

The Effect of a Combination of Probiotic and (*Averrhoa bilimbi*) Powder Extract on Egg Quality of Laying Hens

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Abstract— This research aims to find out the effect of giving a combination of probiotics and (*Averrhoa bilimbi*) powder extract on the egg white and yolk volume, yolk color, yolk cholesterol, eggshell thickness and eggshell color of laying hens. The method used was experimental method with a Completely Randomized Design (CRD) of 4 treatments, each with 6 replications. The treatments consisted of P0 (-) = basal feed, P1 = 0.8% probiotics + 0.25% *Averrhoa bilimbi* powder extract, P2 = 0.8% probiotics + 0.50% *Averrhoa bilimbi* powder extract and P3 = 0.8% probiotics + 0.75% *Averrhoa bilimbi* powder extract. The variables measured in this research were egg quality including internal quality (egg white and yolk volume, yolk color and cholesterol) and external quality (eggshell thickness and color). The data were then analyzed by using ANOVA and continued with Duncan's Multiple Range Test. The results of this study were the addition of combination probiotics and *Averrhoa bilimbi* powder extract showed highly significant difference ($P < 0.01$) on the egg white and yolk volume, yolk color, yolk cholesterol and eggshell thickness. The conclusion of this study is that a combination of probiotics and *Averrhoa bilimbi* powder extract can be used as an alternative of antibiotic in feed.

Keywords— *Averrhoa bilimbi*, External Quality of Eggs, Internal Quality of Eggs.

I. INTRODUCTION

Egg is one of the poultry products that has a complete and good nutritional content for human body. It is also favored as a source of animal protein because of its affordable price. The average consumption of chicken eggs per capita increases 1-2% every year. The increasing of chicken eggs consumption indicates that the required for egg production also increases. Egg productivity is very dependent on the quality of the poultry feed. Some feed additives are generally added so far, such as antibiotics. In 2018, however, Indonesian government banned the use of antibiotics in animal feed. This ban led the farmers to look for feed natural additives as an alternative of antibiotics.

Probiotics are natural feed additives that usual be used as an alternative of antibiotics in animal feed. They are non-pathogenic microorganisms which are beneficial for the body's health and physiology (FAO / WHO, 2002). (*Averrhoa bilimbi*) can be used as an acidifier to replace antibiotics in increasing egg production and quality, balancing the condition of the digestive tract microflora, increasing nutrients absorption in the small intestine as well as increasing the profits of the laying hen farmer. The combination of probiotics and *Averrhoa Bilimbi* as an acidifier is expected to be able to

improve the digestibility of laying hens, as well as improve the quality of the eggs, both internal and external quality. This study aims to observe the effect of adding a combination of probiotics and *Averrhoa bilimbi* powder extract with various percentages of combinations on the internal and external quality of laying hens' eggs.

II. MATERIAL AND METHOD

A. Materials

The research material used were 240 laying hens Lohman Brown (22 weeks old), other materials were probiotics powder (*Lactobacillus sp* and *Bacillus sp* $5,4 \times 10^7$ CFU/mg) and *Averrhoa bilimbi* powder extract as the feed additives and self-mixed feed consisting of 30% concentrate (non-antibiotic), 50% corn 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. Tools:

1. A metabolic cage with feeding and drinking troughs made from halved pipes.
2. A yolk color fan
3. A tripod micrometer
4. Pipettes
5. Measuring cups
6. A caliper

C. Method

This research was a kind of field experimental research that used a Completely Randomized Design (CRD) of 4 treatments, each with 6 replications and there were 10 laying hens in each replication. The following was the list of the treatments:

P0: Control feed (without feed additive) and

P1: 0,8% Probiotics+ 0,25% *Averrhoa bilimbi* powder extract

P2: 0,8% Probiotics + 0,55% *Averrhoa bilimbi* powder extract

P3: 0,8% Probiotics + 0,75% *Averrhoa bilimbi* powder extract

This field experiment used restricted feeding method. The laying hens were given 120 g of feed hen day. The feed

additive was added in the form of powder extract and mixed into feeding trial. The sample of egg was analyzed in the laboratory. The variables observed were internal quality of eggs (egg white volume, egg yolk volume, yolk color, and yolk cholesterol) and external quality of eggs (eggshell thickness).

The data of this research were analyzed by using Analysis of Variance (ANOVA) for Completely Randomized Design (CRD). If the result was significant ($P < 0.05$) or highly significant ($P < 0.01$), then it continued with Duncan's Multiple Range Test. The purpose of this analysis was to find out the best treatment of the percentage of combination of probiotics and *Averrhoa bilimbi* powder extract on the egg quality of laying hens so the combination can be used further as an alternative of antibiotics as in the control treatment.

III. RESULTS AND DISCUSSION

A. Internal Quality of Eggs

The result analysis of the combination of probiotics and analysis powder extract on the internal quality of the eggs are presented in the table II below:

TABLE II.

| Treatments | Egg White Volume (ml) | Egg Yolk Volume (ml) | Yolk Color | Yolk Cholesterol (mg/100g) |
|------------|------------------------------|----------------------------|-----------------------------|------------------------------|
| P0 | 29,133 ± 1,480 ^a | 12,70 ± 1,016 ^a | 9,433 ± 0,294 ^a | 241,035 ± 1,042 ^a |
| P1 | 31,267 ± 0,816 ^{ab} | 12,00 ± 0,473 ^a | 9,267 ± 0,273 ^a | 215,383 ± 2,313 ^b |
| P2 | 31,017 ± 2,032 ^{ab} | 12,37 ± 0,850 ^a | 9,667 ± 0,413 ^{ab} | 208,488 ± 0,977 ^c |
| P3 | 32,367 ± 0,950 ^b | 15,05 ± 1,078 ^b | 10,03 ± 0,345 ^b | 197,475 ± 1,114 ^d |

Note: The different notation in the same line indicates the highly significant difference ($P < 0,01$)

Table II shows the results of the treatments on the quality of egg white volume. Those results were then compared with the results of the control treatment in which the antibiotics were added. The result of the antibiotic treatment indicated an average egg white volume of the samples that was 31.417 ml. The results also showed that the highest percentage of combination treatment (P3 = 0.8% probiotics + 0.75% *Averrhoa bilimbi* powder extract) gave the best result, that was 32,367 ml ± 0,950 then followed by P1, P2 and P0 (-) and when compared with antibiotics treatment (P0 +), the combination treatments got better results. The results of the research evidenced that the addition of a combination of probiotics and *Averrhoa bilimbi* extract on feed showed a highly significant difference ($P < 0.01$) on egg white volume, because this combination was expected to increase digestibility of laying hens so that the establishment of egg white became more leverage. Schreiweis, Hester, Settler and Moody (2005) state that the volume and height of egg white are influenced by the digestibility of egg white. Orhan, Eyduran, Tatliyer and Saygici (2016) states that the quality of protein digestibility is related to the egg size and protein is the largest component in composing egg whites other than water and fat.

Table II shows the results of the treatments on the quality of the egg yolk volume. Those results were then compared with the control treatment in which the antibiotics were added to the feed. The result of the antibiotic treatment showed an average egg yolk volume in the sample, that was 14.47 ml. The results indicated that the highest percentage of combination treatment (P3 = 0.8% probiotics + 0.75% *Averrhoa bilimbi* powder extract) gave the best result of 15.05 ml ± 1.078, then followed by P2, P0 (-), P1 and when compared with antibiotic treatment (P0 +), the combination treatments got better results. The results of the research showed that the addition of combination of probiotics and *Averrhoa bilimbi* powder extract on feed manifested a highly significant difference ($P < 0.01$) on egg yolk volume, this was allegedly caused by the combination of probiotics and *Averrhoa bilimbi* powder extract as an acidifier that can increase digestibility, especially digestibility of fat and protein of laying hens. Egg yolk actually consists of the yolk membrane (Vitelline) and the yolk itself. The yolk percentage is around 30-32% of the total weight of egg yolk. Egg yolks can be consumed as food and fat sources for embryo development. The composition of the yolk is 50% water, 32-36% fat, 16% protein and 1-2% glucose (Bell and Weaver, 2002).

Table II shows the results of the treatment on the yolk color score. Those results were then compared with the control treatment in which the antibiotics were added. The result of antibiotic treatment showed an average egg yolk score in the sample that was 9,450. The results showed that the treatment with the highest percentage of combination (P3 = 0.8% probiotics + 0.75% *Averrhoa bilimbi* powder extract) indicated the best results of 10.03 ± 0.345, then followed by P2, P0 (-), P1 and when compared with antibiotic treatment (P0 +), the combination treatments got better results. The results of the research also showed that the addition of probiotics and *Averrhoa bilimbi* powder extract combination to the feed showed a highly significant difference ($P < 0.01$) on the yolk color score. It was already predicted in the beginning since *Averrhoa bilimbi* powder extract contains vitamin A which can increase egg yolk pigment. Shafie, Yusof and Gan (2019) states that the chemical content of *Averrhoa bilimbi* contains flavonoids, glycosides, proteins, fats, calcium, phosphorus, iron, vitamins A, B1 and C. Vitamin A has a role to give yellow pigment on yolks of poultry. The results showed that the yolk color score ranged from 9-10, which is the score range favored by consumers. Stadellman (1995) states that a good yolk color is in the range of 9-12.

Table II shows the results of the treatments on cholesterol level of egg yolks. Those results were further compared with the control treatment in which the antibiotics were added. The result of the antibiotic treatment showed an average cholesterol level of egg yolk in the sample that was 233.818 mg / 100g. It can be seen from the results that the highest percentage of combination treatment (P3 = 0.8% probiotics + 0.75% *Averrhoa bilimbi* powder extract) showed the best result, that was 197.475 mg / 100g ± 1.114, and then followed by P2, P1 and P0 (-). When the results of combination treatments were compared with antibiotic treatment (P0 +),

they got better results since the combination could reduce the cholesterol level of egg yolk. The results of the research indicated that the addition of combination of probiotics and *Averrhoa bilimbi* powder extract on feed showed highly significant difference ($P < 0.01$) on egg yolk cholesterol level. It was already expected since *Averrhoa bilimbi* contains flavonoids and vitamin C, which both can reduce the blood cholesterol level of poultry and affect the cholesterol level of egg yolk. Millar, Duclos and Blesso (2017) states that flavonoids can reduce blood cholesterol levels by lowering the absorption of cholesterol and bile acid in the small intestine and then increase excretion through feces. This causes liver cells to increase the bile acid establishment from cholesterol. It will reduce the amount of fat since it is converted into energy. Vitamin C has a role to break down the cholesterol into bile acids and salts to be easily excreted in the digestive tract in the feces. This leads to the inhibition of cholesterol establishment. Probiotics also play a role in reducing the cholesterol level of egg yolk. According to Getachew (2016) the decrease of cholesterol level in egg yolk is due to the large amount of probiotics. The higher the amount of probiotics, the greater the activity of microbes and probiotics in the digestive tract. Zhong, Liu, Zhang, Han, Jia and Xie (2016) state that the addition of *Lactobacillus* sp in poultry feed can reduce cholesterol levels of egg yolk.

B. External Quality of Eggs

The result analysis of the combination of probiotics and analysis powder extract on the external quality of the eggs are presented in the table III below:

TABLE III. The effect of a combination of probiotics and *Averrhoa bilimbi* powder extract on the external quality of eggs.

| Treatments | Eggshell thickness (mm) |
|------------|-------------------------------|
| P0 | 0,395 ± 0,01440 ^a |
| P1 | 0,437 ± 0,02856 ^{ab} |
| P2 | 0,441 ± 0,02765 ^b |
| P3 | 0,421 ± 0,00327 ^b |

Table III shows the results of the treatment on eggshell thickness. Those results were then compared with the antibiotic treatment. The result of the antibiotic treatment showed that the average eggshell thickness in the sample was 0.0397 cm. The results of the research indicated that the P2 treatment (0.8% probiotics + 0.50% *Averrhoa bilimbi* powder extract) showed the best result of 0.0441 cm ± 0.002765, then followed by P1, P3 and P0 (-) and when compared with antibiotic treatment (P0 +), the combination treatments got better results. The results of the research pointed out that the addition of probiotics and *Averrhoa bilimbi* powder extract combination on feed showed a highly significant difference ($P < 0.01$) on eggshell thickness. It is because *Averrhoa bilimbi* powder extract contains phosphor and calcium which can affect the thickness of the eggshell. Bhaskar and Shantaram (2013) state that ripe *Averrhoa bilimbi* is acidic and contains high fiber and minerals such as calcium, phosphorus, iron and potassium. Fernandes, Murakami, Sakamoto, Souza, Malaguido and Martins (2008) state that eggshell quality can be seen from its thickness and structure. The content of Ca and

P in feed affects the quality of eggshell since in the establishment of eggshell, it is important to have enough carbonate and Ca ions to form $CaCO_3$ eggshell. Clunies, Parks and Lesson (1992) state that the higher the consumption of calcium, the better the quality of eggshell.

The treatment also has an effect on eggshell color. The treatments (P3) gave the best color (dark brown) then followed by P2, P1 and P0 (-). The results of the combination treatments were then compared to antibiotic treatment (P0+) and showed that the treatment of probiotics and *Averrhoa Bilimbi* extract combination as acidifier got better results. It was because the treatment increased the eggshell thickness, so that the eggshell color score also increased. Besides being influenced by the type of pigment, the eggshell color is also influenced by the concentration of egg color pigments as well as the structure of eggshell (Hargitai, Mateo and Torok, 2011). Brown color thickness in eggs can be used as an indicator of eggshell thickness (Joseph, Robinson, Renema and Robinson, 1999).

IV. CONCLUSION

The addition of combination of probiotics and *Averrhoa bilimbi* powder extract as feed additive in feed showed highly significant differences on the volume of egg white, yolk color, yolk cholesterol and eggshell thickness and difference on eggshell color of laying hens. The combination of probiotics and *Averrhoa bilimbi* powder extract can be used as an alternative of antibiotic in feed.

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