

Inhibition of Pathogenic Bacteria in Digestive Tract of Chickens Using Lime (*Citrus aurantifolia*) and Probiotics

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Abstract— The purpose of this research was to evaluate the inhibitory of the mixed lime (*Citrus aurantifolia*) as a source of lactic acid and probiotics as a non-pathogenic microorganism to improve the digestive tract of chickens. The methods were squeezed the juice of the lime and mixed with probiotic (1:1), then rejuvenation of salmonella, clostridium, e.colli. The inhibitory test used the petri dish paper. The experiments was divided into five treatments and repeated three times namely (P0: negative control, P1: 2,5 gr zinc bacitracin/ 100 ml distilled water, P2: 25 ml lime+probiotic/ 100 ml distilled water, P3: 50 ml lime+probiotic/ 100 ml distilled water, P4: 75 ml lime+probiotic/ 100 ml distilled water. Data was obtained analyzed by analysis of variance from a completely randomized design. If there were a difference in the treatment, then continued with Duncan's Multiple Range Test. The results show that the use of lime and probiotic 50 gr/100 ml distilled water has the best inhibitory to Clostridium, Salmonella and E. coli. It can be concluded that the use of lime and probiotic can inhibit pathogenic bacteria on the chicken digestive tract.

Keywords— Chickens, digestive tract, inhibitory, pathogenic bacteria.

I. INTRODUCTION

Indonesia as a tropical country has medicinal plants of about 30,000 species, 7,500 species of which beneficial and 1,200 species are already being used as raw material for herbal medicines. The use of plants as medicine herbs have been around for a long time, especially on the Java Island. At this time medicinal plants were also used as poultry feed additives replace antibiotics or antibiotic growth promoters (AGP). Feed additives are ingredients in raw feed that does not contain nutrients or nutrients, which is used for certain purposes (RI Law No. 18/2009). The poultry industry has been a lot using antibiotics or AGP. This antibiotic plays a role increase the efficiency of feed use by reducing the population of pathogenic bacteria in the digestive tract, so that it will be more effective in increasing livestock performance. Since January 1, 2018 prohibition antibiotics as a feed additive, either in the form of a product as well as raw materials for veterinary drugs mixed in the effective feed which is stated in ministry agriculture regulations no 14/2017. Accordingly, non-causing feed additives are needed microbial resistance, including plant bioactive substances. As a tropical country, Indonesia is a granary various types of plants with substance content bioactive diverse and have the ability as antimicrobial, antifungal, antioxidant, immunomodulatory and hypercholesteremic so that it can replace antibiotic. The purpose of this research was to evaluate the use of bioactive

compounds extracted from herbal plants i.e lime combined with probiotic.

II. MATERIAL AND METHODS

A. Place and Time Research

This research was conducted in August 2020 in the Laboratory of feed and nutrition and Laboratory of agriculture University of Brawijaya, and materia medika laboratory Batu, Malang

B. Methods

Lime was squeezed and added lactobacillus bacteria (4:1) then stirred until mixed. After that, the sample was diluted using aquades with negative control concentrations, positive control (zinc bacitracin), 25%, 50% and 75%.

Rejuvenation of *Salmonella*, *E. coli*, *Clostridium perfringens* and *Lactobacillus inoculum*: Pure microorganisms were inoculated separately in oblique tubes containing nutrient media. Then the microorganism was incubated at a temperature of 37°C for 24 hours.

Bacterial starter: *Salmonella*, *E.colli*, *Clostridium perfringens* rejuvenated bacteria were inoculated into a separate 100 mL Erlenmeyer containing 10 mL of sterile Nutrient Broth (NB) media. *Salmonella* culture, *E.colli*, *Clostridium perfringens* were incubated using a waterbath shaker at 37°C for 10 hours.

Inhibitory: Disks was filled with a mixture of lime and probiotic-based on the different concentration. Then the disc was placed on a petri dish that already contains a bacterial culture using sterile tweezers, then the bacterial culture was incubated at a temperature of 37°C for 24 hours.

Clear zone diameter measurement: The mix of lime and probiotic activity was determined by measuring the clear zone produced by bacterial cultures.

III. RESULTS AND DISCUSSION

The results show that the use of the mix between lime and probiotic as an inhibitory pathogenic bacteria can be shown in Table 1.

The addition of lime and probiotic as an inhibitory pathogenic bacteria significantly different ($P < 0.05$) on *Clostridium perfringens*, *Salmonella*, and *E. coli*. The inhibitory pathogenic bacteria on P0 or negative control shown the clear zone. In addition on P1 or positive control (zinc bacitracin) has the higher inhibitory compared the other treatment.

TABLE 1. Effect of the lime and probiotic on the inhibition growth of pathogenic bacteria such as *Clostridium perfringens*, *Salmonella*, and *E. coli*

Treatments	<i>Clostridium</i>	<i>Salmonella</i>	<i>e.colli</i>
P0	0±0.00 ^a	0±0.00 ^a	0±0.00 ^a
P1	8.97±1.66 ^c	9.00±0.93 ^{ab}	9.33±1.15 ^d
P2	3.35±1.52 ^b	2.67±1.26 ^{bc}	3.43±0.51 ^b
P3	5.24±1.25 ^b	3.85±1.03 ^c	4.97±1.05 ^c
P4	3.31±2.51 ^{ab}	3.47±0.50 ^c	3.6±0.36 ^{bc}

^{abcd} in the column shown the significantly different (P<0.05) in the treatments

According to Mariam (2009), antibiotic is a drug which extremely killed the bacteria and it is used to treat infections but generally harmless to the host. The mixed of lime and probiotics can inhibit *Clostridium*, P2: 3.35, P4: 3.31, P3: 5.24; *Salmonella*, P2: 2.67, P4:3.47 and P3: 3.85 and *E. coli* P2: 3.43, P4: 3.6 and P3: 4.97. It can be show that the highest inhibitory pathogenic on P3 (50% concentration). Lime is a source of natural organic acid such as citric acid and ascorbic acid (Ndelekwute and Enyehini, 2017). It was reported that lime also contains bioactive compound such as flavonoids and carotenoids. Probiotics such as *Lactobacillus sp.* are widely used as the promotion and improvement of the health in the gastrointestinal tract. *Lactobacillus* can inhibit pathogenic bacteria in the gastrointestinal tract. The mixed between acid

and lactobacillus can be work together for improve the health of digestive tract.

IV. CONCLUSIONS

It can be concluded that the use of lime and probiotic 50% concentration can inhibit pathogenic bacteria on the chicken digestive tract.

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