT       | 110        | AY518322                           |
| <i>RYS</i>      | F: GTCACGACCTTCAGCAGGAA<br>R: AGACATGTAGAGCGGGTCCT         | 139        | HM367069                           |
| <i>β-actina</i> | F: GAGCAACACGGAGTTCGTTGT<br>R: CATCACCAACTGGGACGACATGGA    | 142        | AF300705.2                         |

**Figure 1.** Effect of diet on the zootechnical performance of *P. vannamei*

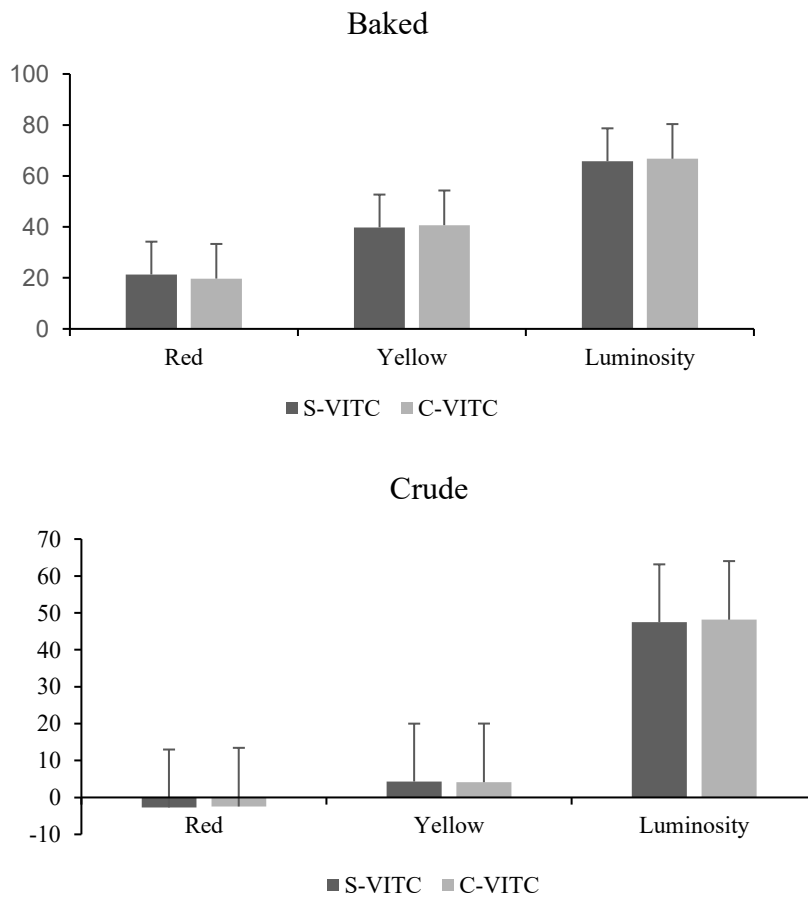


Description: ‡S-VITC: Diet without vitamin C supplementation; C-VITC: Diet with vitamin C supplementation. The results described in the figure are represented as mean and standard error (SE). \*Significant by analysis of variance ( $P < 0.05$ ).

**Figure 2.** Effect of diet on cooking and thawing loss in *P. vannamei*

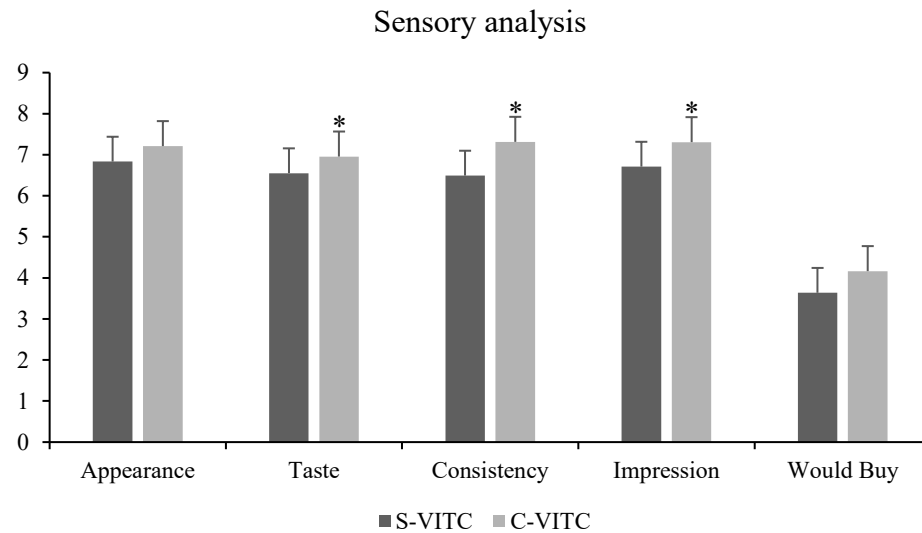


Description: ‡S-VITC: Diet without vitamin C supplementation; C-VITC: Diet with vitamin C supplementation; The results described in the figure are represented as mean and standard error (SE). \*Significant by analysis of variance ( $P < 0.05$ ).

**Figure 3.** Effect of diet on color of raw and cooked *P. vannamei* fillet

Description: ‡S-VITC: Diet without vitamin C supplementation; C-VITC: Diet with vitamin C supplementation. The results described in the figure are represented as mean and standard error bar.

587 **Figure 4.** Evaluation of *P. vannamei* fillets subjected to sensory analysis



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590 Description: S-VITC: Diet without vitamin C supplementation; C-VITC: Diet with  
591 vitamin C supplementation; The results described in the figure are represented as mean  
592 and standard error (SE). \*Significant by analysis of variance ( $P < 0.05$ ).

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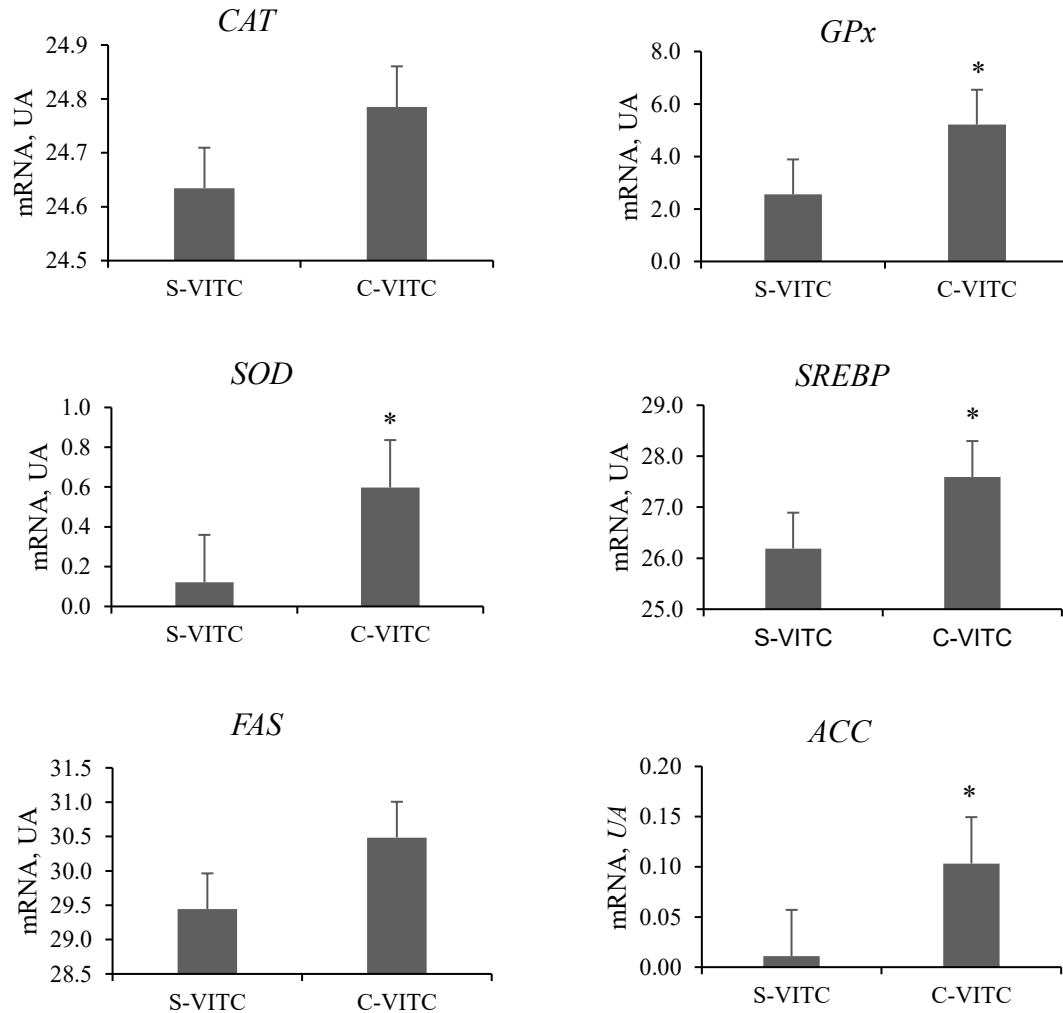
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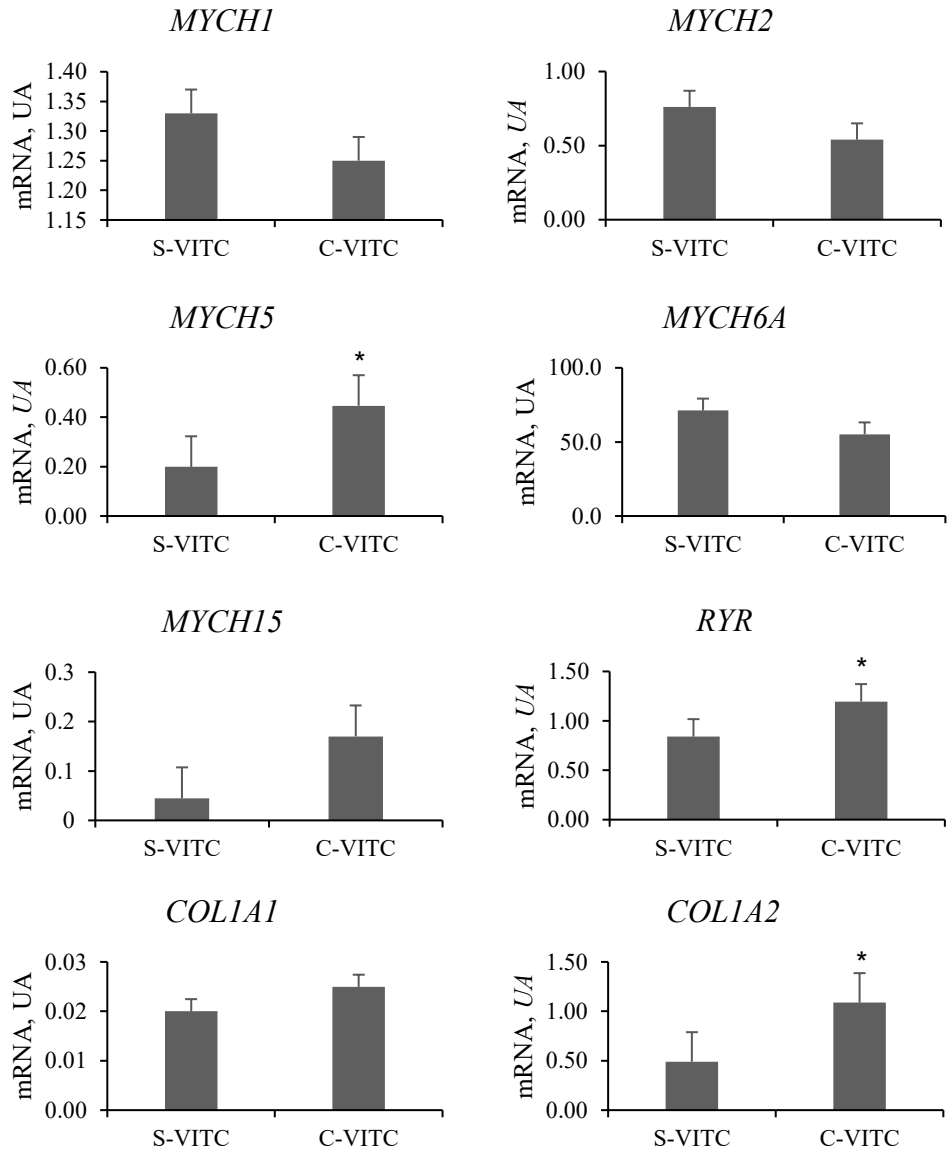
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**Figure 5.** Effect of diet on gene expression in the hepatopancreas of *P. vannamei*



Description: Gene expression of Catalase (*CAT*); Glutathione peroxidase (*GPx*); sterol regulatory element-binding protein (*SREBP*); Superoxide dismutase (*SOD*); Fatty acid synthase (*FAS*); Acetyl CoA carboxylase (*ACC*) in the hepatopancreas of juvenile Pacific white shrimp. S-VITC: Diet without vitamin C supplementation; C-VITC: Diet with vitamin C supplementation. The results described in the figure are represented as mean and standard error (SE). \*Significant by analysis of variance ( $P < 0.05$ ).

**Figure 6.** Gene expression evaluated in the muscle of *P. vannamei*



Description: Gene expression of myosin heavy chain 1 (*MYCH1*); myosin heavy chain 2 (*MYCH2*); myosin heavy chain 5 (*MYCH5*); myosin heavy chain 6A (*MYCH6A*); myosin heavy chain 15 (*MYCH15*); collagen alpha-1 type I (*COL1A1*); collagen alpha-2 type I (*COL1A2*); ryanodine receptor (*RYR*) in muscle of juvenile Pacific white shrimp. S-VITC: Diet without vitamin C supplementation; C-VITC: Diet with vitamin C

621 supplementation. The results described in the figure are represented as mean and standard  
622 error (SE). \*Significant by analysis of variance ( $P < 0.05$ ).

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