Hematological status and egg production of laying hen with probiotic powder as feed supplements

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Abstract. The study aimed to observe the effect of adding probiotic powder on the hematological status and egg production in layer-phase laying hens. This research was conducted for 30 days, located in a layer farms in Sumedang Regency, West Java. The production of probiotic powder was carried out at the Central Laboratory, Padjadjaran University. The blood was analyzed at the Multitest Commercial Laboratory, Margahayu Raya, Bandung City. The variables observed in this study were the levels of erythrocytes, leukocytes, hematocrit, and egg production in layer-phase laying hens. This study used an experimental method with statistical analysis using a completely randomized design (CRD) with four levels of probiotics (0%, 2%, 3%, 4%) and five replications. The research data were analyzed using the 0.05% Variety Print Test. The result of this research showed that the addition of probiotic powder gave the same effect on the levels of erythrocytes, leukocytes, and hematocrits of laying hens. However, probiotic powder increased egg production along with the addition of the dose of probiotic powder. It can be seen that 2%, 3% and 4% probiotic powder increased 64.1%, 41.5% and 118% egg production compared to control.

1. Introduction

The development of laying hen business in Indonesia has good prospects and the development of the laying hen population is currently very rapid. One of the problems in the laying hen business is the decline in egg production along with the age of the chickens. Old chickens will experience a decrease in production because the functions of physiological systems in their bodies have decreased. One of the systems in the body that affects production is health status which can be measured by the hematological value. Animal health status can be observed from the comparison of erythrocyte, leukocyte, and hematocrit levels because blood is an important component of the physiological regulation of the body. One of the indispensable feed additives is the addition of probiotics in the feed.

Probiotics are a group of microbes that can directly help to increase the body's resistance to pathogenic bacteria in the intestines and prevent diseases. The mechanism of action of probiotics that can optimize nutrient absorption contributes nutrients as ingredients in the blood formation process. Most probiotics on the market are in liquid form, but they are less efficient in terms of storage. In addition, giving liquid probiotics to livestock is difficult because there are always plenty of probiotics
left and the possibility for other bacteria to grow is greater than in powder form. Therefore, it is necessary to make preparations in solid form. One alternative to culture preservation is drying. This research was previously conducted by a group of researchers in dry form with the oven-dry method. However, the study resulted in an unsatisfactory bacterial count of $10^3 - 10^6$ cfu/g. So that further research was carried out using the spray drying method with maltodextrin and skim milk coating. It is expected that this drying technology can increase the viability of these probiotic bacteria. Research by many researchers usually uses probiotics with microbiota such as EM-4. However, this study uses yogurt with a consortium of microbiota which is rarely done on laying hen. Therefore, it is necessary to research with a consortium of microbiota that is usually used in humans, as a novelty.

The research goal is whether culled laying hens can maintain egg production and to check whether there are changes in the levels of erythrocytes, leukocytes and hematocrit values in laying hen phase layers.

2. Materials and methods
In this research, a total of 20 commercial layer Lohman Brown strain at 90 weeks of age were used. Laying hens were given 4 treatments and repeated 5 times so that there were 20 experimental units. The cages used were 20 experimental cage units equipped with feed and drinking water containers. Nutrient content and metabolic energy of feed ingredients showed in Table 1, nutritional content and metabolic energy of research ration showed in Table 2.

| Feed ingredients | ME (kcal/kg) | CP (%) | CF (%) | CFib (%) | Ca (%) | P (%) |
|------------------|-------------|--------|--------|----------|--------|-------|
| Corn             | 3370        | 8.60   | 3.90   | 2.00     | 0.02   | 0.10  |
| Bran             | 1630        | 12.00  | 13.00  | 12.00    | 0.12   | 0.21  |
| Concentrate      | 1921.95     | 37.00  | 6.00   | 8.00     | 12.00  | 1.50  |
| Top mix          | -           | -      | -      | -        | 0.60   | -     |
| Macro minerals   | -           | -      | -      | -        | 32.50  | 1.00  |

Source: Food substance content based on Wahju [1]. Calculation of metabolic energy based on Siswohardjono [2].

| Nutrient Content of Feed Ingredients | Number of Standard Requirements |
|-------------------------------------|---------------------------------|
| Metabolic Energy (kcal/kg)          | Min 2700                        |
| Crude protein (%)                  | Min 16.5                        |
| Crude Fiber (%)                    | Max 7                           |
| Crude Fat (%)                      | Min 3                           |
| Calcium (%)                        | 3.25 – 4.25                     |
| Phosphor (%)                       | Min 0.45                        |

Source: SNI 8290.5:2016. Quantity calculation using AFOS application (Software)

2.1. Probiotic powder
The probiotic powder came from fermented cow's milk. The milk was incubated with a consortium of lactic acid bacteria, including *Lactobacillus*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum*. The probiotic drying process used maltodextrin and skim milk as nutrients for bacteria and aimed to keep lactic acid bacteria alive. Drying was done in a spray dryer with an inlet temperature of 160°C and outlet 65-70°C so that the fermented milk obtained the powder form. The total lactic acid bacteria after the drying process was $1.6 \times 10^7$ CFU/g. The provision of dry probiotics was adjusted to the treatment.
2.2. Parameter
The parameters measured were the levels of erythrocytes, leukocytes, hematocrit, and egg production. The analysis was carried out to determine the levels of erythrocytes, leukocytes, and hematocrit using an automatic method using a hematology analyzer. Egg production was recorded daily for 4 weeks.

2.3. Experiment design and data analysis
The study used an experimental method with a completely randomized design (CRD) with 4 treatments and 5 replications so that there were 20 experimental units. The data obtained were tested using an analysis of variance. The treatments in this study are as follows:
- P0 = Ration without probiotic powder treatment
- P1 = Ration with the addition of 2% probiotic powder
- P2 = Ration with the addition of 3% probiotic powder
- P3 = Ration with the addition of 4% probiotic powder

The data obtained were analyzed by analysis of variance (ANOVA). If the results of the analysis of treatment variance there is a significant difference at the 5% level, then the analysis is continued with Duncan's test [3].

3. Result and discussion

3.1. Effect of treatment on erythrocyte levels
Erythrocytes are red blood cells which function as oxygen carriers from the lungs to all body tissues [4]. The average number of erythrocytes in layer-phase laying hens (Table 3) ranged from 1.84-2.11×10⁶/mm³. Statistically showed that the addition of probiotic powder had no significant effect (P>0.05) on the number of erythrocytes.

Table 3. Average levels of erythrocytes, leukocytes and hematocrit of laying hens at 90 weeks of age.

| Treatments | Parameters | Erythrocytes (× 10⁶/mm³) | Leukocytes (× 10⁵/mm³) | Hematocrit (%) |
|------------|------------|--------------------------|------------------------|---------------|
| P0         |            | 2.11 ± 0.31              | 43.98 ± 0.15           | 24.2 ± 2.86   |
| P1         |            | 1.84 ± 0.17              | 48.32 ± 0.10           | 21.4 ± 1.67   |
| P2         |            | 2.04 ± 0.14              | 41.06 ± 0.45           | 23.4 ± 0.89   |
| P3         |            | 1.95 ± 0.24              | 43.16 ± 0.11           | 22.4 ± 2.88   |

Description: P0 (Without probiotic powder); P1 (Probiotic powder 2%); P2 (probiotic powder 3%); P3 (Probiotic powder 4%)

The number of erythrocytes from all treatments tended to be below the normal range. The number of normal laying hens’ erythrocytes is in the range of 2.0-3.2 × 10⁶/mm³ [5]. The low number of erythrocytes can be caused by age. The age of the chickens used were 90 weeks and has passed the 2nd phase of production. The second production phase of laying hens is between the ages of 42-72 weeks [6]. The factors that affect erythrocytes include age, species, ration consumption, and availability of erythrocyte production materials [7].

Research conducted by Edi et al. [8] on 30-week old laying hens without treatment showed an average erythrocyte count of 2.60 × 10⁶/mm³. Another study conducted by Hidayat et al. [9] in laying hens aged 72-weeks without treatment showed an average erythrocyte count of 2.23 × 10⁶/mm³. While this study was conducted on laying hens aged 90-weeks without treatment, the average number of erythrocytes was 2.11 × 10⁶/mm³. So, through the results of some of these studies indicated that the age of laying hens can affect the decrease in the number of erythrocytes.
The formation of erythrocytes takes place in the bone marrow. In adults, long bone marrow which is active in erythropoiesis begins to contain fat. Only flat bone marrow is active in erythropoiesis and tends to decrease its activity with age [10].

In this study, 2% probiotic powder produced low erythrocyte levels, presumably because laying hens had experienced dysbiosis so that 2% probiotic powder was only able to suppress the number of pathogenic bacteria in the intestines. Changes in the composition of the microbiota will affect the interaction between microbes and their hosts which ends in the emergence of disease [11].

3.2. Effect of treatment on leukocyte levels

The average leukocyte laying hens in the layer phase in table 3 ranged from 41.06 – 48.32 × 10³/mm³. Statistically showed that the addition of probiotic powder gave no significant effect (P>0.05) on the number of leukocytes. The results showed that the average number of leukocytes in each treatment was above the normal limit. The normal leukocyte levels in hen ranges from 16-40 × 10³ /mm³ [12].

The number of leukocytes which is higher than the normal range is called leukocytosis [13]. The incidence of leukocytosis can be physiological or pathological. Physiological leukocytosis can usually occur in a state of fear, noise, a new environment, handling by new people, and pain in animals [14]. Pathological leukocytosis can be caused by pyogenic bacterial infection or anemia due to chronic disease [15].

The age of chickens affects the physiological system of livestock so that it affects the immune system. This causes livestock to be easily infected which eventually leukocytes increase above normal limits. Total leukocytes which describe the level of health are influenced by several internal factors including gender, age, disease, and hormones as well as external factors such as environmental conditions, activities, stress, and feed [5].

Research conducted by Nasrullah et al. [16] on 40-week old laying hens without treatment showed an average leukocyte count of 31.30 × 10³ /mm³. Another study conducted by Titin et al. [17] on 119-week old laying hens without treatment showed an average leukocyte count of 56.92 × 10³ /mm³. While this study was conducted on 90-weeks old laying hens without treatment, the average leukocyte levels was 43.98 × 10³ /mm³. So, through the results of some of these studies indicate that the age of laying hens can affect the increase in the number of leukocytes.

Even though the leukocyte count was above normal, the addition of 3% probiotic powder tended to decrease the mean leukocyte count compared to the control. In the addition of 3% probiotic powder (P2), there was a decrease in the number of leukocytes 6.64% compared to the control. This showed that the addition of probiotics can balance the microflora in the digestive tract. The decrease in leukocyte levels occurs because it was closely related to the decrease in the number of pathogenic bacteria in the intestine. The decrease in the number of leukocytes in the blood is a mechanism for the body’s response to invading pathogens.

The presence of lactic acid bacteria in the intestine can create an acidic atmosphere to suppress the growth of pathogenic bacteria in the small intestine [18,19]. Probiotic bacteria such as Lactobacillus acidophilus and Bifidobacterium can inhibit the growth of pathogenic bacteria in the intestine [20].

3.3. Effect of treatment on hematocrit levels

Hematocrit is the volume of erythrocytes in 100 cubic milliliters of blood expressed in percent [21] The average number of layers on layer-phase hematocrit (Table 3) ranged from 21.4 to 24.2%. Statistically showed that the addition of probiotic powder had no significant effect (P>0.05) on the number of hematocrits.

The average number of hematocrits in this study indicated that laying hens treated with 3% and 4% were able to maintain the hematocrit in the normal range. The number of hematocrits of normal laying hen are in the range of 22%-35% [22]. Hematocrit is influenced by strain, age, type of livestock, production phase, sex of livestock, disease, and local climate [23].

Based on these results, it appears that the hematocrit value with the number of erythrocytes has a relationship. The greater the number of erythrocytes, the greater the hematocrit value in the blood [24].
On the other hand, a decrease in the hematocrit value can be caused by erythrocyte damage, decreased erythrocyte production, or can also be influenced by the number and size of erythrocytes. High or low hematocrit values in the blood will have an impact on blood viscosity. The greater the percentage of cells in the blood, the greater the friction that occurs between the various layers of blood and this friction forms viscosity causing an increase and vice versa will slow down blood flow in the capillaries and accelerate the work of the heart [5,25].

### 3.4. Egg production

The average egg production in layer-phase laying hens (Table 4) ranged from 9.75 to 21.3 eggs. The mean increases successively with increasing doses. The values were P1 = 64.1%, P2 = 41.5% and P3 = 118% compared to control.

**Table 4. Egg production of laying hens in each treatment**

| Week | Treatments | P0    | P1    | P2    | P3    |
|------|------------|-------|-------|-------|-------|
|      |            |       |       |       |       |
| 1    |            | 9     | 17    | 19    | 11    |
| 2    |            | 8     | 19    | 18    | 17    |
| 3    |            | 10    | 24    | 15    | 19    |
| 4    |            | 12    | 20    | 17    | 17    |
|      | Average    | 9.75±1.70 | 16±4.74 | 13.8±3.75 | 21.3±8.61 |

Egg production is influenced by digestive conditions in the body of livestock. Increased digestibility and absorption of nutrients due to the presence of lactic acid bacteria. Lactic acid bacteria increase enzyme activity, lactic acid, short-chain fatty acids, and antimicrobials (bacteriocin, hydrogen peroxide, diacetyl) effectively inhibit the development of pathogenic microbes [26,27]. The decrease in pathogenic bacteria will increase enzyme activity and expand the surface of the intestinal villi which affects the increase in nutrient absorption and digestibility [20,28,29]. Feed that is well absorbed is utilized by chickens to increase egg production. Nutrient absorption is better because the villi in the small intestine increase in length and density which causes more goblet cells [29].

### 4. Conclusions and suggestions

Based on the results of the study, it can be concluded that the addition of probiotic powder (0%, 2%, 3%, 4%) gave the same effect on the levels of erythrocytes, leukocytes, and hematocrit values of layer laying hens. However, the addition of probiotic powder (0%, 2%, 3%, 4%) increased egg production by 64.1%, 41.5% and 118% respectively compared to control. Suggestions for this research are the need for further research on the addition of probiotic powder to laying hens at a younger age and with an additional time of more than one month.

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