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Effect of Lysine Amino Acid in Artificial Feed Substituted with *Moringa* (*Moringa oleifera L.*) Leaves on Feed Efficiency of Bileh Fish Seeds (*Rasbora* sp.)

Pengaruh Pemberian Asam Amino Lisin pada Pakan Buatan yang Disubstitusikan Daun Kelor (*Moringa oleifera L.*) Terhadap Efisiensi Pakan Benih Ikan Bileh (*Rasbora* sp.)

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Abstract

This study aims to test the effectiveness of amino acid lysine in artificial feed in increasing feed efficiency of bileh fish fry (*Rasbora* sp.). The experimental design used was a non-factorial completely randomized design (CRD) with five treatments and three replications. The treatments included: feed without lysine (P0), feed with lysine 1.2%/kg (P1), 1.4%/kg (P2), 1.6%/kg (P3), and 1.8%/kg (P4). The fish fry used amounted to 10 fish per container with a size of 2 cm and a weight of 0.10 grams, reared for 40 days. The results of data analysis using ANOVA showed that the provision of amino acid lysine had a significant effect on specific growth rate, but no significant effect on survival, feed conversion ratio and feed efficiency.

Keywords: *Feed, Feed Efficiency, Lysine, Bileh Fish*.

Abstrak

Penelitian ini bertujuan untuk menguji efektivitas asam amino lisin pada pakan buatan dalam meningkatkan efisiensi pakan benih ikan bileh (*Rasbora* sp.). Rancangan percobaan yang digunakan adalah Rancangan Acak Lengkap (RAL) non-faktorial dengan lima perlakuan dan tiga kali ulangan. Perlakuannya meliputi: pakan tanpa lisin (P0), pakan dengan lisin 1,2%/kg (P1), 1,4%/kg (P2), 1,6%/kg (P3), dan 1,8%/kg (P4). Benih ikan yang digunakan berjumlah 10 ekor per wadah dengan ukuran 2 cm dan berat 0,10 gram, dipelihara selama 40 hari. Hasil analisis data menggunakan ANOVA menunjukkan bahwa pemberian asam amino lisin berpengaruh nyata terhadap laju pertumbuhan spesifik, tetapi tidak berpengaruh nyata terhadap dan kelangsungan hidup, rasio konversi pakan dan efisiensi pakan.

Kata kunci: *Pakan, Efisiensi Pakan, Lisin, Ikan Bileh*.

1. Introduction

Bileh fish is a type of freshwater fish endemic to Aceh that generally lives in the wild, such as rivers, lakes, or swamps. Bileh fish has delicious and distinctive meat, so it is in great demand by the public, which causes its selling price to tend to be high, ranging from Rp.35,000 to Rp.45,000 per kilogram (Zulfadhli, 2022). Currently, the bileh fish that is marketed only comes from the catch of fishermen, because there is still little development of bileh fish cultivation that has been carried out. Therefore, efforts are needed to cultivate bileh fish to preserve it and prevent extinction. This is important to keep the productivity of bileh broodstock and fry high and avoid a decline that could threaten the sustainability of bileh populations in nature.

Bileh fish farming activities have now begun to be carried out by farmers but are constrained by the slow growth of bileh fish. Therefore, efforts are needed to overcome this problem. One way that can be done is to improve the quality of feed given to bileh fish seeds. Increased feed quality is expected to increase feed efficiency so that with increased feed efficiency it will increase the growth of bileh fish. According to Rahmatia (2016) Feed is one of the main roles in the ongoing cultivation to increase fish growth. Therefore, improving the quality of feed can be done by adding natural ingredients and additional supplements that can improve the quality of bileh fish feed.

Moringa leaves (*Moringa oleifera L.*) is one of the plants that can potentially be used as raw material for bileh fish feed. According to Shiriki (2015), fresh Moringa leaves contain 51.66% carbohydrates, 4.65% fat, 22.7% protein, 7.92% fiber, 94.01 water content, calcium (mg) 250-550. The use of moringa leaves in artificial feed generally still lacks amino acids, this happens because the vegetable protein sources used in feed do not always contain sufficient amino acids. Therefore, amino acids in feed are needed to ensure the adequacy of these amino acids.

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The protein contained in feed is composed of several amino acids. Amino acids that make up protein are divided into 2 parts, namely essential amino acids and non-essential amino acids. Essential amino acids are elements of protein formation that support the formation of tissues in the body of fish whose processes cannot be made in the body. Essential amino acids consist of several parts, namely lysine, isoleucine, valine, leucine, methionine, histidine, phenylalanine, threonine, tryptophan, and arginine. While non-essential amino acids are amino acids that can normally be produced in the body to meet the needs of fish. Non-essential amino acids are divided into several parts, namely aspartate, proline, glycine, tyrosine, serine, cysteine, asparagine, glutamine, alanine and glutamate acid (Ediwarman 2021).

Lysine is one of the feed additives that can accelerate fish growth and shorten the production period of cultivars. However, the level of lysine in feed must be optimal; too high levels of lysine can interfere with feed utilization and fish growth (Ebeneezar, 2019). Lysine also acts as an appetite stimulant and improves the sense of smell of fish (Aristasari, 2019). According to Pavalesam (2008), lysine deficiency in feed can occur because vegetable protein sources used in feed do not always contain sufficient lysine. Therefore, the addition of lysine in feed is necessary to ensure the adequacy of this amino acid. Reinforced by the statement of Li *et al* (2012) that it is important to ensure that the amino acid content in the feed is complete and balanced, because amino acid deficiency can interfere with fish growth and health.

Research on the use of lysine amino acids that have been carried out in the past can provide a good response to specific growth rate, feed conversion, and feed efficiency in mud crabs with a dose of lysine and methionine of 3% with a ratio of lysine and methionine 1: 1 (Alissianto 2017). The addition of lysine to commercial feed on growth rate, feed conversion ratio and feed efficiency of king prawns (*Macrobrachium rosenbergii*) Pramana (2017). The results of this study showed the addition of amino acid lysine in commercial feed used in king prawns with doses below 2% showed that it has not increased the growth rate and feed efficiency.

Based on the results of the above research, so far there has been no study on how much influence the amino acid lysine has on artificial feed to see the feed efficiency of bileh fish. So to observe the feed efficiency of bileh fish, research needs to be done to ascertain how the effect of amino acid lysine on bileh fish feed.

2. Materials and Methods

2.1 *Tools and Materials*

The equipment used includes a container box with a capacity of 25 liters, a feed making machine to print feed, a ruler, a digital scale with an accuracy of 0.01 grams, a portable pH meter, and a portable thermometer to measure water quality. The fish used were ikan bileh (*Rasbora* sp.) with a size of 2 cm and a weight of 0.10 grams.

2.2 Research Design

This study used a completely randomized design with five different feed doses, and each feed dose was repeated three times (Table 1). The proximate composition of the test feed can be seen in Table 2.

Table 1.
Treatment design

Code	Treatment
P0	Feed control
P1	Treatment of 1.2% amino acid lysine dose in artificial feed
P2	Dosage treatment of 1.4% amino acid lysine in artificial feed
P3	Dosage treatment of 1.6% amino acid lysine in artificial feed
P4	Dosage treatment of 1.8% amino acid lysine in artificial feed

Table 2.
Proximate composition of test feed

Composition	Treatment				
Nutrient (%)	P0	P1	P2	P3	P4
Protein	30,38	32,72	32,95	33,53	33,49
Carbohydrates	46,38	45,26	45,19	44,73	45,85
Fat	5,69	5,68	5,44	5,33	5,23
Fiber content	2,57	2,54	2,56	2,55	2,58
Water content	9,16	8,05	8,03	7,95	7,97
Ash content	5,82	5,68	5,44	5,33	5,25

2.3 Research Procedure

The container used is a 25-liter container box of 15 pieces. The first step is to wash the containers to remove dirt and odor. Once clean, the containers were filled with 10 liters of water each. Each container was labeled with treatment and replicate randomly and aerated. The process of making feed begins with collecting raw materials formulated according to the needs of each treatment. Raw materials are then calculated for formulation and composition and added according to treatment needs. After that, it is evenly mixed, ground using a feed molding machine, then dried and ready for use. Bileh fish (*Rasbora* sp.) were acclimated for 7 days, selected healthy and active with a size of 2 cm and a weight of 0.10 grams, then measured the weight and length for initial data. Fish were placed into treatment containers at a density of 10 fish per container. Fish were kept for 40 days and fed twice a day (at 09.00 and 15.00 WIB) using the *ad satiation* method. Water quality (temperature, pH) was measured daily, while dissolved oxygen (DO) was measured every 10 days. Sampling was conducted on day 1, day 20, and day 40.

2.4 Research Parameters

2.4.1 Survival Rate

Survival is the ratio between the number of fish at the end of rearing and the number of fish at the beginning of rearing. The survival rate can be calculated using the following formula.

$$KH = \frac{Nt}{No} x\ 100\%$$

Description:
KH = Survival (%),
Nt = Number of test fish at the end of the study (individuals),
No= Number of test fish at the beginning of the study (individuals)

2.4.2 Specific Growth Rate (SGR)

Specific growth rate is the percentage increase in weight and length of fish every day during the study. Specific growth rate is calculated using the following formula:

$$LPS = \frac{Ln\ Wt - Ln\ Wo}{t} x\ 100\%$$

Description:
LPS = Specific growth rate,
Ln Bt = Average weight of fish at the end of the study
Ln Bo = Average weight of fish at the beginning of rearing,

t = Maintenance time (length of study).

2.4.3 Feed Conversion Ratio

Feed conversion ratio is the amount of feed required to produce 1 kg of meat. Feed conversion ratio or FCR can be calculated with the following formula:

$$FCR = \frac{F}{(Bt + D) - Bo}$$

Description:
FCR = Feed Conversion Ratio,
F = Total amount of feed (g),
B_t = Average weight of fish at the end (g),
B_o = Average weight of fish at baseline (g),

2.4.4 Feed Efficiency

Fish feed efficiency can be said if feeding is low with good feed quality but produces rapid fish weight gain. Feed efficiency can be calculated using the following formula:

$$EP = \frac{(Bt + D - B0)}{F} \times 100\%$$

Description:
EP= Feed Efficiency (%)
F= Total feed fed (g)
B_t = Final average weight of test fish (g)
B_o = initial average weight of test fish (g)
D= Total weight of fish that died during the study (g)

3. Results and Discussion

3.1 Survival Rate

The results of the survival rate study on Bileh fish (*Rasbora* sp.) with a maintenance period of 40 days had a value of 86.67 ± 73.33. The results of the study presented in Figure 1, get an average value that does not have a significant influence between the control treatment with P4 treatment (86.67%) which tends to be higher than the average value in the control treatment P0 (76.67%) The results of statistical analysis using ANOVA show that the survival rate of bileh fish does not have a significant influence between treatments.

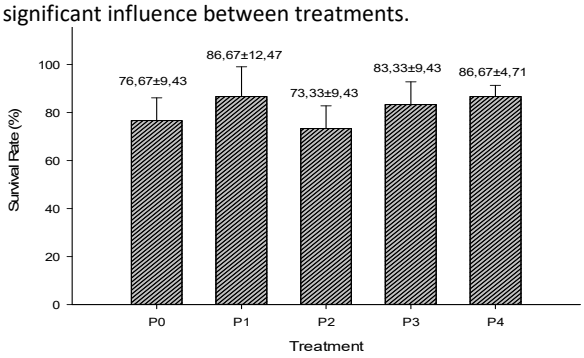

Figure 1. Survival rate of bileh fish (*Rasbora* sp)

Although it did not significantly impact the survival rate of bileh fry, the addition of lysine to the feed still has benefits that need to be considered. This assumption arises because there are various factors and conditions that may affect the survival of bileh fish fry, including environmental factors and fish handling methods. According to Madinawati (2011), survival rate is influenced by two main factors, namely biotic factors and abiotic factors. Biotic factors include competition between species, age, predation, parasitism, population density, and human intervention, while abiotic factors include chemical and physical properties in the aquatic ecosystem. In the context of this study, bileh fish survival was categorized as good. According to Permatasari (2023), the survival rate classified as good is between 50%, while the survival rate between 30-50% is considered moderate, and less than 30% is considered unfavorable.

3.2 Specific Growth Rate

Specific growth rate is the percentage of fish weight and length gain per day. The research findings using ANOVA showed that the addition of lysine to bileh fish fry feed had a significant effect (P<0.05) on the specific growth rate of the fry. The data presented in Figure 2 shows that the mean values of the control treatment and P3 treatment (3.50%) were significantly different, where the mean value of the P3 treatment tended to be higher than that of the control treatment P0 (2.09%).

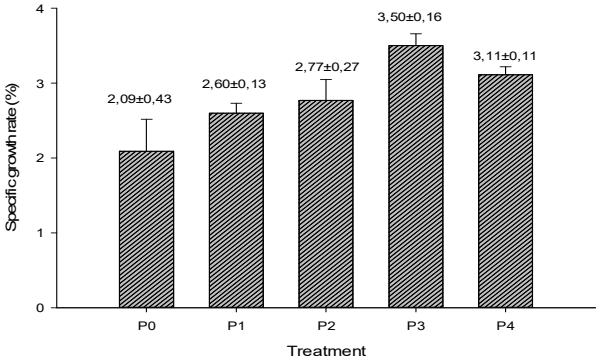

Figure 2. Specific growth rate of billfish (*Rasbora* sp)

The results showed that the addition of lysine to bileh fish fry feed gave a positive response to the specific growth rate of seeds. This indicates that the feed has been utilized effectively, so that the growth of bileh fish seeds increases. This finding is in line with Rachmawati's research (2020) which states that lysine in feed helps balance osmotic and acid-base pressure, so that more energy is used for growth. Pramana (2017) also supports by stating that lysine as a feed additive can increase fish growth and shorten the cultivation production period. Ahmadi (2012) added that good digestion of feed by fish can increase growth, because energy from feed is used optimally by fish.

3.3 Feed Conversion Ratio

Feed utilization can be assessed through the calculation of feed conversion ratio, known as *Feed Conversion Ratio* (FCR). FCR is the ratio between the amount of feed required by the fish to produce one kilogram of meat. In other words, every kilogram of feed given to fish is expected to produce one kilogram of meat. The level of feed efficiency can be known from the low FCR value, the lower the FCR the better the level of feed efficiency. Conversely, if the FCR value is high, the level of feed efficiency is considered poor (Putra, 2020).

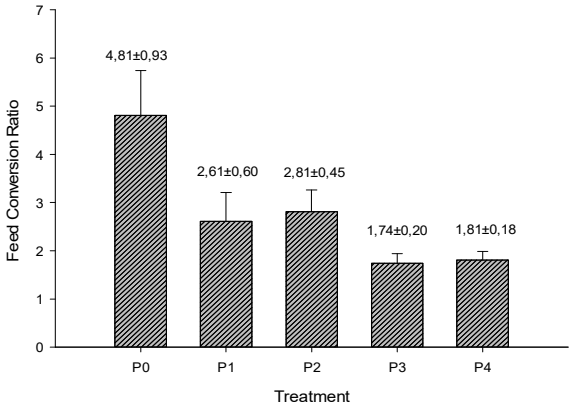

Figure 3. Feed Conversion Ratio of billfish (*Rasbora* sp)

Based on statistical results using ANOVA, the addition of amino acid lysine to the feed does not significantly affect the feed

conversion ratio of bileh fish fry (P>0.05). It can be seen that treatment (P0) has a value of 4.81 ± 0.93, then treatment (P1) with a value of 2.61 ± 0.60, followed by treatment (P2) with a value of 2.81 ± 0.45, treatment (P3) with a value of 1.74 ± 0.20 and treatment (P4) with a value of 1.81 ± 0.18. The feed conversion ratio in the study of lysine provision in bileh fish fry feed was classified as poor. This is thought to be caused by factors such as feeding management and environmental conditions. However, the addition of lysine as a feed additive can increase the protein, fat, and energy content of the feed, resulting in a lower feed conversion ratio compared to the control treatment. This is in line with the opinion of Ardita (2015), which states that the lower the feed conversion value produced, the better the quality of the feed so that it can be used optimally by fish for growth. Conversely, if the resulting feed conversion value is high, then the quality of the feed provided is considered poor.

3.4 Feed Efficiency

Feed efficiency is the ratio between the weight gain of fish and the amount of feed consumed, indicating how effectively feed is utilized by fish. The higher the feed efficiency, the better the utilization of feed by fish.

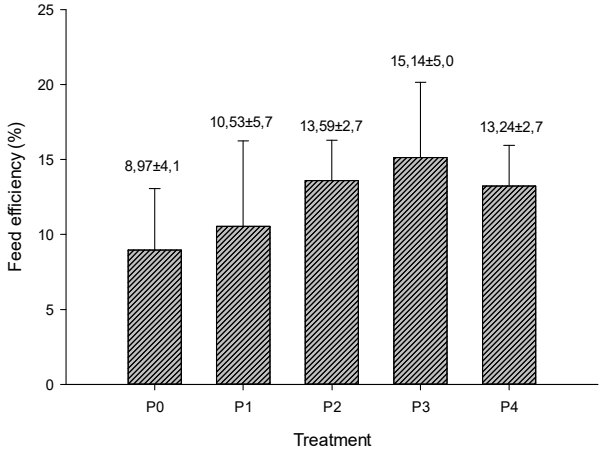

Figure 4. Feed efficiency of bileh fish (*Rasbora* sp)

Based on statistical analysis using ANOVA, based on statistical analysis using ANOVA, the addition of amino acid lysine to the feed did not show a significant effect on feed efficiency of bileh fish fry feed (P>0.05). The feed conversion ratio value for the P0 treatment was 8.97 ± 4.1, for the P1 treatment was 10.53 ± 5.7, for the P2 treatment was 13.59 ± 2.7, for the P3 treatment was 15.14 ± 5.0, and for the P4 treatment was 13.24 ± 2.7. It is suspected that the fish still experience a lack of energy intake from the feed given, where only a small portion of the energy obtained from the feed can be utilized for fish needs. This finding is supported by research by Widodo (2010) which shows that not all energy in feed can be digested and utilized efficiently by fish. This statement is reinforced by Maulidin (2016) which states that a good feed efficiency value depends on the quality of the feed given, thus allowing efficient digestion by fish. Feed efficiency is also closely related to fish digestibility of the feed given. High feed efficiency indicates that the feed consumed is of good quality, so that it can be digested and utilized efficiently by fish. According to Yanti (2013), fish digestibility of feed is influenced by various factors such as water temperature, water quality, type of feed, age and size of fish, nutritional content of feed, frequency of feeding, and the presence of digestive enzymes in the fish digestive tract.

3.5 Water quality

Water quality affects fish production performance. Poor water quality can cause disease, weakened fish health, and even death. During rearing, temperature ranged from 27.1-28.1°C, pH between 8.61-8.63, and dissolved oxygen between 6.57-6.65 mg/l. The water quality in the tilapia rearing containers was still within the proper limits for the survival of tilapia.

Temperature plays an important role in controlling the condition of aquatic ecosystems, affecting fish activities such as reproduction and respiration, and is related to dissolved oxygen and oxygen consumption of aquatic organisms (Sitompul 2020). The optimal temperature for freshwater fish farming media generally ranges from 25-32°C (Mas'ud 2014). The pH of water that is good for fish growth ranges from 7.5-8.5 (Arianto 2018). pH that is too low can cause fish mortality and increase the solubility of heavy metals in aquatic ecosystems, as well as affect the pH of fish blood plasma and their survival.

Dissolved oxygen (DO) is important in aquatic ecosystems, especially in fish farming (Raharjo 2016), as it affects fish growth and survival (Hasim 2015). During rearing, dissolved oxygen ranged from 6.57-6.65 mg/l. According to Indonesian National Standard (SNI) 7550:2009 (March 21, 2013), the optimum dissolved oxygen for freshwater fish is 7 ppm. Thus, water quality during the rearing of bileh fish is still within the limits of feasibility for fish farming media, with water quality test parameters such as temperature, pH, and dissolved oxygen. The research results of water quality parameters during 40 days of rearing can be seen in Table 3.

Table 3. Temperature, pH, and dissolved oxygen range of each treatment.

Treatment	Temperature(°C)	Degrees Acidity (pH)	Oxygen Dissolved (mg/l)
P0	27,1-28,0	8,63-8,61	6,65-6,57
P1	27,2-28,0	8,62-8,65	6,62-6,58
P2	27,1-28	8,63-8,65	6,50-6,51
P3	27,1-28,1	8,63-8,65	6,30-6,45
P4	27-28,1	8,63-8,64	6,48-6,61

4. Conclusion

The addition of amino acid lysine to artificial feed significantly affects the parameters of specific growth rate, but does not significantly affect the parameters of survival rate, feed conversion ratio, and feed efficiency of bileh fish. The best dose in increasing the feed efficiency of bileh fish is artificial feeding with the addition of amino acid lysine at 1.6%/kg feed (P3).

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