

Study of probiotics of yeast and lactic acid bacteria in feeding on culture of larvae shrimp (*Penaeus monodon*)

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Abstract. Aceh is one of province which have a high potential of tiger shrimp hatcheries and is one of the main commodities of marine and fishery resources. Actually, tiger shrimp hatcheries in Aceh have been able to supply the demands of the commercial shrimp farms, but prevalence of diseases and the low quality of the postlarvae have resulted in huge damage to both the hatchery and grow-out sector. Hence, the application probiotics of yeast and lactic acid bacteria (YELAB) from fruit in feeding on shrimp culture have a victorious future to inhibit the growth of pathogenic bacteria. The aims of this study are to examine the effect of probiotics YELAB fruit concentration on the growth, survival, feeding conversion ratio (FCR), development of villy cell in intestine organ and water quality of culture reared larvae of tiger shrimp *P. monodon*. This research will be conducted at Brackish Water Aquaculture Center, Ujong Batee, Aceh Besar district. The concentration of YELAB will be arranged for four treatment which are 0 ppm (control), 50 ppm, 100 ppm, and 150 ppm. The analysis parameters are growth, total length, specific growth rate (SGR), feeding conversion rate (FCR), survival rate (SR), amount of villy cell in intestine, water quality and hystology. The significant effect of YELAB on each parameter will be analysed by ANOVA (analysis of varians) with *post hoc* Duncan comparison of mean scores using SAS software (SAS Inc. Chicago, IL).

1. Introduction

The tiger shrimp (*Penaeus monodon* Fab (1798)), is an indigenous spesies to Indonesian and has contributed significantly to the development of the aquaculture field. *P. monodon* is one of the larger penaeid shrimp in the world which have body length reach 270 mm. Aceh is one of province which have a high potential of tiger shrimp hatcheries and is one of the main commodities of marine and fishery resources in Aceh, especially Aceh Besar Regency and some other areas in Aceh region. It has been able to supply the demand of the commercial shrimp farms. Tiger shrimp in Aceh have high quality, this shrimp known as black tiger shrimp or (prawn). However, lately name of tiger shrimp begins to fade after the introduction of vannamei shrimp which is more dense in one pond and also more resistant to diseases, resulting in higher selling prices [24]. Actually, tiger shrimp exports are still a great opportunity and can be further enhanced, given the potential of availability of vast land for culturing. In addition, tiger shrimp have several advantages, including having a larger harvest size, sweet taste, savory, and high nutritional value [1].



The success of the tiger shrimp hatchery business is the first step in the chain of cultivation system. The success of the hatchery will ultimately support the business of providing quality shrimp seeds. Procurement system of distribution and seed quality is often the main cause of cultivation failure. The availability of the parent and shrimp seeds is increasingly thinning in the free area causing the decrease of shrimp production of the catch, so that the production of shrimp cultivation needs to be improved. The main obstacles encountered are the presence of good disease attacks caused by bacteria and the digestive system is less perfect in the use of feed [4].

In the majority of the successful attempts, the maturation, spawning and reproduction of penaeid shrimp in captivity was accomplished through manipulation of diets nutrition and diets composition. In particular, the role of diets composition in the culture process has been demonstrated for a variety of commercially important penaeid species [7]. Therefore, several efforts are needed to improve the production of tiger shrimp and one of them is through the use of probiotics. One of the findings that has lead to practical feeding regimes is mixed diets with yeast and lactic acid bacteria from fruits (YELAB fruits). Probiotics bacteria have effectiveness to inhibit the growth of pathogenic bacteria as their natural enemies or act as biological controls. Probiotics are defined as any form of supplementary feed of intact (non-living) microbial cells favorable to the host animal by balancing the host's microbiological conditions, modifying the form of association with the host or microbial community of its environment, improving the use of nutrient feed or increasing its nutritional value, increasing the response host immunity to pathogens and improving environmental quality [2, 4, 5, 9, 23].

The use of probiotics will increase production costs because the market price is quite expensive. But this can be suppressed by probiotics reproduction/culture first using certain materials (fermented) so as to increase the population of probiotics bacteria [15]. A number of studies have focused on the use of probiotics in the field of aquaculture has been done on fishery commodities, such as study of probiotics, prebiotics, sinbiotics to improve the growth performance of tilapia (*Oreocormis niloticus*) [16]. Probiotics have good ability to stimulate appetite, improve the nutrient absorption and strengthen the host immunity [20]. Shailender *et al.* [20] reported that probiotics play an important role in growth, survival rate and diseases resistance. Ihsan *et al.* [8] has published the results of his research that increasing the production of tiger shrimp (*Penaeus monodon*) through the application of probiotics showed that probiotics also play an important role in maintaining water quality parameters, managing health and improving survival rate (SR) of shrimp. Similarly, Sivakumar *et al.* [19] has been done research on the probiotic effects of *Lactobacillus acidophilus* against vibriosis in young shrimp, and got the result that *L. acidophilus* as probiotic that able to control *V. alginolyticus* pathogens in shrimp aquaculture. *Bacillus spp* have the ability to potentially inhibit *Vibrio sp* in shrimp hatchery. Probiotics used in this study is a spontaneously fermented fruits that produces yeast and lactic acid bacteria (YELAB). Therefore, this research is focused to study the effect of spontaneous fermentation microbes (yeast and lactic acid bacteria) on the growth and survival of larvae tiger shrimp (*Panaeus monodon*).

The community of shrimp farmers in Aceh is very familiar with the culture of tiger shrimp than other species. But lately, the culture of *P. monodon* is difficult to do on the the intensive scale as before due to the high price of feed and susceptible to diseases. Therefore, YELAB fruit, a positive probiotics bacteria that is easily found and beneficial for aquatic biota. Feeding mixed with probiotics YELAB (yeast and lactic acid bacteria) fruit is expected to improve the growth, survival rate (SR) and digestibility of tiger shrimp and also increase the immunity of shrimp. However, it is not known exactly the concentration of YELAB fruit, the ratio of YELAB fruit to feed to produce the maximum growth and immun of tiger shrimp.

The specific objectives of this research are (1) to examine the growth and total length of larvae tiger shrimp cultured, (2) to know the survival rate (SR), feeding conversion ratio (FCR), and specific growth rate (SGR) of larvae tiger shrimp cultured, (3) to examine the host immunity of larvae tiger shrimp cultured, and (4) to improve the digestibility and development villy cells in intestine organ. The overall goal of this study is to contribute to the sustainable development of the *P. monodon*

farming sector in Aceh through development of feeding techniques by utilization probiotics of YELAB fruit that could lead to the commercialization of tiger shrimp.

2. Materials and Methods

2.1. Time and site

This research was conducted at Brackish Water Aquaculture (*Balai Budidaya Air Payau*, BPBAP), Ujung Batee, Aceh Besar district, Aceh. The study will be run over 10 months starting from Februari 2018 to November 2018.

2.2. Tools and materials

Tools and materials used in this study can be seen in the following table:

Table 1. Tools used in the study

No	Tool	Amount	Function
1.	Jar (volume = 120 L)	16 unit	Container for culture
2.	Plastic tank	1 unit	To Holds water
3.	Thermometer	1 unit	To measure temperature of water
4.	pH meter	1 unit	To measure water acidity
9.	Filter	1 unit	To filter dirt from water
10.	Aeration stones and tubes	32 unit	To supply oxygen levels
11.	Measuring cup	1 unit	To measure water volume
12.	Analytical balance	1 unit	To measure weight of larvae
13.	DO meter	1 unit	To measure dissolved oxygen
14.	Refractometer	1 unit	To measure salinity
18.	Blower	1 unit	To set oxygen levels
19.	Digital caliper	1 unit	To measure length of larvae
20.	Filter bag	1 unit	To filter of waste
21.	Injection 12 ml	1 unit	To feed of YELAB

Table 2. Materials used in this research

No	Materials	Amount	Function
1.	Tiger shrimp (PL 15)	1.920 unit	The biota for the research
2.	Coconut water	0,5L	Medium for bacteria
3.	Rebon flour	Sufficiently	Feed during cultured
4.	Clean water	18 litre	Solvent for treatment
5.	Sea water	Sufficiently	Medium for larvae cultured (PL 15)
7.	YELAB (active microbe)	24 ml	Active YELAB to be cultured/multiply
8.	Molase	0,5L	Nutrition intake for microbe development

2.3 Experimental design

This research used experimental method with Completely Randomized Design (RAL) with 4 treatment levels and 4 replications. Based on preliminary test results with concentrations of 50 ppm and 100 ppm, obtained the best treatment at a concentration of 100 ppm. Therefore, the treatment of advanced tests ranging from the lowest concentration of 50 ppm, 100 ppm and 150 ppm. The tested treatment is the concentration difference of YELAB fruit. The placement of the treatment container will be placed randomly. The treatments are:

Treatment A: without adding YELAB fruit (control)

Treatment B: feeding YELAB fruit 50 ppm 1 times per day

Treatment C: feeding YELAB fruit 100 ppm 1 times per day
Treatment D: feeding YELAB fruit 150 ppm 1 times per day

2.4 Procedure of research

2.4.1 Culturing of YELAB fruit. The raw materials used to culture YELAB fruit are coconut water, pure water, molasses and YELAB fruit (active microbes). YELAB of fruit starts from the provision of water of 18 L, molasses of 0.5 Liter, and coconut water of 0.5 L. Culturing is done by curing for 7 days in order to occur fermentation process.

2.4.2 Preparation of culture process. Container used in this research is a rectangular plastic container volume of 120 liters. Before maintenance, be cleaned first container of dirt. Then the container is filled with sea water through the pumping channel. In the container, there must be a pipe for water inlet and water exit.

Tiger shrimp larvae used in this study is the seed postlarvae with age of 15 days (PL 15) at a density of 1 fish/L. The spread of the larvae PL 15 is done in the morning which aims to avoid too high temperature changes. Spreading is done carefully so the larvae as not to stress with the environment, then acclimatized for 15 minutes in order to the larvae can adapt to their new environment. Feed to be given is artificial feed that is shrimp flour rebon blend until smooth and dried. Feeding is done by feeding the seeds at each aeration point in the container so that the biota is kept to eat evenly. During maintenance there is no water replacement. Frequency of feeding is 3 times a day that is at 8 a.m, 1 p.m and 6 p.m. Feeding with YELAB fruit is carried out by means of being spread at each aeration point. It is intended that the feed is spread evenly throughout the container. Frequency of feeding YELAB is 1 times a day in the morning at 8 a.m [21].

2.5 Research parameter

2.5.1 Total weight and total length. The weight and total length of 10 larvae randomly on each treatment are measured individually. The weight of larvae will be done by weighting using analytical balance. Total length of larvae will be done using digital caliper (in mm).

2.5.2 Growth and survival measurement. All shrimp are individually weighed at the end of each phase and the average weight is recorded for each tank. Growth of the tiger shrimp is calculated in terms of weight gain per day.

2.5.3 Specific Growth Rate (SGR). The daily growth rate (SGR) is the percentage of weight gain of fish each day during culture, the daily growth rate is indicated in percentage units (%). The daily growth of fish / shrimp will be calculated by the formula [11, 25].

2.5.4 Calculating survival percentage (SR). Survival Rate is calculated using Muchlisin et al. (2016).

2.5.5 Feed conversion ratio (FCR). Conversion of observed feed to calculate feed conversion and calculated based on the formula [11].

2.6 Data analysis

The data obtained will be analysed by ANOVA (analysis of varians) with *post hoc* Duncan comparison of mean scores using SAS software.

3. Results and Discussions

In general, the results showed that the administration of YELAB with different concentrations had a significantly different effect ($p < 0.05$) on the growth and survival of tiger shrimp seeds. Weight gain ranged from 0.0842 to 0.1426 g, the increase in length ranged between 2.51-3.29 cm, specific growth

rate (SGR) ranged between 13, 79% -15.55% per day, feed conversion ratio (FCR) ranges from 1.14 to 2.40 and survival (SR) reaches 82.29% - 95, 83% (table 4.1).

Table 3 data of weight gain, length increase, specific growth rate, survival and ratio of feed conversion of tiger shrimp seed (*Penaeus monodon*).

Code	Research parameters				
	weight gain (g)	length increase (cm)	specific growth rate (%)	survival (%)	FCR
A	0,0842 ± 0,003 ^a	2,51 ± 0,07 ^a	13,79 ± 0,12 ^a	82,29 ± 7,21 ^a	2,40 ± 0,23 ^a
B	0,0972 ± 0,010 ^b	2,95 ± 0,17 ^b	14,24 ± 0,37 ^{ab}	85,83 ± 5,73 ^{ab}	1,94 ± 0,13 ^b
C	0,0101 ± 0,006 ^{bc}	26,6 ± 1,19 ^{bc}	14,42 ± 0,21 ^{bc}	91,87 ± 5,74 ^{bc}	1,75 ± 0,17 ^{bc}
E	0,1426 ± 0,05 ^d	3,29 ± 0,17 ^d	15,55 ± 0,12 ^d	95,83 ± 3,96 ^d	1,14 ± 0,076 ^d

Description: Different supercript letters in the same column show significantly different (p <0.05). A = RABAL 0 ppm, B = RABAL 50 ppm, C = RABAL 100 ppm, D = RABAL 150 ppm

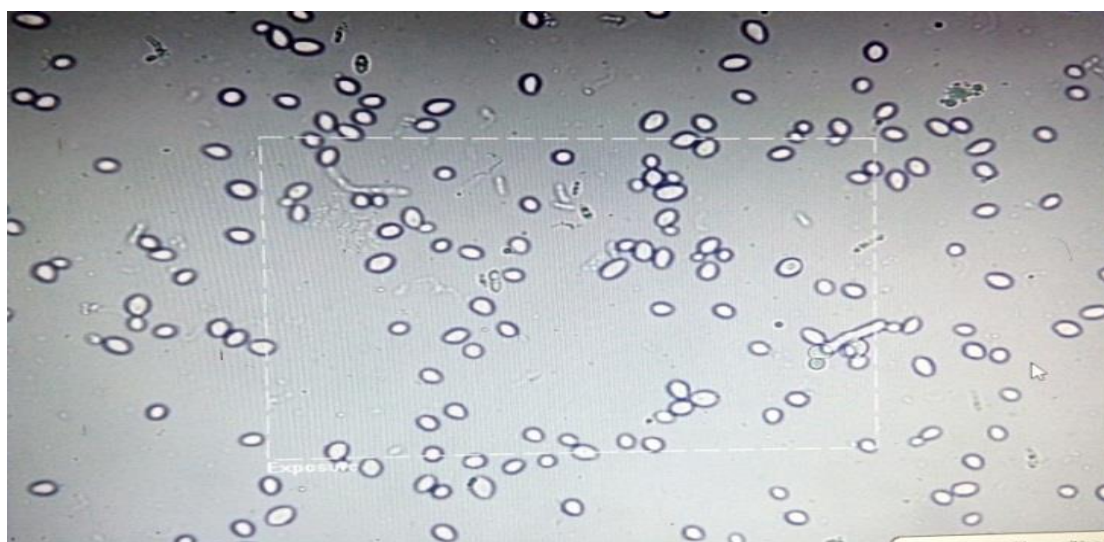


Figure 1. types of microbes (a. *Saccharomyces cerevisiae*, b. *Lactobacillus* sp.)

Results of ANOVA (Analysis of Variant) showed that the difference in concentrations of RABAL given fruit significantly (p <0.05) on weight gain, length increase, specific growth (SGR) feed conversion ratio (FCR) and survival of tiger shrimp seed but not significant effect (p > 0.05) on the physics quality of physics-chemistry (Table 3). The value of weight gain, length increase, specific growth rate and the highest survival rate were found in treatment D, ie at 150 ppm FRA fruit, this value was significantly different from treatment A = 0 ppm, B = 50 ppm, and C = 100 ppm (Table 3) and the lowest FCR value was also found in treatment D so that it can be seen that the best results for growth parameters were obtained in treatment D that was giving a concentration of 150 ppm of fruit RABAL as probiotics. The results of observations of RABAL fruit as probiotics used were yeast and lactic acid bacteria of the *Saccharomyces cerevisiae* microbes, and *Lactobacillus* sp. This type of microbial spontaneous fermentation has an effect on the growth and survival of tiger shrimp seeds (*Panaeus monodon*).

The results of measurements of the physicochemical parameters of water quality during the study. The water quality parameter values are still in a good and normal range for general fish growth (Table 4). Water quality parameters are important factors that must be considered in the maintenance of black tiger shrimp larvae. The water quality is closely related to growth and survival. In our study, the water quality was still within reasonable range of maintenance limits. The measured water temperature

ranges from 27°C-30.7°C, measured water pH ranges from 8-8.9 and DO ranges from 5.00 ppm to 6.08 ppm and salinity ranges from 25-35 ppt (data not shown). The optimal range of temperature, pH, DO and salinity for shrimp farming was 25-31°C; 6.5-8.0; 4-8 mg / l; and 15-35 ppt, respectively. This indicated that the range of values of those parameters of black tiger shrimp culture media were still tolerable range.

Table 4 Value of water quality measurement range

Code	Water quality parameter	Treatment			
		A(0 ppm)	B (50 ppm)	C(100 ppm)	D(150 ppm)
1.	TAN (NH ₃)	0,44	0,54	0,38	0,54
2.	Nitrate(NO ₃ -N)	31,80	25,95	14,80	22,85
3.	Nitrite (NO ₂ -N)	2,41	2,09	2,31	2,57
4.	Temperature (°C)	31,0	30,6	31,0	31,3
5.	DO (Mg/L)	5,3	5,1	5,3	5,2
6.	Salinity (ppt)	30	30	30	30
7.	pH	7,7	7,6	7,7	7,8

4.2. Discussion

The results showed that weight gain, length increase, specific growth rate (SGR), survival rate (SR) and feed conversion ratio (FCR) the best value was found in treatment D, namely giving 150 kg of RABAL concentration. Furthermore, without giving RABAL, fruits on tiger shrimp seeds give the opposite result, where without giving RABAL fruit, the growth of tiger shrimp will be lower.

a. Length- Weight

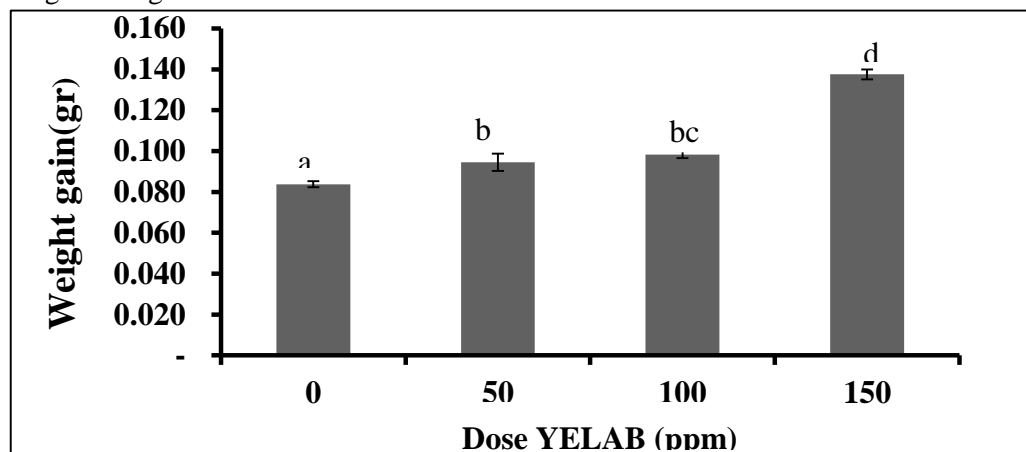


Figure 2 Weight gain diagram of tiger shrimp larvae (*Penaeus monodon*).

This shows that giving RABAL fruit with high concentration gives a positive influence on the growth and survival of tiger shrimp seeds. This is because in treatment B, C, and D contain probiotics that contain beneficial bacteria that can increase growth. Kompiang [10] stated that probiotics are compounds produced by a microorganism that can stimulate the growth of other microorganisms. Treatment of B, C and D contained lactobacillus lactic acid bacteria containing yeast. The content of yeast can also bind a variety of toxic substances that enter food into the body and throw it through the feces, so that fish can grow better because the body is toxic in soluble food that is wasted in feces.

The results of the two graphs above show that the increase in weight and body length of tiger shrimp larvae in treatment D tends to be higher, namely on weight gain (0.1426 g) compared to treatment (without giving RABAL fruit), namely (0.0842 g) and on addition the highest length is also

in treatment D (3.29 cm) and the lowest is in treatment A (2.51 cm). The effect of giving RABAL fruit at different concentrations on growth occurs because of controlling microbial balance in the digestive tract, increasing absorption of feed nutrients and improving the nutritional value of feed. It is also stated by Gullian *et al*, [3] that the presence of probiotics in the digestive tract can increase the synthesis of vitamins and factors that are able to maximize the activity of digestive enzymes in the digestive tract. In addition, an increase in growth can also be caused by an increase in feed nutrition (especially protein).

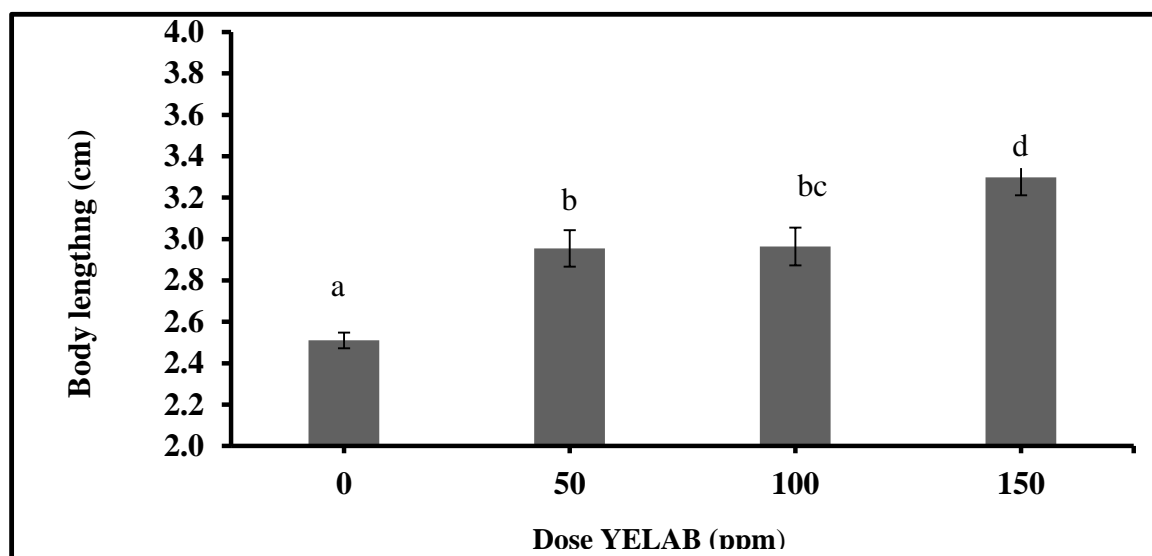


Figure 3 Diagram of body length of tiger shrimp (*Penaeus monodon*)

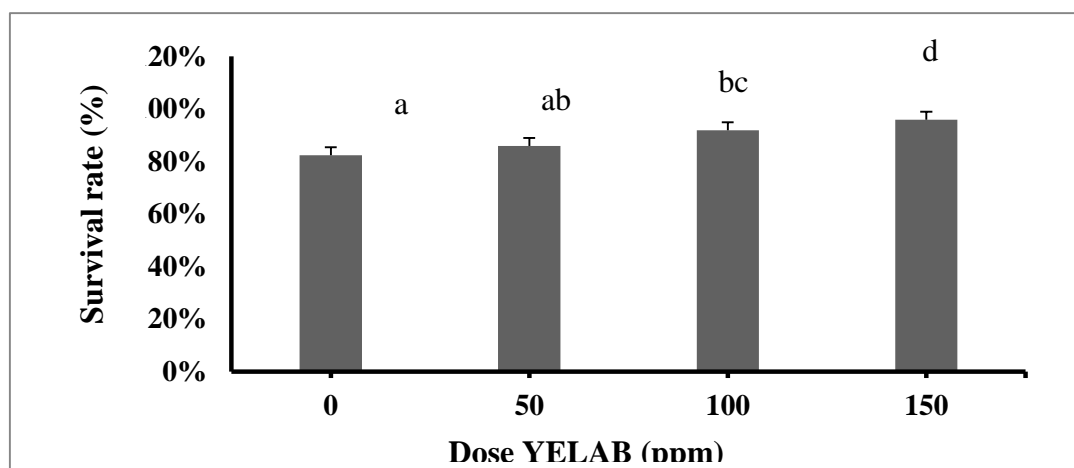


Figure 4 Percentage diagram of survival rate of tiger shrimp (*Penaeus monodon*)

b. survival rate

The survival value of tiger shrimp seed showed a significantly different result ($p < 0.05$) where in each treatment the giving of RABAL fruit got a different value, in treatment A (without giving

RABAL fruit) the value of survival (82.29%), treatment B 50 ppm RABAL fruit (85.83%), treatment C 100 ppm RABAL fruit (91.87%) and treatment D 150 ppm RABAL fruit reached (95.83%). The value of treatment D in giving 150 ppm of RABAL reached a higher value compared to treatment A (without giving RABAL fruit) or probiotics. The survival of tiger prawns tends to increase in treatments using probiotics compared to controls (without probiotic users). This is in line with the results of trials and field observations carried out that by applying probiotics in intensive shrimp culture can have a real influence on the survival of vanname shrimp. Furthermore, it was also explained that the average survival rate of vanname shrimp in the treatment of probiotic applications (79% -80%) tends to be higher when compared to the control treatment without probiotic application) which is 67%. The administration of commercial probiotics with a concentration of 0.5-1.5 mg / L / week in the medium of maintenance of vanname shrimp produced a survival rate of 92.33% -94.33% higher than the control treatment (without probiotics).) with 86.33% syntax. This is because tiger shrimp can adapt to the environment and utilize microbes provided through intermediate water media and water quality during controlled maintenance in optimal conditions.

c. Feeding Conversion Ratio (FCR)

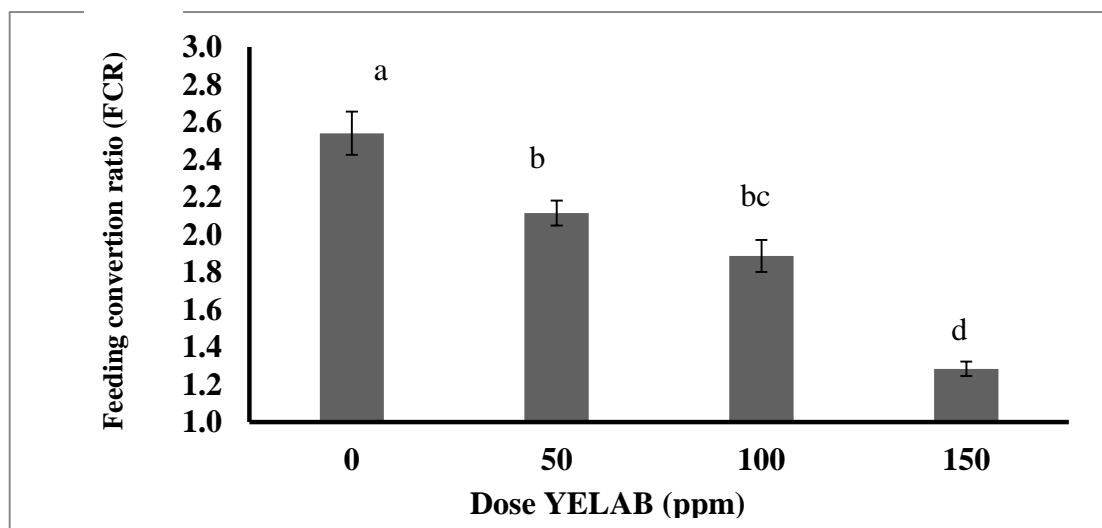


Figure 5 Feed Conversion Ratio Diagram of Tiger Shrimp larva (*Penaeus Monodon*)

Feed conversion ratio (FCR) is an indicator to determine the effectiveness of feed and is one of the parameters used to describe the amount of feed that can be utilized by aquaculture organisms. In (Figure 5) it can be seen that the giving of RABAL fruit on the maintenance water media gives an influence on the FCR value and is significantly different ($p < 0.05$) against treatment A (without giving RABAL fruit). In treatment A (without giving RABAL fruit) got FCR (2.40), B = 50 ppm (1.94), C = 100 ppm (1.75) and D = 150 ppm (1.14). The treatment of D is the lowest feed conversion ratio due to the highest increase in body weight of shrimp seed treatment with other treatments. The comparison of the total amount of feed given with the resulting weight gain is the feed conversion ratio. The value of the feed conversion ratio is inversely proportional to the weight gain, so the lower the value the more efficient the shrimp is in utilizing the food it consumes for growth. The low value of the feed conversion ratio is due to the role of *Bacillus* sp. in the form of probiotics that can produce extracellular enzymes in increasing the digestibility of food ingredients in shrimp intestines so that it is easily absorbed by the body of vanname shrimp.

The addition of RABAL fruits from spontaneous fermentation with different concentrations during maintenance significantly affected ($p < 0.05$) the growth and survival of tiger shrimp. The addition of RABAL through water media shows the active role of microbes in the digestive tract. Probiotics of

yeast and lactic acid bacteria found are *Lactobacillus* sp and *Saccharomyces cerevisiae* microbes which move when they enter the digestive tract, which is growing and colonizing. The difference in concentration of microbes contained in RABAL fruit or probiotics can affect the absorption of nutrients of tiger shrimp because the higher the concentration of *Lactobacillus* sp and *Saccharomyces cerevisiae* in the digestive tract will increase the availability of nutrients that are readily absorbed in the digestive tract through hydrolysis of proteins into simpler compounds, namely amino acids, so that metabolism becomes easier because the absorption of protein is helped by the presence of protease enzymes. This is consistent that enzymes secreted increase in number according to the amount of probiotics present in the digestive tract, which will increase the amount of food digested. This increase in digestibility means that more nutrients are available to be absorbed by the body. This statement is in accordance with Gatesoupe's [4] statement that the activity of bacteria in digestion will change rapidly if there are microbes entering through food or water which cause a change in the balance of bacteria that already exist in the intestinal tract (digestive tract) with incoming bacteria. There is a balance between the bacteria of the digestive tract of fish causing probiotic bacteria to be antagonistic to pathogenic bacteria so that the digestive tract of fish is better at digesting and absorbing feed nutrients. This indicates that the addition of RABAL fruit into the maintenance medium can improve the growth of cultivated tiger shrimp.

The low growth and graduation of tiger shrimp life obtained in treatment A (without giving RABAL fruit) is due to lack of activity of bacteria that break down dissolved organic matter in the maintenance medium that comes from the remaining feed and shrimp stool itself. Thus, fertilization of organic matter occurs which increases with the length of time of maintenance. In addition, organic materials that accumulate cannot be wasted because they are not replaced by water during maintenance. This can cause shrimp stress and affect growth which results in the low graduation of tiger shrimp produced. According to Gunarto [5], the use of probiotics is able to minimize the impact of waste generated from the shrimp culture industry due to the activity of microorganisms contained in probiotics such as *Bacillus* sp. and *Pseudomonas* sp. The microorganisms contained in probiotics are able to decompose organic matter from the remaining feed and feces quickly so that there is no excessive accumulation in the pond bottom.

Water quality parameters are important factors that must be considered in maintaining tiger shrimp seeds, because water quality is closely related to the growth and survival of tiger shrimp seeds. The results of the variance test (ANOVA) showed that the treatment given RABAL fermented fruit was not significantly different ($p > 0.05$) on the water quality parameters of maintenance media, which showed the range of each treatment was relatively the same. The same thing was also done by Basir and Suriati (2013), regarding the use of prebiotics and probiotics in artificial feed to the efficiency of feed and water quality of medium maintenance of vaname shrimp (*Litopenaeus vannamei*) which states that probiotic and prebiotic use does not have a real influence on water quality media maintenance of vanname shrimp, and in this study water quality did not experience significant changes, therefore the water quality in the research container was still in the normal range.

4. Conclusion

Based on the results of the research that has been carried out, it can be concluded that the administration of RABAL (Yeast and Lactic Acid Bacteria) fruit produced by spontaneous fermentation given through water media at different concentrations had a significant effect ($p < 0.05$) on the growth and survival of shrimp seeds windu (*P.monodon*) and can reduce feed conversion, but no significant effect on water quality parameters, where the best fruit RABAL concentration in this study was at a concentration of 150 ppm.

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