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Applications tryptophan and glycine in feed and its effect on the level of post larva cannibalism and survival rate of giant prawn (*macrobrachium rosenbergii*, de mann)

Abstract

The aims of this experiment was to obtain data and information on the percentage of decrease in the levels of cannibalism, growth and survival rate of giant prawn (*Macrobrachium rosenbergii*) by feeding pellet mixed with tryptophan and glycine. This research was conducted in the hatchery of giant prawn on the Research Institute for Fish Breeding, West Java for 40 days using nine aquaria each measuring 60x40x40 m3. Giant prawn juvenille (PL-7) from the hatchery were used for this study, measuring 10,4 ± 0.2 mm in width and 0,02 ± 0.01 g in weight. One hundred juvenile of giant prawn were stocked in each aquarium. The treatments applied were (A): pellet, (B): pellet + tryptophan and (C): pellet + glycine, with three replicates per treatment. Feeding dose was 15% of the total biomass juvenillewhile the dose of tryptophan and glycine were 1.0% of the amount of feed given per aquarium. Feeding was done twice a day in the morning and evening. Variables observed were the growth in total length, weight, level of cannibalism, survival rate and water quality parameters. The results showed that adding the amino acids tryptophan and glycine in pellet feed at 1.0% of total biomass could significantly ($P<0.05$) suppress the level of cannibalism seed without affecting the growth rate (weight and total length) and increase the survival rate of the juvenile giant prawn.

Keywords: Canibalism, Giant prawn, Glycine, Survival rate, Tryptophan, Pellet, Fish breeding, Post Larva, *Macrobrachium rosenbergii* , Prawns, *E. coioides*, Feeding frequencies, Treatment, Pellet feed, Pellets + glycine

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Introduction

Giant prawns is one of freshwater crustacean commercial because it has a delicious taste of meat and high nutrient that contains 65.72% protein, 7.5% minerals and fat 0.88%,[1] but it is growing prawns multiply, responsive to food, fast growing and easily cultivated. Demand for meat increasing prawns needs to be balanced with the production of aquaculture prawns higher. Efforts towards the development of aquaculture prawns, faced with the biological properties of prawns among other territorial, individual growth differences (heterogenous individual growth), aggressive and cannibalism. Cannibalism causes a reduction in the number of individuals due to attacks on individual weak due to moulting.[2,3] Cannibalism is generally related to genetic and lifestyle habits, the difference in size in the group because of genetic variation is a major cause. On the other hand, the habit is determined by environmental conditions such as the availability of food, type of food, the nutritional composition of food, population, density, light intensity, shading and clarity. Minimize cannibalism method such as by manipulating the level of satiety, the optimum frequency of feeding, distribution of feed and determination of the type of feed is preferred. In the studies that have been done, finfish (*Lagodon rhomboids*) is very sensitive to glycine while *Tilapia zilii* very sensitive amino acids that are the alkaline and neutral amino acids.[4]

Tryptophan and glycine is an essential amino acid that serves as a precursor of 5-hydroxytriptamine.[4] In mammals and birds, tryptophan supplementation in the diet can increase the synthesis of 5- hydroxytriptamine in the brain[4-6] and may reduce the level of aggressiveness.[7-10] Previous studies on the decline cannibalism level has been done on the crab, among others, with the addition of various doses of certain amino acids such as glycine addition tryptophan and each with a dose 1.0-1,5% of the total biomass can reduce the level of cannibalism without affecting growth,[11,12] as well as the larvae prawns.[13] From the information above, it is necessary to study the preliminary research application of the tryptophan and glycine in minimizing the level of cannibalism at the seed prawns, especially the addition tryptophan and glycine in the feed pellets are given in the maintenance of prawn seed, which is expected to increase the survival rate in nursery prawns. The purpose of this study was to apply the addition tryptophan and glycine in the feed pellets and their effects on survival and the level of cannibalism prawns.

Materials and methods

Research conducted at the prawns hatchery of Research Institute for Fish Breeding Sukamandi-Indonesia starting from June 30 to July 20, 2015. The treatment to applied is A: pellet feed (Control), (B): Pellets + tryptophan, and (C): Pellet + glycine, each with 3 replications. Dose feeding is 15% of the total biomass,[12] while the addition tryptophan and glycine on pellets is 1.0% of the feed given each aquarium.[11,12] Containers research are nine units aquarium size of 60 x 40 x 40 cm^3 filled with water salinity 10% as much as 30 liters, with the change of water every week as much as 20%. Feeding frequencies twice a day in the morning and afternoon. Every morning do water exchange to remove dirt or remnants of excess feed.

Animal test used is the seed of prawns (PL-7) obtained from the hatchery prawns Research Institute for Fish Breeding, Sukamandi-

Indonesia, measuring the average total length of 10.4 ± 0.2 mm and the average weight was 0.02 ± 0.01 g, seed stocked in each aquarium maintenance with a density of 150 prawns / aquarium or 5 prawn / L. Observations were made every ten days during the 40 days that includes the growth of the seed weight and the total length of prawns by taking 25 seeds prawns. Every day calculation prawn seed that died due to cannibalism and that die naturally. Clinical signs prawn seeds that die from cannibalism is the legs and all its contents are no longer met, encountered only the carapace only, while the seeds of prawns that die naturally all his limbs still intact. Growth in total length was measured by using a ruler accuracy of 0.1 mm. Further growth in weight was measured with a digital scale (Mettler Toledo PL602S) with accuracy of 0.01 g. To calculate the growth rate based on the formula of Zonneveld et al.[14] as follows:

Gr: {(Wt-Wo)/(t)}

Gr: Growth rate (g/day)

Wt: Absolute growth (g)

Wo: Initial growth (g)

t : Rearing period (days)

Survival rate is calculated at the end of the study, by calculating the number living in each treatment. For the percentage of survival of prawns is calculated based on the formula of Effendie[15] as follows:

S:Nt/ No x 100

S: Survival rate (%)

Nt: Amount at the end (individual)

No: Amount at the beginning (individual)

The level of cannibalism seed giant prawns and is calculated based on the formula of Hseu et al.[16] as follows:

KA - KS – KBK

K = ---------- x 100

KA

K: Cannibalism level (%)

KA: Amount at the beginning (individual)

KS: Amount of individual remaining (life)

KBK: Amount of individual dead not by cannibalism

The data of absolute growth rate, survival rate and the rate of cannibalism prawn seeds obtained is calculated and tested by analysis of variance with the pattern completely randomized design (CRD). Then variable water quality include water temperature, pH, dissolved O_2, NH_3-N and NO_2-N, measured every 10 days along with the growth of data retrieval and data obtained are discussed descriptively.

Results and discussion

The observation during the study showed that the growth of the weight and width of the carapace prawns from start to finish stocking the study, each treatment continues to rise (Figure 1 & 2). On the first day until day 10 seed growth prawns relatively equal, then day 20 to day 30 starting no difference in growth. Treatment C (pellet + tryptophan) visible growth rate of prawn seed weight slightly better when compared with the growth rate of weight treatment A (pellets) and B (pellets + glycine) although not significant (P> 0,05). The increasing rate of growth of both the weight and the total length of the seed prawns during the study, indicating that the seeds of prawns in decent condition. It is highly influenced by the transport from the hatchery to the location of research and acclimatization process is running perfectly before the container is stocked to the study, so the seeds prawns do not experience stress as a result of changes in aquarium environment especially salinity, because of salinity in the aquarium at 10%. The range of salinity for life prawn seed in the pond is 8-10 %, This is evidenced by no death of seeds at the time of stocking in aquarium maintenance. Besides, the seed of prawns enough to respond to the feed given. It is seen every conducted feeding prawn seeds directly approaching the feed and to consume them (Figure 1).

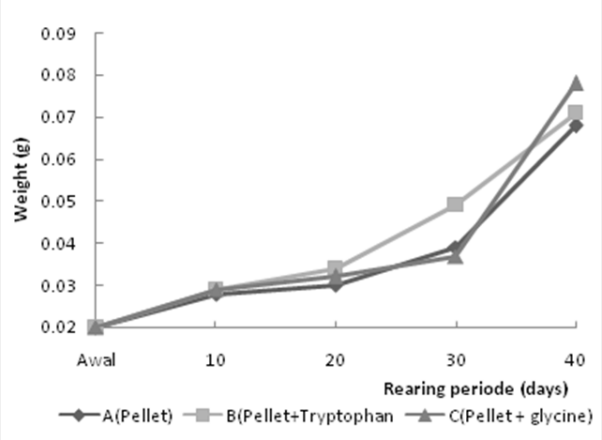

Figure 1 Growth of giant prawn juvenile weight during 40 days.

The growth rate of the total length of treatment B (pellets + tryptophan) a little better when compared to treatment A (pellets) and C (pellet + glycine) although not significant (P> 0.05). Differences in the rate of growth of the seed weight and width of prawns caused by prawns in response to the feed (Figure 2). It is seen that the seeds of prawns that consume feed given to respond quickly, this is related to the eating habits of giant prawns themselves were already accustomed to eating pellets, so the growth rate of each treatment was relatively good.[13] Differences in the rate of growth is also thought to be caused by differences in the digestibility of the feed in the digestive tract related to the amount of feed needed and timely opportunity to digest. According to Carlos[17] states that, frequency of feeding in fish will increase the flow rate of food in the digestive tract. While in this study the frequency of feeding only done two times a day in accordance with that recommended by Suharyanto et al.[13] proper feeding frequency is two to three times a day and provide optimum growth for seed prawns.

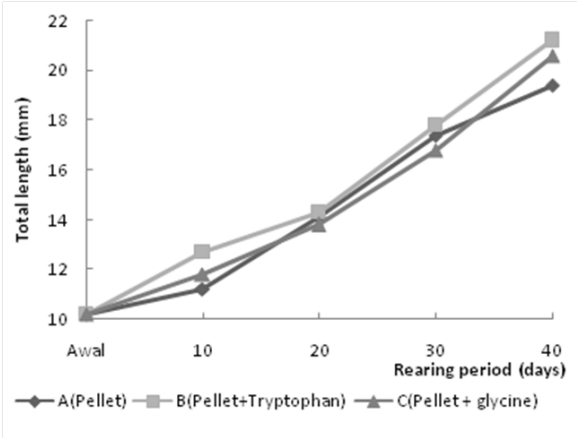

Figure 2 Growth of giant prawn juvenile total length during 40 days.

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The highest survival rate prawn seeds in this study, the treatment C (Pellets + glycine) which is equal to 79.7 ± 1.9%, then treatment B (pellets + tryptophan 76.2 ± 1.2% and was significantly different ($P < 0.05$) on treatment A (pellet / control) which is equal to 50.7 ± 1.3%. This is due to the high level of cannibalism prawn seed in the treatment of feed pellets (A) which is equal to 10.6 ± 0.3%. The highest death prawn seeds in each aquarium treatment occurred in the second week of a study, in which the role of tryptophan and glycine in the feed that is eaten is not optimum (Table 1). It is relatively common also reported by Hseu et al.,[16] Kamaruddin et al.,[18] Suharyanto[11] and Suharyanto et al.[13]

The low survival rate in each treatment is also as a result of the nitrite content of the element is high enough, ie 0.1164 ± 0.1431 to 0.1660 ± 0.1334 mg/L (Table 2), while according to Schmittou,[19] nitrite concentration of 0.1 mg/L can cause stress on aquatic organisms and if the concentration reaches 1.00 mg/L can cause death. Nitrite concentration is high enough that the seed of prawns mostly experience stress, so that the activity of life is also impaired.

Table 1 Growth of weight, total length, survival rate and cannibalism level of giant prawn juvenile during 40 days

Variable	Treatments		
	A (Pellet)	B (Pellet + Tryptophan)	C (Pellet + Glycine)
Cannibalism Level (%)	10,6±0,3[a]	9,8,0±0,4[a]	8,2,0±0,4[a]
Initial Weight (g)	0,02 ± 0,01	0,02 ± 0,01	0,02 ± 0,01
Final Weight (g)	0.068±0,02[a]	0.071±0,02[a]	0.071±0,03[a]
Absolute Weight Gain (g)	0,048±0,01[a]	0,51±0,01[a]	0,51±0,02[a]
Initial Length (mm)	10,4 ± 0,2	10,4 ± 0,2	10,4 ± 0,2
Final Length (mm)	19.4±0,8[a]	21.2±1,7[a]	20.6±1,7[a]
Absolute Length Gain (mm)	9,0±0,6[a]	10,8±1,5[a]	10,2±1,5[a]
Survival Rate (%)	50,7±1,3[a]	76,2±1,2[b]	79,7±1,9[b]

*The value followed by similar superscript in the same row were not significantly different (p>0,05).

Table 2 Water quality on the each treatment during research

Variable	Treatment		
	A (Pellet/Control)	B (Pellet + Tryptophan)	C (Pellet + Glycine)
Water Temperature (oC)	28,6 ± 1,2	28,6 ± 1,2	28,6 ± 1,1
Salinity (‰)	10,0 ± 0,3	10,0 ± 0,3	10,0 ± 0,3
pH	8,2 ± 0,2	8,2 ± 0,2	8,2 ± 0,2
Oxygen (mg/l)	5,4 ± 1,1	5,3 ± 1,0	5,2 ± 0,8
Nitrate (mg/l)	0,1164 ± 0,1431	0,1660 ± 0,1334	0,1229 ± 0,1107
Ammonia (mg/l)	0,0137 ± 0,1070	0,0828 ± 0,0642	0,0732 ± 0,0600

Addition tryptophan and glycine in the feed pellets to 1.0% is not significant effect ($P > 0.05$) on the growth rate and weight gain prawn seed and still relatively equal to feed pellet. While Hseu *et al*. (2003) reported the results of that juvenile grouper *E. coioides* were given additional tryptophan 1% in feed fish tend to have a smaller size than without the addition of tryptophan. On the observations made by Hseu et al.[16] showed the addition of up to 1% tryptophan in the diet tends to reduce feed consumption of juvenile grouper *E. coioides*. While in this study, the amount of seed prawn feed consumption is still likely to be relatively similar between treatments, except for treatment without the addition of tryptophan and glycine are relatively lower, but feed consumption increases again at tryptophan addition of 1% (Figure 3).

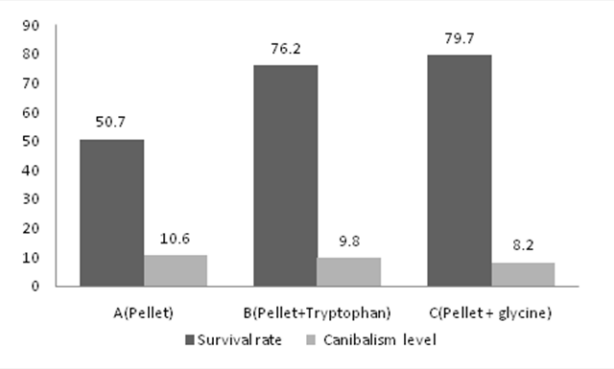

Figure 3 Cannibalism levels and survival rate of giant prawn juvenile on the final research.

The observation level indicates that the level of cannibalism prawn seeds was highest in the treatment of feed pellets (A) which is equal to 10.6 ± 0.3%, and the lowest was in treatment C (pellet + glycine) of 8.2 ± 0, 4%, subsequent treatment B (pellets + tryptophan) of 9.8 ± 0.4%. Thus, it seems that the addition of 1.0% tryptophan and glycine in feed pellets have no effect and can reduce the level of cannibalism seed prawns although statistically not significant ($P > 0.05$) with the treatment of feed pellets (A). Results of this study was slightly higher when compared with tryptophan dose given on the type grouper, *Epinephelus coioides*, Hseu et al.[16] reported that the addition of 0.5% tryptophan in feed has been able to lower the level of the fish cannibalism significantly compared to no additional tryptophan was given. Then Kamaruddin et al.[18] reported the addition of a dose of 1% tryptophan into feed for tiger grouper (*Epinephelus fuscoguttatus*) can reduce the level of the fish cannibalism. There is little difference in the results of these two studies can be caused by different species of fish, or as a result of differences in feed formulation. Tryptophan and glycine is one of the essential amino acids that are important for the growth of fish, other than that tryptophan and glycine is also a precursor for the synthesis of serotonin in the brain. The higher consumption and glycine tryptophan by fish, it tends to the production of serotonin in the brain also increases.[16] Munro[20] and Maler & Ellis[21] reported that the higher the levels of serotonin in the brain, then the level of aggressive fish tends to decrease. In this study, it can be estimated that the aggressiveness of giant prawn seed tends to decrease with the addition of tryptophan and glycine as much as 1.0% in the feed.

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According to the observations Van Damme et al.[22] the larval and juvenile carp, *Cyprinus carpio*, victims of cannibalism can be divided into two types: type I cannibalism, the victims were preyed upon only on the tail and the body alone while the head removed, Cannibalism type II ie, prey eaten can begin of the head (IIa) or from the tail (IIb) and swallowed and digested (IIa). In observation of the seed of this giant prawns, commonly found cannibalism type I. Seed larger prawns and healthy always beat and prey on smaller and weaker. The same thing was also reported in the mud grouper.[16] But sometimes prey torn from the direction of the tail, no one can escape, but generally has been injured and is unable to survive for long. However Hseu et al.[16] reported that the grouper *Epinephelus coioides* not found any dead fish as a result of the bite wound. This could happen because of the size of the fish in this study is relatively larger than that used by Hseu et al.[16] In this study, also often found between prey and predator to die together, and this generally occurs if the size of the seed prawns almost as they were spotted together.

Results of water quality measurements presented in Table 2. The element nitrogen in a body of water is an important element in the formation ProRes protoplasm. The elements of the measurement results showed that the nitrite content is quite high at 0.1164 ± 0.1431 to 0.1660 ± 0.1334 mg / L. The high nitrite content of nitrite is caused not by the utilization of phytoplankton due to lack of sunlight that goes into the research container, so that the photosynthesis process is disrupted. Though the content of nitrogen is necessary because the nitrogen in a body of water is an important element in the formation process protoplasm. According Schmittou[19] nitrite concentration in a body of water should not exceed 0.1 mg / L, if in excess of 0.1 mg / L, the metabolic processes of the organism will be disrupted.

Ammonia content in each treatment are presented in Table 2, are still within acceptable limit for the life of giant prawn seed. Ammonia content in this study is quite low, it can be said ammonia that arises in this study comes from the feces of giant prawn seeds are removed from the body. According to Boyd,[23] ammonia in nature comes from manure, fish feces and from the weathering of microbial nitrogen compounds. According MNLH,[24] the content of ammonia water to biota is 0.3 mg / l. More than 0.3 mg / l is already toxic. Boyd Boyd[23] states that the ammonia content in water causes increased excretion of ammonia by the fish decreases and the content of ammonia in the blood and tissues increases. The result is increased blood pH and adversely affects the reaction of the enzyme catalyst and membrane stability. High ammonia in the water also increases oxygen consumption by the tissues, damages the gills, and reduces the blood's ability to transport oxygen. Then for temperature, pH, salinity and dissolved oxygen still within reasonable range for the life of the seed prawns.

Conclusion and suggestions

The level of cannibalism seed prawns which consume pellets + tryptophan, and pellets + glycine is relatively low when compared with the seed giant prawns which consume pellets. Mixing the pellets with tryptophan and glycine did not affect the growth rate of prawn seed. However pellets plus tryptophan and glycine effectively influence the survival rate and the rate of cannibalism. Seed survival rate of prawns which consume pellets + tryptophan, and pellets + glycine is relatively better when compared with the seed giant prawns which consume pellets. Based on the research results obtained by considering the observed biological variables, it is suggested in the seed nursery prawns (PL-7) seed size 0.02 ± 0.01 g, can use the pellets were mixed with 1.0% tryptophan and or glycine of total biomass.

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Conflicts of interest

None.

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