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Effect of Curcumin-Enriched Silkworms on the Survival Rate of Catfish Infected with *Aeromonas hydrophila*

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ABSTRACT

Intensive catfish farming often faces challenges related to outbreaks of Motile Aeromonas Septicemia (MAS). Currently, farmers treat MAS with antibiotics. However, the use of antibiotics poses significant risks as it can lead to antibiotic residues in fish tissue. Therefore, alternative treatments using natural ingredients are needed. The purpose of this study was to evaluate the effects of curcumin-enriched surta worms on the survival rate of catfish infected with *Aeromonas hydrophila*. The research methods included enriching silkworms with different turmeric powders, namely 0% (K), 0.25% (P1), 0.5% (P2), and 0.75% (P3), feeding silkworms with and without curcumin, diagnosing *Aeromonas hydrophila* infection, and observing survival rate. Parameters observed included curcumin absorption, silkworm biomass, liver and kidney histology, and survival rate. The results showed that surta worms were able to absorb curcumin through fermentation media, with the highest absorption observed at a 0.75% concentration, yielding a curcumin level of 23.8 mg/kg. Different curcumin content in the cultivation media did not affect silkworm biomass. The results also showed that catfish treated with feed without curcumin had damage to the histology of the liver and kidneys after being infected with *Aeromonas hydrophila*. Meanwhile, catfish fed with 0.75% turmeric powder were shown to inhibit *Aeromonas hydrophila* infection; this was evidenced by healthy liver and kidney histology. An independent t-test showed that feeding surta worms with and without curcumin did not affect the survival rate of juvenile catfish infected with *Aeromonas hydrophila*.

1. INTRODUCTION

Catfish are one of the most popular commodities in Asian, especially Indonesia [1, 2]. Market demand for catfish has been increasing annually. This trend encourages catfish farmers to adopt intensive farming practices to increase production [3, 4]. Intensive fish farming is often associated with disease outbreaks. One of the diseases that attack catfish is Motile Aeromonas Septicemia (MAS) caused by *Aeromonas hydrophila* bacteria [5, 6]. This disease can cause up to 100% mortality in juvenile catfish [7, 8]. Currently, most farmers use chemical drugs and antibiotics in the treatment of MAS disease. However, this is strictly prohibited because it can cause bacterial mutations and residues in fish meat [9, 10]. The use of natural ingredients containing antioxidants, antibacterials, and immunostimulants in feed is one solution in reducing the use of antibiotics [11]. One natural ingredient that possesses these properties is turmeric.

Turmeric contains curcumin which acts as an immunostimulant that can increase the body's resistance to disease infection [12]. Curcumin in turmeric also acts as an antibacterial, anti-inflammatory, and antiviral agent, enhancing fish immunity and providing protection against Aeromonas hydrophila infection [13, 14]. The application of turmeric powder in feed also does not cause side effects to fish [10]. For example in the research of Syari et al. [15] which states that the curcumin content in turmeric can increase the immune system against disease in fish and shrimp. This proves that the curcumin content in turmeric can increase fish immunity. However, its application in fish feed, especially for juvenile fish, still requires further investigation. This can significantly impact the water quality of fish ponds. Therefore, enriching natural feed with curcumin is necessary to minimize this impact. One of the natural feeds that can be enriched with turmeric curcumin is silkworms.

Silkworms, as a natural feed source, are highly preferred by catfish fry. In addition, the nutritional content of silkworms is very high [16-18]. According to Herawati et al. [19], silkworms contain protein (50-55%), fat (8-10%), crude fiber (2-5%), ash content (4-7%) and water (8-10%). Enriching silkworms with curcumin is intended to enhance the growth, survival, and immunity of fish larvae [20]. Further explained by Heltonika et al. [21] that the addition of curcumin to silkworms provides significant growth and 100% survival rate

in betok fish. Curcumin enrichment in feed is the most effective and efficient method in terms of making and giving it to catfish compared to other methods, especially for mass-scale cultivation activities [22]. This study was conducted to evaluate the effects of curcumin-enriched surta worms on the survival rate of catfish infected with *Aeromonas hydrophila*.

2. MATERIALs AND METHODS

2.1 Time and location

The research was conducted from December 2022 to January 2023 at the Cultivation Laboratory, AUP Jakarta Polytechnic. Fish histology and proximate content were conducted at the Nutrition Laboratory, Aquatic Organism Health Laboratory, Bogor Agricultural Institute, and BUSKIPM in East Jakarta. Turmeric powder and silkworms were tested for curcumin at Saraswanti Indo Genetech (SIG) Bogor, and *Aeromonas hydrophila* bacteria were tested at HPIK Bacteria Laboratory, Balai KIPM Jakarta II.

2.2 Enrichment of silkworms with curcumin

Curcumin enrichment in silkworms is done by adding turmeric powder through silk worm cultivation media [21]. Silk worm enrichment was carried out using the experimental method of Completely Randomized Design (CRD) consisting of 4 treatments and 3 replicates. The enrichment dose refers to the modified research of Rahmadani et al. [23], namely 0; 2.5; 5; and 7.5g kg^{-1} (Table 1). This enrichment was carried out to see the best dose of curcumin absorption derived from turmeric powder in silkworms. Curcumin enrichment is carried out on silk worm cultivation media which is a place of life and a source of nutrition for silkworms. Silk worm cultivation media consists of tofu dregs, rice bran and fermented fishmeal.

Table 1. Dosage of adding turmeric powder to silkworms cultivation media

Treatment Code	Information
K	Fermentation media without adding turmeric powder
P1	Fermentation media with the addition of 0.25% turmeric powder
P2	Fermentation media with the addition of 0.5% turmeric powder
P3	Fermentation media with the addition of 0.75% turmeric powder

The fermented silk worm cultivation media was then put into a container in the form of a plastic tray measuring 40cm×30cm×10cm as much as 100g. Turmeric powder was added to the cultivation media according to the predetermined dose, then homogenized. Furthermore, the silk worm starter that has been quarantined for 2 days is spread evenly in each cultivation container as much as 100 grams/container. Maintenance of silkworms was carried out for 21 days [24] with the provision of treatment media every 3 days. Harvesting of silkworms was carried out on day 22 after maintenance was completed. The harvested silkworms were then tested for curcumin absorption and silk worm biomass.

2.3 The feeding of silkworms to catfish

The catfish used in this study were fingerlings with a length of 1.6±0.08cm and a weight of 4.20±0.50 grams. Fish were kept for 21 days in an aquarium with a water volume of 2 liters and a stocking density of 30 fish/liter. Feeding silkworms that have been enriched with curcumin content is carried out by the experimental method of Completely Randomized Design (CRD) consisting of 2 treatments and 5 replicates. The treatment of catfish fed with silkworms can be seen in Table 2. Feeding of silkworms is carried out to satiation at 9:00, 13:00 and 17:00 [25].

2.4 In vivo test

In vivo testing was carried out using *Aeromonas hydrophila* obtained from the Fish and Environmental Disease Examination Institute (LP2IL) in Serang, Banten. The bacteria were rejuvenated by inoculating 1 loop onto Trypticase Soy Broth (TSB) medium, then incubated for 24 hours at 28°C [26]. The prepared bacteria were then diluted to a density of 104 CFU/ml. The process of infection with *Aeromonas hydrophila* is carried out by the immersion method, where 60 catfish/container are immersed in the maintenance media containing *Aeromonas hydrophila* at a density of 104 CFU/ml for 60 minutes. Observation of the test fish was carried out from the first day to the seventh day after immersion [25].

2.5 Internal organ observation

Internal organ observations were carried out on the liver and kidneys histologically before and after in vivo testing on fish that did not experience clinical symptoms. The histology method uses Hematoxylin and Eosin (HE) staining with working procedures including fixation, trimming, tissue dehydration, and results preparations [27].

2.6 Survival rate observation

The survival rate was observed during the 21 day rearing period after being infected with *Aeromonas hydrophila*. The survival rate for catfish seeds was calculated using the formula by Rachmawati et al. [28]:

$$SR(\%) = \frac{Nt}{N0} \times 100$$

where,
SR: Survival Rate (%)
Nt: ∑ fish at the end of rearing (ind)
N0: ∑ fish at the beginning of rearing (ind)

2.7 Data analysis

The results of the proximate test data, curcumin absorption, and liver and kidney histology were analyzed descriptively using tables and figures. Biomass data were analyzed using Analysis of Variance (ANOVA) at the 95% confidence level. If the data showed differences, it was continued with the BNT test using the SPSS IBM version 26 application. The survival rate data were analyzed using an independent t test to see the differences between each treatment.

Table 2. Treatment dose given by silkworms feed

Treatment Code	Information
K	Test fish fed cultivated silkworms did not contain curcumin
P	Test fish fed cultivated silkworms contained the best curcumin

3. RESULTS

3.1 Proximate test results for cultivation media

The proximate test was carried out before fermentation and after adding turmeric powder. The proximate composition of each raw material for silkworm cultivation media before fermentation can be seen in Table 3. The highest protein content and ash content is found in fish meal, namely 53.12%. The highest fat content (11.84%), crude fiber (14.49%) and EMWN (41.85) are found in rice bran. Meanwhile, the highest water content was found in tofu dregs, namely 42.89%. The nutritional content of each raw material is high enough to be used as a medium for cultivating silkworms.

Based on the results of the proximate composition test in Table 4, it shows that the highest protein content was in treatment K, namely 10.61%, and the lowest in treatment P3 was 9.89%. The highest carbohydrate content was found in the P3 treatment, namely 4.92% (Crude fiber) and 12.75% (EMWN), the lowest carbohydrate value was found in treatment K, namely 3.42% (Crude fiber) and 9.24% (EMWN). The highest fat content was found in treatment K which was 5.27% and the lowest in treatment P3 which was 4.91%. Clearly, the silkworms cultivation media contain high nutritional content, showing that the fermentation process is successful.

3.2 Curcumin content

According to the results of testing the curcumin content in turmeric powder, silkworms were able to absorb the compound. The curcumin levels that could be detected in each treatment were 3.04mg/kg, 17.98mg/kg and 23.8mg/kg. The detected curcumin levels were not linear when compared with the amount of turmeric powder added to the silkworms media, namely 0.25%, 0.5% and 0.75%. The results of curcumin absorption in silkworms are shown in Table 5.

3.3 Silkworms biomass

The growth of silkworms is expressed in biomass calculated at the end of the 21 day rearing period. The results of the ANOVA test showed that the fermentation media without turmeric powder and the fermentation media with the addition of turmeric powder had no effect on silkworms biomass. Fertilization in silkworms cultivation aims to provide a new source of nutrition during silkworms cultivation. Media as feed for silkworms with higher carbohydrate content compared to protein content did not affect silkworms biomass. The final results of silkworms biomass can be seen in Table 6.

Table 3. Proximate composition of each fermentation media raw material

Type of Raw Material	Protein	Fat	Carbohydrate		Water Content	Ash Content
			Crude Fiber	EMWN		
Fish flour	53.12	7.44	0.48	1.72	16.92	20.32
Rice bran	9.89	11.84	14.49	41.85	10.22	11.71
Tofu dregs	14.89	10.54	9.44	20.86	42.89	1.38

In wet weight (%) (Fish Nutrition Laboratory Test Results, FPIK, IPB, 2023)

Table 4. Treatment proximate composition

Treatment	Protein	Fat	Carbohydrate		Water Content	Ash Content
			Crude Fiber	EMWN		
K	10.61	5.27	3.42	9.24	68.12	3.34
P1	10.03	5.03	4.45	11.32	65.8	3.37
P2	10.19	5.18	4.88	11.68	64.79	3.28
P3	9.89	4.91	4.92	12.75	64.21	3.32

In wet weight (%) (Fish Nutrition Laboratory Test Results, FPIK, IPB, 2023)

Table 5. Absorption of curcumin in silkworms

Test Type	Treatment			Method
	P1	P2	P3	
Curcumin (mg/kg)	3.04±0.04	17.98±0.28	23.80±0.20	18-5-12/MU/SMM-SIG (HPLC-PDA)

(Saraswanti Indo Genetech Laboratory Test Results, 2023)

Table 6. Silkworm biomass

Treatment	Biomass (g/m^2)
K	313.33+9.02[a]
P1	314.33±9.02[a]
P2	315.33±25.01[a]
P3	316.00±14.18[a]

Note: The same uppercase letters behind the mean values (± standard deviation) indicate that they are not significantly different (P>0.05).

3.4 Liver and kidney histology of catfish

Histological observations of catfish fry treated with and without curcumin before being infected with *Aeromonas hydrophila* bacteria showed no necrosis or abnormal liver cells in either case (Figure 1). The liver parenchyma or hepatocytes, is located between the sinusoids containing blood and the bile ducts. Liver cells are generally polyhedral in shape with six or

more surfaces with a round core with lots of endoplasmic reticulum. In normal conditions, hepatocyte cells are round and sinusoids are clearly visible.

Based on liver histology observations of infected catfish fry fed silkworms lacking curcumin after being infected with *Aeromonas hydrophila*, catfish were unable to inhibit infection. This is indicated by the presence of hemorrhage and necrosis in the liver tissue. Hemorrhage and necrosis which are characterized by empty space between the nuclei and the presence of several vacuoles indicate inflammation and damage to the hepatopancreas. Meanwhile, the liver histology of catfish fry fed with silkworms contained curcumin after being infected with *Aeromonas hydrophila* bacteria. The observation results showed that there was no hemorrhage or necrosis. This is thought to be caused by the addition of turmeric powder which is thought to be able to inhibit *Aeromonas hydrophila* bacterial infections. The results of observations of the liver histology of catfish fry after being infected with *Aeromonas hydrophila* bacteria can be seen in Figure 2.

The results of kidney histology observations of catfish fry treated with curcumin and without curcumin before being infected with *Aeromonas hydrophila* bacteria showed normal kidney cells (Figure 3). This can be seen from the condition of the kidney cells, such as the tubules, glomerulus and Bowman's capsule, which show that they are in normal condition. The structure of normal fish kidneys is neatly arranged by the glomerulus which is surrounded by Bowman's capsule.

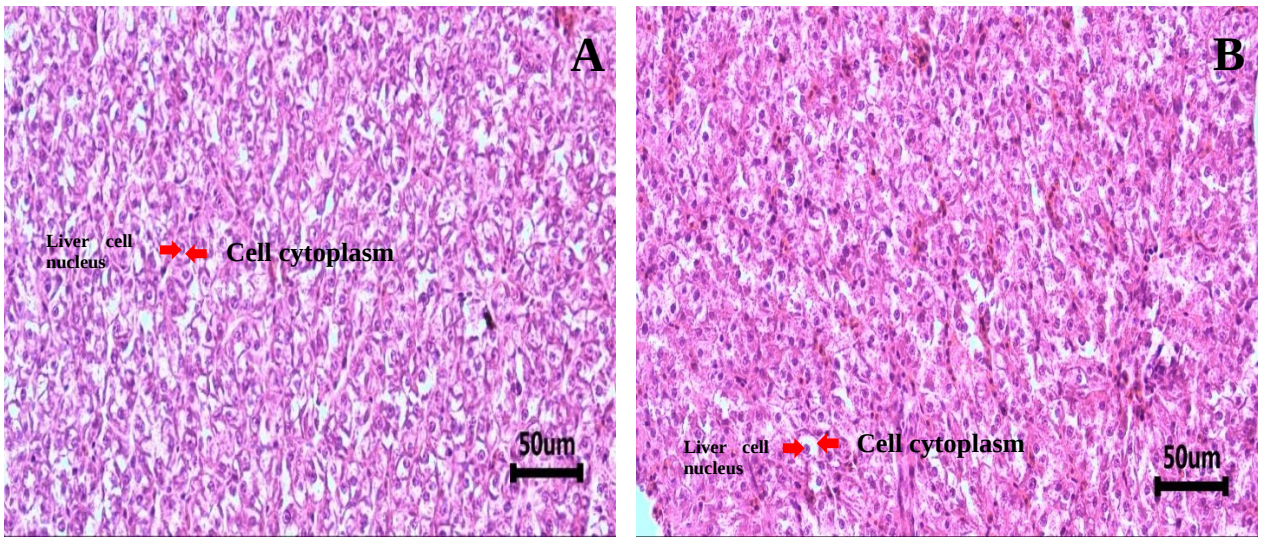

Figure 1. Liver histology of catfish fry before being infected with *Aeromonas hydrophila*
Note: A: silkworms do not contain curcumin; B: Silkworms contain curcumin

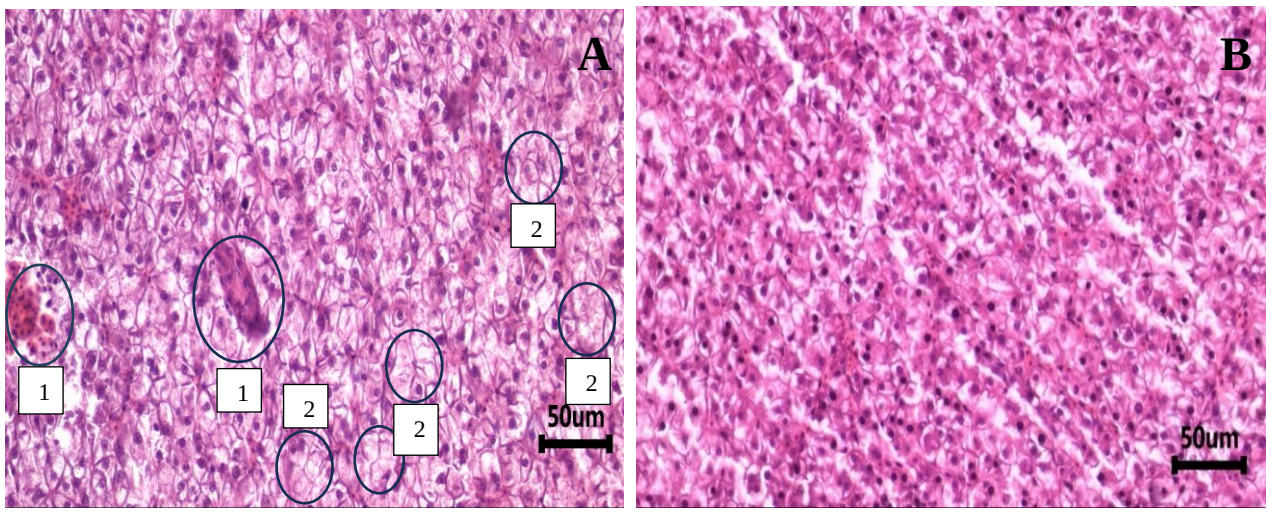

Figure 2. Liver histology of catfish fry after being infected with *Aeromonas hydrophila*
Note: A: silkworms do not contain curcumin; B: Silkworms contain curcumin; 1: Haemorrhage; 2: Necrosis

The results of histological observations of the kidneys of catfish fry fed with silkworms that did not contain curcumin after being infected with *Aeromonas hydrophila* showed necrosis and narrowing of Bowman's capsule. *Aeromonas hydrophila* infection is thought to cause these cell abnormalities. Necrosis occurs due to an inflammatory reaction that causes tissue damage followed by cell death due to infection with the *Aeromonas hydrophila*. Meanwhile, histology of the kidneys of catfish fry fed with silkworms containing curcumin showed that there were no cell abnormalities. The results of histological observations of the kidneys of catfish fry after being infected with *Aeromonas hydrophila* can be seen in Figure 4.

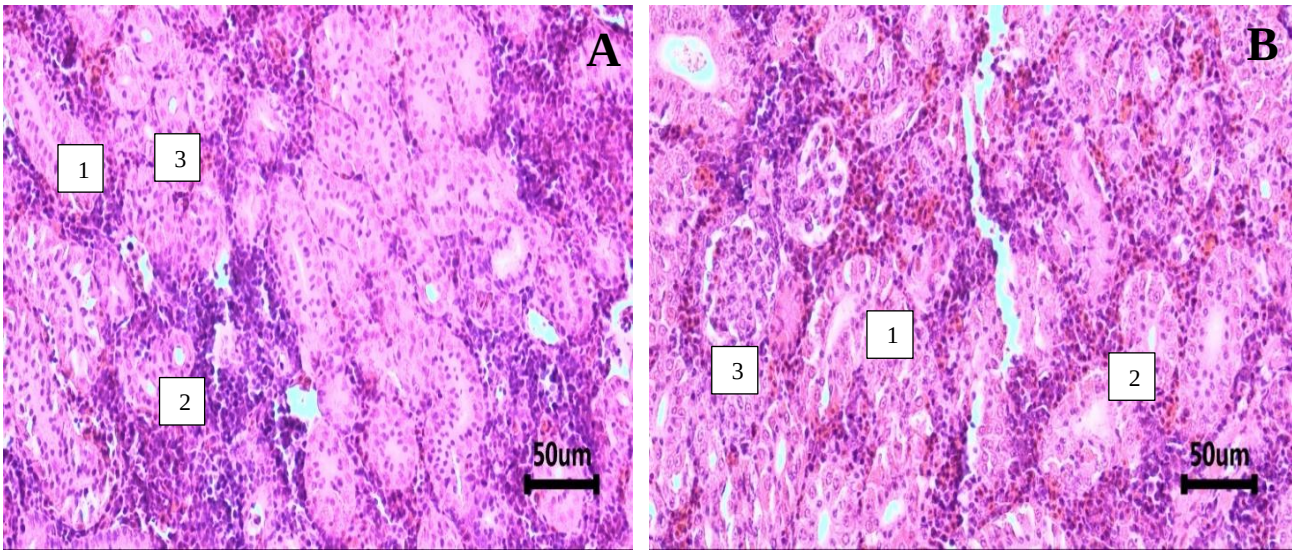

Figure 3. Kidney histology of catfish fry before being infected with *Aeromonas hydrophila* bacteria
Note: A: silkworms do not contain curcumin; B: Silkworms contain curcumin; 1: Tubules; 2: Glomerulus; 3: Bowman's capsule

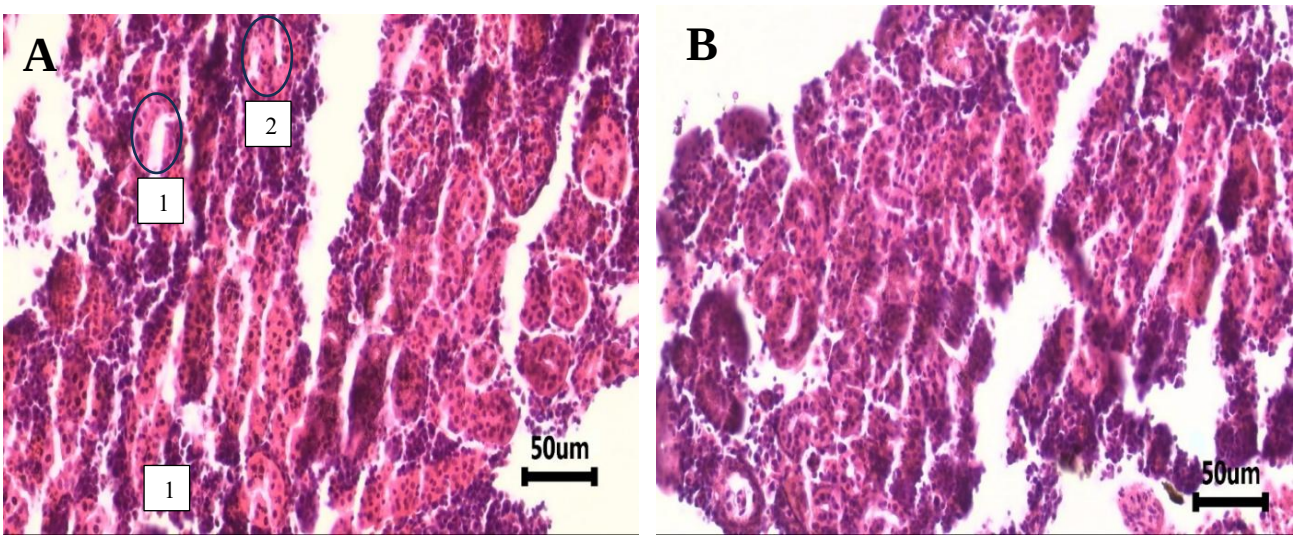

Figure 4. Kidney histology of catfish fry after being infected with *Aeromonas hydrophila*
Note: A: Silkworms do not contain curcumin; B: Silkworms contain curcumin; 1: Necrosis; 2: Narrowing of Bowman's capsule

3.5 Catfish survival

The survival rate of catfish seed fed with silkworms containing curcumin after being infected with *Aeromonas hydrophila* bacteria was 71.7%±11.7% and silkworm feed without curcumin content was 66%±5.7%. The results of the independent t test stated that there was no effect on the survival rate of catfish seeds infected with *Aeromonas hydrophila* bacteria by feeding silkworms with and without containing curcumin. Survival rate measurement results of catfish can be seen in Table 7.

Table 7. Catfish survival rate

Treatment	Survival Rate (%)
K	66%±5.7%
P	71.7%±11.7%

4. DISCUSSIONS

The silkworms were able to absorb curcumin from turmeric powder provided through the media as well as substrate and feed. However, the levels of curcumin detected were not linear when compared to the amount of turmeric powder added to the silk worm media. Based on the results of the proximate composition test, silkworms contain quite highwater content, namely 82.52-87.94%. This shows that curcumin is not optimally absorbed in silk worm cells because of the solubility of curcumin in water and the absorption of curcumin to cells tends to be low [29]. Further explained by Riyadi et al. [30] which states that curcumin is nonpolar in nature where curcumin has low solubility in water and has low cell absorption.

Fermentation media without turmeric powder and fermentation media with the addition of turmeric powder had no effect on silk worm biomass. This is due to the nutrient content, especially protein contained in the fermentation media which is quite high and the nature of turmeric powder which has a low absorption of cells. The protein content in silk worm cultivation media is more easily absorbed so that it can increase the biomass of silkworms [31]. This is further explained by Suminto and Hutabarat [32], who state that the growth of silkworms is influenced by the balance of energy and protein in the cultivation media. The content of nutrients

contained in silk worm cultivation media during the maintenance period greatly affects biomass. Lack of nutrients in the cultivation media can cause a lack of food intake, causing low biomass and nutrient content in silkworms [33].

Histological observations of the treated catfish organs were carried out on the liver and kidneys. These two organs are an important part of fish metabolism and immunity. The liver acts as a metabolic center where it makes bile, stores fat and glycogen, and emulsifies fat during food digestion so that the intestinal walls can absorb it [34]. Meanwhile, the kidneys play a role in the blood-producing system and fish immunity. The majority of the forebrain is home to hematopoietic tissue cells, which produce blood cells and function as a melanomacrophage interface, which is part of the defense system of teleost fish [35]. According to Safratilofa [34], *Aeromonas hydrophila* infection damages liver cells by entering the liver through the fish's digestive metabolism. When a bacterial infection appears, the liver is one of the signs to watch out for [36]. The kidneys become infected with *Aeromonas hydrophila* infection through the circulation. Kidney cells can be damaged by bacteria that grow and consume nutrients for kidney metabolic function [34]. In our study, the histology of the liver and kidneys of catfish before being infected with *Aeromonas hydrophila* bacteria showed normal cells. According to Faccioli et al. [37], normal liver structure is characterized by the presence of round-shaped liver cells (hepatocytes), clearly visible sinusoids and round-shaped lobules. Meanwhile, the structure of normal fish kidneys is neatly arranged by the glomerulus which is surrounded by Bowman's capsule. The glomerulus and Bowman's capsule are shaped like a six with the same size. The glomerulus, whose shape still looks real, is not completely round but resembles the number six [38].

When treated with silkworm feed that does not contain curcumin, hemorrhage and necrosis occurred in the catfish liver histology. *Aeromonas hydrophila* infection in catfish can cause liver cell abnormalities in the form of hemorrhage and necrosis [36]. Hemorrhage is the process of bleeding due to damage to blood vessels. Bleeding in cells is caused by rupture of blood vessels, causing blood to flow in inappropriate places, both out of the body and into body tissues. Hemorrhage caused by congestion in the liver is so severe that it causes blood vessels to become damaged. Hemorrhage can be caused by bacterial infection [39]. Necrosis in liver cells is caused by the presence of extracellular products such as hemolysin, protease and ethalase produced by *Aeromonas hydrophila* [40]. Treatment of silkworm feed containing curcumin showed no hemorrhage or necrosis. This is caused by the content of turmeric which acts as an antibacterial and anti-oxidant such as tannins, flavonoids, alkaloids and saponins [41]. The antibacterial activity of flavonoid compounds has the ability to damage the plasma membrane. In low concentrations, these compounds can damage the composition and permeability of bacterial cell walls, but in high concentrations, they can cause coagulation, resulting in bacterial death [42]. Tannin compounds are one of the secondary metabolite compounds that plants can use to protect against bacterial and fungal attacks. Tannin has the ability to act as an antibacterial by denaturing proteins. Denatured proteins will inhibit the way enzymes work. If the enzyme's work is hampered, it will cause the metabolic process to be hampered. By hampering the metabolic process, the growth and development of bacteria will also be hampered [43, 44]. Turmeric also contains alkaloids that are active compounds. Alkaloids as antibacterial agents cause bacteria peptidoglycan (part of the cell wall) to be disrupted, resulting in the bacterial cell wall not being formed fully and the bacteria dying [45].

It was found that silkworm feed that does not contain curcumin caused necrosis and narrowing of Bowman's capsule. Necrosis is characterized by the appearance of cell boundaries and cell nuclei being unclear or even disappearing [38]. Fish infected with *Aeromonas hydrophila* experience damage such as necrosis and narrowing of the Bowman's capsule [36]. *Aeromonas hydrophila* enters through the gills, liver, spleen, then to the kidneys. *Aeromonas hydrophila* is trapped in the interstitial hematopoietic tissue of the kidney and then reacts with monocytes, macrophages and neutrophils, causing inflammation and inducing degeneration and necrosis [46]. Kidney cell abnormalities are thought to occur because there is no curcumin to inhibit *Aeromonas hydrophila* infection. When treated with silkworms containing curcumin, there were no cell abnormalities. The addition of turmeric is thought to be able to inhibit *Aeromonas hydrophila* bacterial infections. The active ingredients in turmeric powder include curcumin, essential oils, saponins and flavonoids which function as antibacterials by denaturing proteins and damaging bacterial cell membranes. The main mechanisms of antibacterial action are blocking the synthesis of cell walls, blocking the permeability of bacterial walls, inhibiting bacterial protein synthesis, inhibiting bacterial nucleic acid synthesis, and inhibiting bacterial metabolism [47].

The survival rate of catfish fry fed with silkworms containing curcumin during the 21-day rearing period after being infected with *Aeromonas hydrophila* bacteria was 71.7%±11.7% and with silkworms containing curcumin was 66%±5.7%. The results of the independent t test stated that there was no effect on the survival rate of catfish seeds infected with *Aeromonas hydrophila* bacteria by feeding silkworms with and without containing curcumin. Based on observations of liver histology following infection with the *Aeromonas hydrophila* bacteria, curcumin appears to improve liver cell performance. The effect of curcumin is as an antibacterial, stimulating liver cell regeneration and improving liver function. According to Dewi et al. [48], curcumin content is able to improve liver function by stimulating liver cell regeneration and the ability to protect liver cells from the effects of toxins that can damage liver cells. Curcumin is an active ingredient in turmeric with activity as a hepatoprotector to prevent damage to liver cells so that it can improve liver function. Other ingredients in turmeric, such as flavonoids, can also improve kidney performance. Flavonoids are able to inhibit enteretoxin production so that the organs that produce blood (kidneys) return to normal. According to Wahyuni et al. [38], giving turmeric flour which contains flavonoids can prevent damage to the structure of fish kidney tissue due to attacks by *Aeromonas hydrophila*. Providing quality feed affects the survival rate of fish. Turmeric containing the active ingredient curcumin can be used as a feed supplement to provide quality feed.

5. CONCLUSION

According to the findings of this research, silkworms can absorb curcumin through fermentation media used as feed and substrate, with the best results achieved at a 0.75% dose containing 23.8 mg/kg of curcumin. The addition of 0.75% turmeric powder has an effect in inhibiting *Aeromonas*

hydrophila infections. Based on the findings obtained, further research is needed on the effects of silkworms enriched with 0.75% turmeric powder on the growth, survival, and immunity of catfish, both at the cultivation scale and on a larger scale.

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REFERENCES

[1] Budiariati, V., Susmiati, T., Munawaroh, S., Putri, R.C.A., Widayanti, R.W. (2021). Genetic diversity of indigenous catfish from Indonesia based on mitochondrial Cytochrome Oxidase Subunit II gene. Biodiversitas, 22(2): 593-600. https://doi.org/10.13057/biodiv/d220210

[2] Islam, M.S., Lamid, M. (2020). The maggot flour substitution potency (Hermetia illucens) in artificial feed formulation on growth and survival rates of African catfish (Clarias gariepinus). IOP Conference Series: Earth and Environmental Science, 441(1): 012016. https://doi.org/10.1088/1755-1315/441/1/012016

[3] Bulkini, A., Satria, A., Mulyati, H. (2018). Analysis of the catfish (Clarias sp.) value chain and its problems in Bogor, Indonesia. Independent Journal of Management & Production, 9(1): 262-273. https://doi.org/10.14807/ijmp.v9i1.628

[4] Prananingtyas, D., Rahardja, S. (2019). Effect of different salinity level within water against growth rate, survival rate (FCR) of catfish (Clarias sp.). IOP Conference Series: Earth and Environmental Science, 236(1): 012035. https://doi.org/10.1088/1755-1315/236/1/012035

[5] Bartie, K.L., Ngô, T.P.H., Bekaert, M., Hoang Oanh, D.T., Hoare, R., Adams, A., Desbois, A.P. (2023). Aeromonas hydrophila ST251 and Aeromonas dhakensis are major emerging pathogens of striped catfish in Vietnam. Frontiers in Microbiology, 13: 1067235. https://doi.org/10.3389/fmicb.2022.1067235

[6] Ulkhaq, M.F., Budi, D.S., Rahayu, N.N. (2020). The effect of temperature, salinity and antimicrobial agent on growth and viability of Aeromonas hydrophila. IOP Conference Series: Earth and Environmental Science. IOP Publishing Ltd, 441(1): 012020. https://doi.org/10.1088/1755-1315/441/1/012020

[7] Olga, O., Aisiah, S., A Tanod, W., Risjani, Y., Nursyam, H., Maftuch, M. (2020). Immunogenization of heat-killed vaccine candidate from Aeromonas hydrophila in catfish (Pangasius hypophthalamus) using strain of Banjar, South Kalimantan, Indonesia. Egyptian Journal of Aquatic Biology and Fisheries, 24(4): 1-13. https://doi.org/10.21608/ejabf.2020.93528

[8] Kusdarwati, R., Dinda, N.D., Nurjanah, I. (2018). Antimicrobial resistance prevalence of Aeromonas hydrophila isolates from motile Aeromonas septicemia disease. IOP Conference Series: Earth and Environmental Science, 137(1): 012076. https://doi.org/10.1088/1755-1315/137/1/012076

[9] Lulijwa, R., Rupia, E.J., Alfaro, A.C. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: A review of the top 15 major producers. Reviews in Aquaculture, 12(2): 640-663. https://doi.org/10.1111/raq.12344

[10] Yuan, X., Lv, Z., Zhang, Z., Han, Y., Liu, Z., Zhang, H. (2023). A review of antibiotics, antibiotic resistant bacteria, and resistance genes in Aquaculture: Occurrence, contamination, and transmission. Toxics, 11(5): 420. https://doi.org/10.3390/toxics11050420

[11] Ivanova, S., Sukhikh, S., Popov, A., Shishko, O., Nikonov, I., Kapitonova, E., Krol, O., Larina, V., Noskova, S., Babich, O. (2024). Medicinal plants: A source of phytobiotics for the feed additives. Journal of Agriculture and Food Research, 101172. https://doi.org/10.1016/j.jafr.2024.101172

[12] Manurung, U.N., Mose, N.I. (2019). Utilization of turmeric (Curcuma domestica Val) as an immunostimulant in pomfret fish (Colossoma macropomum). Aquaculture E-Journal, 7(1): 21-25. https://doi.org/10.35800/bdp.7.1.2019.24842

[13] Hăbeanu, M., Gheorghe, A., Dinita, G., Mihalcea, T. (2024). An in-depth insight into the profile, mechanisms, functions, and transfer of essential amino acids from mulberry leaves to silkworm *Bombyx mori* L. Pupae and Fish. Insects, 15(5): 332. https://doi.org/10.3390/insects15050332

[14] Enyidi, U.D., Ayogu, I. (2023). Substitution of Hibiscus sabdariffa with Curcuma longa in the Diets of Clarias gariepinus and the Effects on the Growth, Nutrient, and Hematobiochemistry. Journal of Aquaculture & Fish Health, 12(2): 269-280. https://doi.org/10.20473/jafh.v12i2.31675

[15] Syari, R., Saputra, S., Elvitasari, T., Herawati, V.E., Pinandoyo, P. (2021). Effects diets with additional turmeric (Curcuma longa LINN) extract of growth and survival rate in cobia (Rachycentron canadum). Aquacultura Indonesiana, 22(2): 51-60. https://doi.org/10.21534/ai.v22i2.234

[16] Kustiawan, T.P., Satyantini, W.H., Mubarak, A.S. (2011). The effect of remanuring dry chicken manure in Tubifex tubifex population. Jurnal Ilmiah Perikanan dan Kelautan, 3(2): 177-182. https://doi.org/10.20473/jipk.v3i2.11604

[17] Ismawati, H., Sulmartiwi, L., Dewi, N.N. (2023). The effectiveness of additional silage of fish ofal and bagasse fermentation on biomass and population of silk worm (Tubifex tubifex). IOP Conference Series: Earth and Environmental Science. IOP Publishing Ltd, 1273(1): 012048. https://doi.org/10.1088/1755-1315/1273/1/012048

[18] Febrianti, L., Sulmartiwi, L., Dewi, N.N. (2023). Application of combination feed of fish viscera silage and fermented rice bran on biomass and population of silkworms (Tubifex tubifex). IOP Conference Series: Earth and Environmental Science, 1273(1): 012042. https://doi.org/10.1088/1755-1315/1273/1/012042

[19] Herawati, V.E., Hutabarat, J., Pinandoyo, Karna Radjasa, O. (2020). Growth performance of tilapia (Oreochromis niloticus Linnaeus, 1758) larvae with feeding Tubifex tubifex (Müller, 1774) from different fermentation of animal manures. Iranian Journal of Fisheries Sciences, 19(4): 2039-2052. https://doi.org/10.22092/ijfs.2018.123936.1005

[20] Joshua, W.J., Kamarudin, M.S., Ikhsan, N., Yusoff, F.M., Zulperi, Z. (2022). Development of enriched Artemia and Moina in larviculture of fish and crustaceans: A

review. Latin American Journal of Aquatic Research, 50(2): 144-157. https://doi.org/10.3856/vol50-issue2-fulltext-2840

[21] Heltonika, B., Sukendi, Hartati, R. (2016). The effect of adding curcuma on Tubifex sp. on the growth of betok fish larvae (Anabas testudinius). Berkala Perikanan Terubuk, 44(1): 33-39. https://festiva.ejournal.unri.ac.id/index.php/JT/article/view/3495.

[22] Galina, J., Yin, G., Ardó, L., Jeney, Z. (2009). The use of immunostimulating herbs in fish. An overview of research. Fish Physiology and Biochemistry, 35(4): 669-676. https://doi.org/10.1007/s10695-009-9304-z

[23] Rahmadani, T.B.C., Jusadi, D., Setiawati, M., Hastuti, Y.P. (2020). The evaluation of turmeric (Curcuma longa) supplementation within feed as an antioxidant towards growth performance of catfish Clarias gariepinus Burchell 1822 in zero water exchange condition. Jurnal Iktiologi Indonesia, 20(2): 105-115. https://doi.org/10.32491/jii.v20i2.518

[24] Umidayati, U., Rahardjo, S., Ilham, I. (2020). Pengaruh perdedaan dosis pakan organik terhadap pertumbuhan cacing sutra (Tubifex sp). Sains Akuakultur Tropis: Indonesian Journal of Tropical Aquaculture, 4(1): 31-38. https://doi.org/10.14710/sat.v4i1.7230

[25] Wahjuningrum, D., Astrini, R., Setiawati, M. (2013). Prevention of Aeromonas hydrophila on catfish juvenile using garlic and shatterstone herb. Jurnal Akuakultur Indonesia, 12(1): 86-94. https://doi.org/10.19027/jai.12.86-94

[26] Fitriadi, R., Nurhafid, M., Kasprijo, K., Ryandini, D., Riady, R.M., Sukardi, P. (2023). Antibacterial activity of Proteus spp. isolated from the rice-fish farming system cultivation area against A. hydrophila. Iraqi Journal of Veterinary Sciences, 37(4): 929-934. https://doi.org/10.33899/ijvs.2023.138764.2836

[27] Feldman, A.T., Wolfe, D. (2014). Tissue processing and hematoxylin and eosin staining. Histopathology: Methods and Protocols. Humana Press, New York, NY., 31-43. https://doi.org/10.1007/978-1-4939-1050-2_3

[28] Rachmawati, D., Samidjan, I., Nurhayati, D. (2025). The effect of taurine in artificial feed on protein digestibility, feed utilization efficiency, and growth of Java barb (*Barbonymus gonionotus*) seeds. *AACL Bioflux*, *18*(1): 364-372.

[29] Suprihatin, T., Rahayu, S., Rifa'i, M., Widyarti, S. (2020). Senyawa pada serbuk rimpang kunyit (Curcuma longa L.) yang Berpotensi sebagai Antioksidan. Buletin Anatomi dan Fisiologi, 5(1): 35-42. https://doi.org/10.14710/baf.5.1.2020.35-42

[30] Riyadi, S., Abdullah, F.F., Fadhilah, F., Assidiqiah, N. (2022). Anticancer activity of curcuminoids against B16-F10 melanoma cell lines. Jurnal Ilmiah Farmaco Bahari, 13(2): 152-163. https://doi.org/10.52434/jfb.v13i2.1458

[31] Chilmawati, D., Suminto., Yuniarti, T. (2015). Utilization of fermented organic wastes of tofu, rice bran, and chicken manure to increase the culture production and quality of silk worm (Tubifex sp). Pena: Jurnal Ilmu Pengetahuan dan Teknologi, 28(2): 186-201. https://doi.org/10.31941/jurnalpena.v28i2.142

[32] Suminto, M., Hutabarat, J. (2014). The effect of the addition of chicken manure, silage fish and tapioca flour in culture medium on biomass, population and nutrition content of silk worm (Tubifex sp.). Journal of Aquaculture Management and Technology, 3(4): 151-157. https://ejournal3.undip.ac.id/index.php/jamt/article/view/6652

[33] Puspitasari, D., Lutfiyah, L., Sulmartiwi, L. (2023). The effect of the innards silage of fish and coconut pulp fermentation on the biomass and population of silkworm (Tubifex tubifex). IOP Conference Series: Earth and Environmental Science, 1273(1): 012001. https://doi.org/10.1088/1755-1315/1273/1/012001

[34] Safratilofa, S. (2017). Histopathology of liver and kidney of catfish (*Pangasionodon hypopthalmus*) injected with *Aeromonas hydrophila*. Jurnal Akuakultur Sungai dan Danau, 2(2): 83-88. https://doi.org/10.33087/akuakultur.v2i2.21

[35] Dezfuli, B.S., Lorenzoni, M., Carosi, A., Giari, L., Bosi, G. (2023). Teleost innate immunity, an intricate game between immune cells and parasites of fish organs: who wins, who loses. Frontiers in immunology, 14: 1250835. https://doi.org/10.3389/fimmu.2023.1250835

[36] Semwal, A., Kumar, A., Kumar, N. (2023). A review on pathogenicity of *Aeromonas hydrophila* and their mitigation through medicinal herbs in aquaculture. *Heliyon*, *9*(3): e14088. https://doi.org/10.1016/j.heliyon.2023.e14088

[37] Faccioli, C.K., Chedid, RA., Bombonato, M.T.S., Vicentini, C.A., Vicentini, I.B.F. (2014). Morphology and histochemistry of the liver of carnivorous fish *Hemisorubim platyrhynchos*. International Journal of Morphology, 32(2): 715-720. https://doi.org/10.4067/S0717-95022014000200055

[38] Wahyuni, S., Riauwaty, M., Windarti, W. (2020). Histopathology of kidney of striped catfish (Pangasiodon hypophthalmus) feed with turmeric flour. Jurnal Perikanan dan Kelautan, 25(3): 232-237. https://doi.org/10.31258/232-237

[39] Irshath, A.A., Rajan, A.P., Vimal, S., Prabhakaran, V.S., Ganesan, R. (2023). Bacterial pathogenesis in various fish diseases: Recent advances and specific challenges in vaccine development. Vaccines, 11(2): 470. https://doi.org/10.3390/vaccines11020470

[40] Mohamad, N.F.A., Mohd Daud, H., Manaf, S.R. (2022). Pathogenicity of Aeromonas hydrophila in cultured African catfish (Clarias gariepinus). Journal of Smart Science and Technology, 2(1): 34-45. https://doi.org/10.24191/jsst.v2i1.25

[41] Jyotirmayee, B., Mahalik, G. (2022). A review on selected pharmacological activities of Curcuma longa L. International Journal of Food Properties, 25(1): 1377-1398. https://doi.org/10.1080/10942912.2022.2082464

[42] Shamsudin, N.F., Ahmed, Q.U., Mahmood, S., Ali Shah, S.A., Khatib, A., Mukhtar, S., Alsharif, M.A., Parveen, H., Zakaria, Z.A. (2022). Antibacterial effects of flavonoids and their structure-Activity relationship study: A comparative interpretation. Molecules, 27(4): 1149. https://doi.org/10.3390/molecules27041149

[43] Sunani, S., Hendriani, R. (2023). Journal review: Classification and pharmacological activity of tannin active compounds. Indonesian Journal of Biological Pharmacy, 3(2): 130-136. https://doi.org/10.24198/ijbp.v3i2.44297

[44] Bamidele, M.O., Bamikale, M.B., Cárdenas-Hernández, E., Bamidele, M.A., Castillo-Olvera, G., Sandoval-Cortes, J., Aguilar, C.N. (2025). Bioengineering in solid-

state fermentation for next sustainable food bioprocessing. Next Sustainability, 6: 100105. https://doi.org/10.1016/j.nxsust.2025.100105

[45] Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B., Li, M. (2021). Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. Antibiotics, 10(3): 318. https://doi.org/10.3390/antibiotics10030318

[46] Kim, K.T., Lee, S.H., Lee, K.K., Han, J.E., Kwak, D. (2021). Enhanced virulence of *Aeromonas hydrophila* is induced by stress and serial passaging in mice. Animals, 11(2): 508. https://doi.org/10.3390/ani11020508

[47] Eshboev, F., Mamadalieva, N., Nazarov, P.A., Hussain, H., Katanaev, V., Egamberdieva, D., Azimova, S. (2024). Antimicrobial action mechanisms of natural compounds isolated from endophytic microorganisms. Antibiotics, 13(3): 271. https://doi.org/10.3390/antibiotics13030271

[48] Dewi, C.D., Ekastuti, D.R., Sudrajat, A.O., Manalu, W. (2018). The role of turmeric powder supplementation in improving liver performances to support production of siam catfish (Pangasianodon hypophthalmus). Omni-Akuatika, 14(1): 44-53. https://doi.org/10.20884/1.oa.2018.14.1.484