Jurnal Sain Peternakan Indonesia
Available at https://ejournal.unib.ac.id/index.php/jspi/index
DOI: https://doi.org/10.31186/jspi.id.16.3.283-287

P-ISSN 1978-3000
E-ISSN 2528-7109
Volume 16 Issue 3 July-September 2021

Eco-enzyme Disinfection in Pig Housing as an Effort to Suppress *Esherechia coli* Population

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ABSTRACT

Pig farms are susceptible to infection by *Escherechia coli* bacteria which causes Colibacillosis. A dirty cage is one of the causes. This can be prevented by disinfection. Eco- enzymes are fermented fruits that contain enzymes and are anti-microbial. This study aims to find a dilution of Eco- enzyme that can suppress the population of *Escherechia coli* in pig pens. The study was carried out in two stages, first was in *in vitro* to find the best treatment and second was in *in vivo* to determine the decrease in the population of *E. coli* in pig pens after disinfection with Eco-enzymes. The in vitro treatments were P1: Eco enzyme diluted 1:10; P2 1:20 and P3 1:30. Parameters are the area of the inhibition zone and the antimicrobial index. In an *in-vivo* study, the data taken were the population of *E. coli* on the floor of the cage and in the waterways before and after disinfection. The results showed that Eco enzyme diluted 1:30 had the best inhibition zone and antimicrobial index. Disinfection of pig pens using Eco-enzyme dilution of 1:30 was successful in reducing the population of *E.coli* in pig pens.

Key words: biodisinfectant, colibacillosis, eco- enzyme, pig pen, zone of inhibition.

INTRODUCTION

In North Sumatra Province, almost all 33 districts/cities have non-Muslim communities raising pigs. The population of pigs is around 1,274,904 heads (Department of Food Security and Animal Husbandry of North Sumatra, 2019). Pig farming is still carried out traditionally, which has not received good attention on aspects of cages, feed, health, growth, and reproduction. Pig farming is a part-time business to be a savings account, converting organic waste, materials for traditional ceremonies, religion, and culture as well as a source of fertilizer. Currently, farmers have started to make more efforts to gain economic benefits by raising pigs rather than raising pigs for religious and social activities. Its reproductive characteristics are unique compared to other cultivated livestock because pigs are productive animals, mainly due to the high number of births (8-14 heads/birth), and the distance between one birth and the next birth is about four months so that in a year the pig gives birth twice (Sihombing, 2006).

Pig rearing in North Sumatra is mostly done by the community. Productivity is not optimal, because of the diseases such as Colibacillosis or infection with *Escherichia coli*, which is one of the main diseases affecting the pig industry. Most cause deaths occured in newborn pigs and newly weaned pigs (Fairbrother and Nadeau, 2019).

Colibacillosis can be caused by poor environmental conditions such as dirty cages that stimulate the growth and reproduction of *Escherechia coli* bacteria which survive outside the host's body and can infect other individuals and cause serious health problems (Dyantoro, 2017). One effective way to prevent and minimize colitis is to regularly disinfect the cage. Disinfection plays an important role in controlling pathogenic bacteria (Gosling, 2018).

Disinfection is not carried out routinely, especially by small farmers. This is partly related to the ignorance of farmers about the importance of disinfection due to the high price of the commercial disinfection process (Collineau and Stark, 2018). Therefore, it is necessary to find an alternative disinfectant formula that is effective in suppressing the growth of pathogenic bacteria and at an affordable price.

Eco-enzymes are produced from fruit fermentation which can also be combined with vegetables, non-chlorinated water, and molasses (Chelliah and Palani, 2015). Ecoenzymes contain complex organic substances from protein chains, minerals, and salts (Tang and Tong, 2011). Fermentation lasts for three months. Eco-enzymes contain protease, amylase, and lipase enzymes which are antimicrobial/pathogenic. In addition, Ecoenzymes can control gram-negative and positive bacteria (Rahman et al., 2020). Furthermore, Rahman et al., (2020) conducted an Eco-enzyme antagonist test against *E.coli* and found a clear zone of 8mm. Ginting (2020) stated

that due to the low cost of producing Eco enzymes, it is feasible to be used by the farming community as a bio-disinfectant. Ecoenzymes also contains several organic acids (acetic acid, lactic acid, malic acid, oxalic acid, and citric acid), and after three months the concentration of acetic acid increases because the raw materials used in this anaerobic fermentation are fruits (Arun, 2015A; Arun, 2015B).

This study aimed to determine the ability of the Eco-enzyme which was diluted and then tested *in vitro* as an antibacterial against *E. coli*. Furthermore, the results of in-vitro testing were continued with *in vivo* research, which was used as a bio-disinfectant in pig pens and applied by flushing the floor of the cage.

MATERIALS AND METHODS

The *in vitro* research was conducted at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Sumatra Utara University. The *in vitro* research was started by conducting a diluted Eco-enzyme antagonist test against *E.coli*. The study used a completely randomized design. The research treatments were as follows: P1 Eco- enzyme diluted 1:10; P2 1:20 and P3 1:30 with five replicates. The research parameters were the area of the inhibition zone and the antimicrobial index. The dilution ratio was determined after surveying the pigsty that the farmer needed about 20 liters of water to clean a 2x2 m^2 pig pen containing about 6 piglets. The best dilution is applied to pig pens as a biodisinfectant. Liquid samples were taken on the floor of the cage and in the drainage channel before and after the disinfection treatment. *E. coli* population was calculated.

Research Materials and Tools

The research material is extract of Eco-enzyme and pathogenic bacteria *E. coli* ATCC25922 Remel Apogeat from the Medan Veterinary Center. The medium used was Nutrient Gell, Muller Hinton Agar (MHA), MRSA and MRSB. The tools used in this research are a cork borer, needle nose incubator, autoclave, microscope, micro-pipette, vortex, pH meter, paper disc, caliper and digital camera.

Eco-enzyme Preparation

An amount of 300 g of molasses is mixed with 3000 cc of non-chlorinated water in a 10-liter plastic jerry can. Fruit waste consisting of pineapple, banana, and papaya each weighing 300 g, a total of 900 g chopped 3 cm was put into a plastic jerry can which already contains a mixture of molasses and non-chlorine water. The mixture in the container was left anaerobic for 100 days. After 100 days, the mixture was filtered and Eco-enzyme extract was produced. Furthermore, the research process was carried out using Eco-enzyme extract. Eco-enzyme dilutions were prepared by adding the extraction described above with distilled water. Then all treatments were sterilized.

Preparation of Mueller Hinton Agar (MHA) Media

An amount of 19 grams of Mueller Hinton Agar (MHA) media was dissolved in 500 ml of distilled water, sterilized using an autoclave at 1210C at 1 atm pressure for 15 minutes. The sterile MHA media waited until the temperature dropped to 50-60^{0}C then poured into sterile Petri dishes.

Preparation of Nutrient Agar (NA) Media

In this step, 2 g of NG medium was dissolved in 100 ml of distilled water and then heated until completely dissolved. Each 5 ml was put into a test tube and then sterilized using an autoclave at 121^{0}C with a pressure of 1 atm for 15 minutes. The sterile medium was kept at a tilted position.

Table 1. Eco-enzyme dilution with distilled water

Treatment	Comparison of eco- enzymes with distilled water	Eco -enzyme dilution
P1	1:10	1ml Eco- Enzyme added to 10 ml of distilled water
P2	1:20	1 ml Eco- enzyme added to 20 ml distilled water
P3	1:30	1 ml Eco- enzyme added to 30 ml of distilled water

Bacterial rejuvenation

E.coli was rejuvenated by inoculating the bacteria by scraping it from the main strain onto a slanted NA medium that has been sterilized, then incubated at 35^0C for 18-24 hours.

Preparation of bacterial suspension

E.coli bacteria were taken one needle nose from the bacterial stock then dissolved in 5 ml of sterile distilled water, then the turbidity was measured using McFarland where the turbidity of the McFarland solution was 0.5 so that the number of bacteria obtained was equivalent to 1x108 CFU/mL. The McFarland standard is used to visually estimate the concentration of cells present in suspension.

Preparation of positive control using the antibiotic Chloramphenicol

E.coli samples were isolated in test tubes containing 5 ml of physiological NaCl. Homogenized using vortex with standard Mc.Farland 0.5. Furthermore, the bacteria were distributed evenly on the entire surface of the Mueller Hinton Agar (MHA) media then continued by placing the Chloramphenicol antibiotic disc on the media. Bacterial cultures were incubated at 37^OC for 24 hours.

Antibacterial Activity Test

Antibacterial effectiveness was tested using the disc diffusion method and inhibition zone measurement. The sterile disc paper was dripped with 20-micron liters of Eco-enzyme and allowed to stand for approximately 3 hours. Bacterial scraping was carried out using a sterile cotton swab. Discs that have been dripped with Eco-enzyme are inserted into agar media that has been filtered with bacterial suspension. After being incubated for 18-24 hours, the antibacterial activity of Eco-enzyme was observed and the diameter of the clear zone formed around the paper disc was measured.

RESULTS AND DISCUSSION

Eco-enzyme Antagonist Test against *E.coli*

Based on the antagonist test using Ecoenzyme solution against pathogenic bacteria *E.coli* using MRSA media, data on the area of inhibition zones and antimicrobial index were obtained as shown in Table 2. Rahman et al. (2020) found an 8 mm inhibition zone through a pure Eco-enzyme antagonist test against *E.coli.*

Table 2. The results of the Ecoenzyme antagonist test against Escherechia coli

Treatment	Average inhibition zone (mm)	Average Antimicrobial Index (mm)
P1	6.65[a]	0.108[a]
P2	6.85[a]	0.142[a]
P3	6.90[a]	0.150[a]

Same superscript shows no significant differences

The research of Rahman et al. (2020) used the raw materials of pumpkin skin, cabbage, potatoes, and molasses in the manufacture of Eco-enzymes. Further said that protease, amylase, and lipase enzymes from Eco -enzymes showed antimicrobial activity.

In this study, it was found that the inhibition zone and antimicrobial index were not significantly different between treatments, although there was a tendency for P3 to have a better inhibition zone and antimicrobial index than other treatments. The raw materials for making Eco-enzymes in this study were papaya, pineapple, and banana. Bananas contain the enzyme pectinase while papaya and pineapple contain protease enzymes. The content of protease enzymes in papaya and pineapple is high so that local people in Sumatra have used them as a starter in the process of fermenting foods such as curd from milk due to the presence of protease enzymes (Ginting, 2018). The presence of these enzymes also strengthens the substrate in suppressing the development of pathogenic bacteria. This is in accordance with Fleming (2017) who stated that protease enzymes were able to degrade bacterial biofilms by hydrolyzing them.

Neupane and Khadka (2019) stated that different fruit wastes showed differences in enzyme activity and antimicrobial activity. Enzymes produced from fruit waste show antimicrobial properties against gram-negative bacteria such as *E.coli*. In the manufacture of Ecoenzymes in his research, yeast (*Saccharomyces cerevisiae*) and bacterial suspension of *Bacillus* species were added. The antagonist test conducted on *E.coli* found an inhibition zone of 14 mm using papaya extract, 27 mm using pineapple extract, and 19 mm using mixed fruit extract. Neupane and Khadka (2019) said that pineapple, papaya, and mixed fruits have protease enzyme activity.

Saramanda and Kaparapu (2017) found that the antimicrobial activity of Eco-enzymes from orange peel extract showed a good inhibition zone against *E. Coli* of 11 mm. Orange peel contains the enzyme pectinase. In this study, an antibacterial test was also carried out using the antibiotic chloramphenicol and obtained an inhibition zone of 29.9 mm or an antimicrobial index of 3.65 mm. Chloramphenicol is a broad-spectrum antibiotic that fights against various bacterial pathogens such as Staphylococcus, Escherichia coli, Salmonella, and many others. Meanwhile, Rahman et al., (2020) using Gentamicin found an inhibition zone of 8.5 mm.

After doing the in-vitro test, it was concluded that P3 was the best. This was because of the three treatments, P3 had the largest inhibitory zone and antimicrobial index while P3 was 1:30 dilution, meaning that fewer Eco enzymes were added than other treatments. Since cleaning the pigsty requires a lot of water, it is more appropriate to use a 1:30 dilution.

The survey results, before the research was conducted, it was known that the dominant pig breeders do not carry out disinfection in the pig pens. The process of cleaning the pigsty is only by separating the solid waste and then piling it in the yard. If water is available, the cage is doused with water. People's pig farmers are reluctant to use disinfectants because they do not have the budget to buy disinfectants. Cases of pigs with diarrhea often occur, possibly related to dirty cages so that they are infected with *Escherichia coli* (Elisha et al., 2017). Therefore, pig farmers must know about pen disinfection. One possible action is to teach the community to make their organic disinfectant. In this study, an organic Eco-enzyme disinfectant was tested on the floor of the pigsty and in the drain of the cage's laundry water.

Table 3. Population of *E. coli* without and with Eco-enzyme biodisinfectant

Location	Populasi *E Coli*	
	No disinfection	Disinfection
Cage floor	$1x\ 10^7$	$2x10^2$
Cage flushing	17.5×10^4	$1x10^4$

Data were collected from the cage before and after disinfection. Disinfection was carried out by sweeping the floor of the cage using Eco-enzyme disinfectant which was diluted 1:30. Data were taken after the cage was watered consecutively for five days (Sulistyarsi, 2016). Before disinfection, the presence of *E. coli* on the floor of the cage was much higher than in the cage's laundry drain. This can be understood because the floor of the research cage is not smooth, that is, there are many small holes even though it is from the cement floor. The uneven floor of the cage still harbors *E. coli* from pig feces.

After disinfection, there was a decrease in the population of *E. coli* on the floor of the cage. This is because the biodisinfectant is flushed on the floor of the cage which then flows into the cage's laundry channel. There was also a decrease in the *E. coli* population in the cage laundry. Abuoun et al. (2020) stated that disinfection treatment to reduce the population of *E.coli* in pig pens was prioritized over-treating sick pigs due to Colibacillosis by injecting antibiotics. Antibiotics provide opportunities for antibiotic resistance to occur.

CONCLUSION

The results obtained in this study confirmed that the Eco-enzyme diluted at a concentration of 1:30 was able to suppress the development of *E.coli.* In addition, the application of Eco-enzymes diluted 1:30 was able to reduce the population of *E.coli* on the floor of the pig pens and in the waterways. This study shows promising potential for microbial disinfection to prevent cases of Colibacillosis.

ACKNOWLEDGEMENT

The author expresses his gratitude for the financial support from the University of North Sumatra, under the TALENTA 2020 Research Grant No. 441/UN5.2.3.1/PPM/SPPTALENTA USU/ 2020.

REFERENCES

AbuOun, M., H. M.O'Connor, E. Stubberfield, J. Nunez-Garcia, E. Sayers, D.W.Crook, R. R. Smith and M. F. Anjum. 2020. Characterizing antimicrobial resistant *Escherichia coli* and associated risk factors in a cross-sectional study of pig farms in Great Britain. Frontiers in Microbiology, 11: 861. doi: 10.3389/fmicb.2020.00861. eCollection

Arun, C., P. Sivashanmugam. 2015[a]. Investigation of biocatalytic potential of garbage enzyme and its infuence on stabilization of industrial waste activated sludge. Process Saf Environ Prot. 94: 471–478.

Arun, C., Sivashanmugam. 2015[b].Identifcation and optimization of parameters for the semi-continuous production of garbage enzyme from pre-consumer organic waste by green RP-HPLC method. Waste Manage 44: 28–33.

Chelliah, A. and S. Palani. 2015[c]. Investigation of biocatalytic potential of garbage enzyme and its influence on stabilization of industrial waste activated sludge. Process Safety and Environmental Protection 94: 471-478.

Collineau, L. and K. D. C. Stark. 2018. How to motivate farmers to implement biosecurity measures. In book : Biosecurity in animal production and veterinary medicine. ACCO UITgeverij.

Dinas Ketahanan Pangan dan Peternakan Provinsi Sumatera Utara. 2019. Renstra.

Dyantoro and S. Wulandari. 2017. Deteksi antibodi Salmonella pullorum mycoplasma gallisepticum pada anak ayam (DOC) pedaging beberapa perusahaan yang dijual di kabupaten Lamongan. Jurnal Agro Veteriner 5 (2): 152-157.

Elisha, I. L., F. S. Botha, L. J. McGaw and J. N. Eloff. 2017. The antibacterial activity of extracts of nine plant species with good activity against Escherichia coli against five other bacteria and cytotoxicity of extracts. BMC Complementary and Alternative Medicine 17:133. doi: 10.1186/s12906-017-1645-z.

Fairbrother, John M., E. Nadeau. 2019. Deseases of Swine, Eleventh Edition. Wiley Online Library.

Fleming, D. and K. P. Rumbaugh. 2017. Approaches to dispersing medical bioflms. Microorganisms 5 (2) : 15.

Gosling, R. J. 2018. A review of cleaning and disinfection studies in farming environments. Livestock 23 (5): 232-237.

Neupane, K. and R. Khadka. 2019. Production of Garbage Enzyme from Different Fruit and Vegetable Wastes and Evaluation of Its Enzymatic and Antimicrobial Efficacy. TUJM. 6 (1) 113-118.

Ginting, N. 2018. Dadih Bamboo Ampel (*Bambusa vulgaris*) and Bamboo Gombong (*Gigantochloa verticilata*) 2 and 3 days fermented : effect on salad dressing hedonic quality. IOP Conf. Series: Earth and Environmental Science 130 (2018) 012029 doi :10.1088/1755-1315/130/1/012029.

Ginting, N. 2020. Financal Analysis of GE (Garbage Enzyme) Application at Universitas Sumatera Utara Campus. Sustainable Campus Effort during the Covid-19 Pandemic. In press.

Rahman, S., I. Haque, R. C. D. Goswami, P. Barooah, K. Sood, B. Choudhury. 2020. Characterization and FPLC Analysis of Garbage Enzyme : Biocatalytic and Antimicrobial Activity. Waste and Biomas Valorization.

Saramanda, G. And J. Kaparapu. 2017. Antimicrobial activity of fermented citrus fruit peel extract. Int Journal of Engineering Research and Application 7: 25-28.

Sihombing, 2006. Sihombing, D.T.H. 2006. Ilmu Ternak Babi. Ed.2. Gadjah Mada University Press. Bulaksumur, Yogyakarta 55281.

Sulistyarsi, A., Pujiati, M. W. Ardhi. 2016. Pengaruh Konsentrasi dan Lama Inkubasi terhadap Kadar Protein Crude Enzim Selulase dari Kapang *Aspergillus niger*. Proceeding Biology Education Conference (ISSN: 2528-5742), 781-786.

Tang, Fu E. and C. W. Tong. 2011: A Study of the Garbage Enzyme's Efects in Domestic Waste water. World Academy of Science, Engineering and Technology International Journal of Environmental and Ecological Engineering 5(12): 887–892.