

Effect of Maggot-Tofu Dreg Mixed Flours with Addition of Lysine on Formulation of Fish Feed to Accelerate the Growth of Tilapia (*Oreochromis niloticus*)

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Submitted: 20 June 2023; Revised: 23 September 2023; Accepted: 30 October 2023

ABSTRACT Maggot flour, which contains high protein has potential in the development of fish feed. The focus of this study was to investigate the formulation of fish feed based on mixed flours (maggot and tofu dregs flours) with the addition of lysine to improve feed quality and feeding efficiency. The Pearson Square Method (PSM) was used in the formulation process of fish feed with a total protein target of 35%. The formulated fish feed was applied on tilapia fish (*Oreochromis niloticus*). The fish weight and ammonia levels in the water were also measured to determine the effect of fish feed on water quality. The highest protein content (33%) was obtained from ratio of mixed flours of 3:1 (%(w/w)). The highest floatability value of fish feed was 194 ± 2 sec. The optimum results for fish growth were obtained from mixed ratio of maggot flour and tofu dregs of 3:1 % (w/w). with the addition of 2 % (w/w) of lysine. The optimal growth rate of fish was $0.041 \text{ g} \cdot \text{day}^{-1}$ after 30 days of the experiment. While the level of ammonia in the water was not affected by the fish feed formulation or the addition of lysine in the fish feed. Thus, the amino acid lysine greatly influences the feed conversion ratio and specific growth rate so that it has the potential to accelerate the growth of tilapia.

Keywords: Lysine; maggot flour; tilapia; tofu dregs

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is one of the commercial freshwater fish commodities due to it has high economic value. Tilapia has several advantages, including being an omnivorous fish that easily adapts to the environment and is easy to cultivate. In addition, tilapia is popular with Indonesian people due to its nutritional content and delicious taste of meat. Romaneli *et al.* (2021) reported that tilapia, which many people eat as a source of animal protein, has a nutritional content of 17.7 % (w/w) protein and 1.3 % (w/w) fat.

Mo *et al.* (2020) and Prajayati *et al.* (2020), stated that feed is an important factor in fish farming activities both during hatchery and rearing. The availability of feed greatly influences the growth and survival of fish. In the enlargement process in fish farming, feed makes the biggest contribution, more than 60% of production costs are used for feed availability. Prajayati *et al.* (2020) also stated that the high price of feed ingredients is the main reason for the high price of fish feed because 60-70% of raw materials come from imports. Raw materials for feed, such as fish, meat, and soybean meal, the main protein sources for commercial fish feed, are still obtained through imports.

Recently, researchers are interested to explore alternative proteins as raw material in the formulation of fish feed. They highlighted different advantages of alternative proteins to accelerate the growth of fish (César-Chaves *et al.*, 2015; Rana *et al.*, 2015; Gachango *et al.*, 2017). The use of local raw materials with alternative protein as feed ingredients is an alternative strategy to reduce reliance on expensive imported fish meals. Abdul Kari *et al.* (2021) and Prajayati *et al.* (2020) reported that tofu dregs and maggot flour were affordable and sustainable availability as fish meals.

According to Lestari *et al.* (2013), feed formulation is a calculation of the number of raw materials used to make fish feed. Lestari *et al.* (2013) also added that an important thing in compiling a fish feed formulation was the nutritional content needed for fish, namely around 20-60 % (w/w) protein, 4-18 % (w/w) fat, carbohydrates consisting of less than 8 % (w/w) crude fibre and 20-30 % (w/w) NFE (Nitrogen Free Extract), vitamins and minerals about 2-5 % (w/w). Furthermore in SNI 01-7242-2006, complete artificial feed must be composed of proteins, carbohydrates, fats, vitamins, and minerals needed for optimal fish growth and survival. Most fish farmers, in this case, tilapia, use feed containing protein (>30 % (w/w)), fat (>5 % (w/w)), ash (<13 % (w/w)), phosphorus (<1.2 % (w/w)), water (<12 % (w/w)), vitamins, and minerals.

In terms of preparing fish feed, the additional essential amino acid is commonly used to accelerate fish growth. Michelato *et al.* (2016) used lysine to increase the feed conversion ratio value in fish feed. The addition of lysine is expected to reduce the value of the feed conversion ratio and increase feed efficiency. The quality of protein in the fish feed is closely related to the content of amino acids (Michelato *et al.*, 2016). According to Hua *et al.* (2019), commercial feed available today usually contains 1.41 % (w/w) lysine, and the lysine requirement for omnivore fish is 2.07 % (w/w). Hua *et al.* (2019) also added that lysine is an amino acid that is very useful for the body because it is a basic ingredient for blood antibodies, can strengthen the blood circulation system and maintain the growth of normal cells, proline and vitamin C which will form collagen tissue, and can reduce excessive blood triglyceride levels.

In this study, additional lysine was used to investigate the formulation of fish feed based on mixed flours (maggot flour and tofu dregs flour). In addition, the effect of lysine

addition in the fish feed to improve feed quality and increase feeding efficiency was also used to investigate the quality of fish feed.

MATERIALS AND METHODS

Materials

The main raw materials used in this study, maggot flour and tofu dregs were obtained from maggot breeders and the local tofu home industry located in Sugjmanik, Tanggungharjo, Grobogan. Soybean flour, fine bran, corn flour and tapioca flour were purchased from the local market in Semarang. Lysine, fish oil, multivitamin and other supporting chemicals were purchased from CV. Indrasari, Semarang. The tilapia fish seeds were obtained from local farms in Semarang City, Central Java.

Methods

Fish feed formulation

Fish feed formulations based on mixtures of maggot flour and tofu dregs flour with the addition of the amino acid lysine followed the research of [Prajayati *et al.* \(2020\)](#), [Rachmawati *et al.* \(2020\)](#), and [Romaneli *et al.* \(2021\)](#) with some modification. In this study, fish feed was formulated using the Pearson Square Method, with the calculation of the desired feed containing 35% protein and the formulation of maggot flour and tofu dregs (45%), soybean flour (22%), fine bran (14%), corn flour (14%), fish oil (2%), tapioca flour (2.5%), and multivitamin (0.5%). All ingredients were mixed using 4 in 1 Cosmos Blenz (SB-802) mixer and put into mixing bowl stainless-steel. Moisturize the fish feed mixture by spraying with 50 mL hot water and continue mixed with gently for 15 min. After the fish feed ingredients are evenly distributed, put the mixture into the moulding machine. The moulded feed is cut according to the size of the fish's mouth and dried under open sun drying for a day. The research design used a completely randomized (CRD) with two variables (mixed ratio of maggot flour-tofu dregs and essential amino acid lysine). The first variable compares maggot flour and tofu dregs, consisting of 3 treatment levels. The second variable is the concentration of the essential amino acid lysine, which consists of 3 treatment levels. Thus, this study had 3x3 combinations or nine combinations. In addition, each was carried out in 3 repetitions, resulting in 27 treatment combinations, namely 3x9 treatment combinations or 3x3x3 experimental units. The mixed flour ingredients were presented in table below:

Table 1. Mixed flour ingredients and formulation used as fish feed.

Mixed ratio of maggot flour and tofu dregs % (w/w)	Formulation % (w/w)						
	Maggot flour-tofu dregs mixture	Soybean flour	Fine bran	Corn flour	Fish oil	Tapioca flour	Multivitamin
A1(1:3)	45	22	14	14	2	2.5	0.5
A2(1:1)	45	22	14	14	2	2.5	0.5
A3(3:1)	45	22	14	14	2	2.5	0.5

All ingredients were mixed using a 4-in 1 Cosmos Blenz (SB-802) mixer and put into a mixing bowl stainless-steel. Moisturize the fish feed mixture by spraying with 50 mL hot water and continue mixed with gently for 15 min. After the fish feed ingredients are evenly distributed, put the mixture into the moulding machine. The moulded feed is cut accor-

ding to the size of the fish's mouth and dried under open sun drying for a day. The research design used a completely randomized (CRD) with two variables (a mixed ratio of maggot flour-tofu dregs and essential amino acid lysine). The first variable compares maggot flour and tofu dregs, consisting of 3 treatment levels. The second variable is the concentration of the addition of the essential amino acid lysine, which consists of 3 treatment levels. Thus, this study had 3x3 combinations or nine combinations. In addition, each was carried out in 3 repetitions, resulting in 27 treatment combinations, namely 3x9 treatment combinations or 3x3x3 experimental units. [Table 2](#) shows the details of combinations of treatment levels in the formulation of fish feed.

Table 2. Combination treatment of mixed flour and the addition of lysine in the formulation of fish feed based on diet formulation percentage.

Mixed of maggot flour and tofu dregs	Concentration of lysine		
	B1 1.5%(w/w)	B2 1.7%(w/w)	B3 2%(w/w)
A1(1:3(w/w))	Sample 1	Sample 2	Sample 3
A2(1:1(w/w))	Sample 4	Sample 5	Sample 6
A3(3:1(w/w))	Sample 7	Sample 8	Sample 9

Analysis of protein content

Total protein analysis was performed to measure the protein of the formulated fish feeds and compared them to the target protein content in the desired feed. The analysis procedure of fish feed formulations was performed by titration as referred in SNI 01-2354.4-2006. 10 g of the formulated fish feed was diluted into a 100 mL measuring flask and added with distilled water up to the mark. A 10 mL of the sample solution was put in the beaker glass. 20 mL of distilled water containing 0.4 mL of saturated potassium oxalate and 2-3 drops of PP indicator was added into the sample solution. The solution was settled for about 2 min until the color changed. The sample was titrated with a 0.1 N NaOH solution until changed the colour. Then added 2 mL of a 40% formaldehyde solution than continued the titration and recorded the volume used of NaOH solution. Repeated the steps using a blank solution. Protein content was calculated using eq. 1:

$$\text{Total Protein (\%)} = \frac{\text{mL NaOH} \times N \times 14.008 \text{ g/mL}}{\text{Sample weight} \times 10 \text{ mL/L}} \times 100\%$$

Analysis of floatability value

The floatability value analysis on the formulated fish feed was followed by [Felix & Oscar \(2018\)](#) with some modifications. 100 mL of fresh water was prepared in a beaker glass. Formulated fish feed was put into the rearing medium at the same time as turning on the stopwatch. The formulated

fish feed was observed until the fish feed sank and touched the surface of the rearing medium and recorded the time.

Water quality measurement

In this study, the concentration of ammonia was presented to measure the water quality during fish feeding. Analysis of the ammonia content was carried out using the Fenat method using a UV-VIS spectrophotometer (Biobase Bk-V1000), which refers to SNI 06-6989.30-2005. The stages of ammonia content analysis are as follows: Preparing ammonia standard solution by making a standard ammonia solution with concentrations 0.1, 0.2, 0.3, 0.4, 0.5 mg/L and plotting into the standard curve. A 25 mL of the test sample was put into a 50 mL test tube, then 1 mL of phenol solution was added and homogenized. A 1 mL of sodium nitroprusside was added and gently homogenized. 2.5 mL of the oxidizing solution was added to the solution and shaken until gently mixed. The test tube was closed and left in a dark room for 1 hr for colour development. The solution was put into a cuvette on a spectrophotometer and analyzed the absorption value at 640 nm wavelength. The concentration of ammonia was determined using a standard curve.

Fish Feeding

Twenty tilapia seeds (approximately weight: 1 g with size ±5 cm) were given into a rearing container with a size of pxlxt = 50x30x30 cm³ and 4 L of water level. The seeds were acclimatized for 7 days for obtaining the best condition. Feeding was carried out twice time per day at 09:00 and 16:00 with a daily dose of 3% of the average weight of the fish for 30 days. Fish weight was measured every 10 days. Feed conversion ratio (FCR) and specific growth rate (SGR) were used to analyze the performance of formulated fish feed in Tilapia fish.

Following [Prajayati et al. \(2020\)](#), the feed conversion ratio (FCR) was defined as the ratio or level of measurement of feed uses converted to livestock weight. The value of the feed conversion ratio shows the ratio of the amount of feed given to livestock with the addition of weight to livestock. FCR was calculated by the following equation:

$$FCR = \frac{F}{(W_t + D) - W_0}$$

where is the amount of feed given (g), is the final fish weight (g), is the weight of dead fish (g), and is the initial fish weight (g).

Specific growth rate is as used as a parameter for tracing growth of fish limiting substances on fish feed. SGR was calculated using the following equation:

$$SGR = \frac{\ln W_t - \ln W_0}{t} \times 100\%$$

where is the final average weight of fish (g), is initial average weight of fish (g), and is time (days).

Statistical analysis

The data were analyzed statistically using Excel 2016, Microsoft office professional plus software. All measurements were done with at least 3 replicates. The results are shown as the value of averages ± standard deviations. One way analysis of variance (ANOVA) with post hoc Tukey (HSD) test

was done to identify the significant differences (p < 0.05) between data sets.

RESULTS AND DISCUSSION

Effect of mixed flour on the protein content of the formulated fish feed

The protein content obtained in each fish feed formulation. This result was not met the desired protein target of 35%. From all fish feed formulated, the protein content closest to the target of 35% was in the samples 7, 8 and 9, which of those variables obtained the same protein content of 33% as shown in table 3 below:

Table 3. Effect of mixed flour on the total protein percentage of the formulated fish feed.

Sample	Protein content (% w/w)
Sample 1	22±0.38 ^a
Sample 2	22±0.12 ^a
Sample 3	22±0.20 ^a
Sample 4	28±0.14 ^b
Sample 5	28±0.26 ^b
Sample 6	28±0.15 ^b
Sample 7	33±0.08 ^c
Sample 8	33±0.25 ^c
Sample 9	33±0.12 ^c

Noted: Data was presented at confidence level of 95%. Different letters indicated significant differences at P < 0.05 using Tukey's test.

The result above also indicated that adding the amino acid lysine did not significantly affect the protein content of the formulated fish feed. Conversely, comparing the use of substitute ingredients or the comparison between maggot flour and tofu dreg flour significantly affects the protein content in the formulated fish feed. In samples 7, 8, and 9, more maggot flour was used with a ratio of 75 % (w/w) : 25 % (w/w), or in other words, the ratio of maggot flour and tofu dregs flour was 3:1. The discrepancy with the targeted protein content was due to the lack of accuracy in assuming the protein content in tofu dregs. Compare to [Xiao \(2011\)](#), soy pulp (tofu dregs) has a protein nutrient content of 24.5–37.5%. Therefore, it assumed that the protein contained in the tofu dregs used follows the references that have been taken. Thus, it turns out that the protein content of the tofu dregs used was higher than others. The protein content of each tofu dreg from different industries has different protein levels. It may affect to the different protein content in the formulated fish feed. In this study, the use of more maggot flour influenced the protein content in the formulated fish feed.

Effect of mixed flour and addition of lysine on fish feed floatability

The floatability value of the formulated fish feed was carried out by measuring the length of time it takes for the feed to move from the surface of the water to the bottom of the rearing medium ([Felix & Oscar, 2018](#)). The formulated fish feed was measured for the floatability value of each ammonia design, which aims to determine how much the feed's floatability in the water. The floatability value of the feed

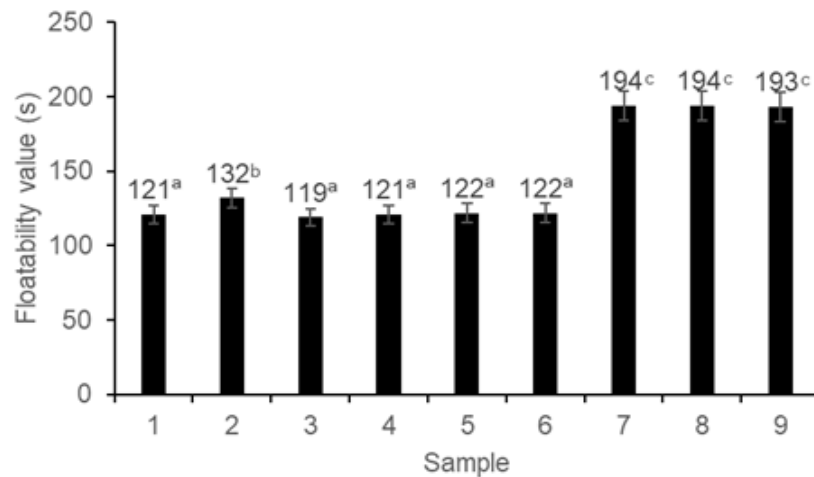


Figure 1. Effect of mixed flour and additional lysine on the floatability of formulated fish feed. Vertical bars represent the standard error SE (n = 10). Different letters indicated significant differences at P < 0.05 using Tukey's test.

was very influential on the application of feed to fish. Fish, especially tilapia, tend to be more attracted to feed those floats on the surface of the water than feed that has sunk. Sinking this feed will affect water quality, and feed that has sunk will usually not be eaten. can eventually lead to the accumulation of ammonia (Romaneli *et al.*, 2021).

Figure 1 shows that the addition of amino acid lysine has no significant effect on the floatability value of fish feed. In contrast, the use of substitute materials or the comparison between maggot flour and tofu dreg flour significantly affects the floatability value of fish feed in sample variables 7, 8 and 9, providing significant results over the others. The maggot flour content in fish feed could affect the floatability value of fish meals due to the edible oil content in maggot flour. Samples 7, 8 and 9, which have the highest maggot flour content, resulted in almost the same floatability value, namely above three minutes, or more precisely 194±2 s. Compared to other samples 1, 2, 3, 4, 5 and 6 had an average floatability value of only 120±4 s.

Effect of mixed flour and addition of lysine on the growth of tilapia fish

Table 4. Effect of mixed flour and additional lysine during formulation on the growth fish for 30 days. Data was presented at confidence level of 95%. Different letters indicated significant differences at P < 0.05 using Tukey's test.

Sample	Fish growth ratio (%)		
	Day-10	Day-20	Day-30
Sample 1	0.461±0.037 ^a	1.211±0.049 ^a	1.980±0.021 ^a
Sample 2	0.503±0.028 ^b	1.306±0.039 ^b	2.108±0.006 ^b
Sample 3	0.542±0.025 ^c	1.351±0.022 ^c	2.148±0.012 ^b
Sample 4	0.464±0.031 ^a	1.213±0.025 ^a	1.971±0.017 ^a
Sample 5	0.568±0.038 ^c	1.423±0.017 ^d	2.300±0.015 ^c
Sample 6	0.591±0.045 ^d	1.452±0.014 ^d	2.348±0.010 ^d
Sample 7	0.501±0.041 ^b	1.291±0.010 ^b	2.136±0.006 ^b
Sample 8	0.570±0.045 ^c	1.397±0.023 ^c	2.283±0.016 ^c
Sample 9	0.619±0.034 ^e	1.494±0.015 ^e	2.421±0.010 ^d

Fish feed formulation was applied for feeding in fish. The

experiment was carried out for 30 days to measure the growth and weight gain of the fish.

Table 4 shows that all variables have a significant effect on fish growth ratio. The addition of the amino acid lysine and comparing the use of substitutes or the comparison between maggot flour and tofu dreg flour has a significant effect on protein levels in the formulated fish feed. Sample 9 has the largest average weight of fish, namely 5.25 g on day 30, whereas on day 0, it had an average weight of 1.54 g, which can be calculated as the weight gain of fish within 30 days, which occurred at 3.71 g. This result was the optimum value compared to other variables. In sample 9, more maggot flour was used with a ratio of 75 % (w/w) : 25 % (w/w), or in other words, the ratio of maggot flour and tofu dregs flour was 3:1, and 2 % (w/w) of the amino acid lysine was added. This shows that the ratio of maggot flour (Rachmawati *et al.*, 2020) and the addition of the amino acid lysine (Romaneli *et al.*, 2021) greatly affect the weight gain of the fish.

Effect of formulated fish feed on the ammonia level in fish-rearing media water

The level of ammonia contained in aquaculture waters was the result of fish metabolism in the form of dissolved feces. Solid manure or feces and unconsumed feed residue are organic materials that contain high levels of protein, which are then broken down into polypeptides, amino acids, and ammonia as the final product in cultivation media. Maulina & Widaryati (2020) stated that the best ammonia for fish was less than 1 mg/L. Maulina & Widaryati (2020) also added that if the free ammonia level was more than 0.2 mg/L, the waters are toxic or toxic to several types of fish; in other words, it was dangerous for some fish. In this study, analysis of ammonia level contained in the water of the rearing medium refers to SNI 06-6989.30-2005.

Table 5 shows ammonia values obtained during the study. The results indicated that ammonia levels in water increased every day. However, the level of ammonia was still within reasonable limits. According to Maulina & Widaryati (2020), the best ammonia for fish was less than 1 mg/L, or as quoted from other sources SNI 8119:2015, acceptance of ammonia level was in the range of 0.1-0.6 mg/L. Ammonia could affect the growth of fish, and high levels of ammonia can be toxic and harmful to aquatic organisms. The design

Table 5. Effect of formulated fish feed on the ammonia levels in fish rearing media water.

Sample	Ammonia levels			
	Day 0	Day 10	Day 20	Day 30
Sample 1	0.0046±0.0015	0.0453±0.0012	0.1323±0.0012	0.2599±0.0025
Sample 2	0.0042±0.0009	0.0443±0.0003	0.1319±0.0023	0.2596±0.0015
Sample 3	0.0047±0.0012	0.0463±0.0012	0.1319±0.0029	0.2597±0.0009
Sample 4	0.0046±0.0023	0.0428±0.0007	0.1285±0.0019	0.2582±0.0017
Sample 5	0.0046±0.0018	0.0418±0.0018	0.1291±0.0023	0.2587±0.0012
Sample 6	0.0046±0.0012	0.0426±0.0018	0.1291±0.0029	0.2583±0.0009
Sample 7	0.0045±0.0009	0.0400±0.0025	0.1278±0.0007	0.2571±0.0015
Sample 8	0.0046±0.0022	0.0406±0.0012	0.1279±0.0023	0.2567±0.0025
Sample 9	0.0046±0.0006	0.0411±0.0015	0.1274±0.0027	0.2569±0.0015

variable of the comparison of maggot flour and tofu dregs or the addition of lysine did not significantly affect the ammonia content in the water after 30 days of feed application. Judging from each variable, it showed fluctuating results but increased every day.

Effect of formulated fish feed on the fish growth of fish

Figure 2 shows the feed conversion ratio of the formulated fish feed with different mixed flour and the addition of the

amino acid lysine. The treatment given significantly affects the tilapia feed conversion ratio. This shows that the addition of lysine to feed formulations reduced the value of the feed conversion ratio. According to [Prajayati et al. \(2020\)](#), feed conversion and growth rate also depend on the nutrient content found in the feed. To obtain optimal growth, fish feed should contain the adequate nutrition. This shows that the addition of lysine to the feed reduced the value

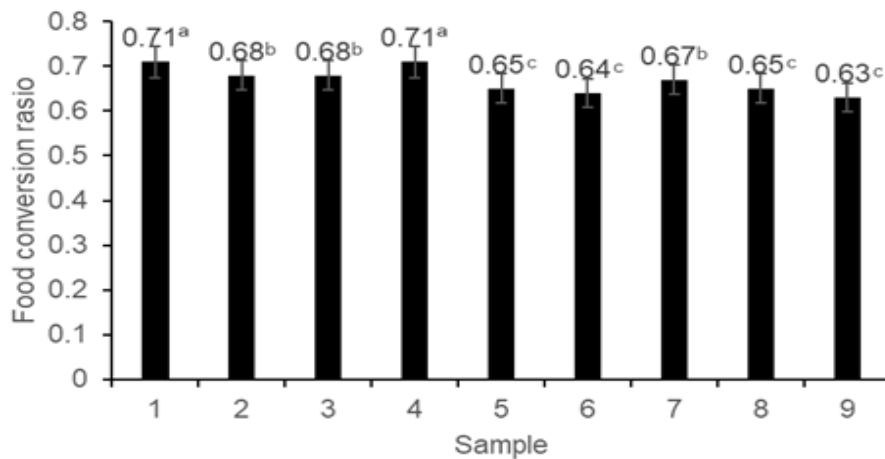


Figure 2. Effect of mixed flour and additional lysine on the feed conversion ratio. Vertical bars represent the standard error SE (n = 10). Different letters indicated significant differences at P < 0.05 using Tukey's test.

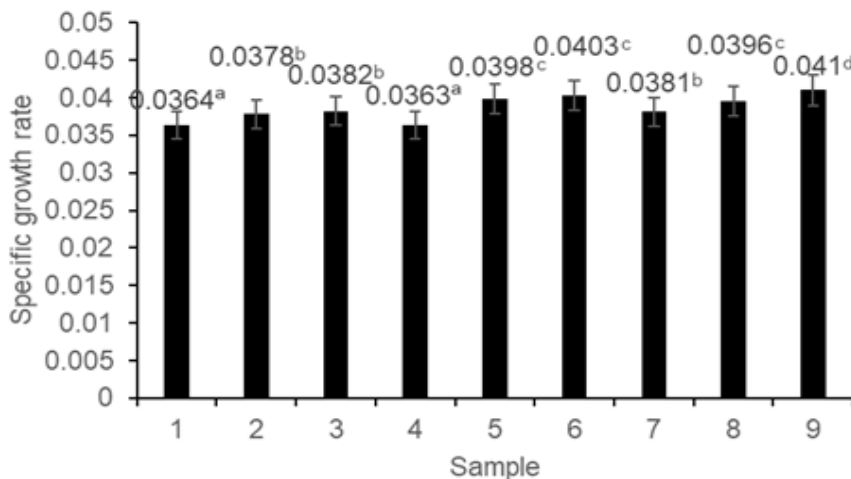


Figure 3. Effect of mixed flour and additional lysine on the specific growth rate. Vertical bars represent the standard error SE (n = 10). Different letters indicated significant differences at P < 0.05 using Tukey's test.

of feed conversion in all samples. Feed quality with the addition of lysine was better, effectively reducing the feed conversion value. This result was similar to Rachmawati *et al.* (2020) that the addition of lysine to feed decreased the feed conversion ratio value. The feed conversion ratio was also an indicator of feed quality, where the farmer's ability to manage feed as well as the cost efficiency used are seen from the existing feed conversion ratio value. The lower feed conversion ratio may be caused the lower cost of feed emitted.

Figure 3 shows that the addition of lysine to the feed formulation had a very significant effect on the SGR of tilapia. The effect of the addition of the amino acid lysine on the growth rate was suspected because the amino acid lysine influenced as a counterweight in maintaining the osmotic and acid-base pressure of the body, therefore not much energy was used to adjust the osmotic pressure of tilapia. Michelato *et al.* (2016) state that lysine is important in maintaining osmotic pressure and acid-base balance in body fluids. In addition, fish fed diets deficient in essential amino acids decreased the growth of fish. Furthermore, it may be due to one of the functions of the amino acid lysine in the formation of carnitine. The function of carnitine to metabolize fat to form or produce energy was essential to the growth of fish. These results were similar to the research conducted by Rachmawati *et al.* (2020) and Prajayati *et al.* (2020), which stated that supplementing the amino acid lysine in protein feeds could improve the growth performance of fish or other livestock.

CONCLUSION AND RECOMMENDATION

Conclusion

In this study, the largest protein content was in the formulation with a ratio of maggot flour, and tofu dregs flour of 3:1, where the protein obtained was 33 wt.%. This formula rapidly accelerated the growth of tilapia fish with additional lysine in feed ingredient. The results of applying the feed to the fish showed that the largest weight of the fish was the fish given the formula feed with a ratio of maggot flour and tofu dregs flour of 3:1 with the addition of 2 wt.% lysine, the weight of the fish after 30 days was 5.25 g with an additional weight of 3.71 g. The fish feed with the longest floatability value was a fish meal with a formulation with a ratio of maggot flour and tofu dregs flour of 3:1, namely for 193 sec (± 1 second). At the same time, the level of ammonia in the water is not affected by the feed formulation or the addition of amino acids. The amino acid lysine greatly influences the feed conversion ratio, survival rate, and specific growth rate of tilapia.

Recommendation

In future research, it is possible to apply feed to fish over a longer time span or with a greater number of fish, in other words, on a larger scale. In addition, variations of the ingredients used in the formulation can also be added.

AUTHOR'S CONTRIBUTIONS

AR formulated the fish feed and carried out the whole experiment. VP designed the analysis method. DIA revised the manuscript and analyzed the research data. HDA designed the study, wrote the manuscript, supervised the experiment and reviewed the manuscript.

ACKNOWLEDGEMENT

Akbar Rahmatullah would like to acknowledge the Department of Industrial Chemical Engineering Technology for providing their equipment during this research.

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