

Evaluation of total bacteria in small intestine ileum of the Indonesian crossbreed chickens fed probiotic bacillus bacteria

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Abstract. The purpose of this study was to determine the number of lactic acid bacteria, Coliform and Enterobacteria in the small intestine ileum of the Indonesian indigenous crossbreed chickens. The material used is 200 super unisex super native day old chick (DOC). The Indonesian indigenous crossbreed chickens received antibiotic and probiotic Bacillus treatment for 10 weeks mixed with rations. The research design that is proposed is a completely randomized design (CRD) consisting of 4 treatments with 5 replications, while the treatments applied are T0 (control), T1 (control + 0.04% Antibiotic (Zinc bacitracin)), T2 (control + 0, 01% commercial probiotics (Bacillus subtilis)) and T3 (control + 0.5% probiotics (Bacillus plus vitamins and minerals)). Parameters were taken randomly, 1 chicken from each experimental plot. The parameters observed were the total bacteria lactic acid, Coliform, and Enterobacter, The data were analyzed for their diversity at an accuracy of 5%. The results showed that there was no significant effect ($P > 0.05$) of treatment on the number of Coliform and Enterobacter bacteria, but a significant effect ($P < 0.05$) on the increase in the number of lactic acid bacteria. It was concluded that giving Bacillus probiotics did not have an effect on the number of Coliform and Enterobacter bacteria but increased the number of lactic acid bacteria.

Keywords : *Coliform, Enterobacteria, Ileum, Lactic acid bacteria*

INTRODUCTION

The Indonesian indigenous crossbreed chickens is one of the livestock that has similar characteristics to local chickens, therefore super native chickens is also popular with the community, but super native chickens have low productivity. One of the efforts to support growth and increase productivity is giving feed additives, ingredients or a mixture of several ingredients that are added in small amounts to the feed (Rahmah et al., 2013).

Feed additive commonly used is antibiotics, but its use has been banned. The use of antibiotics can be bad because it can cause antibiotic residues in meat and have negative effects on the health of consumers in long time. Apart from food safety

reasons, prohibiting the use of antibiotics can also increase mortality and decrease productivity of chickens. Probiotics are a substitute solution for using antibiotics.

Probiotics can be defined as additional feed containing live microbes which have a beneficial effect on the host by improving the microbial balance (Hassan, 2006). Probiotics will increase the number of microbes in the small intestine. The function of probiotics is to regulate the balance of microbes in the digestive tract, to increase immunity, support growth, increase efficiency, feed conversion, and help optimize nutrient absorption (Kompang, 2004). Some benefits of giving probiotics in the mixture feed to chickens are maintaining beneficial microflora in the digestive tract and inhibiting the growth of pathogenic bacteria, increasing digestive enzyme activity,

reducing bacterial enzyme activity and ammonia production, increasing food intake and digestion and neutralizing enterotoxins and stimulates the immune system (Hassan, 2006).

Bacillus is a probiotic that is often used because it has better stability and ability to survive than other probiotics in non-beneficial environmental conditions. Bacillus has antimicrobial properties which function to inhibit the growth of pathogenic bacteria in the small intestine of chickens. Bacillus is able to form spores, produce cellulase, amylase, lipase, and proteases and other enzymes that can help the digestive process in the digestive tract of the host (Haryati, 2011). The working mechanism of Bacillus is to affect cell wall synthesis, disrupt or damage membrane function, inhibit nucleic acid synthesis, and inhibit protein synthesis in pathogenic bacteria (Yulika, 2009).

This study purpose to determine the effect of the addition of Bacillus probiotic additives in super native chicken rations on the number of lactic acid bacteria, Coliform and Enterobacter sp in the small intestine ileum of super native chickens. The benefit of this research is to provide information about the use of probiotic feed additive Bacillus ration of super native chickens to total lactic acid bacteria, Coliform and Enterobacter sp. in the ileum of super native chickens.

MATERIALS AND METHODS

Materials

The material used in this study was 200 day old chickens (DOC) of super native chickens imported from Boyolali, with a body weight of 37.81 ± 0.184 g / head. The rations given does not contain antibiotics. Other ingredients are antibiotics, commercial probiotics (Bacillus subtilis), biotemics and disinfectants for biosecurity, MRS agar and MacConkey agar as a medium for breeding lactic acid bacteria, Coliform and Enterobacter sp, aquades and anaerocult. The composition of the diets and their calculated or analyzed nutrient content are shown in (Table 1).

Methods

The cage of each pen is filled with 10 chickens with 4 treatments and 5 replications. Each pen is equipped with 1 place to feed and 1 place to drink, a 60 watt bulb. Other tools are digital scales, analytical scales, autoclaves, oven, test tubes, erlenmeyers, pipettes, petri dishes, incubators and colony counters. The experimental design used in this study was a completely randomized design (CRD), with 4 treatments and 5 replications so that there were 20 experimental units, each unit had 10 chickens so that there were 200 the Indonesian indigenous crossbreed chickens. The treatments used were T0 (control), T1 (control + 0.04% Antibiotic (Zinc bacitracin)), T2 (control + 0.01% commercial probiotic (Bacillus subtilis)) and T3 (control + 0.5% Probiotic (Bacillus plus vitamins and minerals)).

Sampling was carried out when the Indonesian indigenous crossbreed chickens were 10 weeks old by taking 20 chickens as samples, 1 for each treatment and replication. The sample used to calculate the total lactic acid bacteria, Coliform and Enterobacter sp was digesta from the ileum of the Indonesian indigenous crossbreed chickens. Digestion ileum samples were collected from each experimental unit and then analyzed the total lactic acid bacteria, Coliform and Enterobacter sp. Total calculation of lactic acid bacteria, Coliform and Enterobacter sp was calculated using the Total Plate Count (TPC) method.

Data Analysis

All of the data were analyzed by one-way ANOVA of completely randomized design (CRD), then followed by Duncan Multiple Range Test (DMRT) for testing the significance level of 5%.

RESULTS AND DISCUSSION

Total Bacteria in Ileum

The total number of bacteria in the ileum digesta of the Indonesian indigenous crossbreed chickens after giving Bacillus probiotic in the ration is presented in (Table 2).

Table 1. The composition of the diets and their calculated or analyzed nutrient content

Ratio Ingredients	Composition (%)
Corn	45.5
Soybean Meal	17.0
Wheat Powder	10.0
Bread Powder	5.00
Rice Bran	4.45
Crude Palm Oil	3.50
Corn Gluten Meal	3.60
Distiller Dried Grains	3.00
Meat Bone Meal	2.80
Chicken Feather Meal	2.00
Bone Meal	1.50
Lysine	0.55
Methionine	0.37
L-Threonine	0.08
Salt	0.15
Premix	0.50
Total	100
Nutrients Content	Compositions
Metabolic Energy (kcal/kg) ²	3286
Dry Matter (%)	91.4
Crude Protein (%)	21.7
Crude Fat (%)	5.90
Crude Fiber (%)	6.79
Ash (%)	10.9

Table 2. Average of total bacteria in the Indonesian indigenous Crossbreed Chickens ileum

Bacteria	Treatments			
	T0	T1	T2	T3
	------(log cfu/g)-----			
Lactic acid bacteria	7,19 ^b	7,16 ^b	7,49 ^{ab}	7,76 ^a
Coliform	5,10	5,05	5,07	4,83
Enterobacter	3,86	3,10	3,10	2,94

Different superscripts on the same line indicated a significant difference ($P < 0.05$). T0 (Basal Feed), T1 (Basal Feed + 0.04% Zinc Bacitracin), T2 (Basal Feed + 0.01% Bacillus subtilis) and T3 (Basal Feed + 0.5% Bacillus plus vitamins and minerals).

Number of Lactic Acid Bacteria

The results of statistical analysis showed that there was an effect ($P < 0.05$) on the treatment given to the total lactic acid bacteria in the ileum digesta of Indonesian indigenous crossbreed chickens. Giving antibiotics in feed has no effect in increasing the amount of LAB because antibiotics can kill pathogenic bacteria and good bacteria in the digestive tract. Pelczar (2008) states that antibiotics can kill or inhibit the development of pathogenic bacteria and other

organisms. Probiotics based on Bacillus in feed has a positive effect on the amount of LAB because probiotics can improve the balance of other microorganisms and can eliminate pathogenic bacteria from the host.

Probiotic Bacillus subtilis which is a commercial probiotic is also effective in increasing lactic acid bacteria but not as significant as the results of the increase in lactic acid bacteria given Bacillus plus vitamins and minerals. This can be due to the giving vitamins and minerals aimed at avoiding stress on Indonesian indigenous crossbreed chickens so that the balance of the bacterial population in the intestine can be maintained. According to Kusnadi et al. (2006) certain vitamins and minerals in large quantities are needed if the body is under stress due to emotional stress or environmental stress to maintain productivity and growth by maintaining the balance of the microflora in the intestine.

Number of Coliform Bacteria

The results of statistical analysis showed that there was no effect ($P > 0.05$) on the total coliform bacteria in the ileum digesta of Indonesian indigenous crossbreed chickens. Even so, there was a tendency that adding Bacillus to the feed was able to reduce total coliform bacteria compared to other treatments. Bacillus probiotics will decrease the growth of coliform bacteria because of the presence of antimicrobial compounds in Bacillus. According to Kompiang et al (2004) Bacillus can reduce and inhibit the growth of pathogenic bacteria that attack chicken health. If the number of pathogenic bacteria in the digestive tract exceeds the maximum limit, it can cause inflammation of the small intestine and cecum. According to Sun (2004) that inflammation occurs in the digestive tract will inhibit the absorption of essential nutrients for poultry growth and decrease the growth of microbes that can synthesize vitamins.

Number of Bacteria Enterobacter sp

The results of statistical analysis showed that there was no effect ($P > 0.05$) on each treatment given to the total bacteria enterobacter sp in the ileum digesta of Indonesian indigenous crossbreed chickens. The total of Enterobacter sp bacteria in the ileum of Indonesian indigenous crossbreed chickens which was given Bacillus probiotic was expected to reduce the number of pathogenic bacteria such as

Enterobacter sp. According to Safingi et al (2013) that giving probiotics in the ration can increase the number of bacteria that are useful for chicken digestion and reduce the number of pathogenic bacteria that are detrimental to chicken digestion so that it will increase the digestibility level of the chicken. The fluctuation in the number of Enterobacter sp was related to the number of LAB, the less number of Enterobacter bacteria tended to increase the number of LABs. This shows the occurrence of competitive exclusion, competition between LAB and Enterobacter to get space and nutrition in the small intestine. Nevy and Tafsir (2008) state that one of the factors affecting the bacterial population in the small intestine is the ability to compete in obtaining nutrients and space in the small intestine.

CONCLUSION

The conclusion of this study is that the provision of Bacillus probiotics did not affect the number of Coliform and Enterobacter bacteria but increased the number of lactic acid bacteria.

CONFLICT OF INTEREST

We certify no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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