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Research Paper

ACIBIOTIC (ACIDIFIER, PHYTOBIOTIC, PROBIOTIC) AS AN INHIBITORY *Salmonella sp., Clostridium* AND *E. colli* ON DIGESTIVE TRACT OF BROILER CHICKENS

Nuningtyas, Y. F., M. H. Natsir, and O. Sjofjan

Department of Animal Science,
Universitas Brawijaya, Malang, East Java,
Indonesia.

Abstract

The purpose of this research was to evaluate the used of acibiotic (acidifier, phytobiotic, probiotic) combination as an inhibitory pathogenic bacteria as usually on digestive tract of broiler chickens like salmonella, e.colli and clostridium. This research was divided into two stages, firstly was determined plants such as lime and celery, then continued with mixed between lime (acidifier), lactobacillus sp (probiotic) and celery (phytobiotic) to determinated inhibitory test of these bacteria used T0 (negative control-aquadest), T1 (positive control-2,5 gr zinc bacitracin/100 mL aquadest), T2 (25 gr acibiotic/100 mL aquadest), T3 (50 gr acbiotic/100 mL aquadest) and T4 (75 gr acibiotic/100 mL aquadest). The variables were measured the inhibition diameter zone using the disc method. Data obtained were tabulated with Microsoft Excel program, then analysed using analysis of variance (ANOVA) from a completely randomized design (CRD). If there were any differences by the treatment, then continued with Duncan's Multiple Range Test. The results showed that the ability of acibiotic to inhibit pathogenic bacteria such as salmonella, e colli and clostridium is lower than antibiotics. However, the addition of acibiotics up to a concentration of 75% was able to expand the zone of inhibition against salmonella, e colli and clostridium. It can be conclude that acibiotic up to concentration 75 % has the ability to replace the used of antibiotic on feed.

Key words: *acidifier, pathogenic bacteria, phytobiotic, probiotic*.

INTRODUCTION

The development of livestock in Indonesia is growing rapidly in line with increasing consumer demand for animal protein requirements. Animal protein sources are from eggs, chicken, beef, and milk. However, animal feed cannot be separated from the addition of antibiotics. Antibiotics as a feed additives are substances that useful as

growth promoters and increase feed efficiency. However, the use of antibiotics as a feed additive has the negative effect on health of livestock and caused residues on animal products, which are consumed by humans. Based on ministry agriculture regulations No.14 year 2017 concerning the classification of veterinary drugs, it is stated that the use of antibiotics in feed has been prohibited except for treatment and using a veterinarian's prescription. The use of antibiotics in animal feed is considered killing pathogenic bacteria in the digestive tract of poultry such as salmonella sp, escherichia colli and clostridium perfringens [1]. The use of natural feed additive is an alternative to decrease antibiotics . Natural feed additive can be obtained from phytobiotics, probiotics, and acidifiers. Herbal plants that can be used as natural antibiotics are lime (citrus aurantifolia). Lime is a plant that used in everyday life for drinks or food mixtures which are known to have properties as an inhibitor of bacterial. Lime fruit contains many useful chemical compounds such as citric acid, amino acids and essential oils as well as vitamins A, B1 and C [2]. The use of lime is very potential as a natural antibiotic. In addition, celery is plants that source of bioactive compounds like apin and apigenin. Probiotics and phytobiotics can be combined with lime extract to increase of the additives functions. The aims of this study to evaluate the acibiotics (acidifiers, probiotics, and phytobiotics) as an alternatives to replace antibiotics on broiler chickens by inhibitory to pathogenic bacteria..

MATERIALS AND METHODS

Determination of Lime and Celery Plants

Determination of lime and celery plants were used to identify the active substances using the Thin Layer Chromatography (TLC) method.

Extraction

The dried celery leaves were ground, then 10 g of material was weighed and extracted using ethanol 96% with the ratio of ingredients: ethanol (1: 20 w / v) for 24 hours at room temperature. The waste was then extracted again using water with a ratio of material: water (1: 10 w / v) for 24 hours at room temperature. The resulting filtrate from ethanol and water was mixed and centrifuged for 15 minutes. Then concentrated using a rotary evaporator at a temperature of 40 0 C for 2 hours under vacuum pressure to obtain a concentrated extract of celery leaves. The juice of the lime is taken and added to the celery extract, after which probiotics are added.

Inoculum rejuvenation of salmonella, E.colli, and Clostridium perfrinens

cultures: Pure cultures of microorganisms are separately inoculated in slanted tubes containing nutrient medium to be pure. Then the culture was incubated at 37 0 C for 24 hours. Preparation of bacterial starter: Salmonella, E.colli, Clostridium perfrinens bacteria which have been rejuvenated are inoculated into 100 mL erlenmeyer which are separated containing 10 mL of sterile Nutrient Broth (NB) media. Salmonella, E.colli, Clostridium perfrinens cultures were then incubated using a waterbath shaker at 37 o C for 10 hours..

Treatments of acibiotic

The disc was filled with aquadest (negative control) (T0) ,zinc bacitracin concentrations 2,5% (positive control) (T1) and acibiotic concentrations of 25% (T2), 50% (T3) and 75% (T4). Then the disc was placed on a petri dish that already contained the bacterial culture using sterile tweezers, then the bacterial culture was incubated at 37 0 C for 24 hours.

Measurement of clear zone diameter: Antibiotic activity was determined by measuring the clear zone produced by bacterial cultures that had been given acibiotic using a caliper [3].

Statistical analysis

Data obtained were tabulated with Microsoft Excel program, then analyzed using analysis of variance (ANOVA) from a completely randomized design (CRD). If there were an differences by the treatment, then continued with Duncan's Multiple Range Test [4].

RESULTS

Plant Determination

Acibiotic is a combination of acidifier derived from lime, probiotic and phytobiotic derived from celery. Plant determination aims to identify the clumps of plants used in the study. In detail, the determination of celery and lime plants is as follows:

Celery plant determinations are as follows:

: 1b-2b-3b-4b-12b-13b-14b-17b-18b-19b-20b-21b-22b-23b-24b-25b26b-27a-28b-29b-30b-31a-32b-74a-75b-76a -77a-78b-103c-104b-106b-107a-108b-109a-110b-115a116a-117b-118b .... 148. Apiace 1a-2a-3b-4b-6b-7a-8b-10b. [1]

The determinations in lime are as follows:

1b - 2b - 3b - 4b - 6b - 9b - 10b - 11b - 12b - 13b - 14a - 15b (group 9) 197b - 208b - 219b - 220b - 224b - 225b - 227b - 229a (62. Rutaceae) 1a - (1. Citrus) 1b - 3b (Citrus auranttifolia Swigle).

Inhibition against pathogenic and non-pathogenic microorganisms

Based on the inhibition test of acibiotic against pathogenic microorganisms, namely salmonella, e.colli, clostridium perfringens can be seen in the table below.

Table 1 The inhibitory of acibiotics against salmonella, e.colli, and clostridium

Treatments	*salmonella*	*e.colli*	*Clostridium perfringens*
T0	0^{a}±0	0^{a}±0,0	0^{a}±0,0
T1	$9,8^{c}$±1,28	$13,01^{cd}$±2,50	$9,8^{c}$±1,73
T2	$3,46^{ab}$±0,58	$3,9^{ab}$±0,60	$1,23^{ab}$±0,54
T3	$2,98^{b}$±1,17	$6,13^{abc}$±0,75	$4,4^{b}$±1,40
T4	$4,4^{b}$±0,60	$4,96^{abc}$±1,05	$2,98^{b}$±1,99

Note: T0: negative control, T1: positive control (zinc bacitracin), T2: 25% concentration of acibiotic,T3: acibiotic concentration 50%, and T4: acibiotic concentration 75%

Acibiotics with a concentration of 25%, 50% and 75% can inhibit the microorganisms salmonella, e colli, clostridium perfringens. According to Davis and Stout (2009), the antibacterial strength in inhibiting is divided into four categories, namely weak (<5 mm), moderate (5-10 mm), strong (10-20 mm) and very strong (> 20 mm). The acibiotic zone of inhibition against salmonella is T1: 9,8; T4: 4,4; T2: 3,46; T3: 2,98 and T0: 0 mm; the acibiotic zone of inhibition against e colli is T1: 13.01; T3: 6,13; T4: 4,96; T2: 3,9 and T0: 0 mm. The zones of inhibition against clostridium perfringens are T1: 9,8; T3: 4,4; T4: 2,98; T2: 1,23 and T0: 0. The use of acibiotics had a very significant effect (P> 0.01) on the inhibition of salmonella, e.colli, clostridium perfringens.

DISCUSSION

An imbalance total microorganisms in the small intestine of broilers will be caused the low absorption of nutrients. This is because the number of pathogenic microorganisms more higher than non-pathogenic. To overcome this problem, breeders usually providing antibiotics growth promotors in the feed. However, with the rules relating to

the use of antibiotics as feed are made to replace with feed alternative by using natural ingredients such as phytobiotics, probitics and prebiotics. Alternatives that can be used as feed additive consist of lactobacillus as a probiotic, lime as an acidifier and celery leaves as a phytobiotic. This combination can have a good effect on the digestive system of poultry. The results showed that the inhibition zone was formed from a concentration of 25% to 75%. The addition of acibiotics showed a smaller inhibitory power against salmonella, e.colli, clostridium perfringens and lactobacillus sp bacteria than the use of antibiotics (zinc bacitracin). This is because the metabolite compounds contained in acibiotics are natural substances derived from plants and non-pathogenic bacteria which have the ability to slowly inhibit pathogenic bacteria. The ability of antimicrobials to inhibit microorganisms depends on the high and low number and type of microorganisms. According Munita and Arias [5] state that antimcrobial compounds naturally molecules and envolved mechanism to survive. This is not in accordance with the opinion of Rastina et al. [6] which states that the inhibition of microorganisms is based on the concentration of antimicrobial substances. This is because high concentrations do not necessarily have high amounts of antimicrobials. At a concentration of acibiotics as much as 75% showed a wider zone of inhibition against salmonella bacteria compared to other treatments. This is because at a concentration of 75% it shows the high metabolite compounds and lactobacillus bacteria which result in faster diffusion so that the antibacterials get bigger and make the resulting inhibition zone diameter wider. concentration of 50% to 100% can inhibit pathogenic bacteria such as salmonella because at this concentration the filtrate can work effectively in inhibiting pathogenic bacteria [7]. At concentrations of acibiotics as much as 50% or P3 provides a wider zone of inhibition compared to other treatments. According to Hajar and Ninik opinion[8]states that the content of raffinose in probiotics can increase the number of lactic acid bacteria such as bifidobacterium which lowers the pH so that pathogenic bacteria such as E.colli are stunted. At concentrations of acibiotics as much as 50% or P3, it gives a broader picture of the inhibition zone compared to other concentrations, but smaller than that of antibiotics. This is because the active substances contained in plants in the form of flavonoids are allegedly able to act as antibacterials. At a concentration of 25% or P2 shows a smaller inhibition zone compared to other acibiotic treatments. The ability of probiotic bacteria in acibiotics is able to inhibit pathogenic bacteria and increase the number of lactobacilli.

CONCLUSIONS

The ability of acibiotics to inhibit pathogenic bacteria such as *salmonella*, *e colli* and clostridium is lower than that of antibiotics. However, the addition of acibiotics up to a concentration of 75% was able to expand the zone of inhibition against salmonella, e colli and clostridium.

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