Effect of Vitomolt supplements in feed on growth and survival rate of white shrimp (*Litopenaeus vannamei*) seeds.

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Abstract- Feed is a very important factor in vaname shrimp aquaculture. The addition of phytoecdysteroid supplements (vitomolts) to the feed can increase protein retention, thus the shrimp growth will be better. This study aimed to determine the appropriate dose of Vitomolt supplement in feed for growth and survival of vaname shrimp seeds. The results of this study are expected to be useful in determining the best Vitomolt supplementation in the vaname shrimp feed formulation. The experimental animal used was the vaname shrimp seeds with an average initial weight of 1.5 g per individual. The research design used was a completely randomized design (CRD) with 4 treatments, namely treatment A without Vitomolt supplement, B 1.5 g Vitomolt /kg feed, C 3.0 g Vitomolt/kg feed, and D 4.5 g Vitomolt /kg feed. Each treatment had 3 replications, hence there were 12 experimental units. The experimental feed application was carried out for 40 days of maintenance. The results showed that the treatment of Vitomolt supplement dosage in the feed had a significant effect on the daily growth rate and survival rate of vannamei shrimp. The results of further tests showed that the vitomolt dose of 4.5 g/kg of feed was significantly different from the treatment without vitomolt, but did not differ from those with 1.5 g and 3.0 g /kg of feed Vitomolt dose on the daily growth rate of vaname shrimp seeds. Furthermore, the Vitomolt dose of 3.0 g /kg of feed was significantly different from the treatment without Vitomolt but not significantly different from 1.5 g and 4.5 g /kg of feed Vitomolt dose, on the survival rate of the vaname shrimp seeds. Based on the results of this study, it is recommended to add 3.0 g /kg of vitomolt for vaname seeds feed.

Index Terms- feed, growth, survival rate, Vitomolt

I. Introduction

In an intensive vaname shrimp culture system in ponds, feed is one of the strategic components that determine the success of the business. In these activities, nearly 60-70% of the total production costs are used for the purchase of feed (Haryati et al. 2009; Haliman and Dian, 2005). The high price of commercial shrimp feed is due to the fact that almost 100% of the fish meal used as a protein raw material is imported from other countries. Therefore, the amount of protein in the feed must be limited, protein is optimized only for growth, while energy needs are met from carbohydrates (*protein-sparring effect by carbohydrates*) which are cheaper (Rosas et al. 2001). However, the problem is when the protein content of the feed is low, the potential for protein retention by cultured organisms is also low. It is in this condition that the vitomolts as phytoecdysteroids play an important role in increasing protein retention (Klein 2004, Fujaya et al. 2011).

According to Aslamyah (2000), one of the efforts to reduce protein composition in feed, without disturbing the growth of cultivated organisms is to use steroid hormones. This occurs, because steroid hormones are receptors that carry protein into cells, so that they can activate protein metabolism. Spinach extract, which is a phytoecdysteroid including the steroid class, when added to feed, in addition to accelerating molting and growth, is also expected to increase the efficiency of feed protein utilization. The results showed that vitomolts can retain protein up to> 40%. Thus, vitomolts not only have the potential to reduce feed prices and aquaculture management costs, but also play a direct role in increasing production. Experience with crab can be applied to shrimp with a few adjustments. Because the growth of crab and shrimp are both controlled by the molting hormone in the form of ecdysteroid.

The results of research by Wahyuningsih (2008) and Susanti (2009) suggest that the right dose of ecdysteroid will stimulate crustaceans to molt optimally, while low doses cannot stimulate molting, otherwise too high doses cause inhibition. Molting is an important initial phase for crabs and shrimp because by molting they can experience growth. This indicates that the ecdysteroid dose plays an important role in vitomolt supplementation in the feed. Until now, the dose of vitomolts as a supplement in vaname shrimp feed has never been studied, so it is necessary to do research on this matter. It is hoped that this research will produce low protein vaname shrimp feed with vitomolt supplements as ecdysteroid so that shrimp growth can be faster and the impact of nitrogen waste into the environment is minimal.

II. Methodology

The research was conducted at the Mini Hatchery, Faculty of Marine Sciences and Fisheries Hasanuddin University. The containers used in this study were 12 glass aquariums with a

capacity of 72 liters and filled with 56 L of brackish water for each aquarium. The water used is sea water that has been diluted to reach a salinity of 25-27 ppt. The test animal used in this study was juvenile shrimp with a weight of 1.5 g per individual. Each container is scattered with 20 vaname shrimps. Before giving the test feed, the shrimp were adapted to commercial feed for a week. This is so that the shrimp gets used to eating pellets. After the adaptation period is complete, the test shrimp are fed according to the treatment.

This study used a formulated feed with a low protein composition. The feed raw materials for the formulations are sourced from fish meal, soybean flour, corn flour, sweet potato flour, CMC, vitamin, mineral mix and fish oil as well as vitomolts as a supplement. Feed making begins by pulverizing all the dry ingredients used. All materials are weighed as needed and placed in plastic bags.

All dry feed ingredients are mixed starting from small amounts of fine ingredients followed by large amounts of raw materials, then stirring until they are well blended. Furthermore, added fish oil, vitamin and mineral mix. After it is evenly mixed, 400 mL of warm water is added to the mixture of 1 kg of feed raw materials to form a dough/paste. The feed dough is stirred until it doesn't stick to the hands. Then the dough is put into a feed molding device and molded into pellets. The pelleted feed is spread regularly on trays and dried in the sun to dry (Zainuddin et al. 2019). The dry pelleted feed is mixed with vitomolts according to the treatment dose. The feed that has been mixed with vitomolt is air dried and stored in a plastic jar and labeled according to treatment.

The formulated feed which was tested according to the treatment was randomly placed into the seed rearing medium. The amount of feed given is 10% of the body weight of the seeds (Zainuddin et al. 2020). Feeding is done with a frequency of four times every day, namely 06.00 AM, 10.00 AM, 14.00 PM and 18.00 PM. The test feed application was carried out for 40 days of maintenance to see the response to be measured.

Shrimp growth data were taken per week by sampling shrimp in each aquarium. Shrimp weight was weighed using a digital scale with a capacity of 500 g. Growth sampling was carried out every week to determine the increase in body weight of shrimp and to adjust feed. The method used is an experimental method by applying vitomolt supplementation at various doses as a treatment. The experimental design used was a completely randomized design (CRD) with 4 treatments, namely A without vitomolt supplementation, B 1.5 g vitomolt / kg feed, C 3.0 g vitomolt / kg feed, D 4.5 g vitomolt / kg feed. Each treatment had 3 replications in order to obtain 12 experimental units.

The measured research parameters measured in this study are:
1. Survival Rate (SR)
SR = Nt / Nox100
where :
Nt = number of individuals at the end of the study (individu)
No = number of individuals at the beginningt of the study (individu)
2. Specific growth rate (SGR)

$$SGR = (Ln\ Wt - Ln\ Wo) / tx100$$

Where:
Wt = average individual weight at the end of the study (g)
Wo = average individual weight at the beginning of the study (g)
t = maintenance time (days)

Data were analyzed using analysis of variance (ANOVA) using SPSS version 24 software.

III. Results and Discussion

Daily Growth Rate

The average daily growth rate of vaname shrimp at the end of the study is presented in Table 1.

Table 1. Daily growth rate (DGR) of vaname shrimp during the study

Vitomolt dose (g/kg of feed)	DGR (%/day)
(A) Control	2.11 ± 0.29^{a}
(B) 1.5	2.39 ± 0.41^{ab}
(C) 3.0	3.04 ± 0.51^{ab}
(D) 4.5	3.38 ± 0.32^{b}

Note: Different letters in the DGR column indicate significant differences between treatments ($P < 0.05$)

The results of analysis of variance (ANOVA) showed that the addition of vitomolt to pelleted feed had a significant effect ($p < 0.05$) on the daily growth rate of vannamei shrimp. Furthermore, the results of W-Tuckey's further test showed that the vitomolt dose of 1.5 g / kg of feed and 3.0 g / kg of feed was not significantly different from the control treatment, but the vitomolt dose of 4.5 g / kg of feed was significantly different from the control. The three treatments of adding vitomolts in the feed were not significantly different from the daily growth rate of vaname shrimp during the study.

The results of this study indicated that the higher the dose of vitomoltt added to the feed, the higher the daily growth rate of vaname shrimp (Table 1). This shows that the addition of vitomolts as phytoecdysteroid plays an important role in the growth of vaname shrimp. As it is known that molting is an important initial phase for crabs and shrimp because by moulting they can experience growth. Soumoff & Skinner (1983), the ecdyson hormone is able to increase the moulting process in crustaceans where the stimulation process of the moulting hormone in the premolt phase has an impact on the rapid moulting period and the synchronization of the moulting period. The ecdyson hormone thus has a good impact on metabolism and the moulting period in crab larvae. Gunamalai et al. (2003) also stated that ecdysteroid is the main steroid hormone in arthropods that regulates physiological functions, such as growth, metamorphosis, and reproduction.

Burdette (1962) in Klein (2004) also states that ecdysteroid apart from being a molting hormone also plays a role in increasing protein formation through increased mRNA synthesis. Donalson et al. (1978) stated that the most prominent metabolic action of steroids is the activation of protein metabolism. Protein synthesis is the most basic growth process, without large-scale protein production, growth will not occur (Jobling et al., 2001). The results of this study also showed that even though the protein content in the diet was low, only about 30%, the presence of

ecdysteroid supplementation, the body's protein formation was still high. This is relevant to Burdette (1962 in Klein 2004) explanation that ecdysteroid plays a role in increasing protein synthesis. According to Turner & Bagnara (1976), hormones not only play a role in stimulating action but also inhibiting action. There is a negative feedback mechanism in hormonal action. High hormone concentrations in the circulation give an indication for cells to perform inhibition in order to maintain balance (homeostasis).

IV. Survival Rate

The average survival rate (SR) of vaname shrimp at the end of the study can be seen in Table 2.

Table 2. Average survival rate of vaname shrimp at the end of the study

Vitomolt dose (g/kg of feed)	SR (%)
(A) Control	48.33 ± 20.82^{a}
(B) 1.5	75.00 ± 5.00^{ab}
(C) 3.0	80.00 ± 5.00^{b}
(D) 4.5	76.67 ± 7.64^{ab}

Note: Different letters in the SR column show significant differences between treatments ($P < 0.05$)

The results of analysis of variance (ANOVA) showed that the addition of vitomolts to pelleted feed had a significant effect ($p < 0.05$) on the survival rate of vaname shrimp. Furthermore, the results of W-Tuckey's further test showed that the vitomoltt dose of 3.0 g / kg of feed was significantly different from the treatment without vitomoltt but not different from the dose of 1.5 g and 4.5 g / kg of feed against the survival rate of vaname shrimp seeds.
The role of vitomolt as a supplement in feed is also seen in the survival rate of vaname shrimp. It can be seen that the vitomolt dose of 3.0 g / kg of feed is the optimum dose and when the dose is increased to 4.5 g / kg of feed, the survival rate of vaname shrimp decreases (Table 2). This shows that excess vitomolt supplementation actually has a negative impact on the survival rate of vaname shrimp. The results of research by Wahyuningsih (2008) and Susanti (2009) suggest that the right dose of ecdysteroid will stimulate crustaceans to molt optimally, while low doses cannot stimulate molting, otherwise too high doses cause inhibition.

V. Conclusions and Suggestions

The results showed a good response of the test shrimp with the application of vitomolts in feed. Vitomolt dose 3.0 g / kg of feed is the best treatment compared to other treatments. Based on the results of this study, it is recommended that the addition of vitomolts of 30 mg / kg of vaname shrimp seed feed is recommended.

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