Aloe (*Aloe barbadensis* Miller) flour in broiler ration towards the ratio of Heterophyl Lymphocyte (H/L) and percentage of the relative weight of the lymphoid organ

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Abstract. Phytobiotics had the immunomodulatory effects which known to increase the broiler immunity. Aloe contains acemannan and flavonoids with the effect of immunomodulator. The purpose of this study was to determine the optimal level of *Aloe barbadensis* Miller flour to improve broiler immunity. This study used a completely randomized design consisting of four treatments and four replications, T0: ration without aloe flour (control group), T1: ration + 0.075% aloe flour, T2: ration + 0.100% aloe flour, and T3: ration + aloe flour 0.125%. Eighty broilers were reared intensively and treatments given for 42 days. The ratio of heterophil/lymphocyte (H/L) and the percentage of the relative weight of lymphoid organ was determined at day-42. Results showed that aloe flour had a significant effect (P<0.01) on the ratio of heterophil/lymphocyte (H/L) and (P<0.05) on the percentage of the relative weight of bursa of Fabricius, but did not significantly affect the percentage of the relative weight of the spleen. The lowest (H/L) heterophil/lymphocyte ratio was T3 (0.29), and the highest was T0 (0.65). The highest relative weight percentage of the bursa of Fabricius was T3 (0.09%) and the lowest was T0 (0.05%). The relative weight percentage of the spleen was similar in group T0, T1 and T2, 0.12%, while T3 was 0.13% of the body weight. The addition of *Aloe barbadensis* Miller flour with a level of 0.125% (T3) in broiler ration tend to increase immunity through a decrease in the ratio of heterophil/lymphocytes (H/L) and increase in weight of bursa of Fabricius and spleen.

1. Introduction
Broilers are still the main source for animal protein in Indonesia since its fast and efficient growth in converting feed into meat. Along with the increasing demand for chicken meat, various methods have been tried to optimize the production of broilers. Antibiotics administration on the farm was needed in preventing and eradicate diseases. However, antibiotics might cause residues in the body. Alternative substitutes for antibiotics were needed to maintain the health of livestock by the increase of immunity. Studies of the use of phytobiotics as a substitute for antibiotics have been widely carried out, one of which was *Aloe barbadensis* Miller flour. Acemannan and flavonoids in aloe were polysciosida compounds that play a role in the immune system, as an immunomodulatory [1]. Immunomodulators are substances that can affect the function and activity of the immune system [2]. The content of immunomodulatory bioactive substances such as acemannan and flavonoids in Aloe was thought to increase the immunity of broilers.
Immunity of chicken as a response to stressors could be identified from the ratio of heterophil/lymphocyte (H/L). The H/L ratio is the most easily known indicator of stress. The higher H/L showed higher stress level of chicken [3]. In addition, indicators of immunity can also be identified from the weight of several lymphoid organs. The higher levels of stress and infection in chickens result in a decrease in the weight of several lymphoid organs such as the bursa of Fabricius, spleen, and thymus. Thus, reduced lymphocytes count and consequently decreased antibody production by these organs. However, not clearly known the optimum amount of aloe flour in the ration to increase broilers immunity. This study was purposed to determine the optimal level of \textit{Aloe barbadensis} Miller flour to improve broiler immunity.

2. Materials and methods

2.1. Experimental animals and diets

A total of 80 cobb strain broilers with an average weight of 35 grams used as experimental animals. The cage used was a postal enclosure (litter system) within a plot of 100 cm × 100 cm × 50 cm was made. The ration was prepared based on National Research Council standards, with ingredients were as follow corn, rice bran, fish flour, soybean meal, coconut cake meal, coconut oil, dicalcium phosphate, meat bone meal, and aloe flour.

| Feed ingredients          | Treatments | T0     | T1    | T2    | T3    |
|---------------------------|------------|--------|-------|-------|-------|
| Corn (%)                  | 57         | 57     | 57    | 57    |
| Rice bran (%)             | 5          | 5      | 5     | 5     |
| Fish flour (%)            | 8          | 8      | 8     | 8     |
| Soybean meal (%)          | 13.5       | 13.5   | 13.5  | 13.5  |
| Coconut meal cake (%)     | 4          | 4      | 4     | 4     |
| Coconut oil (%)           | 0.4        | 0.4    | 0.4   | 0.4   |
| Dicalcium phosphate (%)   | 0.1        | 0.1    | 0.1   | 0.1   |
| Meat bone meal (%)        | 12         | 12     | 12    | 12    |
| **Total**                 | 100        | 100    | 100   | 100   |
| Aloe barbadensis Miller flour (%) | 0       | 0.075  | 0.100 | 0.125 |

| Chemical composition      |            | T0     | T1    | T2    | T3    |
|---------------------------|------------|--------|-------|-------|-------|
| Metabolism energy (kcal/kg)| 2930       | 2930   | 2930  | 2930  |
| Crude protein (%)         | 22.52      | 22.52  | 22.52 | 22.52 |
| Crude fibre (%)           | 5.3        | 5.3    | 5.3   | 5.3   |
| Crude fat (%)             | 3.7        | 3.7    | 3.7   | 3.7   |
| Lysin (%)                 | 1.37       | 1.37   | 1.37  | 1.37  |
| Methionine (%)            | 0.44       | 0.44   | 0.44  | 0.44  |
| Ca (%)                    | 1.7        | 1.7    | 1.7   | 1.7   |
| P (%)                     | 1.5        | 1.5    | 1.5   | 1.5   |

\(T0: \) ration without aloe flour (control group), \(T1: \) ration+0.075% aloe flour, \(T2: \) ration+0.100% aloe flour, and \(T3: \) ration+0.125% aloe flour.

This study used a completely randomized design (CRD) with four treatments and four replications. The treatment groups were as follow, T0: ration without aloe flour (control group), T1: ration +
0.075% aloe flour, T2: ration + 0.1% aloe flour, and T3: ration + aloe flour 0.125%. The study design consisted of 16 experimental units, with five broilers of each. The animals were kept for 42 days with two phases: starter and finisher. The starter phase occurred at the age of 1–14 days and the finisher phase occurred at the age of 15–42 days. The nutrient content of ration for each phase presented in table 1 and table 2. The feed was given ad libitum, twice a day. The data obtained were statistically analyzed using ANOVA then followed by the Duncan test [4].

### Table 2. Composition and nutrient content of finisher phase (day 15–42) ration.

| Feed ingredients             | T0  | T1  | T2  | T3  |
|------------------------------|-----|-----|-----|-----|
| Corn (%)                     | 58  | 58  | 58  | 58  |
| Rice barn (%)                | 9   | 9   | 9   | 9   |
| Fish flour (%)               | 6   | 6   | 6   | 6   |
| Soybean meal (%)             | 11  | 11  | 11  | 11  |
| Coconut meal cake (%)        | 6.5 | 6.5 | 6.5 | 6.5 |
| Coconut oil (%)              | 0.4 | 0.4 | 0.4 | 0.4 |
| Dicalcium phosphate (%)      | 0.1 | 0.1 | 0.1 | 0.1 |
| Meat Bone Meal (%)           | 9   | 9   | 9   | 9   |
| **Total**                    | 100 | 100 | 100 | 100 |
| Aloe barbadensis Miller flour (%) | 0   | 0.075 | 0.100 | 0.125 |

**Chemical composition**

| Metabolism energy (ME) (kcal/kg) | 2900 | 2900 | 2900 | 2900 |
|----------------------------------|------|------|------|------|
| Crude protein (%)                | 19.85| 19.85| 19.85| 19.85|
| Crude fibre (%)                  | 5.8  | 5.8  | 5.8  | 5.8  |
| Crude fat (%)                    | 3.4  | 3.4  | 3.4  | 3.4  |
| Lysin (%)                        | 1.1  | 1.1  | 1.1  | 1.1  |
| Methionine (%)                   | 0.4  | 0.4  | 0.4  | 0.4  |
| Ca (%)                           | 1.3  | 1.3  | 1.3  | 1.3  |
| P (%)                            | 1.4  | 1.4  | 1.4  | 1.4  |

T0: ration without aloe flour (control group), T1: ration + 0.075% aloe flour, T2: ration + 0.1% aloe flour, and T3: ration + aloe flour 0.125%.

2.2. Blood sampling for the ratio of heterophil/lymphocyte (H/L) parameter

Blood sampling for H/L parameter was conducted at day 42. Blood samples were obtained from pectoral vein aseptically using vacutainer with EDTA anticoagulant. For total leukocytes count, the blood samples were diluted with Turk solution. Heterophil and lymphocyte were counted by blood smear preparation stained with Giemsa. The number of heterophils and lymphocytes then multiplied by the number of total leukocytes. Determination of the ratio of heterophils/lymphocytes (H/L) was obtained by dividing the percentage of heterophils by the percentage of lymphocytes.

2.3. Lymphoid organ sampling

One bird from each experimental unit was taken for lymphoid organ sampling. Each bird was weighed prior to cut. A total of 16 birds were cut for organ sampling. Bursa of Fabricius and spleen from each bird were weighed. The relative weight percentage of the lymphoid organ was determined from the comparison of organ weights to live weight.
3. Results and discussion

3.1. The ratio of heterophil/lymphocyte (H/L)

The ratio of heterophil/lymphocyte (H/L) is the most easily known indicator of stress. Leukocytes are the basic components in the cellular immune system. Heterophils are a form of neutrophils in chicken and responsible as the first-line defense. Heterophils are formed in the bone marrow of myelocytes. Lymphocytes are the most abundant type of leukocytes in chicken [5]. Lymphocytes play a role in forming antibodies (humoral immunity) and cellular immunity. Circulating lymphocytes are able to produce immunoglobulins (IgG, IgM, and IgA) [6]. The average H/L in broilers aged 42 days after the administration of aloe flour with different levels presented in table 3.

Table 3. The average ratio of heterophile/lymphocyte (H/L) in broilers aged 42 days

| Treatments | (H/L)  |
|------------|--------|
| T0         | 0.65<sup>a</sup> |
| T1         | 0.55<sup>ab</sup>  |
| T2         | 0.37<sup>bc</sup>  |
| T3         | 0.29<sup>c</sup>  |

<sup>a,b,c</sup>Superscript with different notation letter on the same column showed highly significant difference (P<0.01). T0: ration without aloe flour (control group), T1: ration + 0.075% aloe flour, T2: ration + 0.100% aloe flour, and T3: ration + aloe flour 0.125%.

Duncan’s test results showed that the administration of aloe flour had a significant effect (P <0.01) on the ratio of heterophil/lymphocyte (H/L). The P0 group was significantly different (P<0.01) from the group T2 and T3 but was not significantly different from group T1. T2 treatment was not significantly different from treatment T1 and T3. The results of this study indicated that a higher level of aloe flour showed a lower H/L. Low H/L value showed low-stress levels.

Administration of aloe flour had a significant effect on the H/L value in broilers. Acemannan can increase macrophage activity through mannose receptors found on the cell surface, and dendritic cells through increased expression of the major histocompatibility complex (MHC) molecule. Increased activity of macrophage cells will affect the process of antigen destruction in the chicken body [2]. Acemannan in aloe flour increased lymphocyte proliferation, thus causing an increase in the number of lymphocytes in the blood. Acemannan in aloe released cytokines such as IL-1, IL-6, IL-12, and TF-α (tumor necrosis factor) from macrophages. This could stimulate the growth of B-lymphocytes and increase the number of T-lymphocytes, thus increasing the total lymphocyte count [7]. The ratio of heterophil/lymphocyte (H/L) indicated the immunity of chicken, [8] stated that the range of H/L value was 0.2 (low), 0.5 (normal), and 0.8 (high) to environmental adaptation.

3.2. Percentage of the relative weight of lymphoid organs

Bursa of Fabricius is a lymphoid organ responsible for differentiation and maturation of B-lymphocyte. B cell in the secondary lymphoid organ can respond to antigen and produce humoral immunity. B lymphocyte cells that have been exposed to antigens will differentiate into immunoglobulin-producing plasma cells [9]. The spleen is the secondary lymphoid organ with the role related to the immunological response to antigens that enter the blood circulation to resist the invasion of organisms or toxins. Spleen also responsible for maturation of antibody-producing cells [10].

The percentage of the relative weight of bursa of Fabricius and spleen at aged 42 days after administration of Aloe flour were presented in table 4. Duncan's test on the percentage of the relative weight of bursa of Fabricius showed that administration of 0.125% aloe flour had a significant effect (P<0.05) on T0 (control group). Higher content of aloe flour in broiler ration increased the bursa of
Fabricius weight and indicated an increasing number of lymphocyte cells. Glick [11] suggested that more exposure of antigen would increase antibodies production by bursa of Fabricius which led to the depletion of lymphoid follicle and reduction of bursa weight, resulted decreasing of the relative weight of the lymphoid organ. The average relative weight of bursa of Fabricius obtained in this study were 0.05–0.09% of the body weight. The normal relative weight of bursa of Fabricius in broiler aged 42 days was 0.09% [12].

Flavonoids caused the increase of lymphocyte proliferation in the bursa of Fabricius, thus protecting the bursa of Fabricius from the effects of glucocorticoids which excreted under stressed conditions. Research conducted by [13] showed that flavonoids in aloe could increase the production of interleukin-2 (IL-2), which stimulate proliferation and differentiation of B-cells. Increasing of corticosteroids and glucocorticoids would disrupt the immune system by inhibiting lymphocyte proliferation and causing depletion of lymphoid follicle through apoptosis, further would inhibit the immunoglobulin production [14].

Table 4. Percentage of the relative weight of bursa of Fabricius and spleen in broilers aged 42 days

| Treatments | Lymphoid Organ |
|------------|----------------|
|            | Bursa of Fabricius (%) | Spleen (%) |
| T0         | 0.05 ± 0.01<sup>a</sup> | 0.12 ± 0.03 |
| T1         | 0.06 ± 0.02<sup>ab</sup> | 0.12 ± 0.04 |
| T2         | 0.07 ± 0.03<sup>ab</sup> | 0.12 ± 0.02 |
| T3         | 0.09 ± 0.01<sup>b</sup>  | 0.13 ± 0.01 |

<sup>a,b</sup>Superscript with different notation letter on the same column showed highly significant difference (P<0.05).

Analysis of variance showed that treatments did not significantly (P>0.05) affect the percentage of the relative weight of the spleen (table 4). The relative weight percentage of the spleen was similar in group T0, T1, and T2, 0.12%, while T3 was 0.13% of the body weight. The normal relative weight of spleen in broiler aged 42 days was 0.13–0.16% [12]. The relative weight of the spleen in all treatments were still in the normal range (table 4). This indicated that the broiler immunity worked well. The antigens that enter the body of the broiler were unable to reach blood circulation, as of reducing the work of the spleen. An increasing in spleen work would cause changes in the consistency and size of the lymphoid organ, and the spleen will swell [15].

Increased humoral immunity could be associated with polysaccharides (acemannan) in aloe flour. Darabighane [7] suggested that polysaccharides can increase cytokines and antibodies, and improve the natural killer performance of B and T-lymphocytes. According to [2] as a polymer formed by mannose, acemannan can attach to mannose receptors in macrophages and increase macrophage activity to kill intracellular bacteria, non-specific parasites, and neoplastic cells. Thus, in general, polysaccharides can affect the humoral and cellular immune responses.

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