Journal of Fish Health
Volume 4, Issue 4, November 2024
https://doi.org/10.29303/jfh.v4i4.5814

Effectiveness of Sanitation Treatment for Natural Bloodworms (*Tubifex* sp.) in Breeding Siamese Catfish (*Pangasius hypophthalmus*)

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Received : 09-29-2024
Accepted : 10-29-2024

Keywords:
Bloodworms, Fermentation, Growth Rate, Hygiene, Siamese Catfish Seeds

ABSTRACT

The demand for Siamese catfish seeds continues to increase every year. Therefore, it is essential to maintain high-quality seeds in adequate quantities, supported by the availability of natural feed such as bloodworms (*Tubifex* sp.). One of the efforts to improve hygiene and reduce pathogenic bacteria is through the sanitation treatment of feed using fermented tofu waste and mustard greens waste. The objective of this research is to analyze the effectiveness of using hygienic bloodworms treated with fermented tofu waste and mustard greens waste on the performance of Siamese catfish seed breeding. The method employed is a Completely Randomized Design (CRD) with four treatments and three replications. The research data were analyzed using ANOVA statistical tests and Tukey's post-hoc test at a 5% significance level. The sanitation treatment of feed with the addition of fermented tofu waste and mustard greens resulted in the highest nutritional content and the highest abundance of beneficial bacteria on the fifth day of maintenance. The absolute length growth of the Siamese catfish seeds was 9.37±0.044 mm, while the absolute weight growth was 0.08±0.0169 grams. The specific length growth rate of the Siamese catfish seeds was 7.10±0.017%, and the specific weight growth rate was 18.35±0.025%. The survival rate of Siamese catfish seeds across all treatments reached 98%. The sanitation treatment of natural bloodworms with fermented tofu waste and mustard greens is recommended to enhance the nutritional content of bloodworms and accelerate the growth of Siamese catfish seeds.

INTRODUCTION

The potential for freshwater aquaculture in Indonesia is highly promising, with one notable example being the cultivation of Siamese catfish (*Pangasius hypophthalmus*). The demand for Siamese catfish fry continues to grow each year, necessitating the production of high-quality fry in sufficient quantities (Fani *et al*., 2018). One of the key factors supporting the survival of high-quality fry is the availability of natural feed. Tubifex worms (*Tubifex sp.*) are a natural feed commonly used in the fry production of Siamese catfish (Nuraini *et al*., 2019). These worms have several advantages for fry growth: their slow movement makes them easy for fry to catch, their small size matches the fry's mouth opening, and they are easy to digest (Syahendra *et al*., 2016). Fry growth is faster when fed Tubifex worms compared to artificial feed, as Tubifex worms contain very high nutrient levels (Agustina *et al*., 2020). The nutritional composition of *Tubifex sp.* worms is 57% protein, 13.30% fat, and 2.04% carbohydrates (Syahputra and Isma, 2020).

A common challenge in fry production is slow growth, often attributed to poor hygiene in Tubifex worms. Therefore, efforts to reduce pathogenic bacteria in Tubifex worms are essential, such as feed sanitation treatments using fermented tofu dregs and mustard green waste. The fermentation of tofu dregs and mustard green waste, which have high protein content, can enhance the nutritional value of Tubifex worms. Balanced nutrition in Tubifex worms plays a crucial role in supporting the growth of Siamese catfish fry. The nutritional content of Tubifex worms can be improved by supplementing them with fermented tofu dregs and vegetable waste. The nutritional composition of tofu dregs includes approximately 23.39% crude protein, 19.44% crude fiber, 9.96% crude fat, 4.58% ash, and 30.48% nitrogen-free extract (Agustina *et al*., 2020). Additionally, vegetable waste, which consists of leftover materials, also contains a relatively high protein content. Mustard green waste can be utilized as fish feed material (Agustina *et al*., 2020), containing 16.24% protein, 4.26% fat, 4.94% ash, and 74.56% carbohydrates (Umidayati *et al*., 2020). The high protein levels serve as a source of nitrogen that microorganisms can utilize, which then become a food source for Tubifex worms (Cahyono *et al*., 2015).

This study aims to analyze the effectiveness of using hygienic Tubifex worms (*Tubifex sp.*) treated with feed sanitation involving fermented tofu dregs and mustard green waste on the performance of Siamese catfish (*Pangasius hypophthalmus*) fry production.

METHODS

Time and Place of Research

This research was conducted from February 25, 2023, to May 20, 2023, at the Telanaipura Ornamental Fish Installation located at Jalan Letjen Suprapto No.26, Telanaipura, Telanaipura District, Jambi City, Jambi Province, Indonesia.

Research Design

The data collection method employed in this research used an applied research approach with a Completely Randomized Design (CRD) consisting of four treatments and three replications. The treatments tested in this study were as follows:

1. **Treatment A**: Untreated *Tubifex* worms used as a control
2. **Treatment B**: *Tubifex* worms soaked in $KMnO_4$ (2 mg/L) and fed with fermented tofu dregs amounting to 30% of the worm biomass
3. **Treatment C**: *Tubifex* worms soaked in $KMnO_4$ (2 mg/L) and fed with fermented mustard green waste amounting to 30% of the worm biomass

4. **Treatment D**: *Tubifex* worms soaked in $KMnO_4$ (2 mg/L) and fed with a mixture of fermented tofu dregs and mustard green waste (15% + 15%) based on worm biomass

A total of 12 research units were used. The placement arrangement for each aquarium unit was randomized using Microsoft Excel software. The position of each rearing tank did not influence the research outcomes, as the study was conducted under controlled conditions. The containers used in the research consisted of rectangular glass aquariums measuring 1 x 0.4 x 0.4 m^3 with a water height of 20 cm and a total water volume of 80 liters. Twelve units were equipped with aeration and placed inside a hatchery. The Siamese catfish (*Pangasius hypophthalmus*) fry used in this study was 7 days old and stocked at a density of 3,500 fry per container, following the SNI 01-6483.4-2000 standard.

RESULTS

Nutritional Content

The nutritional content of *Tubifex* worms was determined by conducting a proximate analysis. The tests were carried out on the first and fifth days of *Tubifex* worm maintenance. The testing method followed the procedures outlined by the Association of Official Analytical Chemists (AOAC, 2005). The results of the proximate analysis for *Tubifex* worms are presented in Table 1.

Table 1. Nutritional Content of *Tubifex* Worms

Treatment	Nutritional Content (%)									
	Ash		Fiber		Fat		Protein		Moisture	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
A	5.05	4.95	5.20	4.98	8.30	8.25	52.77	51.75	16.75	16.65
B	5.30	5.27	5.25	4.65	8.55	7.95	53.57	54.50	18.01	19.20
C	5.15	5.32	5.65	4.57	8.75	8.10	52.55	54.53	17.33	19.13
D	5.90	5.45	5.35	4.55	9.01	8.35	53.65	55.03	17.67	19.77

Note: Testing was conducted at the Laboratory of Batanghari University. Jambi.

Bacterial Abundance

The bacterial colony count was determined by performing a Total Plate Count (TPC) test on *Tubifex* worms. The tests were conducted on the first, third, and fifth days of *Tubifex* worm maintenance. The testing method followed the SNI 2332.3:2015 standard. The results of the bacterial abundance test on *Tubifex* worms are presented in Table 2.

Table 2. Bacterial Abundance of *Tubifex* Worms

Treatment	Bacterial Abundance (CFU/mL)		
	Day 1	Day 3	Day 5
A	1.43×10^6	1.45×10^6	1.48×10^6
B	1.72×10^6	1.89×10^6	2.07×10^6
C	1.44×10^6	1.94×10^6	2.35×10^6
D	1.88×10^6	2.22×10^6	2.67×10^6

Note: Testing was conducted at SKIPM Jambi.

Performance of Siamese Catfish Fry Breeding

Absolute Length Growth

The growth rate in length is one of the key parameters in the breeding process of Siamese catfish (*Pangasius hypophthalmus*). The graph showing the absolute length growth of Siamese catfish fry is presented in Figure 1.

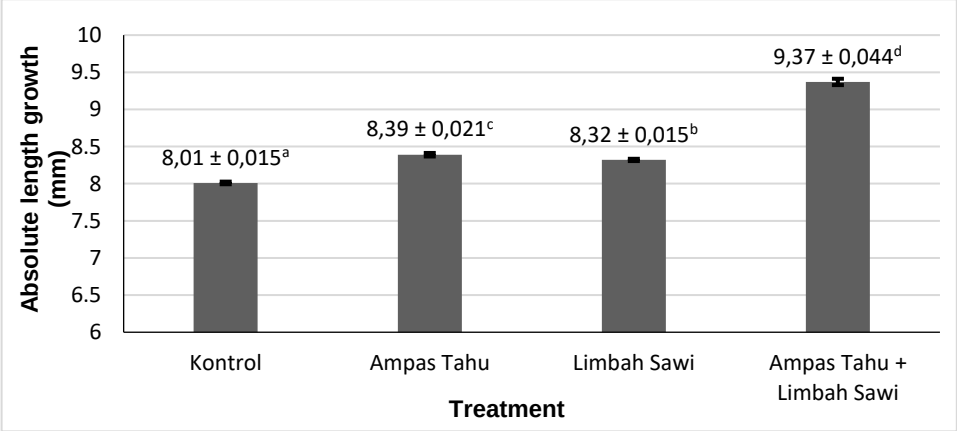

Figure 1. Graph of Absolute Length Growth of Siamese Catfish Fry

Note: Values with different superscripts indicate significant differences ($p < 0.05$). The displayed values represent the mean and standard deviation.

The highest absolute length growth of Siamese catfish fry was observed in Treatment D at 9.37 ± 0.044 mm, followed by Treatment B at 8.39 ± 0.021 mm. Treatment C at 8.32 ± 0.015 mm, and the lowest in the control Treatment A at 8.01 ± 0.015 mm. Based on statistical analysis using SPSS 26.0 with ANOVA, it was found that the feed sanitation treatment for *Tubifex* worms using fermented tofu dregs and mustard green waste had a significant effect on the absolute length growth of Siamese catfish fry ($p < 0.05$, sig. = 0.000). This indicates that H0 is rejected, and H1 is accepted, necessitating a post hoc Tukey test.

Absolute Weight Growth

Weight growth is one of the key aspects of the breeding process. One of the factors influencing weight growth is the feed provided (Nawawi *et al*., 2015). The graph of absolute weight growth of Siamese catfish fry is presented in Figure 2.

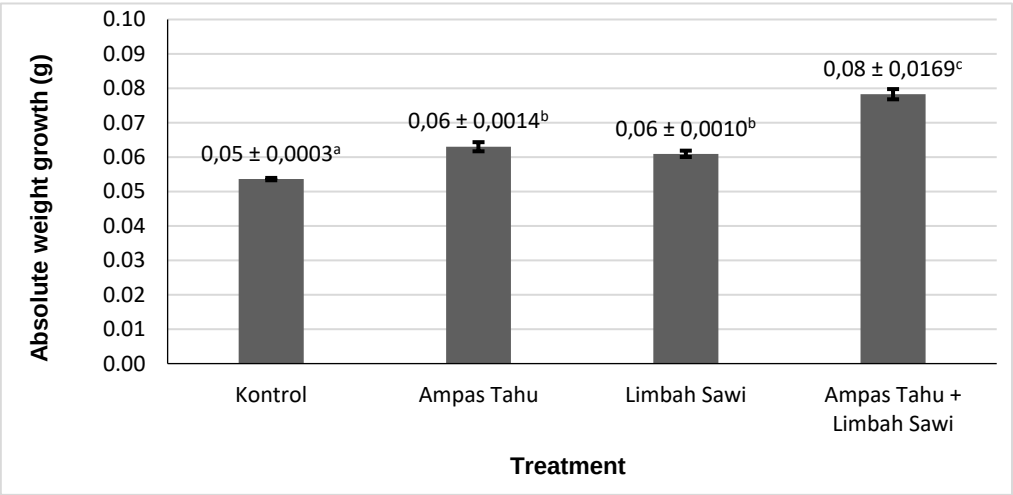

Figure 2. Graph of Absolute Weight Growth of Siamese Catfish Fry

The maintenance of Siamese catfish fry fed *Tubifex* worms supplemented with fermented tofu dregs and mustard green waste resulted in the highest absolute weight growth in Treatment D at 0.08 ± 0.0169 grams. This was followed by Treatment B at 0.06 ± 0.001 grams and Treatment C at 0.06 ± 0.0010 grams, while the lowest absolute weight growth was observed in Treatment A at 0.05 ± 0.0003 grams. Statistical analysis using SPSS 26.0 and ANOVA showed a significant difference ($p < 0.05$, sig. = 0.000) requiring further analysis. Post hoc testing using Tukey's test revealed that Treatment D was significantly different from Treatments A, B, and C. Treatments B and C did not differ significantly from each other but were significantly different from Treatment A.

Specific Length Growth Rate

The specific length growth rate of Siamese catfish fry reflects the percentage increase in fry length over time. The specific length growth rate of Siamese catfish fry from this study is presented in Figure 3.

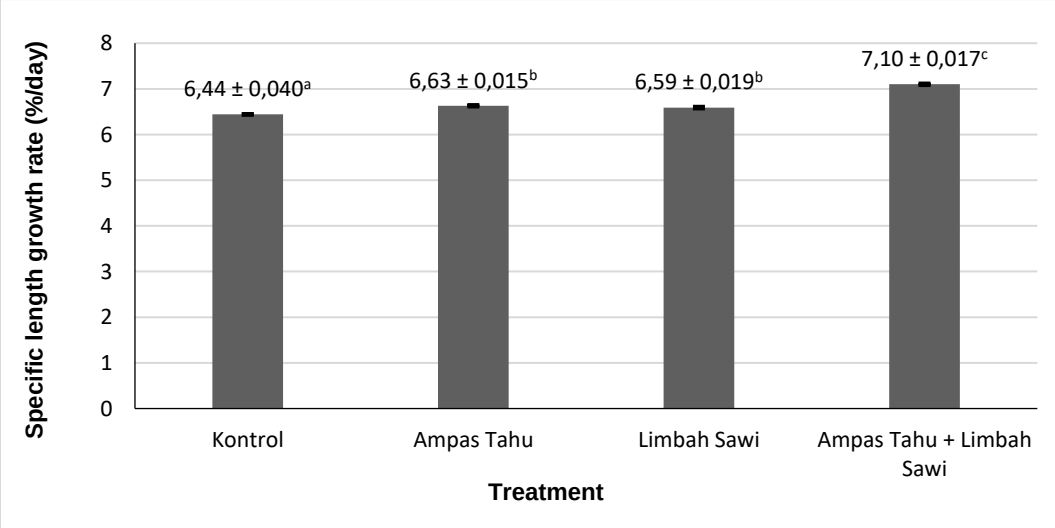

Figure 3. Graph of Specific Length Growth Rate of Siamese Catfish Fry

Note: Values with different superscripts indicate significant differences (p < 0.05). The displayed values represent the mean and standard deviation.

The highest specific length growth rate of Siamese catfish fry was observed in Treatment D with a value of 7.10 ± 0.017%, while the lowest was in Treatment A at 6.44 ± 0.040%. Statistical analysis using SPSS 26.0 and ANOVA showed a significant difference ($p < 0.05$, sig. = 0.000) requiring further analysis. Post hoc testing using Tukey's test revealed that Treatment D was significantly different from Treatments A, B, and C. Treatments B and C did not differ significantly from each other but were significantly different from Treatment A.

Specific Weight Growth Rate

The specific weight growth rate increased during the study period. The specific weight growth rate is expressed as a percentage (%). The graph of the specific weight growth rate of Siamese catfish fry is presented in Figure 4.

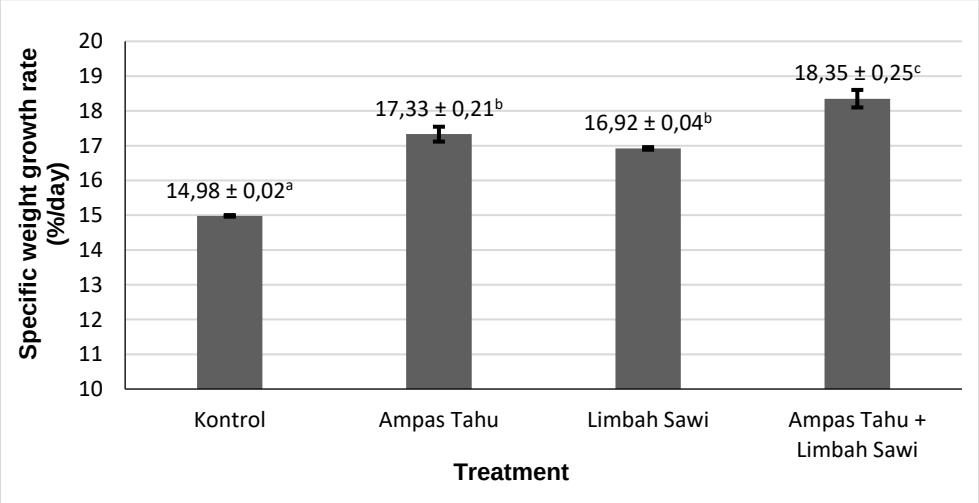

Figure 4. Graph of Specific Weight Growth Rate of Siamese Catfish Fry

Note: Values with different superscripts indicate significant differences ($p < 0.05$). The displayed values represent the mean and standard deviation.

The highest specific weight growth rate was observed in Treatment D, with a value of 18.35 ± 0.025%, followed by Treatment B at 17.33 ± 0.021%, and Treatment C at 16.92 ± 0.04%. The lowest specific weight growth rate was recorded in Treatment A at 14.98 ± 0.02%. Statistical analysis using SPSS 26.0 and ANOVA showed a significant difference ($p < 0.05$, sig. = 0.000) requiring further analysis. Post hoc testing using Tukey's test revealed that Treatment D was significantly different from Treatments A, B, and C. Treatments B and C did not differ significantly from each other but were significantly different from Treatment A.

Survival Rate

The survival rate of Siamese catfish fry calculated from a total of 3, 500 fry per aquarium during the study, showed the highest value in Treatment D at 98.6 ± 0.29%, followed by Treatment B at 98.5 ± 0.30%, and Treatment C at 98.1 ± 0.14%. The lowest survival rate was recorded in the control treatment at 98.0 ± 0.14%. The graph showing the survival rate percentage of Siamese catfish fry is presented in Figure 5.

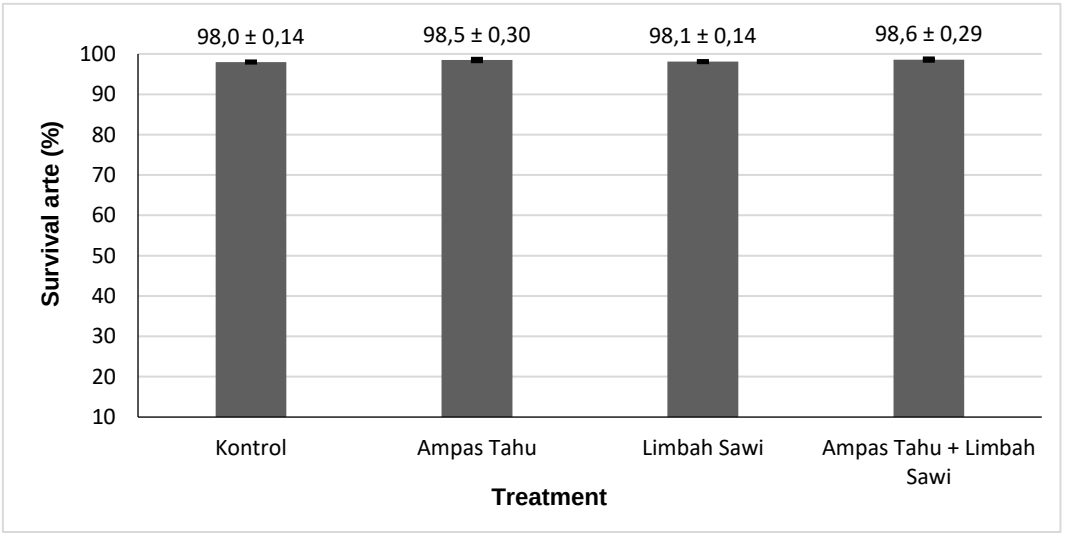

Figure 5. Graph of Survival Rate of Siamese Catfish Fry

Based on statistical analysis using SPSS 26.0 with ANOVA, it was determined that the feed sanitation treatment for *Tubifex* worms using fermented tofu dregs and mustard green waste did not have a significant effect on the survival rate of Siamese catfish fry ($p > 0.05$, sig. = 0.086). This indicates that H0 is accepted, and H1 is rejected, meaning no further post hoc testing was required.

DISCUSSION

Nutritional Content

Each treatment was observed to have an average ash content of 5%, which can be categorized as good because the required ash content for fish fry ranges between 3–7%, as stated by Iskandar and Fitriadi (2017). The ash content decreased in each treatment due to the fermentation process, which increases organic matter as a result of substrate degradation by microbes. This aligns with Setiawati *et al*. (2013), who explained that the reduction in ash content significantly affects organic matter content, with less ash reduction corresponding to an increase in organic matter. The optimal crude fiber level to support the growth of fish fry is 4–8% (Iskandar and Fitriadi, 2017). The crude fiber analysis results for all treatments fell within the optimal range and met the nutritional needs of the fish fry. Treatment A showed the highest crude fiber content because no fermentation was applied to the *Tubifex* worms, resulting in lower feed digestibility and reduced digestion efficiency for the fish fry. The reduction in crude fiber in treatments involving feed sanitation using fermented tofu dregs and mustard green waste was due to enzymes breaking down cellulose into glucose during fermentation. which serves as a source of carbon and energy (Mulia *et al*., 2015).

Table 1 shows that in Treatment A, moisture content decreased due to the absence of feed sanitation using fermented tofu dregs or mustard green waste. In contrast, Treatments B, C, and D exhibited increased moisture content. This aligns with Setiawati *et al*. (2013), who noted that adding fermented tofu dregs and mustard green waste in feed sanitation can increase protein and moisture levels due to the ability of the crude fiber in these materials to retain water. The fat content required for freshwater fish fry ranges from 2–10%. In all treatments, fat content decreased but remained within the optimal range. The addition of feed sanitation using fermented tofu dregs and mustard green waste produced enzymes utilized as nutrients for metabolic activities, leading to a faster reduction in fat content. This is consistent with Mulia *et al*. (2015), who noted that lipase enzymes produced during fermentation can break down fats into fatty acids and glycerol (Santosa *et al*., 2017).

According to Idawati *et al*. (2018), the minimum protein requirement for fish fry is 30%. In Treatment A, protein content decreased by 1.02% over 5 days of maintenance due to the absence of fermented feed, but it remained within the optimal range for Siamese catfish fry. In contrast, Treatments B, C, and D showed increased protein content in *Tubifex* worms due to the fermentation process utilizing tofu dregs and mustard green waste. The fermentation process enriches the nutrient content, which can be utilized by microbes and bacteria to produce proteins (Akhril *et al*., 2019). Agustina *et al*. (2020) stated that tofu dregs have higher protein content (23.29%) compared to mustard green waste (16.24%). Thus, combining fermented tofu dregs and mustard green waste results in higher protein content and provides optimal growth for Siamese catfish fry.

The proximate analysis results in Table 1 indicate that *Tubifex* worms treated with feed sanitation using fermented tofu dregs and mustard green waste have balanced nutritional content. Balanced nutrients in *Tubifex* worms facilitate digestion in fish fry and play a role in

supporting the growth of Siamese catfish fry (Fajri and Hutabarat, 2014). The *Lactobacillus* bacteria present in fermented feed play an active role in enhancing the nutritional quality of *Tubifex* worms, enabling the fish to digest and absorb the feed more effectively (Yuriana *et al*., 2017).

Bacterial Abundance

Soaking *Tubifex* worms in Potassium Permanganate ($KMnO_4$) aims to suppress pathogenic bacteria in wild *Tubifex* worms, allowing beneficial bacteria introduced through fermentation to proliferate within the worms. The feed sanitation treatment, which includes fermented tofu dregs and mustard green waste, is enhanced with EM4 and molasses. These additives contain *Lactobacillus casei*, a lactic acid bacterium, and *Saccharomyces cerevisiae*, a yeast, which functions to suppress harmful microorganisms and dominate the fish's digestive system. Faster bacterial growth accelerates the decomposition of organic matter, leading to quicker formation of food for *Tubifex* worms. The addition of simple sugars such as sucrose, glucose, and fructose found in molasses can increase the population of beneficial bacteria, which serve as a nutritional resource (Handajani and Hastuti, 2013).

Table 2 shows that the bacterial colony count increased across all treatments, with the highest bacterial abundance observed in Treatment D. This is because the greater the amount of fermentation added, the higher the protein content in *Tubifex* worms, which in turn increases the bacterial population as a food source, as stated by Mi'raizki and Chilmawati (2015). The presence of bacteria in the fermentation of tofu dregs and mustard green waste leads to higher bacterial activity in the digestive system, which can positively impact the growth of fish fry. The balanced activity of beneficial bacteria creates an antagonistic effect against pathogenic bacteria in fermented feed preparations. This improves the digestive tract's condition, making it more effective at nutrient absorption from the feed (Arief *et al*., 2014).

Performance of Siamese Catfish Fry

The feed sanitation treatment for *Tubifex* worms using fermented tofu dregs and mustard green waste positively impacted the growth in length of Siamese catfish (*Pangasius hypophthalmus*) fry. *Tubifex* worms treated with fermented tofu dregs and mustard green waste had balanced nutritional content, which likely contributed to optimal growth due to the presence of *Lactobacillus* sp*.* bacteria. These bacteria improve the fish's digestive tract (Simamora *et al*., 2021). Siamese catfish fry has an excellent ability to absorb nutrients from *Tubifex* worms. which supports the growth in length of the fry (Chilmawati *et al*., 2015).

The feed sanitation treatment with fermented tofu dregs and mustard green waste resulted in higher and more balanced protein and fat levels compared to other treatments. Growth in Siamese catfish fry can be hindered if dietary fat does not meet the fry's needs, as energy for activity would then be derived from protein instead. According to Bokings *et al*. (2020), Siamese catfish are carnivorous fish capable of digesting protein and fat more efficiently than carbohydrates, making high protein and fat content essential for weight growth in Siamese catfish.

Siamese catfish fry fed *Tubifex* worms treated with fermented tofu dregs and mustard green waste showed the best growth in length because the feed contained more complex nutrients compared to other feed types. According to Anggraeni and Abdulgani (2013), adequate protein and nutrients in feed are closely linked to the growth rate of Siamese catfish fry. The feed sanitation treatment for *Tubifex* worms using fermented tofu dregs and mustard green waste improved growth rates in fry, as bacteria from the fermented feed helped enhance the fish's digestive tract.

The increase in weight of Siamese catfish fry was attributed to the feed sanitation treatment with fermented tofu dregs and mustard green waste, which was well-utilized by the fry for metabolic processes. This is consistent with Islama and Najmi (2019), who stated that growth rates reflect daily weight gain when fish are fed sufficiently nutritious feed. Higher protein levels in fry feed correlate with faster growth rates, as noted by Bokings *et al*. (2020), who found that protein-rich feed resulted in a positive linear relationship with weight gain in fish fry.

An internal factor influencing the growth rate of fish fry is their ability to convert feed into growth energy. which is partly determined by digestibility (Mahardhika *et al*., 2017). Feed sanitation treatment with fermented tofu dregs and mustard green waste provides balanced nutrients that make digestion easier for the fry. According to Fajri and Hutabarat (2014), balanced nutrition plays a significant role in supporting the growth of Siamese catfish fry.

Survival Rate

The survival rate of fish is highly dependent on their ability to adapt to food and the environment, health status, stocking density, and water quality that supports fry survival (Yusapri and Sarifatun, 2020). Based on Figure 5, the survival rate of Siamese catfish (*Pangasius hypophthalmus*) fry reached 98%, indicating that the fry was in a suitable living condition and likely had relatively strong immunity. Stocking density also affects the survival rate. The stocking density used in this research adhered to the SNI 01-6483.4-2000 standard, which is 40 fry per liter. The water quality during the study was well-controlled and remained within ideal conditions for Siamese catfish fry, ensuring no negative impact on survival rates. This aligns with Handayani *et al.* (2014), who stated that maintaining optimal water quality is crucial for the survival of fish fry.

CONCLUSION

The feed sanitation treatment of wild *Tubifex* sp. worms with the addition of fermented tofu dregs and mustard green waste resulted in the highest nutrient levels and the highest abundance of beneficial bacteria. This showed a significant effect ($p < 0.05$) on the absolute growth and growth rate of Siamese catfish (*Pangasius hypophthalmus*) fry. The absolute length growth of the Siamese catfish fry was 9.37 ± 0.044 mm, while the absolute weight growth was 0.08 ± 0.0169 grams. The specific length growth rate of the fry was 7.10 ± 0.017%, and the specific weight growth rate was 18.35 ± 0.025%. The survival rate of Siamese catfish fry in all treatments reached 98%.

ACKNOWLEDGEMENT

The author expresses gratitude to the academic community of the Politeknik Ahli Usaha Perikanan and the leadership and staff of the Telanaipura Ornamental Fish Installation for their support. which made this research possible.

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