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The Effect of Bromelin in Artificial Feed on Feed Utilization Efficiency, Growth, and Survival of Gourami Seeds *(Osphronemus goramy)*

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Abstract
The relatively slow body growth of gourami (*Osphoronemus goramy*) compared to other fish can be overcome by adding bromelain to the feed. The research aims to determine the effect of bromelain on feed utilization efficiency, protein efficiency ratio and seed growth rate. The research was conducted at the Muntilan Loka for 42 days using gourami seeds measuring 7.92±0.18 cm with a weight of 7.36±0.34g. The research used an experimental method with a completely randomized design consisting of 4 treatments with 3 replications, namely treatments A, B, C, D with doses of 0; 0.75; 1.50; and 2.50g/kg feed. The results showed that bromelain had a significant effect (p<0.05) on the variables EPP, PER, RGR and had no significant effect (p>0.05) on the TKP and SR variants. The optimum dose of bromelain for gourami seeds is 1.49-2.37g/kg feed and produces a maximum EPP value of 76.47%, PER 1.63% and RGR 4.81%/day.

Keywords: Feed, growth, gourami, bromelain

1. Introduction
Gourami (*Osphronemus goramy*) is a type of freshwater fish commodity that experiences increasing demand every year (DKP 2022). The advantage of gourami is that it contains 18.93% protein and 2.43% fat (Firmansyah *et al*., 2021) [5]. The relatively slow growth of gourami seeds is one of the obstacles in their cultivation activities. Factors that determine fish growth are the nutritional content contained in the feed and the fish's ability to digest the nutrients in the feed provided. Gurami is a fish whose digestibility of feed is not optimal so that the nutrients in the feed provided cannot be utilized optimally and growth is relatively slow compared to other fish (Mareta *et al*., 2018) [12].
The bromelain enzyme is one of the enzymes included in the sulfhydryl protease enzyme which has the function of breaking down proteins by hydrolysis of peptide bonds in amino acids (Andini and Widaryati, 2020) [1]. Bromelain can be found from pineapple plants (*Ananas comosus*) in the fruit, leaves, skin, stalks and stems. Bromelin can break down the protein content in feed into simpler ones, which makes it easier for gourami to digest and absorb protein into their bodies. The application of bromelain in feed can increase feed utilization and growth of several types of fish such as nilem (Nasution *et al*., 2017) [15], catfish (Novita *et al*., 2017) [18], tilapia (Yuangsoi *et al*., 2018) [25], tiger prawns (Rachmawati *et al*., 2018) [21], white snapper catfish (Kartina *et al*., 2023) [10], and eels (Maharani *et al*., 2023) [11].
Based on research that has been carried out on several fish, it is necessary to carry out research regarding the effect of bromelain to increase feed utilization, growth and survival in gourami seeds (*O. goramy*).

2. Materials and Methods
This research was conducted for 42 days in October-November 2023 at Loka Muntilan, Magelang Regency, Central Java. The tools used were 12 hapa measuring (0.6x0.6x0.6) m^3, bamboo, digital scales, millimeter block, plastic bottles, thin wall, thermometer, DO meter, pH meter, stationery, camera and labels. The test fish used namely gourami seeds measuring

7.92±0.18 cm with an average weight of 7.36±0.34 g, totaling 120 individuals with a stocking density of 10 individuals/hapa.

Test fish were obtained from gourami seed farmers in Paremono Village, Mungkid, Magelang Regency. The test feed used was the commercial feed Ms Prima Feed PF1000 in pellet form with a size of 0.5-0.7mm produced by PT. Matahari Sakti with a protein content of 39%. The feed was mixed with bromelain produced by PT. Great Giant Pineapple with an enzyme activity of 50,000 U/g and can be used up to a temperature of 65 °C. Adding bromelain to the feed using the coating method.

2.1 Experimental design

The research method applied in this series of research is the experimental method. The experimental design used was a completely randomized design (RAL) consisting of 4 treatments with 3 repetitions. The treatment applied refers to research by Subandiyono *et al*., (2018) [23] who added brome lain for the growth of tawes seeds at the following concentration:

A: bromelain content in artificial feed at a dose of 0 g/kg;
B: bromelain content in artificial feed at a dose of 0.75 g/kg;
C: bromelain content in artificial feed at a dose of 1.50 g/kg;
D: bromelain content in artificial feed at a dose of 2.25 g/kg.

The basis for determining the dose for adding bromelain in this research is a modification of the research results of Subandiyono *et al*., (2018) [23], that the best dose for adding bromelain for the growth of Tawes seeds is 1.5%. This study used 5 treatments with bromelain doses of 0%, 0.75%, 1.50%, 2.25, and 3%. The results of adding 1.50% bromelain showed an EPP value of 37.63±5.99%, PER 1.14±0.18%, RGR 1.94±0.39%, and relative length growth rate of 0.84±0.13%. Feeding is carried out ad satiation with a frequency of feeding twice a day, namely at 08.00 and 16.00 WIB. According to Andini and Widaryati (2020) [1], the addition of bromelain at a dose of 10g/kg feed showed the best results for tilapia seeds with absolute weight growth values of 1.26g, absolute length growth of 2.13 cm, FCR 4.02, EPP 23.26%, and SR 95.5%.

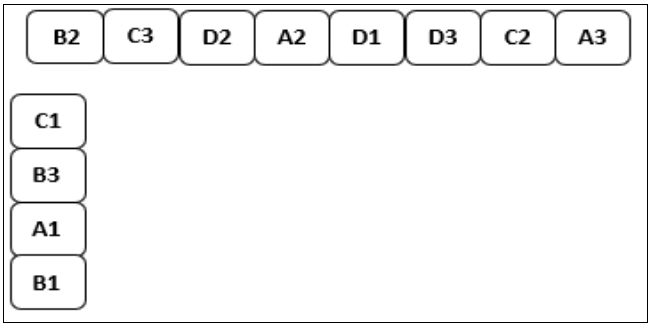

Fig 1: Design of placement of test containers for gurami (*Osphronemus goramy*) maintenance during the study.

2.2 Variables

Different variabels are Total Feed Consumption (TFC), Efficiency Feed Utilization (EFU), Protein Efficiency Ratio (PER), Relative Growth Rate (RGR) and Survival Rate (SR) were analyzed to investigate the effects of bromelain on the growth of the experimental fishes.

2.2.1 Total Feed Consumption (g)

$$TFC = F1 + F2 + \ldots + Fn$$

2.2.2 Efficiency Feed Utilization (%)

$$EFU = \frac{Final\ weight - Initial\ weight}{The\ amount\ of\ feed\ consumed}$$

2.2.3 Protein Efficiency Ratio (%)

$$PER = \frac{Final\ weight - Initial\ weight}{The\ amount\ of\ feed\ consumed \times Protein\ content\ of\ feed}$$

2.2.4 Relative Growth Rate (%/day)

$$RGR = \frac{Final\ weight - Initial\ weight}{Initial\ weight \times Time\ experiment}$$

2.2.5 Survival Rate (%)

$$SR = \frac{Final\ count}{initial\ count} \times 100\%$$

3. Results

The results of observations of total feed consumption (TKP), feed utilization efficiency (EPP), protein efficiency ratio, relative growth rate (RGR), and survival (SR) of gourami seeds during the study are presented in Table 1.

The water quality parameters measured during the research were temperature, degree of acidity (pH), and dissolved oxygen (DO). Water quality measurements such as temperature, pH and DO are carried out every day, namely morning and evening. Meanwhile, ammonia measurements were carried out at week 1, week 3 and week 6 of the study. Ammonia measurements were carried out at the Aquaculture Laboratory at Diponegoro University, Semarang. The results of water quality measurements can be seen in Table 2. The results of water quality measurements show that the water quality parameter values during the research were classified as optimal for use as a medium for rearing gourami seeds. This is based on literature about optimal water quality conditions for gourami seeds.

Table 1: Growth performances of the gurami (*Osphronemus goramy*) during the study period

Treatment	Observational Variables				
	TFC (g)	EFU (%)	PER (%)	RGR (%/day)	SR (%)
A	190.80±6.49^{a}	53.75±1.29^{a}	1,37±0.03^{a}	3.06±0.03^{a}	90.00±10.00^{a}
B	200.72±4.94^{a}	61.51±0.50^{b}	1.44±0.01^{b}	3.71±0,04^{b}	96.67±5.77^{a}
C	204.64±4.50^{a}	79.80±2.30^{d}	1.74±0.05^{d}	4.94±0.16^{d}	96.67±5.77^{a}
D	207.38±7.06^{a}	74.56±0.79^{c}	1.51±0.01^{c}	4.73±0.03^{c}	93.33±5.77^{a}

Table 2: Results of measurement of average water quality in indigo maintenance media gurami (*Osphronemus gorm*) during the study

Water Quality Parameters	Range of Water Quality Parameter Values		Eligibility
	Morning	Evening	
DO (mg/l)	6.1-7.5	6.2-7.5	6.3-7[a]
Temperature ^{0}C	25.1 – 26.9	27 – 29.2	25-30[b]
pH	7.1 – 7.8	7.6 – 7.9	6,5-8[b]
NH_3 (mg/l)	<0.012-0.036		<1[b]

Information: [a] Usman *et al.* (2022) [24]; [b] Jumaidi (2017) [9]

For each variable that was significantly different, an orthogonal polynomial test was carried out to determine the optimum dose of bromelain in feed. The optimum dose of bromelain to produce maximum EFU values can be seen in Figure 2, PER in Figure 3, and RGR in Figure 4.

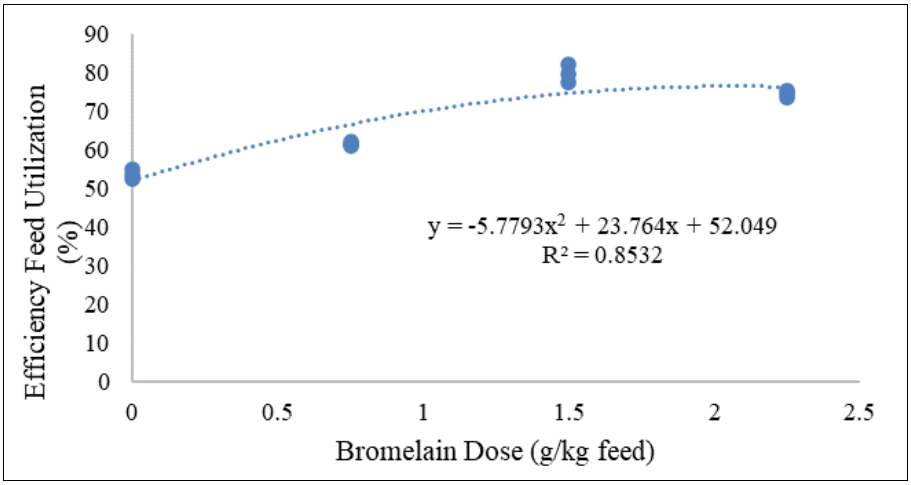

Fig 2: Polynomial orthogonal efficiency feed utilization of Gurami (*Osphronemus goramy*) during the study

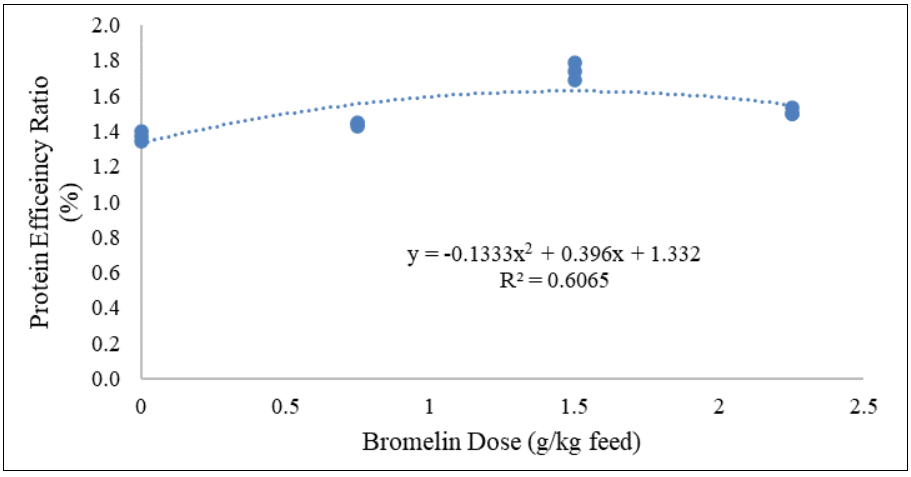

Fig 3: Polynomial orthogonal protein efficiency ratio of Gurami (*Osphronemus goramy*) during the study

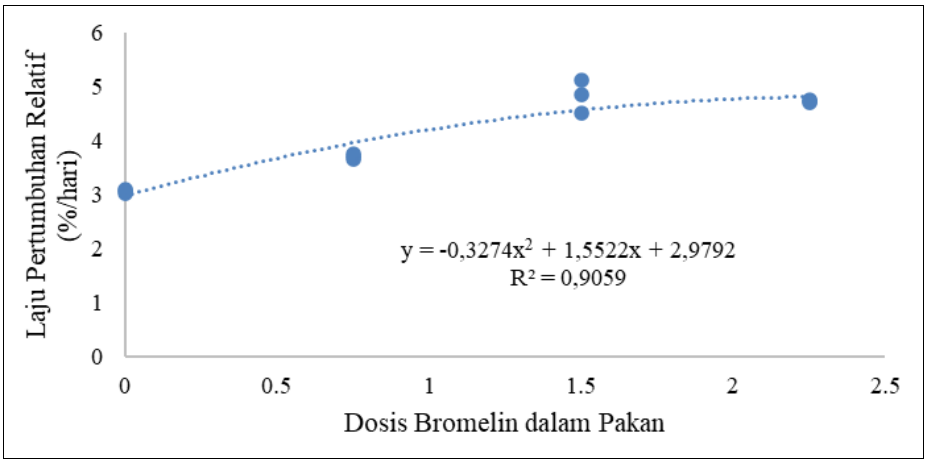

Fig 4: Polynomial orthogonal relative growth rate of Gurami (*Osphronemus goramy*) during the study

4. Discussion

4.1 Total Feed Consumption

The results of the analysis of variance in total feed consumption showed that the addition of bromelain in artificial feed at different doses had the same effect (p<0.05) on the total feed consumption of gourami seeds (*O. goramy*). The best total feed consumption value in treatment C (1.50 g/kg) was 204.64±4.50 g. The total value of feed consumption for gourami seeds can be caused by various factors, both internal and external factors. According to Putra *et al*. (2020) [20], stated that total fish feed consumption is influenced by several factors including nutritional content in feed, feed palatability, fish size, fish stage, fish stomach capacity, water quality, and fish maintenance.

The addition of bromelain in artificial feed at different doses showed similar feed consumption values between treatments. This shows that the addition of bromelain does not affect the palatability of the feed so it does not change the amount of feed consumed by gourami seeds. Bromelain is a type of protein, so it does not increase feed consumption and feed taste but does have an influence on feed utilization and protein absorption in the fish body (Delima *et al*., 2017) [4]. The same TFC value indicates that the total energy and metabolism of the gourami body is relatively the same in each treatment. This is confirmed by Hanief *et al.* (2014) [7], stated that the level of feed consumption provided can be influenced by the energy level in the feed, so that protein energy that exceeds the body's optimal needs cannot be utilized for growth but is excreted in the form of feces.

4.2 Efficiency Feed Utilization

Based on the research results, it shows that treatment C (1.50 g/kg) produces the highest feed utilization efficiency value, namely 79.80±2.30%. This value is very significantly different from the EFU value in treatment a (0 g/kg) which produces an EFU value of 53.75±1.29%. This shows that there is a difference between giving bromelain in commercial feed and not giving bromelain in feed. Purwadi (2019) [19], bromelain as an exogenous enzyme can help hydrolyze protein into simpler amino acids so that they are easily digested by fish. Bromelain has the ability to increase the absorption of protein contained in feed into the body, thereby increasing the utilization value of feed (Novita *et al*., 2017) [18]. According to Nisrinah *et al.* (2013) [17], bromelain can break down proteins into peptide bonds and amino acids which are more easily digested by the body, thereby increasing the efficiency of feed utilization. Peptide bonds and amino acids from decomposed proteins are easier for fish to digest compared to complex proteins.

An increase in the EFU value occurred in treatment at (0 g/kg), treatment B (0.75 g/kg), and treatment C (1.50 g/kg), however, there was a decrease in the EFU value in treatment D (2.25 g/kg). The decrease in EFU value is thought to be caused by excessive increase in bromelain which can reduce protein quality so that the profile of essential amino acids in feed will change. The addition of bromelain to produce the best EFU value in gourami seeds during the research was 79.80% with a dose of 1.50 g/kg. Then an orthogonal polynomial test was carried out to obtain the optimum dose of bromelain for EFU, obtained by the equation $Y = -5.7993x^2 + 23.764x + 52.049$ and R2 = 0.85. Based on this equation, the optimal dose of bromelain from this equation is 2.05 g/kg. This optimal value is able to produce an optimal EPP of 76.47%.

Based on the correlation analysis of the use of bromelain in artificial feed on the efficiency of utilization of gourami seed feed, the resulting value of R2 = 0.85 indicates that 85% of the efficiency of feed utilization is influenced by the addition of bromelain in artificial feed, while 15% of the efficiency of feed utilization is influenced by other factors, namely the environment. This value is higher than research with bromelain by Anugraha *et al.* (2014) [3] with an EFU value of 33.9% for goldfish, Andini and Widaryati (2020) [1] with an EFU value of 23.26% for tilapia with an EFU value of 61.1% for vannamei shrimp, Delima *et al*. (2017) [4] with an EFU value of 37.63% for tawes, Masniar *et al.* (2016) [13] with an EPP value of 40.2% for betok, Nisrinah *et al.* (2013) [17] with an EFU value of 60.88% for catfish, and Novita *et al.* (2017) [18] with an EFU value of 59.11% for pangas catfish.

4.3 Protein Efficiency Ratio

The results of analysis of various protein efficiency ratios showed that the addition of bromelain in artificial feed at different doses had a significant effect (p>0.05) on the protein efficiency ratio of gourami seeds (*O. goramy*). The highest and lowest PER values were found in treatments C (1.50 g/kg) and A (0 g/kg) which produced an average protein efficiency ratio of 1.74±0.05% and 1.37±0.03%. This result is thought to be influenced by the level of protein consumption in the feed and fish growth. Bromelain includes protease enzymes which will facilitate the digestion of protein in the body so that the amount of protein in the body will be greater. Adequate protein in the body will produce balanced energy for growth. This is confirmed by Davis *et al*. (1998), stated that bromelain contains proteases which can break down proteins into simple compounds so that proteins are easily digested and fish body growth is more optimal.

The decrease in PER value from the best value in the study occurred in treatment D (2.25 g/kg). The decrease in the PER value is thought to be caused by a decrease in the amount of protein in the feed due to a decrease in the efficiency value of protein utilization that occurs in the fish intestine.

The addition of bromelain to produce the best PER value in gourami seeds during the research was 1.74±0.05% with a dose of 1.50 g/kg. Then an orthogonal polynomial test was carried out to obtain the optimum dose of bromelain for PER, obtained by the equation $Y = -0.1333x^2 + 0.396x + 1.332$ and $R^2 = 0.61$. Based on this equation, the optimal dose of bromelain is 1.49 g/kg. This optimal value is able to produce an optimal PER of 1.63%.

Based on the correlation analysis of the addition of bromelain in artificial feed to the protein efficiency ratio, the resulting value of R2 = 0.61 indicates that 61% of the feed conversion ratio is influenced by the addition of bromelain in artificial feed, while 39% of the protein efficiency ratio is influenced by other factors, namely the environment. This value is higher than research with bromelain by Anugraha *et al*. (2014) [3] with a PER value of 0.81±0.12% for goldfish, Delima *et al.* (2017) [4] with a PER value of 1.14% for tawes but lower than research with bromelain by Nisrinah *et al.* (2013) [17] with a PER value of 1.87±0.05% for catfish and Novita *et al.* (2017) [18] with a PER value of 1.84±0.14% for catfish.

4.4 Relative Growth Rate

Based on the research results, it shows that the addition of bromelain in commercial feed can increase the relative growth rate. The highest and lowest RGR values were found in treatments C (1.50 g/kg) and A (0 g/kg) which produced an

average RGR of 4.94±016%/day and 3.06±0.03%/day. Bromelain acts as a biocatalyst which can influence fish growth if consumed in appropriate amounts, however if it exceeds the optimum point it can cause negative effects in the form of reduced growth (Masniar *et al*., 2016) [13]. The decrease in gourami growth in the study occurred in treatment D (2.25 g/kg). The decrease in growth is thought to be caused by changes in the amino acid profile so that the essential amino acids in the feed are not used for growth but are used as an energy source.

The RGR value with the best dose of bromelain for gourami seeds during the research was 4.94±016%/day with a dose of 1.50 g/kg. Then an orthogonal polynomial test was carried out to obtain the optimum dose of bromelain for RGR, obtained by the equation $Y = -0.32742x^2 + 1.5522x + 2.9792$ and R2 = 0.90. Based on this equation, the optimal dose of bromelain from this equation is 2.37 g/kg. This value is able to produce an optimal RGR of 4.81%/day. Based on the correlation analysis of the addition of bromelain in artificial feed to the daily growth rate, the resulting value of R2 = 0.90 indicates that 90% of the feed conversion ratio is influenced by the addition of bromelain in artificial feed, while 10% of the daily growth rate is influenced by other factors, namely the environment. This value is higher than research with bromelain conducted by Anugraha *et al.* (2014) [3] with an RGR value of 0.67±0.11%/day for goldfish, Delima *et al.* (2017) [4] with an RGR value of 0.84%/day for tawes, Masniar *et al.* (2017) [13] with an RGR value of 0.97±0.02%/day for betok, Nisrinah *et al.* (2013) [17] with an RGR value of 2.85±0.02%/day for catfish, and Novita *et al.* (2017) [18] with an RGR value of 1.47±0.15%/day for catfish.

4.5 Survival Rate

The results of the analysis of survival variance showed that the addition of bromelain in artificial feed at different doses had the same effect ($p<0.05$) on the survival of gourami seeds (*O. goramy*). The survival value in treatment a (0 g/kg) was 90.00±10.00%, treatment B (0.75 g/kg) was 96.67±5.77%, treatment C (1.50 g/kg) was 96.67±5.77%, and treatment D (2.25 g/kg) was 93.33±5.77%. The addition of bromelain in artificial feed at different doses caused the survival rate of gourami seeds between treatments to be the same. This shows that the survival of gourami seeds reared during research is not directly influenced by feed. This was confirmed by Sawitri *et al*., (2018) [22] that high survival rates which were not significantly different in each treatment could be caused by appropriate water quality and appropriate stocking density.

Based on the research results, it shows that the highest survival values were found in treatment B (0.75 g/kg) and treatment C (1.50 g/kg) which resulted in an average survival of 96.67±5.77%. The lowest SR value was in treatment a (0 g/kg) which produced an average survival of 90.00±10.00%. According to Mulyani *et al*., (2014), fish survival >50% is included in the good category for cultivation activities. Survival rates can be influenced by biotic and abiotic factors. Biotic factors that influence survival value include fish stage and the fish's ability to adapt to new environments, while abiotic factors include food availability, water quality, fungi, diseases and pests (Firmansyah *et al*., 2021) [5]. Another factor that can cause death in fish is the body's ability to resist stress (Hadijah *et al*., 2015) [6]. Stress in fish can occur when sampling to measure body length and weight (Novita *et al*., 2017) [18].

4.6 Water Quality

The water quality measured during the 42 days of research was temperature, dissolved oxygen (DO), acidity (pH), and ammonia. The results of measuring water quality parameters during the research showed that the water quality of the gourami seed rearing pond was in a proper and suitable condition. The results of measuring the water temperature in the gourami seed rearing pond ranged from 25-29 °C. The water temperature value during the rearing period is still considered adequate to support the survival and growth of gourami seeds. This is confirmed by Jumaidi *et al.* (2017) [9] that gourami seeds can grow well in waters with water temperatures ranging from 25-30 °C. The water temperature in fish rearing ponds can affect the level of food digestibility in the body, the efficiency of feed utilization, and fish growth. A higher water temperature will increase the metabolic rate of the fish's body so that the fish's appetite will increase, while a low water temperature will reduce the metabolic rate of the fish's body so that its appetite will decrease (Usman *et al*., 2022) [24]. According to Jumaidi *et al*. (2017) [9] high water temperatures can affect respiration, thereby reducing the oxygen concentration in the water which can cause stress and even death in fish.

The results of dissolved oxygen (DO) measurements during the maintenance period for gourami seeds ranged from 6.1-7.5 mg/L. The DO value obtained during the research is considered adequate to support the life and growth of gourami seeds. This is confirmed by Usman *et al.* (2022) [24] that the optimal dissolved oxygen level for gourami seeds ranges from 6.3-7. The dissolved oxygen content in water functions to help the process of oxidation and burning of food consumed by fish as a source of energy for the survival and growth of gourami seeds. Gurami have the ability to live in waters with less dissolved oxygen because they have an artificial breathing apparatus in the form of a labyrinth (Jumaidi *et al*., 2017) [9]. Low levels of dissolved oxygen in water can cause fish stress, anorexia, tissue hypoxia, and even mass death (Usman *et al*., 2022) [24]. Decreasing dissolved oxygen levels can also reduce feed consumption levels, because oxygen is needed by fish to oxidize the nutrients contained in the feed.

The results of measuring the degree of acidity (pH) during the maintenance period for gourami seeds showed values ranging between 7.1-7.9. The acidity value obtained during the research is considered adequate to support the survival and growth of gourami seeds. This is confirmed by Jumaidi *et al.* (2017) [9] that gourami seeds can grow optimally in waters with pH levels ranging from 6.5-8.5. An acidity value that is too low can inhibit the growth of fish seeds and can cause fish to be easily attacked by bacteria and disease (Usman *et al*., 2022) [24]. According to Sabrina *et al*., (2018) that a pH value that is too high (>9) can also inhibit fish growth.

The ammonia (NH3) value in the gourami seed rearing medium for 42 days produced values ranging from 0.012-0.036 mg/L. The ammonia value obtained during the research is considered adequate to support the survival and growth of gourami seeds. This is confirmed by Jumaidi *et al.* (2017) [9] that gourami seeds can grow optimally in waters with ammonia levels <1 mg/L. Ammonia levels that are too high can cause a decrease in appetite. According to Usman *et al*., (2022) [24] that high levels of ammonia in water can cause an increase in blood pH which has an impact on enzyme reactions and membrane stability. The impact of increasing blood pH is damage to the gills which causes a reduction in the capacity of the blood to carry oxygen and damage to red

blood cells.

5. Conclusion

Based on the research results, it can be concluded that the optimal dose of adding bromelain in artificial feed for gourami seeds (*O. goramy*) is 2.05 g/kg of feed which is capable of producing an optimal feed utilization efficiency value of 76.47%, adding bromelain of 1.48 g/kg of feed is capable of producing efficient protein. The optimal ratio is 1.63% and the addition of bromelain of 2.37 g/kg feed can produce optimal growth of 4.81%/day.

6. Suggestion

Based on the research that has been carried out, the following suggestions can be given:

1. It is best to add bromelain to artificial feed at a dose of 1.48-2.37 g/kg feed which can be applied to cultivating gourami seeds (*O. goramy*) to increase growth;
2. It is recommended that further research be carried out using optimal doses of bromelain in different rearing locations or containers for gourami (*O. goramy*) at the rearing stage.

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