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

Effect of Probiotic and Acidifier Combination as an Alternative to Antibiotic Growth Promoters on Digesta pH and Intestinal Microflora of Laying Hen

Desna Ayu Wijayanti[1], Irfan H. Djunaidi[1*], Osfar Sjofjan[1]
[1]Faculty of Animal Science, University of Brawijaya, Malang, Indonesia (65145)
*Corresponding author: irjuna@gmail.com

Abstract**—***The research was conducted to determine the effect of using a mixture of probiotic (Lactobacillus sp. and Bacillus sp.) and acidifier (bilimbi powder) as an alternative to antibiotic growth promoter (bacitracin) on digesta acidity (pH) and intestinal microflora of laying hen. The research material used were 240 laying hens (Lohmann brown), which raised until 22 wks, designed by 4 treatments and 6 replications. The treatments applied on feed, which were consisted of T0: 0%, T1: 0.8% probiotic + 0.25% bilimbi powder, T2: 0.8% probiotic + 0.50% bilimbi powder, T3: 0.8% probiotic + 0.75% bilimbi powder. The observed variables were the pH of digesta (duodenum, jejunum and ileum) and the population of intestinal microflora on ileum. Data were analyzed by Analysis of Variance (ANOVA) using Completely Randomized Design (CRD), then tested by Duncan's Multiple Range Test (DMRT) if had a different effect. The results indicate that the treatments gave highly significant effect (T<0.01) on pH (duodenum, jejunum and ileum), the population of Salmonella sp. and Escherichia coli then gave significant effect (T<0.05) on the population of Lactic Acid Bacteria. In conclusion, a combination of probiotic and acidifier can improve the stability of intestinal microflora population efficiently.*

Keywords**—** *Antibiotic, acidifier, laying hen, probiotic.*

I. INTRODUCTION

Laying hen is one of the livestock commodities in Indonesia, which produced egg as a common demand for society with the low price. Currently, the price of eggs in the market increased continuously up to IDR 28,000/ kg. This condition has been affected by the imbalance between supply and demand. Furthermore, one thing that the low supply due to the case of a high number of mortality in laying hen, which related by the prohibition of using AGP (Antibiotic Growth Promoter). AGP is commonly used by farmer as a growth promoter in term to eliminate all harmful bacteria in the digestive tract, so the production reached optimum performance. Long-term use of AGP exactly caused a negative effect on the stability of intestinal microflora and makes antibiotic become resistance (Corcionivoschi et al., 2016).

In term of the negative effect that emerged by using antibiotic, it is necessary to have an alternative growth promoter which can optimize the condition of intestinal health through improving the stability of microbial population in the digestive tract. Related to the digestive tract, absorption of nutrients can be optimum only when the intestine was healthy, so the stability of microbial populations both pathogens and non-pathogens greatly affected to function path of digester feed. Alternative growth promoter is expected to be able to suppress the propagation of pathogenic microbes called *Salmonella* sp. and *Escherichia coli* also increase *Lactobacillus* growth in intestinal of laying hen. Non-pathogenic strain of *Escherichia coli* can be changed become pathogens when immunosuppression and disorders occurred in the digestion host (Sharma et al., 2011). Substitution of Antibiotic Growth Promoter namely as Alternative Growth Promoter can be a method to keep health condition on intestine by using a natural substance like a combination of probiotic and acidifier.

The application of probiotic in Indonesia was familiar for farmers, so far probiotic was able to prevent digestive tract disorders by utilizing probiotic microbes to do propagation on non-pathogenic microbes, then facilitate a stable of pathogenic and non-pathogenic microbes in the digestive tract. Probiotic improved the performance of consumption and digestibility of laying hen (Youssef et al., 2017) through competitive-exclusion mechanism or competition between pathogenic and non-pathogenic microbes (Shivani et al., 2017). Inhibiting substances have a synergistic role by maximizing the activity of probiotic to make optimum growth of non-pathogenic microbes, so it is combined, which was called as organic acid or acidifier.

The principle of acidifier is increase acidity of the digestive tract to inhibit the growth of pathogenic microbes. Bilimbi (*Averrhoa bilimbi*) is often used as a source of organic acid in Indonesia. Bilimbi contains various types of organic acids such as acetic acid, citric acid, formic acid, lactic acid, oxalic acid and ascorbic acid (Roy et al., 2011). Organic acids are known as strong antibacterial effects and can be used as a feed protection which is from microbes and fungi (Hedayati et al., 2013). So, it has the potential to be additive feed and alternative to antibiotics because it can eliminate *Salmonella sp.* and other pathogenic bacteria in the digestive tract. Acidic characteristic derived from bilimbi is possible to affect pH of the digestive tract so that it can improve the activity of the enzyme and its digestive tract become optimum performance. The study above is expected to support conducting research and providing a positive effect on digestive tract health of poultry, includes the intestinal acidity (pH) and the stability of the intestinal microflora population

Desna Ayu Wijayanti, Irfan H. Djunaidi, and Osfar Sjofjan, "Effect of Probiotic and Acidifier Combination as an Alternative to Antibiotic Growth Promoters on Digesta pH and Intestinal Microflora of Laying Hen," *International Research Journal of Advanced Engineering and Science*, Volume 4, Issue 2, pp. 1-4, 2019.

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

II. Materials and Methods

A. Materials

The research materials used were 240 laying hens (Lohmann browns) aged 22 wks. Each laying hen housed using battery cage, which sized 50 cm x 20 cm x 50 cm, then was equipped by feeder and drinker. Other materials were probiotic powder, which contains *Lactobacillus* sp. (5.4×10^7 CFU/ mg) and *Bacillus* sp. (2.3×10^8 cfu / mg), bilimbi powder and self-mixing feed (50% corn, 30% concentrate and 20% rice bran). The composition and its nutrient content of feed are shown in Table I.

TABLE I. Nutrient content of basal feed

Nutrient Content	Self mixing
Dry Matter (%)	91.65
Crude Protein (%)	17.65
Crude Fiber (%)	10.89
Crude Fat (%)	3.18
Ash (%)	16.31

B. Methods

The research used in vivo experiment, conducted in laying hens farms where located in Malang Regency. Rearing period was conducted for 5 wks, starting 23rd – 27th wks. This method of this research was Completely Randomized Design (CRD). This experiment consisted of 4 treatments 6 replications (10 laying hens each). The treatment given as follow: T0= basal feed (without addition of feed additive); T1= basal feed + 0.8% probiotic + 0.25% bilimbi powder; T2= basal feed + 0.8% probiotic + 0.50% bilimbi powder; T3= basal feed + 0.8% probiotic + 0.75% bilimbi powder.

The production of bilimbi powder is selected from fresh half-riped bilimbi (*Averrhoa bilimbi*), which had been seen from physical uniformity (yellowish green, maximum on large fruit shape). Then, make a simplicia oven at 55^0C and then grinded become powder. First, the bilimbi powder was mixed with probiotic powder. After that, a combination both probiotic and acidifier are added into feed, which based on the percentage of treatments.

Feeding treatment used the restricted-feeding method, which adjusted by 120 g/ hen/ day of laying hens, but the water was given as ad libitum. The composition and nutrient content of feed are shown in Table I. The sample of digesta liquid was taken at the end of raising (5 wks of raising during research) or when the laying hen age was 27 wks. This sample put in the film pot and stored in a cold box for maintenance. Then, this sample was analyzed in the laboratory. The variables observed were:

a). pH of the digestive tract

The pH measurement conducted on digestive tract part include duodenum, jejunum, and ileum. Each part of digesta was taken 10 ml and put into beaker glass. Then, measured by pH meter.

b). Total population of *Escherichia coli*, *Salmonella* sp. and *Lactic Acid Bacteria* (LAB)

Calculation of total bacteria used the Total Plate Count (TPC) method. TPC calculation used a method of Fardiaz (1993):

Colonies per mL or g = Total of Colonies x 1/(Dilution factor)

Data were analyzed by ANOVA, if the results showed significant differences ($p < 0.05$) or highly significant differences ($p < 0.01$), then continued with Duncan's Multiple Range Test (DMRT).

III. Results and Discussions

A. Effect Combination of Probiotic and Acidifier on pH of Small Intestine

The additional effect of probiotic and acidifier combinations of feed on pH of duodenum, jejunum and ileum in laying hen is shown in Table II.

TABLE II. The pH value of different part of small intestine

Treatment	Duodenum	Jejunum	Ileum
T0	$5{,}83 \pm 0{,}14^b$	$6{,}58 \pm 0{,}16^b$	$7{,}35 \pm 0{,}26^b$
T1	$5{,}87 \pm 0{,}12^b$	$6{,}48 \pm 0{,}15^{ab}$	$7{,}30 \pm 0{,}13^b$
T2	$5{,}73 \pm 0{,}26^{ab}$	$6{,}40 \pm 0{,}28^{ab}$	$6{,}95 \pm 0{,}41^{ab}$
T3	$5{,}45 \pm 0{,}22^a$	$6{,}15 \pm 0{,}20^a$	$6{,}80 \pm 0{,}28^a$

Notes: The different superscripts in the same row showed highly significant differences ($T<0.01$)

Table II showed the data of average of pH from each part of the small intestine (duodenum, jejunum and ileum). The highest pH average of all parts is shown by T0 (without the addition of feed additive) with an average were 5.83 ± 0.14 for duodenum, 6.58 ± 0.16 for jejunum and 7.35 ± 0.26 for ileum. While, the lowest pH average value is shown by T3 which has average were 5.45 ± 0.22 for duodenum, 6.15 ± 0.20 for jejunum and 6.80 ± 0.28 for ileum.

The result of variance analysis in Table II showed that the addition of probiotic and acidifier gave highly significant effect ($T<0.01$) on digesta pH in the duodenum, jejunum and ileum. Addition of probiotic and acidifier combination has capable to decrease the pH of digestive tract, especially in the small intestine of laying hen. The normal digesta pH value of each part of the small intestine has differences. Commonly, range of pH of each part of small intestine was 5-6 for duodenum, 6.5-7 for jejenum with an average 6.6 and 7-7.75 for ileum with an average 7.2 (Gauthier, 2002).

Citric acid and lactic acid are the most compositions of an organic acid, which contained in bilimbi. Either citric or lactic acid, it has a role to improve health of digestive tract on laying hen. Bilimbi contains 92.6 - 133.8 mEq citric acid and 0.4-1.2 mEq lactic acid (Carangal et al., 1961). Citric acid and lactic acid play a major role in reduction and defense mechanism on acidic condition in the digestive tract. Citric acid as an acidifier is capable to enhance growth and rate of digestion through decreasing pH condition of the digestive tract. So, these conditions can support the performance of digestive enzymes and reduce the growth of pathogenic microbes in the intestine (Chaveerach et al., 2002). Similar to citric acid, lactic acid also capable to create suitable pH condition on feed digestion, which entered to the digestive tract and increase the growth of non-pathogenic microbes (Hyden, 2000).

Reverse to antibiotics, a combination of probiotic and acidifier is an appropriate combination because the synergy of its mechanism activity can maintain health and stability of digestive tract microflora. By producing antimicrobial substances from Lactic Acid Bacteria (LAB) and organic acid,

Desna Ayu Wijayanti, Irfan H. Djunaidi, and Osfar Sjofjan, "Effect of Probiotic and Acidifier Combination as an Alternative to Antibiotic Growth Promoters on Digesta pH and Intestinal Microflora of Laying Hen," *International Research Journal of Advanced Engineering and Science*, Volume 4, Issue 2, pp. 1-4, 2019.

probiotic can support to decrease pH, so that acidic condition created and lead to suppressing harmful microbial growth (Rossland et al., 2005).

B. Effect Combination of Probiotic and Acidifier on Intestinal Microflora

The additional effect of probiotic and acidifier combination on the population of intestinal microflora in laying hen is shown in Table III.

TABLE III. Population of intestinal microflora (*Lactobacillus, Escherichia coli* and *Salmonella* sp.)

Treatments	*Lactobacillus* (CFU/ ml)	*Escherichia coli* (CFU/ ml)	*Salmonella* sp. (CFU/ ml)
T0	7.52 ± 0.50^{a}	6.26 ± 0.58^{b}	5.42 ± 0.42^{b}
T1	7.89 ± 1.30^{a}	6.11 ± 0.51^{b}	5.38 ± 0.72^{b}
T2	8.03 ± 0.79^{a}	5.81 ± 0.42^{ab}	4.86 ± 0.59^{ab}
T3	9.39 ± 0.89^{b}	5.08 ± 0.46^{a}	4.26 ± 0.58^{a}

Notes: The different superscripts in the same row showed highly significant differences ($p<0.01$)

Table III showed an average total of pathogenic bacteria (*Escherichia coli and Salmonella* sp.) and non-pathogenic bacteria (*Lactobacillus*). The highest population of *Escherichia coli* bacteria is shown by T0 with an average was 6.26 ± 0.58 CFU/ ml, meanwhile, the lowest population is shown by T3 with an average was 5.08 ± 0.46 CFU/ ml. The highest number of *Salmonella* sp. bacteria is shown by T0 with an average was 5.42 ± 0.42 CFU/ ml, but the lowest population is shown by T3 with an average was 4.26 ± 0.58 CFU/ ml.

The result of variance analysis showed a highly significant effect (T<0.01) due to the addition of probiotic and acidifier combination in the feed to the population of pathogenic bacteria, especially *Escherichia coli* and *Salmonella* sp. At first, the number of pathogenic bacteria from both types of gram-negative bacteria was 11.68 CFU / ml then reduced to 9.34 CFU/ ml. This is similar to the report from Gunal et. al. (2016) that the addition of probiotic and acidifier can reduce the number of pathogenic microbes in the ileum and caecum. The total of gram-negative bacteria was 7.95 CFU / ml in basal then reduced to 7.28 CFU/ ml in the feed using probiotic and acidifier combination. These result still further than a comparison of antibiotics application, which reduced bacteria up to 6.06 CFU/ ml. However, the application result of probiotic and acidifier combination still capable to inhibit pathogenic bacteria on the digestive tract if would be used for a long time.

Reduction of total gram-negative bacteria cannot be separated from the antibacterial mechanism of probiotic and acidifier. Antibacterial effect of probiotic comes from a modification of microflora, there was nutrition competition in preventing of pathogenic microbes, which attached to the intestine and nutritional competition in surviving of non-pathogenic microbes (Hemaiswarya et al., 2013). Whereas for acidifier, the antibacterial mechanism which associated in decreasing pH can limit the growth of acid intolerant microbes (Hedayati et al., 2013). Meanwhile, antibiotics are designed to inhibit most of the microbial growth both pathogens and non-pathogens, probiotic and acidifier were focused to reduce harmful microbes and to assist beneficial microbes to dominate the digestive tract, through competition and reduction of pH in the digestive tract (Mathew et. al., 1996).

In addition, the reduction of intestinal bacteria is affected by intestinal pH value. Intestinal pH value plays a major role in bacterial growth because a high-low pH value can assist the growth of bacteria. Pathogenic bacteria unable to survive in acidic condition, so according to the result, probiotic and acidifier combination created acidic environmental condition and reduced the total of *Salmonella* sp. from 5.42 ± 0.42 CFU/ ml to 4.26 ± 0.58 CFU/ ml. *Salmonella* sp., which is optimally propagate at pH above 6.8 or around 6.8-7.2 (Holt et al., 1994). Similar to *Salmonella* sp., a total of *Escherichia coli* reduced from 6.26 ± 0.58 CFU/ ml to 5.08 ± 0.46 CFU / ml. According to Murry et al (2004), *Escherichia coli* bacteria only can survive at pH 6-8.

The stable population of intestinal microflora can protect the host from pathogenic microbial colonization. So the presence of non-pathogenic microbes was important to maintain intestinal health. Table III also showed that the total average of non-pathogenic bacteria (*Lactobacillus*). The highest total of non-pathogenic bacteria is shown by T3 which has an average was 9.39 ± 0.89 CFU/ ml, meanwhile, the lowest total of pathogenic bacteria is shown by T0 which its average was 7.52 ± 0.50 CFU / ml.

The result of ANOVA showed that the application of probiotic and acidifier combination in feed gave a highly significant effect (T<0.01) to the population of non-pathogenic bacteria (*Lactobacillus*). Average data from the microflora population showed that the higher application of probiotic and acidifier combination lead to increase of a total of *Lactobacillus* bacteria. According to some previous research, the study stated that chicken given probiotic could increase a total of *Lactobacillus* along decreasing of pathogenic bacteria in the digestive tract. The growth of *Lactobacillus* bacteria depends on the pH environment because non-pathogenic bacteria tend to grow in an acidic environment. The stability of a total of *Lactobacillus* bacteria requires an appropriate environmental conditions, such as an acidic pH environment (Hangoor et al., 2013).

IV. Conclusion

The addition of probiotic and acidifier combination from bilimbi powder can reduce pH value, lead to reduce a total of pathogenic bacteria and increase a total of *Lactobacillus* or non-pathogenic bacteria. So that, combination of probiotic and acidifier can be used as an alternative to antibiotic growth promoter.

References

[1] Carangal, A. R., L. G. Gonzalez dan I. L. Daguman. 1961. The Acid Constituents of Some Philippines Fruits. In: Subhadrabandhu.Under-utilized Tropical Fruit of Thailand. Food and Agricultural Organization of The United Nations Regional Office of Asia and The Pacific. Bangkok.

[2] Chaveerach, P., D. A. Keuzenkamp, H. A. Urlings, L. J. Lipman and F. V. Knapen. 2002. In Vitro Study on the Effect of Organic Acids on Campylobacter Jejuni/coli Populations in Mixtures of Water and Feed. Poultry Science. 81(5): 621-628.

Desna Ayu Wijayanti, Irfan H. Djunaidi, and Osfar Sjofjan, "Effect of Probiotic and Acidifier Combination as an Alternative to Antibiotic Growth Promoters on Digesta pH and Intestinal Microflora of Laying Hen," *International Research Journal of Advanced Engineering and Science*, Volume 4, Issue 2, pp. 1-4, 2019.

[3] Corconivoschi, N., D. Drinceanu, I. M. Pop, D. Stack, L. Stef, C. Julen and B. Bourke. 2010. The Effect of Probiotics on Animal Health. Papers: Animal Animal Science and Biotchnologies. 43(1): 35-41.
[4] Fardiaz, S. 1993. Food Microbiology Analysis. Ed.1. Jakarta. Raja Gravindo Persada.
[5] Gauthier, R. 2002. Intestinal Health, The Key to Productivity (The case of organic Acid). XXVII Convention ANECA-WPDC. Puerto Vallarta. Mexico.
[6] Gunal, M., G. Yayli, O. Kaya, N. Karahan and O. Sulak. 2006. The Effects of Antibiotic Growth Promotor, Probiotic or Organic Acid Supplementation on Performance, Intestinal Microflora and Tissue of Broilers. Int Journal of Poultry Science. 5(2): 149-155.
[7] Hangoor, E.H., J. M. B. M. Vossen, F. H. J. Schuren, M. W. A. Verstegen, J. E Oliveira, R. C. Montijn and W. H. Hendriks. 2013. Ileal Microbiota Comosition of Broilers Fed VariousCommercial Diet Compositions. Poultry Science. 92: 2713-2723.
[8] Hedayati, M., M. Manafi, M. Yari and P. Vafei. 2013. Effects of supplementing diets with an acidifier on performance parameters and visceral organ weights of broilers. European Journal of Zoological Research. 2(6): 49-55.
[9] Hemaiswarya, S., R. Raja, R. Ravikuman and I. S. Carvalho. 2013. Mechanism of Action of Probiotic. Int. Journal of Brazilian Archives of Biology and Technology. 56(1): 113-119.
[10] Holt, J. G., N. R. Krieg, P. H. A. Sneath and J. T. William. 1994. Bergeys Manual of Determinative Bacteriology. 9th edition. William and Wilkin. USA.
[11] Hyden, M. 2000. "Protected" Acid Additives. Feed International. July. 2000
[12] Mathew, A. G., M. A. Franklin, W.G. Upchurch, S. E. Chattin. 1996. Influence of weaning age on ileal microflora and fermentation acids in young pigs. Nutrition Research. 16(5): 817-827.
[13] Murry, A. C., A. Hinton and M. Morrison. 2004. Inhibition of Growth of Escherichia coli, Salmonella typhimurium and Clostridia perfringens on Chicken Feed Media by Lactobacillus salivarius and Lactobacillus plantarum. Int. J. Poult. Sci. 3: 603-607.
[14] Rossland, E., T. Langsurd, P.E.Granum and T. Sorhaug. 2005. Production of Antimicrobial Metabolites by Strains of Lactobacillus or Lactococcus co-cultured with Bacillus cereus in Milk. Int J Food Microbiol. 98(2):193-200.
[15] Roy, A., R. V Greetha, T. Lakshmi. 2011. Averrhoa bilimbi linn Nature's Drug Store A Pharmacological Review. International Journal of Drug Development and Research. 3(3): 101-106.
[16] Sharma, K. G., V. K. Vidyarthi, K. Archana and Zuyie. 2016. Probiotic Supplementation in the Diet of Rabbits. Livestock Research International Journal. 4(1): 1-10.
[17] Shivani, K., D. Sidharath and R. Rajesh. 2017. Effect of Probiotic Supplementation in Broiler Birds Offered Feed Formulated with Lower Protein Densities. International Journal of Livestock Research. 7(2): 1-13.
[18] Youssef, A. W., H. M. A.Hassan, H. M. Ali and M. A. Mohamed. 2017. Effect of Dietary Inclusion of Probiotic and Organic Acids on Performance, Intestinal Microbiology, Serum Biochemistry and Carcass Traits of Broiler Chickens 7(2): 57-71.

Desna Ayu Wijayanti, Irfan H. Djunaidi, and Osfar Sjofjan, "Effect of Probiotic and Acidifier Combination as an Alternative to Antibiotic Growth Promoters on Digesta pH and Intestinal Microflora of Laying Hen," *International Research Journal of Advanced Engineering and Science*, Volume 4, Issue 2, pp. 1-4, 2019.