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The Effect of Adding Papain Enzyme and TCR Calcium to Commercial Feed on The Growth of Giant Freshwater Prawns (*Macrobrachium rosenbergii*)

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Abstract

Giant freshwater prawn (Macrobrachium rosenbergii) is one of the freshwater commodities in Indonesia with high market demand. In order to increase the growth rate of these prawns, their feed is supplemented with papain enzyme and calcium. The former is a proteolytic enzyme that plays a role in breaking down proteins into simpler compounds of amino acids, allowing it to be more easily absorbed into the prawn's body. The latter, i.e., calcium, plays a role in the molting process by accelerating the gastrolization process, which eventually can accelerate the growth rate. This study aims to test the effect of providing papain enzyme and calcium from crab shell flour (TCR) to the reprocessed commercial feed. The subjects tested in this study were 6-to-9-gram giant freshwater prawns about 9-cm long, at a density of 1 prawn/2 liters of water. The Completely Randomized Design (CRD) was used with 5 treatments and repeated 3 times. The treatments were referred to as P0 (Papain 0%; TCR 0%), P1 (Papain 2.25%; TCR 3%), P2 (Papain 3.375%; TCR 3%), P3 (Papain 5%; TCR 3%), and P4 (Papain 6%; TCR 3%). The best treatment was P3 (Papain 5%; TCR 3%) that had the values of EPP, PER, absolute weight growth, absolute length growth, RGR, and SR of 52.44%, 9.99, 3.74g, 2.08cm, 0.78%/day, and 91%, respectively.

Keywords: *Macrobrachium rosenbergii*; Growth; Papain enzyme; Crab Shell Flour;

1. Introduction

Indonesia is one of the countries that has the potential of developing giant freshwater prawn farming. The demand for these giant freshwater prawns at national scale could hit 10-20 ton/day (Samadan *et al*. 2023). This high market demand for Giant freshwater prawns in Indonesia makes it feasible for further development. For this reason, it is necessary to increase its production to meet the market demand. One of the attempts to increase the giant freshwater prawn production is by increasing the prawns' growth rate. The main external factors capable of affecting fish growth are water quality and feed. The giant freshwater prawns' growth can be affected by the feed nutrition (both macro and micro nutrition) given to them, such as protein absorption that can increase the prawn's weight growth and calcium mineral absorption that can help accelerate the molting process in prawns.

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Crab shell flour (TCR) is a by-product of export crab meat production processing and contains calcium and phosphor at 19.97% and 1.81% respectively (Kurnia *et al*. 2016; Mahary *et al*. 2022). Adding this crab shell flour may increase the absolute growth of koi weight and length (Wahyu & Chadijah 2017). Supporting this, Tarigan (2022) in their research finds that adding crab shell flour at 6% may increase both weight and length absolute growth and food conversion ratio (FCR) of tilapia fish. Calcium can also help accelerate the gastrolization (shell re-hardening) process after the molting process and increase the molting frequency (Handayani & Syahputra 2018). According to Zufadhillah et al. (2018), adding nano-calcium from giant mud crab shell at 3% to the feed can increase the weight and length growths by 2.58 g and 2.29 cm in giant freshwater prawns.

Papain is a proteolytic enzyme capable of breaking down protein that can be obtained from papaya extracts. This enzyme serves to break down protein into peptide and to further break down peptide into

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amino acid and can also increase growth (Mareta et al. 2017; Prihatini & Dewi 2021). According to Robi et al. (2020), adding papain enzyme at 4.5% to the feed of striped snakehead seeds is the most optimal. Meanwhile, Rostika et al. (2021) find that adding the papain enzyme dose at 3.75% and 5% has significant effect on the growth of Cantang grouper. According to Patil & Singh (2014), adding papain enzyme from LOBA CHEMIE at 0,1% to the feed of post larvae of giant freshwater prawns is the most optimal by resulting maximum of length gain 18,36%; weight gain 81,15%; specific growth rate 1,41% and survival rate 59,33%.

Based on the information above, it is clear that adding calcium can increase the somatic growth (weight and length) and adding papain enzyme to the feed can increase the feed efficiency (EPP), protein efficiency ratio (PER) and relative growth rate (RGR). For this reason, further study is needed on other cultivars at different and or higher doses. In previous studies on the growth of giant freshwater prawns, researchers commonly used papain enzyme extracted from papaya latex and synthesized nano-calcium from scratch. This approach however is costly and difficult for farmers to replicate, limiting it's accessibility and practical application. To improve the accessibility and affordability for the local farmers, it's essential to explore alternative sources of papain enzyme and calcium that are both readily available and cost-effective. Therefore, this research will test the effect of combination of crude papain enzyme and commercial calcium derived from crab shell flour at a 3% inclusion rate in commercial feed on the growth rate of giant freshwater prawns. By utilizing more practical and affordable sources, this study seeks to provide a feasible feeding strategy that can be adopted by farmers to enhance prawns growth efficiently and economically.

2. Materials And Methods

2.1. Research Time and Location

This research was carried out on Monday, 24 July 2023 through Friday, 22 September 2023 or 60-day maintenance period. It was conducted at Balai Benih Udang Galah (BBUG) Pamarican, Ciamis Regency, West Java.

2.2. Research Method

The test prawns were 6-to-9-gram giant freshwater prawns about 9 cm long at a density of 1 prawn/2 liters of water. This was experimental research with Completely Randomized Design (CRD) and 5 treatments repeated 3 times. The five treatments were P0 (Papain 0%; TCR 0%), P1 (Papain 2.25%; TCR 3%), P2 (Papain 3.375%; TCR 3%), P3 (Papain 5%; TCR 3%), and P4 (Papain 6%; TCR 3%). The feeding was conducted three times daily with a dosage 6% of the mass weight of giant freshwater prawns: in the morning from 8:00 to 9:00 a.m., in the afternoon from 3:00 to 4:00 p.m., and in the evening from 8:00 to 9:00 p.m.

The commercial feed used in this study, from the brand Feng Li type Prima Feed PF-100, which after being tested, the proximate analysis conducted by the Laboratory of Ruminant Nutrition and Animal Feed Chemistry, Faculty of Animal Science, Padjadjaran University, could be know it's contain crude protein of 38,11%. Papain enzyme and calcium TCR were incorporated into commercial feed by mixing with progol solution, tapioca flour, 3% calcium TCR and crude papain enzyme powder at the appropriate dosage. The mixture was then reformed using a feed molding machine with an 8 mm size. After molding, the feed was dried in a conventional oven at 50°C for 12 hours.

The parameters observed include absolute weight growth (GR), absolute length growth (L), relative growth rate (RGR), feed efficiency (EPP), protein efficiency ratio (PER), survival rate (SR), and water quality. Each data will be tested using the F-test (ANOVA) at a 95% trusted level or 0,05% error level. If significant differences are found between treatments, a Duncan's multiple range test will be used at a 95% trusted level. The water quality will be analyzed descriptively.

2.3. Research Parameter

2.3.1 Absolute weight growth

The absolute weight growth (GR) could be calculated using Effendie (1997) formula as follows:

$$GR = (W_t - W_0)$$

Where:

GR = Test animal's absolute weight growth (g)

W_t = Test animal's average weight at the end of research (g)

W_0 = Test animal's average weight at the beginning of research (g).

2.3.2 Absolute Length Growth

The absolute length growth (L) could be calculated using the Effendie (1997) formula below:

$$L = (L_t - L_0)$$

Where:

L = Test animal's absolute length growth (cm)

L_t = Test animal's average length at the end of research (cm)

L_0 = Test animal's average length at the beginning of research (cm)

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2.3.3 Relative Growth Rate (RGR)

The relative growth rate (RGR) could be calculated using the formula based on Takeuchi (1988) as follows:

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

Where:
RGR= Relative growth rate (% / hari)
W_t = Test animal's biomass at the end of research (g)
W_0 = Test animal's biomass at the beginning of research (g)
t = Experiment duration (days)

2.3.4 Feed Efficiency (EPP)

The feed efficiency (EPP) could be calculated based on the Tacon (1987) formula as follows:

$$EPP\ (\%) = \frac{W_t - W_0}{F} \times 100\%$$

Where:
EPP = Feed efficiency (%)
W_t = Test animal's biomass at the end of research (g)
W_0 = Test animal's biomass at the beginning of research (g)
F = The amount of feed consumed during research (g)

2.3.5 Protein Efficiency Ratio (PER)

The protein efficiency ratio (PER) could be calculated based on Zonneveld (1991) formula as follows:

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

Where:
PER = Protein efficiency ratio (%)
W_t = Test animal's biomass at the end of research (g)
W_0 = Test animal's biomass at the beginning of research (g)
P_i = Protein weight of the consumed feed (g)

2.3.6 Survival Rate (SR)

The survival rate of giant freshwater prawns was observed once in 10 days during the maintenance period. To figure out the survival of giant freshwater prawns, the following formula was used:

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

Where:
SR = Survival rate (%)
N_t = Number of test animals at the end of experiment
N_0 = Number of test animals at the beginning of experiment

2.3.7 Water Quality

Each parameter of water quality was measured using different tools. DO meter was used to measure DO, pH indicator (litmus) to measure pH, thermometer to measure temperature, and SERA test kit to measure ammonia.

3. Results and Discussion

The growth of giant freshwater prawn (*Marcobranchium rosenbergii*) seeds during research was observed from their absolute weight growth, absolute length, relative growth rate, feed conversion ratio, feed efficiency, protein efficiency ratio, survival rate and molting frequency. In terms of the giant freshwater prawn seeds' growth, the results can be seen in Table 1.

Table 1. Data on Growth Observation Results of Giant Freshwater Prawn Seeds

Variable Value	Treatment				
	P_0	P_1	P_2	P_3	P_4
Absolute weight growth (g)	2.34 ± 0.74a	2.84 ± 0.83b	3.07 ± 0.39c	3.74 ± 0.2e	3.37 ± 0.6d
Absolute length growth (cm)	1.46 ± 0.1a	1.53 ± 0.25ab	1.70 ± 0.32b	2.08 ± 0.02c	1.71 ± 0.14b
RGR (%)	0.55 ± 0.06a	0.58 ± 0.04a	0.69 ± 0.06b	0.78 ± 0.02bc	0.83 ± 0.07c
EPP (%)	44.52 % ± 0.01c	46.36 % ± 0.02c	49.34 % ± 0.01b	52.44% ± 0.002a	51.69 % ± 0.01a
PER	6.14 ± 0.22e	7.58 ± 0.24d	8.10 ± 0.24c	9.99 ± 0.09a	8.94 ± 0.11b
SR (%)	44% ± 0.58a	71% ± 0.58b	82% ± 0.58c	91% ± 0.58d	84% ± 0.58cd

3.1. Absolute Weight Growth

Treatment P_3 showed the highest absolute weight average value of around 3.74 g and treatment P_0 showed the lowest absolute weight growth at 2.34 g as indicated in Table 2. This meant that the commercial feed added with papain enzyme at 5% and calcium TCR at 3% was the most optimal to be digested by the giant freshwater prawn seeds, making it possible to increase the prawn's weight.

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Table 2. Absolute Weight Growth of Giant Freshwater Prawns upon Varied Additions of Papain Enzyme at a Fixed TCR calcium level of 3% (P_1 to P_4) in Commercial Feed

Papain Enzyme Treatment	Absolute Weight Growth (g)
P_0 (0%)	2.34 ± 0.74^a
P_1 (2.25%)	2.84 ± 0.83^b
P_2 (3.375%)	3.07 ± 0.39^c
P_3 (5%)	3.74 ± 0.20^d
P_4 (6%)	3.37 ± 0.46^e

The weight growth of juvenile giant prawns increased significantly with increasing papain enzyme levels up to 5% added at a fixed TCR calcium level of 3%, whereas the P_4 treatment showed a decrease relative to the P_3 treatment. The weight growth of juvenile giant prawns in P_4 was 3.37 ± 0.46 g, and in P_3 it was 3.74 ± 0.20 g. The decrease in weight growth can be attributed to the papain enzyme reaching saturation, where all active sites are bound to the substrate, and increasing the substrate concentration will not significantly increase the reaction rate, as measured experimentally by calculating the Michaelis-Menten kinetic parameters (Elsson et al. 2019).

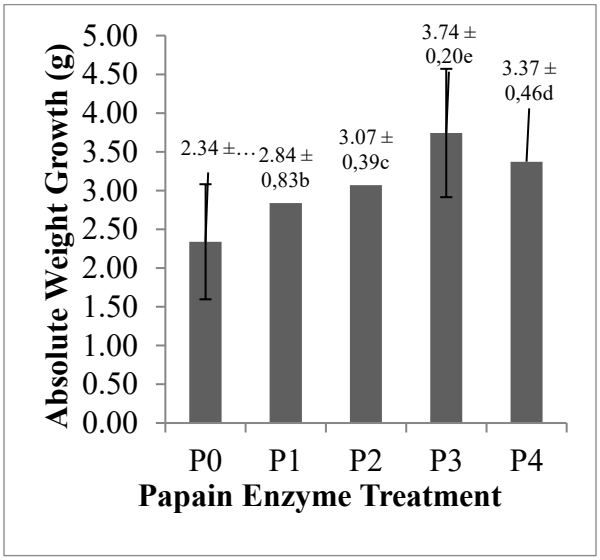

Figure 1. Absolute Weight Growth of Giant Freshwater Prawns upon Varied Additions of Papain Enzyme at a Fixed TCR calcium level of 3% (P_1 to P_4) in Commercial Feed

3.2. Absolute Length Growth

Treatment P_3 generated the highest absolute length growth value of 2.08 cm and the lowest absolute length growth value was found in treatment P_0 of around 1.46 cm.

Table 3. Absolute Length Growth of Giant Freshwater Prawn upon Varied Additions Papain Enzyme at a Fixed TCR calcium level of 3% (P_1 to P_4) in Commercial Feed

Papain Enzyme Treatment	Absolute Length Growth (cm)
P_0 (0%)	1.46 ± 0.10^a
P_1 (2.25%)	1.53 ± 0.25^{ab}
P_2 (3.375%)	1.70 ± 0.32^b
P_3 (5%)	2.08 ± 0.02^b
P_4 (6%)	1.71 ± 0.14^c

The greater the amount of papain enzyme was added to the commercial feed, the higher the length growth value would be in the giant freshwater prawns. This was different from treatment P_0 in which no papain enzyme was added to the commercial feed and the lowest length growth of giant freshwater prawns was found. The protein absorption and digestion rate could increase thanks to the addition of papain enzyme which was a protease one, capable of hydrolyzing protein into simpler form, namely peptide and amino acid. This was consistent with Rostika et al. (2018) who found that supplementing papain enzyme into feeds significantly increased the protease enzyme activity, EPP, REP, and LPH. The papain enzyme optimal dose added to the commercial feed was 3.75% for Nile tilapia to increase the EPP to 48.31%, REP to 2.13%, and LPH to 2.07.

3.3. Relative Growth Rate (RGR)

Table 4 showed that the greater the papain enzyme was added, the higher the increase in RGR value (%/day) would be. This could be seen from the fact that the highest RGR value was found in treatment P_4 at 0.83%/day as compared to P_0 in which neither papain enzyme nor calcium TCR was added to the feed and the RGR was found to be 0.55%/day.

Table 4. Relative Growth Rate (RGR) of Giant Freshwater Prawns upon Varied Addition of Papain Enzyme and a fixed TCR calcium level of 3% (P_1 to P_4) in Commercial Feed

Papain Enzyme Treatment	Relative Growth Rate (%/day)
P_0 (0%)	0.55 ± 0.06^a
P_1 (2.25%)	0.58 ± 0.04^a
P_2 (3.375%)	0.69 ± 0.06^b
P_3 (5%)	0.78 ± 0.02^{bc}
P_4 (6%)	0.83 ± 0.07^c

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3.4. Feed Efficiency (EPP)

According Puspasari (2015), EPP was classified as good when it was over 50%. EPP was closely related to the prawns' ability to digest the feed given to them.

Table 5. Feed Efficiency (EPP) of Giant Freshwater Prawns upon Varied Addition of Papain Enzyme and a fixed TCR calcium level of 3% (P_1 to P_4) in Commercial Feed

Papain Enzyme Treatment	Feed efficiency (%)
P_0 (0%)	44.52% ± 0.011^c
P_1 (2.25%)	46.36% ± 0.017^c
P_2 (3.375%)	49.34% ± 0.012^b
P_3 (5%)	52.44% ± 0.002^a
P_4 (6%)	51.69% ± 0.006^a

Based on Figure 2, the least EPP was found in treatment P_0 in which neither papain enzyme nor TCR calcium was added. Meanwhile, when the feed was added with some enzyme and TCR calcium, an increase in EPP was observed. Also from Figure 2, it could be seen that the greater the papain enzyme was added to the giant freshwater prawns' feed, the higher the EPP value would be. From P_3 to P_4 feed treatment, a decrease of 0.75% was spotted. Yet, since the value was still higher than 50%, it was still considered as an optimal treatment in terms of feed provision efficiency. This was consistent with the previous study conducted Rostika et al. (2018) who added papain enzyme to the commercial feed for Nile tilapia growth. Their research showed that supplementing papain enzyme to the feed could significantly increase EPP by 48.31% with the optimum dose of papain enzyme addition being 3.75% into the commercial feed.

3.5. Protein efficiency ratio (PER)

Table 6 showed that the most optimum composition of papain enzyme added to the commercial feed was found when the papain enzyme was added at 5%. This could be seen from the fact that the PER value obtained from this composition was the highest at 9.99. When the papain enzyme added to the feed was greater at 6% of PER, a decrease re-occurred from the previous one at 8.94. Meanwhile, the previous study carried out by Rachmawati et al. (2020) that tested the addition of papain enzyme to the Vaname prawn feed found that the PER value was 1.64. In another previous study by Rostika et al. (2018), a PER value of 2.13 was found for the 3.75% papain enzyme addition to the commercial feed for Nile tilapia. This showed that the PER value we obtained was higher than the previous studies.

Table 6. Protein Efficiency Ratio (PER) of Giant Freshwater Prawns upon Varied Additions of Papain Enzyme and a Fixed TCR Calcium Level of 3% (P_1 to P_4) in Commercial Feed

Papain Enzyme Treatment	Protein Efficiency Ratio (%)
P_0 (0%)	6.14 ± 0.22^e
P_1 (2.25%)	7.58 ± 0.24^d
P_2 (3.375%)	8.10 ± 0.24^c
P_3 (5%)	9.99 ± 0.09^b
P_4 (6%)	8.94 ± 0.11^a

3.7. Survival Rate (SR)

Treatment P_3 had the highest SR value at 91% and was followed by treatment P_4 at 84%, P_2 at 82%, P_1 at 71% and the treatment with the lowest SR value was treatment P_0 at 44%.

Table 7. Survival Rate (SR) of Giant Freshwater Prawns upon Varied Addition of Papain Enzyme and a Fixed TCR Calcium Level of 3% (P_1 to P_4) in Commercial Feed

Papain Enzyme Treatment	Survival Rate (%)
P_0 (0%)	44% ± 0.58^a
P_1 (2.25%)	71% ± 0.58^b
P_2 (3.375%)	82% ± 0.58^c
P_3 (5%)	91% ± 0.58^{bc}
P_4 (6%)	84% ± 0.58^d

Based on Table 7, the giant freshwater prawns fed with commercial feed that had been supplemented with papain enzyme and TCR calcium had a greater survival rate than those fed with commercial feed with no addition of papain enzyme or TCR calcium. The high survival rate indicated that the feed given to them could meet their needs. According to Hoseinifar et al. (2024), the right combination of feed could support growth, prevent infection and improve survival rate.

According to Bahri et al. (2022), the survival rate can be considered good when the SR value was greater than 70%, and at 50%-60% it could be considered adequate. However, when it was less than 50% it could be classified low or inadequate. Based on these criteria, the commercial feed added with papain enzyme and TCR calcium was classified as good because its SR value was over 70%. Providing feed that had been added with papain enzyme and TCR calcium could prevent cannibalism during molting process in giant

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freshwater prawns and eventually increased the SR value.

3.8. Water Quality

During the 60-day maintenance period, the water quality was observed and measured in terms of its temperature, pH, ammonia and DO in all treatments in aquariums of 80 cm in length by 40 cm in width by 40 cm in height. Based on Table 8, it was found that during the maintenance period the water quality, except for temperature, was still within a feasible range to support the growth and survival of giant freshwater prawns.

Table 8. Measurement of Quality of Media for Giant Freshwater Prawn Maintenance

Parameter	Result	Water Quality Standard for Giant Freshwater Prawn Cultivation (SNI 01-7244-2006)
Temperature	24.2-25.5 ℃	28 - 30 ℃
Acidity level (pH)	7 - 7.5	6.5 - 8.5
Ammonia	0.006 - 0.009 mg/L	≤0.02 mg/L (Suriyani 2010)
Dissolved Oxygen (DO)	7.0 - 8.5 mg/L	≥4 mg/L

During the giant freshwater prawn cultivation period, the media temperature ranged from 24.2 to 25.5°C. This value is below the water quality standard for giant freshwater prawn cultivation according to SNI 01-7244-2006. This is influenced by the building's open ventilation and the maintenance carried out during the rainy season, which resulted in subnormal temperatures.

4. Conclusion

The provision of re-pelleting feed made of commercial feed added with papain enzyme at different dose and TCR calcium at a fixed level of 3% could affect the length growth and absolute weight, relative growth rate, feed efficiency and protein utilization ratio of giant freshwater prawns. The effective condition for adding papain enzyme to the feed was found in treatment P3 at 5% papain enzyme addition. In this treatment P3, it was found that the values of EPP, PER, absolute weight growth, absolute length growth, RGR and SR were 52.44%, 9.99, 3.74 g, 2.08 cm, 0.78%/day, and 91%, respectively. The temperature during the cultivation period was below the standard according to SNI 01-7244-2006, that influenced by the building's open ventilation and the maintenance carried out during the rainy season; even so, it's still within a feasible range to support the growth and survival of giant freshwater prawns.

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