

# The effect of different additions of curcuma extract (*Curcuma xanthorrhiza* Roxb) in artificial feed on the growth and immunity of Juvenile Tiger Shrimp (*Penaeus monodon*)

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## ABSTRACT

The objective of this study was to determine the effect of curcuma extract addition in artificial feed on growth and immunity of juvenile tiger shrimp (*Penaeus monodon*). The research material used was juvenile tiger shrimp at PL30 size. The study was conducted using a completely randomized design with 5 treatments, they are: without the addition of curcuma extract (A), addition of 1 mL of curcuma extract (B), addition of 1.5 mL of curcuma extract (C), addition of 2 mL of curcuma extract (D), addition of 2.5 mL of curcuma extract (E). On the 21<sup>st</sup> day a challenge test for *Vibrio* sp. bacteria was carried out with a concentration of LC 50. The results of the study showed that the addition of immunostimulants gave a significant effect on the survival rate. The highest value to the lowest value was B treatment was  $90.00 \pm 3.33\%$ ; C treatment was  $88.89 \pm 1.92\%$ ; D treatment was  $80.00 \pm 3.33\%$ ; E treatment was  $77.78 \pm 1.92\%$  and A treatment was  $54.44 \pm 3.85\%$ .

**Key words :** *Curcuma extract, Immunostimulants, Tiger Shrimp, Vibriosis bacteria.*

## Introduction

Shrimp is one of the leading commodities in the field of fisheries. In Indonesia, shrimp is a prima donna of non-oil and gas exports, especially tiger shrimp (*Penaeus monodon* Fab.). Tiger shrimp is still a mainstay commodity in the fisheries sector, even though Indonesia's last production only reached 50,000 tons or year. It was reported that exports of fish or shrimp aquaculture were 90,500 tons, taking into account the condition of the existing coastal areas, the opportunity to develop fish or shrimp aquaculture was still very high, as of the end of 1999 the utilization of fish and shrimp cultivation in ponds only amounted to 393,193 hectares (Director-

ate General of Aquaculture, 2011).

The "luminous bacteria" disease of shrimp is found in the rainy season. Outbreak occurs when changes in salinity cause the shrimp to be more easily stressed so that bacterial attacks are more easily spread. Vibriosis transmission is quite fast so it can increase the value of mortality in a pond within 1-2 days (Amri, 2003).

Of the various types of medicinal plants that have many benefits, curcuma is a plant that is very much beneficial because it has various useful substances. Curcuma (*Curcuma xanthorrhiza* Roxb) is an annual plant that can increase appetite and increase the immunity of a living thing (Ozaki, 1990).

The purpose of this study was to determine the

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effect of curcuma extract addition in artificial feed for tiger shrimp on survival and growth of juvenile tiger shrimp PL30-PL50 and its effect on immunity of the juvenile tiger shrimp.

## Materials and Methods

The experimental design used in this study was a Completely Randomized Design (CRD) with 5 treatments and 3 replications for each of the treatment. The treatment given is the difference in protein doses, they are:

1. Treatment A, addition of 0 mL of curcuma extract dose
2. Treatment B, addition of 1 mL of curcuma extract dose
3. Treatment C, addition of 1.5 mL of curcuma extract dose
4. Treatment D, addition of 2 mL of curcuma extract dose
5. Treatment E, addition of 2.5 mL of curcuma extract dose

Parameters observed included relative growth rate (RGR), feed conversion ratio (FCR), feed utilization efficiency (FUE), protein efficiency ratio (PER), survival rate (SR), and water quality. Analysis of these parameter variables is first tested for normality, homogeneity, and additivity to determine the variance of data, if it meets the assumptions, then analysis of the variance would be done to determine the effect of treatments. If a significant effect is obtained, the Dunnett's multiple comparisons test is carried out to find out which treatment has different effects towards particular parameters. The composition of feed ingredients is presented in Table 1.

### Relative growth rate (RGR)

Relative growth rate in this study was calculated using the formula by Tacon (1987) as follows:

$$RGR = \frac{W_t - W_0}{W_0 \times t} \times 100\%$$

Description:

RGR = Relative growth rate (%/day)

$W_0$  = Biomass weight of tested shrimp at the beginning of the experiment (g)

$W_t$  = Biomass weight of tested shrimp at the end of the experiment (g)

t = Length of experiment (day)

**Table 1.** The composition of feed ingredients

Number	Ingredients	100 g
1.	Soybean Meal	37.43
2.	Fish Meal	3.89
3.	<i>Acetes indicus</i> Meal	5.99
4.	Milkfish innards meal	11.67
5.	Dextrin Meal	24.02
6.	Fish Oil	6
7.	Corn Oil	8
8.	Lecithin	1
9.	Vitamin Mix	1
10.	Mineral Mix	0.5
11.	CMC	0.5

### Feed conversion ratio (FCR)

Feed conversion ratio in this study was calculated using the formula from Tacon (1987) as follows:

$$FCR = \frac{F}{(W_t + D) - W_0}$$

Description:

FCR = Feed conversion ratio

F = The amount of feed given to tested shrimp during the experiment (g)

$W_0$  = Biomass weight of tested shrimp at the beginning of the experiment (g)

$W_t$  = Biomass weight of tested shrimp at the end of the experiment (g)

D = The weight of died shrimp during experiment (g)

### Feed utilization efficiency (FUE)

Feed Utilization Efficiency can be determined using the formula by Tacon (1987), as follows:

$$FUE = \frac{W_t - W_0}{F} \times 100\%$$

Description:

FUE = Feed Utilization Efficiency (%)

$W_0$  = Biomass weight of tested shrimp at the beginning of the experiment (g)

$W_t$  = Biomass weight of tested shrimp at the end of the experiment (g)

F = The amount of test shrimp feed consumed during the experiment (g)

### Protein efficiency ratio (PER)

Protein efficiency ratio can be determined using the formula by Tacon (1987), as follows:

$$PER = \frac{W_t - W_0}{P_i} \times 100\%$$

Description:

PER = Protein efficiency ratio (%)

W<sub>0</sub> = Biomass weight of tested shrimp at the beginning of the experiment (g)

W<sub>t</sub> = Biomass weight of tested shrimp at the end of the experiment (g)

Pi = The amount of test feed consumed is multiplied by the protein content of the test feed

**Survival rate (SR)**

Survival Rate (SR) calculated to determine the survival rate of test animals during experiment, Survival Rate can be determined using the formula by Effendi (1997):

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

Description:

SR = Survival Rate (%)

N<sub>0</sub> = Number of test shrimp at the beginning of the experiment (shrimp)

N<sub>t</sub> = Number of test shrimp at the end of the experiment (shrimp)

**Curcuma Extract**

To determine the effectiveness of curcuma extract which has been given, a challenge test was carried out on 21<sup>st</sup> day by using the LC<sub>50</sub> *Vibrio harveyi* bacteria (the amount of virus concentration to kill 50% of tested shrimp population).

**Results**

**Relative Growth Rate**

The relative growth rate for each treatment is presented in Figure 1.

Based on the results of the tests that have been carried out, the relative growth rate of juvenile tiger shrimp spreads in normality, homogeneous, and additive so that it meets the requirements for further

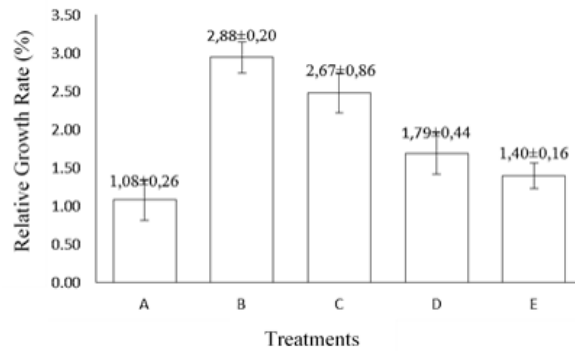


Fig. 1. Relative Growth Rate of juvenile tiger shrimp

analysis of variance.

Variance analysis results of relative growth rate of juvenile tiger shrimp showed that the addition of curcuma extract in artificial feed gave a different effect on the relative growth rate of tiger shrimp juvenile seed. Furthermore, to find out the differences between treatments, Dunnett's multiple comparisons test can be carried out which is presented in Table 3.

Dunnett test results revealed that treatment B was significantly different (P ≥ 0.81) for treatment A. Treatment C was significantly different (P ≥ 0.81) against treatment A. Treatment D was not signifi-

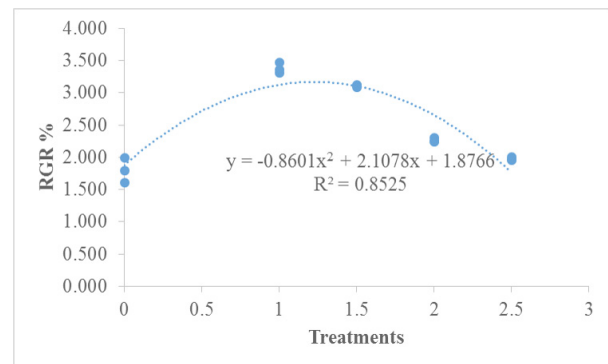


Fig. 2. Orthogonal Polynomial graph of Relative Growth Rate (%) of juvenile tiger shrimp

Table 2. Relative growth rate analysis of variance of juvenile tiger shrimp

SK	DB	JK	KT	F value	F table	
					0.05	0.01
Treatments	4	7.43	1.86	8.62	3.48	5.99
Error	10	2.15	0.22			
Total	14	9.58				

Description:

F value > F Table, so that H1 is accepted (significantly different)

**Table 3.** Dunnett’s multiple comparisons test for relative growth rate of juvenile tiger shrimp

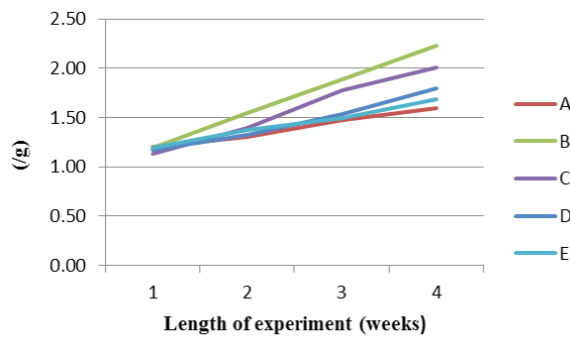
Control (k)	Treatments (p)	k-p	Notation
A	B	1.81*	b
	C	1.53*	b
	D	0.70**	a
	E	0.33**	a

Description:

\* If  $|k-p| > 0.81$ , it is significantly different.

\*\* If  $|k-p| \leq 0.81$ , it is not significantly different.

Conclusion: treatments which significantly different to control variable were treatment B and C



**Fig. 3.** Cultivan Growth Rate on each week

cantly different ( $P \leq 0.81$ ) against treatment A. Treatment E was not significantly different ( $P \leq 0.81$ ) against treatment A.

**Feed conversion ratio (FCR)**

The value of Feed Conversion Ratio (FCR) for each treatment is presented in Figure 4.

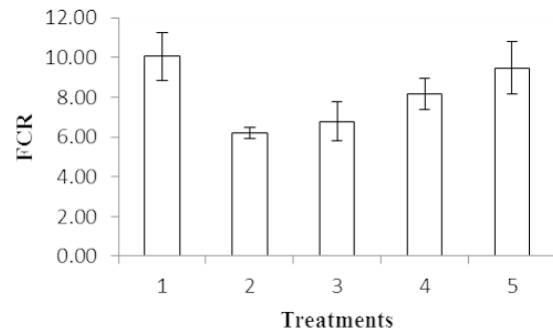
Based on the results of the tests that have been carried out, the feed conversion ratio data of juvenile tiger shrimp spreads in normality, homogeneous, and additive, so that it meets the requirements for analysis of variance. The results of feed conversion ratio analysis of variance of juvenile tiger shrimp is presented in Table 4.

**Table 4.** Feed Conversion Ratio (FCR) analysis of variance of juvenile tiger shrimp

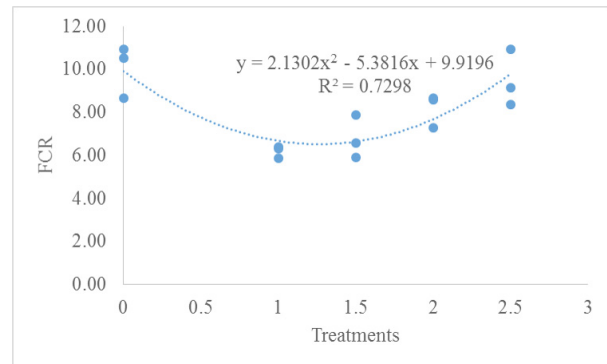
SK	DB	JK	KT	F value	F table	
					0.05	0.01
Treatments	4	172.55	43.14	68.30	3.48	5.99
Error	10	6.32	0.63			
Total	14	178.87				

Description:

F value < F table, so that H1 is rejected (had not significantly different)



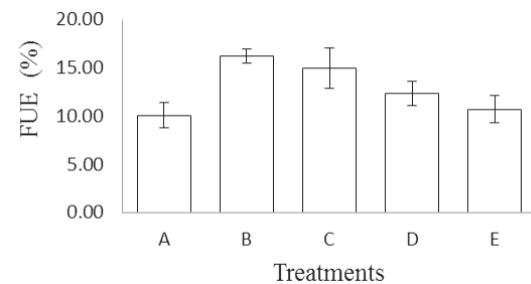
**Fig. 4.** The value of Feed Conversion Ratio (FCR)



**Fig. 5.** Orthogonal Polynomial graph of Feed Conversion Ratio of juvenile tiger shrimp

**Feed utilization efficiency (FUE)**

The histogram of the feed utilization efficiency during the study is presented in Figure 6.



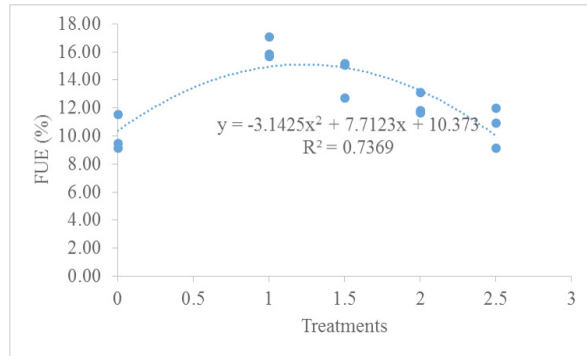
**Fig. 6.** Feed Utilization Efficiency of juvenile tiger shrimp

**Table 5.** Feed Utilization Efficiency (FUE) analysis of variance of juvenile tiger shrimp

SK	DB	JK	KT	F value	F table	
					0.05	0.01
Treatments	4	85.08	21.27	10.23	3.48	5.99
Error	10	20.80	2.08			
Total	14	105.87				

Description:

F value > F table, so that H1 is accepted (significantly different)



**Fig. 7.** Orthogonal Polynomial Graph of Feed Utilization Efficiency of juvenile tiger shrimp

Based on the results of the tests that have been carried out, the data of feed utilization efficiency of juvenile tiger shrimp is spread in normality, homogeneous and additive so that it meets the requirements for analysis of variance.

The results of the analysis of variance data on the utilization of tiger shrimp juvenile feed above shows that the addition of curcuma extract in artificial feed has a significantly different effect on the immunity of juvenile tiger shrimp. Furthermore, to find out the differences between treatments, the result of Dunnett's multiple comparison test is presented in Table 6.

**Table 6.** The result of Dunnett's multiple comparison test of Feed Utilization Efficiency of juvenile tiger shrimp

Control (k)	Treatments (p)	k-p	Notation
A	B	6.14	b
	C	4.89	b
	D	2.25	a
	E	0.63	a

Description:

\* If |k-p| > 2.52, it is significantly different

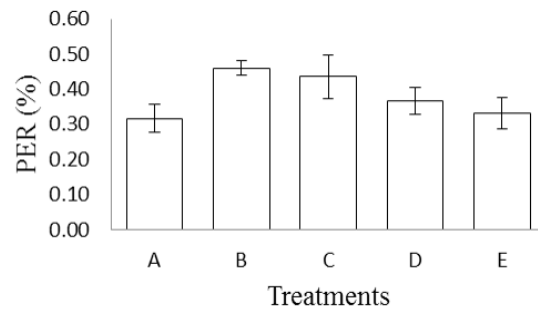
\*\* If |k-p| ≤ 2.52, it is not significantly different

Conclusion: treatments which significantly different to control variable were treatment B and C

Dunnett test results showed that treatment B was significantly different ( $P > 2.52$ ) against treatment A. Treatment C was significantly different ( $P > 2.52$ ) against treatment A. Treatment D was not significantly different ( $P \leq 2.52$ ) against treatment A. Treatment E was not significantly different ( $P \leq 2.52$ ) against treatment A.

**Protein Efficiency Ratio (PER)**

Histogram of protein efficiency ratio during the study is presented in Figure 8.



**Fig. 8.** Protein Efficiency Ratio (PER) of Juvenile tiger Shrimp.

Based on the results of the tests that have been carried out, the protein efficiency ratio of juvenile tiger shrimp spreads in normality, is homogeneous and has additives so that it meets the requirements for analysis of variance. Protein Efficiency Ratio (PER) analysis of variance of juvenile tiger shrimp is presented in Table 7.

The analysis results of the protein efficiency ratio variance data of the juvenile tiger shrimp above showed that the addition of curcuma extract in artificial feed gave a very significant different effect on the protein efficiency ratio of juvenile tiger shrimp. Furthermore, to find out the differences between treatments, the Dunnett multiple comparison test is presented in Table 8.

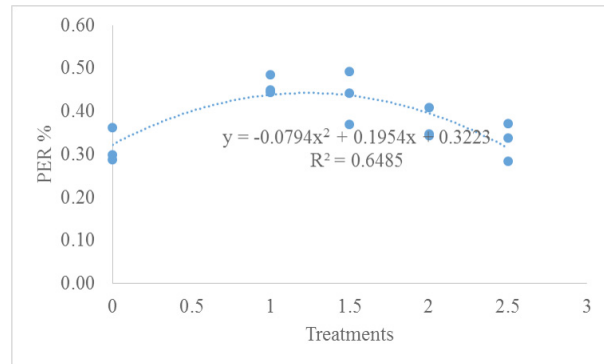
Based on Dunnett test results, it was found that

**Table 7.** Protein Efficiency Ratio (PER) analysis of variance of juvenile tiger shrimp

SK	DB	JK	KT	F value	F table	
					0.05	0.01
Treatments	4	0.0483	0.012	6.49	3.48	5.99
Error	10	0.0186	0.002			
Total	14	0.0669				

Description:

F value > F table, so that H1 is accepted (significantly different)



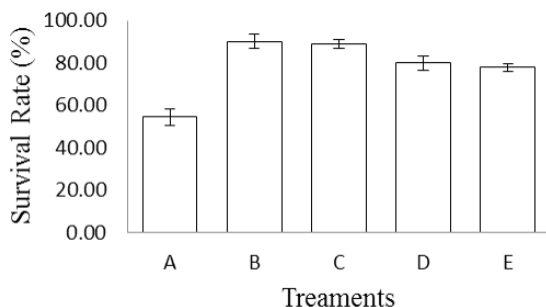
**Fig. 9.** Orthogonal Polynomial Graph of Protein Efficiency Ratio of juvenile tiger shrimp.

treatment B was significantly different ( $P \geq 0.08$ ) to treatment A. Treatment C was significantly different ( $P \geq 0.08$ ) to treatment A. Treatment D was not significantly different ( $P \leq 0.08$ ) to treatment A. Treatment E was not significantly different ( $P \leq 0.08$ ) against treatment A.

**Survival Rate (SR)**

Based on observations which has been done for 40 days, the value of the survival rate of juvenile tiger shrimp is presented in Figure 10.

Based on the results of the tests that have been carried out, the data on the survival of juvenile tiger shrimp spreads in normality, is homogeneous and has additives so that it meets the requirements for



**Fig. 10.** Survival Rate (SR) of Juvenile tiger Shrimp.

**Table 8.** The result of Dunnett’s multiple comparison test of Protein Efficiency Ratio of juvenile tiger shrimp

Control(k)	Treatments (p)	k-p	Notation
A	B	0.14	b
	C	0.12	b
	D	0.05	a
	E	0.01	a

Description:

\* If  $|k-p| > 0.08$ , it is significantly different

\*\* If  $|k-p| \leq 0.08$ , it is not significantly different

Conclusion: Treatments which significantly different to control variable were treatment B and C

variance analysis. The results of survival rate analysis of variance of juvenile tiger shrimp is presented in Table 9.

Variance analysis results showed that the addition of curcuma extract in artificial feed had no significant effect on the survival rate of juvenile tiger shrimp.

**Water Quality**

Water quality parameters observed during experiment included: salinity, temperature, pH, dissolved oxygen and ammonia content. The result of observation is presented in Table 10.

**Challenge Test**

Challenge test was carried out for about 20 days during experiment. The result of challenge test is presented in Table 11.

**Discussion**

**Relative growth rate (RGR)**

The results of the observation showed that the highest RGR value in juvenile tiger shrimp was treatment B, which was  $2.88 \pm 0.20\%$  / day, and give a significantly different effect. This is presumably be-

cause the nutritional content of the feed given is in accordance with nutritional needs so that it can spur growth. Basically the growth of tiger shrimp depends on the available energy and how the energy used in the body (Yang *et al.*, 2015). In order for the organism to grow it needs sufficient amount of nutrient energy. Energy nutrients that enter the body

of the shrimp are used for various processes in the body such as growth, metabolism, tissue formation, physical activity, and maintenance.

Growth rate can be accelerated if the feed given has good nutritional value. Nutrition is used to produce energy and replace damaged body cells. The feed provided is not only sufficient and timely, but

**Table 9.** Survival Rate (SR) analysis of variance of juvenile tiger shrimp

SK	DB	JK	KT	F value	F table	
					0.05	0.01
Treatments	4	2,463.70	615.93	69.29	3.48	5.99
Error	10	88.89	8.89			
Total	14	2,552.59				

Description:

F value < F table, so that H1 is rejected (had not significantly different)

**Table 10.** Water quality data during experiment

Treatments	Parameters			
	Temperature	pH	DO	NH <sub>3</sub>
A	27-28	7-8.5	6.46-7.77	0.14-0.18
B	28-30	7-8.5	6.46-7.77	0.16-0.21
C	28-31	7-8.5	6.46-7.77	0.18-0.22
D	28-32	7-8.5	6.46-7.77	0.17-0.21
E	28-30	7-8.5	6.46-7.77	0.18-0.22
Unit	°C	-	mg/L	mg/L
References	28-30 <sup>(A)</sup>	6.5-8.7 <sup>(A)</sup>	> 4 <sup>(C)</sup>	< 1 <sup>(B)</sup>

(A) Ghufran *et al.* (2010)

(B) Ghufran *et al.* (2010)

(C) Pescod (1973)

**Table 11.** Cultivan death data after challenge test

Number	Treatments	Replications	Every 5 days in 20 days			
			I	II	III	IV
1.	A	1	5	2	3	3
		2	4	3	3	3
		3	6	4	2	3
2.	B	1	2	-	-	-
		2	2	-	1	-
		3	3	1	-	-
3.	C	1	3	-	-	-
		2	1	2	-	-
		3	2	1	1	-
4.	D	1	3	1	1	1
		2	2	2	1	-
		3	4	2	1	-
5.	E	1	2	2	1	1
		2	3	2	1	1
		3	4	2	-	1

must have adequate nutrition and energy (Ramezani, 2009). The nutrients needed are proteins, fats, carbohydrates, vitamins and minerals. Fish consume feed with low energy content causing stunted growth and symptoms that are called malnutrition (Wang *et al.*, 2006).

#### Feed Conversion Ratio (FCR)

The difference in the addition of curcuma extract to artificial feed for tiger shrimp juveniles had no significant effect ( $P \leq 1.39$ ) on the feed conversion ratio, where the average FCR results for each treatment were treatment A was  $10.05 \pm 1.21\%$ ; treatment B was  $6.19 \pm 0.29\%$ ; treatment C was  $6.79 \pm 1.00\%$ ; treatment D was  $8.18 \pm 0.79\%$ ; and treatment E was  $9.48 \pm 1.32\%$ . The results of the observation showed that the highest RGR value in juvenile tiger shrimp was treatment A at  $10.05 \pm 1.21\%$ . According to Juancey (1982), feed efficiency depends on the adequacy of nutrition and feed energy, if the nutrient feed is not sufficient like high or low energy, then the resulting weight gain is low.

#### Feed Utilization Efficiency (FUE)

The results of the observation showed that the highest FUE value in juvenile tiger shrimp was treatment B, which was  $16.19 \pm 0.77\%$ , and give a significantly different effect. According to Tacon (1987), feed utilization efficiency is the ratio between body weight gain and the amount of feed given to tested shrimp during the study. High feed efficiency shows that the use of feed is efficient so that only a few feed substances are overhauled to meet energy needs and the rest is used for growth (Huet, 1970).

Slow growth is caused by insufficient protein content for the body of shrimp juveniles that require a lot of protein to replace damaged cells in growth. The excessive amount of feed is not good because there would be any leftover feed which results in excessive ammonia content from feed that evaporates in the water so that it can cause fish to die or be stressed (Juancey, 1982).

#### Protein Efficiency Ratio (PER)

The results of the observation showed that the highest PER value in juvenile tiger shrimp was treatment B, which was  $0.46 \pm 0.02\%$ ; and gave a significantly different effect. This shows that the energy of fat and nitrogen-free extract in the test feed contributes energy that is relatively greater than protein energy. The optimal use of protein is achieved if most of the

fish's energy needs are met from non-protein components such as fat and nitrogen-free extract, so that the protein energy contained in feed can be utilized optimally for growth, because shrimp are able to utilize fat energy and nitrogen-free extract for metabolism (Helper and Pruginin, 1981).

#### Survival Rate (SR)

The difference in dosage of curcuma extract given to tiger shrimp juveniles had no significant effect ( $P > 0.05$ ) on SR, where the average SR result of each treatment were treatment B which was equal to  $90.00 \pm 3.33$ ; treatment C was  $88.89 \pm 1.92\%$ ; treatment D was equal to  $80.00 \pm 3.33\%$ ; treatment E was equal to  $77.78 \pm 1.92\%$ ; and treatment A is  $54.44 \pm 3.85\%$ .

The level of juvenile tiger shrimp survival rate in the study was very good, treatment B was  $90.00 \pm 3.33$ . The degree of survival rate is influenced by the chemical and physical properties of water media and feed quality. Water media quality parameters during maintenance in treatments A, B, C, D and E are still in a decent range. This is because every two days, a cleaning to remove dirt and leftovers had been done, and the continuous aeration system causes water media quality to remain stable in a range that is suitable for fish growth.

#### Water quality

Decent quality in shrimp maintenance is one of the determinants of success in the growth of a cultivar. During this study the water quality factors measured were temperature, pH, DO, ammonia. Water temperature greatly affects the shrimp's living environment, especially related to metabolism, dissolved oxygen content, growth and appetite of shrimp (Murtidjo, 2003). The temperature range measured during the study was 27-28 °C. Temperature range that is good for growth and shrimp life is between 28-30 °C, although tiger shrimp can live at a temperature of 18 °C and 26 °C Ghufran *et al.* (2010).

The pH range measured during the study was 7-8.5. This condition is very feasible for the life and growth of tiger shrimp. Shrimp cultivation will be good in water with a pH of 6.5-9.0 and the optimal pH range for shrimp aquaculture is 7.5-8.7 (Ghufran *et al.* (2010). Ammonia content measurements show that the highest value was in treatment A of 0.14-0.18; treatment B was 0.16-0.21; treatment C was 0.17-0.22; treatment D was 0.19-0.23; and treatment



E was 0.21-0.25. Ammonia concentration above 0.45 ppm can inhibit shrimp growth up to 50%. Tiger shrimp can grow well if the concentration of ammonia in water is not more than 0.1 ppm (Buwono, 1993).

### Challenge Test

After the challenge test was done, the average value of each treatment can be obtained, they were treatment B, which was equal to  $90.00 \pm 3.33$ ; treatment C was  $88.89 \pm 1.92\%$ ; treatment D was equal to  $80.00 \pm 3.33\%$ ; treatment E was equal to  $77.78 \pm 1.92\%$ , and treatment A was  $54.44 \pm 3.85\%$ . One way to deal with diseases is by immunoprophylaxis, which is to increase the body's immune system against disease, immunity to the disease can be stimulated by giving immunostimulants including vaccinations and vitamins (Anderson, 1992).

### Conclusion

The addition of temulawak extract to artificial feed with different doses had a significant effect on Protein Efficiency Ratio (PER), Feed Utilization Efficiency (FUE), Relative Growth Rate (RGR), and the addition of different curcuma extracts had no significant effect on Survival Rate (SR) and Feed Conversion Ratio (FCR) in tiger shrimp juveniles. Based on the study conducted, further study is needed on several combinations of types of immunostimulants and repeated vaccine administration in order to improve the body's defense system in tiger shrimp juveniles, in an effort to suppress death from attacks by *Vibrio harveyi* bacteria and other attacks by bacteria or viruses.

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