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4 The Effect of Dosage Combination and Feeding Frequency on Growth and Survival Rate of Vannamei Shrimp Juveniles in Ponds

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Abstract. Vannamei shrimp production in Indonesia has experienced a rapid development in the last decade. One of the main problems in the cultivation of vannamei shrimp in ponds is the high use of artificial feed. This study aimed to examine the combination of feed dose and feeding frequency on the growth and survival rate of vannamei shrimp farmed in ponds. Vannamei shrimp juveniles were kept in square floating net cages (1 m x 1 m x 1.5 m) called *hapa* suspended in the ponds. The feed formulation used corn flour substituted with 50% sweet potato flour as a carbohydrate source. The research used a factorial Completely Randomised Design (CRD). The first factor was the feed dose (3%, 6% and 9% of shrimp bodyweight) and the second factor was the feeding frequency (3, 4 and 5 times per day). There were 3 replicates of each combination of treatments giving a total of 27 experimental units. The experiment lasted 8 weeks. The results showed a significant influence of treatment applied on the growth and survival of juvenile vannamei shrimp. The highest daily growth rate of shrimp was 3.98% obtained with the 3% feed dose treatment combined with the feeding frequency of 4 times per day. The highest survival rate of vannamei shrimp was 80%, obtained with a combination of 3% feed dose and feeding frequencies of 3 and 4 times per day.

1. Introduction

Vannamei shrimp is a species of penaeid shrimp which tends to grow rapidly and is easy to culture. Nowadays, vannamei shrimp production in Indonesia continues to increase significantly. Indonesian vannamei shrimp production in 2011 was 246,420 tons and increased to 406,795 tons in 2015 [1]. Feed is a strategic factor in vannamei shrimp farming because it accounts for up to 50-60% of total production costs. Therefore, cost effective and environmentally friendly feed is crucial for the future of vannamei shrimp farming [2].

One approach to formulating environmentally friendly feed is to reduce the protein content and increase the carbohydrate content in the composition of vannamei shrimp feed. Under this approach, the protein content in the feed must be limited in quantity and optimised only for growth, while energy requirements are met from other more cost effective sources including carbohydrates (protein-sparing effect by carbohydrates). Zainuddin et al. [3] found that sweet potato flour could be substituted for corn flour up to a ratio of 50%.

Shrimp possesses a limited ability to digest and utilise carbohydrates [4] and low regulation of plasma glucose concentration [5]. The low digestibility of carbohydrates by shrimp is related to the



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availability of the enzyme α -amylase, while the low regulation of plasma glucose concentration is assumed to be caused by a deficiency of the insulin hormone [6–8]. Guided by recommendations for people suffering from diabetes [6] suggested that, with more frequent feeding, the ability to utilise carbohydrates could be improved. Furthermore, Silas et al. [6] suggested that continuous feeding can increase the use of carbohydrates and increase fat reserves through increased lipogenesis. There is therefore a need to accurately determine the dose and frequency of feeding to promote the optimal utilisation of carbohydrates by shrimp in order to acceleration growth and promote the survival of vannamei shrimp.

2. Research Methods

2.1. Research time, site and facilities

The shrimp grow-out trials were carried out from June to November 2018 in the shrimp ponds of Nisombalia Village, Marusu District, Maros Regency, South Sulawesi, Indonesia. The experimental facilities used in this research were 27 square net cages placed in the experimental pond and used for shrimp grow-out. The dimensions of these cages, called *hapa*, were 1x1x1.5 meters (length x width x height). The salinity of the pond water was 30-35 ppt.

2.2. Experimental Animals

Experimental animal used in this research were juvenile vannamei shrimp (*Litopenaeus vannamei*) with average initial weight of 0.5 g obtained from a shrimp hatchery in Pangkep Regency. Each of the 27 net cages was stocked with 40 shrimp/m² giving a total of 1080 juveniles.

2.3. Experimental Feed

The study used a low protein feed formulation comprising 20% fish meal, 20% soy flour, 10% shrimp head flour, 20% corn flour, 20% sweet potato flour, 4% fish oil, and 3% each of vitamin and mineral mixes. The experimental feed ingredients were weighed and placed in plastic bags. The dry feed ingredients were mixed starting from fine ingredients in small quantities followed by raw materials in larger quantities, and stirred until evenly mixed. Fish oil, vitamins and mineral mix were added to the dry ingredients mixture and evenly mixed, and then about 100 ml of hot water was added to the mixture to form a dough or paste and stirred until it was not sticky to the touch. The feed mixture was put into a feed template to make pellets, which were spread evenly on trays and sun-dried until the pellets were dry, with a water content of about 10%. Dried feed was placed in labelled plastic bags and stored in a dry place. Proximate analysis yielded a feed nutritional content of 7.94% water, 24.97% crude protein, 11.71% crude fat, 5.40% crude fibre, 38.62% Nitrogen Free Extract (NFE) and 11.36% ash content. The experimental feed was used throughout the 8 week grow-out period.

2.4. Sampling and variables measured

Sampling was conducted once a week during the shrimp grow-out by taking 10 shrimp from every cage. The sampled shrimp were weighed using digital scales with 0.1 g precision. Sampling was carried out for 8 times during the research period. At the end of the research, all remaining shrimp were weighed to evaluate the absolute biomass growth as well as to counting the live shrimp to calculate the survival rate.

2.5. Treatments and Experimental Design

The experimental design applied was a factorial completely randomized design (CRD). The first factor was the feed dose and the second factor was the frequency of feeding. There were three replicates of each treatment combination (27 units) with randomised placement of each treatment.

- First Factor (daily feed dose):

(A1) 3% of body weight
(A2) 6% of body weight

(A3) 9% of body weight

- Second Factor (daily feeding frequency):

- (B1) Three times (06:00 AM, 12:00 PM and 18:00 PM)
- (B2) Four times (06:00 AM, 10:00 AM, 14:00 PM, and 18:00 PM)
- (B3) Five times (06:00 AM, 09:00 AM, 12:00 PM, 15:00 PM, and 18:00 PM).

2.6. Research Variables

The variables observed in this study were Absolute Biomass Growth (W, in g), Specific Growth Rate (SGR in g/day) and Survival Rate (SR in %). These parameters were calculated as follows:

$$1 = B_t / B_o \quad (1)$$

$$SGR = \frac{\ln W_t - \ln W_o}{t} \times 100 \quad (2)$$

$$SR = N_t / N_o \times 100\% \quad (3)$$

where:

- B_t = Final shrimp biomass (g)
- B_o = Initial shrimp biomass (g)
- W_o = Initial shrimp weight (g)
- W_t = Final shrimp weight (g)
- t = Grow-out time (days)
- N_t = Initial number of shrimp
- N_o = Final number of shrimp

2.7. Data Analysis

Data were analysed using the Analysis of Variance (ANOVA) function in SPSS version 22 software.

3. Results

3.1. Absolute Biomass Growth

The analysis of variance showed a significant effect of treatment on the absolute biomass growth of the shrimp (Table 1). Feed dose caused a significant difference in absolute biomass growth of vannamei shrimp juvenile, while the effect of feeding frequency and the interaction of both variables were not significantly different. The highest absolute biomass growth of vannamei shrimp was 122.88 g on 3% dose per day, and the lowest was 54.83 g on 9% dose per day.

Table 1. Average Absolute Biomass Growth of vannamei shrimp juveniles during the research (g).

Dose (A) \ (B) Frequency	B1	B2	B3	\bar{A} Tukey 0.05
A1	123.09±9.12 ^a	130.35±32.67 ^a	122.09±12.12 ^a	128.88 ^c
A2	104.67±13.42 ^a	76.58±8.45 ^a	89.33±18.94 ^a	90.23 ^b
A3	47.90±5.04 ^a	45.42±6.11 ^a	71.62±2.25 ^a	54.83 ^a
\bar{B} Tukey 0.05	91.79 ^a	83.78 ^a	94.38 ^a	

Note: values with different superscripts were significantly different (P<0.05)

3.2. Specific Growth Rate

Data on shrimp weight gain during the grow-out period (Figure 1) show that during the grow-out period the vannamei shrimp juveniles gained a significant amount of weight. Growth patterns in all treatments seemed to have a linear tendency.

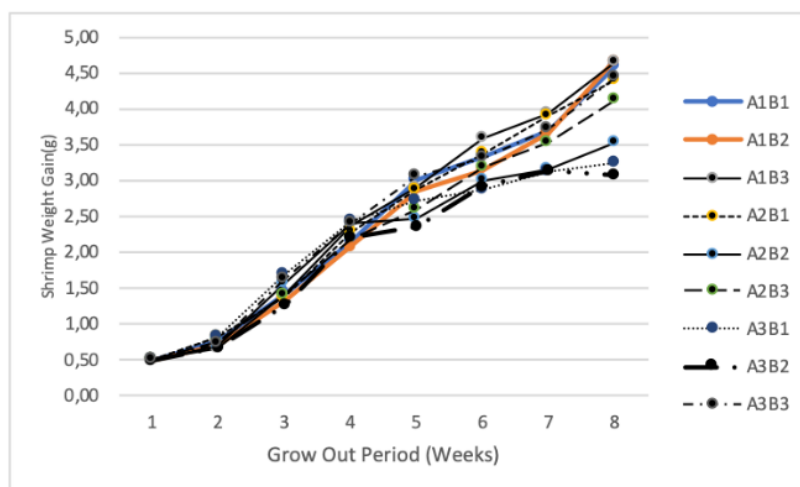


Figure 1. Mean shrimp weight gain under each treatment during the research period.

The combination of 3% feed dose and 5 times (A1B3) feeding frequency and 3% feed dose and 4 times (A1B2) feeding frequency were the best treatments based on final growth rate. The analysis of variance showed that the feed dosage, frequency of feeding and interaction of both had a significant effect on the specific growth rate of vannamei shrimp juveniles (Table 2). The specific growth rate of vannamei shrimp juvenile under the 3% feed dose treatment was significantly different than those fed a 9% feed dose but not significantly different from those fed a 6% feed dose.

The specific growth rate of vannamei shrimp juveniles fed 4 times per day was not significantly different from those fed 3 times per day, but was significantly lower than those fed 5 times per day. The specific growth rate of vannamei shrimp juvenile obtained from the interaction between feed dose factors and feeding frequency demonstrate a significant difference. The growth rate of combinations A1B2 and A1B3 were significantly higher than those of A3B1 and A3B2 treatments but were not significantly different from other treatment combinations.

Table 2. Average Specific Growth Rate of Vannamei shrimp juveniles during the research.

Dose (A) Frequency (B)	B1	B2	B3	\bar{A} Tukey 0.05
A1	3.95±0.10 ^c	3.98±0.45 ^c	3.98±0.16 ^c	3.95 ^c
A2	3.88±0.21 ^{bc}	3.49±0.20 ^{abc}	3.76±0.22 ^{abc}	3.70 ^{ab}
A3	3.33±0.13 ^{ab}	3.24±0.07 ^a	3.9±0.12 ^{bc}	3.49 ^a
\bar{B} Tukey 0.05	3.72 ^{ab}	3.55 ^a	3.87 ^b	

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 3.3. Survival Rate

The mean survival rates of vannamei shrimp under each treatment combination at the end of the study (Table 3) shows that there was a significant effect from the treatments applied on the survival of vannamei shrimp (P < 0.05). The data in the table clearly demonstrate that the feed dose factor was the sole factor that had a significant effect on the survival rate of vannamei shrimp, while the frequency of administration and the interaction between the two factors did not have a significant effect. Tukey's post-hoc tests showed that the 3% feed dose significantly contributed to a higher shrimp survival (78.61%) followed by the 6% feed dose (67.61%) survival rate and the lowest survival rate with 9% dose (52.50%).

Table 3. Average Survival Rates of Vannamei shrimp juveniles at the end of the research.

Dose (A) / Frequency (B)	B1	B2	B3	Ā Tukey 0.05
A1	78.33±2.89 ^a	80.83±3.82 ^a	76.67±2.89 ^a	78.61 ^a
A2	70.83±1.44 ^a	68.33±1.44 ^a	66.67±2.89 ^a	68.61 ^b
A3	52.5±4.33 ^a	53.33±5.77 ^a	51.67±2.89 ^a	52.50 ^c
B Tukey 0.05	67.22 ^a	67.50 ^a	65.00 ^a	

Note: values with different superscripts are significantly different (P<0.05).

4. Discussion

The results of this study showed that generally the feed dosages and feeding frequency had a significant effect on the specific growth rate of vannamei shrimp growth in ponds. The feed dose of 3% per day gave better results in terms of biomass increase and survival rate compared to the 6% and 9% per day doses. This shows that the feed formulation with 50% sweet potato flour substitution for corn flour can meet the requirement of shrimp for biomass growth at a dose of 3% per day. [9] stated that increased growth was also influenced by food factors, especially inorganic nitrogen and carbohydrate levels. Growth will occur if the amount of food consumed is greater than what is required for maintenance. The results are consistent with the opinion of Suarez et al. [10] that the rate of growth and survival of shrimp is influenced by food availability. The amount of feed provided must be appropriate so that the shrimp do not experience food insufficiencies [11]. The high value of specific daily growth rate in the A1B2 treatment was due to the more effective feed utilization by vannamei shrimp with a feeding frequency of 4 times per day.

The composition of feed with sweet potato and corn flour as carbohydrate sources is important for shrimp growth [9]. It is stated that carbohydrate sources play an essential role in reducing total ammonia nitrogen and reduce the use of protein as an energy source although carbohydrate intake has no effect on survival in shrimp. In terms of the quality of carbohydrate raw materials, [3] found that sweet potato flour had higher glucose and fructose content (4.49% and 4.23%) compared to other raw materials such as cassava, sago, and corn flour. According to Cahyoko [12] the digestibility of feed containing glucose and sucrose is higher than that of feed containing starch and dextrin. This high digestibility is due to glucose being a simple sugar so that absorption by the digestive tract is easier compared to other types of carbohydrates that have more complex molecular structures such as starches.

The results of this study illustrated that increasing the frequency of feeding from 3 times per day to 4 or 5 times per day had a positive influence on shrimp growth, especially in the 3% feed dose treatment. One of the determining factors in increasing the digestibility of carbohydrates in feed is the frequency of feeding. [2] found that the frequency of giving feed four times a day had a positive influence on the growth and survival of vannamei shrimp. Increased growth is also influenced by food

factors and juvenile ability to take food, while the rate of shrimp growth and survival is influenced by the availability of food, age, water quality, the ability of shrimps to utilize the feed provided [13], the quality of the feed, and frequency of feeding.

Treatments A3B2 and A3B1 performed poorly. This was thought to be due to the combination of high feed dose and low frequency of feeding, hence the shrimp's ability to digest the feed was not optimal. According to studies of Sunfish Black Sea Trout [14] and tiger shrimp [15], feeding with optimal frequency can increase growth and improve feed conversion ratio. This indicates that feeding frequency in treatments A3B2 and A3B1 was not yet optimal, while treatment of A3B3 with a high dose and a high frequency of 5 times per day resulted in faster growth.

Feed is a dominant factor affecting the growth of aquatic animals (fish and crustaceans) since feed functions as an energy supplier to promote growth and maintain survival [16]. The high survival rate at 3% of feed dose validated that juvenile vannamei shrimp can meet their nutritional requirements for survival at this feeding rate. When the food provided is mostly consumed and the process of digestion is optimum, there are fewer faeces produced.

The lowest survival rate obtained in this study was 52.50% under the 9% feed dose treatment. This was presumably due to the presence of high cannibalism in vannamei shrimp in this treatment. Vannamei shrimp are aggressive, healthy shrimp will attack weak shrimp, especially when moulting or sick shrimp [17]. Furthermore, moulting is a complicated process where a substantial death rate is inevitable [18], and mortality due to cannibalism on shrimp that are moulting can be high when the moulting process is not concurrent between one shrimp and the other shrimp [19]. Low survival of vannamei shrimp can also occur due to environmental factors and stress such as water replacement and poor water quality.

8 5. Conclusion

Based on the results of the study it can be concluded that the highest growth rate, feed conversion ratio (FCR), and survival rate during vannamei shrimp grow-out in ponds were obtained with a 3% of feed dose treatment and feeding frequency of 4 times per day. In order to further increase the digestibility of carbohydrates in shrimp feed, further research is recommended on the use of microorganisms to fermentation feed ingredients.

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