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Chaetoceros ceratosporum Diatomae in Feed Formula To Increase Growth and Post Larvae Immunity of Tiger Shrimp (*Penaeus monodon* Fab.) to *Vibrio harveyi* infection

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Abstract

This experiment aims to determine the effect and the best dose of *Chaetoceros ceratosporum* diatomae utilization in feed formula for post larvae of tiger shrimp (*Penaeus monodon* Fab.) growth and immunity to *Vibrio harveyi* infection. This research applied Completely Randomized Design (CRD) with 4 treatments and 3 replications. The treatment use *Chaetoceros ceratosporum* diatomae in feed formula (*iso* protein 39.02% and *iso* energy 3.58 kcal/g diet) in different doses, i.e. treatment A = 0 %; B = 3.04 %; C = 6.0 8%; D = 9.12 %. Observed parameters were Survival Rate, Growth Rate, Food Conversion Ratio (FCR) and Protein Efficiency Ratio (PER). Result showed that *Chaetoceros ceratosporum* diatomae utilization in feed formula affect the increase of growth and immunity of post larvae of tiger shrimp (*Penaeus monodon* Fab.) to *Vibrio harveyi* infection. The best dose in feed formula ranged from 5,75% – 5,95%.

Key words: *Chaetoceros ceratosporum,* balance energy, feed conversion, protein efficiency, *Vibrio harveyi,* tiger shrimp

INTRODUCTION

In the decade of 1991, Indonesia experienced a peak production of shrimp; ranked second after Thailand, nevertheless post 1991, one of the problem is bacterial infection [1].

Several species of *Vibrio* bacteria that can be isolated from diseased shrimp are *Vibrio parahaemolyticus, Vibrio harveyi, Vibrio alginolyticus* and *Vibrio vulvificalis,* while *Vibrio damsela, Vibrio anguillarum* and *Vibrio fluvialis* rarely found. From the various types of *Vibrio, Vibrio harveyi* is often leading to > 100% death at larvae, post-larvae, juvenile, sub-adult and adult phase [2].

Various attempts to control the disease in shrimp farming has been carried out, i.e. health environment management, selection of healthy fry and feed processing as needed. Prevention and control of disease in shrimp should be done through an integrated approach to the factors that influence the onset of disease. These factors were associated with bacterial virulence and the shrimp's immunity.

The immune system is not separated by the availability of nutrients consumed by organisms, including shrimp. However, information on the association between the nutrients composition balance with the immune system of shrimp was not completed explored yet. An increase in immunity against disease not only by feeding with a balanced nutrient composition, but also by the provision of immunostimulant feed. Immunostimulant directly connected to immune system cells which make them more active. Immunostimulant addition (*bacterin vibrio* and *yeast glucan*) into shrimp (*Penaeus monodon* Fab.) enhanced the system activity of pro-phenoloxidase (pro-PO)[3]. Diatomae of *Chaetoceros ceratosporum* as natural feeding also improved shrimp's larvae immunity to *Vibrio harveyi* exposure [4; 5] but whether it acts as an immunostimulant diatomaceous or not was still need to be investigated. Is the formula feed usage also promotes growth and post larvae immunity of shrimp against *Vibrio harveyi* infection or not.

This research aimed to determine the effect and best dose utilization of diatomaceous *Chaetoceros ceratosporum* in feed formula to increase growth and immunity of shrimp post-larvae (*Penaeus monodon* Fab.) towards the infection of *Vibrio harveyi*.

MATERIALS AND METHODS

Materials

Materials used in this research are : Post larvae (PL) 20 Shrimp (*Penaeus monodon* Fabricus), compounds for feed formula, dried *Chaetoceros ceratosporum*, *Vibrio harveyi* bacteria and sea water with 30 ppt salinity as culture media. Chemical compound for natural feed, bacteria culture, feed compound proximate analysis and chemical compound to alnalyze immune responses.

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Equipments

Equipments for this research are : natural feed culture containers, shrimp container and maintenance equipments (aeration), bacteria culture equipments, feed compund proximate tools, feed manufacturing, water quality assessment. This research was conducted in two stages:

Phase I:

Chemical composition Evaluation of standard feed (*rebon* flour and tapioca flour) and dried *Chaetoceros seratosporum* comprise: water content with Oven, Protein by Kjedhal method, fat by soxhlet method, ashing by 600°C.

Feed formulation for tiger shrimp (*Penaeus monodon* Fab.) by the addition of *Chaetoceros seratosporum* known chemical composition with various doses. Feed formula manufactured with 39.02 % protein content and 3.58 k kalg^{-1} energy content according to previous research [6] as basic feed formula and using *Chaetoceros ceratosporum* as one of feed formula compound in different amount. Compositions of feed and feed formula were shown in Table 1 and 2. Re-proximate analysis was conducted on feed and feed size was made suitable to shrimp size (*crumble*).

Phase II:

Feed formula assessments in laboratory scale were conducted to analyze the growth and the immunity of tiger shrimp (*Penaeus monodon* Fab.) to *Vibrio harveyi* infection with experimental Complete Randomized Design method.

In Vivo Experiment Feed Assessment

Used post Larvae (PL 20) in this experiment was from hatchery. Shrimps were nurtured in 45 x 45 x 45 cm^3 aquarium filled with 30 ppt salinity sea water for 30 cm height. Each aquarium filled with 30 shrimps. Pada Table 2 explain the different dose of *Chaetoceros seratosporum* in each treatment, i.e. A = 0 %; B = 3.04 % C = 6.08 %; D = 9.12 %. Each treatmenr were replicated three times. Feeding in 15% of body weight given in every day, at 08.00, 16.00, and 21.00 GMT/UTC+7, for 30 %, 30 % and 40 % respectively. Hatchery took 30 days. Aquarium placement were shown in Fig. 1.

D	A	C	B	A	B	C	D	B	C	A	D
2	3	1	3	1	2	3	3	1	2	2	1

Figure 1. Aquarium Experiment Placement
Explanation: A, B, C, D = treatments

Tabel 1. Composition of experiment feed ingredients

Analysis	*Rebon* Flour	Plankton Flour	Tapioca Flour
Dried content (%)*	86,34	85,38	89,4
Protein (%)*	62,98	3,99	-
Fat (%)*	1,59	0,29	-
Ash content (%)*	17,05	66,84	0,59
Rugged fiber (%)*	3,01	2,61	-
BETN **	15,37	26,26	99,41
Energy (kkal/gr) **	327,69	123,65	397,64

Explanation :
* : Result Analysis of Laboratory Quality and Food Safety Assessment, Dept. of Agricultural Technology, Faculty of Agricultural Technology, Brawijaya University, Malang.
** : BETN = 100 – Protein – Fat – Ash content – rugged fiber.
*** : Energy = (4 x Protein) + (9 x Fat) + (4 x BETN).

Tabel 2. Experiment Feed Formula of tiger shrimp (*Penaeus monodon* Fab.)

Ingredients	Treatment Dose			
	A (0 %)	B (3,04 %)	C (6,08 %)	D (9,12 %)
Rebon flour	61,96	61,96	61,96	61,96
Tapioca flour	15,77	14,38	13,88	12,93
Flour of *C. ceratosporum*	-	3,04	6,08	9,12
Fish Oil	3,75	3,75	3,75	3,75
Corn oil	6,50	6,50	6,50	6,50
Vitamin mix	2,70	2,70	2,70	2,70
Mineral mix	2,00	2,00	2,00	2,00
CMC	7,32	5,22	3,13	1,02
Total	**100**	**100**	**100**	**100**

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Observed parameter are growth for every 10 days, water quality of maintenance media (temperature, pH, solved Oxygen and ammonia) and shrimp survival rates at the end of research. Furthermore, maintenance media filled with 20 shrimps infected with 10^5 cellml^{-1} *Vibrio harveyi* and 1 week survival rate was observed.

Bacteria Culture of Vibrio harveyi

Ose sterilized by heated above the Bunsen till incandescent. After ose ascertained being cold, *Vibrio* bacteria taken by touching the ose tip in stock. The surface of TCBSA media scratched with the quadrant streaking method to obtain separate colonies. Then it incubated in 30^0C for 24 hours. Growing pure colonies identified to ascertain the species of bacteria. Having proven the species is *Vibrio harveyi*, enrichment cultures taken to produce in large quantities.

Enrichment procedure

Pure colonies were taken by sterile Ose, then putted into Erlenmeyer containing liquid medium of TSB +. Erlenmeyer re-closed with sterile cotton, and then putted in water bath shaker. It incubated then in 30^0C shaking speed 100 rpm for 2 x 24 hours. The culture results observed to make sure there is no contamination with gram staining and viewed by microscope. Then the bacterial culture density assessed with Mc Farland. Of the OD (*Optical Dencity*) measurement, diluted to get the desired bacterial density.

Data Analysis

Shrimp Survival Rate

Survived shrimp (indv.) compared to initial number of shrimp x 100%.

$$\text{Survival rate} = \frac{\sum survived\ shrimp\ (indv.)}{\sum initial\ number\ of\ shrimp\ (indv.)} \times 100\ \%$$

Specific Growth Rate

Specific growth rate calculations based on the average individual weight of shrimp during the study [7]:

$$\mathbf{SGR} = \frac{\ln \overline{Wt} - \ln \overline{Wo}}{t} x100\%$$

Explanation:

SGR : Specific Growth Rate

$\overline{Wt}$: average weight of individu at time t (g)

$\overline{Wo}$: average weight of individu at time t = 0 (g)

t : time (days)

Feed Conversion Ratio (FCR)

$$\text{FCR} = \frac{\text{Given Feed}}{\text{weight gain}}$$

Protein Efficiency Ratio (PER)

$$\text{PER} = \frac{\text{weight gain}}{\text{given protein}}$$

Survival rate, Spesific Growth Rate, Feed Conversion Ratio (FCR), and Protein Efficiency Ratio (PER) anylzed using ANOVA. The response were tested with F Test [8].

RESULT AND DISCUSSION

Survival rate, Spesific Growth Rate, Feed Conversion Ratio (FCR), and Protein Efficiency Ratio (PER) of tiger shrimp (*P. monodon* Fab.) showed in Table 3 as follow. Water quality range during study showed in Table 4. Survival rate of post larvae of tiger shrimp (*Penaeus monodon* Fab.) were maintained for 30 days by experiments feeding that utilize plankton flour of *C. ceratosporum* were not significantly different, 78,90 – 84,43% (Table 3). Survival rate of shrimp survival is mainly determined by the physical-chemical properties of water and adequate feed. Physical-chemical properties of water in these experiments are in a good range for the survival and growth of shrimp in accordance with some expert advice (Table 5). Feed management is a key factor that affects the water quality [9] and economically produced in cultivation [10].

Feed management is an attempt to control and use feed in the cultivation in order to address these needs with the optimum feed residue and minimize environmental impact; improve the feed conversion ratio (FCR) as well as growth and maximum production [11]. Number of feeding in this research is 3%BB^{-1}hari^{-1} with the frequency of 3 times hari^{-1}. It corresponds with Wyban and Sweeney [12] that frequency of viable feeding for the hatchery is 2 – 4 times depends on shrimp's size. Although feeding manipulation had no effect on poduction, but it improve feed conversion value [13].

In this study, growth pattern of post-larval shrimp which weighted average of 0.021 ± 0.001 g is exponential. This reflects sufficient feed for the growth of post-larval shrimp.

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Tabel 3. Survival Rate, Growth Rate, Feed Conversion Ratio (FCR), and Protein Efficiency Ratio (PER) of Post Larval of Tiger shrimp (*Penaeus monodon* Fab.)

Parameter	Treatments			
	A	B	C	D
Pre-infection of *V. harveyi:*				
Survival Rate (%)	80 ±3,3a	78,90±1,91a	84,43±1.96a	81,10±3,81a
Growth Rate (%BW/day)	6,51±0,33a	7,40±0,25b	8,23±0,25c	7,39±0,20b
FCR	1,64±0,11a	1,37±0,07b	1,18±0,05c	1,37±0,05b
PER	1,55±0,11a	1,84±0,09b	2,20±0,10c	1,89±0,07b
Post Infection of *V. harveyi:*				
Survival Rate (%)	18,33±5,77a	56,67±5,77b	81,67±7,64c	53,33±5,77b

Explanation: Same notation indicates indifference, whereas different notations indicate difference between treatments with confidence level 95%

Table 4. Range of water media quality parameters of tiger shrimp (*Penaeus monodon* Fab.)

Treatments	Water Quality Parameter			
	Temperature (ºC)	pH	DO (mgL^{-1})	NH_3 (mgL^{-1})
A (0 %)	27 – 29	7,84-8,44	5,4-6,5	0,018-0,038
B (3,04 %)	27,5 - 29	7,81-8,45	5,5-5,7	0,018-0,044
C (6,08 %)	27,5 - 29	7,77-8,25	6-6,5	0,012-0,026
D (9,12 %)	27 - 29	7,67-8,21	5,6-6	0,014-0,044

Table 5. Experiment Media Quality of Tiger Shrimp (*Penaeus monodon* Fab.)

Water Quality Parameter	Water Quality Value	
	Study	References
Suhu (ºC)	27 – 29	25 – 30 [14] ; 25 – 31 [15]
pH	7,67 – 8,45	7,5 – 9,0 [16] ; 6,8 – 8,7 [17]
DO (mgL^{-1})	5,4 – 6,5	5 – 8 [18]
NH3 (mgL^{-1})	0,012 – 0,44	< 0,1 [19]

Survival rate on shrimp treatment B, C and D after infected by *Vibrio harveyi* showed different results compared to control without the use of *C. ceratosporum* (treatment A) (Table 5). Correlation between the numbers of *C. ceratosporum* in feed formula (X) with a survival rate of shrimp post-larvae (Y1) after infected *V. harveyi* patterned a quadratic equation:

$$Y\,1 = -1{,}128\,X^2 + 12{,}97\,X + 23{,}85\;;\;\; R^2 = 0{,}90.$$

This equation showed that number of *C. ceratosporum* in feed formula that produced the highest survival rate (61.12 %) after infected *V. harveyi* was 5.75%.

This indicates that the use of *C. ceratosporum* in feed formula increase immunity of shrimp. It is assumed that *C. ceratosporum* contain β-(1-3)-glucans as an immunostimulant. Storseth *et al.* [20; 21] have proved the existence structures of β-D-(1-3)-glucan on *Chaetoceros mulleri*. Furthermore, prove the structure of β-D-(1-3,1-6)-glucan on *Chaetoceros debilis* [22]. Results concluded that different diatom species has a structure of β-D-(1-3)-glucan and different molecular weight. This study also explained the use of *C. mulleri* can improve the survival and growth of Cod fish larvae. In line with the role of *C. ceratosporum* to increase immunity, then followed by an increase in specific growth rate, feed conversion ratio and protein efficiency ratios.

Growth is closely related to feed, because feed provide nutrients and energy needed for growth. Correlation between the number of *C. ceratosporum* in feed (X), growth rate (Y2), feed conversion (Y3) and efficiency ratio (Y4) post-larval shrimp with patterned quadratic equation:

$$Y2 = -0{,}046\,X^2 + 0{,}538\,X + 6{,}432\;;\; R^2 = 0{,}81$$
$$Y3 = \;\; 0{,}012\,X^2 \; - \; 0{,}147\;\; X + 1{,}653;\; R^2 = 0{,}79$$
$$Y4 = -0{,}016\,X^2 \; - \;\; 0{,}194\;\; X + 1{,}515;\; R^2 = 0{,}80$$

Based on these equations derived that the best rate of growth, feed conversion and protein efficiency ratio (7, 99 $\%BW^{-1}day^{-1}$, 1,22 and 2,02 respectively), obtained the use of *C. ceratosporum* in feed formula are 5.78 %, 5.86 % and 5.95 % respectively. Growth rate is influenced by the balance of energy/protein in the feed. In this study, a proportion of energy/protein feed is 10,89 kka g^{-1} protein. Balance energy/protein in the optimal feed for *Litopenaeus vannamei* is 11,9 kkal g^{-1}protein [23]. If the balance of feed energy/protein is 3.37 kkal g^{-1}protein then growth rate declined. With the increase of growth rate will be followed by an

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increase in feed conversion and the efficiency of protein utilization.

The feed conversion ratio is affected by several factors but the most important is the quality and quantity of feed, species, size and quality of water [24]. Low feed conversion value indicates better utilization and well absorbed by the body to promote growth. The improvement of feed conversion values is caused by high nutrients that is not used optimally by the body or in other words wasted in the form of feces.

Feed Conversion in this study is 1,22. This value is better than the results of Venero [25], i.e. 1,8 with balance energy/protein in feed 9kkalg^{-1} protein. It showed the roles of *C. ceratosporum* in increasing immunity, means energy for surviving was more efficient and the rest energy used for growth process. Furthermore, by the increase in growth rate, then feed utilization will be more efficient. Similarly, protein efficiency ratio will also be more efficient because the protein will be used for growing. These results are supported by the results of tests on the immune response of shrimp.

CONCLUSION

Based on the results, this study concluded that the use of diatomaceous *Chaetoceros ceratosporum* in feed formula increase growth and immunity of post-larvae shrimp (*Penaeus monodon* Fab.). The best dose is 5.75% - 5.95% in the feed formula.

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