International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

Microbial Characteristics of Laying Hens in the Production Phase with the Addition of a Combination of Moringa (*Moringa oleifera*) and Ginger (*Zingiber officinale*) Leaves as Phytobiotik

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Abstract**—** *This study aimed to evaluate the effect of adding Moringa flour and ginger as an antimicrobial in feed to the intestinal microflora population consisting of Lactic Acid Bacteria, Escherichia coli and Salmonella sp. The material used in this study was laying hens aged 32 weeks strain Isa brown produced by PT. Malindo Feedmill Tbk Gresik, East Java with a coefficient of uniformity of egg mass before the study of 4.8%. The method used in this study was a completely randomized design with 4 treatments with 4 replications, the variables observed were the population of Lactic Acid Bacteria, Escherichia coli and Salmonella sp. in the small intestine of laying hens, if there is a significant effect, it will be tested using the Duncan's Multiple Range Test Method. The treatments tested consisted of P0 = control feed, P1 = control feed (without addition), P2 = 025% moringa flour + 0.25% ginger flour, P3 = 0.50% moringa flour + 0.50% ginger flour, P3 = 0.75% Moringa flour + 0.75. The results showed that the addition of Moringa flour and ginger flour to the feed had a very significant effect (P<0.01) on the number of microbes Lactic Acid Bacteria, Escherichia coli and Salmonella sp. The addition of 0.75% Moringa flour and 0.75% ginger gave the best results in reducing the population of Lactic Acid Bacteria, Escherichia coli and Salmonella sp*.

Keywords**—** *Laying hen, Moringa oleifera, Zingiber officinale, microflora, chicken intestine.*

I. INTRODUCTION

Feed is the most important component that needs to be considered because quality feed can improve the performance of livestock production, because optimal feed can affect production. One of the efforts to improve feed quality is by adding feed additives. In general, the addition of feed additives can be in the form of antibiotics, prebiotics, probiotics, enzymes, organic acids, and phytobiotics. However, the use of antibiotics for feed additives has been prohibited as stated in Article 16 paragraph 2 of the Minister of Agriculture of the Republic of Indonesia Number 14 of 2017 which states that veterinary drugs that have the potential to endanger human health are prohibited from being used on livestock whose products are consumed by humans.

One alternative that can be done to reduce the use of antibiotics is to use feed additives in the form of phytobiotics, because antibiotics are not at risk for human health, which is caused by antibiotic residues in livestock products that can lead to resistance in the livestock body and then consumed by humans. According to [1] phytobiotics are herbal plants that have active ingredients that can be antibacterial and can improve the condition of the digestive tract, balance pH and the number of microflora, feed conversion and increase production performance. Provision of phytobiotics to poultry can increase growth and feed efficiency, improve intestinal histomorphology and can function as an antimicrobial so that it increases the immunity of poultry [2].

One of the spice plants that have the potential as an antibacterial is ginger. Ginger is one of the herbal plants that is easily obtained by the community and has high production, including export commodities [3] The content of flavonoids in ginger has antibacterial activity that inhibits bacterial growth. Ginger generally can inhibit the growth of pathogenic bacteria including Escherichia coli and Staphylococcus aureus [4]. Moringa leaves (Moringa oleifera) and ginger (Zingiber officinale) have the potential to reduce fat and cholesterol content in eggs, can inhibit the growth of pathogenic bacteria and can improve egg quality.

II. MATERIAL AND METODS

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 UPT. Quality Testing and Development of Marine and Fishery Products and Surabaya.

The material used was Isa Brown laying hens produced by PT. Malindo Feedmill Tbk Gresik, East Java as many as 168 chickens aged 35 weeks with a coefficient of diversity egg mass before the study was 4,8%. Moringa flour and ginger flour were obtained from PT. Jaya Makmur Poultry, Tulungagung. 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.

A. 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

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ISSN (Online): 2455-9024

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.

B. 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 moringa flour and ginger at 0.25, 0.50 and 0.75% levels. 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.

C. Research Metods

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 5 replications, where each replication consisted of 7 laying hens. Levels of giving moringa flour and ginger flour on laying hens feed are:

P0 = Commercial feed (without treatment)
P1 = Commercial feed + moringa flour 0.25% + ginger flour 0.25%
P2 = Commercial feed + moringa flour 0.50% + ginger flour 0.50%
P3 = Commercial feed + moringa flour 0.75% + ginger flour 0.75%)

The addition of Moringa flour and ginger was done by mixing directly with basal feed which had been adjusted to the percentage of treatment then stirred manually until homogeneous. The variables taken in this study were the population of Lactactic Acid Bacteria, *Escherichia coli* and *Salmonella* sp.

D. 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.

E. 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:

Colonies per mL or g = Total colonies x (1 : dilution factor)

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

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

Colonies per mL or g = Total colonies x (1 : dilution factor)

3. Lactactic Acid Bacteria

Counting the total colonies of Lactatcid Acid Bacteria based on the method of calculation the totalcolonies of bacterial by Fardiaz (1992). The formula is:

Colonies per mL or g = Total colonies x (1 : dilution factor)

F. 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:

$$Yij = ÿ + ÿi + ÿij$$

Information:
Yij = observations on the treatment of all replicates all i and j
I = 1, 2, 3, 4, 5
J = 1, 2, 3, 4, 5
jÿ = the midpoint population
ÿi = treatment effect
ÿij = errors (error) in the treatment of all replicates all i and j

III. RESULT AND DISCUSION

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

Variables	Total Colony Bacteria (log cfu/g)			
	P0	P1	P2	P3
Escherichia coli	6,29±0,75^{a}	7,4±1,13^{ab}	5,64±0,20^{b}	5,11±0,33^{c}
Salmonella sp.	6,49±0,94^{a}	5,32±0,90^{b}	4,91±0,69^{b}	4,00±0,21^{c}
Lactactic Acid Bacteria	7,40±0,51^{a}	7,40±1,13^{ab}	8,02±0,26^{b}	9,11±0,92^{c}

Note: Different notation values on the same line indicate a significant difference (P<0.01) between treatments.

The effect of treatment toward the total colonies of Escherichia coli bacteria. Based on the results showed that the addition of Moringa flour and ginger gave a very significant effect (P<0.01) with the best treatment, namely P3 (5.11 ± 0.33) it affected the decrease in the number of pathogenic microbes Escherichia colli the higher the concentration of addition of flour Moringa and ginger reduce the number of pathogenic bacteria in the small intestine, Moringa flour and ginger can block E. Coli from receptors so that their ability to stick to the intestinal mucosa becomes weak, on the other hand, the ability of LAB is increasing. This is in line with the results of research using dahlia tuber extracts conducted by [5] which showed that the higher the LAB population, the lower the number of Escherichia coli in chicken intestines. The success of beneficial bacteria in competing with pathogenic bacteria is determined by the initial step of their success attached to the surface of intestinal epithelial cells.

The effect of treatment toward the total colonies of *Salmonella* sp. Bacteria. The results of observations and analysis of laying hens with the combination treatment of Moringa flour and ginger added to the feed showed a significant difference (P>0.01) to the number of colonies of Salmonella sp. Population of Salmonella sp. The higher the

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level of flour administration, the lower the number of *salmonella* sp. Sequentially the average population of Salmonella sp. from the highest to the lowest, namely P0 (6,49±0.94), P1 (5,32±0,90), P2 (4.91±0.69), P3 (4,00±0,21) log cfu/g. The lower the population 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 intestine is not balanced, it can interfere and even cause disease. The higher the number of LAB population in the small intestine, it tends to suppress the growth of pathogenic bacteria such as Escherichia coli and Salmonella sp. because LAB can produce bacteriocins that can suppress the growth of pathogenic bacteria in competition to stick to the small intestinal mucosa so that natural body resistance is formed and prevents pathogenic bacteria from penetrating the small intestine cells so that the digestive process becomes healthier [6].

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.

The effect of treatment toward the total colonies of Lactactic Acid Bacteria. The addition of Moringa flour and ginger at different concentrations gave a very significant effect ($P<0.01$) on the number of microbes in the small intestine of laying hens. The results of the lowest LAB value in this study were P0 with an average value (7.4 cfu/g) and the highest was P3 (9.11 cfu/g), this indicates that the administration of Moringa flour and ginger tends to increase the LAB population. According to research conducted by Abdel-Raheem et al. (2012) the total lactic acid bacteria in the small intestine of chickens in general was 8.19 log cfu/g, while in the cecum it was 8.81 log cfu/g. The total lactic acid bacteria in the small intestine and cecum indicates the balance condition of the digestive tract microflora. According to [7] fluctuations in the number of LAB are related to the number of coliform bacteria, namely the less the number of coliform bacteria tends to increase the number of LAB and vice versa. This shows the occurrence of competitive exclusion, namely the competition between bacteria (in this case LAB and coliform bacteria) to get space and nutrients in the small intestine.

IV. Conclusion

The results showed that the addition of Moringa flour and ginger flour to the feed had a very significant effect ($P<0.01$) on the total population of Lactic acid bacteria, Escherichia coli and Salmonella sp. The addition of Moringa flour and ginger to the feed as much as 0.75% gave the best results in reducing the number of microbial populations.

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