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Research Article
Effect of Immunostimulant on Growth Performance of Whiteleg Shrimp (*Litopenaeus vannamei*) Reared at Different Stocking Densities

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Abstract
Objective: This study aimed at investigating the effect of immunostimulant administered at different times on the growth performance of whiteleg shrimp reared at different stocking densities. **Materials and Methods:** This study used a randomized complete block design (RCBD) with two factors. Factor A was administration times (7th, 14th, 21st and 28th days) and factor B was stocking densities (10 larvae/15 L, 15 larvae/15 L and 20 larvae/15 L). Data in terms of specific growth rate (SGR), feed efficiency (FE) and feed conversion ratio (FCR) were calculated and compared among treatments using Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test to find a significant difference among treatments. **Results:** The results showed that SGR, FE and FCR were significantly affected by stocking density and administration time, $p<0.05$. The highest specific growth rate was obtained from shrimp with a stocking density of 20 shrimps/15 L at the administration of the 7th day. While the best FE is in a stocking density of 15 shrimps/15 L given on the 21st day, then the best FCR was in a stocking density of 20 shrimps/15 L given on the 28th day. **Conclusion:** SGR, FE and FCR of white shrimp were significantly influenced by the administration time of immunostimulant (whole protein of *Zoothamnium penaei*) and stocking densities.

Key words: Feed efficiency, feed conversion ratio, immunostimulant, stocking density, specific growth rate, whiteleg shrimp

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Whiteleg shrimp (*Litopenaeus vannamei*) has important economic value and high demand by the world market. According to the Directorate General of Aquaculture[1], whiteleg shrimp has many advantages over other aquaculture species, such as: able to be cultivated with high stocking densities and preferred by consumers because of the high protein content (52%). However, Amir *et al.*[2]also explained that whiteleg shrimp cultivation has several obstacles that generally occurred in post-larval stages such as uneven growth and susceptible to environmental changes, including the availability of feed quality.

Besides the feed quality, it is also necessary to pay attention to the health condition of shrimp during maintenance because these factors are early indications of an attack of the disease. One way to overcome the disease is by giving immunostimulants, immunostimulants that enter the body of the shrimp will stimulate the hemocytes to do degranulation and phagocytic activity. Immunostimulant used in the present study is a membrane protein of *Zoothamnium Penaei* which has a molecular weight higher than 1,000 daltons so that it is capable of being immunogenic. Briggs *et al.*[3] also says that proteins that have a molecular weight of more than 1,000 daltons and have complex structures will be able to be immunogenic so that they can be used as immunostimulants. According to Mahasri *et al.*[4], the use of immunostimulants on shrimp seeds before stocking is useful for enhancing the resilience of the shrimp body. Immunostimulants used are from immunogenic membranes proteins *Zoothamnium Penaei*. According to Darwatin *et al.*[5], the growth of shrimp decrease with increasing age. The objectives of this study were as follows: (1) Knowing the effect of different administration time of immunostimulant on the specific growth rates (SGR), feed efficiency (FE) and feed conversion ratio (FCR) of whiteleg shrimp (*Litopenaeus vannamei*), (2) Determine the effect of different stocking densities on SGR, FE, FCR, (3) knowing the interaction between administration time and different stocking densities on SGR, FE and FCR.

This study was expected to provide information and insights on the effect of administration time, different stocking densities and interactions between the two factors on SGR, FE and FCR of whiteleg shrimp (*Litopenaeus vannamei*) exposed to immunostimulants from whole protein *Zoothamnium penaei* via commercial feed.

MATERIALS AND METHODS

Materials and tools: The material used in this study was PL 40-50 whiteleg shrimp with 7-10 cm length, Seawater with a salinity of 22-23 ppt, immunostimulant from whole protein *Zoothamnium penaei* and Commercial shrimp feed with a protein content of 30.29%.

The study also used 18 plastic containers (45×38.5×34.5 cm) with a capacity of 43 L, 18 aerators, hosesaeration, digital balance, rulers, tubs, DO meters, pH meter and refractometer.

Method: The study used a randomized complete block design consisting of factor A (3 levels of stocking density) and factor B (delivering time).

Factor A (spread density):
Treatment A1: Density ten shrimp in 15 L of water
Treatment A2: Density stocking 15 shrimp in 15 L of water
Treatment A3: Density stocking 20 shrimp in 15 L of water

Factor B (delivering time):
Treatment B1: Day 7
Treatment B2: Day 14
Treatment B3: Day 21
Treatment B4: Day-to-28

Feeding and administration of immunostimulants: Administration of the immunostimulant was performed by *dipping* the shrimp according to Mahasri *et al.*[4]. In brief, *Zoothamnium penaei* was homogenized with PBS and concentration was adjusted at a dose of 3 ppm. Then, the shrimp was immersed for 10-15 min and transferred to the rearing containers at three stocking densities. The administration of whiteleg shrimp feed was done three times a day, namely in the morning, afternoon and evening. The amount of feed given is 3-5% of the total weight of the shrimp[6]. Uneaten feed in the bottom of rearing containers was siphoned manually using a series of aeration hoses.

Culturing of whiteleg shrimp: The rearing containers were previously cleaned and dried. Thereafter, the containers were filled with brackish water. Shrimps were stocked at three stocking densities 10, 15 and 20 shrimps/15 L. Density of 15 larvae/15 L or 1,000 shrimps/m3 was considered as an intensive system according to Indonesia national standard (SNI 8037.1)[7]. which states that the density of shrimp stocking in intensive cultivation is 1,000 shrimps/m^3. The whiteleg shrimp were cultured for 28 days.

RESULTS

Specific growth rate (SGR): The results showed that SGR of the whiteleg shrimp significantly influenced by stocking density ($F = 3.35$, df 2, 72, $p = 0.04$) and administration times of immunostimulant ($F = 11.45$, df 3, 72, $p<0.01$). Based on the results of Duncan's Multiple Range Test, the best SGR was obtained from shrimp reared in the highest stocking density and received immunostimulant at day 7th and shrimps reared at the lowest stocking density with immunostimulant administration time at day 28th, ($p<0.05$), (Table 1).

In general, the results showed that SGR of whiteleg shrimp tended to be higher when they received immunostimulant at day 28th in two stocking densities (S1 and S2) compared to SGR of those shrimps received immunostimulant at day 7, 14 or 21, $p<0.05$. However, the highest SGR was observed from shrimp fed on immunostimulant at day 7th in the highest stocking density (S3). Among the treatments, the best SGR was obtained from shrimps reared in a stocking density of 20 shrimps/15 L (S3), which was 1.25% body weight day^{-1}.

Feed efficiency (FE): Table 2 shows the average value of feed efficiency during the 28 experimental periods of whiteleg shrimp reared in 3 different stocking densities and four different administration times of immunostimulant. Statistically, FE of shrimps was not significantly influenced by stocking density ($F = 2.42$, df 2, 72, $p = 0.09$) but was significantly affected by the administration time of immunostimulants ($F = 3.47$, df 3,72, $p = 0,022$). The best FE was observed from shrimps reared at a stocking density of 20 shrimp/15 L and given immunostimulant on day 28.

Feed conversion ratio (FCR): In terms of feed conversion ratio, shrimps reared at three different stocking densities (600 and 1,000 and 1,330 shrimps/m3) had no significant influence on FCR, ($F = 2.75$, df 2, 72, $p = 0.072$). However, the result showed that there was a significant influence of administration time of immunostimulant ($F = 11.52$, df 3, 72 $p<0.01$). The best FCR was observed from shrimp with a stocking density of 20 shrimps/15 L and received immunostimulant at day 28 (Table 3).

Water quality: Other results showed that water quality parameters: dissolved oxygen, temperature, salinity, pH, ammonia and nitrite during the experimental period were all in a range of safe levels for shrimp aquaculture (Table 4).

Table 1: The average of SGR whiteleg shrimp in each treatment

	Specific growth rate (%) (factor A)		
Administration time (factor B)	Stocking density of 10 shrimps/15 L (S1)	Stocking density of 15 shrimps/15 L (S2)	Stocking density of 20 shrimps/15 L (S3)
Day 7th	1.03 ± 0.77^{bc}	0.91 ± 0.12^{cde}	1.25 ± 0.10^{a}
Day 14th	0.90 ± 0.13^{cde}	0.79 ± 0.09^{de}	0.97 ± 0.12^{bcd}
Day 21st	0.85 ± 0.10^{cde}	0.80 ± 0.31^{de}	0.76 ± 0.12^{e}
Day 28th	1.12 ± 0.14^{ab}	1.00 ± 0.19^{bc}	0.82 ± 0.22^{de}

$^{a-e}$Different superscripts represent significant differences ($p<0.05$)

Table 2: The average of feed efficiency recorded from shrimps reared for 28 days at different stocking densities and administration times of immunostimulant

	Feed efficiency (%) (factor A)		
Maintenance time (S2) (factor B)	Stocking density of 10 shrimps/15 L (S1)	Stocking density of 15 shrimps/15 L	Stocking density of 20 shrimps/15 L (S3)
Day 7	0.92	88.8 ± 1.00^{abc}	90.3 ± 1.48^{abc}
14th Day	87.8 ± 1.83^{cd}	$90.4\pm3{,}00^{ab}$	88.3 ± 1.63^{bc}
21st Day	88.2 ± 1.84^{bc}	91.0 ± 2.34^{a}	89.3 ± 2.60^{abc}
28th Day	$90.2\pm1{,}37^{abc}$	88.2 ± 2.19^{bc}	85.3 ± 1.17^{d}

$^{a-d}$Different superscripts showed significant differences ($p<0.05$)

Table 3: The value of the conversion of whiteleg shrimp feed ratios in each treatment combination

	FCR (%) (factor A)		
Maintenance time (factor B)	Stocking density of 10 shrimps/15 L (S1)	Stocking density of 15 shrimps/15 L (S2)	Stocking density of 20 shrimps/15 L(S3)
Days	1.09 ± 0.67^{ab}	0.90 ± 0.09^{cdef}	1.07 ± 0.04^{bc}
14th Day	1.25 ± 0.10^{a}	1.12 ± 0.75^{ab}	1.13 ± 0.73^{ab}
21st Day	1.05 ± 0.11^{bcd}	0.85 ± 0.11^{ef}	1.00 ± 0.27^{cde}
28th Day	0.90 ± 0.14^{def}	1.10 ± 0.68^{ab}	0.80 ± 0.18^{f}

$^{a-f}$Different superscripts showed significant differences ($p<0.05$)

Table 4: Water quality data water quality

Parameters	Stocking density of 10 shrimps/15 L (S1)	Stocking density of 15 shrimps/15 L (S2)	Stocking density of 20 shrimps/15 L(S3)	Optimal value (KEP. 28/MEN/2004)
Dissolved oxygen (ppm)	3.19-4.45	3.31-4.11	2.90-3.95	3-7.5
Temperature (°C)	28.7-31.1	28.9-31	28.7-31.2	28.5-31.5
Salinity (ppt)	22-23	22-23	22-23	15-25
pH	7.1-7.8	7.1-7.7	7.1-7.8	7.5-8.5
Ammonia (ppm)	0.5-0.75	0.5-0.75	0.75-1.25	0.01-0.05
Nitrite (ppm)	1.25	1.25	1.25	0.1

DISCUSSION

This study was conducted to determine the effect of administration time of immunostimulant on the SGR, FE and FCR of whiteleg shrimp reared at different stocking densities, as well as the interaction between administration time of immunostimulant and stocking densities of whiteleg shrimp. The result showed that SGRis the daily growth rate or percentage weight gain of fish each day[8]. The growth of whiteleg shrimp is indicated by the increase in weight and length. The results of statistical analysis showed that maintenance time had significant differences ($p<0.05$) on the specific growth rates of whiteleg shrimp then there were significant differences ($p<0.05$) between stocking densities different from the specific growth rates of whiteleg shrimp, there were also time factors maintenance and stocking density factors differ significantly ($p<0.05$) so that there are interactions between the two factors. The interaction shows that between maintenance time and different stocking densities have a relationship with the specific growth rate of whiteleg shrimp (*Litopenaeus vannamei*). The interaction occurred on day 14 which was indicated by the decrease in the value of the specific growth rate from day 7.

The statistical analysis showed that whiteleg shrimp cultivation with the highest value was found in a 7-day maintenance time with a stocking density of 20 (1.25% BW day^{-1}) while the lowest value was found in treatment with a maintenance time of 21 days in a stocking density of 20 shrimp (0.76% BW day^{-1}). This is because, on the 7th day, the shrimp is still in the juvenile stage where the shrimp body's defense cells increase in activity and amount so that growth tends to be faster. Then on the 21st day, the shrimp experienced a decline in the value of growth because the shrimp had entered the adult stage where at that stage, shrimp had decreased activity of the body's defense cells so that the growth of shrimp began to slow down.

The average weight of shrimp at the beginning of maintenance obtained 10 g/shrimp and on the 7th day it was found that the average shrimp weight was 11.4 g/shrimp which showed that the shrimp had experienced a growth period, then on the 14th day there was a decrease in the growth rate until the day 28. This decrease is due to the poor quality of water with high levels of ammonia and nitrite in the maintenance media so that shrimp use the nutrients contained in the feed for their hemostatic activity in adjusting to the less optimal conditions. Immunostimulants given will stimulate the defense cells of the shrimp body so that it responds to the shrimp immune system to survive.

Feed efficiency (EP) is defined as the value of comparison between fish weight gain and weight of feed consumed during the maintenance period is expressed in percent[9]. From the results of the study note that the highest feed efficiency value was found in the treatment of the 21st day maintenance time with a stocking density of 15/15 L which was 91.0% and the lowest value was found in the 28 day maintenance time with a stocking density of 20/15 L which was 85.3%.

These results indicate that cultivation at the 21st day of maintenance provides better feed efficiency (EP) compared to the 28th day maintenance time because, on the 21st day, the shrimp are in a stable condition so they can respond to feed well and grow with well. The higher feed efficiency values indicate that the feed consumed is more optimally used by fish for growth. The stocking density of 20 shrimp/15 L on the 28th day shows a decrease in feed efficiency; it is not in accordance with the graph of the specific growth rate at the same stocking density and the same day. Decrease the specific growth rate of shrimp on day 28 because shrimp uses nutrients on feed not for growth but reproduction. The results obtained in this study tend to be dimmed when compared to day 7 but classified as good because feed efficiency reaches values of 85-91%. This is in accordance with Ahmadi *et al.*[10] who explained that feed is said to be good if the feed efficiency value is more than 50% or even close to 100%.

Feed conversion ratio (FCR) is a comparison value between the amount of added weight produced by the amount of feed given. From the results of the study, it was found that the lowest feed conversion ratio was found in the 28 day maintenance time with a stocking density of 20 shrimp/15 L which was 0.8 while the highest feed conversion ratio was found in the 14 day maintenance time with 10 stocking densities/15 L. These results indicate that the treatment in immunostimulant cultivation of whole protein *Zoothamnium Penaei* in the stocking density of 20 shrimp/15 L results in a better feed conversion ratio (FCR) compared to the low stocking density of 10 shrimp/15 L. Provision of immunostimulants from whole protein *Zoothamnium Penaei* helps shrimp to survive in less optimal environmental conditions, it is indicated by the results of measurements of high ammonia and nitrite so that in addition to being used for reproduction, shrimp uses feed nutrients to be tolerant to the environment so that the value of feed efficiency in the day 28th also shows a decline. In accordance with that at the juvenile stage, most of the energy is used for the growth process, while most of the energy is used for reproduction and maintenance at the adult stage. This is supported by the decreasing value of feed efficiency on day 28.

The whiteleg shrimp that is cultivated so that it can grow properly is not only fed according to its nutritional needs but also from environmental conditions of maintenance. The body and gills of shrimp are directly related to substances dissolved in water. Therefore water quality directly affects the health and growth of cultivated organisms[11]. Water quality plays an important role in determining the success of a culture. This is because water quality will affect the health of cultivated fish, therefore the quality of water during the cultivation process must always be considered at the optimal range.

The temperature in this study is between 31.2-28.7°C, this temperature is quite ideal for aquaculture activities as well as salinity ranging from 22-23 ppt. This is in accordance with Briggs *et al*.[3]. who stated that white leg shrimp is able to grow well in a temperature range of 23-30°C and in salinity of 15-25 ppt. In addition to temperature and salinity, other water qualities that must be considered are pH and dissolved oxygen. The measurement results in this study obtained a pH value ranging from 7.1-7.8. This value is ideal for maintaining shrimp. This is in accordance with the statement of Amir *et al*.[2] who stated that for a good pH in whiteleg shrimp cultivation ranges from 7.5-8.5.

Another factor which then becomes a concern in shrimp farming is dissolved oxygen. The availability of oxygen dissolved in water bodies as a factor in supporting the growth, development and life of shrimp. The results of DO measurements in this study ranged from 2.90-4.45 ppm (mg L^{-1}), according to Haliman and Adijaya[12] stating that the dissolved oxygen content ranged from 4-6 mg L^{-1} will affect shrimp metabolism well, then the data Dissolved oxygen obtained in this study is considered ideal.

Ammonia values in this study ranged from 0.5-1.25 mg L^{-1} and nitrite was at 1.25 mg L^{-1}. Ammonia and Nitrite come from organic materials that accumulate in maintenance containers. However, this value can still be tolerated by shrimp. That is according to Boyd[13] who reported that ammonia content of 0.45 mg L^{-1} can inhibit the growth rate of shrimp up to 50%, while ammonia at the level of 1.29 mg L^{-1} can kill some species of Penaeus, ammonia content 0.05-0.2 mg L^{-1} affects the growth disturbance in general aquatic organisms. Kilawati and Yunita[14] mentioned that the causes of high nitrite levels include too high a density so that a lot of decay from dirt or feces and the rest of the feed.

CONCLUSION

There is an effect of different maintenance times ($p<0.05$) shown on day 28 with a decrease in specific growth rate, feed efficiency and increasing the feed conversion ratio of whiteleg shrimp (*Litopenaeus vannamei*) given commercial feed and exposed to immunostimulants from the whole protein *Zoothamnium Penaei*. There is no effect of different stocking densities ($p>0.05$) as indicated by stocking density 10 heads, 15 shrimp and 20 shrimp/15 L decline unspecific growth rate, feed efficiency and feed conversion ratio shrimp whiteleg (*Litopenaeus vannamei*) which given commercial feed and exposed to immunostimulants from the whole protein *Zoothamnium Penaei.* The existence of the effect of interactions between different maintenance and stocking density on the specific growth rate, feed efficiency and conversion ratio feed whiteleg shrimp (*Litopenaeus vannamei*) fed with commercial and exposed to immunostimulatory than whole protein *Zoothamnium penaei*.

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