IOP Conf. Series: Earth and Environmental Science **137** (2018) 012059 doi:10.1088/1755-1315/137/1/012059

The effect of amino acid lysine and methionine addition on feed toward the growth and retention on mud crab (*Scylla serrata*)

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Abstract. High market demand of mud crab (*Scylla serrata*) encourages farmers to increase the production of mud crab. However, mud crab can not synthesize essential amino acids, so it is necessary to supply essential amino acids such as lysine and methionine in the diet. This study aims to determine the effect of lysine and methionine on feeds to increase growth and retention of mud crabs (*Scylla serrata*). In this study the amount of lysine amino acid and methionine added to the trash fish diet were: P0 (0: 0%); P1 (0.75: 0.75%); P2 (1: 1%); P3 (1.25: 1.25%); P4 (1.5: 1.5%) with the ratio of lysine and methionine 1: 1. The parameters observed in this study were Survival Rate (SR), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Efficiency Feed (EF), protein retention and energy retention. The results of the 35-day maintenance study showed significant differences (P <0.05) against Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Efficiency Feed (EF), protein retention and no significant effect (P> 0.05) on energy retention and Survival Rate (SR) on mud crab. The best results in this study were found in P4 treatment with addition of lysine amino acids and methionine (1.5: 1.5%).

1. Introduction

Mud crab (*Scylla serrata*) is an important economic fishery commodity. The demand of the global mud crab market is increasing every year. The demand for mud crab increases because of its high nutritional content so that it is demanded by many people [1]. The high market demand encourages farmers to increase the production of mud crab (*Scylla serrata*). Mud crabs (*Scylla serrata*) have the potential to be cultivated because of their advantages, including rapid growth, larger size than other species, disease resistance, and high selling prices [2].

Feed is a factor that must be met to achieve maximum production in the cultivation of mud crabs. The use of trash fish feed is more advantageous because the price is relatively inexpensive, easy to obtain, and suitable with the eating habit of mud crab (*Scylla serrata*) [3]. Essential amino acids are amino acids derived from additional food ingredients. Because they cannot be produced by the body, they need to be obtained in order to be able to meet the daily needs of amino acids [4].

Some of the essential amino acids needed by mud crabs (*Scylla serrata*) are lysine and methionine [5, 6]. Lysine is an amino acid that makes proteins accelerate growth [7]. Methionine is an essential amino acid containing sulfur, a substance necessary for natural antioxidants [8]. The presence of lysine and methionine amino acids in the diet can improve the utilization balance of other amino acids in

order to increase growth [9]. Research on the addition of lysine and methionine amino acids to determine the growth, energy retention and protein retention in mud crab has not been done. So, further research is required.

2. Methodology

2.1. Sample preparation

The samples were male mud crabs (*Scylla serrata*) with the size of 80-90 gram suitable for enlargement [9]. Male mud crabs (*Scylla serrata*) can grow faster than the female ones, because the feed consumed by male crabs is only used for growth process and not for egg maturation process [10].

2.2. Feed feeding

Sampling was carried out once every 7 days to determine the amount of feed given during maintenance. The amount of feed required for mud crab was 5% of its body weight in order to grow optimally [11]. Feed given in the form of trash fish that had been mixed with lysine and methionine amino acids with a dose of P0 (0: 0 %); P1 (0.75: 0.75 %); P2 (1: 1 %); P3 (1.25: 1.25 %); P4 (1.5: 1.5 %).

2.3. Feed making

In the process of making the feed, the composition of each material needed was calculated and weighed in accordance with the needs of the feed. Then, the type of yellow fish was cut and smoothed using chopper. Further, lysine and methionine were added to the amino acids, further binded in the form of tapioca starch with a predetermined dose into the trash fish feed. It should be noted that the mixing of feed ingredients should be done gradually, starting from the smallest to largest materials needed. Trash fish feeds that had been added with lysine and methionine amino acids and tapioca starch were aerated about 5-10 minutes before being given to mud crabs (*Scylla serrata*) [12].

2.4. Proximate analysis

The mud crab samples were tested in terms of their protein content using Kjeldahl method. The principle of kjeldahl method includes 3 stages of destruction, distillation, and titration. At the stage of destruction, the sample was taken as much as 1 gram and then put into kjeldahl flask. HgO 40gr, K_2SO_4 1.9 mg and H_2SO_4 2 ml were added. Then, the gourd was heated in the acid chamber until the solution became clear. The result of the destruction was diluted with 10-20 ml of distilled water. The distillation was carried out by washing and rinsing the destruction sample with distilled water on the distillation apparatus, then 8-10 ml of sodium thiosulfate was added. The result was then diluted to about 50 ml. Prepare a 125 ml containing 5 ml of boric acid and 2-4 drops of indicator of (mixture of 2 parts methyl red 0.2 % in alcohol and 1 part methylene blue 0.2 % in alcohol). Titrations were performed by dripping HCl 0.02 N from the burette on the sample until the solution color turned pink [13].

2.5. *Protein and energy retention*

Protein and energy retention can be calculated based on the following formula of [14]:

Protein retention:

$$\frac{Final\ body\ protein\ (gram) - initial\ body\ protein\ (gram)}{Total\ protein\ consumption\ (gram)}\ x\ 100\ \% \quad (1)$$

Energy retention:

$$\frac{Final\ energy\ (kkal) - Initial\ energy\ (kkal)}{Total\ energy\ consumption\ (kkal)}\ x\ 100\ \% \quad (2)$$

2.6. *Statistical analysis*

Statistical analysis was conducted using Analysis of Variance (ANOVA) to determine the effect of the treatment given. If there were significant results, then the calculation was continued with Duncan Multiple Range Test.

3. Results and Discussion

The results showed a significantly different on each treatment and the highest growth yield was in P4 treatment with the addition of lysine and methionine of 1.5: 1.5%. The lowest crab growth was found in P0 treatment without the addition of lysine and methionine in feed. Meanwhile, for SR, it is not significantly different in each treatment (table 1).

Table 1. The average growth data.

Treatment	SR ± SD	GR± SD	SGR ± SD	FCR ± SD	EF± SD
P0	1 ± 0.000	0.47^{a} ± 0.041	0.53^{a} ± 0.04	2.80^{b} ± 0.321	0.36^{a} ± 0.037
P1	1 ± 0.000	0.52^{ab} ± 0.034	0.59^{ab} ± 0.03	2.92^{b} ± 0.210	0.34^{a} ± 0.024
P2	1 ± 0.000	0.55^{bc} ± 0.040	0.61^{bc} ± 0.04	2.78^{b} ± 0.205	0.36^{a} ± 0.025
P3	1 ± 0.000	0.56^{bc} ± 0.015	0.62^{bc} ± 0.14	2.72^{b} ± 0.152	0.36^{a} ± 0.021
P4	1 ± 0.000	0.59^{c} ± 0.055	0.65^{c} ± 0.06	2.04^{a} ± 0.193	0.49^{b} ± 0.046

Note: The results ($p < 0.05$) were significantly different, and the results ($p > 0.05$) were not significantly different. P0: Lysine dose and methionine (0.0 %) P1: Lysine dose and methionine (0.75:0.75 %); P2: Lysine dose and methionine (1:1 %); P3: Lysine and methionine dosage (1.25:1.25 %); P4: Lysine dose and methionine (1.5:1.5 %). Survival Rate (SR), Growth Rate (GR), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Efficiency Feeds (EF).

The results of protein retention showed significantly different results on each treatment with different dose. Protein retention increased in P1 (0.75: 0.75 %), P2 (1: 1 %), P3 (1.25: 1.25 %), and significantly in P4 treatment with lysine and methionine doses of 1.5: 1.5%. The results of energy retention showed no significant difference on each treatment with different dose. The highest energy retention at P4 treatment was 148.1375 kcal/kg with the addition of lysine amino acid and methionine in feed at 1.5 %: 1.5 % methionine (table 2).

Table 2. The average retention data.

Treatment	Protein retention (%) ± SD	Energy retention (Kkal/kg) ± SD
P0	20.280^{a} ± 7.652	143.775 ± 27.601
P1	36.485^{ab} ± 17.181	118.612 ± 6.761
P2	46.517^{b} ± 12.719	132.585 ± 6.452
P3	50.040^{b} ± 11.419	125.022 ± 13.010
P4	84.137^{c} ± 21.841	148.137 ± 19.839

Note: The results ($p < 0.05$) were significantly different, and the results ($p > 0.05$) were not significantly different. P0: Lysine and methionine dosage (0.0 %) P1: Lysine and methionine dose (0.75:0.75 %); P2: Lysine dose and methionine (1:1 %); P3: Lysine and methionine dosage (1.25:1.25 %); P4: Lysine dose and methionine (1.5:1.5 %).

The Increase of GR and SGR occured because the food consumed can be digested and absorbed well by the body. This is indicated by the increased weight of mud crab (*Scylla serrata*) at the end of treatment [15]. The highest increase of GR and SGR were in P4 treatment with lysine and methionine dose of 1.5: 1.5 %. The presence of lysine and methionine in the diet can improve the balance of other

amino acids so that the process of absorption of nutrients into the body become faster, leading to the increase of growth [9].

The lowest FCR and the highest EF were identified in P4 treatment with lysine and methionine dose of 1.5: 1.5 %. Good quality feed has low FCR and highest EF [3]. Undigested or undesirable feed is a factor that causes a high Feed Convertion Ratio (FCR) [16]. Factors influencing the value of EFF are feed type, feed quality, and feeding technique [17]. Lysine and methionine are simple compounds. When entering the stomach, they will be rapidly metabolized by digestive enzymes and absorbed by the body quickly [18]. Addition of lysine and methionine may increase the utilization of feed consumed, resulting in a low feed conversion ratio [19].

The level of SR was not significantly different in each treatment because lysine and methionine absorbed by the body only affected the weight gain of mud crab (*Scylla Serrata*) [20, 21]. Increased protein content of mud crabs is due to lysine and methionine, an important amino acid in protein synthesis (transcription process, which translates the sequence of nitrogenous bases in DNA to form RNA) because the methionine code is the same as the initial code for one set of RNAs. The protein content in the body of its existence is influenced by the retention of feed and the pile of proteins that are positively correlated with the protein content of the feed [22]. Retention of protein has increased from the control treatment. This is because trash fish added with lysine and methionine have fulfilled the nutritional needs of feed as well as absorption of essential amino acids [23]. The study of Palavesam *et al*. [24], in pearl spotted fish also showed an increase in protein metabolism after feeding was given L-Lysine. For example, fish given 25 % L-Lysine had higher consumption, assimilation and protein production compared to the control treatment.

The decrease of energy in mud crabs caused by excessive protein supply without the addition of energy resulted in the increase of energy for the deamination process of lysine and methionine amino acids so that it will reduce the portion of energy for vital growth [25, 26]. Energy retention is not significantly different from control treatment. This is because the size of the crab is too small. So, most of the energy absorption from lysine and methionine is used for growth [23]. Crustaceans use energy savings in feed for growth, metabolism, maintenance needs, ammonia excretion, feces and molting [27].

4. Conclusion

this study has demonstrated that amino acid lysine and methionine can potentially be used in addition on feed toward the growth and retention on mud crab (*Scylla serrata*).

5. References

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