

Growth performance and nutritional impact of *Moringa oleifera* leaf extract as supplemental diets in the juvenile pacific white shrimp (*Penaeus vannamei*)

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Abstract. Moringa (*Moringa oleifera*) is widely recognized for its potent medicinal properties and immunostimulant effects. This study aimed to investigate the impact of a hot water extract of moringa leaves on the growth performance, feed conversion ratio, and survival of pacific white shrimp, *Penaeus vannamei*. Four different diets were used in this experiment, each containing varying levels of moringa leaf extract: 0, 2.5, 5, and 7.5 g/kg of the diet. A total of 1,200 shrimp, with an initial body weight of 0.01 g/individual, were carefully selected to ensure they were free from *Vibrio* infestation. One hundred shrimp were placed in each circular tank, with a volume of 750 L or 133 individuals per cubic meter. The shrimp were fed the diets four times daily, with a protein content ranging from 13% to 39%, over a period of 30 days. The results of the study revealed that the growth performance and survival rate of the shrimp in the groups that were fed diets supplemented with moringa were significantly higher than those in the control group (without moringa supplementation). The highest growth performance, in terms of specific growth rate, weight gain, and final weight, was observed in the groups of shrimps that were fed diets containing either 2.5 g or 5 g of moringa leaf extract per kilogram of the diet ($p < 0.05$). Furthermore, the diet containing 2.5 g of moringa leaf extract per kilogram resulted in the highest survival rate compared to the other treatment groups. Therefore, it can be concluded that the optimal level of moringa in the diet of vannamei shrimp is 2.5 g/kg of the diet.

1 Introduction

The white shrimp, *Penaeus vannamei* (still referred to as *Litopenaeus vannamei* by some authors), is a highly valuable commercial shrimp species. Apart from being caught in the wild, it is also cultivated in aquaculture for human consumption [1]. Consequently, aquaculturists, particularly in Southeast Asia, continually strive to enhance the key aspects for this species in efforts to increase production and improve yields [2]. One aspect of such improvements lies in influencing the performance of *P. vannamei*, which can lead to better shrimp health and increased survival rates for instances is by nutritional manipulation [3].

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To ensure the well-being of the shrimp during their growth phase, aquaculturists have formulated an economical and nourishing food regimen that includes the usage of moringa (*Moringa oleifera*). Moringa represents a compelling substitute, not solely because of its cost-effective and environmentally friendly antimicrobial characteristics with minimal adverse effects, but also because it improves growth performance [4]. This study explores the utilization of moringa to enhance the growth performance, survival rate, and feed conversion ratio of these shrimps.

Moringa oleifera, a member of the Moringaceae family, is a rapidly growing plant found abundantly in tropical and subtropical regions, holding significant economic importance in both the food and medical industries [5]. With all its parts possessing nutritional and pharmacological properties, Moringa is considered one of the world's valuable trees. The leaves, abundant in protein, vitamins, and minerals, are widely consumed by both humans and animals [6]. Similar findings have been observed with inclusion of Moringa leaf meal in formulated fish diets [7].

The addition of moringa leaf powder ranging from 1.5% to 5% in the diet significantly enhanced immune response and managed infections in tilapia (*Oreochromis niloticus*) [8,9]. Other investigations have demonstrated that dietary inclusion of moringa meal as feed ingredients is optimum at 10% level. Including *M. oleifera* leaf meal above 10% leads to reduced growth performance of tilapia and bocourti's catfish [10–12] that resulted in increased serum enzymes, indicating potential cellular damage [13]. Studies with its extract suggested that incorporating moringa leaf extract into their diets can improve growth and immunological performance for freshwater prawns (*Macrobrachium amazonicum* and *M. rosenbergii*) [14,15] and pacific white shrimp (*P. vannamei*) [16,17].

Although moringa offers numerous advantages, it also carries toxicological characteristics that display a correlation with the dosage administered [18]. Thus, determining the safe concentration of moringa for white shrimp is crucial. Moreover, it is vital to ensure that the utilization of moringa does not impede growth, as certain herbs could potentially inhibit growth while concurrently boosting immunity, or vice versa. [19,20].

2 Materials and Methods

2.1 Preparation of *Moringa oleifera* extract

Moringa oleifera leaves were sourced from a traditional local market located in Jepara, Central Java, Indonesia. These leaves underwent a cleaning process and were subsequently air-dried over the course of 3 days at a temperature of 40°C. After drying, the leaves were finely ground to create a powder, and this powder was filtered using muslin cloth [21].

The powdered material was then combined with distilled water that had been brought to a boil, maintaining a ratio of 1 part powder to 9 parts waters. The mixture was stored at room temperature for a duration of 24 hours. Subsequently, the solution underwent further filtration using a nylon mesh. The resulting filtrate was frozen at -80°C and subjected to freeze-drying for a period of 3 days until the solution underwent transformation into a powdered state. This powder was subsequently stored at a temperature of 20°C until it was ready for future utilization [22].

2.2 Experimental shrimps

A total of 4,000 shrimp (*P. vannamei*) individuals were procured from a local hatchery located in Jepara, Central Java, Indonesia. These shrimps were gradually acclimatized to laboratory conditions within a temperature range of 29 to 32°C and a salinity of 28 to 30 psu, over the course of a week. The acclimation process took place in a circular tank with a capacity of 1 m³. During this period, the shrimp were provided with a commercial powdered diet, which was administered four times daily at a feeding concentration of 5 to 10 grams per cubic meter per day. For subsequent treatments, only shrimp in the intermoult stage that appeared healthy (showing no signs of disease, normal feeding behavior, and possessing a hardened carapace) were chosen for participation. These selected shrimps had an average body weight of 0.01 grams.

2.3 Experimental Diet

The experimental diet was formulated by incorporating moringa extract at amounts of 2.50, 5.0, and 7.50 grams, which were bound by calcium lignosulfonate (Progol™), per kilogram of the commercial shrimp feed. These formulations are henceforth referred to as ME2.50, ME5.0, and ME7.5, respectively. In contrast, the control diet was produced using the same base diet but did not include any moringa extract. The composition of the commercial feed consisted of approximately 32% crude protein, 6% lipid, 3% fiber, 13% ash, and 12% moisture.

2.4 Feeding trial

A sum of 1,200 robust shrimp, each weighing approximately 0.01 grams on average, were arbitrarily allocated into 12 tanks. These tanks were divided into four groups, each consisting of three replicates. Each replication consisted of 100 shrimp housed in a 750-liter tank, with a daily water exchange rate of 50%. Over a period of 30 days, the shrimp were fed three times a day, with their daily intake ranging from 13% to 39% of their body weight. To ensure proper nutrition, the amount of food provided was adjusted every three days based on the shrimp's weight.

Routine maintenance included daily siphoning of organic sediment, along with regular monitoring of water quality. Aeration systems were in place to maintain a dissolved oxygen concentration above 4.5 mg/L. At the conclusion of the feeding trial, the shrimp were counted to determine the survival rate, and the biomass was calculated by measuring the total weight of the shrimp and dividing it by the number of shrimps to obtain the average final body weight. Various parameters were then calculated using specific formulas.

Average final weight is determined by dividing the total biomass by the number of shrimps. Feed conversion rate (FCR) is calculated as the ratio of consumed diet (in grams) to the gain in biomass (in grams). Specific growth rate (SGR), expressed as a percentage per day, is computed using the formula: $SGR = ((\ln(\text{final weight in mg}) - \ln(\text{initial weight in mg})) / \text{days}) \times 100$. Survival rate is calculated as a percentage: $\text{Survival rate} = ((\text{number of individuals at the end of the trial} / \text{initial number of individuals stocked})) \times 100$.

2.5 Data analyses

The data gathered for initial weight, final weight, daily weight gain, SGR, FCR, and survival were expressed as means \pm standard deviation for all respective treatments. These parameters underwent analysis through one-way analysis of variance (ANOVA). To ascertain significant differences among treatments, the Duncan posthoc test was conducted at a significance level of 0.05. Prior to analysis, normality and data heterogeneity were assessed. The statistical analysis was performed using SPSS (IBM statistical software, v.25.0).

3 Results and discussion

As indicated in Table 1, the application of moringa leaf extract (ME) has a notably beneficial influence on the biological performance of vannamei shrimp. The extract has resulted in an elevated growth rate, reduced feed conversion ratio, and improved shrimp survival rates. In general, the shrimp experienced a growth rate of approximately 300 times its original size. Notably, the administration of ME at doses of 2.5 and 5 grams per kilogram of feed yielded the most favorable results. However, when considering variables related to feed conversion and survival, only the 2.5-gram ME dose demonstrated the most favorable outcome. This treatment notably reduced the feed conversion ratio (FCR) by 25% and increased the survival rate by 14%.

Table 1. Biological effects of juvenile Pacific white shrimp consuming varying concentrations of moringa leaf extracts in their diet.

Parameter	Diet			
	control	ME2.5	ME5	ME7
Initial weight (g)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Final weight (g)	2.87 ± 0.21 ^b	3.33 ± 0.21 ^a	3.22 ± 0.15 ^{ab}	2.94 ± 0.25 ^{ab}
Daily weight gain (% Day ⁻¹)	926 ± 64 ^b	1084 ± 57 ^a	1030 ± 71 ^{ab}	951 ± 72 ^b
SGR (% Day ⁻¹)	18.76 ± 0.23 ^b	19.29 ± 0.18 ^a	19.12 ± 0.23 ^{ab}	18.85 ± 0.25 ^{ab}
FCR	1.98 ± 0.26 ^b	1.49 ± 0.14 ^a	1.67 ± 0.02 ^b	1.93 ± 0.09 ^b
SR (%)	80.00 ± 5.00 ^b	91.33 ± 3.20 ^a	84.00 ± 3.00 ^b	79.33 ± 4.04 ^b

The outcomes are displayed as means ± standard error. Varied superscripts among data within the identical row signify noteworthy variations $p < 0.05$, $n_0 = 100$

Growth encompasses the increase in cell numbers as well as weight and volume. The growth of cells occurs because they receive sufficient building materials. Adequate availability of these materials comes from the nutrients obtained by the shrimp [23]. Nutritional sufficiency for growth can also be attributed to the efficiency of its utilization. When shrimp are free from diseases, the energy that would have been used to combat illness becomes more available [24]. The microbial conditions in the intestines also play a crucial role. Nutrients digested in the shrimp's intestines may not be utilized by pathogenic bacteria, or some of these nutrients may not be used for the growth of intestinal bacteria [3].

Moringa extract can have various effects. Firstly, moringa acts as an antimicrobial, inhibiting the growth of bacteria that might consume the feed and, thus, serves as a natural antimicrobial growth promoter [25]. Additionally, moringa also modulates the intestine's conditions, thereby supporting the development of beneficial microbes for shrimp health. Furthermore, moringa can function as an antioxidant, neutralizing free radicals produced by metabolism, thus preventing damage to shrimp cells and reducing the energy required for tissue regeneration [6]. Moreover, moringa leaf extract proved to be a valuable dietary supplement for fish. It contains active compounds capable of enhancing the function of internal organs, safeguarding them from harm, including organs like the pancreas. This, in turn, positively impacts metabolic processes and the absorption of nutrients (such as carbohydrates, fats, and proteins) in the livestock's body [26].

The presence of antinutrients in moringa could significantly disrupt the absorption of nutrients from the diet, ultimately leading to growth inhibition. A substantial concentration of moringa extract in the diet could impede the activity of digestive enzymes and the breakdown of complex dietary proteins due to the occurrence of compounds like tannins,

saponins, and other secondary metabolites. These elements, when present in excessive levels, do not contribute to nutrition [27]. Nevertheless, when introduced in suitable proportions, the inclusion of herbal extracts in the diet of aquatic animals triggered the release of digestive enzymes such as protease, amylase, and lipase. Additionally, these extracts served as appetizers, resulting in enhanced growth and more effective utilization of the diet [28].

4. Conclusion

The utilization of moringa leaf extract has been demonstrated to improve the growth performance and survival rate while reducing the feed conversion ratio in young Pacific white shrimp.

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