



Total of *Escherichia coli* Excreta Broiler Given *Enterococcus* sp. as Probiotics Candidate of Poultry

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Abstract

The purpose of this study was to see the effect of giving *Enterococcus* sp. bacteria at *Escherichia coli* of excreta broiler. The research design used was a completely randomized design consisting of four treatments, three replications, and each replication had four broilers. The treatment given consisted of T1 (0 mL/L), T2 (1 mL/L), T3 (3 mL/L), and T4 (5 mL/L) control treatments. The number of colonies of *Enterococcus* sp. given through drinking water every day, namely 10^7 CFU / mL. The results of the study respectively showed the number of *Escherichia coli* in the treatment of T1 (Log 7.54 CFU/g), T2 (Log 7.53 CFU/g), T3 (Log 7.48 CFU/g), and T4 (Log 6.78 CFU/g). The colony total of *Escherichia coli* of broiler excreta decreases with increasing doses of *Enterococcus* sp. It is shown that *Enterococcus* sp. has an antimicrobial compound activity which can inhibit pathogen growth in broiler digestive tract so that it has the potential to be developed as a probiotic.

Keywords: *Enterococcus* sp., *Escherichia coli*, probiotic, Excreta of Broiler

A. Introduction

The digestive tract of the broiler is a habitat for various living microorganisms. Some organisms had beneficial and detrimental the productivity of broilers. Among groups of microbes that live in the digestive tract of broiler chickens, namely the *Escherichia coli* bacteria. These bacteria have been reported to have the potential of pathogens in the gastrointestinal tract of livestock if the population continues to grow. According to Kumar, Jindal, Shukla, Asrani, Ledoux, & Rottinghaus (2004) and Alonso, Padola, Parma, & Lucchesi (2011) colibacillosis, caused by enterotoxigenic *Escherichia coli*, results in considerable economic losses in poultry production. It will provide a financial decline in broiler cultivation.

Various serotypes of *Escherichia coli* can infect most mammals and poultry. Especially for chickens called Avian Pathogenic *Escherichia coli* (Arne, Marc, Bree, Scholer, & Moulin, 2000). Young birds, in which the protective immune system is not fully developed, are more vulnerable. *Escherichia coli* serotypes O78: K80, O1: K1, and O2: K1 is the most commonly found in domestic breeds with colibacillosis (Sharma, Jakhar, & Dahiya, 2016).

An approach that can be done to control the development of *Escherichia coli* in the digestive tract, namely maintaining a balance between beneficial and harmful bacteria. Probiotic giving of bacteria is an alternative that can be done to maintain beneficial bacterial populations to remain stable in the digestive tract of broilers.

Lactic acid bacteria are one group of bacteria that have long been used as probiotics. Lactic acid bacteria are useful groups of microorganisms because they do not have toxic properties for the host and are known as microorganisms that are not at risk for health. The potential of lactic acid bacteria to be used as probiotics because it produces compounds that can inhibit the growth of pathogenic bacteria (Klaenhammaer, 2001).

Enterococcus sp. is one of the lactic acid bacteria that can be developed as a probiotic in broiler cultivation. One of the criteria for bacteria to be used as a candidate for probiotics, which could inhibit the growth of pathogenic bacteria in the digestive tract. According to Bednorz, Guenther, Oelgeschager, Kinnemam, Pieper, Hartmann, Tedin, Semmler, Neumann, Schierack, Bethe, & Wieler (2013), various strains of bacteria have been used as probiotics, and the most commonly used species include *Bacillus*, yeast and lactic acid-producing bacteria such as *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, and *Enterococcus*. Therefore, in this study, we will look at the effectiveness of giving *Enterococcus* sp. which has been isolated from the digestive tract Day-Old Chicken (DOC) broiler on the number of *Escherichia coli* colonies in broiler excreta.

B. Methodology

1. The Material

A total of 48 DOC broiler strain Cobb 500 mixed sex (male and female) were placed randomly in the experimental unit according to complete randomized design. The cages used were 12 experimental units with a size of 75 cm x 100 cm x 50 cm. The floor of the cage was given with rice husk with a thickness of 1.7 cm. Each cage of the experimental unit is equipped with a 40-watt lamp as a heater for a week. The research chickens were treated with *Enterococcus* sp from the age of 1 to 35 days and given feed ad-libitum.

2. Research Procedures

Four treatments were consisting of 3 replications, each replication containing four DOC broiler. Probiotics were used as treatments, namely *Enterococcus* sp (10^7 CFU/mL). The dose of probiotic treatment consists of T1 (0 mL/L/day), T2 (1 mL/L/day), T3 (3 mL /L / day), and T4 (5 mL/L /day). The treatment of probiotics was given for 35. The composition of the ration is presented in Table 1.

Table 1. Composition of basal rations during research.

Feed ingredients	Amount (%)
Corn	58
Pollard	6
Fish flour	10
Soybean Meal	9
Rice Bran	4
Coconut cake	3
Meat and Bone mash	9
Premix	0.5
	100
Crude protein	20.27
Energy Metabolism (kcal / kg)	3026
Fat	6.90
Coarse fiber	3.49
Phosphor	1.05
Calcium	1.65

Source: Composition of rations based on calculation results

3. Calculation of the number of *Escherichia coli* colonies

At the end of the stud, 2 grams of broiler chicken manure was taken for each repetition in the treatment put in a plastic bottle and taken to the laboratory as a research sample to calculate the amount of *Escherichia coli*. The tools and media used were sterilized by autoclave at 121 °C for 20 minutes.

Eosin Methylene Blue Agar (EMBA) media, weighed 9.5 grams then dissolved with 250 mL distilled water in Erlenmeyer. Furthermore, it is homogenized by heating with a magnetic stirrer. After being homogenized then sterilized in an autoclave for 15 minutes at 121 °C. Moreover, the temperature of the EMBA media and BPW is cooled to about 40-45 °C in the water bath, after the cold, the EMBA media is poured into a petri dish of 15 mL. While the BPW media is poured into a test tube 10 mL each. As much as 1 gram of broiler excreta is weighed from each treatment T0 (0 mL/L/day), T2 (1 mL/L/day), T3 (3 mL/L/day), and T4(5 mL/L/day) inserted into the test tube and in the stomacher until homogeneous. Calculation of the number of *Escherichia coli* colonies was carried out in the last two dilutions in duplicate. Each one ml piped into a petri dish, then poured sterilized EMBA media. Next, the Petri plates were incubated at 37 °C for 18-24 hours in reverse. Counter colonies calculate the number of *Escherichia coli* by calculating the number of colonies that grow between 30-300 CFU/gram.

4. Data Analysis

The results of the study were analyzed using variance analysis according to the design of a completely randomized design. Duncan's multiple region tests will identify the effect of treatment differences at the level of 5% ($P < 0.05$).

C. Result and Discussion

The media used to grow *Escherichia coli* bacteria in this study, namely Eosin Methylene Blue Agar (EMBA), which is a selective medium for these bacteria. Colonies that grow were round, smooth, convex, red-black, or shiny green or blackish blue to brown with a metallic sheen on EMBA media (Quinn, Markey, Carter, Donnelly, & Leonard, 2002). The results of the study of the number of *Escherichia coli* in broiler excreta given the treatment of *Enterococcus* sp. are presented in Table 1 illustration 1.

Table 1. The average number of *Escherichia coli* colonies in broiler excreta at 35 days of age

Variable	Treatment			
	T1	T2	T3	T4
<i>E.coli</i> (Log CFU/mL)	7.54 ^a	7.53 ^a	7.48 ^a	6.78 ^b

^{a,b} Mean value on the same row with different superscripts shows significantly different ($P < 0.05$). T1 (*Enterococcus* sp. 0 mL/L/day), T2 (*Enterococcus* sp. 1 mL/L/day), T3 (*Enterococcus* sp. 3 mL/L/day), and T4 (*Enterococcus* sp. 5 mL/L/day).

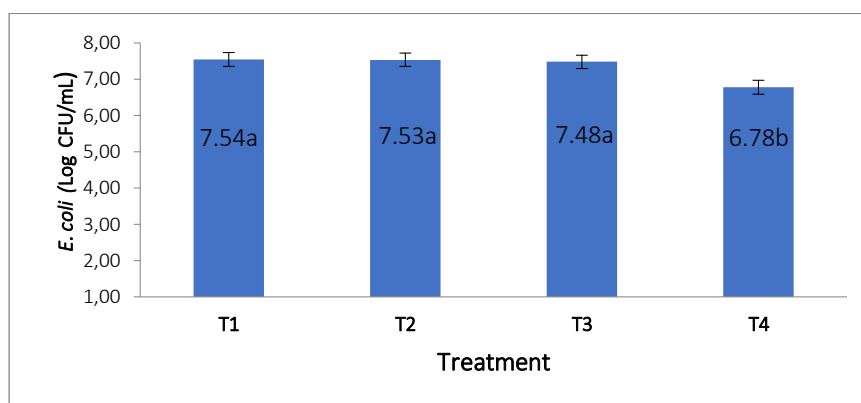


Figure 1. The colony total of *Escherichia coli* on broiler excreta at the age of 35 days. Mean value^b with different superscripts shows significant differences ($P < 0.05$).

The results of variance showed that the treatment had a significant effect ($P > 0.05$) on the number of *Escherichia coli* in broiler excreta maintained for 35 days. There is a tendency for the amount of *Escherichia coli* to decrease with increasing administration dose of *Enterococcus* sp. in broiler drinking water. The highest number of *Escherichia coli* in control treatment (0 mL/L) was not given *Enterococcus* sp. (Log 7.54 CFU/g) and the lowest in the treatment of *Enterococcus* sp. 5mL/L of drinking water per day (Log 6.78 CFU/g). According to Kabir (2010), In chickens, there are about 10^9 colony forming units (CFU) of bacteria per gram of excreta, and these, 10^6 CFU is *Escherichia coli*.

In this study, the treatment of 5 mL/L of drinking water per day was a treatment that gave an optimum response to the amount of *Escherichia coli* excreta broiler. The ability of *Enterococcus* sp., decreasing the amount of *Escherichia coli* excreta broiler in this study will have a good impact on the health conditions of the broiler digestive tract. The health of the digestive system is an important thing that must always be maintained. It was because the gastrointestinal tract is the place to pass and the entry of various nutrients needed for the survival of the body. In addition to increasing the absorption of food substances, the large surface of the digestive tract is also often exposed to various foreign elements or materials, including pathogenic agents. The presence of pathogens in the gastrointestinal tract can cause different disease, including diarrhea. According to Savkovic, Villanueva, Turner, Mathowskyj, & Hecht (2005), pathogens that often cause interference in the digestive tract, especially in the small intestine, namely Enteropathogenic *Escherichia coli* (EPEC). EPEC, which is found in the gastrointestinal tract, can potentially damage the mucosa of the digestive tract.

The digestive tract of chickens is known to be one of the principal reservoirs of the *Escherichia coli* bacteria that need to be watched out for besides cattle as the main reservoir (Heuvelink, Zwartkruis, Beumer, & Boer, 1999). Avian pathogenic *Escherichia coli* (APEC) causes various diseases, collectively termed colibacillosis, in chickens, and these diseases are responsible for significant economic loss in the chicken industry (Hammerum & Heuer, 2009 and Mohamed, Shehata, & Rafeek, 2014). Moreover, poultry products contaminated with APEC are potential sources of foodborne extraintestinal pathogenic *Escherichia coli* infections for humans, posing a threat to human health (Bergeron, Prussing, Boerlin, Daignault, Dutil, Reid-Smith, Zhanel, & Manges, 2012)

The results of this study indicated that *Enterococcus* sp. can inhibit the development of *Escherichia coli* found in the digestive tract of broilers. The ability of *Enterococcus* sp. in inhibiting the growth of *Escherichia coli* in the broiler digestive tract because it is caused, these bacteria have antimicrobial compounds. One of the metabolites produced by *Enterococcus* sp., which is lactic acid, can reduce the pH conditions of the digestive tract of broilers. At low pH conditions, some pathogenic bacteria can be inhibited of growth. The research Peng, Zeng, Zhu, Wang, Liu, Hou, Thacker & Qiao (2016) had shown that lactic acid bacteria (*Lactobacillus Plantarum* B1) decreased the number of fecal *Escherichia coli* in broilers due to its ability to produce lactic acid and short chain fatty acids. Furthermore, the study of Hidayat, Malaka, Agustina, & Pakiding (2018) showed the strength of lactic acid bacteria *Lactobacillus* in inhibiting the growth of pathogenic bacteria *Escherichia coli* on excreta of the broiler.

The group of lactic acid bacteria can inhibit the growth of pathogenic bacteria, through several antimicrobial compounds produced, such as organic acids, hydrogen peroxide, diacetyl and bacteriocin (Abdelbasset & Djamila, 2008). Enterococci may produce antimicrobial peptides named bacteriocins (enterocins) capable of inhibiting the growth of specific pathogens and spoilage microorganisms, with great potential for food preservation (Franz, van Belkum, Holzappel, Abriouel, & Gálvez, 2007). Bacteriocins are ribosomally synthesized peptides produced as a defense mechanism against closely related bacteria Drider, Fimland, Héchard, McMullen, & Prévost (2006).

The ability of bacteria *Enterococcus* sp. in reducing the population of *Escherichia coli* in broiler excreta in this study could be the reason that they could be used as one of the candidates for probiotics in broilers. Several previous studies have shown the results of using a group of *Enterococcus* bacteria as probiotics in poultry. These results research Cao, Zeng, Chen, Zhou, Zhang, Xiao, & Yang (2013) suggest that *Enterococcus faecium* can promote growth performance, improve intestinal morphology, and beneficially manipulate the cecal microflora in broilers challenged with *Escherichia coli* K88. However, to ensure that the *Enterococcus* sp bacteria can be used as probiotics, it is still necessary to test several criteria for a bacterium to be used as a probiotic.

Enterococci are extensively studied as potential candidate probiotics. Considerations for strain selection include several criteria such as molecular identification using genetic typing techniques, safety, capacity to survive intestinal transit, manufacturing, distribution, and the targeted application. The functional requirements of probiotics include tolerance to gastric juice and bile, adherence to epithelial surfaces, persistence in the gastrointestinal tract (GIT), immune stimulation, antagonistic activity toward intestinal pathogens and the capacity to stabilize and modulate the intestinal microbiota (Hanchi, Mottawea, Sebei, & Hammami, 2018)

D. Conclusion

The results showed that administration of *Enterococcus* sp. bacteria could reduce the number of *Escherichia coli* colonies in Broiler excretions, especially at doses of 5 mL/L of drinking water per day. This shows that *Enterococcus* sp. used in this study can be developed as one of the probiotic candidates.

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