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The Impact of Lysine and Probiotic Supplementation on Local Artificial Feed Toward Growth, Feed Efficiency, and Survival of Bilih Fish (*Rasbora maninjau*)

Dini Islama[1*], Sufal Diansyah[1], Khairul Samuki[1], Irhami S.[1] and Agusriati Muliyana[1]

[1]Aquaculture Study Program, Faculty of Fisheries and Marine, Teuku Umar University, Jl. Alue Peunyareng, Gunong Kleng, West Aceh, Aceh, 23681, Indonesia

*Correspondence :
diniislama@utu.ac.id

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Abstract

Rasbora maninjau is a native fish of Aceh that is commonly found in the rivers of Nagan Raya Regency, Aceh. Although this fish has been domesticated, it is severely hampered by its slow growth. Lysine and probiotics are introduced to local feed to maintain the proper balance of amino acids in the feed and improve the quality of the protein feed produced. This makes it possible for the feed to act in a different way to promote the growth of the fish. The purpose of this study is to examine how *R. maninjau* growth, feed efficiency, and survival are affected by the addition of lysine and probiotics to local feed. A randomized design with three replications and five treatments was used in this study. The formulation uses local feed as a reference (P0), local feed with lysine added at a rate of 1.2% and probiotics at a concentration of 15 ml/kg (P1), 1.4% and probiotics of 15 ml/kg (P2), 1.6% and probiotics of 15 ml/kg (P3), and 1.8% and probiotics of 15 ml/kg (P4). Three times a day, 3% of body weight is fed. The study was raised for 40 days. The findings indicated that adding lysine and probiotics to a locally manufactured meal had a substantial ($P < 0.05$) effect on *R. maninjau*'s growth performance but had no significant effect on survival ($P > 0.05$). The best dosages for *R. maninjau* growth performance in the locally manufactured feed are 1.6% lysine supplementation and 15 ml/kg probiotics (P3).

INTRODUCTION

Bilih fish (*Rasbora maninjau*) is one of the endemic fish species in Indonesia; whereas *R. tawarensis* is widespread in several freshwater lakes in Central Aceh, its populations have significantly decreased and are in danger of going extinct (Muchlisin, 2013). This fish is commonly found in rivers and lakes, which are its natural environment. Due to its significant economic value—it sells for between Rp 35,000 and Rp 45,000/kg on the market—efforts to successfully domesticate it are currently ongoing (Zulfadhli *et al*., 2023). Numerous studies have been conducted in an attempt to support the success of this fish cultivation, such as growing bilih fish in different containers (Zulfadhli and Fadhillah, 2019), providing different feed (Zulfadhli *et al*.,

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2023), adding candlenut oil (Islama *et al*., 2020), and adding water hyacinth flour (Islama *et al*., 2023) in artificial feed to speed up *Rasbora* growth.

Farmers frequently encounter the challenge of slow growth during the domestication stage of *R. maninjau*. Farmers endeavor to promote growth by supplying commercial feed. However, this directly correlates with the amount of money required to buy feed, thereby increasing feed expenses. Farmers are quite concerned about this issue since their earnings do not correspond with the price at which fish are sold. Aquaculture output growth is hindered by the high feed costs farmers must pay for their operations, which can account for 70% to 90% of operational expenditures (Firdus *et al*., 2020; Islama *et al*., 2021).

Feed formulations that use local materials are being supplemented with lysine and probiotics to increase aquaculture productivity. The fish has to get lysine from its diet because its body cannot make it on its own. Because local feed lacks several important amino acids, including lysine, lysine supplementation in feed composition is necessary to compensate. According to Khalida *et al*. (2017), lysine can have an impact on feed quality since it limits the amount of important amino acids in fish feed. One way to keep the balance of amino acids in artificial feed and enhance the quality of the protein in the feed generated so that it may satisfy fish's nutritional needs is to add lysine to it. Lysine contributes to the growth and repair of bodily tissue. Fish development and feed efficiency are negatively impacted by the absence of lysine in local raw materials, which are primarily composed of vegetable protein (NRC, 2011; Rachmawati *et al*., 2021).

Prior studies have demonstrated that adding lysine to feed can effectively boost fish growth. For example, adding 1.2% lysine to feed can boost GIFT tilapia growth without compromising fish production performance (Prabu *et al*., 2017). On the other hand, juvenile tilapia need higher lysine levels, specifically 1.56% in feed, for the best growth performance (Diógenes *et al*., 2016). Supplementing feed with lysine can improve feed consumption and *P. hypophthalmus* and Javanese barb growth (Rachmawati *et al*., 2021; Rachmawati and Nurhayati, 2022). According to many other studies, adding lysine to food can boost growth in many fish species, including *Litopenaeus vannamei* (Xie *et al*., 2012), *Heteropneustes fossilis* (Farhat and Khan, 2013), *Macrobrachium rosenbergii* (Pramana *et al*., 2017), and *Trachinotus blochii* (Ebeneezar *et al*., 2019).

Given the high crude fiber content of vegetable raw materials, adding probiotics to artificial feed is also crucial to enhancing feed quality. Fish growth and endurance have been scientifically examined when probiotics are added to their diet. According to research, local snakehead fish (Saputra and Ibrahim, 2021), tilapia (Pangaribuan and Sembiring, 2022), depik fish (*R. tawarensis*) (Fratiwi *et al*., 2018), and goldfish (Tarigan and Meiyasa, 2019) can all grow more when probiotics are added to their diet. The addition of lysine and probiotics to local feed to promote *R. maninjau* growth, however, has never been the subject of any research. The purpose of this study is to examine the effects of lysine and probiotics added to local feed on the survival, growth, and feed efficiency of bilih fish.

METHODOLOGY

Ethical Approval

Not Applicable.

Place and Time

This research was conducted at the Hatchery and Aquaculture Systems and Technology and Environment Laboratory, FPIK, Teuku Umar University. Proximate analysis of feed was carried out at the Food and Agricultural Products Analysis Laboratory, Syiah Kuala University, Banda Aceh. This study was conducted from July to August 2023.

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Research Materials

The materials used in this study were *R. maninjau* measuring 2-3 cm, a fish meal with 50% protein content, moringa leaf flour with 24.14% protein content, soybean flour with 47.77% protein content, polishing with 12.90% protein content, corn flour with 7.90% protein content, L-lysine with a protein content of 99%, L-methionine with a protein content of 99%, probiotics (EM4®), fish oil with a lipid content of 99%, palm oil with a lipid content of 99%, phytase enzyme Bio Phytase 5000®, premix (Aquavita®), and CMC binder. The apparatus used in this study included a 30 x 30 x 25 cm aquarium, a pH meter (ATC digital tester®) with an accuracy of 0.1 pH units, a thermometer (Oxygen meter®) with an accuracy of 0.1°C, a DO meter (Oxygen meter®) with an accuracy of 0.01 mg/L, a digital scale (Mini Digital Platform Scale I-2000®) with an accuracy of 0.01 g, a sieve with a size of 12–20 mesh, a basin with a volume of 10 L, a feed flouring machine (Maksindo FFC-15®) with a capacity of 55 kg/hour, a feed mixer machine (Mixer Planeteri SL-B20A®) with a dough capacity of 4 kg, and a feed pelleting machine (MKS-PLT15®) with a capacity of 20 kg/hour.

Research Design

A non-factorial fully randomized design (CRD) including three replications and five treatments was employed in this study. The formulation uses local feed as a reference (P0), local feed with lysine added at a rate of 1.2% and probiotics at a concentration of 15 ml/kg (P1), 1.4% and probiotics at a concentration of 15 ml/kg (P2), 1.6% and probiotics at a concentration of 15 ml/kg (P3), and 1.8% and probiotics at a concentration of 15 ml/kg (P4). The results of earlier studies, which show that 1.2% lysine supplied in feed can boost the growth of tilapia (Prabu *et al*., 2017) and *P. hypophthalmus* (Rachmawati and Nurhayati, 2022), are used to determine the dosage of lysine supplementation. To maximize feed efficiency and development, *Puntius javanicus* seeds need 1.58% to 1.70% lysine (Rachmawati *et al*., 2021). Goldfish (Karel *et al*., 2019), *Clarias* sp. (Izzah *et al*., 2019), and tilapia (Fahrizal and Nasir, 2017) can all grow more when given a probiotic supplement at a dose of 15 ml/kg diet.

Work Procedure

Preparation and Production of Test Diet

The local diet is made using several local raw materials, such as moringa leaf flour, local fish meal, and polishing. Local raw materials such as Moringa leaf flour are treated first through a fermentation process using 25% EM4 mixed with 2% brown sugar (molasses) and 10% water and left for 10 days (Nainggolan *et al*., 2018). Feed formulations are prepared by calculating the composition of raw materials based on guidelines for a list of feed raw materials and their nutritional content. In the formulation process, we also pay attention to the nutritional needs of farmed fish, namely *Rasbora maninjau*, so that the feed is formulated to have a protein content of ≥ 30% to meet the needs of *Rasbora maninjau* (Cyprinidae) at the growth stage. Lysine substitution was carried out to reduce the use of soy flour, and then enrichment was carried out through the addition of probiotics using the spraying method. The test diet formulation can be seen in Table 1.

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Table 1. Formulation of test feed.

Raw materials	Composition (%)				
	P0	P1	P2	P3	P4
Fish meal	40	40	40	40	40
Moringa leaf flour	20	20	20	20	20
Soybean flour	14	12.8	12.6	12.4	12.2
Polishing	18.17	18.17	18.17	18.17	18.17
Corn flour	5	5	5	5	5
Fish oil	0.5	0.5	0.5	0.5	0.5
Palm oil	0.5	0.5	0.5	0.5	0.5
L-Lysine	-	1.2	1.4	1.6	1.8
L-Methionine	1	1	1	1	1
Phytase Enzym	0.03	0.03	0.03	0.03	0.03
Premiks	0.5	0.5	0.5	0.5	0.5
CMC	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100

The first step in creating a diet is combining raw ingredients that have been weighed under the feed formulation's dosage. To increase their homogeneity, all raw ingredients are combined using a feed mixing machine. Under the feed formulation, raw materials with lower proportions are mixed first, and then raw materials with larger proportions are mixed progressively after that. After all the raw materials are combined evenly and left to sit for approximately fifteen minutes, feed additives, including L-lysine (based on the treatment dosage), L-methionine, phytase enzymes, vitamins, and minerals are added last. After the material is uniformly spread, a pelleting machine is used to print it. The *R. maninjau* is used as the test fish, and the size of the food is tailored to fit its mouth opening (Islama *et al*., 2023). The spraying approach was used to add the probiotics, and they were subsequently dried in an oven.

Maintenance of Test Fish

R. maninjau, which is 2-3 cm long, was employed in this study as test subjects to examine the impact of adding probiotics and lysine to meals prepared using locally sourced ingredients. In Nagan Raya, West Aceh, this bilih fish was purchased from nearby farmers. Test fish were housed for 40 days at a stocking density of one fish per liter. Feeding occurs three times a day, at 08.00 WIB, 12.00 WIB, and 17.00 WIB, till satiation. Thirty percent of test fish from each treatment container were sampled to measure growth parameters. After using a scoop to remove the fish from the container, the sample is put in a bucket. The sample's weight and length were then measured.

Test Parameters

To see the fish's response to the test feed provided, several parameters were measured as follows:

Survival Rate

Survival rates were calculated based on the formula of Putra *et al*. (2019):

$$SR = \frac{Nt}{No} x100\%$$

Information:
SR = survival (%)
Nt = Number of live fish at the end of the experiment (fish)
No = Number of fish at the start of the experiment (fish)

Absolute Length Growth and Absolute Weight Growth

Absolute length growth and Absolute weight growth can be determined by calculating the difference in length/weight of fish at the beginning and end of the experiment based on the formula of Islam *et al*. (2023):

$$ALG = Lt - Lo$$

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Information:
ALG = Absolute length growth (cm)
Lt = Length of fish at the end of the experiment (cm)
Lo = Length of the fish at the start of the experiment (cm)

$AWG = Wt - Wo$

Information:
AWG = Absolute weight growth (g)
Wt = Fish weight at the end of the study (g)
Wo = Fish weight at the beginning of the study

Specific Growth Rate

The specific growth rate was calculated using the formula of Muchlisin *et al.* (2016):

$$SGR = \frac{(LnWt - LnWo)}{t} x100\%$$

Information:
SGR = Specific growth rate (% day-1)
Wo = Fish biomass weight at the start of the study (g)
Wt = Fish biomass weight at the end of the study (g)
t = Length of maintenance (days)

Feed Efficiency

Feed efficiency can be calculated using the following formula (Tacon, 1993):

$$FE = \frac{(Wt + D)}{F} x100\%$$

Information:
FE = Feed Efficiency (%)
Wt = Fish biomass weight at the end of the study (g)
Wo = Fish biomass weight at the start of the study (g)
D = Weight of dead fish (g)
F = Amount of feed consumed (g)

Data Analysis

Analysis of variance at a 95% confidence interval was used to examine the collected data (Fahlevie *et al.*, 2023). Additional tests, such as the Duncan test, will be administered if a substantial difference is found.

RESULTS AND DISCUSSIONS

According to the analysis of variance (ANOVA) results, *R. maninjau* survival was not significantly impacted by the addition of lysine and probiotics to locally produced feed ($P > 0.05$). *R. maninjau* had the highest survival rate in treatments P1, P3, and P4 (86.67%), while control treatments P0 and P2 had the lowest (83.33%). This is believed to be the case since environmental factors related to upbringing and handling during the research also affect the survival rate of *R. maninjau* during rearing, in addition to the test feed that is supplied.

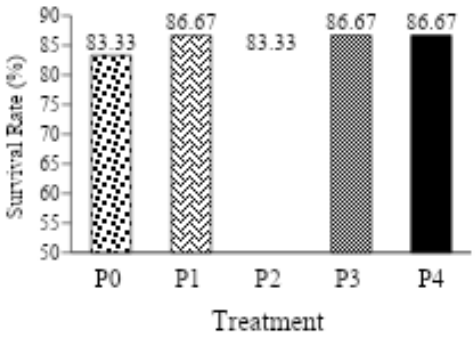

Figure 1. Survival rates of *Rasbora maninjau* after 40 days of rearing.

The physiological condition of fish, their environment, the level of stress they experience, and the infections they encounter are all critical to their survival (Apriliani *et al.*, 2018; Fajriyani *et al.*, 2017). To survive, fish need to have exceptional physiological flexibility, according to Wangni *et al.* (2019). The *R. maninjau* survival rate in this study was >80%, which makes it a respectably good rate for all therapies. Rachmawati and Nurhayati (2022) state that aquaculture operations are considered effective if they provide a survival rate higher than 80%.

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Another study about the addition of lysine in feed also did not significantly affect the survival rate of catfish (Aristasari *et al*., 2020) and baung fish (Kusuma *et al*., 2022).

The findings demonstrated that *R. maninjau* growth performance metrics, including absolute weight growth, absolute length growth, specific growth rate, and feed efficiency, were significantly impacted by the addition of lysine and probiotics to locally produced feed ($P < 0.05$). According to Duncan's test results, adding lysine and probiotics to locally manufactured feed resulted in notable variations across treatments in *R. maninjau* weight gain, length increase, specific growth rate, and feed efficiency. *R. maninjau's* growth performance was lowest in the control treatment (P0), and highest in the P3 treatment (1.6% lysine supplementation and 15 ml/kg probiotics in locally produced feed). Table 2 displays *R. maninjau*'s growth performance.

Table 2. Growth performance of *R. maninjau* for all levels of lysine and probiotic supplementation in local diets.

Treatment	Parameter			
	AWG (g)	ALG (cm)	SGR (%/day)	FE (%)
P0	$0.10^{a} \pm 0.04$	$0.82^{a} \pm 0.10$	$0.75^{a} \pm 0.25$	$18.35^{a} \pm 3.43$
P1	$0.15^{b} \pm 0.02$	$1.09^{b} \pm 0.15$	$1.24^{b} \pm 0.20$	$28.23^{b} \pm 1.14$
P2	$0.17^{b} \pm 0.03$	$1.11^{b} \pm 0.14$	$1.29^{b} \pm 0.15$	$29.72^{b} \pm 2.35$
P3	$0.29^{c} \pm 0.01$	$1.57^{c} \pm 0.12$	$1.98^{c} \pm 0.16$	$38.14^{c} \pm 1.19$
P4	$0.22^{c} \pm 0.02$	$1.32^{c} \pm 0.10$	$1.66^{bc} \pm 0.18$	$32.68^{bc} \pm 2.13$

Note: Values with different superscript letters in the same column indicate significantly different results ($P<0.05$). P0 = diet from local raw materials as a control, P1= diet local with lysine supplementation of 1.2% and probiotic 15 ml/kg, P2= diet local with lysine supplementation of 1.4% and probiotic 15 ml/kg, P3= diet local with lysine supplementation of 1.6% and probiotic 15 ml/kg, P4= diet local with lysine supplementation of 1.8% and probiotic 15 ml/kg. AWG = absolute weight growth; ALG = absolute length growth; SGR = specific growth rate; FE = feed efficiency.

Laboratory proximate feed testing revealed that treatments P1, P2, P3, and P4 (locally produced feed supplemented with lysine and probiotics) had higher protein content than those without these supplements (P0). Table 3 displays the feed-proximate test results for each treatment.

Table 3. Proximate analysis of experimental diet.

Treatment	Water Content (%)	Crude Ash (%)	Fat (%)	Protein (%)	Crude Fiber (%)	Carbohydrate (%)
P0	9.16	5.82	5.69	30.38	2.57	46.38
P1	8.05	5.68	5.75	32.72	2.54	45.26
P2	8.03	5.44	5.83	32.95	2.56	45.19
P3	7.95	5.33	5.91	33.53	2.55	44.73
P4	7.97	5.25	5.86	33.49	2.58	44.85

When lysine and probiotics are introduced to the locally produced feed, *R. maninjau* develops more successfully. The fact that *R. maninjau* in treatments P1, P2, P3, and P4 had greater growth values than the control (P0) indicates this. This is likely because the locally produced meal included a lysine supplement, which can promote the development of energy, muscle, and bone. Probiotics also can enhance feed digestion. According to Yusuf *et al*. (2016), lysine serves as a precursor during the carnitine synthesis pathway. Carnitine helps transport long-chain fatty acids across the mitochondrial membrane for the fatty acid β-oxidation pathway. Because carnitine

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production boosts the β-oxidation process, which produces the energy metabolism required for fish growth, adding lysine to the diet can help fish grow. Lysine increases muscle growth in fish by producing hyperplasia and hypertrophy, which rapidly increase the size and length of muscle fibers (Michelato *et al*., 2016). Furthermore, lysine facilitates fish's better absorption of calcium, which is essential for bone formation and growth (Ovie and Eze, 2013).

According to this study, adding lysine, an amino acid, to feed made from local raw resources should increase its nutritional value. Proximate assays, which demonstrate that the test feed's protein content is larger than the control feed's due to the addition of lysine, support this. According to Nunes *et al*. (2014), lysine can be added to feed that is poor in protein to increase its nutritional quality.

R. maninjau growth is believed to be stimulated by the higher feed protein value in the test meal. According to Xie *et al*. (2012), lysine found in feed is utilized in metabolic processes more readily and rapidly than other amino acids. Yang *et al*. (2011) found that adding lysine and methionine to the diet of grass carp (*Ctenopharyngodon idella*) could increase body weight. The findings of this study support that finding. According to other studies, tilapia (Ovie and Eze, 2013), catfish (Aristasari *et al*., 2020), and catfish (Rachmawati and Nurhayati, 2022) can raise their ultimate body biomass when fed lysine supplementation.

The effects of adding probiotics to fish feed on fish endurance and development have also been studied. By either producing antimicrobial compounds or fostering nutritional competition, probiotic therapy aims to reduce the amount of harmful bacteria and enhance the absorption process in the fish gut (Daten and Ardyati, 2018). Adding 10–30 ml/kg of probiotics has been shown in numerous previous studies to increase the specific growth rate of depik fish (Fratiwi *et al*., 2018) and asang fish seeds (Bulanin *et al*., 2021). Additionally, goldfish seed growth can be enhanced by supplementing the feed with 15 ml/kg of probiotics.

Prior research has also examined the potential for supplementing fish meals with probiotics to increase fish growth and endurance. By producing antimicrobial compounds or nutritional competition, probiotics aim to reduce the amount of pathogenic bacteria in the fish gut and enhance the absorption process (Daten and Ardyati, 2018). According to certain studies, adding 10–30 ml/kg of probiotics might increase the specific development rate of depik fish (Fratiwi *et al*., 2018) and asang fish seeds (Bulanin *et al*., 2021). Furthermore, it has been demonstrated that adding 15 ml/kg of probiotics will improve goldfish seed growth. According to research by Rachmawati *et al*. (2021), the lysine requirement for *P. javanicus* seeds optimizes feed efficiency and growth ranges from 1.58% to -1.70%. This suggests that the P3 treatment produced the largest increase of *R. maninjau* and that this is the ideal dose for growth. Similar to treatments P1 and P2, which received lower doses of lysine, the results of treatment P4, which received a larger dose of lysine, indicated a decrease in bilih fish growth. However, due to toxicity from either an excess of lysine in the feed or a lysine shortage in the feed, this argument cannot be made (Farhat and Khan, 2013). *R. maninjau* fish may grow less than P3 because of either an excess or a shortage of lysine in their diet.

Further evidence that lysine and probiotics given to locally produced feed can promote *R. maninjau* growth comes from the feed efficiency of the *R. maninjau* in this study. *R. maninjau* growth and feed efficiency are directly correlated: the higher the feed efficiency value, the greater the fish growth value. It is thought that the addition of probiotics and lysine to the meal can increase the ileum's (intestine's) digestibility, allowing the fish to grow quickly, become well-fed, and absorb nutrients more quickly. This is why treatments P1, P2, P3, and P4 have higher feed efficiency values than treatment P0

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(Aristasari *et al*., 2020). Higher feed efficiency values are indicative of higher-quality feed, according to Trisnasari *et al*. (2020). It is believed that lysine and probiotic supplements improve *R. maninjau*'s ability to use protein in a diet made from local raw materials. Prior studies have demonstrated that feeding lysine supplementation to sea bass (Salama *et al*., 2013) and tilapia (Ovie and Eze, 2013) results in an increase in the protein efficiency ratio. If the protein efficiency ratio value is higher, fish can effectively use the protein in their feed (Li *et al*., 2013). A greater protein efficiency ratio value suggests that the fish can use feed effectively for growth, according to Pinandoyo *et al*. (2021).

CONCLUSION

The addition of lysine and probiotics to a local artificial feed had a substantial ($P < 0.05$) effect on *Rasbora maninjau's* growth performance but had no significant effect on survival ($P > 0.05$). The optimal dosages for *Rasbora maninjau* growth performance in the local artificial feed are 15 ml/kg of probiotics and 1.6% lysine supplementation (P3).

CONFLICT OF INTEREST

There is no conflict of interest between authors in writing and publishing the manuscript. This research was carried out honestly.

AUTHOR CONTRIBUTION

Each author had the following contributions: Irhami participated in collecting data, Khairul Samuki participated in analyzing data, Dini Islama participated in the conception, experimental design, and drafting of the manuscript, and Sufal Diansyah assisted in the manuscript preparation and revision process.

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