Effect of Application of Propolis as Feed Supplement and Preservation Agent to Pathogenic Microbes Contamination of Local Chicken Meat

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Abstract. Chicken meat is one of the primary protein sources for the Indonesian market. However, due to the warm and humid climate, chicken meat is subject to microbial contamination, i.e., *Escherichia coli* dan *Salmonella* sp. The source of the contamination is the chicken gastrointestinal environment during rearing and post-harvest handling. One of the approaches to solve this problem is applying the natural product, which is safe for human consumption, as an antibiotic and preservation agent. This study used propolis of *Tetragonula laeviceps* as a feed supplement and preservation agent for local chicken meat. Chickens were divided into three groups (1) group I, in which chicken did not provide with propolis supplement and the harvested meat did not dip into propolis (2.5%), (2) group II in which chickens provided with propolis supplement and the harvested meat did not dip into propolis (2.5%), (3) group III in which chicken provided with propolis during rearing and the harvested meat did not dip into propolis solution, and (4) group IV in which chicken provided with propolis supplement (propolis 3%) during rearing and harvested meat dipped into propolis. Observation of *Escherichia coli* dan *Salmonella* sp. Infestation conducted at 0, 4, and 8 h. The result indicated that 2.5% propolis solution suppressed *E. coli* for all observation periods. Still, the application of propolis during the chicken-rearing period did not significantly reduce the *E. coli* population in the meat. Both Triple-Sugar-Ion (TSI) and Lysne Iron Agar (LIA) tests did not detect any *Salmonella* sp infestation. However, the disc diffusion method showed antibacterial activity of propolis against *Salmonella thyprinum*. The present finding suggested that Indonesia local chicken is more susceptible to *E. coli* infestation than *Salmonella* and the application of propolis, as immersion agent, may slowing the population growth on the meat.

Keywords: Chicken meat · *Escherichia coli.* · *Propolis* · *Salmonella sp.* · *Trigona sp*

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1 Introduction

Chicken is one of Indonesia's main protein sources, and market demand for this product increases rapidly due to population growth and change in consumption patterns [1]. In 2015, the consumption rate of chicken was 4.50 kg/capita/year and increased to 5,201 kg/capita/year in 2016 [2].

However, the high ambient temperature in a tropical country like Indonesia is one of the most severe problems poultry producers face [3, 4]. Heat stress produces several problems, such as low body weight due to inadequate feed intake and feed conversion rate [5–9] changes in internal organ condition [10,11] decreasing abdominal fat weight [12], sudden mortality due to suppressed immunity [12] and the worst of all (in term of public health) is the possible proliferation of harmful foodborne pathogens including *Escherichia* and *Salmonella* [14].

Studies showed the association between foodborne pathogen infection and the consumption of poultry products worldwide [15, 16]. This condition could significantly impact the economy and public trust in local poultry products. Furtherly, bacteria infestation in highly perishable foods such as meat and meat products shorten the shelf life through off-doors, off-flavors, discoloration, gas, and slime production [17].

Reports showed that some meat producers applied formaldehyde as a postharvest treatment to prevent spoilage by bacteria [18]. This practice significantly reduces food health and safety for public consumption. Another concern in local chicken meat is applying synthetic antibiotics at the chicken farm to prevent disease while improving feeding efficiency and body weight. This practice usually produces residues of antibiotics which may harm the health if consumed continuously [19]. Applying natural substances as a substitution for synthetic chemical substances may act as an alternative method. However, most substances are applied only for one purpose, such as antibiotics during farming or post-harvest treatment, due to the characteristic of the essence. This condition leads to higher production costs, limitation of use, and waste. In this study, we selected one substance with a broad application: propolis.

Propolis originated from plant exudate, which collected by bees as nest protective material [20, 21, 22]. Studies showed that propolis rich content of flavonoid, tannin, oil, steroid, triterpenoid, alkaloid, and glicosyd of propolis [23, 24]. Propolis is also known for its properties as antibacterial, anti-tumor, anti-fungal, antioxidant, anti-allergic, and anti-inflammatory activity [25–28].

Natural antibiotics such as propolis may effectively prevent *Salmonella* and *E. coli* contamination. There are studies on the application of this substance for food protection. Observation of physical and chemical characteristics of Tangerine cultivar Garut fruit showed that 10% propolis extract coating maintained endocarp firmness, diameter, and vitamin C level for also delayed the decaying process 20% longer than the control [29], another study show that 2,5% propolis extract could maintain the albumen and yolk condition up to 21 days [30]. However, most studies only applied during pre-harvest or post-harvest, and there still needs to be a report on how it affects if used during both periods. Therefore, this study aimed to investigate the effect of propolis application during the rearing period and post-harvest on the antibacterial activity against *Salmonella* and *E. coli.*

2 Methods

2.1 Propolis Extraction

Crude propolis was obtained from local stingless bees (*Tetragonula laeviceps*) farm in Subang, Jawa Barat. Crude propolis was immersed in 70% ethanol and stirred steadily for seven days at + 150 rpm in the darkroom to produce an ethanolic extract of propolis (EEP). Then, the solution was filtered and distilled by a rotary evaporator. Remain of ethanol was removed by keeping the distilled solution in an 80 °C water bath.

2.2 Pre-harvest Application of Propolis Extract

Two weeks old chickens were used in this study. All chickens were divided into two groups, (1) without propolis as a feed supplement and (2) the group received 1 mL of propolis extract, 3%, as a feed supplement, for 14 days. All chickens were slaughtered at 35 days old, and breast meat was collected. All harvested breast meats were divided into two groups, (1) meat that did not immerse in propolis extract 2.5%, and (2) meat that was immersed in propolis extract 2.5% for 5 min. All meats were kept in the open air at room temperature and became subject to *E. coli* and *Salmonella* sp. Infestation tests after 0, 4, and 8 h.

2.3 Measuring E. Coli Population

About 2.5 g of chicken meat samples were collected from control and application groups. Samples were diluted in 22.5 mL peptone water sterile, homogenized, and cut to 10–10. Inoculation of presumptive *E. coli* isolate mixed with 20 mL melted MacConkey agar, which already cooled to 45 °C (a technique known as the pour plate method). The mixture was incubated for 24 h at 37ºC. The number of *E. coli* growth in the medium was manually calculated, and the population of *E. coli* was determined by formulae [31]:

$$Population\ Size\left(\frac{Cfu}{g}\right) = \frac{Number\ of\ calculated\ colonies}{Inoculation\ volume\ x\ dilution\ factor}$$

2.4 Identification of the Salmonella Isolates

In this study, the presumptive *Salmonella* isolates were identified by two biochemical tests, the Triple-Sugar-Iron (TSI) agar test and the Lysine Iron Agar (LIA) test. The presumptive *Salmonella* colonies collected from chicken meat were stabbed into the TSI agar and LIA slant. The inoculated samples were incubated at 35 °C for 24 h [32].

2.5 Disc Diffusion Method

Aseptically, Nutrient Agar plates were swabbed by *S. tgyprinum*. Sterile paper discs (3.5 mm) were dipped in 20 μL Steril aquadest (as control) and 20 μL propolis extract 2.5% (as application) and placed on swab plates for *S. tgyprinum* and *E. coli* in specific

dilutions and placed in the agar plate. All agar plates were incubated at 37 °C for 24 h. Measured inhibition zones from each paper disc were measured by a caliper in the nearest mm. The size of the inhibition zone can be determined by subtracting the disc diameter from the total diameter of the disc and the inhibition zone. If the test organism grows on the disc, it may be assumed that it is resistant to propolis.

3 Result and Discussion

3.1 The Antibacterial Activity of EEP Against E. Coli

The result showed a significant effect of EEP application as an immersion agent to prevent chicken meat spoilage by *E. coli* infestation (Anova, $P < 0.05$). On the other hand, the application of propolis as a feed supplement did not prevent *E. coli* infestation (Anova, $P > 0.05$). The population of *E. coli* in the meat increased with observation time. Among all groups, meat immersed with and originating from chicken that did not receive propolis as a feed supplement showed the lowest *E coli* population (Table 1).

P0. TC = No EEP supplement, no EEP. Immersion.

P0.C = No EEP supplement, EEP immersion.

P1. TC = EEP supplement, no EEP immersion.

P1.C. = EEP supplement, EEP immersion.

A, B = Values with the different capital letters indicated significant differences in the *E. coli* population during observation time ($P < 0.05$).

a,b,c = Values with the other small letters showed significant differences in *E. coli* population among treatment regimes ($P < 0.05$).

Results showed that supplementation of EEP did not show the effect of total coliforms, *E. coli* which agrees with Rahman et al. [33], Mahmoud et al. [34, 35], although a study by Abdel-Mohsin et al. [36] and Sheif & El-Saadany [37] showed the contradictive result. *E. coli* belongs to the gram-negative bacteria, and some studies showed the weak response of EEP to gram-negative bacteria [38].

Some hypotheses explain this result: (1) Differences in the bacterial wall structure. Gram-negative bacteria have a complex outer membrane consisting of two lipid bilayers [39, 40]. These layers provide a physical barrier that prevents bacterial cell interactions with harmful substances. On the other hand, Gram-positive bacteria only have one

Table 1. The population size of *E.coli* in chicken meat treated

Treatment	Observation after (log_{10} cfu/g)		
	0h	4h	8h
P0.TC	2.68^{Ba}	8.02^{Bb}	11.72^{Bc}
P0.C	1.83^{Aa}	5.09^{Ab}	9.15^{Ac}
P1.TC	3.01^{Ba}	6.51^{Bb}	12.01^{Bc}
P1.C	0.49^{Aa}	5.41^{Ab}	11.07^{Bc}

relatively permeable membrane, making them more susceptible to exchange with the environment [41]; (2) solvent used for extraction. To produce EEP, ethanol was used as a solvent. *E.coli* population growth in ethanol-rich environments will activate the regulation of lipid to protein ratio of the plasma membrane, allowing them to produce a more rigid plasma membrane that improves their survival [42]. The solvent effect is quite significant as the study showed potent antibacterial activity of propolis extract by acetone to *E. coli* [43]; (3) Variation of the chemical composition of EEP. Propolis originated from substances produced by plants. Some active ingredients may easily penetrate bacteria, while others may fail to interact with the bacteria cell. The variations of propolis's chemical contents depend on several factors, such as age, time of harvest, and plant origin [44, 45].

Application of EEP as post-harvest showed that meat originating from chicken fed on propolis supplement was more likely to harbor a large *E. coli* population after 4 h at room temperature. However, immersion of meat with EEP reduced *E. coli* population as reported by previous studies [46]. We hypothesized the possibility of development of propolis resistance during administration of propolis supplements to chickens. Further study is required to test this hypothesis by observing the resistance level of *E. coli* in the fecal and digestive systems. Another possibility is the concentration of propolis applied was too low to produce significant effect to reduce *E. coli* population [47].

3.2 The Antibacterial Activity of EEP Against *Salmonella Sp.*

The possibility of *Salmonella* infestation in the chicken meat showed Both Triple-Sugar-Ion (TSI) and Lysne Iron Agar (LIA) tests did not detect any *Salmonella* sp in all groups (Table 2).

P0. TC = No EEP supplement, no EEP immersion.
P0.C = No EEP supplement, EEP immersion.
P1. TC = EEP supplement, no EEP immersion.
P1.C. = EEP supplement, EEP immersion.

The negative result of the *Salmonella* test indicated a lack of Salmonellosis disease in the chicken used in this study, and it can describe the condition of the chicken and good sanitation during rearing. Because of that, secondary data collection was carried out to see the ability of the EEP in suppressing the growth of *Salmonella* sp. as an antibacterial using the disc diffusion method (Table 3).

Table 2. The *Salmonella* test in chicken meat treated

Treatment	Observation after		
	0h	4h	8h
P0.TC	Negative	Negative	Negative
P0.C	Negative	Negative	Negative
P1.TC	Negative	Negative	Negative
P1.C	Negative	Negative	Negative

Table 3. Inhibition zone formed due to EEP

Disc	Replication (mm)			Average (mm)
	1	2	3	
1	2.40	2.50	2.90	2.60
2	2.40	3.10	2.50	2.66
Average (mm)				2.63

The disc diffusion method confirmed antibacterial activity of local propolis to *Salmonella* by producing an inhibition zone with an average size of 2.63 mm. Furthermore, the result also showed that standard practices of local chicken farming could maintain the health of the farm environment. Thus, applying propolis as an antibacterial furtherly improves the health of chicken meat for human consumption.

4 Conclusion

Application of propolis extract during rearing and at harvested chicken meat suppressed the growth of *E. coli* and *Salmonella* sp. Until 8 h after application.

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