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The Effect of Coconut Shell Liquid Smoke in Commercial Feed Towards Total *Pseudomonasaeruginosa* Bacteria on Gastrointestinal Tract Tilapia (*Oreochromis Niloticus*)

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The Effect of Coconut Shell Liquid Smoke in Commercial Feed Towards Total *Pseudomonas aeruginosa* Bacteria on Gastrointestinal Tract Tilapia (*Oreochromis Niloticus*)

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Abstract. Bacteria *Pseudomonas aeruginosa* is one of the pathogenic bacteria that can be found in the gastrointestinal tract of fish and can affect cultivation. Bacterial disease in fish can be overcome by antibiotics, but antibiotics cause negative effects. The use of coconut shell liquid smoke in the feed is needed to decrease the growth of harmful bacteria, the liquid coconut shell contains compounds such as phenols and acids that can be used as antibacterials. Result of research of total bacterium of *Pseudomonas aeruginosa* on gastrointestinal tract of tilapia fish have an effect significantly different ($P < 0,05$) to decrease of total bacteria *Pseudomonas aeruginosa* on gastrointestinal tract tilapia (*Oreochromis niloticus*). The use of coconut shell liquid smoke in the feed is most effective for decrease *Pseudomonas aeruginosa* bacteria in the gastrointestinal tract of tilapia (*Oreochromis niloticus*) in treatment with a concentration of 1.5% ie $2,11 \times 10^5$ CFU/g.

Keyword. *Pseudomonas aeruginosa*, tilapia, coconut shell liquid, gastrointestinal tract.

1. Introduction

Tilapia (*Oreochromis niloticus*) is one of the leading commodities of freshwater fish that is easily cultivated, grows rapidly using relatively easy cultivation techniques, and tolerant to the rather bad environment (Widyastuti and Eri, 2013). Tilapia aquaculture business develops intensively so that the changes in the aquaculture environment occur due to the high pollution and improper handling of cultivations, such as the inefficient use of feed which triggers the emergence of disease problems (Aniputri et al., 2014).

One type of fish disease that is often encountered is bacterial disease (Meidiza et al., 2017). *Pseudomonas aeruginosa* is one of the pathogenic bacteria that affects tilapia cultivation and can be found in the gastrointestinal tract of fish (Sivakami et al., 1996). ***Pseudomonas aeruginosa*** bacteria can cause swelling of the stomach, exophthalmia, opacity, wounds on the body and tail eroded, bleeding in the internal organs, around the mouth, opercula and necrosis in the tissues of the spleen



and kidneys (Manurung and Santie, 2017; Hardi et al., 2012). Measures to prevent and overcome the attack of *Pseudomonas aeruginosa* bacteria in tilapia aquaculture activities include administration of antibiotics (Aniputri et al., 2014). Delduca et al. (2013), explained that the use of antibiotics is less efficient because it is not economical and can have a negative impact, including bacterial resistance to these antibiotics and can pollute the environment.

Materials that can be used as a substitute for antibiotics are coconut shell liquid smoke. One of the factors that influence the feed growth and efficiency is the presence of a population of pathogenic bacteria. The use of coconut shell liquid smoke in rations is needed to reduce the development of harmful bacteria (Permatahati et al., 2014). Coconut shell liquid smoke exhibits excellent antibacterial activity for *Pseudomonas aeruginosa* (Anisah, 2014).

Based on the stated problem background, coconut shell liquid smoke contains phenolic compounds and organic acids which can be used as antibacterial, especially *Pseudomonas aeruginosa* bacteria. Therefore, this study was conducted to show the effect of giving coconut shell liquid smoke in commercial feed on the total bacteria of *Pseudomonas aeruginosa* in the gastrointestinal tract of tilapia (*Oreochromis niloticus*).

2. Methodology

2.1 Place and time of research

The research was performed from May to July 2018 at the Faculty of Fisheries and Marine of Universitas Airlangga, Surabaya.

2.2 Equipments and materials

The material used in this research was tilapia (*Oreochromis niloticus*) originating from the Technical Implementation Unit for the Development of the Umubulan, Pasuruan Freshwater Cultivation, at the age of 1.5 months with a size of around 7-9 cm/tail and weighing at 6-10 grams. The bacteria *Pseudomonas aeruginosa* used in this research was from the Testing Service Unit of Universitas Airlangga, Surabaya. Coconut shell liquid was from Yogyakarta Al-Madaniah grade 1 and Hi-Pro-Vite 781 commercial feed. Other materials used were feed Tryptone Soya Agar (TSA), Tryptone Soya Broth (TSB), Cetrimide agar, glycerine, alcohol 70%, 0.9% NaCl and distilled water.

The equipment used in this research included 20 aquariums with a size of 50*30*30 cm³, aerators, 20 pieces of aeration, hose squeezing, aeration hose, bucket, analytic balance (Ohaus Scout-Pro), ruler, scissors, pH test, thermometer, DO meter (YSI 550A), ammonia test kit, reservoir, net, microscope, hotplate and magnetic stirrer (AM4 VUP Scientifica), microtube, micropipette (0.5 - 1000 pl), vortex (VM-1000), centrifuge, cotton, plastic wrap, measuring cups (100 ml and 500 ml), test tubes (10 ml and 15 ml), bulbs, beaker glass (100 ml and 250 ml), petri dishes, Erlenmeyer tubes (100 ml, 250 ml and 500 ml), glass object, volume pipette (1 ml and 100 ml), opaque paper, autoclave (Hirayama, Japan), Laminar Air flow, refrigerator, incubator, tray, tweezers and syringe.

2.3 Method

Completely Randomized Design (CRD) was used because there was only one source of diversity in this study, namely the concentration of coconut shell liquid smoke mixed in feed. Completely randomized design only had one source of diversity, namely treatment in addition to random influences, so the results of differences between treatments were only caused by the effects of treatment and random effects.

3. Work procedures

3.1 Rejuvenation of *pseudomonas aeruginosa* bacteria

Sterilization of test tubes, petri dishes, measuring pipettes and glass cups were done using autoclave at a temperature of 121°C and a pressure of 1 atm for 15 minutes. The bacteria *Pseudomonas aeruginosa* ATCC 27853 used in this research came from the Testing Service Unit of Universitas Airlangga, Surabaya. Pure culture of *Pseudomonas aeruginosa* ATCC 27853 rejuvenated on TSA media to tilt as

much as one colony was scratched using the scratch method on tilted TSA media and incubated for 24 hours at 30°C. Bacterial planting was performed by taking one ose of bacterial colonies using an ose loop from bacteria that had been grown on the TSA media to tilt, then rejuvenated in a test tube that had been filled with TSB media. Pure culture was incubated for 24 hours at 30°C.

Having been cultured from rejuvenating cultures on grown Triptone Soya Broth (TSB), the *Pseudomonas* bacteria was then centrifuged at 3000 rpm for 20 minutes (Hossain, 2006). Then the supernatant was discarded and added with physiological NaCl solution of 0.9% until the volume was the same as the initial volume, then homogenized using vortex and centrifuged again three times. Furthermore, density calculations were performed using turbidimeters (Kelley et al., 2014). After knowing the number of bacteria, when the bacteria were too dense, dilution was performed to obtain a density of 2×10^7 CFU/ml.

3.2 Testing Feed Production

The ingredients used were commercial feed and coconut shell liquid smoke. Pellets to be used as feed were formed into flour beforehand and then mixed with coconut shell liquid smoke according to the concentration of treatment. Liquid smoke was measured by measuring cup according to the dose in use: 10 ml/kg of feed, 15 ml/kg of feed and 20 ml/kg of feed. After mixing the feed and liquid smoke, it was then reprinted until it was in the form of pellets measuring 2-3 mm in size.

3.3 Cultivating Tilapia

Tools that would be used in cultivating were $50 \times 30 \times 30$ cm³ aquariums, aeration hoses, aerated stones are sterilized by chemical means, soaked with 400 ppm chlorine solution for 24 hours, then rinsed using water and dried (BBL, 2003). The dried aquarium was placed on a wooden shelf, filled with 25 liters of fresh water and given aeration.

Tilapia (*O. niloticus*) was put into an aquarium of 10 tails and acclimatized for 5 days with the aim of environmental adaptation and test feed. After adaptation, the fish were fasted for 24 hours with the aim of eliminating the effect of remaining food in the body of the fish. After the fish were fasted the length and weight of the body were measured as data for the initial length and weight of the fish body (Mulyani et al., 2014). Feed was given twice a day as much as 5% of the total body weight of the test fish. Feeding was performed at 09.00 and 16.00 WIB (Soedibya, 2013). Growth measurements were performed every 7 days.

Water quality in cultivation media was squeezed the remaining food waste and metabolism in the aquarium every 3 days. This squeezing was performed by replacing water as much as 50% of the previous water. Measurement and recording of water quality was performed every day in the morning and evening including pH, oxygen solubility and temperature of water and ammonia measured and recorded once a week during treatment (Centyana, 2014).

3.4 Infection of bacteria in tilapia

The infusion of *Pseudomonas aeruginosa* with 0.2 ml/CFU/ml density of 0.2 ml/head was injected intraperitoneally with 1 ml syringe in 10 tilapia each replicate (Hardi, 2014). In the control treatment, 0.9% NaCl was injected at 0.2 ml/head intraperitoneally with a 1ml syringe. The position of the intraperitoneal injection was between the pelvic and anal fins, and the injected tilapia was then put into the aquarium and maintained for 21 days (Saad et al., 2014). Observation of clinical symptoms of fish infected with *Pseudomonas aeruginosa* bacteria was performed for 1 week and water quality measurements.

3.5 Calculation of the number of bacteria in the gastrointestinal tract

Selective media were used to isolate *Pseudomonas aeruginosa*, namely cetrimide agar media (Brown and Lowbury, 1965). The Total Plate Count (TPC) method was performed by making multilevel dilutions. dilutions from 10^{-3} to 10^{-5} (Kamal et al., 2016).

TPC calculation was performed using the spread method. The spread method was performed by

taking 1 ml of the diluted sample using a micropipette from the dilution tube and transferred into two petri dishes containing Cetrimide Agar, which had been hardened in duplo and flattened using drigalski. Then it was put in an incubator at 30°C for 24 hours.

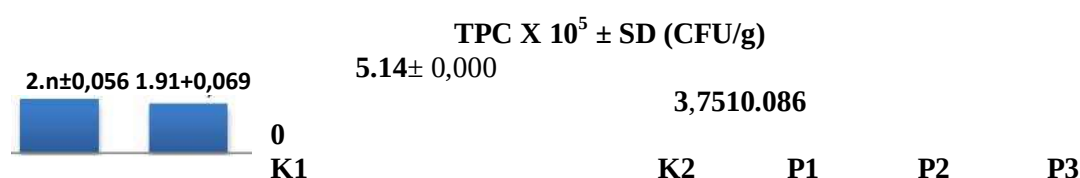
The counting of the number of bacterial colonies was by means of a petri dish placed on a black background. The count of colonies was petri dishes which have bacterial colonies between 25-250 colonies (Indonesian National Standardization Agency, 2006). In duplo dilution (one dilution using two petri dishes) the amount used was the average of the two plates. The calculation results are multiplied by 10 to one according to the dilution.

3.6 Data analysis

Data analysis was performed experimentally, in which the collected data were analyzed using ANOVA (Analysis of Variance) test. In the event of any difference, the analysis was then followed by Duncan's multiple test with a real level of 5% (Kusriningrum, 2012).

4. Results and discussion

The average of Total Plate Count (TPC) of *Pseudomonas aeruginosa* bacteria in the gastrointestinal tract of tilapia (*Oreochromis niloticus*) (CFU/g)



The highest total value of *Pseudomonas aeruginosa* bacteria in the gastrointestinal tract of tilapia (*Oreochromis niloticus*) during the research is K2 treatment of 5.14×10^5 CFU/g, then followed by lower total bacteria in a row, that are P1 3.75×10^5 CFU/g, P2 2.11×10^5 CFU/g P3 1.91×10^5 CFU/g and K1 0.00×10^5 CFU/g. The total statistical test of the *Pseudomonas aeruginosa* bacteria on the gastrointestinal tract of tilapia (*Oreochromis niloticus*) shows that there are significant differences ($P < 0.05$), so that it can be said that coconut shell liquid smoke affects the total decrease of *Pseudomonas aeruginosa* bacteria. Therefore, it was continued by Duncan's Multiple Range Test.

The P2 treatment on feed mixed with coconut shell liquid smoke can reduce the total bacterial *Pseudomonas aeruginosa* in the gastrointestinal tract because liquid smoke had an active substance namely acid and phenol. Phenol is the dominant chemical component in coconut shell liquid smoke, which can be bacteriostatic and bactericidal (Karseno et al., 2002). The mechanism of phenol as an antibacterial is that it passes through the cell wall and damages the cytoplasmic membrane resulting in disturbed membrane permeability (Anisah, 2014). Acidic components in liquid smoke can acidify the cytoplasm, damage the membrane surface tension and lose the active transport of nutrients through the membrane so that it can cause various functions and structural components of cells to become unstable (Aisyah et al., 2013). The ability of phenol as an antibacterial increase as it synergizes with acidic compounds.

Infection of *Pseudomonas aeruginosa* 2×10^7 CFU/ml in fish will cause an imbalance of the normal intestinal flora. This is according to statement of (Widowati et al., 2014) who claimed that *Pseudomonas aeruginosa* produces enterotoxins which are bacterial extracellular toxins specifically attacking the gastrointestinal tract. Bacteria contained in the gastrointestinal tract can cause inflammation and destruction of the intestinal lining. Besides, the toxin will also interfere with the process of absorption of nutrients. Hardi et al. (2014) stated that *Pseudomonas sp.* produce exotoxin and endotoxin which will damage the intestinal blood vein endothelial.

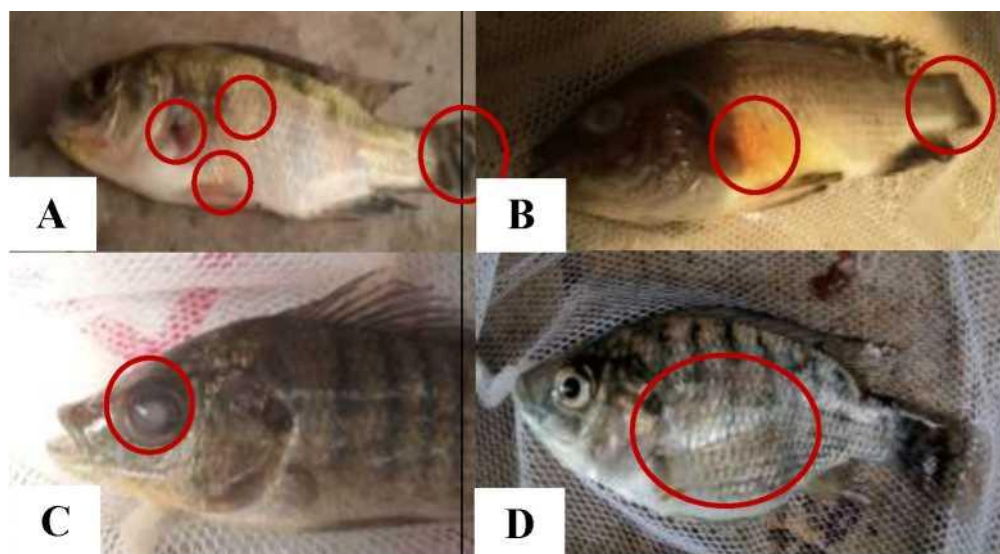


Figure 1. Clinical symptoms.

Description: red spots and flaky tails (a), red spots and flaky tails (b), opacities (opacity) (c), swollen abdomen (d).

The clinical symptoms in this research are the supporting parameters. The changes that occur include red spots, opacities (opacity), flaky fins and fluid filled abdomen. This is consistent with the report that *Pseudomonas sp.* infecting tilapia will produce extracellular products by showing symptoms such as flaky fins, bleeding on the skin, wounds on the surface of the body, opacities (opacity), changes in internal organs that appear runny and a decrease in consistency in the gastrointestinal tract (Hardi et al., 2012; Hardi et al., 2014; Nurjanah et al., 2014). According to Herfiani et al. (2011), the occurrence of death in fish is closely related to bacterial pathogenicity, speed of propagation of pathogens, and host defenses against pathogens.

Table 1. Water quality.

Parameter	Range
Temperature(°C)	27-29.4
pH	7
Dissolved Oxygen (mg/l)	3.99-5.96
Ammonia (mg/l)	0-0.5

Water quality parameters at the beginning and end of the observation shows a reasonable range for tilapia cultivation media. The temperature range during the research is still in the normal range for tilapia maintenance, which is at 27-29.4°C. Tilapia can live at temperatures of 25-30°C (Ardita et al., 2015). Athirah (2013) stated that temperature affects metabolism and growth of organisms and influences the amount of feed consumed by aquatic organisms. Drastic temperature changes can cause physiological disorders of fish that can lead to stress. During the research on the pH value of tilapia maintenance which shows 7, the pH is still ideal for the survival of tilapia. This is based on BSNI (2009) which stated that the pH range of water for tilapia is 6.5-8.5. The pH value in a waters can affect the growth of tilapia in aquaculture and can cause death if the pH value was incompatible with living media (Centyana et al., 2014).

Dissolved oxygen levels in the research aquarium range from 3.99 - 5.96 mg/l. This is in accordance with BSNI (2009) who claimed that dissolved oxygen levels in tilapia culture media must be higher than 3.0 mg/l. Fujaya (2008) stated that oxygen as a respiratory material needed by cells for

metabolic reactions, reduced levels of dissolved oxygen in the waters will affect the physiology of fish respiration. Ammonia in the research aquarium ranges from 0-0.5 mg/l. This is in accordance with the statement of Centyana et al. (2014), that ammonia in tilapia does not exceed 0.5 mg/L. The high ammonia level of a waters is caused by the metabolism of fish and the rest of the feed, the decomposition of metabolites and the remaining food remaining will increase the concentration of ammonia in the culture so that the ammonia rate increases (Nugroho et al., 2013). Based on these results, the condition of water quality in the research is almost the same as the normal range of tilapia fish habitat, allowing tilapia to live well. The provision of coconut shell liquid smoke does not affect the quality of the waters.

5. Conclusion and suggestion

5.1 Conclusion

The conclusion of this research is that the use of coconut shell liquid smoke in feed affects the total decrease in *Pseudomonas aeruginosa* in the gastrointestinal tract of tilapia (*Oreochromis niloticus*) and the use of coconut shell liquid smoke in the most effective feed to reduce the *Pseudomonas aeruginosa* bacteria in the tilapia's (*Oreochromis niloticus*) gastrointestinal tract in the treatment at a concentration of 1.5% is 2.11×10^5 CFU/g.

5.2 Suggestion

It is suggested that a further studies need to be conducted for further research on histopathology, calculation of erythrocytes and leukocytes of tilapia on coconut shell liquid smoke with different concentrations mixed on commercial feed. The concentration of 1.5% of coconut shell liquid smoke on feed can be applied to tilapia cultivators.

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