Open Access

Int. J. Trop. Vet. Biomed. Res.
Vol. 8 (1): 1-6; May 2023

www.jurnal.usk.ac.id/IJTVBR
E-ISSN : 2503-4715

The application of asam sunti as feed additives for bacterial infection control of *Edwardsiella tarda*

Zulhan Efendi[1], Suhartono[2*], Firdus[3]

[1]*Fish Quarantine, Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia*
[2]*Department of Biology, Faculty Mathematics and Natural Science, Universitas Syiah Kuala, Banda Aceh*
[3]*Department of Biology, Faculty Mathematics and Natural Science, Universitas Syiah Kuala, Banda Aceh*
**Corresponding author: suhartono@usk.ac.id*

Abstract

Cultivating catfish is one of the businesses that are of interest in Indonesia. The success of cultivation begins to be determined by the provision of fries. Healthy fries lead to a high survival rate, and unhealthy fries due to disease will cause a high mortality rate, causing losses in the cultivation business. Bacterial infectious diseases *Edwardsiella tarda* can cause a low survival rate of catfish fries, and even the death rate can reach 100%. This study aimed to evaluate and determine the best dosage of asam sunti in feed to control the pathogenic bacteria *E. tarda* infection in catfish. Asam sunti was given through feed with different treatment doses, with concentrate 0% (K), 0.5% (P1), 1% (P2), 2% (P3), and 4% (P4) for 14 days after being infected with pathogenic bacteria. The effect of giving asam sunti was measured by observing the survival of fish. The results showed that the administration of asam sunti could control the bacterial diseases of *E. tarda* bacteria sequentially control (K) is 0%, (P1) is 16.67%, (P2) 40%, (P3) 66.67%, and (P4) 90%. From these results, it can be concluded that the 4% asam sunti dose is the best dose for the survival of catfish fry, with the survival rate for pathogenic bacteria *E. tarda* at 90%.

Keywords: Asam sunti*, Clarias gariepinus, Edwardsiella tarda*

Citation: Efendi, Z., Suhartono, S., & Firdus, F. (2023). The application of asam sunti as feed additives for bacterial infection control of *Edwardsiella tarda*. *The International Journal of Tropical Veterinary and Biomedical Research*, 8(1), 1-6. https://doi.org/10.21157/ijtvbr.v8i1.32105.

Background

Catfish (*Clarias gariepinus*) is one of the fisheries in Indonesia which is in great demand by fish cultivators. Disease attacks often constrain the increase in fish production from microorganisms, one of which is a group of bacteria. This condition impacts decreased production, so it is insufficient for market demand and increases in selling prices and affects consumer consumption (Ryan et al., 2012; Mathew et al., 2014; Tran et al., 2017). Clarias gariepinus (*C. gariepinus*) inhabits almost all zones of freshwater bodies, including rivers, lakes, floodplains, large and shallow rivers. In addition, *C. gariepinus* is a basic omnivorous eater, eating almost anything it comes into contact with (Ogueji et al., 2019).

Bacterial infectious diseases can cause low fish survival, even the death rate can reach 100% (Sri, 2022). Increasing catfish production is often done by increasing feed efficiency and adding antibiotics. However, in recent years, strains of pathogenic microbes have emerged that are resistant to certain antibiotics (Widodo, 2019). The use of antibiotics for a long time can cause resistance to pathogenic bacteria in the body, both in fish and in environments polluted by antibiotics which result in non-target organisms being killed (Scientific, 2012).

Natural ingredients that can potentially control bacterial infectious diseases in catfish fries include the star fruit plant (*Averrhoa bilimbi*). Wijayanti and Safitri (2018) said that starfruit leaves had antibacterial activity at concentrations of 2.5%, 5%, and 10% against the growth of *Staphylococcus aureus*. Research results from Maryam et al., (2015) showed that the ethanol extract of star fruit which are called 'belimbing wuluh' at a concentration of 0.4% had antibacterial activity for *Shigella dysenteriae*, *Salmonella typhi*, *Pseudomonas*

aeruginosa, *Vibrio cholera, Escherichia coli, and S. aures*. Thus, a way of handling it is needed by looking for natural products that have antimicrobial properties (Chotiah, 2013) and can be added to feed (feed additives), as a substitute for synthetic antibiotics to control disease in catfish farming.

Materials and Method

Experimental Design

This study used a completely randomized design (CRD) with one treatment factor (sunti acid administration) with treatment levels of 0%, 0.5%, 1%, 2%, and 4%, which were mixed with commercial feed.

Preparation of Containers and Test Fish Fries

The place or container used to maintain catfish fry is an aquarium with a size of 30 x 30 x 20 cm totaling 5 pieces. The initial stage is to clean the aquarium and arrange it on the shelf where the aquarium is, then fill it with 10 liters of fresh water and add airase. The aquarium was filled with 10 test catfish fries before treatment, followed by acclimatization for three days. During the research, the water quality conditions with a pH between 7–8.5, water temperature between 23–30 °C, and dissolved oxygen (DO) of 3.5 mg/L-6 mg/L (Nikhlani et al., 2022). Fish were fed using the at satiation method with a frequency of twice a day during rearing, around 08.00 and 17.00 WIB. During the treatment, every two days siphoning was carried out, and 50% water replacement of the total volume was carried out to maintain water quality.

Preparation of *Edwardsiella tarda* Cultures

Edwardsiella tarda cultures were obtained from the Aceh KIPM Station. One ose of bacteria was cultured in 10 mL of Tryptic Soy Broth (TSB) medium and incubated for 24 hours. After incubation, 1 mL of culture medium was re-cultured on Tryptic Soy Agar (TSA) medium and then incubated for 24 hours.

Pathogenicity Test

The pathogen test is to see or confirm that pathogenic bacterial isolates can infect healthy catfish fry with the same clinical symptoms (Ariani et al., 2009). Target bacterial isolates were cultured into 10 mL of Tryptone Soya Broth (TSB) and adjusted or equalized to 0.5 McFarland standard (10^8 CFU/mL). As much as 0.05 mL of a bacterial suspension with a density of 10^8 CFU/mL was injected intraperitoneally using a syringe into 10 catfish fries, and previously the catfish fry were inactivated in water with a temperature of 25 °C for 30 seconds by dipping (Wibowo et al., 2010).

After injection, the catfish fry were then put back into an aquarium containing 10 liters of water and observed for seven days or until clinical symptoms and death appeared in the catfish fry. The presence of pathogenic bacteria was then isolated from the liver and kidneys on Tryptic Soy Agar (TSA) media and incubated for 24 hours at 30 °C, and re-identified by biochemical tests (Wibowo et al., 2010). Pathogenic bacteria that can be deadly or cause clinical symptoms in catfish fry are used again in the next test stage.

Preparation of Suspension of *Edwardsiella tarda* and LC_{50} Test

Target bacterial colonies growing on TSA media suspension, equivalent to the McFarland 0.5 standard (Ariyanti et al., 2012). The way to test LC_{50} in this method is to inactivate catfish fry at 25 °C for 30 seconds by dipping (Wibowo et al., 2010), then as much as 0.05 mL of pathogenic bacteria (bacterial suspension) with a dilution of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} were injected intraperitoneally into 50 catfish fry. After the injection, the catfish fries were transferred to the maintenance aquarium. The number of catfish fry was observed from one day after the injection of pathogenic bacteria until the seventh day. Probit analysis using Microsoft Excel to calculate the LC_{50} determination.

Preparation of Feed with Asam Sunti

This study used commercial feed in crushed granules containing 30% protein,

3% crude fat, 12% moisture, and 4% fiber. Then the feed is added with commercial asam sunti at a predetermined dose and adjusted according to the treatment (Widanarni et al., 2012).

Asam Sunti Test in Controlling *Edwardsiella tarda* Infection

In each aquarium, 10 fish/aquarium of catfish fry were kept for seven days to control pathogenic bacteria. For feeding with a frequency of twice a day at satiation, namely at 08.30 and 17.00 WIB according to the treatment dose, namely treatment 0% (K), 0.5% (P1), 1% (P2), 2% (P3), and 4% (P4).

Catfish fry (test fish), inactivated by immersion in water at 25 °C for 30 seconds, were infected intraperitoneally with pathogenic bacteria (density according to LC_{50} test). Then the test fish were placed in a maintenance aquarium by providing a feed without asam sunti two times a day at satiation, namely at 08.30 and 17.00 WIB. Fish maintenance and treatment for 14 days (Dinamella et al., 2013).

Testing The Number of *Edwardsiella tarda* in The Liver and Kidneys of Fish

The determine of total plate count (TPC) of each pathogenic bacteria were determined to determine the number of pathogenic bacteria present in catfish fry (test fish). The method is that 1 g of liver and kidney of the test fish was taken aseptically and put into 10 mL of sterile butterfield's phosphate buffered (BFP), then homogenized and serial dilutions were carried out up to 10^{-6}. As much as 1 mL was inoculated from each dilution into a cup containing TSA media. At 30 °C, incubation was carried out for 48 hours. After 48 hours of incubation, the number of colonies was read using a colony counter.

Observation of Survival Rate

According to Agung et al. (2022), the stages of reducing and preventing pathogenic bacterial infection on survival using a formula to know the effect of treatment. The formula is**:**

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

With: SR = *Survival Rate* (%);
No = Initial population (tail)
Nt = Final population (tail)

Research Parameters

The parameters of this study included the level of pathogenicity of pathogenic bacteria, LC_{50} concentration of bacterial infections, control of pathogenic bacterial infections, TPC of pathogenic bacteria, absolute weight gain of fish, and environmental parameters, including temperature, and pH.

Data analysis

Data from observations of the pathogenicity of pathogenic bacteria, environmental parameters were analyzed descriptively presented in tabular form, LC_{50} observations of the concentration of bacterial infections were analyzed with probit. For controlling bacterial infection, TPC of pathogenic bacteria and absolute weight gain were analyzed using analysis of variance (ANOVA) with a 95% degree of confidence ($P<0.05$) using Statistical Package for the Social Science (SPSS) version of IBM 25 if there are significant differences significant, further tested by Duncan's Test 95% ($P<0.05$).

Results and Discussion

Pathogenicity of *Edwardsiella tarda* in Catfish Fry

Pathogenicity of *Edwardsiella tarda* in catfish fry was seen in the death of *E. tarda* treatment. The results showed that catfish infected with *E. tarda* had a mortality rate of 50% each, while in the control treatment, there was a mortality rate of 10% (Table 1).

The death of catfish due to infection with *E. tarda* showed that the bacterium was indeed a disease that attacked catfish fries. Nur (2019) said that *E. tarda* bacteria are pathogenic, which can cause death in catfish fry. According to Firma et al. (2012) *E. tarda* bacteria can cause disease in catfish fries, these two types of bacteria can infect the liver and kidneys so that their function is disrupted. Wahidi et al. (2022) explained that impaired liver function in *Clarias*

gariepinus causes fish to become susceptible to disease because the liver is unable to carry out the detoxification process and disrupts the phagocytosis of foreign bodies that enter the body, as a result of this disturbance, the catfish fries become weak accompanied by reduced appetite until it ends with death. Meanwhile, the death of control catfish fry was thought to be due to stress conditions and environmental factors (Hendri, 2022). Mortality of over 50% of catfish fries in a very short time was caused by *E. tarda* where the bacteria have a very high ferocity, major losses in catfish fry cultivation can be caused by *E. tarda* (Qosimah & Khotimah, 2020).

Table 1. The number of test catfish fry died in the tail/day pathogenicity test (n = 10)

Bacterial isolate	Days to-							Total	Percentage (%)
	1	2	3	4	5	6	7		
E. tarda	-	-	-	1	1	1	2	5	50%
Control	1	-	-	-	-	-	-	1	10%

LC_{50} Concentration of *Edwardsiella tarda* Infection in Catfish Fries

The higher number of dead catfish fry caused by the higher density of isolates of pathogenic bacteria was shown from the LC_{50} test results (Table 2.). These results are consistent with previous studies where isolates of the pathogenic bacteria *E. tarda* with higher densities increased fish mortality in a relatively short time of death (Narwiyani, 2011; Wahidi, 2022). Probit analysis using Microsoft Excel generated data where the LC_{50} value generated for *E. tarda* was 10^{-8}, this result is to the findings of Mufidah et al. (2022).

Table 2. Percentage of mortality rate in test catfish fry in LC_{50} test

Density (CFU/mL)	The average percentage of deaths (%)
	E. tarda
10^{-4}	90
10^{-5}	83
10^{-6}	70
10^{-7}	60
10^{-8}	50

Controlling *Edwardsiella tarda* Infections with Sunti Acid Treatment

Control of bacterial infections in catfish fries can be done in various ways, including by improving feed quality. The results showed that fish infected with *E. tarda* bacteria could survive, which was marked by increased survival in the feed treatment with the addition of sunti acid. Table 3 shows a significant difference between the treatments of adding asam sunti in feed, with the best treatment being 4%.

Table 3 shows that increasing the concentration of asam sunti increased the average survival rate of catfish fry in the presence of both pathogenic bacteria *E. tarda*. The survival of catfish fry in the presence of *E. tarda* showed a significant difference between the control treatment (K) and other treatments (P1, P2; P3 and P4).

Table 3. Percentage of survival of test catfish fries in infection control tests

Bacteria	The average survival of each treatment (%)				
	0% (K)	0.5% (P1)	1% (P2)	2% (P3)	4% (P4)
E. tarda	0.00 ± 0.00^{a}	16.67 ± 0.58^{b}	40.00 ± 0.00^{c}	66.67 ± 0.58^{d}	90.00 ± 1.00^{e}

Note: Control (K) *E. tarda* significantly differed in each treatment (P1, P2, P3, and P4).

From this test, it can also be concluded that the highest concentration results were in the treatment of sunti acid with a concentration of 4% (P4), with the survival of catfish fries reaching 90% in the presence of *E. tarda*. These results also show that the addition of asam sunti at a concentration of 4% is more effective in controlling pathogenic bacterial infections by the treatment of adding asam sunti to feed for catfish fries, these findings are also by the research of Misrahanum (2022) and Pertiwi et al. (2020) where the administration of asam sunti was able to prevent the occurrence of pathogenic bacterial infections. According to Amelia (2020), the content of asam sunti is useful for strengthening immunity in fish where the accumulation of primary metabolites (acetic acid, ethanol, carbon dioxide, and lactic acid) present in asam sunti can inhibit bacteria.

Total Plate Count of *Edwardsiella tarda*

At the end of the infection reduction test, the number of *E. tarda* in catfish fries was known by calculating the total plate count (TPC) of each *E. tarda* (Table 4).

Table 4. TPC value of pathogenic bacteria in the tested catfish fries

Bacteria	TPC (CFU/g)				
	0% (K)	0.5% (P1)	1% (P2)	2% (P3)	4% (P4)
E. tarda	1.8×10^6	0.1×10^5	8.6×10^4	3.6×10^4	9.0×10^3

Note: Control (K) *E. tarda* showed significantly different in each treatment (P1, P2, P3, and P4)

After treatment, pathogenic bacteria *E. tarda* were still found in the liver and kidneys of live catfish fry. In general, it was found that there was a decrease in the number of *E. tarda* in the treatment of 4%, 2%, and 1% as compared to the treatment of 0.5% and 0%. The data in Table 4 shows that sunti acid can inhibit or prevent the growth of bacterial pathogens. In accordance with Pertiwi et al. (2020) cited by Misrahanum et al. (2022) found that sunti acid produces flavonoids, terpenoids and saponins which have the potential as antimicrobials. Then according to Misrahanum et al. (2022) asam sunti contains organic acids that can prevent bacterial growth.

Conclusion

The infectivity of *Edwardsiella tarda* in the pathogenicity test showed survival in catfish fries with a mortality rate of up to 50%. Adding asam sunti to feed effectively controls pathogenic bacteria *E. tarda* in catfish fry. The concentration of 4% asam sunti in the feed shows the highest effectiveness in controlling pathogenic bacterial infections, whereas the *E. tarda* bacteria shows a figure of 90%.

References

Amelia, D., Irfannur, I., Baihaqi, B., & Akmal, A. (2020). The effect of giving wuluh starfruit juice (*Averrhoa bilimbi*) to increase the growth of the king danu goldfish (*Cyprinus carpio*). *Arwana: Jurnal Ilmiah Program Studi Perairan*, 2(1), 6-12. https://doi.org/10.51179/jipsbp.v2i1.343.

Chotiah, S. (2013). Potency of bacteriocin for animal health and food safety. *Wartazoa*, 23(2), 94-101. https://doi.org/10.14334/wartazoa.v23i2.719.

Firma, Amalia, R.R., Sari, U., Chusbul C., & Siregar, A.G. (2012). Detection of edwardsiella tardain catfish (*Clarias* sp.) by fluorescent antibody technique (FAT). *Jurnal Akuakultur Indonesia*, 11(1), 96-102. https://doi.org/10.19027/jai.11.96-102.

Hendri, A., Samuki, K., Mahendra, M., & Diana, F. (2022). Survival and growth of sangkuriang catfish (*Clarias gariepinus*) juvenile on electric shock 10, 15, 20 volt. *Jurnal Akuakultura*, 6(1), 28-38. https://doi.org/10.35308/ja.v6i1.6319

Ilmiah, Sukenda, Widanarni, & Harris, E. 2012. Selection of probiotic bacteria from the coral reef and tiger grouper fish (*Epinephelus foscoguttatus*) farming

environment. *Jurnal Akuakultur Indonesia*, 11(2), 109-117. https://doi.org /10.19027/jai.11.109-117

Maryam, St., Baits, M., & Nadia, A. (2015). Measurement of antioxidant activity and ethanol extract of moringa leaves (*Moringa oleifera* Lam.) using the frap method (ferric reducing antioxidant power). *Jurnal Fitofarmaka Indonesia*, 2(2), 115-118. https://doi.org/10.33096/jf fi.v2i2.181.

Mathew, C., Mdegela, R.H., Mwamengele, G.L., & Kassuku, A.A. (2014). Prevalence and mean intensity of ectoparasite infections in pond reared nile tilapia (*Oreochromis niloticus*) in Morogoro, Tanzania. *Tanzania Veterinary Journal*, 29(1), 63-71.

Misrahanum, M., Ayuningrum, N., & Helwati, H. (2022). Phytochemical and activity test asam sunti (*Averrhoa bilimbi L.*) activity as antimicrobial. *Jurnal Ilmiah Ibnu Sina*, 7(1), 155-164. https://doi.org/10.36387/jii s.v7i1.854.

Mufidah, T., Sukenda, S., Widanarni, W., Darusman, H.S., & Lusiastuti, A.M. (2022). Analysis of the pathogenesis of *Aeromonas hydrophila* in the african catfish, *Clarias gariepinus* and involvement of the TNF-α in response to the infection. *Indonesian Aquaculture Journal*, 17(1), 73-85.

Narwiyani, S. (2011). Lethal concentration 50% (LC-50) of four isolates *Edwardsiella tarda* four isolation freshwater fish from Indonesia. *Jurnal Sains Veteriner*, 29(1), 51-54. https://doi .org/10.15578/iaj.17.1.2022.73-85.

Nur, I. (2019). *Penyakit Ikan*. Deepublish Publisher. Daerah Istimewa Yogyakarta. ISBN 978-623-209-694-3

Ogueji, E., Iheanacho, S.C., Mbah, C., Yaji, A.J., & Ezemagu, U. (2019). Effect of partial and complete replacement of soybean with discarded cashew nut (*Anacardium occidentale L*) on liver and stomach histology of *Clarias gariepinus* (Burchell, 1822). *Aquacult. Fish*, 5(2), 86-91. https://doi.org/10.1016/j.aaf.2019. 10.005.

Pertiwi, D., Hafiz I., Jannah, W., Winata, H.S., Sari, M., & Suroyo, R.B. (2020). Antibacterial activities of belimbing wuluh (*Averrhoa bilimbi L.*) ethyl acetate extract on gel formulated against *Propionibacterium acnes* and *Staphylococcus aureus*. *International Journal of Applied Pharmaceutics*, 224-228. https://doi.org/10.22159/ijap.2020v1 2i6.39406.

Qosimah, D., and Khotimah, H. 2020. *Pengendalian dan Diagnosis Penyakit Ikan: Kausa Bakteri Dan Jamur*. Universitas Brawijaya Press.

Ryan, A., Iskandar & Taofiqurohman, A. (2012). Effectivity of commercial feed added with probiotic *Bacillus* sp. isolated from digestive tract of pangasius on survival and growth of red tilapia (*Oreochromis niloticus*). *Jurnal Perikanan dan Kelautan*, 3(3),75-83.

Sri, A.P.J., Hafrijal, S., & Azrita, A. (2022). Analysis of the use of gambir extract (*Uncaria gambir roxb*) on survival and growth of pearl catfish (*Clarias gariepinus*) infected with *Aeromonas hydrophila* bacteria (Doctoral dissertation, Universitas Bung Hatta).

Tran, N., Rodriguez, U.P., Chan, Y.C., Phillips, M.J., Mohan, J.G. Henriksson, Koeshendrajana, S., Suri, S., & Hall, S. (2017). Indonesian aquaculture futures: an analysis of fish supply and demand in Indonesia to 2030 and role of aquaculture using the asia fish model. *Marine Policy*, 79, 25–32. https://doi.org/10.1016/j.marp ol.2017.02.002.

Wahidi, B.R., & Prihartini, N.C. (2022). Histopathological of *Clarias gariepinus* infected with *Edwardsiella tarda* in Kediri, East Java. *Jurnal Akuakultur Rawa Indonesia*, 10(2), 186-198. https://d oi.org/10.36706/jari.v10i2.18863.

Widodo, E., Natsir, M. H., & Sjofjan, O. (2019). *Poultry Feed Additives Substitute Antibiotics: Responses to the Indonesian Government's Antibiotic Ban*. Universitas Brawijaya Press.

Wijayanti, T. R. A. & Safitri, R. 2018. Antibacterial test of starfruit leaf extract (*Averrhoa bilimbi Linn*) against the growth of *Staphylococcus aureus* bacteria that causes puerperal infection. *Jurnal Ilmiah Ilmu Kesehatan*, 6(3), 277-285. https://doi.org/10.33366/jc.v6i3.999.