



## Beneficial Effects on Growth Performance of Brown Shrimp (*Penaeus aztecus*) Fed Dietary Inulin and Vitamin C

Dogukan KAYA<sup>1\*</sup>, Ercument GENC<sup>2</sup>

<sup>1</sup>Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Animal Science, Tokat

<sup>2</sup>Ankara University, Faculty of Agriculture, Department of Fisheries and Aquaculture Engineering, Ankara,

Corresponding author's email: [dogukan.kaya@gop.edu.tr](mailto:dogukan.kaya@gop.edu.tr)

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**Abstract:** This study evaluated the effects of different dietary doses of inulin, vitamin C and combinations on the growth performance of brown shrimp (*Penaeus aztecus*). The experiment was conducted over a period of 84 days using 27 tanks (20 juvenile shrimp/tank, initial weight: 0.85 g) with a total of 540 individuals. Nine dietary treatments were formulated: I0C0 (control, basal diet); I3C0 (0.3% inulin); I4C0 (0.4% inulin); I0C0.1 (0.1% vitamin C); I0C0.2 (0.2% vitamin C); I3C0.1 (0.3% inulin + 0.1% vitamin C); I4C0.1 (0.4% inulin + 0.1% vitamin C); I3C0.2 (0.3% inulin + 0.2% vitamin C); and I4C0.2 (0.4% inulin + 0.2% vitamin C). Shrimp fed with the I4C0.2 diet exhibited the highest growth performance, achieving the best values for final body weight, weight gain, specific growth rate, protein efficiency ratio, and feed conversion ratio. While the I4C0.2 group showed superior feed efficiency, intermediate improvements were observed in the I3C0.2, I4C0.1, and I0C0.2 groups, which also demonstrated significantly higher performance metrics compared to the control ( $P < 0.05$ ). These findings highlight the potential of inulin and vitamin C as effective feed additives for improving growth performance in brown shrimp, either individually or in combination. The remarkable synergistic effects observed in the I4C0.2 group suggest that this combination promotes better growth and feed efficiency. Future studies should explore the physiological and molecular mechanisms underlying these effects to optimize the application of inulin and vitamin C as functional feed additives.

**Keywords:** Prebiotic, dietary additives, shrimp, growth, sustainability

### İnülin ve C Vitamini ile Beslenen Kahverengi Karideslerin (*Penaeus aztecus*) Büyüme Performansı Üzerine Faydalı Etkiler

**Öz:** Bu çalışma, farklı diyet dozlarında inülin, C vitamini ve bunların kombinasyonlarının kahverengi karideslerin (*Penaeus aztecus*) büyüme performansı üzerindeki etkilerini değerlendirmiştir. Deneme, 84 gün boyunca toplam 540 birey ile 27 tankta (20 karides/tank, başlangıç ağırlığı: 0.85 g) yürütülmüştür. Dokuz diyet grubu formüle edilmiştir: I0C0 (kontrol, bazal diyet); I3C0 (%0,3 inülin); I4C0 (%0,4 inülin); I0C0.1 (%0,1 C vitamini); I0C0.2 (%0,2 C vitamini); I3C0.1 (%0,3 inülin + %0,1 C vitamini); I4C0.1 (%0,4 inülin + %0,1 C vitamini); I3C0.2 (%0,3 inülin + %0,2 C vitamini); ve I4C0.2 (%0,4 inülin + %0,2 C vitamini). I4C0.2 diyetiyle beslenen karidesler, final ağırlık, ağırlık kazancı, spesifik büyüme oranı, protein etkinlik oranı ve yem değerlendirme oranı açısından en yüksek büyüme performansını sergilemiştir. I4C0.2 grubu üstün yem etkinliği gösterirken, I3C0.2, I4C0.1 ve I0C0.2 gruplarında da orta düzeyde iyileşmeler gözlenmiş ve bu gruplar kontrol grubuna kıyasla anlamlı olarak daha yüksek performans değerleri sergilemiştir ( $P < 0,05$ ). Bu bulgular, inülin ve C vitamininin kahverengi karideslerde büyüme performansını iyileştiren etkili yem katkı maddeleri olarak potansiyelini vurgulamaktadır. I4C0.2 grubunda gözlenen diğkate değer sinerjik etkiler, bu kombinasyonun daha iyi büyüme ve yem etkinliğini desteklediğini göstermektedir. Gelecekteki çalışmalar, bu etkilerin altında yatan fizyolojik ve moleküler mekanizmaları araştırarak inülin ve C vitamininin işlevsel yem katkıları olarak uygulanmasını optimize etmeyi hedeflemektedir.

**Anahtar Kelimeler:** Prebiyotik, diyet katkı maddeleri, karides, büyüme, sürdürülebilirlik

#### 1. Introduction

Crustaceans, particularly penaeid shrimps, hold a significant position in the global aquaculture industry (Haris et al., 2024). Among these, Pacific white shrimp (*Penaeus vannamei*) leads global shrimp production, followed by tiger shrimp (*Penaeus monodon*). However, the dominance of a few species underscores the need for increasing species diversity in aquaculture to ensure food security and enhance resilience in global food

systems (FAO, 2024; Chan et al., 2024). Addressing this need has spurred research efforts to identify alternative penaeid species that hold promise for aquaculture development.

The brown shrimp (*Penaeus aztecus*), a penaeid species native to the Western Atlantic, has emerged as a viable candidate due to its high economic value and adaptability. Recent studies have investigated its potential in aquaculture systems, reporting encouraging

findings regarding its growth performance and market potential (Al-Badran et al., 2019; Uludağ & Aktaş, 2021; Durmuş & Aktaş, 2021; Genc et al., 2024a, 2024b). These studies form a basis for exploring how nutritional strategies could optimize the cultivation of brown shrimp and unlock its full potential.

Functional feed additives have gained attention for their role in enhancing productivity and sustainability in aquaculture (Karataş 2024; Kaya et al., 2024). Prebiotics, such as inulin, are known to selectively promote the growth of beneficial gut bacteria, improving nutrient absorption and supporting growth (Gibson et al., 2004; Rohani et al., 2022). Similarly, vitamin C plays a critical role in physiological processes, including growth promotion and maintaining homeostasis (Darias et al., 2011). Studies have consistently demonstrated that optimal dietary levels of vitamin C enhance growth metrics such as final body weight, weight gain, and specific growth rates in various aquatic species (Kong et al., 2021; Cai et al., 2022; Song et al., 2023).

Efforts have been accelerated to strengthen the concept of sustainability in the aquaculture sector. In this context, the use of environmentally friendly feed additives that meet the needs of aquatic animals, prioritize welfare, and support reliable and responsible production is emphasized. Feed additives under investigation are expected to align with the United Nations Sustainable Development Goals (SDGs), which represent a holistic approach to addressing urgent challenges and achieving measurable progress by 2030. The introduction of functional, animal-compatible growth promoters into the feed industry has the potential to help the aquaculture sector minimize waste, improve health and welfare, ensure clean water and sanitation, and mitigate the effects of climate change—all while preventing harm to aquatic life and the environment. These contributions directly support SDGs such as Responsible Consumption and Production. Additionally, this approach could foster partnerships for global and local goals, increasing the likelihood of advancing Decent Work and Economic Growth (United Nations, 2015).

This study is the first application of an alternative shrimp species, *P. aztecus*, for culture, and therefore focuses primarily on growth parameters. In this context, we focus on the potential synergy between inulin and vitamin C, two well-documented feed additives, when combined in basal diet. Although their individual benefits are well-established, limited research has explored their combined effects at varying doses. By

investigating these synergistic interactions, this study aims to bridge a critical knowledge gap and provide insights into optimizing the growth performance of brown shrimp.

## 2. Material and methods

### 2.1. Basal and supplemented diets

A basal diet and experimental diets supplemented different doses of inulin (Orafti®) and vitamin C (Aromel®) to the basal diet were prepared separately and in combinations. The diets were named as follows: IOC0 (basal diet); I3C0 (basal diet + 0.3% Inulin); I4C0 (basal diet + 0.4% Inulin); IOC0.1 (basal diet + 0.1% vitamin C); IOC0.2 (basal diet + 0.2% vitamin C); I3C0.1 (basal diet + 0.3% Inulin + 0.1% vitamin C); I4C0.1 (basal diet + 0.4% Inulin + 0.1% vitamin C); I3C0.2; (basal diet + 0.3% Inulin + 0.2% vitamin C) and I4C0.2 (basal diet + 0.4% Inulin + 0.2% vitamin C). The ingredients and proximate composition of the basal diet are presented in Table 1.

**Table 1.** The composition and proximate analysis of the basal diet

#### *Çizelge 1. Bazal diyetin bileşimi ve besin madde kompozisyonu*

Ingredients	Basal diet (%)
Fish meal	24
Soy protein concentrate	2
Soybean meal	8
Collagen	1
Corn gluten	7
Pea protein concentrate	2
Wheat flour	47.1
Fish oil	3.7
Soy oil	3.7
Vitamins mineral premix	1
Vitamin C	0.1
Cholesterol	0.4
Total (~)	100
Proximate composition (%)*	
Crude protein	35.33±0.10
Crude fat	10.63±0.23
Ash	5.89±0.19
Crude cellulose	1.57±0.06
Moisture	10.82±0.41

### 2.2. Experimental culture conditions

The study was conducted in the Ankara University fisheries unit using 27 fiberglass tanks with a water volume of 45 L each. Shrimps were obtained from Iskenderun Technical University and transported to Ankara in plastic tanks (200 L) under aerated conditions. After transportation, the shrimps were fed with the basal diet for 15 days to acclimate to the experimental conditions. Five hundred and forty juvenile shrimp were randomly distributed in triplicates

to the experimental tanks (20 individuals each). The shrimp were fed at 08.00, 13.00, and 18.00 h every day during the experiment. The feeding regime was 6% of body weight for days 1-14, 5.4% for days 15-28, 4.8% for days 29-42, 4.2% for days 43-56, 3.6% for days 57-70 and 3% for days 71-84. During the trial, water temperature, salinity, pH and dissolved oxygen indices

(YSI® 556, YSI Inc., Yellow Springs, OH, USA) were measured daily at  $28.60 \pm 0.24^\circ\text{C}$ ,  $34 \pm 1$  ppt,  $7.99 \pm 0.10$ ,  $6.49 \pm 0.24$  mg/L, respectively.

### 2.3. Growth parameters

At the end of the trial, the Equations 1-5 were used to calculate the growth indices of the shrimps.

$$\text{Weight gain (WG; g/shrimp)} = \text{Final weight (g)} - \text{Initial weight (g)} \quad (1)$$

$$\text{Specific growth rate (SGR; \% / day)} = ((\ln(\text{final weight}) - \ln(\text{initial weight})) / \text{days}) \times 100 \quad (2)$$

$$\text{Protein efficiency ratio (PER)} = \text{Weight gain (g)} / \text{Protein intake (g)} \quad (3)$$

$$\text{Feed conversion ratio (FCR; \%)} = \text{Feed intake (g)} / \text{Weight gain (g)} \quad (4)$$

$$\text{Survival rate (SR; \%)} = (\text{Shrimp final number} - \text{shrimp initial number}) \times 100 \quad (5)$$

### 2.4. Statistics

The data sets were analyzed using the SPSS 27 statistical software (Chicago, IL, USA) for parametric analyses. Before performing parametric tests such as analysis of variance and Tukey tests, assumptions were evaluated. Normality assumptions were checked by examining skewness and kurtosis statistics for all groups, and the homogeneity of variances was assessed using the Levene test. The data were reported as mean  $\pm$  standard deviation. A P-value of less than 0.05 was considered statistically significant.

### 3. Results

Over an 84-day period, the growth performance of brown shrimp subjected to dietary treatments revealed statistically significant differences between groups (Tukey test, Table 2). Each zootechnical parameter demonstrated varying effects of dietary inulin and vitamin C supplementation.

For final body weight (FBW), the I4C0.2 group achieved the highest value ( $9.59 \pm 0.20$  g), significantly outperforming all other groups ( $p < 0.05$ ). This was followed by the I3C0.2 ( $9.27 \pm 0.03$  g) and I4C0.1 ( $8.97 \pm 0.04$  g) groups, which also exhibited significantly higher FBW compared to the control group ( $7.78 \pm 0.01$  g). Intermediate improvements were observed in the I4C0 ( $8.50 \pm 0.09$  g) and I0C0.2 ( $8.70 \pm 0.18$  g) groups, which formed a statistically similar subset. The lowest FBW was recorded in the control group, representing shrimp fed a basal diet without supplementation.

Weight gain (WG) followed a similar pattern, with the I4C0.2 group achieving the highest WG ( $8.74 \pm 0.20$  g), significantly exceeding all other groups ( $p < 0.05$ ). This was followed by the I3C0.2 ( $8.42 \pm 0.03$  g) and I4C0.1 ( $8.13 \pm 0.05$  g) groups, both of which showed substantial gains compared to the control group

( $6.94 \pm 0.01$  g). Intermediate WG values were observed in the I4C0 ( $7.66 \pm 0.09$  g) and I0C0.2 ( $7.85 \pm 0.18$  g) groups, which formed another distinct subset. The control group exhibited the lowest WG.

Specific growth rate (SGR) was highest in the I4C0.2 group ( $2.88 \pm 0.02$ ), which significantly surpassed all other groups ( $p < 0.05$ ). The I3C0.2 ( $2.84 \pm 0.01$ ) and I4C0.1 ( $2.82 \pm 0.01$ ) groups also demonstrated significantly higher SGR values compared to the control group ( $2.64 \pm 0.01$ ). Moderate but statistically significant increases in SGR were recorded in the I4C0 ( $2.75 \pm 0.02$ ) and I0C0.2 ( $2.77 \pm 0.03$ ) groups. The control group exhibited the lowest SGR.

Protein efficiency ratio (PER) was highest in the I4C0.2 group ( $1.80 \pm 0.04$ ), significantly exceeding all other groups ( $p < 0.05$ ). High PER values were also observed in the I3C0.2 ( $1.73 \pm 0.01$ ) and I4C0.1 ( $1.68 \pm 0.01$ ) groups, forming a statistically distinct subset compared to the control group ( $1.45 \pm 0.00$ ). Intermediate values were recorded in the I4C0 ( $1.58 \pm 0.02$ ) and I0C0.2 ( $1.62 \pm 0.04$ ) groups, which were statistically similar but significantly higher than the control. The control group exhibited the lowest PER.

The feed conversion ratio (FCR) was most efficient in the I4C0.2 group ( $1.59 \pm 0.04$ ), indicating optimal feed utilization. This value was significantly better than all other groups ( $p < 0.05$ ). The I3C0.2 ( $1.65 \pm 0.01$ ) and I4C0.1 ( $1.70 \pm 0.01$ ) groups also demonstrated improved FCR values, outperforming the control group ( $1.97 \pm 0.00$ ). Intermediate improvements were observed in the I4C0 ( $1.81 \pm 0.02$ ) and I0C0.2 ( $1.77 \pm 0.04$ ) groups. The control group exhibited the least efficient FCR, highlighting the absence of dietary supplementation.

In terms of survival rate (SR) did not exhibit significant differences between groups ( $p > 0.05$ ). The highest SR values ( $73.33 \pm 7.64\%$  to  $73.33 \pm 12.58\%$ )

were observed in the I3C0.2, I4C0.1, I3C0.1, and I0C0.2 groups. The I4C0.2 group (73.33±10.41%) showed slightly lower SR values but remained statistically similar to these groups. The control group recorded the

lowest SR (65.00±5.00%), suggesting that dietary treatments may have marginally improved resilience or health conditions, although these effects were not statistically significant.

**Table 2.** Growth parameters of brown shrimp grown in tanks with different feed additive groups for 84 days

**Çizelge 2.** Farklı yem katkı gruplarında 84 gün boyunca tanklarda yetiştirilen kahverengi karideslerin büyüme parametreleri

P/G*	I0C0	I3C0	I4C0	I0C0.1	I0C0.2	I3C0.1	I4C0.1	I3C0.2	I4C0.2
<b>IBW</b>	0.85±0.01	0.85±0.01	0.84±0.02	0.85±0.01	0.86±0.02	0.85±0.02	0.85±0.02	0.85±0.01	0.85±0.02
<b>FBW</b>	7.78±0.01 <sup>a</sup>	7.89±0.07 <sup>a</sup>	8.50±0.09 <sup>b</sup>	7.80±0.02 <sup>a</sup>	8.70±0.18 <sup>bc</sup>	8.63±0.10 <sup>b</sup>	8.97±0.04 <sup>c</sup>	9.27±0.03 <sup>d</sup>	9.59±0.20 <sup>e</sup>
<b>WG</b>	6.94±0.01 <sup>a</sup>	7.04±0.06 <sup>a</sup>	7.66±0.09 <sup>b</sup>	6.95±0.03 <sup>a</sup>	7.85±0.18 <sup>bc</sup>	7.78±0.10 <sup>b</sup>	8.13±0.05 <sup>cd</sup>	8.42±0.03 <sup>d</sup>	8.74±0.20 <sup>e</sup>
<b>SGR</b>	2.64±0.01 <sup>a</sup>	2.65±0.01 <sup>a</sup>	2.75±0.02 <sup>b</sup>	2.65±0.01 <sup>a</sup>	2.77±0.03 <sup>bc</sup>	2.77±0.02 <sup>b</sup>	2.82±0.01 <sup>cd</sup>	2.84±0.01 <sup>de</sup>	2.88±0.02 <sup>e</sup>
<b>PER</b>	1.45±0.00 <sup>a</sup>	1.47±0.01 <sup>a</sup>	1.58±0.02 <sup>b</sup>	1.45±0.01 <sup>a</sup>	1.62±0.04 <sup>bc</sup>	1.61±0.02 <sup>b</sup>	1.68±0.01 <sup>cd</sup>	1.73±0.01 <sup>d</sup>	1.80±0.04 <sup>e</sup>
<b>FCR</b>	1.97±0.00 <sup>e</sup>	1.94±0.02 <sup>e</sup>	1.81±0.02 <sup>d</sup>	1.97±0.01 <sup>e</sup>	1.77±0.04 <sup>cd</sup>	1.78±0.02 <sup>d</sup>	1.70±0.01 <sup>bc</sup>	1.65±0.01 <sup>ab</sup>	1.59±0.04 <sup>a</sup>
<b>SR</b>	65.00±5.00	66.67±7.64	68.33±10.41	70.00±5.00	71.67±12.58	73.33±7.64	73.33±7.64	73.33±12.58	73.33±10.41

\***Parameters and groups:** I0C0 (baal diet); I3C0 (basal diet + 0.3% Inulin); I4C0 (basal diet + 0.4% Inulin); I0C0.1 (basal diet + 0.1% vitamin C); I0C0.2 (basal diet + 0.2% vitamin C); I3C0.1 (basal diet + 0.3% Inulin + 0.1% vitamin C); I4C0.1 (basal diet + 0.4% Inulin + 0.1% vitamin C); I3C0.2; (basal diet + 0.3% Inulin + 0.2% vitamin C) and I4C0.2 (basal diet + 0.4% Inulin + 0.2% vitamin C). **IBW**, initial body weight (g); **FBW**, final body weight (g); **WG**, weight gain (g); **SGR**, specific growth rate (%/day); **PER**, protein efficiency ratio; **FCR**, feed conversion ratio; **SR**, survival rate (%). The data correspond to the mean ± standard deviation. Means within the same row with different superscript letters indicate significant differences at the level of  $P < 0.05$ . No significant differences were observed among groups for IW and SR ( $P > 0.05$ ).

#### 4. Discussion

Dietary prebiotics have been extensively studied as growth promoters in aquatic organisms, including shrimp (Wee et al., 2024). For example, dietary supplementation with 0.2–0.4% inulin significantly improved the growth performance of *Penaeus vannamei* after an 8-week feeding trial (Zhou et al., 2020). Similarly, in a 56-day trial, dietary inulin at doses of 4 and 8 mg g<sup>-1</sup> enhanced growth parameters in *P. vannamei* (Li et al., 2021). Beyond prebiotics like inulin, dietary vitamin C has also been widely recognized for its ability to promote growth in aquatic species such as red swamp crayfish (*Procambarus clarkii*) (Kong et al., 2021) and Pacific white shrimp (*P. vannamei*) (Niu et al., 2009). These studies highlight the potential of functional feed additives to improve aquaculture productivity. Consistent with these findings, the present study demonstrated that dietary supplementation with inulin or vitamin C significantly enhanced growth performance in brown shrimp. The most remarkable finding, however, was that the combination of these supplements provided superior results compared to their individual use. Shrimp fed diets supplemented with 0.4% inulin and 0.2% vitamin C (I4C0.2) exhibited significantly higher growth performance, feed efficiency, and survival rates compared to the control group. Other groups, including I4C0, I3C0.1, I3C0.2, I4C0.1, and I0C0.2, also showed notable improvements, highlighting the versatility of these dietary supplements, particularly when combined or administered at optimal doses.

The superior growth indices observed in the I4C0.2 group likely stem from the synergistic effects of inulin and vitamin C in enhancing nutrient absorption and utilization. This combination appears to promote better growth rates and feed efficiency, a finding consistent with prior studies suggesting that combining functional feed additives can yield synergistic benefits by positively influencing gut microbiota and improving overall health conditions (Yamamoto et al., 2020; Li et al., 2021). As a prebiotic, inulin supports the proliferation of beneficial gut microbiota, improving digestive efficiency and general health (Escamilla-Montes et al., 2021; Wang et al., 2024). Vitamin C, meanwhile, contributes to growth by supporting metabolic processes, immune function, and stress resilience (Ibrahim et al., 2010).

The enhanced specific growth rate (SGR) and protein efficiency ratio (PER) observed in the I4C0.2 group further emphasize the advantages of combining inulin and vitamin C. This combination not only promotes better growth but also facilitates more efficient protein utilization, which is critical for reducing production costs in penaeid aquaculture. Additionally, the low feed conversion ratio (FCR) in the I4C0.2 group supports the hypothesis that this combination improves the shrimp's capacity to convert feed into body mass, thereby optimizing production performance. The improved survival rate (SR) in the I4C0.2 group suggests a potential for enhanced resilience against environmental stress or disease, possibly by maintaining gut health and immune balance.

However, this hypothesis warrants further investigation through detailed studies.

These present study findings underscore the potential of dietary inulin and vitamin C as effective feed additives for enhancing growth in brown shrimp, either individually or in combination. The synergistic effects observed in the I4C0.2 group are particularly noteworthy and highlight the value of integrating these functional additives into aquaculture feeds. Future research should focus on elucidating the physiological and molecular mechanisms underlying these benefits to optimize their application as functional feed additives.

This study, conducted on brown shrimp (*P. aztecus*), a species tested for its performance as a candidate for aquaculture production, demonstrates its alignment with broader topics supporting sustainable aquaculture. Perhaps most importantly, the adoption of these functional feed additives is consistent with global sustainability initiatives. The United Nations' Sustainable Development Goals (SDGs) emphasize the importance of addressing food security, environmental sustainability, and responsible production systems (United Nations, 2015). By improving feed efficiency, growth performance, and resilience in aquatic species, these strategies directly support SDGs such as Zero Hunger (Goal 2), Good Health and Well-being (Goal 3), Life Below Water (Goal 14), and Responsible Consumption and Production (Goal 12). Additionally, FAO's Blue Transformation roadmap underscores the need for sustainable aquaculture practices to meet the growing global demand for aquatic products while maintaining ecological integrity (FAO, 2022). The findings of the present study, which demonstrate improvements in feed efficiency and survival rates in shrimp farming, can be considered consistent with these goals, contributing to sustainable aquaculture practices. Furthermore, the European Green Deal promotes carbon-neutral production practices and sustainable food systems to combat climate change and environmental degradation (European Commission, 2019). The preliminary data from this study suggest that the use of environmentally friendly feed additives can enhance resource efficiency while supporting the reduction of the environmental footprint of aquaculture systems.

## 5. Conclusion

In conclusion, based on the results of this study, the combined supplementation of inulin and vitamin C is recommended for penaeid shrimp farming. These findings align with international goals that prioritize

improving global welfare, encouraging the development of sustainable aquaculture systems that are efficient, resilient, and environmentally responsible through the use of functional feed additives.

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