

## Hematology Profile and Liver Histopathology in *Escherichia coli* Infected Layers Treated with Combination of *Phyllanthus* (*Phyllanthus niruri* L.) and Turmeric (*Curcuma domestica*)

### *Profil Hematologi dan Histopatologi Hati pada Ayam petelur yang Diinfeksi Escherichia coli dengan Terapi kombinasi Meniran (Phyllanthus niruri L.) dan Kunyit (Curcuma domestica)*

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#### Abstrak

Kolibasilosis merupakan penyakit disebabkan oleh *Escherichia coli* (*E. coli*) yang dapat mengakibatkan kerugian ekonomi dan masih menjadi masalah. Herbal banyak dikembangkan untuk keperluan terapi. Meniran (*Phyllanthus niruri* L.) dan kunyit (*Curcuma domestica*) merupakan herbal yang dapat digunakan sebagai imunomodulator. Studi ini bertujuan untuk mengetahui keamanan kombinasi meniran (*Phyllanthus niruri* L.) dan kunyit (*Curcuma domestica*) terhadap profile darah dan gambaran histopatologi pada hati ayam petelur yang diinfeksi *E. coli*. Ayam dibagi menjadi 5 kelompok yaitu a. Kelompok ayam yang diinfeksi *E.coli* tanpa perlakuan (kontrol), b. kelompok ayam yang diinfeksi *E.coli* yang diberi perlakuan meniran dengan dosis 500 mg/kg bb. c. kelompok ayam yang diinfeksi *E.coli* yang diberi perlakuan kunyit dengan dosis 300 mg/kg bb., d. Kelompok ayam yang diinfeksi *E.coli* yang diberi perlakuan kombinasi meniran dan kunyit dengan perbandingan 1:1, e. kelompok ayam yang diinfeksi *E.coli* yang diberi perlakuan kombinasi meniran dan kunyit dengan perbandingan 1:2. Sampel darah dan hati diambil setelah 21 hari perlakuan. Profil hematologi (hemoglobin, hematokrit, eritrosit, leukosit,) dan gambaran histopatologi hati diamati. Hasil dianalisa dengan Anova dan deskriptif. Hasil penelitian menunjukkan, pada kelompok perlakuan meniran: kadar hemoglobin, hematokrit dan eritrosit lebih tinggi dibanding kelompok kontrol, walaupun tidak berbeda signifikan dengan antar kelompok. Gambaran histopatologi hati pada kelompok kontrol tanpa perlakuan menunjukkan adanya sel radang heterofil, sedangkan pada kelompok perlakuan herbal meniran dan kunyit tidak menunjukkan adanya sel radang. Kesimpulan dari penelitian menunjukkan bahwa kombinasi herbal meniran dan kunyit mempunyai efek terapi terhadap infeksi *E.coli* dan tidak berpengaruh terhadap gambaran darah maupun hati sehingga aman digunakan.

**Kata kunci:** ayam petelur; *Escherichia coli*; hati; hematologi; kunyit (*Curcuma domestica*); Meniran (*Phyllanthus niruri* L.);

## Abstract

Colibacillosis is a disease caused by *Escherichia coli* (*Escherichia coli*) which can cause economic losses and is still a problem. Herbs have been developed for therapeutic purposes. *Phyllanthus niruri* L and *Curcuma domestica* are herbs that can be used as immunomodulators. This study aims to determine the safety of the combination of *Phyllanthus niruri* L and *Curcuma domestica* on the blood profile and histopathological features of the livers of layers infected with *E. coli*. Chickens were divided into 5 groups, namely: a. Group of chickens infected with *E. coli* without treatment, b. Group of chickens infected with *E. coli* and treated with *Phyllanthus niruri* L at a dose of 500 mg / kg BW. c. Group of chickens infected with *E. coli* and treated with turmeric (*Curcuma domestica*) at a dose of 300 mg / kg BW., d. Group of chickens infected with *E. coli* were treated with a combination of *Phyllanthus niruri* L and *Curcuma domestica* with a ratio of 1: 1, e. Groups of chickens infected with *E. coli* were treated with a combination of *Phyllanthus niruri* L and *Curcuma domestica* in a ratio of 1: 2. Blood and liver samples were taken after 21 days of treatment. Haematological profile (hemoglobin, hematocrit, erythrocyte, leukocyte) and liver histopathology were observed. The results were analyzed using ANOVA and descriptive. The results showed that the *Phyllanthus niruri* L treatment group had higher hemoglobin, hematocrit and erythrocyte levels than the control group, although not significantly different between groups. Liver histopathology in the control group without treatment showed heterophyl inflammatory cells, whereas the herbal treatment groups *Phyllanthus niruri* L did not show inflammatory cells. The conclusion from this study shows that the herbal combination of *Phyllanthus niruri* L and *Curcuma domestica* have a therapeutic effect on *E. coli* infection and does not affect the profile of the blood and liver so it is safe to use.

**Key words:** *Curcuma domestica*; *Escherichia coli*; hematologi; layer; liver; *Phyllanthus niruri* L,

## Introduction

Poultry farms have excellent prospects for both large and small scale community farms including easy access to input production, rapid capital turnover and increasing demand for chicken meat, all of which support the development of poultry farms. In Indonesia, the carcasses per capita consumption was predicted to increase from 8.6 kg per capita in 2013 to 14.49 kg per capita in 2017. Similarly, predicted egg consumption will continue to increase over the next five years. In 2012, egg consumption was 74 kg per capita and increased to 162 kg per capita in 2017 (Suwanti, 2015).

Colibacillosis is one of the diseases that affects poultry and causes economic losses in the chicken farming industry worldwide (Kabir, 2010). In addition to causing death, it can cause growth failure and falling egg production. Incorrect antibacterial use leads to antibacterial resistant bacteria that prove difficult to treat. For in these cases, second or third line antibacterial drugs should be used. However, that treatment do not rules out the occurrence of immunity (Hasan *et al.*, 2011; Solà-Ginés *et al.*, 2015).

Pharmacologically, phyllanthus can serve as an immunomodulator (Bagalkotkar *et al.*, 2006). Kamruzzaman and Hoq (2016) have reported that phyllanthus showed some level of antimicrobial activity, particularly toward *E. coli*. Turmeric (*Curcuma domestica*) has long been used, namely as a spice in cooking, herbs and cosmetics. Turmeric has been reported to be an efficacious treatment for hemorrhoids, menstruation, cholesterol, bacterial infection and aging (Çikrikçi *et al.*, 2008). Today, the research and development of medicinal plants both at home and abroad is growing rapidly. The development of both pharmacological and phytochemical studies based on medicinal plants has been conducted by some communities with empirically tested efficacy. The results of these studies have solidified the usefulness of medicinal plants.

The number of blood cells is an indicator of production and quality. Several studies have shown that erythrocytes play a role in immune function (Tian *et al.*, 2013). Therefore, this study aimed to determine whether phyllanthus and turmeric can be used as a therapeutic alternative for colibacillois in layers.

## Materials and Methods

Twenty-five layers of Day-Old-Chicken were used in this study. All layers were vaccinated following a one-week adaptation. One week after the vaccinations, the layers were infected with *E. coli* at  $10^8$  CFU/mL. After contracting colibacillosis, the layers were divided into the following 5 groups: 1) control group, colibacillosis group without treatment (group A); 2) colibacillosis group treated with 500 mg/kg BW of phyllanthus (group B); 3) colibacillosis group treated with 300 mg/kg BW of turmeric (group C); 4) colibacillosis group treated with phyllanthus and turmeric combination (1:1) (group D) and 5) colibacillosis group treated with phyllanthus and turmeric combination (1:2) (group E). The herbal treatments were given for 21 days.

The experiments presented here received approval from the Ethical Clearance Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, with number: 0006 / EC-FKH / Int. / 2017. Blood and organ samples were collected after the herbal treatment. Blood was collected through the right brachial veins. Hemoglobin (HB), hematocrit

(PCV), erythrocyte (RBC) and leukocyte (WBC) concentrations in the blood sample were assayed with a Semi-Auto Chemistry Analyzer. The layers were euthanized by intracardiac administration of saturated MgSO<sub>4</sub> solution (Matin *et al.*, 2017). A thorough post-mortem examination of all the dead layers was carried out. Liver samples were collected and fixed with formalin. Organ samples were processed via the paraffin-embedded method and visualized using Hematoxylin-Eosine staining.

The hematology profile means and standard deviation (SD) were calculated using descriptive statistical procedures with the SPSS 16 program (SPSS, Chicago, USA). The liver histology samples were analyzed qualitatively.

## Results and Discussion

The result of the macroscopic examination of the layers organs 21 days post-therapy showed that the control group had positive infections of *E. coli*, which continuously occurred for the following two weeks. The same results were found for the the groups given turmeric (Table 1).

Fig. 1 showed the macroscopic alterations associated with positive *E. coli* infection including

Table 1. The results of the macroscopic examination of the organs infected by *E. coli* 21 days post-treatment

No	Group A	Group B	Group C	Group D	Group E
1.	++	-	+	-	-
2.	++	-	-	-	-
3.	++	-	+	-	-
4.	++	-	+	-	-
5.	++	-	+	-	-

Note:

(+) : infected

(-) : recovered

Control group, colibacillosis group without treatment (group A), colibacillosis group treated with 500 mg/kg BW of phyllanthus (group B), colibacillosis group treated with 300 mg/kg BW of turmeric (group C), colibacillosis group treated with phyllanthus and turmeric combination (1:1) (group D) and colibacillosis group with phyllanthus and turmeric combination (1:2) (group E).

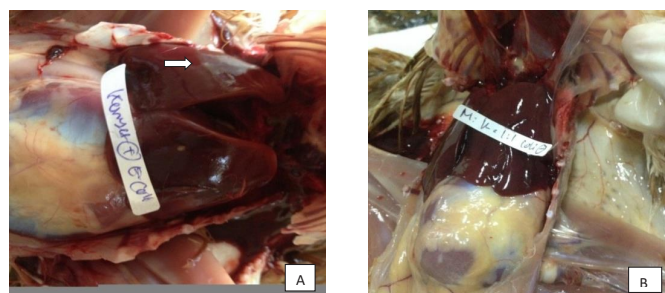


Figure 1. Macroscopic view of the liver infected by *Escherichia coli*, A: control group, positive for colibacillosis showing fibrinous perihepatitis (→) and B: colibacillosis group treated with phyllanthus (*Phyllanthus niruri*) and colibacillosis group treated with the combination herbal therapy, no lesion in liver

air sacculitis, perihepatitis, pericarditis, and even serositis. These symptoms are in accordance with Abalaka *et al.* (2017), who stated that *E. coli* can cause either local or systemic infection, including air sacculitis, fibrinous polyserositis (pericarditis, perihepatitis, and peritonitis), coligranuloma, coliseptisemia, and swollen head syndrome. Notably, the group treated with phyllanthus alone or with combination of phyllanthus and turmeric showed no sign of *E. coli* infection.

Histopathologic liver examination of the colibacillosis control group showed infiltration of heterophil cells around the hepatocytes, whereas the colibacillosis group treated with phyllanthus alone and herbal combination therapy did not showed this infiltration (Fig. 2).

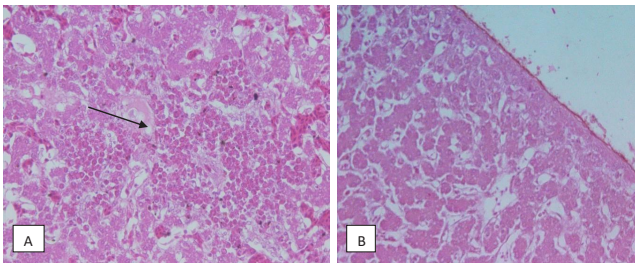


Figure 2. Histopathology of the liver infected by *Escherichia coli*, A : control group, positive for colibacillosis, showing infiltration of heterophil cells around the hepatocytes (arrow) and B: colibacillosis group treated with phyllanthus (*Phyllanthus niruri*) and colibacillosis group treated with the combination of herbal therapy, negative for colibacillosis and showing no heterophil cell reaction in the liver tissue.

Alterations associated with colibacillosis pathology include: a) acute septicemia consisting of spleen in which multiple necrosis, some lymphoid follicles related to fibrinous exudate and bacteria colony, trombifibrinuos in sinusoid

hepar, and hepatocyte necrosis exist but there is no serosal lesion; b) related to sub-acute serositis, serositis fibrinopurulent and granulomataous lesion are dominant lesis; spleen and hepar lesion not only have resemblance with acute septicemia but also involve their serosa in their functions; mesothelial cell of epicardium has necrosis and contains exudate fibrinous; submesothelial tissue has edema and infiltrating heterophil cells. In more serious cases, granulomataous lesions and eosinophilic debris come surrounded by macrophages and giant cells. In this case, adhesion in both the pericardium and epicardium frequently occurs (Nakamura *et al.*, 1985).

The macroscopic examination of the layers' organs post-treatment showed that the control group had positive infections of *E. coli*, which continuously occurred for the following two weeks. Histopathological observation of the colibacillosis group found infiltration of heterophil cells around the hepatocytes (Fig.2). The same results were also found the groups treted with turmeric (Table 1). The main chemical content of curcuminoid and essential oils in *Curcuma longa* could be antibacterial. Curcumin in *Curcuma longa* can change the permeability of the cytoplasmic membrane, which can damage the cell membrane, disrupt cell metabolism and inhibit bacterial growth (Li *et al.*, 2013). The natural product curcumin has low bioavailability and therefore therapeutically ineffective (Shen and Ji, 2012, Hartati *et al.*, 2018). This is in accordance with the results presented here.

Table 2 shows the hematological profile. The mean values of hemaglobin, hematocrit and erythrocyte content of the colibacillosis group

Table 2. Density average of hemoglobin, hematocrit, erythrocytes and leukocytes 21 days post-treatment

Group	Hemaglobin (HB) (gr/dL)	Hematocrit (PCV) (%)	Erythrocyte (Million/UI)	Leukocyte (Thousand/mm <sup>3</sup> )
Group A	10.15±1.07	28.25±2.2	2.47 ± 0.16	20.9 ± 5.6
Group B	10.95±0.25	30.75± 2.2	2.64 ± 0.23	12.2 ± 1.20
Group C	10.45±0.53	30.50±2.5	2.16 ± 0.70	17.0 ± 5.56
Group D	9.84±0.41	29.00±1.2	2.32 ± 0.55	13.8 ± 6.95
Group E	9.92±0.46	29.80±1.3	2.17 ± 0.19	12.3 ± 5.42

Note:

Control group, colibacillosis group without treatment (group A), colibacillosis group treated with 500 mg/kg BW of phyllanthus (group B), colibacillosis group treated with 300 mg/kg BW of turmeric (group C), colibacillosis group treated with phyllanthus and turmeric combination (1:1) (group D) and colibacillosis group treated with phyllanthus and turmeric combination (1:2) (group E).

treated with *phyllanthus* (group B) were higher than the control group (group A) and the other groups. The colibacillosis group displayed higher levels of leukocytes ( $20.9 \pm 5.6$  thousand/ $\text{mm}^3$ ). Statistical analyses, however, did not reveal significant differences between the treatment groups ( $P > 0.05$ ).

As shown in Table 2, the colibacillosis layers treated with the combination of *phyllanthus* and turmeric showed a higher level of hemoglobin, hematocrits and erythrocytes than the control group. Normal levels for hemoglobin, hematocrit, erythrocytes, and leukocytes for chickens are 7.0 - 13.0 g/dL (Jain, 1993), 22.0 - 35.0% (Nakamura *et al.*, 1985), 1.3 - 4.5 million/UI, and 7.0 - 32.0 thousand/mL (Li *et al.*, 2013), respectively.

Hemoglobin is an erythrocyte pigment consisting of a conjugating protein and a simple protein. The proteins are globulin and heme cells or iron atoms that give the red pigment. The synthesis of hemoglobin is influenced by the presence of nutrients in the diet. Hemoglobin serves to transport oxygen, and is therefore an indicator of oxygen adequacy. The hemoglobin levels in the group treated with turmeric were higher than the other groups due to the content of curcumin in turmeric that serves as an antioxidant and protects hemoglobin from oxidation. Oxidation reactions can damage hemoglobin (Nakamura *et al.*, 1985).

Hematocrit or packed cell volume shows the ratio of red blood cells to total blood volume (Nakamura *et al.*, 1985). Increased levels of hematocrit indicate dehydration and edema due to the release of fluid from the blood vessels. Conversely, the decrease levels of hematocrit indicate blood cell deficiency or anemia. Hence, the hematocrits values is related to the blood viscosity. Normally, the hematocrit values are positively correlated with the number of erythrocytes, erythrocyte size, and blood volume. The hematocrit value was higher in the turmeric-treated group. All of the groups had a normal mean value of hematocrit; demonstrating that the animal health status was good.

The number of blood cells is an indicator of production and quality. The number of blood cells that are more or less than normal, it can cause health problems for animals. Therefore, the number of blood cells can be used for the diagnosis,

treatment and prognosis of disease (Meyer and Harvey, 2004). The function of erythrocytes is generally related to the function of hemoglobin, which plays a role in gas exchange and oxygen distribution into the cell, which is required by the cells for metabolic processes. Several studies have shown that erythrocytes play a role in regulating the immune system, ranging from identification, adhesion, and killing of pathogens (Tian *et al.*, 2013).

The number of erythrocytes and hemoglobin levels will rise if the amount of oxygen in the blood is low. Which stimulate an increase in erythrocyte and hemoglobin levels. The formation of erythrocytes is stimulated by the hormones glycoprotein and erythropoietin found in the kidneys. Factors that affect the number of erythrocytes in the circulation include the hormone erythropoietin which serves to stimulate erythropoiesis by triggering the production of proerythroblasts from hemopoietic cells in the bone marrow. Vitamin B12 and folic acid affect erythropoiesis at the final erythrocyte maturation stage, whereas hemolysis can affect the number of erythrocytes in the circulation. The results showed that the number of erythrocyte in the turmeric-treated group was higher than the other groups. This effect may be to curcumin contained in turmeric having antioxidant activity for hemolysis and lipid peroxidation in erythrocytes induced by hydrogen peroxide as previously shown in rats.

The leukocyte content of the colibacillosis group and colibacillosis group treated with turmeric was higher than the other groups. All groups displayed a mean leukocyte count in the normal range. Normal levels of leukocytes for chickens are 7.0 - 32.0 thousand/mL (Li *et al.*, 2013). A decrease in the number of leukocytes in many species is derived from a decrease in the number of neutrophils and/ or decreases in lymphocytes count. The decrease in the number of lymphocytes is thought to be caused by the differentiation of lymphocytes into plasma cells to produce antibodies. Turmeric contains curcumin-specific compounds that can increase T cell proliferation, and can therefore boost the immune system. In addition, the content of filastin in *phyllanthus* also serves as an immunomodulator.

## Conclusion

Based on the studies presented here, treatment of colibacillosis in layers with the combination of phyllanthus and turmeric did not affect the hematological profile (the levels of hemoglobin, hematocrit, erythrocyte and leukocyte) or the liver histology. The results suggests that the administration of herbal medicine is safe and can be used as an alternative colibacillosis therapy.

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