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Effectiveness of *Phyllanthus niruri* and *Andrographis paniculata* Extracts on Egg Quality in Laying Hens with Avian Pathogenic *Escherichia coli*

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Abstract

Providing sufficient and high-quality eggs plays an essential role in accommodating food safety and food security for consumers. The purpose of this study was to determine the effect of *Phyllanthus niruri* and *Andrographis paniculata* extracts on egg yolk index and haugh units (HU) value in laying hens with Avian Pathogenic *Escherichia coli*. This type of study was experimental using a complete randomized design with 100 experimental units of five treatments (P-) placebo, (P+) infected with Avian Pathogenic *E. coli* without *P. niruri* and *A. paniculata* extract, then groups of chickens infected with Avian Pathogenic *E. coli* and administration of (P1) 10% of *P. niruri* and 30% of *A. paniculata*, (P2) 20% of *P. niruri* and 20% of *A. paniculata*, (P3) 30% of *P. niruri* and 10% of *A. paniculata* then egg yolk index and HU value were evaluated. The data obtained were analyzed using ANOVA followed by Duncan's test. The results showed a significant difference between the treatment groups based on the egg yolk index and the HU value. It can be concluded that P2 can be recommended for laying hens with avian pathogenic *E. coli*.

Keywords: *Andrographis paniculata*, egg quality, *Escherichia coli*, food safety, *Phyllanthus niruri*

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INTRODUCTION

Colibacillosis is a disease in poultry caused by *Escherichia coli* (*E. coli*) pathogenic strains, which can affect chickens at all ages, but more often at a young age (Niyatri, 2010). Colibacillosis is considered one of the most important poultry diseases caused by Avian Pathogenic *E. coli*, which is responsible for several extra-intestinal pathological conditions in laying hens (Paixao *et al*., 2016). Laying hens exposed to *E. coli* show a decrease in daily egg quality production by 60–80%. Colibacillosis should be treated with good sanitation and antibacterial given. Inadequate farmer awareness and knowledge regarding proper antibiotic usage will negatively impact the development of microbial resistance. The poultry farm is of considerable potential importance in the supply of animal protein. Any disease is a constraint on the farm that causes enormous economic losses (Pratama *et al*., 2021).

One of the most common infectious bacterial diseases in the layer industry is Colibacillosis. Colibacillosis is an infectious bacterial disease in birds caused by pathogenic *E. coli*. The infectious disease is called *E. coli* or Colisepticaemia (Wardiana *et al*., 2021). This pathogen has been reported worldwide in broilers and breeders of all ages, as well as in other avian species like turkeys and ducks. Farmers frequently use antibiotics in the treatment of *E. coli*. However, the inappropriate use of antibiotics can cause problems, like antibiotic residues in meat meant for human consumption. Inappropriate antibiotic usage can cause allergies in consumers due to antibiotic residues in meat or eggs, imbalance of microorganisms in the digestive tract, and antibiotic microbial resistance. This is a matter of concern not only in Indonesia but in other developing countries as well. As reported by

Schierack *et al*. (2009) 72.1% of *E. coli* samples isolated from the field are resistant to ampicillin, chloramphenicol, kanamycin, streptomycin, sulfamethoxazole, and tetracycline.

One of the bacterial disease preventive measures that is considered safe is by using herbs. Indonesia is a tropical country with a wealth of potential herbs. Many types of plants contain compounds with antimicrobial characteristics because it contains bactericidal, and bacteriostatic, as well as an immunomodulator. Meniran (*Phyllanthus niruri*) is a plant that can be used for the alternative prevention and treatment of diseases caused by *E. coli*. The chemicals contained in *P. niruri* include flavonoids and tannins (Hamid *et al*., 2018). Flavonoids act as an immunomodulator and can be used to boost the immune system and improve its function. Tannins act as an antiseptic and hemostatic (Mathiyanan *et al*., 2006).

The main content of sambiloto (*Andrographis paniculata*) plants is diterpenoide lactones (andrographolide), paniculides, farnesols and flavonoids. According to various studies, the content found in this plant is believed to be able to fight disease is andrographolide. In addition, *A. paniculata* plants contain saponins, alkaloids, and tannins. Other chemical contents found in plants are lactone, paniculin, and kalmegin (Dalimunthe, 2009). Pharmacologically, *A. paniculata* plants have analgesic, anti-inflammatory, antibacterial, antimalarial, antiviral, immunostimulatory, hepatoprotective, cardiovascular, and anticancer properties (Jarukamjorn *et al*., 2009; Schierack, 2009).

According to Bagalkotkar *et al*. (2006), *P. niruri* leaves contain various kinds of secondary metabolites, including flavonoids, alkaloids, lignans, tannins, and saponins. Almost all parts of the *P. niruri* plant are medicinal. *P. niruri* herbal extract contains alkaloids, flavonoids, saponins, steroids, tannins, and phenolic compounds (Rivai *et al*., 2013). This study aimed to determine the effect of *P. niruri* and *A. paniculata* extracts of egg quality, namely the yolk index and haugh units (HU) value in laying hens infected with Avian Pathogenic *E. coli*.

MATERIALS AND METHODS

Ethical Approval

This study was approved by Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Universitas Airlangga, Ethical Clearance No: 2.KE.142.08.2018.

Study Period and Location

This study was conducted for 1 month at the commercial chicken layer farm in Balongpanggang, Gresik, where the experimental animals and sample collection took place. The extraction of *P. niruri* and *A. paniculata* was performed at the laboratory of the Department of Pharmacology, Faculty of Veterinary Medicine, Universitas Airlangga. The examination of egg quality was conducted at the laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Universitas Airlangga.

Extraction of *P. niruri* and *A. paniculata*

The extract used in this study was prepared based on the methanol extraction (Sasidharan *et al*., 2011). *P. niruri* and *A. paniculata* were dried and grind, soaked with methanol 96% solution, and mixed for three days. Drained the product and evaporated using a rotary evaporator until a concentrated extract was produced.

Preparation of Experimental Animals

A total of 100 laying hens, aged 26-week-old were used. These were housed in a battery cage system. Chickens were adapted for five days by feeding commercial feed, ad libitum, without antibiotics. Data collection for egg quality was performed in the last week. On day six, at the age of 27 weeks, the chickens were infected with *E. coli* intramuscularly with a concentration of 108 cells/kg weight and then observed clinical symptoms for three days. The *P. niruri* and *A. paniculata* extracts were administered starting on the 9th day and mixed in the feed with different doses of each treatment group.

Experimental Design

This study was experimental using a complete randomized design with five treatments

i.e. (P-) placebo group, without being infected with Avian Pathogenic *E. coli* and without *P. niruri* and *A. paniculata* extract, (P+) infected with Avian Pathogenic *E. coli* without *P. niruri* and *A. paniculata* extract, then groups of chickens infected with Avian Pathogenic *E. coli* and administration of (P1) 10% of *P. niruri* and 30% of *A. paniculata*, (P2) 20% of *P. niruri* and 20% of *A. paniculata*, (P3) 30% of *P. niruri* and 10% of *A. paniculata* then egg yolk index and HU value were evaluated.

Egg Quality Examination

To check the egg quality, the following variables were evaluated as follow, an egg yolk separator was used to separate egg yolks and albumin; the base with a width of 20 cm and a length of 15 cm was used as the base for measuring the albumin diameter and egg yolks; the sliding term was used to measure the diameter of albumin and egg yolk; the spherometer was used to measure the thickness of the albumin and the yolk.

Sample Collection

The yolk index was a comparison between the height of the egg yolk and the yolk diameter. According to the National Standardization Agency (2008) on SNI 3926: 2008 stated that the index of fresh egg yolks ranged from 0.33–0.52. HU value was calculated using the formula (Bagalkotkar, 2006), HU (%) = 100 x log ($H^{+}7.57 - 1.7W^{0.37}$), where H is the height of albumin and W is the weight of the egg. Eggs with good quality have a minimum HU value of 72. Eggs that are not worth consuming have less than 30 HU value (Koswara, 2009).

Data Analysis

The data obtained were analyzed using the ANOVA test and Duncan's follow-up test at a 95% confidence level.

RESULTS AND DISCUSSION

Results of data analysis on egg yolk index showed results significantly different between the treatment groups occurred decrease in the value of the yolk index caused by the water content of the albumin around the yolk absorbed into the yolk; reduced permeability of the vitelline membrane causing the yolk to experience pressure. The egg yolk osmotic pressure is bigger than albumin, so water from the albumin moves towards the yolk. Continuous water transfer will cause the viscosity of the yolk to decrease, so the yolk gets flattened and later will break because this water transfer process depends on the thickness of the albumin and the egg yolk index decreases, then the vitellin membrane will be damaged and cause broken egg yolk. The average egg yolk index value in group P- of 0.44 with quality II, group P + of 0.41 with quality II, group P1 of 0.43 with quality II, P2 group is 0.46 with quality I, and the P3 group is 0.44 with quality II (Table 1 and Figure 1) based on the National Standardization Agency (2008) states that the yolk index quality level is 0.458–0.521 (Quality I), 0.394–0.457 (Quality II), and 0.330–0.393 (Quality III). The results of data analysis of the HU value showed a significant difference between the treatment groups. Melia *et al*. (2008), stated that the process of depletion of albumin occurred due to the interaction between the enzyme lysozyme and ovomucin. Acidity level (pH) rises due to CO_2 coming out during storage and bacterial Avian Pathogenic *E. coli* infection that results in reduced solubility of ovomucin and the thickness of the damaged albumin.

Egg quality is determined by its consumer acceptance concerning several characteristics, including cleanliness, freshness, surface area, mass, volume and coefficient of packaging, egg weight, shell quality, yolk index, albumen index, HU value, and chemical composition factors that cause egg damage includes the occurrence of evaporation, loss of CO_2 through the pores egg shells, and the entry of microorganisms into the eggs which will decompose that protein found in the eggs (Yuwanta, 2010).

The egg yolk index is a method to determine the condition of eggs in general in the form of measured calculations. The egg yolk index is the ratio between the height of the yolk and the diameter of the yolk. Fresh eggs have an egg yolk index of 0.33–0.50, with an average value of 0.42.

Table 1. Egg yolk index and HU value in all treatment groups

Variables	Treatment groups				
	P-	P+	P1	P2	P3
Egg yolk index	0.44 ± 0.01[b]	0.41 ± 0.01[a]	0.43 ± 0.01[b]	0.46 ± 0.01[c]	0.44 ± 0.01[bc]
HU value	99.40 ± 5.88[ab]	93.16 ± 3.34[a]	96.08 ± 0.68[ab]	102.10 ± 5.19[b]	98.43 ± 1.01[ab]

The same superscript in the same row showed no significant difference ($p > 0.05$).

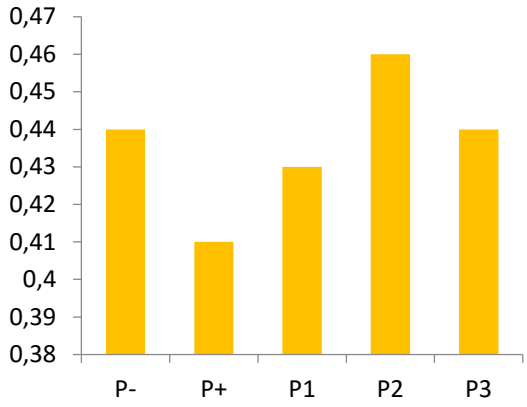

Figure 1. Egg yolk index in all treatment groups.

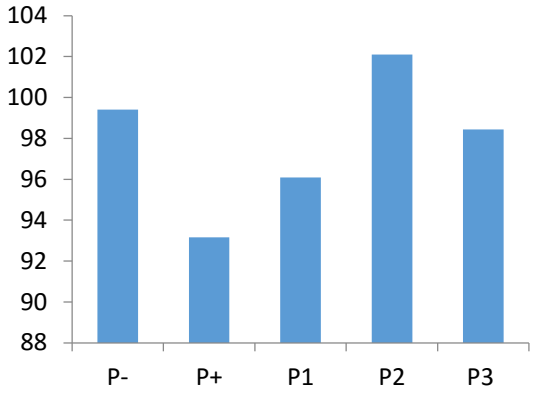

Figure 2. HU value in all treatment groups.

As the age of the eggs increases, the egg yolk index will decrease due to the increase in the size of the yolk due to water transfer (Swacita and Cipta, 2011).

Results of data analysis of the HU value showed a significant difference between the treatment groups. Level acidity (pH) rises due to CO_2 coming out during storage and a bacterial infection that results in reduced solubility of ovomucin and the thickness of the damaged albumin. The HU average value of group P- of 99.40 with AA quality, the P + group was 93.16 with AA quality, the P1 group was 96.08 with AA quality, the P2 group 102.10 with AA quality, and the P3 group 98.43 with AA quality (Table 1 and Figure 2). United States Department of Agriculture (2000) states that the HU value is less out of 31 classified quality C, HU value between 31–60 are classified as quality B, HU value between 60–72 are classified quality A, and HU value more than 72 are classified as AA quality.

The HU value are one of the important criteria for determining egg quality. According to Keshavarz (2003), egg quality also refers to the weight of the eggs produced. It also shows that the egg weight can affect the egg HU value because the higher the egg weight, the HU value is also higher, and it is so because the HU value is the determinant of the egg albumen quality. As a result, it can be concluded that egg weight and HU value have a role in determining the egg quality. The egg yolk index is influenced by several factors, including the oviduct channel muscle, albumen volume and the size of the isthmus, type, offspring, the initial period of laying, and the phase of egg production. The HU value is one of the quality parameters of albumin. The HU value is a measure to determine the freshness of eggs because the longer the time the eggs are stored will affect the decrease in albumin (Harmayanda, 2016; Lovela *et al*., 2023).

The hen's reproductive tract is susceptible to various bacterial infections. Bacterial infections may cause not only functional disorders of these organs but also internal contamination of the eggs by microorganisms. The infection attacks the reproductive tract, namely: the ovary, infundibulum, uterus, magnum, isthmus, and vagina (Wales and Davies., 2011). As the Avian Pathogenic *E. coli* bacteria attacks the magnum in the reproductive tract, the formation of the egg is affected and manifests in egg production. Infected eggs are produced with low quality, as shown by poor egg yolk index and HU value (Kabir *et al*.,

2017). Tannins are phenol compounds that serve to inhibit bacterial growth by bringing up protein denaturation and decreasing the surface tension, so bacterial permeability increases, calcium ion concentration decreases, enzyme production is inhibited, and there is disruption of the enzymatic reaction process in bacteria, thus inhibiting its occurrence plasma coagulation (Purnama *et al*., 2021). Tannin's antibacterial effect lies in protein precipitation (Pramestya *et al*., 2021). Tannins will react with the protein contained in eggshells that have similar properties to animal skin collagen form of brown sediment which can close the eggshell pores be impermeable to gas and air, leading to reduced evaporation of water as well as loss of CO_2 through the eggshell (Fajarwati *et al*., 2020).

CONCLUSION

According to these results, can be concluded that there were differences between the treatments. The best treatment used was P2, a dosage of 20% of *P. niruri* and 20% of *A. paniculata* which can be used on laying hens against the Avian Pathogenic *E. coli*.

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AUTHORS' CONTRIBUTIONS

SH: Conceptualization and drafted the manuscript. EKS, SR, and N: Treated the animal laboratory. ARK: Validation, supervision, and formal analysis. NN: Performed the statistical analysis and the preparation of table and figures. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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