

Effect of Herbal Formulas and LAB as Antimicrobials on Microflora (*Escherichia Coli* and *Salmonella Sp.*) in the Intestines of Layer Chicken

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Abstract—This study aimed to evaluate the effect of adding herbal formulas and Lactic Acid Bacteria (LAB) as antimicrobials in feed on the population of microflora in the intestines consisting of *Escherichia coli* and *Salmonella sp.* The material used in this study was chicken aged 35 weeks strain Isa brown produced by PT. Malindo Feedmill Tbk Gresik, East Java with an egg mass uniformity coefficient prior to the study of 5.92%. The method used in this study was a completely randomized design with 4 treatments with 6 replications, the variables observed were the population of *Escherichia coli* and *Salmonella sp.* in the small intestine of laying hens, if there is a significant effect, it will be tested by Duncan's Multiple Range Test Method. The treatments tested consisted of P0 = control feed, P1 = control feed + 0.25% herbal formula + 0.2% LAB, P2 = control feed + 0.50% herbal formula + 0.2% LAB, P3 = control feed + 0.75% herbal formula + 0.2% LAB. The results showed that the addition of herbal formulas and LAB in feed had a significant effect ($P < 0.05$) on the population of *Escherichia coli* and *Salmonella sp.* The addition of the levels of herbal formulas and LAB in the feed as much as 0.25-0.50% can reduce the population of *Escherichia coli* and *Salmonella sp.*

Keywords—Laying hens, Herbal Formula, LAB, microflora, chicken intestine.

I. INTRODUCTION

Feed in chicken farming business activities is the highest production cost component reaching 70-80%, so the use of feed must be used efficiently, but does not interfere with livestock production. One of the efforts to improve feed quality is the addition of feed additives. In general, the addition of feed additives can be in the form of antibiotics, prebiotics, probiotics, enzymes, organic acids, phytobiotics or plant bioactives and essential oils (Magdalena et al., 2013). However, the use of antimicrobials for feed additives has been prohibited through Law 18 of 2009 concerning Livestock and Animal Health Article 51 paragraph (3) which states that everyone is prohibited from using certain veterinary drugs on livestock whose products are for human consumption. In addition, there is Regulation of the Minister of Agriculture 14 of 2017 Article 4 which states that veterinary drugs that have the potential to endanger human health are prohibited from being used on livestock whose products are for human consumption. According to Mulyadi (2013) the use of antimicrobials as a growth promoter that continuously results in residues in livestock products produced so that it affects food safety.

Efforts made to overcome this need to be done with feed manipulation. Manipulation carried out is to replace from chemicals (antimicrobials) that is to replace them with herbal or phytobiotic materials, where these materials must have the same role so that they can replace the role of the use of antibiotics in feed. The types of herbal plants that have good properties include ginger and ginger. Ginger contains flavonoid compounds, phenols and essential oils as antibacterial, *Escherichia coli* and *Bacillus subtilis* bacteria which are pathogenic to the digestive tract (Sulistyoningsih, Dzaky and Nurwahyunani, 2014) and Temulawak contains components of flavonoid compounds, phenols and curcumin which act as antioxidants (Jayaprakhasha)., 2006). The use of phytobiotics already has good quality as a substitute for AGP, but it takes a long time so that other feed additives such as LAB are needed to help maximize the digestive process in the intestine.

Lactic Acid Bacteria is a type of probiotic group with live microbes that is given as a dietary supplement with the aim of improving microbial health and development. While the addition of probiotics such as LAB can reduce the ability of pathogenic microorganisms to produce toxins, reduce the negative effects caused by feed barriers (in the form of anti-nutrients) because probiotics are able to stimulate an increase in the availability of food substances, stimulate the production of digestive enzymes and produce vitamins and antimicrobial substances so as to improve health status. digestive tract (Sumarsih et al, 2012). This study aimed to evaluate the effect of herbal formulas and LAB as antimicrobials in feed on populations of *Escherichia coli* and *Salmonella sp.*

II. MATERIAL AND METHODS

This research was carried out at the laying hens farm owned by Mr. Ahmad Fauzi, located in Joho Village, Kalidawir, Tulungagung. Proximate analysis of the treated feed was carried out at the Nutrition and Animal Husbandry Laboratory, University of Muhammadiyah Malang. Intestinal microflora analysis was carried out at the Laboratory of Biology and Technology, Airlangga University, Surabaya.

A. Materials

The material used was Isa Brown laying hens produced by PT. Malindo Feedmill Tbk Gresik, East Java as many as 168 chickens aged 42 weeks with a coefficient of diversity egg mass before the study was 5.92%. Temulawak and ginger

flour were obtained from PT. Jaya Makmur Poultry, Tulungagung. The LAB used in the study was obtained from Bengkel Farmers (BeTa) Malang. The control feed used was in the form of mash, namely a mixture of corn, rice bran, concentrate. The concentrate used is commercial feed with code KLKS 36 SPR from PT. Japfa Comfeed Indonesia Tbk.

B. Cages and Equipment

The cage used in the study was a sealed battery cage with a size of 30 x 30 x 40cm. Each cage unit is filled by 1 chicken. The battery cage is made of bamboo and is equipped with a pipe for eating and drinking. Drinking water flows from water reservoirs. The roof of the cage is made of esbes and the base is made of bamboo. The equipment used in the study included ration mixing containers, digital scales for weighing eggs and leftover feed, observation sheets and writing instruments.

C. Feed and Drinking

The control feed used was in the form of mash, namely a mixture of corn, rice bran, concentrate. The concentrate used is commercial feed with code KLKS 36 SPR from PT. Japfa Comfeed Indonesia Tbk. The basal diet was added with ginger flour and temulawak at 0.25, 0.50 and 0.75% levels, respectively, and Lactic Acid Bacteria (LAB) 0.2% respectively. Feeding refers to Isa Brown (2010), the feed consumption of laying hens aged 30-40 weeks is 120 g/head/day. Feed is given 2 times a day, in the morning at 08.00 WIB and in the afternoon at 15.00 WIB. Provision of drinking water ad libitum.

D. Research Methods

The data obtained were tabulated with the Microsoft Excel program and then statistical analysis was performed using analysis of variance (ANOVA). If there is an effect between treatments, then Duncan's Multiple Distance Test is continued. The experimental design used was a completely randomized design (CRD), with 4 treatments and 6 replications, where each replication consisted of 7 laying hens. Levels of giving ginger flour, temulawak and LAB on laying hens feed are:

- P0 = Control feed (without treatment)
- P1 = control feed + herbal formula (*Zingiber officinale* 0.25% + *Curcuma xanthorrhiza* roxb 0.25%) + LAB 0.2%
- P2 = Control feed + herbal formula (*Zingiber officinale* 0.50% + 0.50%) + LAB 0.2%
- P3 = Control feed + herbal formula (*Zingiber officinale* 0.75% + *Curcuma xanthorrhiza* roxb 0.75%) + LAB 0.2%

The addition of herbal formulas and Lactic Acid Bacteria (LAB) is done by mixing directly with basal feed which has been adjusted to the percentage of treatment and then manually stirred until homogeneous. The variables taken in this study were the population of *Escherichia coli* and *Salmonella* sp.

E. Media for calculating TPC (Total Plate Count)

The media used to calculate the TPC of the intestinal microflora were XLD (Xylose Lysine Deoxycholate Agar) media for *Salmonella* sp. test bacteria, EMB (Eosin Methylene Blue Agar) media for *Escherichia coli* test bacteria.

F. Observed Variables

The variables which was observed in this research is the total colonies of bacteria that was contained inside native chicken crossbreed's intestine involve :

1. *Escherichia coli* (log CFU/g)

Counting the total colonies of *Escherichia coli* bacteria based on the method of calculation the total colonies of bacterial by Fardiaz (1992). The formula is :

$$\text{Colonies per mL or g} = \text{Total colonies} \times (1 : \text{dilution factor})$$

2. *Salmonella* sp. (Log CFU/g)

Counting the total colonies of *Salmonella* sp. bacteria based on the method of calculation the total colonies of bacterial by Fardiaz (1992). The formula is :

$$\text{Colonies per mL or g} = \text{Total colonies} \times (1 : \text{dilution factor})$$

G. Data Analysis

Data that was obtained then was tabulated using Microsoft Excel Application and was continued with statistical analysis using Analysis of Variance (ANOVA). If the result that was obtained was different between treatments then the analysis will be continued using Duncan Multiple Range Method. The mathematic model of Completely Randomized Design Pattern is:

$$Y_{ij} = \bar{y} + \bar{y}_i + \bar{y}_j$$

Information :

Y_{ij} = observations on the treatment of all replicates all i and j

I = 1, 2, 3, 4, 5

J = 1, 2, 3, 4, 5

\bar{y} = the midpoint population

\bar{y}_i = treatment effect

\bar{y}_j = errors (error) in the treatment of all replicates all i and j

III. RESULT AND DISCUSSION

TABLE 1. The effect of Herbal Formulas and LAB on Microbial Pathogens *Salmonella* sp. and *Escherichia coli*

Variables	Total Colony Bacteria (log cfu/g)			
	P0	P1	P2	P3
<i>Escherichia coli</i>	4,72±1,02 ^{bc}	5,64±0,29 ^c	3,52±0,15 ^a	4,12±0,20 ^{ab}
<i>Salmonella</i> sp.	5,72±0,54 ^c	3,67±0,34 ^a	4,63±0,23 ^{ab}	5,48±0,24 ^{bc}

Note: Different notation values on the same line indicate a significant difference (P<0.05) between treatments.

A. The effect of treatment toward the total colonies of *Escherichia coli* bacteria.

The results of observations and analysis of variance in *Escherichia coli* laying hens with the treatment of a combination of herbal formulas and Lactic Acid Bacteria (LAB) added to the feed can be seen in the Appendix. The data analysis in the table shows that the population average of *Escherichia coli* in the intestines of laying hens starts from the highest to the lowest, P1 (5.64±1.02), P0 (4.72±0.20), P3 (4, 12±0.15) and P2(3.52±0.29) log cfu/g. The results of the analysis of variance in the attachment showed that the administration of herbal formulas and LAB were significantly different (P<0.05) on the total colony of *Escherichia coli* bacteria in the intestines of laying hens. The decrease in the population of colonies was thought to be due to the antibacterial compounds contained in the herbal formula and

LAB which produced gram-positive bacteria so that the combination of feeding treatments worked optimally. The decrease indicates that these compounds are able to inhibit the growth and metabolism of pathogenic bacteria will be disrupted. The herbal formula contains essential oils, flavonoids and curcumin which can kill pathogenic bacteria such as *Salmonella* sp, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas fluorescens* (Setiawan, 2002).

B. The effect of treatment toward the total colonies of Salmonella sp. bacteria

The results of observation and analysis of laying hens with combination treatment of herbal formulas and LAB added to the feed showed a significant difference ($P > 0.05$) on the number of colonies of *Salmonella* sp. Population of *Salmonella* sp. increases with the increase in the level of treatment given. Sequentially the average population of *Salmonella* sp. from the highest to the lowest, namely P1 (5.64 ± 0.29), P0 (4.72 ± 1.02), P3 (4.12 ± 0.20), P2 (3.52 ± 0.15) log cfu/g. The lower the number populations of pathogenic bacteria in the intestines of laying hens, the more balanced the process of digestion of food in it. Because if the number of bacteria in the intestines is not in balance, it can interfere and even cause disease. This is supported by Yegani and Corver (2008) who stated that harmful bacteria in the digestive tract can be involved in local or systemic infections, intestinal decay and toxin formation.

Salmonella sp. can cause disease in livestock through 3 stages, namely colonization of the intestine, absorption of the intestinal epithelial layer and stimulating fluid secretion, the presence of non-pathogenic bacteria LAB will help inhibit the growth of *Salmonella* sp. Colonization of these bacteria is influenced by peristalsis in the intestine, where if the intestinal peristalsis decreases it will easily colonize in the intestine (Lay and Hastowo, 2000).

Non-pathogenic LAB bacteria have a positive role for livestock, while *Escherichia coli* bacteria are bacteria that are normally found in the digestive tract, if the number is above normal limits it can cause diarrhea in chickens, but if the numbers are balanced then the livestock will be in good health and their growth can be maximized. It can be concluded that the addition of herbal formulas and LAB if more and more are added to the feed, the effectiveness in inhibiting the microbial population decreases, which can be caused by an unbalanced pH ratio in the body of the chicken. According to Hardiningsih (2006), LAB can grow optimally in suitable environmental pH conditions ranging from 2-6.5. Lactic acid bacteria can produce organic acids in lowering the pH and can inhibit the growth of the pathogen *Salmonella* sp. and *Escherichia coli*. LAB can also produce bacteriocin which functions to suppress the growth of pathogenic bacteria in the competition to stick to the small intestinal mucosa or is called competitive exclusion so that natural body resistance is formed and prevents pathogenic bacteria from penetrating small intestinal cells so that the digestive process becomes healthier (Imam 2018).

The average results of the treatment of herbal formulas and LAB on the population of pathogenic microbes in the chicken intestines were best found in the P1 treatment with a 0.25% level of administration, the total colonies of *Salmonella* sp and *Escherichia coli* bacteria were directly proportional to the increasing level of administration, this is It can be concluded that the shogaol content contained in the herbal formula can increase the pH of the body of chickens, which if the environmental pH also increases, the chickens will become dehydrated, the intensity of feed consumption decreases and they drink more often so that the performance of LAB is not optimal. The pH level in the digestive tract of laying hens, especially in the ileum, is very important in making the area an ideal environment for non-pathogenic bacteria so that digestion and absorption of food is maximized. According to Lee (2005), non-pathogenic bacteria that are beneficial for the digestive tract in an acidic environment are in the pH range of 5.8-8.2.

IV. CONCLUSION

The results showed that the addition of herbal formulas and LAB in feed had a significant effect ($P < 0.05$) on the number of *Escherichia coli* and *Salmonella* sp. The addition of the levels of herbal formulas and LAB in the feed as much as 0.25-0.50% can reduce the number of *Escherichia coli* and *Salmonella* sp

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