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Research Article

Nutritional evaluation of plant-based diets containing corn and soy protein sources on growth performance and survival rate of *Litopenaeus vannamei*

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Abstract

The aquaculture industry faces increasing challenges in developing sustainable and cost-effective feeding strategies. Plant-based protein sources, such as soybean meal and corn gluten, present promising alternatives to traditional fishmeal in shrimp diets. This study aimed to investigate the effects of varying levels of plant protein substitution on the growth performance, survival rate, and economic feasibility of Pacific white shrimp (*Litopenaeus vannamei*), while also determining the optimal level of plant protein replacement in shrimp feed. Six experimental diets were formulated with plant protein replacement levels of 0% (T0), 20% (T20), 40% (T40), 60% (T60), 80% (T80), and 100% (T100) relative to fishmeal. A 60-day culture period was implemented to evaluate the impact of these dietary treatments on growth indices, survival rates, and nutritional parameters. Additionally, cost analyses were conducted for each treatment group. The results indicated that growth indices, including weight gain and specific growth rate, did not significantly differ from the control group at replacement levels up to 60% ($p\leq0.05$). However, substituting plant protein at levels of 80% and 100% resulted in significant negative effects on growth performance ($p\leq0.05$). Survival rates remained stable across all dietary treatments throughout the culture period ($p>0.05$). The nutritional indices, such as feed conversion ratio and protein efficiency ratio, were significantly higher in the T0 and T60 groups compared to those with greater plant protein inclusion ($p\leq0.05$). This study concludes that partial substitution of fishmeal with plant proteins (up to 60%) is both nutritionally effective and economically advantageous. However, complete replacement at 80% and 100% levels significantly impairs growth performance in *L. vannamei*. The consistent survival rates across all dietary treatments suggest that the substitution of the plant-based proteins does not compromise shrimp health.

Introduction

The aquaculture industry has rapidly expanded, becoming a crucial sector for global food security and protein production over recent decades (Yeganeh *et al*., 2020). This expansion has not only enhanced food availability but has also contributed significantly to economic development, job creation, and poverty alleviation (Ayisi *et al*., 2017). However, the sustainability of this growth is increasingly threatened by the overexploitation of aquatic resources, intensifying seafood demand, and the adverse effects of overfishing (Borgeson *et al*., 2006; Sharifinia *et al*., 2023a). Consequently, there is an urgent need for innovative and sustainable feeding strategies that can meet the rising production demands without further depleting marine ecosystems.

Among the various species cultivated in aquaculture, the Pacific white shrimp (*Litopenaeus vannamei*) has emerged as a dominant player, accounting for approximately 53% of all farmed crustaceans in 2018. The production of this species has surged from 2.64 million tons in 2010 to 4.96 million tons in 2018 (FAO, 2020), solidifying its status as the most significant species in global shrimp farming. This remarkable growth can be attributed to the species' adaptability to high-density farming conditions, disease tolerance, and relatively low protein requirements (NRC, 2011). Nevertheless, the sustainability of shrimp farming practices faces challenges, particularly in light of rising feed costs and the pressing need for environmentally responsible production methods.

Historically, fishmeal has been the primary protein source in aquaculture feeds, prized for its superior nutritional profile, which includes essential amino acids, fatty acids, vitamins, and minerals (Davis and Arnold, 2000; Sharifinia *et al*., 2023b). However, the production of fishmeal has experienced a significant decline over the past decade (Kobayashi *et al*., 2015). This trend poses substantial challenges for the aquaculture industry, particularly regarding the rising costs of feed, which constitute the largest expense in high-density aquaculture systems (Ahmad *et al*., 2021; Verdegem *et al*., 2023). As protein is a critical component of aquaculture diets, its cost directly impacts the overall economic viability of shrimp farming.

In response to the urgent need for sustainable aquaculture practices, researchers have increasingly focused on alternative protein sources to replace fishmeal (Sharifinia, 2024). Plant proteins, such as soybean meal and corn gluten, have emerged as viable candidates due to their availability and potential cost-effectiveness. However, the nutritional limitations of plant proteins, including deficiencies in essential amino acids and the presence of anti-nutritional factors, pose challenges for their efficient incorporation into shrimp diets (Chen *et al*., 2024; Olaleru *et al*., 2024).

Extensive research has evaluated the feasibility of replacing fishmeal with various plant protein sources. For instance, Oujifard *et al.* (2012) demonstrated that rice protein could replace over 50% of fishmeal in the diets of *L. vannamei* without adversely affecting survival rates, while improving the digestibility of crude protein, fat, and energy. Similarly, Ghorbani Vaghei *et al.* (2017) found that replacing 70% of fishmeal with soybean meal reduced feed costs by more than 25% without significantly impacting shrimp production efficiency. Other studies, including those by Bae *et al.* (2020), indicated that alternative diets incorporating soybean meal, fermented soy, and sesame meal did not yield significant differences in growth indicators compared to control diets, highlighting the potential of these plant-based sources in shrimp nutrition.

Moreover, Guo *et al.* (2020) explored the combination of soy protein concentrate and corn in shrimp diets, finding that plant protein concentrates could effectively substitute fishmeal under various farming conditions. Ray *et al.* (2020) further emphasized the benefits of soy protein concentrate in enhancing growth, immunity, and disease resistance in *L. vannamei*. Additionally, Yu *et al.* (2013) demonstrated that incorporating corn protein concentrate into shrimp diets did not adversely affect growth performance or feed conversion ratios (FCR), supporting its use as a viable alternative to fishmeal. Collectively, these studies highlight the potential of plant-based diets in promoting sustainable aquaculture practices while addressing the economic challenges posed by rising fishmeal costs. Therefore, this research aims to systematically evaluate the effect of plant-based diets containing corn and soy protein sources on the growth performance and survival rate of *L. vannamei*. By investigating the effectiveness of these alternative protein sources, this study seeks to contribute to the development of sustainable aquaculture practices that reduce reliance on fishmeal, lower production costs, and promote environmental sustainability and biodiversity conservation.

Materials and methods

Shrimp and experimental conditions

Juvenile *L. vannamei* shrimps were sourced from the wintering ponds of the spawning project at the Shoghab Station. Prior to the commencement of the experiment, the shrimp were acclimatized for one week in 500-liter tanks within the breeding hall of the Persian Gulf Research Station, affiliated with the Shrimp Research Institute of Iran, located in Bushehr Province. This acclimatization period was implemented to minimize stress and ensure the shrimp adapted to the new environment. At the beginning of the experiment, a total of 540 shrimp with an average weight of 6±0.2 grams were randomly distributed into 18 cylindrical tanks, each with a capacity of 300 liters, resulting in a stocking density of 30 shrimp per tank. Each tank was filled with sterilized seawater, and aeration was provided using air blowers to maintain optimal dissolved oxygen levels.

The feeding regimen consisted of providing the shrimp with four meals daily at 08:00, 13:00, 18:00, and 00:00. The amounts of feed were adjusted based on the shrimp's appetite and satiety levels. Salinity was maintained at 41±1 parts per thousand, while the water temperature was kept at 28±2 degrees Celsius throughout the experimental period. Uneaten feed and feces were siphoned from the tanks daily, and 20% of the water volume was replaced with fresh seawater to ensure optimal water quality. Daily mortality rates were recorded, and biometric measurements were conducted every ten days to monitor the growth and health of the shrimp.

Throughout the 60-day experimental period, the physicochemical parameters of the water, including temperature, dissolved oxygen, salinity, and pH, were monitored daily using calibrated instruments: a thermometer (Tefa, Germany), an oxygen meter (WTW OXI 3205, Germany), an optical salinity meter (ATAGO S/Mill, Japan), and a pH meter (Inolab WTW, Germany). These measurements ensured that the environmental conditions remained suitable for the growth and survival of the shrimp.

Preparation of experimental diets

The dietary formulations utilized in this study were meticulously developed by Beyza Fars Company by employing Lindo's linear feed formulation software. The experimental diets comprised six distinct treatments, each replicated three times, featuring varying levels of fishmeal replacement with plant protein sources, specifically soybean and corn protein. The treatments included a control diet that consisted of 25% fishmeal and 43% soy protein, along with five experimental diets where fishmeal was substituted with plant protein at replacement levels of 0 (control; T0) 20% (T20), 40% (T40), 60% (T60), 80% (T80), and 100% (T100) (as detailed in Table 1). These formulations were carefully crafted to ensure that all diets fulfilled the nutritional requirements of the shrimp, as specified by the National Research Council (NRC, 2011).

Table 1: Formulation and proximate analysis of the experimental diets for juvenile *Litopenaeus vannamei*.

Ingredients	Experimental diets					
	T0	**T20**	**T40**	**T60**	**T80**	**T100**
Fishmeal	25	20	15	10	5	0
Soybean Meal	41.3	43.3	45.3	47.3	50.1	52.5
Corn Gluten	0	4	6	8	10	12
Whole Wheat	24	24	23.8	23.8	24	24
Fish Oil	4.4	4.4	4.6	5	5	5.6
Soy Lecithin	2	2	2	2	2	2
Mineral Premix*	1	1	1	1	1	1
Vitamin Premix**	1	1	1	1	1	1
Binder	1	1	1	1	1	1
Choline Chloride	0.2	0.2	0.2	0.2	0.2	0.2
Stay C 35%	0.1	0.1	0.1	0.1	0.1	0.1
Lysine	0	0	0	0.5	0.5	0.5
Methionine	0	0	0	0.1	0.1	0.1
Total	100	100	100	100	100	100
Proximate composition						
Moisture	8.69±0.14	8.57±0.16	8.62±0.11	8.21±0.16	8.04±0.13	7.86±0.10
Protein	37.54±0.34	37.65±0.28	35.52±0.25	35.39±0.31	36.61±0.19	33.53±0.23
Fat	8.36±0.11	8.04±0.14	7.91±0.10	7.94±0.08	7.61±0.13	7.03±0.07
Ash	8.45±0.21	8.06±0.17	7.41±0.14	7.30±0.23	6.76±0.19	6.06±0.22

*The composition of the mineral premix (Kimia Ligo) per liter includes: Phosphorus (140,000 mg), Calcium (20,000 mg), Magnesium (10,000 mg), Sodium (45,000 mg), Zinc (2,500 mg), Manganese (2,500 mg), Iron (2,500 mg), Copper (500 mg), Cobalt (50 mg), Selenium (20 mg).

** The vitamin premix (milligrams per kilogram of diet) includes: 32 mg of Vitamin A; 10 mg of Vitamin K3; 45 mg of Riboflavin; 20 mg of Pyridoxine Hydrochloride; 25 mg of Thiamine; 0.1 mg of Vitamin B12; 20 mg of Folic Acid; 1.2 mg of Biotin; 120 mg of Vitamin E; 800 mg of Inositol; 60 mg of Calcium Pantothenate; 200 mg of Nicotinic Acid; and 5 mg of Vitamin D.

Calculations

Shrimp growth parameters were measured every ten days using a digital scale with an accuracy of 0.01 g. The growth indicators assessed included final weight, body weight gain (WG), weight gain percentage, specific growth rate (SGR), and food conversion ratio (FCR), which were calculated using the following formulas (Sharifinia *et al*., 2023b):

$$\mathrm{WG\ (g)} = \mathrm{Final\ weight} - \mathrm{Initial\ weight}$$

$$SGR(\%) = \left[{lnW_f - lnW_i}/{t}\right] \times 100$$

$$\mathrm{FCR} = {\mathrm{Feed\ given\ (g)}}/{\mathrm{Alive\ weigh\ gain\ (g)}}$$

$$\mathrm{SR\ (\%)} = \left[{\mathrm{Final\ number\ of\ shrimp}}/{\mathrm{Initial\ number\ of\ shrimp}}\right] \times 100$$

Where, W_f is the final weight, W_i is the initial weight, and t is the duration of the experiment in days.

Statistical analysis

The results are expressed as means ± standard error of the mean (SEM). Statistical analyses were performed using SPSS version 21.0 (SPSS, Chicago, IL, USA). Initially, the data were assessed for homogeneity of variances. If the variances were found to be similar, a one-way analysis of variance (ANOVA) was conducted to evaluate the primary effects of the dietary treatments. In cases where significant differences were identified (p<0.05), post-hoc comparisons of group means were carried out using Duncan's multiple range test. Conversely, if the data exhibited non-homogeneous variances, the non-parametric Kruskal-Wallis test was utilized. Should the Kruskal-Wallis test indicate significant differences (p<0.05), all pairwise multiple comparisons were performed to further explore the differences between groups.

Results

Growth performance

The evaluation of growth performance indices indicated significant differences in average WG (g), SGR (%), and SR (%) among the various dietary treatments (Fig. 1). Notably, the T20, T40 and T60 exhibited statistically significant improvements (p≤0.05) in these growth metrics compared to the control group. In contrast, the T80 and T100 treatments resulted in significantly lower average WG and SGR (p≤0.05) when compared to both the control group and the other experimental groups. Importantly, the survival percentage did not reveal any statistically significant differences among the treatment groups in comparison to the control group (p>0.05). The FCR was significantly lower in the T20 replacement group compared to the control and other treatment groups (p≤0.05). In contrast, the FCR values for the T80 and T100 groups were significantly higher than those observed in the control and other experimental groups (p≤ 0.05).

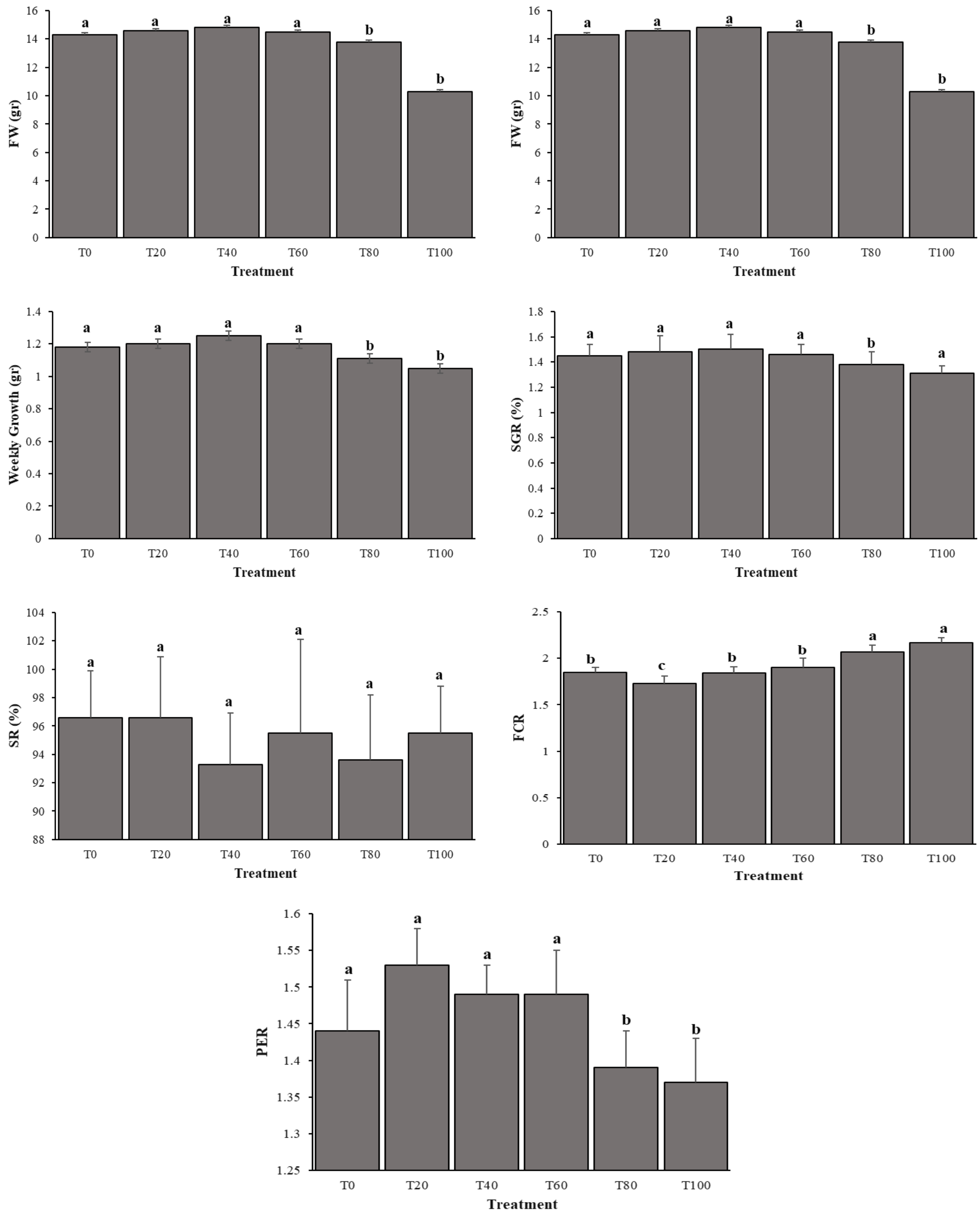

Figure 1: Growth performance and feed efficiency of *Litopenaeus vannamei* fed with various dietary regimens. T0, T20, T40, T60, T80, T100.

Discussion

The results of this study provide critical insights into the implications of substituting fishmeal with plant protein sources in the diet of *L. vannamei*. The significant enhancements observed in growth performance metrics, specifically average WG and SGR, at moderate levels of fishmeal replacement (20%, 40%, and 60%) highlight the viability of incorporating plant proteins into aquaculture diets. These findings are consistent with recent studies that have reported positive growth responses in shrimp when plant protein sources are utilized at appropriate inclusion levels (Zhou *et al*., 2020; Yang *et al*., 2021). The observed growth performance at these moderate substitution levels can be attributed to the adequate provision of essential amino acids and other nutrients that support metabolic functions and growth performance (Naylor *et al*., 2021). However, the notable decline in growth performance at higher replacement levels (80% and 100%) highlights the potential limitations associated with excessive plant protein incorporation, particularly concerning the presence of anti-nutritional factors. Research indicates that high levels of plant protein can lead to nutrient imbalances, particularly in essential amino acids such as methionine and lysine, which are critical for optimal growth (Gatlin III *et al*., 2007; Guo *et al*., 2020). Moreover, the digestibility of certain plant proteins can be lower than that of fishmeal, further exacerbating growth issues when fishmeal is completely replaced (Kumar *et al*., 2020). This suggests that while plant proteins can serve as viable alternatives, careful formulation is essential to ensure that all nutritional requirements are met.

The lack of statistically significant differences in survival percentages across the treatment groups indicates that while growth performance may be influenced by dietary composition, the overall resilience and adaptability of Pacific white shrimp to varying diets remain robust. This observation aligns with the findings Tacon and Metian (2015), who suggested that shrimp can thrive on a range of dietary formulations, provided that essential nutrients are adequately supplied. The ability of shrimp to adapt to diverse diets is critical for the sustainability of aquaculture practices, particularly as the industry seeks to reduce reliance on fishmeal due to environmental and economic concerns.

The analysis of nutritional indices revealed that the FCR was significantly lower in the 20% replacement group, indicating enhanced feed utilization efficiency. Conversely, the significantly higher FCR values observed in the T80 and T100 treatments suggest that excessive substitution of fishmeal with plant proteins may hinder nutrient absorption and overall feed efficiency. This phenomenon can be attributed to the presence of anti-nutritional factors such as oligosaccharides, phytates, and trypsin inhibitors found in soybean meal and other plant proteins, which can adversely affect digestibility and nutrient bioavailability (Gatlin III *et al*., 2007; Guo *et al*., 2020). Recent research by Li *et al*. (2021) further supports this notion, indicating that the presence of these anti-nutritional factors can lead to reduced growth performance and feed efficiency in shrimp. Additionally, the processing methods employed for plant proteins, such as fermentation or enzymatic treatment, have been shown to reduce the levels of these anti-nutritional factors, thereby improving the overall nutritional profile of the feed. The results of this study indicate that while complete replacement of fishmeal with plant proteins is feasible, particularly with soybean meal and corn gluten, it is essential to maintain a careful balance to avoid negative impacts on growth performance and feed efficiency.

The observed decline in growth metrics at high replacement levels suggests the existence of a threshold beyond which the benefits of cost reduction may be outweighed by diminished growth performance. Therefore, it is recommended that future research explore the utilization of processed plant protein products, such as fermented soybean meal, protein concentrates, and enzyme-treated plant meals, to mitigate the anti-nutritional effects associated with raw plant ingredients (Gyan *et al*., 2019; Guo *et al*., 2020).

Conclusion

This study highlights the potential for incorporating plant protein sources into the diets of Pacific white shrimp, demonstrating that moderate levels of substitution can enhance growth performance while simultaneously reducing feed costs. However, careful consideration must be given to the levels of substitution to ensure optimal growth and feed efficiency. Future investigations should focus on the health implications of these dietary changes, the potential benefits of utilizing processed plant protein products, and the long-term sustainability of aquaculture practices. Continued research in this area is essential to develop nutritionally balanced, economically viable, and environmentally sustainable feed formulations that can support the growing demands of the aquaculture industry.

Conflicts of interest

The authors have declared no conflict of interest.

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