Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(4): 555 – 571 (2024)
www.ejabf.journals.ekb.eg

Growth, Enzyme Activity, Score, and Essential Amino Acid Index of the Asian Tiger Shrimp (*Penaeus monodon* Fab. 1798) Supplemented with Multi Enzyme

Fitriwi Arlini[1*], Edison Saade[2], Haryati Tandipayuk[2]

[1]Master Program of Fisheries Science, Faculty of Marine Science and Fisheries, Hasanuddin University, Jl. Perintis Kemerdekaan KM 10, Tamalanrea, Makasar 90245, South Sulawesi, Indonesia
[2]Departemen of Fisheries, Faculty of Marine Science and Fisheries, Hasanuddin University. Jl. Perintis Kemerdekaan KM 10, Tamalanrea, Makasar 90245, South Sulawesi, Indonesia

[*]**Corresponding author**: fitriwiarlini1999@gmail.com

ARTICLE INFO

Article History:
Received: July 11, 2024
Accepted: July 20, 2024
Online: July 24, 2024

Keywords:
Enzyme activity,
Essential amino acids,
Growth,
Multi enzyme,
Tiger shrimp

ABSTRACT

The tiger shrimp (*Penaeus monodon* Fabicius, 1798) is a popular Indonesian fishery product with high market value both domestically and internationally. Its high nutritional value makes it a valuable product that is in high demand. Along with increased production, various challenges appeared in management and feed quality aspects. Multi-enzymes are one method for increasing the tiger shrimp productivity. This study aimed to identify the dose of multi-enzymes in the feed that leads to an optimal growth performance, and optimum activity of digestive enzymes (amylase, protease, lipase), as well as improving the essential amino acids index in the shrimp body. This study used a completely randomized design (CRD) with 4 treatments and 3 replications each, thus 12 experimental units were applied. The treatments used were A at 0%/kg feed, B at 40%, C at 60%, and D at 80%/kg feed. The experimental animal was the tiger shrimp with a mean weight of 0.07g/ ind. The shrimps were reared in the hatchery laboratorium of the Marine Science and Fishery Faculty, Hasanuddin University, Makassar. The parameters examined were essential amino acid index (EAAI), essential amino acid score (EAAS), enzyme type activity, specific growth rate (SGR), relative weight gain (RWG), survival rate (SR), and protein efficiency ratio (PER). The results showed that feed with 80% multienzyme/kg provided the highest EAAI compared to other doses, namely 2.64. In addition, the most EAAS deficiency occurred in methionine EAA, namely 9.29 in multi-enzyme feed with a dose of 40%/kg. SGR, RWG, and PER were not significantly different ($P > 0.05$), but SR showed a significant difference ($P < 0.05$). The activity of amylase, protease, and lipase enzymes in the tiger shrimp also increased with the addition of multienzyme doses to the feed.

INTRODUCTION

Penaeus monodon Fabricius, 1798 is an original Indonesian shrimp, with high demand in domestic and international markets due to its high nutritional value reaching 55.94% proteins of meat **(Liu *et al.*, 2021)**. As an eminent commodity, shrimps assist in promoting the country's foreign exchange **(Rachmawati *et al.*, 2021)**. To increase shrimp

production, management, and feed quality are required. Therefore, it is vital to make various attempts to increase feed quality, e.g. multi-enzyme approaches. The rationale is the availability of enzymes in feed boosting, feed digestibility, and nutrient absorption, allowing feed to be absorbed efficiently and increasing shrimp growth.

Shrimp growth is encouraged by the use of quality feed, which consists of protein, fat, carbohydrates, vitamins, and minerals according to the dietary requirements of the shrimp. Additionally, nutritional requirements should be balanced in order to support physiological and metabolic processes **(Ngoc Anh *et al.*, 2023)**. The tiger shrimp develop optimally on feed containing 35- 40% protein **(Islam *et al.*, 2024)**. According to **Lalopua *et al.* (2022)**, shrimp protein requirements are impacted by environmental factors, and feed prices are currently high. Farmers commonly employ commercial artificial feed, which is manufactured from specific raw materials and is distributed in large **(Jannah, 2020)**. However, commercial feed provides limited protein and is costly if it contains high protein. **García-rodríguez *et al.* (2021)** suggested that integrating multi-enzymes into commercial feed may improve its quality significantly.

Arlini (2022) found that using various enzymes in the tiger shrimp feed improved the protein and carbohydrate content of the shrimp body. Furthermore, multi-enzyme supplementation increases the performance and nutrition value reaching 90% of the protein, 48.88% of the essential amino acid index, and 69.82% of the essential amino acid score of feed. Previous research has demonstrated that administrating several enzymes to feed will improve the catfish fry growth **(Jayanti, 2019)**. The abundance of enzymes in the shrimp digestive system is considered to promote feed digestibility, making nutrient absorption better **(Felix *et al.*, 2018)**. To improve enzyme quality, multi-enzyme products should consist of a variety of enzymes such as xylanase, glucanase, protease, cellulase, amylase, and mannanase that should be added.

Multienzymes given to diet may increase the digestive enzyme activity, consequently, shrimp development was accelerated **(Cahyadi, 2020; Rachmawati *et al.*, 2020)**. The digestive enzyme activity of diverse shrimp species is linked to their feeding behavior. Beneficial proteins for shrimp growth are distinguished by their relationship to enzyme activity in the shrimp body. Moreover, the digestive enzyme activity is higher in the hepatopancreas compared to the stomach or intestines. In addition, the tiger shrimp digestive enzyme activity was discovered to be related to growth due to enzymes improving feed digestibility in the digestive tract and improving amino acid absorption **(Xiao *et al.*, 2023)**.

Protein is a complex compound consisting of amino acids. Protein is composed of two types of amino acidic substances essential and non-essential **(Tomičić *et al.*, 2020)**. Enzyme activity and amino acid concentration are useful to assess feed quality by employing criteria including the chemical score (CS) and the essential amino acid index (EAAI). The chemical score is based on the concept of EAA utilization which is

indicated by the lowest amino acid score. In contrast, the lowest proportion of amino acid requirement reflects the most deficient CS value.

According to **Sefer *et al.* (2021)**, the nutritional value of protein is determined by all necessary amino acids, rather than just one (as in CS calculations). It is because each amino acid is unique and essential. Therefore, determining the nutritional value of a protein requires calculating the integration of all essential amino acids known as the essential amino acids index (EAA-Index). Based on these hypotheses, the enzyme activity, growth, score, and essential amino acid index of the tiger shrimp fed a multi-enzyme feed is extremely important to evaluate.

MATERIALS AND METHODS

1. General description and design experimental

This study was conducted from October to December 2023 at the Hatchery Laboratory, Faculty of Marine and Fisheries Sciences, Hasanuddin University, Makassar. The juvenile shrimps were reared in 12 aquariums with 30L volume measuring 40x36x32cm^3 equipped with a recirculation system. The tiger shrimp samples with a mean weight of 0.07g/ ind were collected from UD Pembibitan Udang Mitra Sejahtera, Barru Regency, and stocked at a density of 1 individual/L making it 30 Individuals in total. The enzyme used is the Biogreen Juara Multi Enzyme obtained from CV. Arjuna Brawijaya was mixed in feed with different doses, namely: 0, 40, 60, and 80cc/ kg. Essential amino acid analysis was carried out at the Integrated Laboratory of the Bogor Agricultural Institute, West Java, while proximate analysis was examined at the Animal Food Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University, Makassar. Enzyme activity evaluation was performed at the Biochemistry Laboratory, Faculty of Mathematics and Science, while ammonia measurement was conducted in the Water Productivity and Quality Laboratory at the Faculty of Marine and Fisheries Sciences, Hasanuddin University, Makassar.

This study used a complete randomized design (CRD) with 4 treatments and 3 replications each, thus 12 experimental units were applied. Feeding (5% v/v) was performed every day with a frequency of 4 times a day. Weighing using analytical scales and length was measured using a ruler. Water quality measurements included temperature, salinity, dissolved oxygen, and pH as supporting data. Enzyme activity and essential amino acids in the tiger shrimp were analyzed at the end of the rearing period.

2. Essential amino acid index (EAAI)

The protein quality of a food composition is determined by the essential amino acid index (EAAI). According to **Muchtadi (2013)**, EAAI is calculated using a formula as follows:

$$\mathbf{EAAI} = n\sqrt{\frac{100a}{a_e} \times \frac{100_b}{b_e} \times \frac{100c}{c_e} \times \ldots\ldots \times \frac{100_j}{j_e}}$$

Where,

a, b, c, ..., j = Percentage of essential amino acids in the feed;
ae, be, ce, …, je = Percentage of essential amino acids required;
n = Number of essential amino acids.

3. Essential amino acid score (EAAS)

The essential amino acid score (EAAS) represents the lowest proportion of the required total essential amino acid content for protein synthesis. According to **Muchtadi (2013)**, the score for each EAA is expressed as a percentage of the concentration contained in the standard protein, using the formula as follows:

$$\text{EAAS (\%)} = \frac{\text{EAA concentration of the sample}}{\text{EAA concentration requirement}} x\ 100$$

4. Amylase enzyme activity

Amylase activity was determined via the method of **Bernfeld (1955)**. An amount of 0.5mL of the enzyme solution was taken, then added to phosphate buffer pH 7, 0.5mL of 1% starch, and 1.5mL of DNS, and then incubated at 37°C for 30 minutes. Then, the solution was heated for 10 minutes, cooled, and measured for absorbance at the maximum wavelength using a UV-Vis spectrophotometer. The maltose standard and blank used are phosphate buffer pH 7. Enzyme activity is expressed in units (U) and defined as the amount of enzyme required to liberate 1µmol of maltose per minute.

5. Lipase enzyme activity

Lipase activity was assessed using the tititation method of **Lindfield *et al.* (1984)**. The amount of 1g of palm oil, 0.5mL of enzyme solution, and 2mL of phosphate buffer solution pH 7.5 were homogenized using a magnetic stirrer for 1 hour at 37°C. Then, 5mL of acetone-alcohol (1:1) was added and stirred until getting homogeneous. 2-3 drops of phenolphthalein indicator were added and titrated using 0.005 N alcoholic KOH until the color turned to pink and disappeared. Then, the titration volume was recorded. The blank solution was prepared in the same way as the sample but the acetone-alcohol solution (1:1) was added at hour 0 before stirring to stop the enzyme activity. Enzyme activity is expressed in units (U), and it is defined as the amount of enzyme that releases 1µmol of fatty acid per minute.

6. Protease enzyme activity

Protease activity was analyzed based on the modified method of **Kwan *et al.*, (1983)**. 1mL of Tris-HCI buffer pH 7 and 1ml of 20mg/ mL casein were added to 0.2ml of enzyme solution sample, then incubated at 37°C for 30 minutes and 1ml of 0.1 M TCA was added. Controls were made using the same procedure, but without the direct incubation process, and TCA was added to stop enzyme activity. Each filtrate of the sample and control solutions was centrifuged at 10,000rpm for 5 minutes. 1.5ml of the protease enzyme filtrate was taken, then added to 2.5ml of 0.4 M Na_2CO_3 and 1mL of 50% follin, and stored for 30 minutes. The absorbance was measured at the maximum

wavelength using a UV-Vis spectrophotometer. Tyrosine standards and tris-HCI pH 7 buffer blanks were prepared using the same procedure as the hydrolysis filtrate. Enzyme activity is expressed in units (U) defining the required amount of enzyme needed to liberate 1µmol of tyrosine per minute.

7. Spesific growth rate (SGR)

According to **Effendie (1997)**, growth represents the length and weight increase of shrimp over a certain period. According to **Opasola *et al.* (2013)**, the specific growth rate of shrimp is calculated using the following formula as follows:

$$SGR\ (\%) = \frac{Ln\ Wt - Ln\ Wo}{T} x\ 100$$

Where:

SGR = Specific growth rate (%/day);
Wt = Final mean weight of shrimp (g);
Wo = Initial mean weight of shrimp (g);
T = Rearing duration (days).

8. Relative weight gain (RWG)

Relative weight gain is the difference in shrimp weight at the end and initial of rearing divided by the initial weight. The weight gain was calculated using the **Effendi (1997)** equation as follows:

$$RWG\ (\%) = \frac{Wt - Wo}{Wn} x\ 100$$

Where,

RWG = Relative weight gain (%);
Wt = Final weight of shrimp (g);
Wo = Initial weight of shrimp (g).

9. Survival rate (SR)

Survival assessments were carried out by counting the number of shrimp at the initial of rearing and the number of survived shrimp at the final rearing. The SR value of shrimp was calculated using the formula according to **Effendie (1979)** as follows:

$$SR\ (\%) = \frac{Nt - No}{Wn} x\ 100$$

Where,

SR = Sintasan (%);
N_o = Number of shrimp at the initial of rearing (ind);
N_t = Number of shrimp at the final of rearing (ind).

10. Protein efficiency ratio (PER)

The protein efficiency ratio was determined using the **Tacon (1987)** formula as follows:

$$PER\ (\%) = \frac{Wt - Wo}{Pi} x\ 100$$

Where,
PER = Protein Efficiency Ratio (%);
Wo = Shrimp biomass at the initial of rearing (g);
Wt = Shrimp biomass at the final of rearing (g);
Pi = Weight of consumed feed × feed protein content (g).

11. Data analysis

The data obtained were tested statistically using the analysis of variance (ANOVA) to determine the effect of treatment and the W-Tukey further test to identify the appropriate treatment. Calculation data for essential amino acid scores and index as well as water quality were described and tabular presented.

RESULTS

1. Digestive enzyme activity

The enzyme activity in the digestive tract of the tiger shrimp increased with the addition of the multi-enzyme dose. The activities of amylase and protease were found to be similar following the multi-enzyme treatment. This suggests that the administration of multi-enzymes does not affect the activity of the amylase and protease enzymes. In contrast, the lipase enzyme increased to 1,209U as the dose of multi-enzyme increased in treatment D. The results of measuring the digestive enzyme activity of the tiger shrimp given various doses of multi-enzyme are presented in Fig. (1).

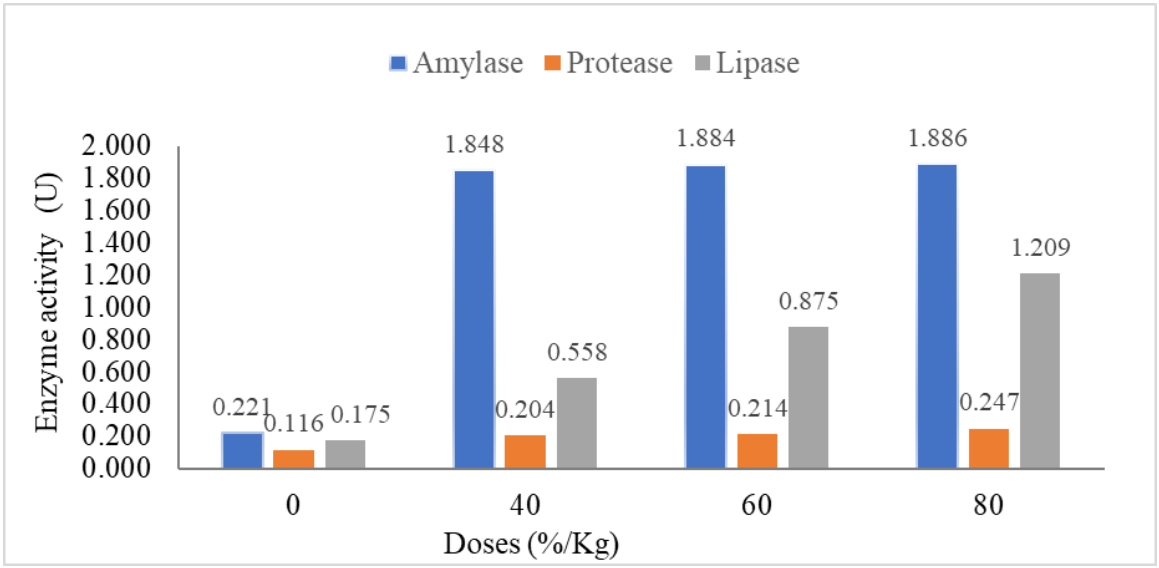

Fig. 1. Enzyme activity in shrimp given multi-enzyme doses in feed

Fig. (1) demonstrates that the amylase, protease, and lipase enzyme activities in treatment A (without multiple enzymes) showed a lower value compared to the mean of

amylase, protease, and lipase enzyme activity of shrimp in treatment D with a dose of 80%.

2. Essential amino acid index (EAAI) and essential amino acid score (EAAS)

The assessment of the essential amino acid index in feed given various doses of multi-enzymes is shown in Table (1). The results also indicate a linear correlation between multi-enzyme doses and increases in essential amino acid index and essential amino acid score.

Table 1. Essential amino acid index (EAAI) and essential amino acid score (EAAS) in feed

The quality of EAA	Dose of multi-enzyme			
	A (0%)	B (40%)	C (60%)	D (80%)
EAAS	03.48	09.29	10.00	10.00
Deficiency*	96.52	90.71	90.00	90.00
EAAI	00.92	01.85	01.91	02.64

Note: *Deficiency EAA = 100%-EAAS (%).

3. Specific growth rate (SGR), relative weight gain (RWG), survival rate (SR), and protein efficiency ratio (PER)

Specific growth rate (SGR), relative weight gain (RWG), survival rate (SR), and protein efficiency ratio (PER) in the tiger shrimp given various multi-enzyme doses to feed are presented in Table (2)**.**

Table 2. Mean of SGR, RWG, SR, and PER on tiger shrimp given various doses of multi-enzymes in feed

Dose (%)	Mean of SGR (%/Day)	Mean of RWG (%)	Mean of SR %	Mean of PER
0% Multi-enzyme	1.07 ± 0.09^{a}	1.58 ± 0.30^{a}	90.00 ± 5.00^{b}	2.55 ± 0.30
40% Multi-enzyme	1.25 ± 0.12^{a}	1.24 ± 0.42^{a}	95.00 ± 5.00^{b}	2.21 ± 0.42
60% Multi-enzyme	1.42 ± 0.32^{a}	1.45 ± 0.47^{a}	81.67 ± 7.64^{ab}	2.54 ± 0.39
80% Multi-enzyme	1.47 ± 0.05^{a}	2.12 ± 0.47^{a}	70.00 ± 5.00^{a}	3.09 ± 0.47

Note: Different letters indicate significant differences between treatments at the 5% level ($P<0.05$).

The results show that the mean of SGR during the study ranged from 1.07± 0.09 - 1.47± 0.05 %/day, while the mean RWG of 1.45± 0.47 - 2.12± 0.47 %, the mean SR of 70.00± 5.00 - 90.00± 5.00, and mean PER of 2.21± 0.42 - 3.90± 0.47 were recorded. These results show that the SGR, RWG, and PER values decreased at a dose of 60% but subsequently increased significantly at a dose of 80%.

DISCUSSION

1. Digestive enzyme activity (amylase, protease, and lipase)

The results showed an increase in the activity of the amylase, protease, and lipase as the multi-enzyme dose was improved. This is likely because the addition of multi enzymes affects the digestibility of protein in the feed consumed. Food undergoes a

hydrolysis process by protease enzymes in the digestive tract (stomach, small intestine) into its constituent units, namely amino acids. The small intestine absorbs these amino acids, which are then transported to the liver and dispersed throughout the body's tissues **(Muchtadi, 2013)**. Amylase, protease, and lipase are well-known hydrolytic enzymes responsible for breaking down carbohydrate, protein, and fat macromolecular compounds.

Assessment of digestive enzyme activity provides data regarding feed digestibility **(El-Shenawy *et al.*, 2020)**. The activity of digestive enzymes such as amylase, protease, and lipase is measured to indicate organisms' ability to digest carbohydrates, proteins, and lipids **(Susilo *et al.*, 2015; Ramadhani, 2021)**. Digestive enzyme activity is a key biological aspect that affects feed utilization and shrimp growth **(Muttharasi *et al.*, 2021)**. As a digestive enzyme, protease degrades proteins into peptides and amino acids, which are absorbed by erythrocyte cells in the inner wall of the intestine and eventually delivered to the blood for shrimp growth and development. Shrimp's ability to utilize feed nutrients is highly dependent on its digestive system, which is represented in enzyme activity **(Sankar *et al.*, 2014)**.

High amylase activity in shrimp indicates an efficient starch digestion, which can be attributed to the high carbohydrate content of the feed. Amylase activity in shrimp is influenced by feed protein levels, namely high protein levels contributing to low enzyme activity, accessibility, and nutrient utilization **(Xiao *et al.*, 2023)**. In addition, during rearing, the digestive system may undergo an anatomical development which is associated with the creation of digestive enzymes. According to **Bakkara *et al.* (2015)**, changes in enzyme activity in the digestive tract are influenced by changes in the digestive tract as well as the quality and quantity of feed provided.

The high activity of this protease enzyme is assumed to be linked to shrimp's higher attempt to digest protein to maximize feed protein utilization **(Paria *et al.*, 2017)**. Increasing protein levels in the intestine to a certain extent will promote the expression of regulatory enzymes in synthesizing protease enzymes and conversely, synthesis will decrease when substrates are reduced. In this case, the presence of feed protein seems to play a role in activating the expression of the enzymes which contribute to the synthesis of protease enzymes **(Komari & Susilo, 2021)**. Optimum protein content improves protease enzyme activity and digestibility of the shrimp juveniles **(Xiao *et al.*, 2023)**.

Based on its physiological function, lipase has an important role in hydrolyzing fats and oils into fatty acids and glycerol which in metabolic processes are required **(Komari & Susilo, 2021)**. Lipase commonly functions in hydrolysis triacylglycerols (triglycerides) to produce long-chain fatty acids and glycerol. Each enzyme has the maximum activity at a certain temperature and will increase with increasing the temperature until the optimum temperature is reached. After that, exceeding the optimum temperature threshold causes the enzyme activity to decrease **(Sholeha & Agustini, 2021)**.

2. Essential amino acid index (EAAI) and aessential amino acid score (EAAS)

The EAAI and EAAS test results indicate that adding multiple enzymes to feed improves digestibility at varied doses. The prevailing assumption is that the crustaceans require a balanced essential amino acid supplementation rather than a specific amount of protein **(Rosyida, 2007)**. Protein quality is determined by the completeness and balance of key amino acids, which optimize shrimp protein absorption, particularly methionine and lysine **(Lante *et al.,* 2015)**. In producing feed, the protein and essential amino acid content must be adequate. According to **Suprijatna *et al.* (2005)** and **Muhsafaat *et al.* (2015)**, the essential amino acid deficit reduces protein use efficiency, particularly for body tissue formation and egg production.

The EAAI and EAAS values are used to evaluate protein in fish feed **(Bunda *et al.,* 2015)**. Protein is a required nutrient for the growth and survival of cultured animals and feed is a source of protein. Consequently, its use must be proportionate to dietary requirements to guarantee optimal growth rates **(Xing *et al.,* 2023)**. EAAI in this study showed that EAA in shrimp increased with the addition of multi-enzyme doses. The results of **Xiaoqing *et al.* (2022)** show that the addition of a multi-enzyme complex improves growth and the availability of exogenous digestive enzymes. Additionally, multi enzymes help aquatic animals break down anti-nutritional compounds, increase feed digestibility, and increase cultivar growth.

EAAS is defined as the lowest ratio of the essential amino acid content in a protein to each amino acid in the protein or to the level required for EAA **(Bunda *et al.,* 2015)**. The results showed that the feed's EAAS was obtained from EAA methionine. According to **Sumitro *et al.* (2022)**, methionine deficiency causes limited growth and low feed efficiency. Methionine promotes bone growth and restores damaged body tissue. Methionine is one of the EAA for protein formation and is needed by the body for growth **(Rohchimawati *et al.,* 2022)**. Methionine and phenylalanine are two of the 10 EAA that can be replaced by non-essential amino acids due to their sparing effect **(Guo *et al.,* 2020)**. According to **Puspita *et al.* (2015)**, feed utilization efficiency should be >50% or close to 100%. Besides, amino acid deficiency causes a limited growth and a weak feed conversion thus the appetite decreases.

The amino acid methionine is important for the assembly of proteins in cells because formylmethionine RNA is required in the initial phase in the nucleus. Protein synthesis in the body is impaired when methionine levels are low **(Andri *et al.,* 2020)**. Shrimp growth is regulated not only by dietary protein concentration but also by amino acid requirements. When low-quality feed is used, a large proportion of protein is broken down to fulfill the requirement for energy, with only a tiny portion being used for growth **(Yao *et al.,* 2019)**. Methionine is an important amino acid utilized in shrimp growth since it is a precursor to nucleic acids, proteins, carnitine, and choline. In addition to growth, methionine is also associated with immunological responses in particular species of fish **(Zannah, 2019)**. Furthermore, limited methionine results in the body's protein formation not taking place optimally **(Azwar & Melati, 2012; Rohchimawati *et al.,* 2022)**.

3. Growth perfomance

Specific growth rate (SGR) measurements showed that the administration of multi-enzymes at certain doses had no significant effect (P>0.05). The 80% multi-enzyme dose provides a specific growth value of 1.47%, and the 60% dose is 0.32%. This is because the shrimps require additional nutrients to grow and perform other body functions. Table (2) illustrates the findings for each treatment. The highest specific growth rate for the tiger shrimp was 1.47%/day at a multi-enzyme dose of 80%, followed by 1.25%/day at a dose of 40%, and the lowest was 0.32%/day at a dose of 60%. **Amalia *et al.* (2022)** stated that the growth rate of the tiger shrimp is 46.9%/week and tends to decrease as they grow. According to **Biswas *et al.* (2020)**, the shrimp growth rate is influenced by several factors including environmental aspect and the availability of quality feed.

However, the analysis of variance revealed that all multi-enzyme doses (0, 40, 60, and 80%) resulted in similar specific growth rates (P>0.05). These results assume that the shrimp growth varies significantly between replications in each treatment, leading to a larger standard deviation. Furthermore, the tiger shrimp fed in indoor recirculating tanks require artificial feed with a complete macro and micro nutritional content, even though the amount of protein in the test feed is adequate..

Feeding containing multi-enzymes had no significant effect (P>0.05) on shrimp RWG. However, adding a multi-enzyme dose to the feed increases the optimum weight gain at a dose of 80%, namely 2.12%. These findings are consistent with those reported by **Sundu and Dingle (2003)**, who found that adding multi enzymes to the ratio increased RWG. This is hypothesized to be ascribed to the potential of the multienzymes to optimize nutritional digestion and absorption thereby improving RWG and protein ingestion **(Berliana *et al.*, 2022)**. This finding is supported by **Iqbal *et al.* (2012)**, who postulated thatthe amount of protein consumed determines body weight gain resulting from the body's protein synthesis.

According to **García-rodríguez *et al.* (2021)**, shrimp weight varies with feeding frequency. As a result, feeding frequency and quantity may have an impact on biomass production. However, energy levels obtained from frequent feeding are critical for immunological performance and other energy-dependent shrimp metabolic functions that aid in shrimp survival **(Wahjuni, 2013)**. Shrimp's ability to digest feed is highly dependent on the completeness of their digestive organs and the availability of digestive enzymes. The digestive tract develops gradually, and after reaching a specific size/age, it achieves completeness **(Yu *et al.*, 2021)**.

The results of survival observations showed that multi-enzyme supplementation in feed had a significant effect (P> 0.05) on the average SR of the tiger shrimp. This shows that multi-enzyme supplementation in feed affects the tiger shrimp survival. Treatment D was much lower than the other three treatments, with treatment A at 90%, treatment B at

95%, and treatment C at 81.67%. Excess enzymes in the feed are considered to contribute to the low SR in treatment D. Excess enzymes cause excessive monosaccharides to be produced and released, which may inhibit development and disturb the shrimp health including hyperglycemia. Hyperglycemia is a condition characterized by high glucose levels, which inhibit insulin secretion and increase insulin retention. This exacerbates hyperglycemia, resulting in less insulin **(Nimrat *et al.,* 2013)**. Hyperglycemia is one of the most important neural peptides produced by the immunological neuro-endocrine regulation network, which plays a vital role in different biological processes, particularly immune function and stress response **(Xu *et al.,* 2019)**.

The observations of shrimp survival rates revealed that the treatment without multi-enzyme administration (control) had a higher percentage than the other treatments. Pressure interventions and stresses influence survival rates. The percentage of the shrimp survival appears to be low as the multi-enzyme dose increases, which is consistent with data on shrimp growth findings. Survival remains in the common category for the shrimp because it is in the range of more than 50%. According to **Situbondo Brackish Aquaculture Fisheries Institute (2021)**, the good category has a survival rate >70%, the moderate category is 50-60%, and the low category is less than 50%. Shrimp length increases with age. Shrimp digestive enzymes grew and functioned similarly among treatments. Additionally, administering multi enzymes to shrimp has a major impact on SR. The survival rates for treatments A, B, C, and D were 90, 95, 81, and 70%, respectively.

Table (2) shows the mean protein efficiency ratio (PER) of feed using various multi-enzyme doses. The research treatment showed a statistically insignificant result (P>0.05). **Wiratmo *et al.* (2013)** found a low PER value of 1.25 ± 0.05 in growing the tiger shrimp with a feed protein level of 39%. Protein efficiency describes the amount of protein used and indicates how well the protein source is absorbed to fulfill the requirements for vital amino acids in shrimp **(Singh *et al.,* 2011)**. Furthermore, **Hardy and Barrows (2002)** defined protein efficiency as the increase in weight of the tiger shrimp following the addition of multiple enzymes. Commonly, multienzymes may improve protein utilization, resulting in better feed efficiency. The use of multi-enzymes is better than utilizing a single one due to their contribution to the capability of breaking down several nutrients simultaneously **(Balcazar *et al.,* 2006)**. Supported by the statements of **Zamini *et al.* (2014)** and **Puspitasari *et al.* (2018)**, the higher feed efficiency value represents the higher feed quality level.

CONCLUSION

This study concluded that an 80% multi-enzyme dose produced the optimum value in terms of digestive enzyme activity, essential amino acid index (EAAI), essential amino acid score (EAAS), survival growth rate (SGR), relative weight gain (RWG), and protein efficiency ratio (PER). However, adding multienzymes to feed leads to a low

survival rate. A more comprehensive study is required to compare the effects of multienzymes to those without multienzymes as well. Furthermore, multi-enzyme application in feed requires a renewal through multiple methods.

REFERENCES

Amalia, R.; Rejeki, S.; Widowati, L. L. and Ariyati , R. W. (2022). The growth of tiger shrimp (*Penaeus monodon*) and its dynamics of water quality in integrated culture. Biodiversitas, 23(1): 593 - 600.

Andri, R. P. H. and Yuli A. T. (2020). Estimation and validation of the amino acids methionine, lysine, and threonine from grain feed as a source of vegetable protein. Jurnal Nutrisi Ternak Tropis, 3(1): 18 - 22.

Arlini, F. (2022). Score and index of essential amino acids for tiger prawns, *Penaeus monodon* which consumes pellets containing multi-enzymes and is reared in recirculating tarpaulin ponds plus fecal chamber. Thesis of Fisheries Science Departemen, Hasanuddin University of Makassar.

Bakkara, O. R.; Aslamyah, S. and Fujaya. Y. (2015). Developmental response of the crab larva *Portunus pelagicus* to accelerate the change from natural food to predigest artificial food. J. Science and Teknologi, 15(1): 74 - 83.

Balcazar, J. L.; Ignacio, D. B.; Imanol, R. Z.; David, C.; Daniel, V. and Jose, L. M. (2006). The role of probiotics in aquaculture. Veterinary Microbiology, 114: 173 - 186.

Berliana, B.; Azis, A.; Sestilawarti.; Yusrizal. and Noferdiman. (2022). The addition of multienzymes in diets containing palm kernel meal on the growth performance and morphometrics of the small intestine of broilers. Jurnal Ilmiah Ilmu-Ilmu Perikanan, 25(1): 1 - 12.

Biswas, G.; Kumar, P.; Ghosal, T. K.; Kailasam, M.; De Debasis.; Bera, A.; Mandal, B.; Sukumaran, K. and Vijayan, K. K. (2020). Integrated multi-trophic aquaculture (IMTA) outperforms conventional polyculture with respect to environmental remediation, productivity and economic return in brackishwater ponds. Aquaculture, 516: 734626. DOI: 10.1016/ J.Aquaculture.2019.734626. DOI: 10.1016/j.aquaculture.2019.734626

Brackish Aquaculture Fisheries Institute, Situbondo. (2021). Farming of vannamei shrimp (*Litopenaeus vannamei*) in millennial ponds : 46.

Bunda, M. G. B.; Tumbokon, B. L. M. and Jr, A. E. S. (2015). Composition, chemical score (CS) and essential amino acid index (EAAI) of the crinkle grass *rhizoclonium* sp. as ingredient for aquafeeds. AACL Bioflux, 8(3): 411 - 420.

Cahyadi, U.; Dedi, J.; Ichsan, A. F. and Sunarma, A. (2020). Supplemental enzyme in artificial diets for north african catfish larvae *Clarias gariepinus* Burchell, 1822. Jurnal Iktiologi Indonesia, 20(2): 155 - 169.

Effendie. (1997). Fisheries biology. Yayasan Pustaka Nusatama, Yogyakarta. 163 Pp.

Effendie, M. (1979). Metode biologi perikanan. First edition. Penerbit Yayasan Dewi Sri. Fakultas Perikanan IPB, Bogor. 112 Pp.

El-Shenawy, A. M.; Abeer, E. K. M.; Alsokary, E. T. and Gad, D. M. (2020). Impact of carbohydrate to lipid ratio and bile salts supplementation on performance, body

gain and body composition of nile tilapia fish. International Journal Fisheries and Aquatic Studi, 8(3):88 - 97.

Felix, N.; Prabu, E.; Kannan, B. And Manikandan, K. (2018). An evidential review on potential benefits of enzymes in aqua feed industry. International Journal of Current Microbiology and Applied Sciences, 7(12): 2053 - 2074.

García-Rodríguez, L. D.; Sainz-Hernández, J. C.; Aguiñaga-Cruz, J. A; Juárez-Acosta, C. G; Santamaría-Miranda, A. and Apun-Molina, J. P. (2021). Enzymatic activity in the shrimp *Penaeus vannamei* fed at different feeding frequencies. Latin American Journal of Aquatic Research, 49(2): 280 - 88 DOI: 10.3856/vol49-issue2-fulltext-2521

Guo, J.; Huang, Y.; Salze, G.; Roy, L. A. and Davis, D. A. (2020). Use of plant-based protein concentrates as replacement for fishmeal in practical diets for the pacific white shrimp (*Litopenaeus vannamei*) reared under high stocking density and low salinity conditions. Aquaculture Nutrition, 26(2): 225 - 232. DOI: 10.1111/anu.12982

Hardy, R. W. and Barrows, F. T. (2002). Diet formulation and manufacture. In Halver, J.E. and Hardy, R.W, (eds). Fish Nutrition. New York: Academic Press. 505 - 600 p.

Islam, M. D. S.; Das, M.; Chakroborty, K.; Lee, J. M.; Islam, M. R. and Rafiquzzaman, S. M. (2024). Aquamimicry improves the growth performance and immunity of black tiger shrimp (*Penaeus monodon*) in low saline ponds. Aquaculture Reports, 36(1): 102082. DOI: 10.1016/j.aqrep.2024.102082.

Iqbal, F.; Atmomarsono, U. and Muryani, R. (2012). Influence of various feeding frequency in feed restriction on protein utilization of broiler chicken. Anim. Agric. J., 1(1): 53 - 64.

Jayanti, D. (2019). The effectiveness of adding multi-enzymes to feed on the growth performance of catfish fry *Pangasius hypophthalmus*. Thesis of Fisheriy and Marine Science, IPB University, Bogor.

Komari, N. and Susilo, T. B. (2021). ENZYMOLOGY (types, functions, and applications of enzymes). CV Banyubening Cipta Sejahtera, Banjarbaru.

Kwan, K. W.; Lai, W. M. and Mow, V. C. (1983) Response of an articular cartilage layer subjected to a spreading pressuredistribution simulating walking. In I983 Bimwchunics Symporirtm, pp. 9-12. ASME. New York.

Lalopua, V. M. N.; Silaban, B. B.; Gaspers, F. F. and Labobar, S. (2022). Amino acidsprofile and protein quality of *panulirus versicolor* fresh. Jurnal Teknologi Hasil Perikanan, 2(1): 21 - 27.

Lante, S.; Usman. and Asda. L. (2015). Effect of feed protein levels on growth and survival of black tiger prawns, *Penaeus monodon* Fab. Transveksi. Jurnal Perikanan Universitas Gadjah Mada, 17(1): 10 - 17.

Linfield, W. M.; O'Brien, D. J.; Serota, S. and Barauskas, R. A. (1984). Lipid- lipase interactions. i. fat splitting with lipase from *Candida rugosa*. J. Am. Oil Chem. Soc., 61(1): 1067 - 1071.

Liu, Z.; Liu, Q.; Zhang, D.; Wei, S.; Sun, Q.; Xia, Q.; Shi, W.; Ji, H. and Liu, S. (2021). Comparison of the Proximate Composition and Nutritional Profile of Byproducts and Edible Parts of Five Species of Shrimp. Food, 10(11): 2603. doi: 10.3390/foods10112603.

Muchtadi, D. (2013). Food supplementation (part 1). Teaching module of Universitas Terbuka, Jakarta. 41 Pp. https://repository.ut.ac.id/4616/1/PANG4311-M1.pdf.

Muhsafaat, L. O.; Heri, A. S. and Suryahadi. (2015). Protein quality and amino acid composition of fermented sago pulp (fsp) by *aspergillus niger* with urea and zeolit addition. Jurnal Ilmu Pertanian Indonesia, 20(2): 12 - 130.

Muttharasi, C.; Gayatri, V.; Muraliskandar, T.; Mohan, K.; Uthayakumar, V.; Radhakrishnan, S.; Kumar, P. and Palanisamy. M. (2021). Growth performance, digestive enzymes and antioxidants activities in the shrimp *Litopenaeus vannamei* fed with *Amphiroa fragilissima* crude polysaccharides encapsulated *Artemia nauplii*. Aquaculture, 545(3): 737263. DOI: 10.1016/j.aquaculture.2021.737263.

Ngoc Anh, T. N.; Huynh, T. T. and Nguyen, V. H. (2023). Evaluating artemia biomass and gut weed (*Ulva intestinalis*) meal as a dietary protein source for black tiger shrimp (*Penaeus monodon*) postlarvae. Egyptian Journal of Aquatic Research, 49(1): 97 - 103. DOI: 10.1016/j.ejar.2022.11.003.

Nimrat, S.; Phattanunt, T.; Kallaya, S.; Traimat, B. and Verapong, V. (2013). Enhancement of growth performance, digestive enzyme activities and disease resistance in black tiger shrimp (*Penaeus monodon*) postlarvae by potential probiotics. Aquaculture International, 21(3): 655 - 666.

Opasola, O. A.; Solomon, O. A. and Olatunde, O. F. (2013). Growth performance and survival rate of *Clarias gariepinus* fed *Lactobacillus acidophilus* supplemented diets. IOSR Journal of Agriculture and Veterinary Science, 3(6): 45 - 50.

Paria, A.; Shoghi, M. and Fereidouni, M. S. (2017). Growth performance and digestive enzymes activities of pacific white leg shrimp (*Litopenaus vannamei*) juveniles fed dietary mixtures of four medicinal plants. Iranian Journal Of Fisheries Sciences, 16(4): 1157 - 1163.

Puspitasari, M. U.; Johannes, H. and Vivi, E. H. (2018). Effect of using flour-fermented *Lemna* sp. in feed on the efficiency of feed utilization, growth, and survival of tilapia (*Oreochromis niloticus*). PENA Akuatika, 17(1): 53 - 75.

Puspita, T.; Andriani, Y. and Hamdani, H. (2015). Utilization of peanut cake in fish feed on the growth rate of tilapia (*Oreochromis niloticus*). Jurnal Perikanan Kelautan, 6(2): 91 - 100

Rachmawati, D.; Elfitasari, T.; Samidjan, I.; Windarto, S. and Sarjito. (2021). Protein digestive performance, feed utilization efficiency and growth of sangkuriang catfish (*clarias gariepinus* var Sangkuriang) through supplementation of *saccharomyces cerevisiae* in commercial feed. Indonesian Journal of Tropical Aquacultute, 5(2): 216 - 222.

Rachmawati, D.; Hutabarat, J.; Dewi, E. N. and Windarto, S. (2020). Supplementation of papain in feed on growth performance, efficiency of feed utilization, and survival rate of whiteleg shrimp (*Litopenaeus vannamei*). Journal of Marine Research, 9(3): 215 - 22.

Ramadhani, S. (2021). Inhibitory activity of angiotensin converting enzyme (ACE) biscuits based on red bean (*Phaseoulus vulgaris*) tempe flour. Thesis of Chemical Study Program, UIN Syarif Hidayatullah, Jakarta.

Rohchimawati, R.; Diana, R. and Rosa. A. (2022). The effect of different doses of methionine in artificial feed on the growth and survival of sangkuriang catfish (*Clarias gariepinus*) fry. Sains Akuakultur Tropis, 6(2): 193 - 201.

Rosyida, E. (2007). Protein / amino acid and carbohydrate requirements of penaeid prawns. Biota, 12(1): 17 - 22.

Sankar, H. H. S.; Jose, J; Varadarajan, R.; Bhanub, S. V.; Joy, S. and Philip, B. (2014). Functional zonation of different digestive enzymes in *Etroplus suratensis* and *Oreochromis mossambicus*. International Journal of Scientific and Research Publications, 4(5): 1 - 10.

Singh, P.; Maqsood, S.; Samoon, M. H.; Phulia, V.; Danish, M. and Chalal, R. S. (2011). Exogenoush supplementation of papain as growth promoter in diet of fingerlings of *Cyprinus carpio*. Faculty of Fisheries, Shere-e-Kashmir University of Agricultural Science and Technology of Kashmir, India. 3: 1 - 9 pp.

Sefer, M.; Petronijevic, R. B.; Trbovic, D.; Ciric, D.; Baltic, T.; Parunovic, V. and Djordjevic, V. (2021). Amino acids in animal feed: significance and determination techniques. IOP Conference Series: Earth and Environmental Science, 854: 012082

Sholeha, R. and Agustini, R. (2021). Seed lipases and its characterization. Unesa Journal of Chemistry, 10(2): 168 - 83.

Sumitro.; Emu, S. and Harsin, L. O. (2022). Effect of addition of amino acid methionine with different dosages in feed on the growth of red tilapia (*Oreochromis niloticus*). Aquamarine, 9(2): 8 - 12.

Sundu, B. and Dingle, J. 2003. Use ofenzymes to improve the nutritionalvalue of palm kernel meal and coprameal. Proceedings of Queensland Poultry Science Symposium, the University of Queensland, Australia. 11: 1 - 15.

Tomičić, Z. M.; Nedeljka, J. S.; Sanja, J. P.; Vojislav, V. B.; Oliver, M. D. and Ružica, M. T. (2020). By products of the oil industry as sources of amino acids in feed. Food and Feed Research, 47(2): 131 - 37. DOI: 10.5937/ffr47-28435.

Tacon. (1987). The nutrition and feeding of farmed fish and shrimp - a training mannual. FAO of The United Nations, Brazil, 4 pp.

Wahjuni, S. (2013). Biochemical metabolism. Udayana University Press, Denpasar. 113 pp.

Webster, C. D. and Lim, C. (2002). Introduction to fish nutrition. nutrient requirements and feeding of finfish for aquaculture. Wallingford Oxon, UK.: CABI Publishing. 27 Pp.

Wiratmo.; Sunaryo. and Bambang. (2013). The effect of using divine cigarettes on the growth rate of black tiger shrimp *Penaeus monodon*. J. Mar. Research, 2(3): 104 - 113.

Xing, S.; Xiaofang, L.; Xiaoran, Z.; Zaitun, A. T.; Hao, H. P.; Min, L.; Kangsen, M.; Min, X. and Sadasivam, J. K. (2023). Essential amino acid requirements of fish and crustaceans, a meta-analysis. Reviews In Aquaculture, (6): 1 - 18. DOI: 10.1111/raq.12886.

Xiaoqing, D.; Xinzheng, N.; Chunchun, Y.; Lai, J; Wenxin, K. and Lichun, Q. (2022). Effects of dietary multienzyme complex supplementation on growth performance, digestive capacity, histomorphology, blood metabolites and hepatic glycometabolism in snakehead (*Channa argus*). Animals (Basel) MDPI, 12(3): 380. DOI: 10.3390/ani12030380

Xu, L.; Luqing, P.; Xin, Z. and Cun, W. (2019). Effects of crustacean hyperglycemic hormone (CHH) on regulation of hemocyte intracellular signaling pathways and phagocytosis in white shrimp *Litopenaeus vannamei*. Fish and Shellfish Immunology 93(2): 559 - 66. DOI: 10.1016/j.fsi.2019.07.051.

Xiao, F.; Wang, J.; Liu, H.; Zhuang, M.; Wen, X.; Zhao, H. and Wu, K. (2023). Effects of Dietary protein levels on growth, digestive enzyme activity, antioxidant capacity, and gene expression related to muscle growth and protein synthesis of juvenile greasyback shrimp (*Metapenaeus ensis*). Animals, 13: 3886. DOI: 10.3390/ ani13243886.

Yao, W.; Xiaoqin, L.; Chowdhury, M. A. K. and Jing, W. (2019). Dietary protease, carbohydrase and micro-encapsulated organic acid salts individually or in combination improved growth, feed utilization and intestinal histology of pacific white shrimp. Aquaculture, 503: 88 - 95. DOI: 10.1016/j.aquaculture.2018.12.064.

Yu, J. Y.; Ning, Y.; Zi-Hao, H.; Jing-Jing, W.; Ting, L.; Lin-Rui, C.; Yan, F. and Dong-Chun, Y. (2021). Research progress on hosts and carriers, prevalence, virulence of infectious hypodermal and hematopoietic necrosis virus (IHHNV). Journal of Invertebrate Pathology, 183(2): 107556. DOI: 10.1016/j.jip.2021.107556.

Zamini, A.; Kanani, H.; Esmaeili, A.; Ramezani, S. and Zoriezahra, S. (2014). Effects of two dietary exogenous multi-enzyme supplementation, natuzyme and beta-mannanase (hemicell), on growth and blood parameters of Caspian salmon (*Salmon trutta caspius*). Comparative Clinical Pathology, 23(1): 187 - 192. DOI 10.1007/s00580-012-1593-4.

Zannah, S. R. (2019). Effect of adding methionine to formulated feed on growth rate and survival of cobia fish (*Rachycentron canadum*) reared in floating net cages. Thesis of Agriculture Faculty, Lampung University, Bandar Lampung.