The effect of papain and bromelain enzymes on the growth and feed utilization of post larvae *Litopenaeus vannamei*

I I Arisa\(^{1,2,3,*}\), Z A Muchlisin\(^{1,2}\), S Purba\(^1\), A A Muhammadar\(^{1,2,3}\) and S Mellisa\(^{1,2,3}\)

\(^{1}\)Department of Aquaculture, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh, Aceh, 23111, Indonesia.
\(^{2}\)Marine and Fisheries Research Center, Universitas Syiah Kuala, Banda Aceh, Aceh, 23111, Indonesia.
\(^{3}\)Animal Histology and Fish Nutrition Laboratory, Marine and Fisheries Faculty, Universitas Syiah Kuala, Banda Aceh, Aceh, 23111 Indonesia.
*Corresponding author: muchlisinza@unsyiah.ac.id*

**Abstract.** The aim of this research was to evaluate the effect of papain and bromelain enzymes in feed on growth and feed utilization of post larvae *Litopenaeus vannamei* shrimp. The research was conducted during 23 January to 2 March 2020 at the Fish Hatchery Laboratory, Faculty of Marine and Fisheries, Syiah Kuala University. The completely randomized design consisting of four treatments and three replications were used in this study (A (feed without enzyme); B (feed + 1% papain enzyme); C (feed + 1% bromelain enzyme), and D (feed + 1% papain enzyme + 1% bromelain enzyme)). The shrimp was fed with the tested feed five times on 07.00 AM, 11.00 AM, 03.00 PM, 07.00 PM and 11.00 PM for 42 days. The ANOVA test results showed the addition of papain and bromelain enzymes in the feed had a significant effect on weight gain, daily growth rate and specific growth rate of vannamei shrimp (\(P<0.05\)). However, there was no significant effect on its survival, feed conversion and feed efficiency (\(P>0.05\)). The best treatment was found in treatment D with the weight gain of 1.15 g, daily growth rate of 0.027 g day\(^{-1}\), specific growth rate of 7.14\% day\(^{-1}\), feed conversion ratio of 1.85, and feed efficiency 54.15\%, but the highest survival was found at treatment A. It is concluded that the best treatment is a combination between of 1% papain enzyme and 1% bromelain enzyme.

1. **Introduction**

The success of vannamei shrimp cultivation is supported by the availability of feed, because it affects growth and survival [1]. The feed given to cultured shrimp must have good quality such as high nutritional content and easily digested by the shrimp gut, which functions as a support for growth and high feed efficiency [2]. One of the ways to increase feed efficiency is optimizing the digestion and absorption of feed.

Optimal digestion and absorption of feed in shrimp can occur with the addition of digestive enzymes. There are several enzymes that are commonly added to feed to increase feed digestibility, including papain and bromelain enzymes. Papain comes from papaya sap which contains protease enzymes, which are proteolytic and function as protein hydrolysis. According to Rostika *et al.* [3] Enzymes in feed can increase protein absorption and the digestive system in the digestive tract. There are several enzymes commonly added to feed to increase feed digestibility, including papain and bromelain enzymes. Ellson *et al.* [4] said papain is a proteolytic enzyme isolated from the papaya fruit sap e tapping, or it could also come from papaya leaves (*Carica papaya* L.). Than according to Manush [5] the performance of protease in the digestive tract is the main thing in terms of digestibility and the efficiency of protein
digestion. The papain enzyme found in feed helps accelerate the digestive process of shrimp by breaking down protein into simpler protein so that it is easily digested and absorbed by the intestines and increases the growth rate. Several other studies regarding the use of the enzyme papain in feed which can increase the growth of fish [6, 7 and 8] and shrimp [9] have also been reported.

In addition to the papain enzyme, the use of the enzyme bromelain also plays a role in the process of digestion of food. Bromelain enzyme can be produced from pineapple extract. Research by Rachmawati and Samidjan [10] stated that the addition of pineapple extract in feed can affect the efficiency of feed utilization and growth of vaname shrimp. Research by Rostika et al. [3] the combination of papain and bromelain enzymes in feed could increase the rate of Daily Growth Rate and Feed Utilization Efficiency of Pangasianodon hypophthalmus. Therefore, the researchers studied the effect of adding the combination of papain and bromelain enzymes in feed in increasing the growth of vaname shrimp. The aim of this research was to evaluate the effect of papain and bromelain enzymes in feed on growth and feed utilization in post larvae Litopenaeus vannamei shrimp.

2. Material and Methods

2.1. Tools and Material

The tools used in this research are DO meters, digital scales, thermometers, pH meters, rulers, filters, plastic containers, aerators. The ingredients used are L. vannamei shrimp, fish meal, shrimp head meal, young pineapple, head meal, tofu waste meal, tapioca flour, bran, corn meal, fish oil, vitamins, mineral, papain enzyme, bromelain enzyme.

2.2. Method

The study was conducted at the fish hatchery Laboratory, Marine and Fisheries Faculty, Syiah Kuala University, Banda Aceh, Indonesia, during 42 days (23 January - 2 March 2020). The L. vannamei was stocked in a plastic bucket (35 L), totaling 35 shrimp/buckets with a weight of 0.06 g/shrimp. Shrimp is kept for 42 days. Sampling is done every 7 days. The frequency of feeding was carried out 5 times a day, namely at 07.00, 11.00, 15.00, 19.00 and 23.00 WIB. Feed is given as much as 5% of the weight of the shrimp biomass.

The design of this study is to use a completely randomized design (CRD) using 4 treatments and 4 replications. The treatments tested in this study are as follows:

A = test feed without enzymes  
B = test feed + papain enzyme 1% 
C = test feed + bromelain enzyme 1% 
D = test feed + papain enzyme 1% + bromelain enzyme 1%

2.3. Bromelain Enzyme Extraction

Pineapple fruit cleaned and mashed in a blender, then homogenized with a cold pH 7.0 phosphate buffer as much as 1: 1. The solution obtained was centrifuged at 3000 g for 15 minutes. Furthermore, the supernatant separated from the sediment or called a pellet. The supernatant obtained was a crude extract of the bromelain enzyme [11].

2.4. Test Feed Formula

The feed formulation as shown in the Table 1.

2.5. Growth Parameter

2.5.1. Absolute growth

The absolute growth was calculated according Effendi [12]: \( W = Wt - Wo \), Where: \( W \) = growth (g) \( Wt \) = final weight of biomass (g) \( Wo \) = initial weight of biomass (g).
2.5.2 Daily weight growth rate
The Daily Weight Growth Rate (DWGR) was calculated according to DeSilva and Anderson [13]:
\[ \text{DWGR} = \frac{W_2 - W_1}{T_2 - T_1}, \]
where: DWGR = Daily weight growth rate (g day\(^{-1}\)); W1 = initial weight of fish (g); W2 = final weight of fish (g); T1 = initial time (day); T2 = final time (day).

2.5.3 Specific growth rate
The specific growth rate (SGR) was calculated according to De Silva and Anderson [13]:
\[ \text{SGR} = \frac{\ln W_t - \ln W_0}{t} \times 100, \]
where: SGR = Specific growth rate (% day\(^{-1}\)); Wt = final weight of biomass; Wo = initial weight of biomass; t = research time (day).

2.5.4 Feed efficiency
Feed efficiency (FE) was calculated according to Tacon [14]:
\[ \text{FE} = \frac{(W_t + D) - W_0}{F} \times 100, \]
where: FE = Feed efficiency (%); Wt = Final fish weight (g); Wo = Initial fish weight (g); D = Dead fish weight (g); F = Feed consumed (g).

2.5.5 Survival rate
Survival rate (SR) was calculated according to [15]:
\[ \text{SR} = \frac{N_0 - N_t}{N_0} \times 100, \]
where: SR = survival rate (%); Nt = number of death fish (ind); No = number of observed fish (ind).

### Table 1. The composition of raw materials for the test feed with 35% protein

| No | The Ingredients of The Test Feed | Amount of Protein (%) | Amount of Feed Ingredients in Treatment (g) |
|----|---------------------------------|-----------------------|-------------------------------------------|
|    |                                 |                       | A  | B  | C  | D  |
| 1  | Shrimp head meal                | 43,2                  | 320| 320| 320| 320|
| 2  | Fish meal                       | 45                    | 420| 420| 420| 420|
| 3  | Tofu flour waste                | 17,72                 | 83 | 78 | 78 | 75 |
| 4  | Bran                            | 11                    | 50 | 50 | 50 | 45 |
| 5  | Fish oil                        | 0                     | 10 | 10 | 10 | 10 |
| 6  | Tapioca flour                   | 0,5                   | 50 | 50 | 50 | 48 |
| 7  | Vitamin                         | 0                     | 10 | 10 | 10 | 10 |
| 8  | Corn meal                       | 3,7                   | 50 | 45 | 45 | 45 |
| 9  | Mineral                         | 0                     | 2  | 2  | 2  | 2  |
| 10 | Papain enzyme                   | 0                     | 0  | 10 | 0  | 10 |
| 11 | Bromelain enzyme                | 0                     | 0  | 0  | 10 | 10 |

### 3. Results and Discussion
The results showed that the addition of papain and bromelain enzymes in the feed had an effect on growth performance, where the best results were obtained in the combination treatment of papain 1% enzymes and 1% bromelain enzymes (P < 0.05). However, the addition of these enzymes in the feed did not affect the utilization of the feed and the survival of the vannamei shrimp larvae (P > 0.05) (Table 2). The results showed that the combination of the two enzymes gave the best results compared to using the enzyme alone. It is assumed that the two enzymes have complementary functions in helping the digestive process of feed. According to Winarno [16] the papain enzyme works more actively to hydrolyze vegetable protein, while the bromelain enzyme works more actively to hydrolyze animal protein. As reported by Kordi [17], vannamei shrimp is an omnivore scavenger and is very greedy. Thus, the role of the use of papain and bromelain enzymes here is thought to help hydrolyze feed protein into simpler molecules so that it can optimize digestion and absorption of feed by shrimp to increase growth.
However, further research is needed to obtain optimal enzyme concentrations for the utilization of vaname shrimp feed.

### Table 2. Absolute growth, daily weight growth rate, specific growth rate, and survival rate of *L. vannamei*.

| Treatments | Absolute rate (g) | Daily weight growth rate (g day⁻¹) | Specific growth rate (% day⁻¹) | Feed efficiency (%) | Feed Convention Ratio |
|------------|------------------|----------------------------------|------------------------------|---------------------|-----------------------|
| A (test feed without enzymes) | 0.88 ± 0.045ᵃ | 0.021 ± 0.0010ᵃ | 6.54 ± 0.115ᵃ | 1.93 ± 0.18ᵃ | 52.23 ± 4.683ᵃ |
| B (test feed + papain enzyme 1%) | 0.92 ± 0.044ᵃ | 0.022 ± 0.0012ᵃ | 6.65 ± 0.104ᵃ | 1.88 ± 0.05ᵃ | 53.32 ± 1.474ᵃ |
| C (test feed + bromelain enzyme 1%) | 1.03 ± 0.038ᵇ | 0.024 ± 0.0058ᵇ | 6.90 ± 0.081ᵇ | 1.87 ± 0.11ᵃ | 53.65 ± 3.212ᵃ |
| D (test feed + papain enzyme 1% + bromelain enzyme 1%) | 1.15 ± 0.030ᶜ | 0.027 ± 0.0058ᶜ | 7.15 ± 0.060ᶜ | 1.85 ± 0.14ᵃ | 54.15 ± 4.093ᵃ |

Description: Different superscript letters in the same column show significantly different (P<0.05)

A report on research on the use of papain and bromelain enzymes in feed has been reported by Taqwdasbriliani *et al.* [18] namely the addition of a combination of papain and bromelain enzymes in commercial feed of the tiger grouper (*Epinephelus fuscoguttatus*) had an effect on fish growth, feed use efficiency and protein efficiency. Ananda *et al.* [19] stated that the addition of the enzyme papain in feed had a better growth rate than without the enzyme papain. Nisrinah [20] also stated that the addition of bromelain enzymes in feed is better than feed that did not contain bromelain. Mo *et al.* [21] reported an increase in the growth performance of *Ctenopharyngodon idellus* in general (feed conversion ratio, protein efficiency ratio and relative weight gain) was observed in feed added 5 g/kg⁻¹ of *Saccharomyces cerevisiae* and enzymes (bromelain and papain, with a ratio of 1: 1).

![Figure 1](image-url)  
**Figure 1.** Graph of survival rate *Litopenaeus vannamei* for 42 days of maintenance (A= test feed; B= test feed + papain enzyme 1%; C= test feed + bromelain enzyme 1%; D test feed + papain enzyme 1% + bromelain enzyme 1%).

Vannamei shrimp survival rates during maintenance were treated A (81.9%), B (73.33%), C (70.48%) and D (76.19%) shown in Figure 1. The best survival rate was obtained at treatment A, but it was not statistically significant with other treatments (P>0.05). This may be due to the fact that survival is not only affected by feed, but also by environmental factors (water quality) and handling during the study. The survival of shrimp is influenced by internal and external factors. Internal factors come from
the shrimp itself. Shrimp experience stress due to careless treatment resulting in high mortality and competition for food. External factors that influence include environmental conditions such as high ammonia and/or due to unfavorable conditions for maintenance.

However, the water quality during the study was still in a good range for shrimp growth. The quality of water obtained during the study, namely temperatures ranging from 26.5-29°C, the value of the degree of acidity (pH) ranged from 7.4 to 8.2, the value of salinity (salinity) was around 20-23 ppt and the value of dissolved oxygen (DO) ranged 4.6-5.38 ppm. The water quality is still in a good quality condition. This is consistent with the statement of Kordi [17], the growth of shrimp both at salinity of 10-30 ppt and temperature of 24-34°C and ideal growth at salinity of 15-25 ppt and temperature of 28-31°C. The optimum range of water quality for DO shrimp maintenance is around ≥ 3 ppm and pH 7.5-8.5 [22].

4. Conclusion

Based on the results of research that has been carried out, the addition of papain and bromelin enzymes affected in *Litopenaeus vannamei* feed had an effect on growth performance, but did not affect feed utilization. The combination of the enzyme papain and bromelain can give better results than using it separately.

References

[1] Soemardjati W dan Suriawan A 2007 *Petunjuk Teknis Budidaya Udang Vanamei (Litopenaeus vannamei) di Tambak* (Situbondo: Departemen Kelautan dan Perikanan Direktorat Jenderal Perikanan Budidaya Balai Budidaya Air Payau) pp 30
[2] Isnahwati N, Sidik R and Mahasri G 2015 *Jurnal Ilmiah Perikanan dan Kelautan* 7 121-124.
[3] Rostika R et al 2006 IOP Conference Series: Earth and Environmental Science 139 012006
[4] Elsson M et al 2019 IOP Conference Series: Earth and Environmental Science 217 012037
[5] Manush S M, Srivastava P P, Kohli M P S, Jain K K, Ayyappan S and Metar S Y 2013 Turkish Journal of Fisheries and Aquatic Sciences 13 479-486.
[6] Muchlisin Z A, Fardin A, Tanzil M, Nur F, Zulkarnain J and Muhammador A A 2016 *Journal Biosaintifika* 8 172-177
[7] Amalia R, Subandiyono and Arini E 2013 *Journal of Aquaculture Management and Technology* 2 136-143
[8] Rachmawati D et al 2020 IOP Conference Series: Earth and Environmental Science 530 012037
[9] Patil DW and Singh H 2014 *Journal International Journal of Fisheries and Aquatic Studies* 1 176-179
[10] Rachmawati D and I Samidjan 2018 Suplementasi Ekstrak Nanas Pada Pakan Terhadap Pemanfaatan Pakan Dan Pertumbuhan Udang Vanamei (Litopenaeus vannamei) Upaya Untuk Meningkatkan Produksi Prosiding Seminar Nasional Kelautan dan Perikanan IV Tahun 2018 278-284
[11] Wuryanti 2004 *J. Kim dan Apt 7* 78-82.
[12] Effendie M I 1997 *Biologi Perikanan* (Yayasan Pustaka Nasutama: Yogyakarta)
[13] De Silva S S and Anderson T A 1995 Fish Nutrition in Aquaculture *Springer Science & Business Media* 320
[14] Tacon A G 1987 The nutrition and Feeding of Farmed Fish and Shrimp- *A Training Manual FAO of The United Nations* Brazil 106-109
[15] Goddard S 1996 Feed Management in Intensive Aquaculture (Chapman and Hall: New York)
[16] Winarno F G 1995 Enzim Pangan (PT Gramedia Utama: Jakarta)
[17] Kordi K M G H 2010 Budidaya Udang Laut (Penerbit ANDI: Yogyakarta)
[18] Taqdwasbriliani E B, Hutarbarat J and Arini E 2013 *Journal of Aquaculture Management and Technology* 2 76-85
[19] Ananda T, Rachmawati D and Samidjan I 2015 *Journal of Aquaculture Management and Technology* 4 47–53
[20] Nisrinah, Subandiyono and Elfitasari T 2013 *Journal of Management an Technology* 2 57-63
[21] Mo W Y, Choi W M, Man K and Wong M H 2020 *Journal Science of the Total Environment* **707** 134954

[22] Standar Nasional Indonesia (SNI) 2006 *Produksi Udang Vannamei (Litopenaeus vannamei) di Tambak Dengan Teknologi Intensif* (Jakarta: Badan Standardisasi Nasional)