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THE APPLICATION OF YEAST AND LACTIC ACID BACTERIA IMPROVE GROWTH AND INTESTINAL STRUCTURE OF TIGER SHRIMP LARVAE *Penaeus monodon* Fab.

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ABSTRACT

This study aims to investigate the effect of the different concentration of RABAL (yeast and lactic acid bacteria) probiotics on the survival, feed conversion, growth and intestinal cells changes of tiger shrimp *Penaeus monodon* Fab. larvae. This study used a Completely Randomized Design (CRD) method with 4 treatments and 4 replications. Tiger shrimp with average size of 0.02 g was randomly stocked in each container containing RABAL with concentration of 0 ppm (A/control), 75 ppm (B), 150 ppm (C), and 225 ppm (D) with frequency once a day for 30 days. Data were analysed using one way ANOVA. The result showed that the application of RABAL with different doses into the shrimp culture media significantly influences ($P<0.05$) the growth and food conversion ratio of tiger shrimp feed but did not significantly affect ($P> 0.05$) on the survival rate. The value of water quality parameters in this study were still at normal levels for tiger shrimp rearing. As conclusion, the application of probiotics RABAL with different concentrations have positive effect on the absolute weight, absolute length, specific growth rate, feed conversion ratio, and intestinal villi surface area of tiger shrimp larvae, without adverse effect on the survival rate of tiger shrimp seed.

Key words: fruit-based lactic acid bacteria (RABAL), tiger prawns, yeast

ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh perbedaan konsentrasi probiotik RABAL (ragi dan bakteri asam laktat) terhadap kelangsungan hidup, konversi pakan, pertumbuhan dan perubahan sel usus udang windu Penaeus monodon Fab. larva. Metode riset menggunakan metode Rancangan Acak Lengkap (RAL) dengan 4 perlakuan dan 4 ulangan. Udang windu ukuran rata-rata 0,02 g ditebar secara acak pada setiap wadah yang berisi RABAL dengan konsentrasi 0 ppm (A/kontrol), 75 ppm (B), 150 ppm (C), dan 225 ppm (D) dengan frekuensi 1 kali sehari selama 30 hari. Data dianalisis menggunakan one way ANOVA. Hasil penelitian menunjukkan bahwa aplikasi RABAL dengan dosis berbeda pada media budidaya udang berpengaruh nyata ($P<0,05$) terhadap pertumbuhan dan rasio konversi pakan udang windu tetapi tidak berpengaruh nyata ($P>0,05$) terhadap kelangsungan hidup. Nilai parameter kualitas air pada penelitian ini masih dalam taraf normal untuk pemeliharaan udang windu. Sebagai kesimpulan, aplikasi RABAL probiotik dengan konsentrasi berbeda berpengaruh positif terhadap berat mutlak, panjang mutlak, laju pertumbuhan spesifik, rasio konversi pakan dan luas permukaan vili usus larva udang windu, tanpa berpengaruh buruk terhadap kelangsungan hidup benih udang windu.

Kata kunci: bakteri asam laktat berbahan dasar buah (RABAL), udang windu, ragi

INTRODUCTION

The coastal area of Aceh province has abundant potential for fisheries resources (Putra 2022a). Shrimp resources are one of the important fishery commodities in Indonesia, especially in Aceh Province. Several species of shrimp found in Aceh including *Penaeus monodon*, *Litopenaeus vanname*, *Metapenaeus ensis*, *Penaeus merguiensis and Exopalaemon styliferus* (Baleta *et al*. 2013; Muhammadar *et al*. 2018; Putra *et al*. 2018a; Putra *et al*. 2018b; Putra *et al*. 2020a). Tiger shrimp, *Penaus monodon* is one of the primadona shrimps of non-oil and gas export commodities from the fisheries sector with the main destination countries such as Japan, Europe and the United States (Rahmitasari 2018). The shrimp nutrition contains of complete essential amino acids and some small amount of fat (Amri 2003). Tiger shrimp cultivation thus becomes one of the popular commodities that have been chosen by shrimp farmers. To support this shrimp culture activity, it is necessary to increase the provision of sustainable shrimp larvae. The success of shrimp culture is determined by the ability of shrimp tolerance to environmental changes, resistance to pest and disease attacks and the suitability of the feed provided (Putra, 2022b). The provision of feed with sufficient nutrition as well as the addition of probiotics is able to facilitate digestion in fish (Ray *et al*. 2012). The use of probiotics can be applied by mixing in feed, in order to balance the condition of microbiota in the digestive tract and to make the absorption and digestion process getting better (Verschuere *et al*. 2000). Probiotics living in intestinal habitat can provide benefits for the host since it can improve the balance of microbes in the digestive tract. One of the bacteria that can be used as a probiotic is Lactobacillus.

Several previous studies related to the application of feed and health to tiger shrimp have been carried out, such as the provision of sea grape, *Caulerpa lentillifera* flour in feed (Putra *et al*. 2019), histopathology of tiger shrimp in traditional ponds culture (Putra *et al*. 2021), the addition of different live feed in shrimp larvae (Putra *et al*. 2020b), the addition of eggshell flour to shrimp feed (Safriani *et al*. 2019) and the supplementation of yeast and lactic acid bacteria fruit based (RABAL) to tiger shrimp larvae (Muhammadar

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et al. 2018). However, the information regarding the addition of fruit-based probiotic particularly RABAL on growth of the tiger shrimp larva is still limited. The effectiveness of probiotics has a beneficial effect on the host because of its non-pathogenic, non-toxic nature as well as its ability to survive and carry out metabolic activities in the intestine (Douglas and Sanders 2008). The application of RABAL probiotics aims to investigate the effect on growth, the ratio of feed and villi of tiger shrimp. Villi is an area of nutrients absorption, the higher the area of villi, the more food substances will be absorbed and will have a good impact on the body growth of shrimp. Growth of shrimp intestinal villi will be disrupted when pathogenic microbes increase rapidly. Hence, this study aims to investigate the effect of dietary RABAL with different doses on the digestibility and changes in intestinal cells of tiger shrimp through histological observations. This study is expected to provide the information about the effect of the RABAL on the growth and intestinal changes of tiger shrimp in laboratory scale.

MATERIALS AND METHODS

Experimental Design

This research was conducted at Center for Brackhiswater Aquaculture (BPBAP) Ujung Batee, Aceh Besar District, Aceh Province. Histomorphometric measurements were conducted at the Pathology Laboratory of the Veterinary Faculty, Syiah Kuala University.

This research used experimental method to completely randomized design (CRD) with 4 treatments and 4 replications. Previous study had been carried out to evaluate the effect of RABAL probiotic with concentrations of 50 ppm, 100 ppm and 150 ppm on tiger shrimp performance which obtained the best result at a concentration of 150 ppm (Muhammadar *et al*. 2018). Therefore, in this study, different concentration was used with concentration of 0 ppm (treatment A), 75 ppm (treatment B), 150 ppm (treatment C) and 225 ppm (treatment D).

RABAL Culture

RABAL culture was conducted by mixing 0.5 L molasses, 0.25 L coconut water and 0.25 L RABAL into 9 L of water, the mixture was then fermented for 4 days. The application of RABAL was carried out by spreading at aeration point in the culture media. Then the RABAL spreaded throughout the container with the frequency of RABAL application once a day at 13.00 WIB (Muhammadar *et al.* 2018).

Shrimp Rearing

A postlarva larvae (PL 24) was obtained from Develoment Center of Brackhiswater Aquaculture (BPBAP) Ujung Batee, Kabupaten Aceh Besar. As many as 60 tiger shrimp seed (0.2 mg) was randomly stocked in a rectangular plastic container measuring 40 x 60 x 60 cm^3 equipped with inlet and oulet pipes, with a stocking density of 1 shrimp /L were. Prior to shrimp stocking, the container was cleaned then filled with sea water through an existing pump channel. Rearing condition and feeding were carried according to Muhammadar *et al*. (2018). Water quality was analysed every 7 days.

Growth Performance Analysis

The growth parameters measured was absolute weight growth, absolute length, Specific Growth Rate (SGR), Survival Rate (SR), and Feed Conversion Ratio (FCR).

Absolute growth is the difference in total body weight of shrimp at the end and beginning of rearing. Calculation of absolute weight growth using the formula of (Huang *et al*. 2008) as follow:

$$\mathbf{W = Wt - Wo}$$

Where in:
W = Absolute weight growth (g)
Wt = Fish weights at the end of the experiment (g)
Wo = Fish weights at the beginning of the experiment (g)

Accroding to Zonneveld *et al*. (1991) calculation of absolute length growth (L) as follows:

$$\mathbf{L = L_t - L_0}$$

L = Absolute total length (cm)
L_t = The average length of the end of the study (cm)
L_0 = The average length of the beginning of the study (cm)

SGR is the percentage weight gain of fish every day during the culturing, the daily growth rate is expressed in units of percentage (%). Daily growth of fish/shrimp will be calculated by the formula given by Kusriani *et al*. (2012).

$$\mathbf{SGR = \frac{Ln\ Wt - Ln\ W0}{t} X\ 100}$$

SGR = Specific Gowth Rate (%)
Wt = Fish biomass test at the end of the study (gr)
Wo = Fish biomass test at the beginning of the study (gr)
T = Time (days)

SR calculated using the formula of Muchlisin *et al.* (2016) as follows:

$$\mathbf{SR = \frac{Nt}{No} x\ 100}$$

SR = Survival Rate (%)
Nt = The number of living fish at the end of the study
No = The number of living fish at the initial of the study

Feed conversion using the formula of Kusriani *et al*. (2012) as follows :

$$FCR = \frac{F}{Wt - W0} \times 100$$

FCR = Feed Conservation Ratio (%)
F = The amount of feed given (g)
Wt = Shrimp biomass weights at the end of culturing (g)
W_0 = Shrimp biomass test weight at the beginning of culturing (g)

Histology Observation

Tiger prawn intestines were removed at the end of the study. Three shrimps from each treatment were collected randomly from the experimental tanks. The intestines were removed with scalpel scissors and preserved in bouin solution. Histological sampling was carried out using the paraffin method based on Putra *et al*. (2012). Samples were dehydrated through an alcohol sequence and cleaned in xylol. Next, gut and gut samples were embedded in paraffin. The paraffin block was cut to 6 µm, and the sections were stained with hematoxylin and eosin. The size (height and width) of the villi was determined using a binocular microscope (Olympus). Every effort is made to reduce harm to animals by adhering to the ethical guidelines for using animals in the research at the University of Syiah Kuala.

Analysis Data

Data were analysed using one way Analysis of Variance (ANOVA) and followed by Duncan Multiple Range Test (DMRT)

RESULT AND DISCUSSION

Statistically, the results showed that the application of RABAL probiotics with different concentrations on tiger shrimp seed significantly affected (P<0.05) the absolute weight, absolute length, specific growth rate, feed conversion ratio and intestinal vili of tiger shrimp, *P. monodon* larvae. However, it did not significantly affect (P>0.05) the survival rate of tiger shrimp seed (Figure 1-6). In this study, the intestinal villi height, weight growth, the absolute length and growth rate of tiger shrimp larvae were significantly increased. Neti (2008) reported that Lactic Acid Bacteria (LAB) is one of the organisms that can ferment food through carbohydrate fermentation and generally produces large amounts of lactic acid. This bacterium contributes significantly to the improvement of flavor, texture, and shelf life of fermented products. Antibacterial agents such as lactic acid and bacteriosin which are produced by probiotic bacteria can inhibit the growth of pathogenic bacteria, moreover this antibacterial agent is able to reduce pH level, and thus the pathogenic bacteria have difficulty in developing their life

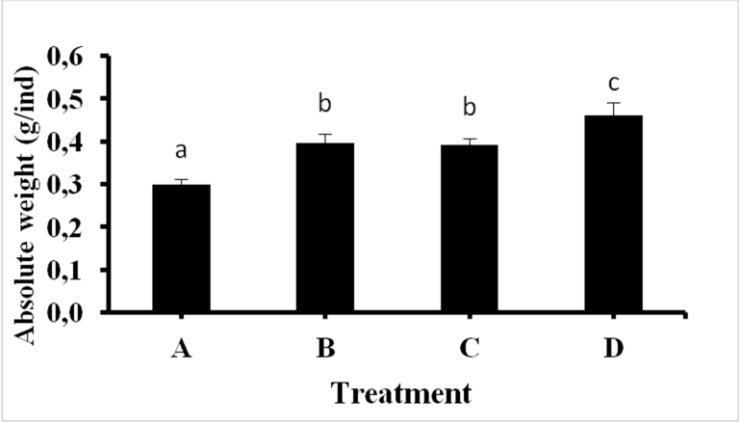

Figure 1. Absolute weight of tiger shrimp (*P. monodon*) in different treatment. [a,b,c]Different alphabet on the bar chart showed a difference (P<0.05) among treatments

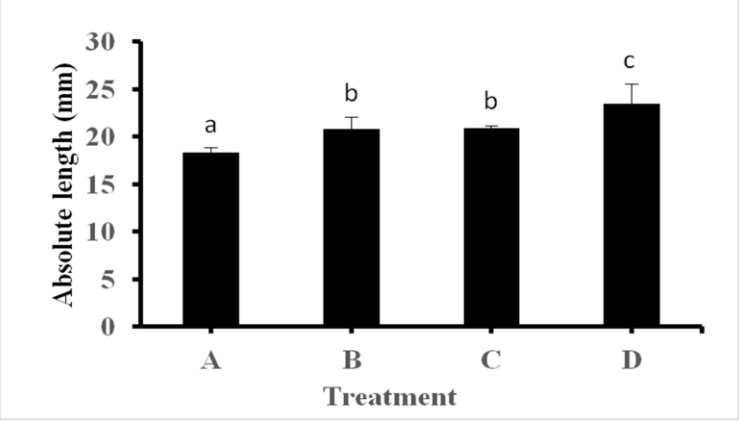

Figure 2. Absolute length of tiger Shrimp (*P. monodon*) in different treatment. [a,b,c]Different alphabet on the bar chart showed a difference (P<0.05) between treatments

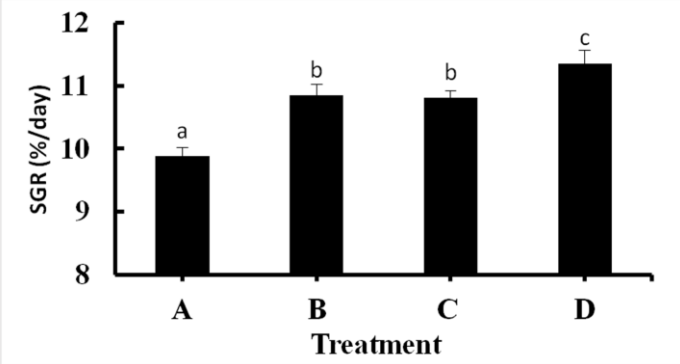

Figure 3. Specific Growth Rate (SGR) of tiger shrimp (*P. monodon*) in different treatment.[a,b,c]Different alphabet on the bar chart showed a difference (P<0.05) between treatments

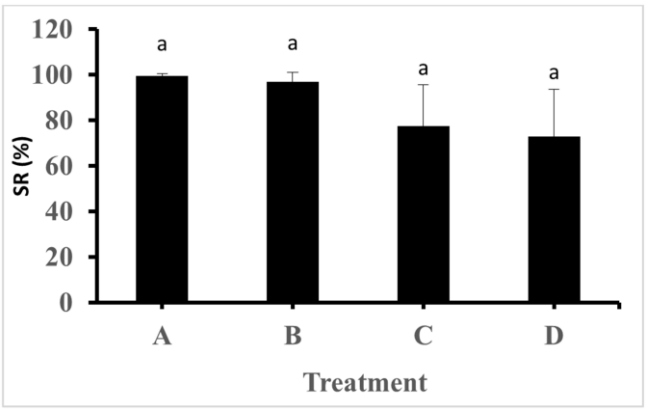

Figure 4. Survival Rate of tiger shrimp (SR) (*P. monodon*) in different treatment. [a]Different alphabet on the bar chart showed a difference (P<0.05) between treatments

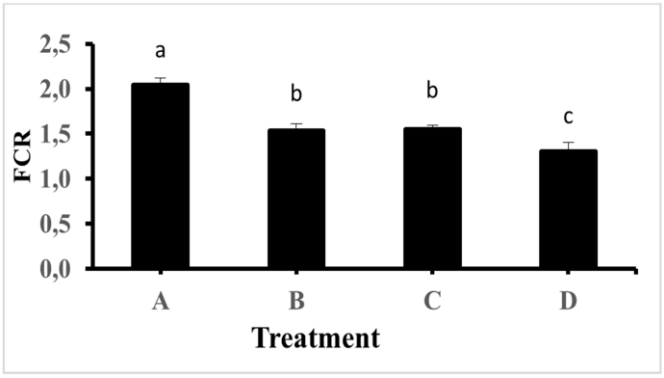

Figure 5. Feed Conversion Ratio (FCR) of tiger shrimp (*P. monodon*) in different treatment. [a,b,c]Different alphabet on the bar chart showed a difference (P<0.05) between treatments

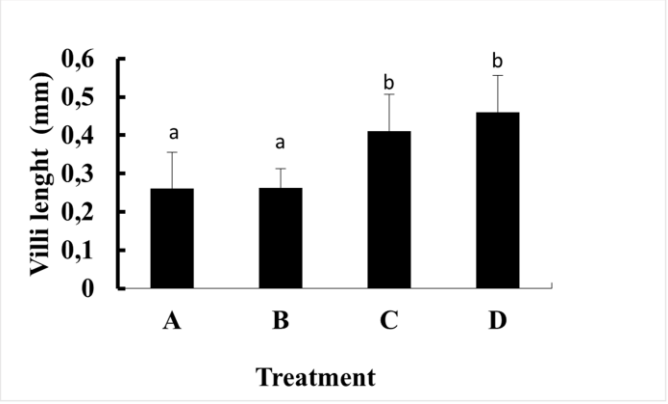

Figure 6. Villi lenght of tiger shrimp (*P. monodon*) in different treatment. [a,b]Different alphabet on the bar chart showed a difference (P<0.05) between treatments

(Tambekar and Bhutada 2010). This proved that the application of RABAL probiotics can improve nutritional content and help the growth of tiger shrimp so that it improves digestive function and increases intestinal villi height.

This study revealed that the application of RABAL with different concentrations significantly affect the vili length of tiger shrimp (Figure 7), which the highest value was found in treatment D (225 ppm). This observation indicated that the higher dose of RABAL, the higher vili lenght of tiger shrimp intestines. It was assumed that RABAL suppressed the growth of pathogens in the digestive tract, thus improved the intestinal work system. As Mansyur and Tangko (2008) stated that the use of probiotics in aquaculture aims to maintain microbial balance and suppress the activity of pathogens in the digestive tract, as well as improves the aquatic environment through the biodegradation process and is able to cause an increase in enzyme activity in the digestive tract, which can increase digestibility. Sari *et al.* (2016) added that the administration of fermented material can increase the proliferation process of epitelial cells in the small intestine villi and as consequence increase the height and width of the villi. Mile *et al.* (2006) investigated that the large area of the absorption of nutrients contained in the bloodstrean is influenced by the extent of the surface in the intestine, therefore increasing the height intestinal villi in line with the increase in digestive function.

The weight gain and length of tiger shrimp larvae in treatment D (225 ppm) tends to be higher over other treatment. Widanarni *et al.* (2010) claimed that the use of probiotics has a positive impact on the length and weight and survival rate of shrimp. Similarly, Muhammadar *et al.* (2018) also observed that the use of RABAL probiotics with high concentrations has a positive impact on the growth and survival of tiger shrimp larvae. In addition, Widanarni *et al.* (2013) found that the length and weight of tiger shrimp post larvae increases with age and dose of probiotics given during the culture period. The effect of fruit-based RABAL application at different concentrations on the length and weight growth of tiger shrimp was assumed due to the control of microbial balance in the digestive tract and increased nutrition. Kaligis *et al.* (2009) explained that the administration of pobiotics can improve the balance of intestinal microflora (Kumar *et al.* 2017), thus providing the benefits of disease protection and improvement of digestibility (Feliatra *et al.* 2004). Moreover, Hai (2015) stated that the provision of probiotics in the culturing of tiger shrimp has many benefits, especially for absorbing nutrients from feed.

The current study also reported that the RABAL probiotic application did not significantly affect the survival of tiger shrimp. The survival rate of tiger shrimp in the culturing postlarva affected by several factors, such as density, quantity and quality of feed, aquaculture environment and other factors. Kholifah *et*

Table 1. Water quality measurement during shrimp rearing

Parameter	Value			
	Treatment A	Treatment B	Treatment C	Treatment D
Temperature (° C)	28.3-29	28.5-29	28.5-29	28.5-29
Salinitas (ppt)	29-31	30-31	30-31	30-31

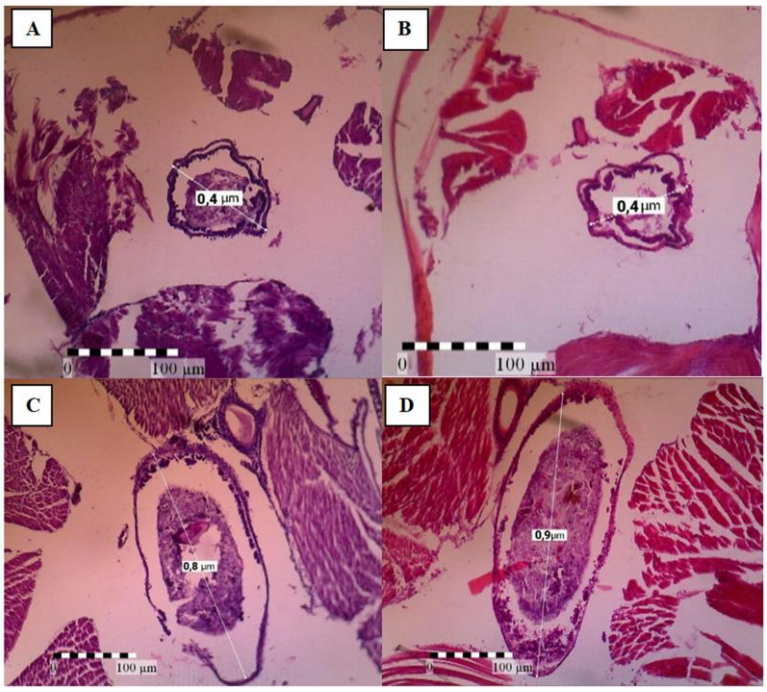

Figure 7. Histological observation of the intestinal villi lenght of tiger shrimp (*P. monodon*) in different treatment. 0 ppm (treatment A), 75 ppm (treatment B), 150 ppm (treatment C) and 225 ppm (treatment D) mag. 40x

al. (2008) stated that the high density of shrimp larvae can cause variations in seed death that is different, as a result of the nature of shrimp cannibals. While Yúfera and Darias (2007) reported that the larval rearing needs serious treatment in terms of feeding and water quality management. Based on the research results, the survival rate of tiger shrimp survival in treatment A, B, C and D were 99%, 97%, 75% and 72%, respectively. The highest survival rate was obtained in the treatment A (without the administration of RABAL), while the lowest was in the treatment D. This resut was in line with Usman and Rochmady (2017) who reported that the administration of probiotics in different doses gave no significant effect on the survival rate of tiger shrimp post.

The feed conversion ratio (FCR) is defined as the ratio between the amount of feed consumed and the weight gain of fish or shrimp. The lowest FCR value was observed in treatment D (225 ppm). The low value of feed conversion ration might be influenced by the role of probiotic bacteria contained in RABAL Another factor that affects the growth and survival of tiger shrimp is water quality. The size of the changes in water quality can affect the functional and structural properties of post larvae of shrimp that are kept. When changes occur, the post-larvae shrimp will perform osmoregulation mechanism to maintain the balance of body fluids to the external environment. Some water quality parameters include temperature and salinity. According to Harefa (1996), the factors that most influence the level of life of post-tiger shrimp larvae life are water quality in the maintainance media and feed quality. Good water quality in maintenance media is a factor that supports metabolic processes in physiological processes and accelerates skin changes that can facilitate the osmoregulation process. ANOVA test results showed that the water quality in RABAL treatments were not significantly different ($P>0.05$) compared to the water quality in treatment A (without RABAL administration), it showed that the range of each treatment was relatively similar (Table 1).

According to Said (2007) the optimal temperature of shrimp larvae growth between 26-32° C, the optimal pH range of tiger shrimp larvae maintenance is 7.8-8.8 and the optimal salinity range is 24 ppt-35 ppt tiger shrimp larvae. Purba (2012) stated that the suitable temperature for shrimp larvae growth is between 25-32° C, the good acidity (pH) for shrimp culture is 7.4-8.9 and the range of good salinity for shrimp hatchery is 15-34 ppt. Added the statement from Komarawidjaja (2006), that the range of water temperature on the growth of tiger shrimp post larvae is around 26-32° C.

CONCLUSION

The application of probiotics RABAL with different concentrations have positive effect on the absolute weight, absolute length, specific growth rate, feed conversion ratio and intestinal villi surface area of tiger shrimp larva, without adverse effect on the survival rate of tiger shrimp seed. A dose of 225 ppm RABAL probiotics is effectively recommended to obtain the optimum growth for tiger shrimp larvae.

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