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Research Article

Effect of Papain Enzyme in Feed on Digestibility of Feed, Growth Performance, and Survival Rate in Post Larvaes of Freshwater Lobster [*Cherax quadricarinatus* (Von Martens, 1868)]

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Abstract: The study aimed to find the impact of additional papain enzyme in feed digestibility, growth performance, and survival rate in post larvaes of freshwater lobster [*Cherax quadricarinatus* (Von Martens, 1868)]. There were five treatments with dosage A (0 % papain enzyme per kg feed), B (0.1 % papain enzyme per kg feed), C (0.2 % papain enzyme per kg feed), D (0.3 % papain enzyme per kg feed) and E (0.4 % papain enzyme per kg feed). Every treatment was repeated three times. The experiment used Completely Randomized Design. EFU (Efficiency of Feed Utilization), FCR (Feed Conversion Ratio), PER (Protein Efficiency Ratio), RGR (Relative Growth Rate), ADCP (Apparent Digestibility Coefficient of Protein), SR (Survival Rate) and water quality parameters were determined. The study found that the impacts of the various dosages of papain enzyme exhibited significant effects ($P < 0.01$) on the EFU, FCR, PER, RGR, ADCP; however, insignificant effects were observed ($P > 0.05$) on the survival rate of freshwater lobster. It was concluded that the incorporation of papain enzyme improved the feed digestibility and the growth performance of post larvae in freshwater lobster. The study found that optimum dosages of papain enzyme on feed digestibility, efficiency of feed utilization and growth rate of freshwater lobster ranged from 0.24 % to 0.31 % papain enzyme per kg feed. The quality of media culture was still in the feasible condition for post larvae of freshwater lobster cultivation.

Keywords: Artificial feed, Diet management, Diet efficiency, Nutrient absorption, Proteolytic enzyme.

1. INTRODUCTION

Freshwater lobsters [*Cherax quadricarinatus* (Von Martens, 1868)] have high economic value in food consumption and decorative purposes. Farming of freshwater lobsters highly depends upon the supply of feed. Akiyama et al. [1] reported that the cost portion of diet for fish cultivation is around 60 % of total cost whether it is reared in semi-intensive or intensive technology. The quality of artificial feed is determined by nutrient composistion, nutrient balance and utilization efficiency. Therefore, better diet management is needed to increase diet utilization efficiency. The efficiency could be boosted by adding enzyme in diet [2] and digestion efficiency of the cultured species can be increased by supplementing the enzymes to the feed [3]. The addition of enzymes breaks down protein into shorter peptides that speeds up the digestion of protein [3–6] favoring the feed utilization. One of the many enzymes used in diet fish industry is papain. Papain is categorized as protease enzyme

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that can hydrolyze protein into short peptide. It is an important factor to boost digestibility of protein and absorption and also to enhance growth [7]. Panigrahi et al. [8] stated that papain enzyme is proteolytic enzyme that can hydrolyze protein into amino acid or peptides. Papain enzyme contains 187 amino acids weighing 21 000 moles. It has sulfhydryl function compound and is able to hydrolyze peptide compound into lysine and glycine amino acids [9]. However, until recently there has been lack of study of papain enzyme incorporated feed on feed digestibility, efficiency of feed utilization and growth rate of freshwater lobster for post larvae of freshwater lobster (*C. quadricarinatus*). Morerever, reference [3, 10–12] suggested that the papain enzyme addition in the diet would help and accelerate nutrient absorption; in turn it can provide enough nutrients for fish growth and survival.

2. MATERIALS AND METHODS

2.1. Animal Test

Animal test used in the study was post larvae of freshwater lobster (*C. quadricarinatus*) with initial body weight averaging (0.43 ± 0.09) g. As many as 150 post larvaes were collected from the Center for Freshwater and Brackish Aquaculture, Jepara, Central Java, Indonesia. They were first acclimated for one week in the 500 L fiber container in order to be able to adjust to the environment and the diet. During acclimatization the post larvaes have been fed with control diet [10]. To avoid stress on the larvaes aeration was installed. Feces and diet waste were discarded by syphoning 25 % water every day and then refill with fresh water.

2.2. Diet Test

Diet test used in the study was in the form of pellets containing 30 % protein [13]. Various doses of papain enzyme [(0; 0.1; 0.2; 0.3; and 0.4) % kg^{-1} feed] were added into the artificial diet. This experiment was modified from [10] that they used papain enzyme with dose of 0.1 % kg^{-1} feed in the post larvae of *M. rosenbergii*. The composed diet consisted of fish meal, soybean meal, corn meal, rice bran, wheat flour, fish oil, corn oil, mineral mix and Cr_2O_3 0.5 % [14] and papain enzyme. The brand of

Table 1. Ingredients (%) and proximate composition (% of dried mass) of the artificial diet for post larvae of *C. quadricarinatus*

Ingredients Diet Composition	Composition (%)				
	A	B	C	D	E
Papain	0	0.1	0.2	0.3	0.4
Fish meal	27.5	27.5	27.5	27.5	27.5
Soybean meal	23.8	23.8	23.8	23.8	23.8
Corn meal	19.3	19.3	19.3	19.3	19.3
Rice bran	19.7	19.7	19.7	19.7	19.7
Wheat flour	4.7	4.6	4.5	4.4	4.3
Fish oil	1.0	1.0	1.0	1.0	1.0
Corn oil	0.5	0.5	0.5	0.5	0.5
Vit Min Mix	2.0	2.0	2.0	2.0	2.0
Cr_2O_3	0.5	0.5	0.5	0.5	0.5
CMC	1.0	1.0	1.0	1.0	1.0
Total	100	100	100	100	100
Proximate Analysis Results					
Protein (%)*	30.04	30.02	30.05	30.15	30.06
Fat (%)*	7.87	7.97	7.84	7.63	7.49
BETN (%)*	36.54	36.51	36.55	37.24	37.68
Energy (kcal) *	260.67	260.82	260.01	260.44	260.06
Ratio E/D (kcal g^{-1} Diet)	8.67	8.69	8.65	8.64	8.65

Notes:

a. The values were calculated based Digestible Energy [27] for 1 g protein equals 3.5 kcal, 1 g fat equals 8.1 kcal, and 1 g carbohydrate equals 2.5 kcal.

b. According [28], the optimal E/P ratio for growth ranges from 8 kcal g–1 to 12 kcal g–1.

c. *Animal Nutrient Laboratory, Faculty of Husbandry and Agriculture, Diponegoro University (2017).

papain enzyme used in the study was “NEWZIME” produced by the Center for Freshwater and Brackish Aquaculture, Jepara, Central Java, Indonesia.

2.3. Elements and Proximate Composition

Mixing the diet was done in order. First was to mix the diet ingredients with the smallest amount and final step was to mix the diet ingredients with the largest amount, except fat ingredients (fish oil and corn oil) added after all ingredients mixed. The ingredients were mixed homogenously then they were manufactured into 1 mm to 2 mm pellets. The pellets were dried in the oven with the temperature ± 40 °C [14]. The diet elements and Proximate Composition is shown in the Table 1. Nitrogen content was measured using Microjedhal method [15], while protein content was measured by multiplying nitrogen with 6.25 constant. Soxhlet extraction method with petroleum ether was used to analyze raw fat content [15]. Ash content was obtained by burning the diet at 550 °C in the furnace for 5 h [15]. Carbohydrate was measured using Remainder method [16].

2.4. Experiment Procedure

There were five treatments which were by adding different doses of papain enzyme. Every treatment was repeated three times. The experiment used Completely Randomized Design. The larvaes were raised in circular plastic containers with the capacity of 30 L each. There were 15 containers and each was filled with 30 L of water. Each container had 10 larvaes of freshwater lobster. To maintain the containers cleaned, the fish feces and the feeding waste were discarded by syphoning 25 % water every day and then refill with fresh water. Feeding time was three times a day at 11:00, 15:00, and 19:00. The larvaes were given diet at 5 % of the mass weight [13]. Since freshwater lobsters are nocturnal, feeding during the night was given more compared to during the day. At the beginning of the study the larvaes were scaled. To evaluate growth the sampled larvaes were scaled every week for 42 d. All data of experiment were recorded [10]. The measurement of water quality was conducted in the morning at 8:00 and in the afternoon at 17:00. It used Water Quality Checker to measure dissolved oxygen and acidity of the media. Thermometer was also used to measure the temperature of the media. Measurement of ammoniac was done at the beginning and at the end of the study.

Parameters used in this study were adopted from Tacon et al. [17]. There were Efficiency of Feed Utilization (EFU), Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER), Relative Growth Rate (RGR), while apparent digestibility coefficient of protein (ADC_p) was adopted from Gul et al. [4], and Survival Rate (SR) was adopted from National Research Council [14]. The modified colorimetric method was used to analyze chromic oxide level in the feed and feces [4]. The values were gauged with a spectrophotometer (540 nm) (Shimadzu UV-2102 PC, UV-visible Scanning Spectrophotometer). After per chloric acid oxidation and a colored complex with diphenyl carbazide (DPC) have been formed, the measurement was done. pH

$$\text{EFU} : \frac{(\text{Final weight} - \text{Initial weight})}{\text{The amount of feed consumed}} \times 100\,\% \quad (1)$$

$$\text{FCR} : \frac{\text{The amount of feed consumed}}{(\text{Final weight} + \text{Total weight fish death}) - \text{Initial weight}} \times 100\,\% \quad (2)$$

$$\text{PER} : \frac{\text{Final weight} - \text{Initial weight}}{\text{The amount of feed consumed} \times \text{Protein content of feed}} \times 100\,\% \quad (3)$$

$$\text{RGR} : \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight} \times \text{Time experiment}} \times 100\,\% \quad (4)$$

$$\text{ADC}_P : 100 \left\{ \frac{\%\, Cr_2O_3 \text{ in the feed}}{\%\, Cr_2O_3 \text{ in the feces}} \times \frac{\%\text{ protein in the feces}}{\%\text{ protein in the feed}} \right\} \quad (5)$$

$$\text{SR} : \frac{\text{Final count}}{\text{Initial count}} \times 100\,\% \quad (6)$$

(Jenway 3510), DO (Jenway 970), temperature and ammoniac (HANNA: HI. 8633) were tested for water quality variables. Each container has an aerator to recirculate the water. The variables were measured by Equation (1) to Equation (6) :

2.5. Statistical Analysis

Before analyzing ANOVA, normality, additivity, and homogeneity tests were conducted to guarantee that the data have normal, homogeneity and additive properties. If the probability results of ANOVA s are $P < 0.05$ or $P < 0.01$, it requires Duncan Test. Polynomial Orthogonal Test with SAS9 and Maple12 [18] were used to measure papain enzyme optimal dose, while water quality analysis was thoroughlyexplained.

3. RESULTS AND DISCUSSION

Data on EFU (Efficiency of Feed Utilization), FCR (Feed Conversion Ratio), PER (Protein Efficiency Ratio), RGR (Relative Growth Rate), ADC_P (Apparent Digestibility Coefficient of Protein), SR (Survival Rate) of *C. quadricarinatus* post larvaes were presented in the Table 2.

Until now, there is no any research on additional papain enzyme in the diet for *postlarvae C. quadricarinatus*, however, there were some studies on the effect of photolytic enzyme for other species, such as study by Sajjadi and Carter [19] reported that *Salmo salar* (Linnaeus, 1758) has better growth due to additional protolithic enzyme in the diet. Singh et al. [3] reported that the feeding fish with the 2 % papain addition has the lowest ratio of feed conversion, higher growth rate, digestibility of protein, and a ratio of protein efficiency of *Chanos chanos* (Forsskål, 1775). Patil and Singh [10] stated that the results of this feeding trial show that the 0.1 % papain enzyme incorporated diet for post-larvae of *Macrobrachium rosenbergii* (De Man, 1879) has better growth and feed utilization. Khati et al. [11] stated that papain enzyme was able to increase digestibility of nutrient and also to improve the health of *Labeo rohita* ((Hamilton, 1822). Muchlisin et al. [12] reported that the papain enzyme optimum dosage of for keureling fish [Tor tambra (Valenciennes, 1842)] was 27.5 mg kg–1 feed.

The addition of papain enzyme in the diet successfully improved the efficiency of feed utilization of postlarvae of *C. quadricarinatus*. It was indicated by the higher feed efficiency. Gatlin [20] reported that nutrient efficiency is an indicator in nutrient utilization by the fish in which low nutrient efficiency ratio show that the nutrient has been digested and optimally absorbed by the fish. The efficiency of feed utilization is the ratio between the growth of body weight and feed during cultivation [17].

ANOVA test shows that the impacts of papain enzyme addition in the feed was significant ($P < 0.05$) on Efficiency of Feed Utilization (EFU) of postlarvae of *C. quadricarinatus*. This study as shown in Table 2 that the D treatment (0.3 % kg–1 feed) had the highest EFU as much as 73.09 % and the lowest was A treatment (0 % papain enzyme per kg feed) as much as 60.82 %. The high EFU value in treatment D (0.3 % papain enzyme per kg feed) show that freshwater lobster only used little protein for regular activities; therefore, the rest was to support growth. It was indicated by the highest of RGR value of D treatment compared to treatments of A (8.48 % d–1), B (9.25 % d–1), C (9.89 % d–1) and E (9.33 % d–1). The high efficiency indicated that the diet has high quality, therefore it can be utilized efficiently [14]. Moreover, Singh et al. [3] also suggested in their studies that the enzyme could help fish to breakdown complex big organic molecules, such starch, cellulose and protein into simpler forms; therefore, it can increase diet efficiency. The higher the efficiency of feed utilization was higher was the growth.

Cubical equation between papain enzyme addition in the feed and EFU of postlarvae of *C. quadricarinatus* was Y = -810.56x3 + 372.02x2 – 2.2127x + 61.22 with R2 = 0.88. The equation was obtained from the orthogonal polynomial test (Figure 1). The papain enzyme optimum dose in the feed on the EFU was 0.31 % kg–1 feed with EFU maximum of 73.51 %.

The effect of papain enzyme addition was significant ($P < 0.05$) on the ratio of feed conversion (FCR) in the post-larvae of *C. quadricarinatus* (Tabel 2). The value of the ratio of feed conversion was better as the dose of the enzyme increased [(0.1 to 0.3) % papain enzyme per kg feed]. Papain enzyme implementation has resulted in an increase in the ratio of feed conversion. It was because of better metabolism in fish fed on papain enzyme

Table 2. Efficiency of Feed Utilization (EFU), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Relative Growth Rate (RGR), Apparent Digestibility Content Protein (ADCP) and Survival Rate (SR) of *C. quadricarinatus post larvae*

Experiment Data	**Treatments**				
	A	**B**	**C**	**D**	**E**
EFU (%)	60.82 ± 0.54^{c}	65.96 ± 0.97^{b}	67.65 ± 0.57^{b}	75.09 ± 0.65^{a}	69.35 ± 0.36^{b}
FCR	2.55 ± 0.10^{c}	2.46 ± 0.24^{b}	2.26 ± 0.24^{b}	1.76 ± 0.07^{a}	2.30 ± 0.22^{b}
PER	1,90 ± 0.04^{c}	2.19 ± 0.23^{b}	2.389 ± 0.23^{b}	3.75 ± 0.05^{a}	2.24 ± 0.25^{b}
RGR (% d^{-1})	8.48 ± 0.20^{c}	9.26 ± 0.15^{b}	9.89 ± 0.20^{b}	11.01 ± 0.23^{a}	9.33 ± 0.24^{b}
ADC_P	60.47 ± 0.02^{c}	78.65 ± 0.05^{b}	79.15 ± 0.05^{b}	83.93 ± 0.05^{a}	77.65 ± 0.04^{b}
SR (%)	93.33 ± 5.77^{a}	90.00 ± 10.0^{a}	90.00 ± 10.0^{a}	96.67 ± 5.77^{a}	93.33 ± 5.77^{a}

Note: The values with the same superscripts in the column show that there was no difference

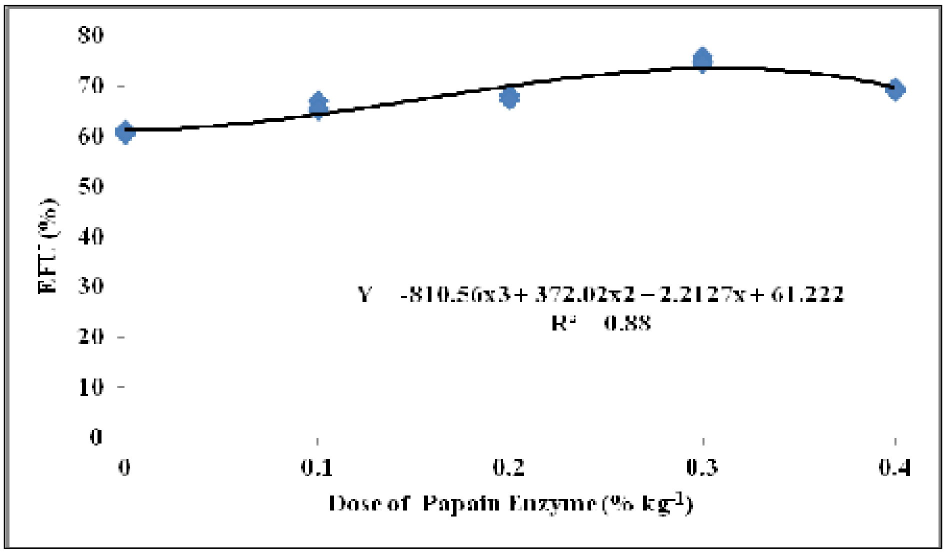

Fig. 1. Graph of the relationship between papain enzyme addition in the feed and EFU of post larvae of *C. quadricarinatus*

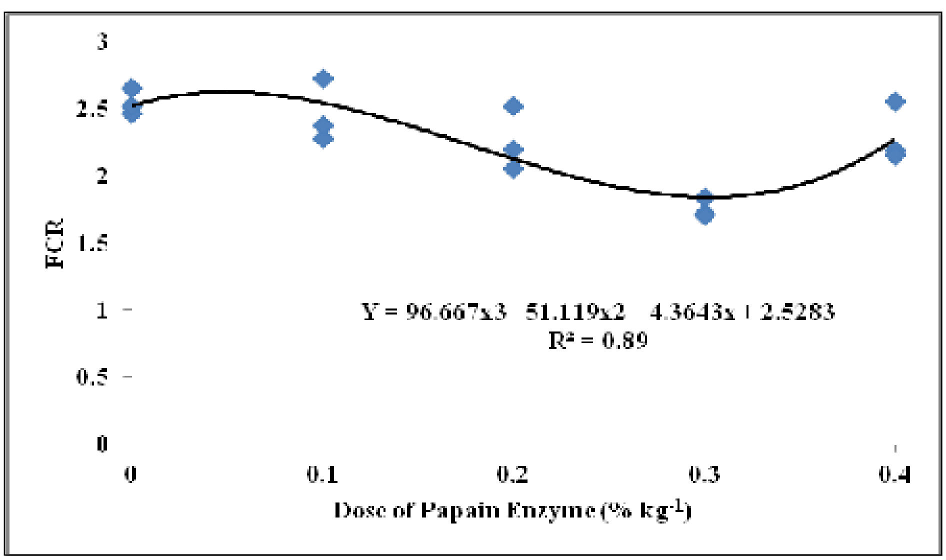

Fig. 2. Graph of the equation between papain enzyme incorporated feed and FCR of post larvae of *C. quadricarinatus*

incorporated diet. In turn, it has better FCR. The lowest FCR with the value of 1.75 was obtained in treatment D in which the dose of the papain enzyme was 0.3 % kg–1 feed and followed by the treatments of C (2.26), E (2.30), B (2.46) and A (2.55). It could be concluded that the papain enzyme addition could improve the feed utilization efficiency and reduce a ratio of feed conversion, as results of the study that treatment D brought about the highest EFU (75.09 %) and the lowest FCR (1.75). Similar results were reported by reference [11, 12, 21].

The cubical equation on the relationship of papain enzyme in the feed and the FCR of Y = 96.667x3 - 51.119x2 + 4.3643x + 2.5283 with R2 = 0.89 was obtained from the orthogonal polynomial test (Figure 2). The papain enzyme optimum dose in the feed on the FCR was 0.31 % per kg feed with FCR maximum of 1.84.

The ratio of Protein Efficiency Ratio (PER) indicates protein sources in diet that provide essential amino acids required by fish [11]. The study showed that a ratio of protein efficiency has increased in all PER treatments that ranged from 2.19 to 3.75 (Table 2). It was always higher than the treatment without papain enzyme. Protein efficiency ratio is the measurement to indicate how good protein in the diet can provide essential amino acids for fish [22]. The highest ratio of protein efficiency was obtained in the D treatment (0.3 % kg–1 feed). It was expected that amino acid composition in the treatment D (0.3 % kg–1 diet) was suitable with the amino acid in the body of postlarvae of *C. quadricarinatus.* Similar results were also reported by reference [3, 11, 12].

Cubical equation between papain enzyme in the feed and the PER was Y = -231.94x3 + 121.74x2 – 10.742x + 1.9791 with R2 = 0.80. ThisThe equation was obtained from the test of the orthogonal polynomial (Figure 3). The papain enzyme optimum dosage in the feed for the PER was 0.29 % papain enzyme per kg feed and the value of PER was 1.84.

The results of variance analysis displayed significant impact (P < 0.05) with incorporating papain enzyme ion relative growth rate (RGR) of freshwater lobster *(C. quadricarinatus).* The lowest RGR was on the treatment A (0 % kg–1 feed). It was suspected that there was no papain enzyme which hydrolyzed polypeptide in the diet. It caused low in diet digestibility and efficiency, in turn, it hindered freshwater lobster growth. The results show that treatment A generated low RGR as well as ADCP and EFU. The highest RGR was obtained in the D treatment (0.3 % papain enzyme per kg feed). It can be concluded that the addition of 0.3 % papain enzyme on every kg feed was the optimal dosage. The papain enzyme was to hydrolyze protein into short chain peptide in order to increase protein digestibility, nutrient efficiency, and growth of freshwater lobster. The results show that treatment D generated high RGR as well as ADCP and EFU. The result was the same as the study conducted by Mo et al. [21]. He found that papain is the protease enzyme that hydrolyzes protein into short chain peptide in the feed. It is the main factor in increasing protein digestibility, fast absorption, and growth. Moreover, Miyamoto et al. [23] stated that papain enzyme can hydrolyze lipid, carbohydrate, and protein. An exogenous enzyme has proved that it can increase feed nutrients as reported by reference [3, 10–12].

Cubical equation between papain enzyme in the feed and the RGR was Y = -220.28x3 + 100.55x2 – 2.8496x + 8.5393 with R2 = 0.90. The equation was derived from the test of an orthogonal polynomial (Figure 4). The papain enzyme optimum dose in the feed for the RGR was 0.29 % per kg feed. The maximum value of RGR was 10.78 %.

Table 2 show that the addition of papain enzyme (0.1 to 0.4) % per kg feed could boost the apparent digestibility coefficient of protein (ADCP). It was because of extensive protein hydrolysis that was induced by papain [11]. The highest coefficient of protein digestibility was obtained in the D treatment (0.3 % per kg feed). The value was 83.93 %. The papain dose was suitable that could increase the protein breakdown, protein digestibility, protein absorption and produce short peptides in diet, which in turn it increased growth. Dawood et al. [24] thought that the addition of proteolytic enzyme would improve protein content. Moreover, Singh et al. [3] found that the addition of papain enzyme into the feed can boost protein digestibility. Similar results were reported in the studies of *C. chanos* [3], *L .rohita* [11], and *T. tambra* [12].

Cubical equation between papain enzyme in the feed and the ADCP was Y = -551.94x3 - 650.07x2

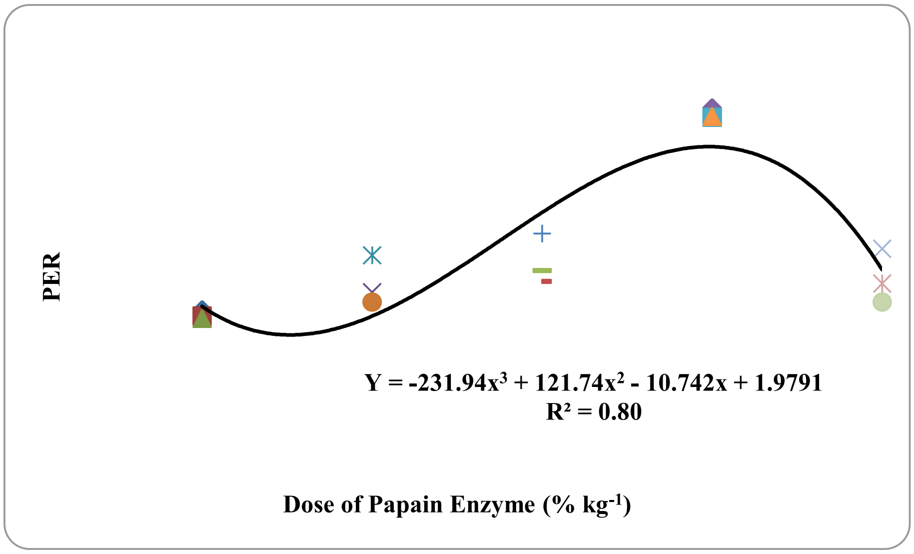

Fig. 3. Graph of the equation between papain enzyme incorporated feed and PER of post larvae of *C. quadricarinatus*

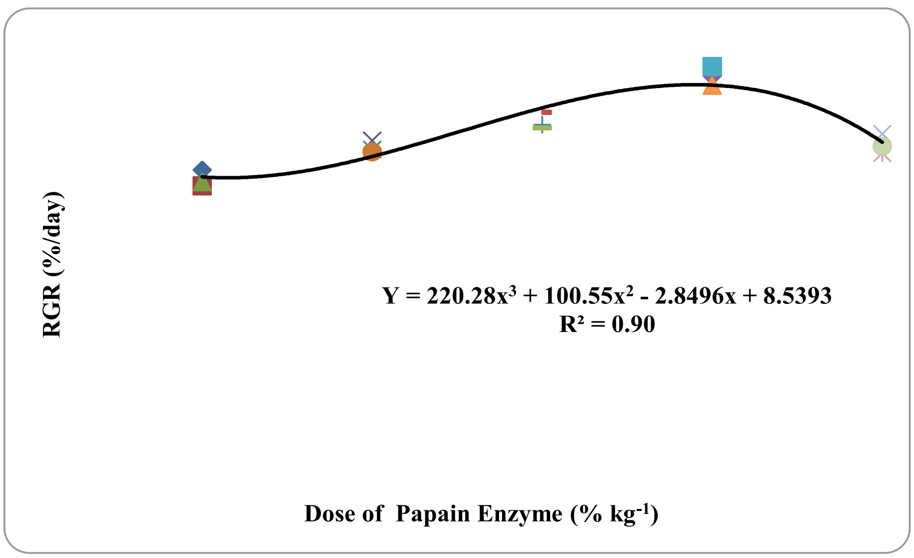

Fig. 4. Graph of the relationship between papain enzyme in the feed and RGR of post larvae of *C. quadricarinatus*

+ 214.68x + 61.003 with R2 = 0.94. The equation was derived from the test orthogonal polynomial (Figure 5). The papain enzyme optimum dose in the feed on the ADCP was 0.24 % per kg feed with ADCP maximum of 82.72 %.

The variance analysis results show that the effect of papain enzyme addition was not significant ($P > 0.05$) on the survival rate (SR) of postlarvae of *C. quadricarinatus*. Yakuputiyage [25] informed that the diet did not affect the survival rate, because the survival rate was influenced by the initial treatment of the fish and water quality. Similar findings were declared in the studies of *C. chanos* [3], *M. rosenbergii* [10] and L. rohita [11]. Hepher [26] found that the survival rate was influenced by the gender of fish, heredity, age, reproductive characteristics, disease prone and external factors such as water quality, density, amino acids in the diet. Water quality was still on condition that overpassed to the cultivation of post larvae of *C. quadricarinatus*. The measurement of water

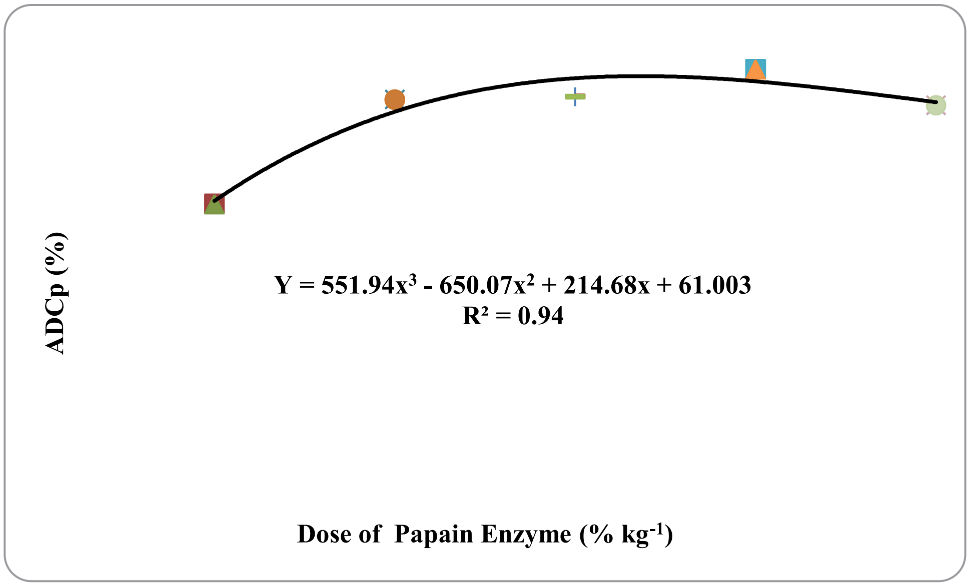

Fig. 5. Graph of the relationship between papain enzyme addition in the artificial feed and ADCP of post larvae of *C. quadricarinatus*

Table 3. Parameters of water quality for cultivation of Freshwater Lobster (*C. quadricarinatus*)

Variables	Values A	B	C	D	Feasibility
Temperature (°C)	25.1 to 28.2	25.1 to 28.2	25.2 to 28.2	25.1 to 28.1	25 to 32*
pH	8.63 to 8.86	8.64 to 8.77	8.70 to 8.79	8.66 to 8.82	7 to 9*
DO (mg L^{-1})	3.01 to 4.0	3.05 to 3.63	3.04 to 3.62	3.00 to 3.63	4 to 6*
Ammoniac (mg L^{-1})	0.25 to 0.6	0.25 to 0.6	0.25 to 0.6	0.25 to 0.6	< 1*

Note: *[29]

parameter during postlarvae of *C.quadricarinatus* cultivation was presented in Table 3.

4. CONCLUSION

The study concluded that the papain enzyme addition could boost the digestibility of feed and the growth performance of post larvae of freshwater lobster (*C. quadricarinatus)*. The optimum doses of papain enzyme on digestibility of feed and the growth performance of freshwater lobster (*C. quadricarinatus)* ranged from (0.24 to 0.31) % kg^{-1} feed.

5. ACKONWLEDGEMENTS

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