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The Application of Asterales (*Chromolaena odorata* L). Leaves on the Feed for Disease Prevention in Tiger Shrimp Cultivation (*Penaeus monodon fabricius*) in Pond

Harlina Harlina¹, Andi Gusti Tantu², Rosmiati Rosmiati³, Kamarudin Kamaruddin⁴

¹Muslim University of Indonesia, Indonesia; ²Makassar-Indonesia Bosowa University, Indonesia; ³Maros Aquaculture and Fisheries Extension Research Center Maros, Indonesia

ABSTRACT

One of the natural materials that need to be evaluated as the natural anti-bacterial in the pond is Asterales leaves (*Chromolaena odorata* L.). Asterales leaves contain quite high flavonoid which is important in disease prevention of tiger shrimp cultivation. This study aims to evaluate the addition of Asterales leaf flour on the feed towards the growth, survival rate, FCR and usage potential for disease prevention of shrimp cultivation. The study was designed using treatments given artificial feed containing Asterales leaves at an optimum dose of 15 g/kg of feed and control (feed without the provision of Asterales leaves). Test animals used were PL-20 fry with an average weight of 0.02 ± 0.02 g/head. The study used 4 ponds measuring 0.4 ha (2 plots) and 0.5 ha (2 plots) with a stocking density of 10 heads/m² so that each 40,000 heads were 0.4 ha ponds and 50,000 hectares for ponds 0.5 ha. Three ponds were used for treatment (A1, A2, A3) and 1 plot (0.5 ha) was used for control (Pond B). Feeding the test was carried out 2 times/day by 50% of biomass/day at the beginning of stocking and decreased to 2% of biomass/day in the last week. The results showed that shrimp given test feed containing Asterales leaf flour (A1, A2 and A3) in the 120 days of the rearing period obtained an average weight of 24.33-24.61 g/head, survival rate > 70%, average production of 746.32-942.37 kg/pond, and FCR of 1.69-1.75. Meanwhile, control (B) was not obtained growth due to mass death at 32 days of the rearing period. Based on the results of the feasibility analysis of the cultivation business, the value of R/C Ratio > 1 is obtained so that cultivation using feed containing Asterales leaf flour is feasible to be developed.

Keywords: *Vibriosis*; *Penaeus monodon* Fabr; Organic Feed; Asterales (*Chromolaena odorata*)

INTRODUCTION

Until now, Tiger Shrimp (*Penaeus monodon fabricius*) becomes the main commodity in the fishery cultivation sector and it is proven to become the main priority especially in South Sulawesi. However, the cultivation of tiger shrimps is still faced with the problem of the low survival rate of tiger shrimp due to mass death and the main cause is the attack of vibriosis disease [1]. This disease is caused by *Vibrio* sp. bacteria and the attack spreads shortly [2]. Some vibrio species found in ponds are *Vibrio harveyi*, and *V. parahaemolyticus*. *Vibrio* infection can occur in all phases (eggs to broodstock) and many cases cause death of cultivated organisms to 100% [3].

The prevention of vibriosis disease has been done either by using antibiotics or chemicals. The Government through the Decree of the Minister of Maritime Affairs and Fisheries of the Republic of Indonesia Number 52 of 2014 permits the use of

some hard drugs as antibiotics for treatment of diseases that attack on aquaculture activities [4]. Hard drugs that are allowed as antimicrobials (antibiotics, non-antibiotic antibacteria, antifungal and antiprotozoa) are tetracyclines with active ingredients in the form of clortetracycline, oxytetracycline, and tetracycline; macrolides with active substances in the form of erythromycin; and quinolones with active substances in the form of enrofloxacin [5]. These chemicals usage in the long run may cause negative impacts including the emergence of strains of bacteria resistant to drugs [6,7]. Therefore, alternative medicines must be sought immediately, which are more efficient and environmentally friendly, and one of them is the utilization of the active ingredient of Asterales (*C. odorata* L.) herbal leaves [8].

The use of copasanda leaf bioactive compounds as natural antibacterial products can indirectly increase growth and survival, increase disease resistance and can modulate immune parameters

Correspondence to: Harlina Harlina, Lecturer at the Muslim University of Indonesia, Indonesia, Tel: 81243868134; E-mail: linausman1965@yahoo.com

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(Total Haemocyte Count (THC), and Differential Haemocyte Count (DHC) [9]. The results of previous studies have proven that *Asterales* leaves contain bioactive compounds which are potential to be used as natural antibacterial namely flavonoids, alkaloids, steroids, saponins and tannins [10].

Based on the antibacterial activity test of *Asterales* leaf bioactive compounds, it is known that *Asterales* leaf extract has a strong antibacterial activity and is a promising candidate to be developed as a source of natural bioactive compounds because it is able to suppress the *V. harveyi* population causing vibriosis in vitro [10-12]. The application of copasanda leaf bioactive compounds through immersion method does not cause toxic effects on hepatopankreas post tiger shrimp larvae through immersion method based on histopathological analysis so that it is safe to use as a natural antibacterial in the prevention of vibriosis in tiger shrimp culture [13].

The application of natural antibacterial of *Asterales* leaves in the pond using immersion method is not effective for the need of bigger antibacterial, and most are wasted in the rearing media, considering the width of pond cultivation. It needs alternative of using natural antibacterial of *Asterales* leaves using feed mixing.

RESEARCH METHODOLOGY

Time and place

The Study was held in village pond of Bonto Langkasa Village of Pangkep District on March to September 2018 using four ponds, they are A1 pond (0.5 ha), A2 pond (0.4 ha), A3 pond (0.4 ha), and B pond (0.5 ha). The A1, A2 and A3 ponds were used for feed with *Asterales* leaf flour additional and B pond was used for control (feed without additional of *Asterales* leaves). The water filling in the pond was 100 cm height was held with the standard operational procedure including: drying, liming and fertilizing [4]. Preparation of the test feed was carried out at the Nutrition Research Laboratory of the Brackish Aquaculture Fisheries and Fisheries Education Center (BRPPBAPPP) Maros.

Pellets feed preparation

Making shrimp feed pellets with some percentage of crude protein 38.21%; crude fat 9.27%; crude fiber 4.52%; ash content of 12.48%; and 32.86% NFE added with *Asterales* leaf flour 15 g/kg of feed for treatment (A) and without the addition of *Asterales* leaf flour as a control (B) with a total energy of 1646 kcal/kg of feed. Feed formulation used was a formulation that is developed by Merican and Sanchez [14] by substituting crab shell flour with shrimp head flour (Table 1). Test pellets that have been in the form of pellets are dried in an oven to a water content of + 8-6%. After drying the feed was stored in a place where the temperature could be constant.

Test animal preparation

Test animals used in the form of shrimp fries are WSSV (*P. monodon*) free based on PCR examination results with PL-20 size with an average initial weight of 0.02 ± 0.02 g. Before the fry are stocked, acclimatize the temperature by floating a bag of fries in the pond and gently watering it. The action was carried out until the water temperature in the plastic package was close to or equal to the water temperature in the plot which was characterized by the

appearance of dew inside the plastic packaging. Acclimatization of salinity was done by opening the bag and given pond water little by little for about 30 minutes.

Feeding and rearing

The stocking was done in the morning, slowly spread to ponds with a density of 10 head/m² so that the initial stocking for ponds measuring 0.4ha is 40,000 and 0.5ha is 50,000 tails. Shrimp rearing was carried out for 120days. During rearing, shrimp in ponds A1, A2 and A3 were given feed containing copasanda leaf bioactive while shrimp in pond B (control) were fed without bioactive copasanda leaves each by 50% of biomass/day at the beginning of stocking and decreased by 2% from biomass/day in the last week. Sampling of shrimp was done after the age of rearing 30 days in the morning. Sampling was done using a net with a span of 30 days. The purpose of sampling is to estimate the population and to know the development of shrimp being cultivated.

Shrimp weight gain

The weight gains of sampling shrimp every month was calculated using the formula (Andrino, Apines-Amar, Janeo, & Corre, 2014) as follows:

$$W = \frac{B}{N}$$

Explanation:

W = Shrimp average weight (g)

B = Total shrimp weight (g)

N = Number of shrimp (head)

Survival Rate

Shrimp survival rate percentage was calculated at the end of study with the following formula [15]:

$$SR = \frac{N_t}{N_0} \times 100\%$$

Explanation:

SR = Survival rate (%)

N_t = Number of survived shrimp at the end of study (head)

N₀ = Number of shrimp in the beginning of the study (head)

Feed Conversion Rate

Feed Conversion Rate (FCR) was calculated with formula used by

Table 1: Formula for artificial shrimp feed (% dry weight).

Feed Material	Composition (%) for Feed Formula	
	A	B
Fish Flour	40	40
Shrimp Head Flour	10	10
Copra Oilcake	9	9
Corn Starch	12	12
Soybean Flour	17	17
Wheat flour	10	10
Vitamins and minerals	2	2
<i>Asterales</i> leaves (g/kg feed)	15	0
Total	100	100

Fry et al. [16] and presented as follows:

$$FCR = \frac{F}{Bt} - Bo$$

Explanation:

Bt = Shrimp biomass at the end of study (g)

Bo = Shrimp biomass at the beginning of study (g)

F = Number of feed given during the study (g)

Tiger shrimp cultivation feasibility

In order to find out the feasibility of shrimp cultivation, the data was analyzed using quantitative R/C Ratio analysis [17]. The formula is as follow:

Profit = Total Revenue (TR) - Total Cost (TC)

Whereas:

TC = Total Cost; TR = Total Revenue

With the business criteria:

TR > TC, means profitable business;

TR = TC, means break even effort

TR < TC, means business loss

R/C = TR/TC

With the criteria:

R/C > 1, then the cultivation business is worth developing

R/C = 1, then the business even

R/C < 1, then the cultivation business is not worth developing

Whereas:

R = Revenue

C = Cost

Water quality

Observations of water chemical-physical parameters include pH, temperature, salinity, DO, alkalinity, BOT, NH₃-N, NO₃-N and NH₂-N and biological parameters, namely the population of *V. harveyi* bacteria, monitored every 2 weeks [18].

Bacterial population

Vibrio harveyi population is calculated according to the method [19], with the following formula:

$$P = \frac{Q}{T} \times \frac{1}{S} \times \frac{1}{V}$$

Explanation :

N = Number of bacteria (CFU/mL)

Q = The total number of bacteria growing in one degree of dilution (colony)

T = Number of plate used

S = Dilution level

V = Volume of planted sample (mL)

Data analysis

The data collected was presented in the form of Tables and Graphs and analyzed descriptively, that was comparing between treatments and controls that are strengthened with other supporting references.

RESULTS AND DISCUSSION

Additional weight of shrimp

Tiger prawns reared in Pond 1, Pond 2 and Pond 3 which were fed with *Asterales* leaf flour were successfully harvested at 4 months of rearing with an average weight of 24.33, 24.46 and 24.61 g/head. While tiger shrimp kept in plot 4 (control) control failed to harvest due to mass death during the 32-day rearing period in the pond. However, in the 30-day rearing period at the time of the first sampling, prior to mass death, tiger prawns stocked in control ponds were found to be an average weight of 6.46 g/head lower than the average weight of tiger prawns reared on Pond 1, Pond 2 and Pond 3 with an average weight of 7.10; 7.13 and 6.75 g/head in the same rearing period. This indicated that the addition of *Asterales* leaf flour to the feed gives a higher shrimp weight gain than without the addition of *Asterales* leaf flour to the feed. Shrimp growth monitoring conducted every month shows that the average weight gain generated at the end of rearing could be more optimal with a growth rate of 1-2.41 g/week. As reported by Alam [20] that shrimp could grow well with growth rates of 1-2.5 g/week (Table 2).

Life sustainability

Shrimp survival affects the value of shrimp production in ponds. The difference in survival in each pond during the 120-day rearing period can be seen in Figure 1.

Figure 1 showed the lowest Survival Rate of 0% in pond B and the highest Survival Rate on pond A3 which was 78%. The low Survival Rate on pond B happened for mass death to 100%. The Pond B mass death was suspected to relate with the high population of *Vibrio* sp. as shown in the population of the bacteria in the body of shrimp during the mass death case (Table 3).

Table 3 showed that *Vibrio* sp. bacteria population in the body of shrimp in the pond A1, A2 and A3 were less than 10⁴ CFU/mL, meanwhile the *Vibrio* sp. population on the shrimp in the pond B are above 10⁴ CFU/mL. According to Ramadhani et al. [21] the population of *Vibrio* sp. >10⁴ CFU/mL can turn into pathogen and cause death to rearing shrimp. Similar research was also reported by Alfiansah et al. [22] Alfiansah et al., (2018) that the population of *Vibrio* sp. bacteria in the water and soil of the pond reached 10³ - 10⁴ CFU/mL and has caused death in the cultivation of vannamei

Table 2: Average shrimp weight (gr/head) during 120 days of rearing period.

Experimental Pond	Average Tiger Shrimp Weight (gr/head) on day				
	0	30	60	90	120
Pond 1	0.019	07,10	11,06	19,95	24,33
Pond 2	0,019	07,13	11,48	21,00	24.46
Pond 3	0,019	06,75	11,97	21,59	24,61
Pond 4	0,019	06,46	-	-	-

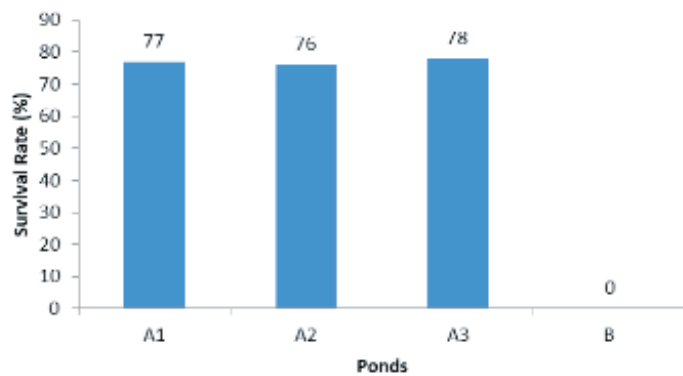


Figure 1: Percentage of shrimp life in each pond on 120 days of rearing period.

Table 3: Total *Vibrio* (TBV) bacteria in the body of shrimp during the mass death of 32 day rearing period.

Pond	Total <i>Vibrio</i> bacteria (CFU/mL)
A1	1.2×10^2
A2	2.6×10^2
A3	1.3×10^2
B	1.3×10^5

shrimp at the age of 60 days rearing in the pond. Bacteria *Vibrio* sp. is one of the triggers for the emergence of disease in ponds. The presence of these bacteria must always be monitored during the rearing period because if the population has reached quorum sensing can result in the emergence of vibrio disease or vibriosis. Some field data indicate that shrimp ponds affected by WSSV contain a total of *Vibrio* bacteria $>10^4$ CFU/mL [23]. So that the mass death of tiger prawns reared in Pond B was allegedly due to WSSV attacks.

The survival rate of tiger prawns maintained with a rearing period of 120 days on Ponds A1, A2 and A3 with the application of feed cooperatives showed survival rates above 70%, with shrimp growth reaching a range of 24.33-24.61 grams/head. According to Widagdo et al. [24] the survival rate of shrimp is categorized well if the SR value $>70\%$, for the moderate category 50-60%, and in the low category the survival value $<50-60\%$. The high survival value of shrimp obtained during the rearing period showed that the application of feed application containing *Asterales* leaves bioactive compounds had given an immune response to the shrimp. This is in line with the results of previous studies which showed that the survival of tiger shrimps had been maintained for 21 days and fed containing natural ingredients of *Asterales* leaves and then infected with *V. harveyi* is higher than the treatment that is fed without natural ingredients of *Asterales* leaves [25]. *Asterales* leaves contain flavonoids and alkaloids which are strong antibacterial against *V. harveyi* in shrimp which can indirectly increase growth and survival, increase disease resistance and can modulate immune parameters (Total Haemocyte Count (THC), Differential Haemocyte Count (DHC) at a dose of 1500 ppm [25]. As it is known that the body's defense system in crustaceans including tiger shrimp is the body's defense which is dominated by hemocytes. If there is a pathogen that enters the body, the shrimp will increase the production of hemolymph. In the hemolymph there will be phagocytic activity such as hyaline and semigranular cells, destruction of the pathogen by phenoloxidase activity and also the activation of antibacterial

by antimicrobial peptides (AMPs) such as hygiene cells and semigranular cells, destruction of pathogens by phenoloxidase activity and also the activation of antibacterial by antimicrobial peptides (AMPs) such as penidinal cells, crustin and antilipopoly saccharide factors (ALFs) [26].

Production

Production is obtained by adding up the total harvest at the end of the rearing period. Production results in each pond can be seen in Figure 2. The lowest production was in pond B, 0 kg and the highest was in pond A1, 942.37 kg. However, if converted to the percentage of survival and weight of shrimp at the end of rearing, the highest production is found in Pond A3. High and low production depends on the weight value of the average shrimp at harvest and the survival rate of shrimp. The higher the production, the better because it will have an impact on the farmer's income.

Feed Conversion Ration (FCR)

Feed Conversion Ratio/FCR is the ratio of how many kilogram feed that can be converted into one kilogram of meat, the low feed conversion happened if the amount of feed given is smaller and if the weight gain of shrimp is produced and conversely the value of feed conversion will be high if the amount of feed given is more many compared to shrimp weight gain [27]. Feed conversion is a comparison between the amount of feed given with the amount of weight of shrimp produced, the smaller the value of feed conversion means the level of utilization of feed is more efficient otherwise if the feed conversion is greater than the level of feed utilization is less efficient [28]. Differences in feed conversion in each pond can be seen in Figure 3.

Figure 3 showed that FCR of the shrimps on the treatment pond 1, A2 and A3 are 1.69 - 1.75. Whereas the Pond Control B experienced 100% death so there is no FCR data. FCR values obtained at 120 days of maintenance period above the maximum number for shrimp culture are 1.4 [29]. FCR values > 1.4 that were found indicate an inaccuracy in feeding. According to Muangkeow et al. [30], FCR that is too high indicates an excessive amount of over feeding which does not always cause high shrimp growth. Efficient feeding is shown in Pond 3 where growth and production are better than in Pond 2 with the same area and initial density of ponds but Pond 3 results have better survival and growth and thus results in higher production with lower FCR. Over feeding will have an impact on the high burden of waste due to residual feed and shrimp dung?

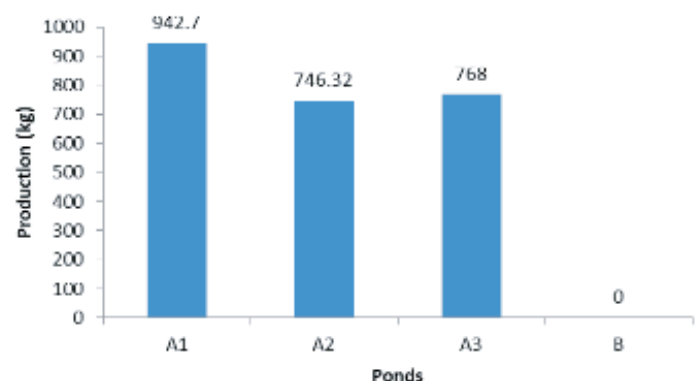


Figure 2: Tiger shrimp production in ponds A1, A2, A3 and control pond B in the 120-day rearing period.

Feasibility analysis shrimp farming business

In order to calculate the feasibility of shrimp pond farming using feed containing copasanda leaf flour, an analysis using R/C (Return Cost Ratio) or known as the comparison between revenues and costs. The R/C values (Table 4) obtained were respectively at Pond A1 (5.27), Pond A2 (5.12) and Pond A3 5.41. Shrimp that are kept in the three ponds are given feed containing Asterales leaf flour show tilapia R/C >1, then the shrimp cultivation business using these feeds is all declared in the feasible category. However,

from the calculation results that the treatment C has a higher R/C ratio (5.41) compared to other treatments, it means that by using Rp. 1,000,000 the revenue will be Rp. 5,410,000 [31].

Water quality

The water quality during the reading on each pond can be seen on Table 5.

Table 5 showed some water quality parameters in the optimal range for growth and some parameters that are less than ideal for shrimp

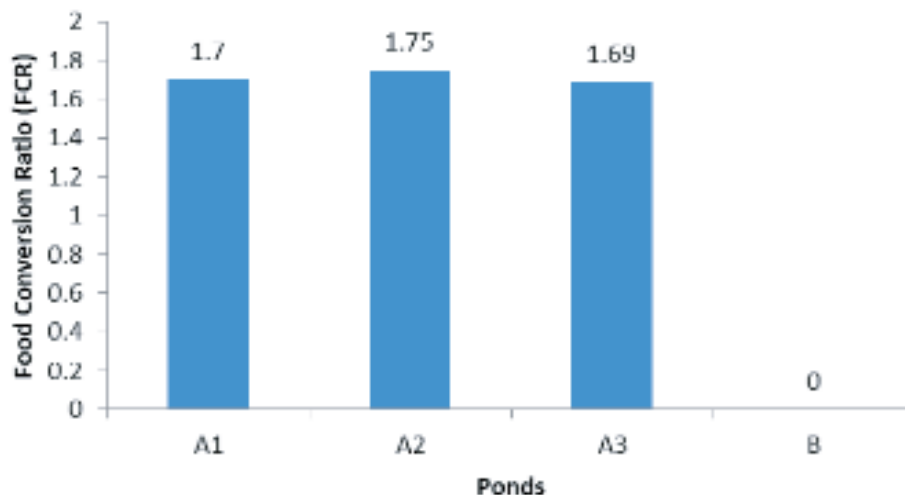


Figure 3: Value of Feed Conversion Ratio (FCR) in the treatment pond A1, A2, A3 and control B in 120-days of rearing period.

Table 4: Feasibility analysis of tiger prawn cultivation.

No	Description	Treatment A			Treatment B			Treatment C			Control		
		Number of unit	Price/ unit (Rp)	Total price (Rp)	Total unit	Price/ Unit (Rp)	Total price (Rp)	Total unit	Price/ Unit (Rp)	Total price (Rp)	Total unit	Price/ Unit (Rp)	Total price (Rp)
1	Shrimp fry	50.000	47	2350000	40.000	47	1880000	40.000	47	1880000	50.000	47	2350000
2	Saponin	5	30000	150000	5	30000	150000	5	30000	150000	5	30000	150000
3	Urea fertilizer	100	1800	180000	100	1800	180000	100	1800	180000	100	1800	180000
4	TSP fertilizer	50	2000	100000	50	2000	100000	50	2000	100000	50	2000	100000
5	Feed	1602	15000	24030000	1306	15000	19590000	1297	15000	19455000	323	15000	4845000
6	Total Cost			26810000			21900000			21765000			7625000
7	Total Income	942,37	150000	141355500	746,32	150000	111948000	786	150000	117900000	170	150000	25500000
8	Total Revenue			114545500			90048000			96135000			17815000
9	R/C- Ratio		5,27			5.12			5.41			3,34	

Table 5: Water quality during the reading on each pond.

Water quality parameters	Experimental Pond					Feasibility
	A1	A2	A3	B		
Temperature (°C)	27-28	26,7-28,1	26,7-28,7	26,8-28,5	26-30	[31]
Salinity (g/L)	10,5-15,6	10,2-16,1	10,4-15,7	10,3-16,2	15-25	[32]
pH	8,1-8,5	8,1-8,6	8,1-8,5	7,9-8,4	7,5 - 9,0	[33]
Dissolved oxygen (mg/L)	4,6-5,6	4,5-5,4	4,1-5,3	5,3-5,6	>5	[34]
BOT (mg/L)	24,40	30,65	25,02	20,02	<20	(Herbeck, Unger, Wu, & Jennerjahn, 2013)
Total Alkalinity (mg/L)	88,44	92,46	88,44	88,44	≥80	[36]
Ammoniac (mg/L)	0,271	0,195	0,224	0,262	<0,1	[37]
Nitrate (mg/L)	0,080	0,030	0,001	0,254	0,4-0,8	[38]
Nnitrite (mg/L)	0,005	0,017	<0,001	0,006	0,1 - 4,5	[39]

life. Water quality in culture media measured such as temperature (26-28°C), pH (7.9 - 8.6), dissolved oxygen (4.1-5.6 mg /L), and alkalinity (88.44-92, 46 mg /L), and BOT (20.02-30.65 mg/L) are in normal condition. However, salinity <15 ppm that has been observed in all ponds is under optimal conditions. Low salinity is thought to be one of the triggers of mass death in controls. According to Chhabra [32-39], low salinity of less than w15 ppt could accelerate shrimp molting, but shrimp were sensitive to disease. The optimum dissolved oxygen content for shrimp farming in ponds is greater than 5 ppm. Dissolved oxygen measurement results (DO) in ponds ranged from 4.1-5.6 ppm. Thus dissolved oxygen is quite good. Although the DO value has been below 5 ppm, it is still below the minimum limit set for shrimp ponds in 3 ppm [40].

The result of Ammonia measurement in ponds was 0.20-0.27 mg/L. This value can still support the growth and survival of farmed shrimp. According to Boyd [41] that the ammonia content in water should not exceed 1.2 mg/L. In general the ammonia concentration that is safe and non-toxic to farm animals is less than 0.1 mg/L [42]. The high concentration of ammonia that was observed in the pond was suspected of overfeeding which caused the accumulation of leftovers. The results of measurements of nitrite levels obtained ranged <0.0010-0.0171 mg/L. This value is below the optimal range of nitrite for cultivation that is 0.01-0.05 mg/L [43]. However, according to Füssel et al. [44] that the recommended nitrite (NO₂-N) content limit for shrimp farming is <0.25 mg/L.

The average nitrate was around 0.0011 - 0.2535 mg/L. The value was sufficient to support natural food that grows in shrimp rearing plots. Nitrate content needed for algae growth in waters is 0.2 - 0.9 mg/L and is optimal in the range of 0.1 - 4.5 mg/L [45].

CONCLUSION

The conclusion of the study is stated that, the feed given with *Asterales* leave was able to increase the growth and life sustainability of tiger shrimp (*P. Monodon* Fabr.). In treatment ponds 1,2 and 3 absolute growth was obtained from the beginning to the end of the study ranging from 24.33 to 24.61 g/head with a survival rate above 70% and production ranging from 746.32 to 942.37 kg addition to the addition of flour *Asterales* leaves in feed can improve feed efficiency from 1.69 to 1.75. Whereas in the control pond (Pond 4), there was no growth due to mass deaths in the 32-day maintenance period. Based on the feasibility analysis of shrimp farming business with the use of formula feed added *Asterales* leaf flour is in the proper category with an R/C ratio >1.

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