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Effectiveness Meniran (Phyllanthus Niruri Linn) as Immunomodulation on Lymphocyte of Broiler Infected with Enterotoxin Escherichia Coli Resistant Antibiotics

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Abstract

Enterotoxin Escherichia coli resistant to antibiotics on broiler. The used of antibiotics should be re-evaluated, as well as over Escherichia coli enterotoxin. Plants meniran (Phyllanthus niruri Linn) is a plant that can be used as an alternative prevention and treatment of diseases caused by Escherichia coli enterotoxin. In this research, broilers at the age of 23 days were divided into six groups. Two groups are positive control was infected by Escherichia coli and negative control was not infected by Escherichia coli. Three groups are extract of Phyllanthus niruri Linn. plant that variety in three dosage: 20%, 25%, 30% and antibiotic. After five days treatment the broiler was to take the sample of blood tissue. The data were tested with Annova and continued by Duncan multiple range test. There was a significant difference of immunomodulation activity between treatment group doses 30% has been able to decrease lymphocyte on blood. The result showed doses 30% can be used as immunomodulation to Escherichia coli.

Keywords: *Phyllanthus niruri Linn. extract, Immunomodulation, Escherichia coli, Broiler, Lymphocyte*

Abstrak

Efektivitas Meniran (Phyllanthus Niruri Linn) sebagai Imunomodulasi pada Limfosit Ayam Broiler yang Terinfeksi Enterotoksin Escherichia Coli Resistant Antibiotics. Enterotoxin Escherichia coli resisten terhadap antibiotik pada ayam pedaging. Penggunaan antibiotik harus dievaluasi ulang, begitu juga Escherichia coli enterotoksin. Tanaman meniran (Phyllanthus niruri Linn) merupakan tanaman yang bisa dijadikan alternatif pencegahan dan pengobatan penyakit yang disebabkan oleh Escherichia coli enterotoksin. Dalam penelitian ini, ayam broiler pada umur 23 hari dibagi menjadi enam kelompok. Dua kelompok kontrol positif terinfeksi oleh Escherichia coli dan kontrol negatif tidak terinfeksi oleh Escherichia coli. Tiga kelompok adalah ekstrak Phyllanthus niruri Linn. Tanamlah varietas itu dalam tiga dosis: 20%, 25%, 30% dan antibiotik. Setelah lima hari pengobatan ayam pedaging tersebut mengambil sampel jaringan darah. Data diuji dengan Annova dan dilanjutkan dengan uji Duncan multiple range. Ada perbedaan yang signifikan antara aktivitas imunomodulasi antara dosis kelompok perlakuan 30% yang mampu menurunkan limfosit pada darah. Hasilnya menunjukkan dosis 30% dapat digunakan sebagai imunomodulasi ke Escherichia coli.

Kata Kunci: Phyllanthus niruri Linn. ekstrak, imunomodulasi, Escherichia coli, broiler, limfosit

INTRODUCTION

Poultry farm is considerable potential importance in the supply of animal protein. The disease is a constraint on the farm that causes enormous economic losses. One of the most common infectious bacterial diseases of the layer industry is Colibacillosis. Colibacillosis is an infectious bacterial diseases in birds caused by pathogenic Escherichia coli. The infectious called Escherichia coli or Colisepticaemia is infected worldwide in broilers and breeders of all ages, and other birds like turkeys and ducks (Charllton et al., 2000).

The cause collibacillosis is originated from E. coli enterotoxin. There is certain E. coli' strains that can produce Shiga toxin so that these strains are known by various names such as Shiga toxin-producing E. coli (STEC) or verocytotoxigenic E.coli (VTEC) or enterohemorrhagic E. coli (EHEC). Enterotoxin which causes diarrhea can be produced by several strains of E. coli such as O157: H7 and O111 or E. coli' non enterictic bacterium. E.coli group that has virulence factors causing diseases, colonization in humans and animals increasing morbidity and mortality.

Farmers frequently use antibiotics in the treatment of Escherichia coli. The use of antibiotics can cause problem which now discovered antibiotic residues in cattles' carcasses consumed by human (Ardiansya et al., 2012). Overdose in using antibiotics is concerned to cause allergies in consumers due to antibiotic residues in meat or eggs, imbalance microorganisms in the digestive tract as well as the resistance of microorganisms to antibiotics (Bogaard and Stobberingh, 1999; Mellor, 2000).

One of the bacterial disease prevention that are safe is by using herbs. Indonesia as a tropical country has a wealth of potential herbs. Many types of plants contain compounds with antimicrobial characteristics because it contains bactericidal (bacteria-killing), and bacteristatic (hold bacterial growth) as well as imunnomodulator. Meniran plants (Phyllanthus niruri Linn) is a plant that can be used as an alternative prevention and treatment of diseases caused by E. coli. The chemicals contained in Meniran include flavonoids and tannins. Flavonoids functioned as imunnomodulator that used to boost immune system and improve its dysfunctional. Tannins functioned as antiseptic (prevent bacterial growth) and hemostatic (stop bleeding) (Mathivanan et al., 2006). Thus, supply meniran's extract per-oral to chickens can influence the function and activity of the immune system as immunostimulatory. It is shown, for example, with the activity of monocytes or macrophages in phagocytic function and chemotaxis and secretion of several cytokines by the immunogenic cells.

Based on the above background, conducted study on the effectiveness of meniran (Phyllanthus niruri Linn) as an immunomodulator to lympocite in broiler chickens infected with antibiotic-resistant's Escherichia coli enterotoxin.

MATERIALS AND METHOD

This study was conducted using experiment on broiler chickens were divided into six treatment groups, each treatment consisted of 5 replicates. The treatment group was prepared as follows:

P0+: (positive control) infected with E. coli without given the meniran's extract

P0-: (negative control) without treatment

P1 : infected with E. coli + given 20 % meniran's extract.

P2: infected with E. coli + given 25 % meniran's extract.

P3: infected with E. coli + given 30 % meniran's extract.

AB: infected with E. coli + given flemequin antibiotics.

Meniran's extract given a day after infected Escherichia coli' in chickens aged 28 days for five days with cages battery adaptation period of five days.

Calculation of lymphocytes obtained from examination of Leukocyte Type Count

Leukocyte type count is an assessment of leukocytes in the blood based on the proportion (%) of each type of leukocytes from the whole number of leukocytes. To get the absolute amount of each type of cell, the relative value (%) multiplied by the total leukocyte count (cells / mL). The results of this examination can specifically describe the occurrence and disease processes in the body, especially infectious diseases. Leukocyte counts is lymphocytes. In doing leukocyte count, first step was making blood smear stained with Wright dye. Observed under a microscope and count the types of leukocytes and came up with 100 cells. Each type of leukocyte written in percent.

The data obtained were analyzed using one-way ANOVA then followed by Duncan's multiple range test.

RESULT AND DISCUSSION

The result by Duncan's test showed if $P < 0.05$ resulted significant difference between treatments.

Table 1 Average Value of Lymphocyte Number Among Treatments

Treatments	Average
P0+	13344.320[a]
P0-	13344.320[ab]
P1	13524.720[a]
P2	13084.840[ab]
P3	11159.720[b]
P antibiotics	9574.840[b]

All blood cells (lymphocytes, granulocytes, erythrocytes and megakaryocytes) derived from a type of cells (stem cells) in the bone marrow. Most of the lymphocyte cells of the newly formed "stem cells" will flow towards the thymus gland. In the thymus lymphocyte cells will experience like maturation process into lymphocytes which will serve in the cellular immune response (cellular immunity). Lymphocytes that have been processed in the thymus gland is called T lymphocyte cell. Lymphocyte cells that do not undergo a process of maturation in the thymus gland, undergo a process of maturation in the bone marrow and may be in the lymph nodes. These cells will have the ability to produce antibodies in the immune response after undergo process of maturation. These cells called B. Newly formed T and B cells

lymphocytes will flow in the blood vessels and lymph vessels.

Most of lymphocytes cells (T and B) will go into the lymph nodes and settled temporarily in it, while others will leave the lymph nodes and come back in circulation. Once it gets into the lymph nodes, lymphocytes will immediately occupy the places that have been determined for each of the T cell and B cell. Lymphocytes B will go into the follicle, while lymphocytes T occupy the area of para-cortex and medulla (Harryadi, 1980)

If there is an antigen entering the body, the lymphocytes T also will be transformed into imunoblast. While on lymphocytes B, antigen stimulation caused the transformation of cells that ultimately produce plasma cells. These plasma cells produce antibodies (humoral immunity reaction). Plasma cells which is the end product of lymphocytes B will no longer have immunoglobulins on their cell surface.

Lymphocytes T is an expression of the TCR (T-Cell Receptor) that provides unique and specific antigens on the cell. Immature lymphocyte cells are removed from the marrow to experience growth and maturation in the thymus. Growth CD4 + lymphocyte cells or CD8 + will leave thymus and spread into peripheral lymphoid tissue, to certain sections of lymph node paracortex, splenic periarteriolar lymphoid sheath or perrifolicular area connection between mucosal tissue and lymphocytes. Lymphocytes T has a need for activation. Intact antigens are generally not able to stimulate T-cell. Activation of T cells requires intracytoplasmic signals transmission after introduced peptide antigen and MHC residues of TCR, whole interaction of APC and T cells on the surface of other molecules, release costimulatory cytokines APC which tied cytokine receptor on T cells. Lymphocytes T leave the bone marrow and grow longer, then migrate toward the thymus. After leaving thymus, these cells circulate in the blood until met with antigens where they have been programmed to recognize it. Once stimulated by antigens, these cells produce chemicals that destroy microorganisms and notice other leukocyte cells of infections occurred (Hand, 2008).

Lymphocytes B are formed in the bone marrow and circulated in the blood until they encounter the antigen which has been programmed to recognize it. At this stage, the lymphocytes B undergo further maturation into plasma cells and produce antibody (Hand, 2008). Each antibody is specific for a particular antigen. This is due to the unique structure of an antibody that is composed of amino acids in the part that can be changed from both light and heavy chains. The amino acid sequence has a different shape for each antigen specificity (Guyton, 1983).

CONCLUSION

Based on the study concluded that the extracts of Meniran plant (Phyllanthus niruri L.) as much 30% concentration has the highest immunomodulatory activity thus decreasing the number of lymphocytes on infected broilers with enterotoxin Escherichia coli's antibiotics resistant.

ACKNOWLEDGE

Based on the study conducted, it can be recommended a suggestion as follows: Meniran's extract can be recommended on farmers to be used as an alternative to prevent colibacillosis in broilers.

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