

Effects of Bamboo Leaf Extract Supplementation on Heterophil, Lymphocyte, and Heterophil to Lymphocyte Ratio of Broiler Chickens

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ABSTRACT

This experiment was conducted to assess the effects of bamboo leaf extract (BLE) supplementation on heterophil, lymphocyte, and heterophil to lymphocyte ratio of broiler chickens. A total of 90 one-day-old Cobb broilers were randomly allocated to 5 treatments with 3 replicates of 6 broilers each. The treatments were T1) BLE-free control diet (control); T2) control + 100 ppm BLE; T3) control + 300 ppm BLE; T4) control + 500 ppm BLE; and T5) control + 700 ppm BLE. The experiment lasted for 35 days. All birds did not get any vaccinations. Blood samples were collected on day 21 and day 35. Results showed that on day 21, T2 had lower heterophil than other treatments with the highest at T1, based on average level ($p > 0.05$). T5 showed higher lymphocyte levels and the lowest at T2 than other treatments. Meanwhile, on day 35, the lowest level of heterophils was still at T2. The highest lymphocyte levels were at T4 and the lowest was at T3. Control diet with 500 ppm bamboo leaf extract (T4) showed that bamboo leaf extract reduced the heterophil to lymphocyte (H/L) ratio. Flavonoids and vitamin C in bamboo leaf extract improved the lymphocyte levels and decreased the heterophil levels which affected the H/L ratio value.

Keywords: Bamboo Leaf Extract, Heterophil, Lymphocyte, H/L Ratio.

1. INTRODUCTION

Broiler chickens face several challenges during their life. One of the big challenges is they have to survive from environment exposure (temperature, feed, biotic and abiotic exposures) that may affect metabolic and health status. Bacteria, virus and other pathogenic microorganism may affect their health and the consequences is low productivity and high mortality. Animal's health including livestock is maintained through a good immune system and it should be supported by feed intake [1]. Broiler diet are formulated based on requirement and mostly contained feed additives such as antibiotic growth promotor [2]. Antibiotics and growth promotors are often used as feed additives to increase the broiler productivity due to their important role in the broiler digestive system as an infection preventer. The use of antibiotics has prohibited due to concerns about side effects, such as resistance to pathogens and accumulation of residues that can affect human's health [3].

The prohibition of antibiotics leaves a gap in comply the needs of animals health, so alternatives are needed. Natural or herbal ingredient often used as an alternative to replace antibiotic [4]. Several studies have been reported that herbal ingredients contain active substances that can act as antibacterial, antiviral, antioxidant, growth and health promoter for broiler [5].

Apus bamboo (*Gigantochloa apus*) is known as a potential plant with many benefits and variously used in Indonesia. Apus bamboo has large leaves, high growth rates, and grows in almost areas. Bamboo plants grows well in Southeast Asia and become an important commodity as a source of food and traditional medicine. Several studies have revealed that bamboo is a natural or herbal ingredients with many active substances [6]. The active substances contained in bamboo leaves are vitamin C and flavonoids, in flavones C-glycoside form which can be obtained through the extraction process [7, 8]. According to several studies, flavonoids can be used as a boost in the immune system, while vitamin C is an important component to maintain a healthy body.

Bamboo leaf extract has the potential use as an immunity booster or immunomodulator. The flavonoids contained in bamboo leaf extract can help the immune response by inducing cells to increase lymphocyte cell proliferation [9]. In addition, flavonoids can stimulate phagocytic cells, i.e. heterophils. This immune mechanism indirectly in line with the increase of phagocytic activity in the non-specific immune system [10]. Meanwhile, vitamin C in bamboo leaf extract is a strong antioxidant that reduces heterophil cells damage caused by phagocytic activity. Vitamin C protect cells from oxidation and support heterophil phagocytic activity, thus increasing the immune response accordingly.

The increase of leukocyte cells along with their differential indicates a health improvement through immunity with better immune response. An increase in heterophil to lymphocyte ratio may exhibit some infections detected by the immune system. Infection will stimulate an inflammatory response till the bone marrow produce more heterophils as the main immune response. The increase of heterophils affect heterophil to lymphocyte ratio respectively. Flavonoids in bamboo leaf extract also known as anti-inflammatory, that can suppress the stimulation of heterophil production. The absence of inflammation in the body implies signal reduction for heterophils, especially in blood. This makes bamboo leaf extract return the number of heterophils to normal levels after an increase in the initial infection as a primary response. In attempt to balance the heterophils to lymphocytes (H:L) ratio, bamboo leaf extract can increase the proliferation of lymphocyte cells. Higher lymphocytes level in the body is expected to improve health status because of strong innate response.

Previous studies reported that the used of Moso bamboo leaf extract (*Phyllostachys* sp.), as much as 0.3 g/kg or 300 ppm increased the health and production in broilers [11]. Furthermore, the active substances in bamboo leaf extract increased phagocytic cells which indicates non-specific immune system improvement [12]. This study aimed to examine the immune status of broiler chickens with supplementation of Apus bamboo (*Gigantochloa apus*) leaf extract. Bamboo leaf extract can affect both body immunity and health due to flavonoids with vitamin C.

2. MATERIAL AND METHOD

This study used Apus bamboo leaves, in the order of 3-4 from the tip, the older leaves provided more active substances [13]. Bamboo leaf extract was obtained through extraction with methanol maceration method for 6 days [14].

A total of 90 one-day-old *Cobb* broilers were used in the study, body weight coefficient of variation below 10 percent. The experimental pens were used litter system and grouped to 5 treatments with 3 replicates of 6 broilers each. Curtains were installed in pens to regulate air circulation.

The experiment used Completely Randomized Design with bamboo leaf extract consisted 5 level of treatments. Each treatment was repeated 3 times. The treatments were applied following: 1) bamboo leaf extract-free as control diet (T1); 2) control diet + 100 ppm bamboo leaf extract (T2); 3) control diet + 300 ppm bamboo leaf extract (T3); 4) control diet + 500 ppm bamboo leaf extract (T4); control diet + 700 ppm bamboo leaf extract (T5)

Table 1. Content of Metabolic Energy and Nutrients in Feed Ingredients

Feed Ingredients	ME	Crude Protein	Crude Fat	Crude Fiber	Calcium	Phosporus	Lisine	Methionine
	kcal/kg%.....						
Corn	3370	8.60	3.90	5.36	0.21	0.17	0.24	0.18
Rice bran	3784	11.55	8.37	12.43	0.11	0.17	0.51	0.22
Soybean meal	2240	45.00	0.90	6.00	0.32	0.29	2.90	0.65
Fish flour	3080	45.00	9.00	0.91	5.50	2.80	5.00	1.80
Coconut oil	8600	0.00	100.00	0.00	0.00	0.00	0.00	0.00
Bone meal	0.00	0.00	0.00	0.00	24.00	12.00	0.00	0.00
L Lisine	0.00	95.80	0.00	0.00	0.00	0.00	78.50	0.00
DL Met	0.00	58.00	0.00	0.00	0.00	0.00	0.00	99.0

Source: Laboratory of Ruminant Animal Nutrition and Animal Feed Chemistry Universitas Padjadjaran (2019)

Broilers were fed control diet on day 0 to day 7, while the experimental doses were applied on the day 8 to day 35 of age. Water was given ad libitum. Bamboo leaf extract was mixed into diet until homogenous. The metabolizable energy (ME) of broiler chickens diet is 3000 kcal/kg and crude protein (CP) is 21-22% based on Cobb-vantress 2018. The nutrient content and metabolic energy of control diet is presented in Table 2.

Blood samples were taken on the day 21 and day 35 of age through the pectoralis vein using a 3 mL syringe. Data were analyzed using one-way analysis of variance (ANOVA). The significant differences among different treatment means were investigated using Duncan's test by considering differences significant at $p < 0.05$ [15].

Table 2. Content of Metabolic Energy and Nutrients in Basal Ration

Metabolic Energy and Nutrients	Amount of Content
metabolizable energy (kcal/kg)	3006
Crude Protein (%)	21.28
Crude Fiber (%)	4.64
Crude Fat (%)	1.47
Lisine (%)	1.34
Methionine (%)	0.49
Methionine+sistine (%)	0.65
Calsium (%)	1.00
Phosporus (%)	0.68

3. RESULT AND DISCUSSION

3.1. Heterophil Levels in Broiler Chickens

The heterophile levels data of broilers chickens supplemented with bamboo leaf extract are presented in Table 3. The average heterophil level of broilers on day 21 ranged from 2% to 4%. Heterophils are part of leukocytes that play a role in the body immunity from infection or inflammation. Heterophil destroys pathogens by phagocytosis [16].

Based on numerical, the highest heterophil averages to lowest are sequentially, T1, T3, T4 with T5, and T2. The highest percentage of heterophil levels was obtained in T1) $4 \pm 1.63\%$ and the lowest in the T2) $2 \pm 0.82\%$. The difference of heterophil levels in each treatment compared to control, each decreased by 50% (T2), 41.75% (T4 with T5), and 25% (T3). Based on the results of the analysis of variance, there was no significant difference. The normal heterophils percentage of broiler

ranges from 20-30% of the total leukocytes [17]. This indicates the levels of heterophils in blood of the experimental broilers are below the normal range. Bamboo leaf extract supplementation showed lower heterophil results than the control treatment. The decrease in the number of heterophils is caused by minimum response to stimulate macrophages and monocytes production in the body [18]. While the percentage of heterophil levels on day 35, ranged from 2% to 3% of the total leukocytes. The highest percentage of heterophil level was showed at T3) $3.00 \pm 0.82\%$ and the lowest was at T2) $2.00 \pm 0.82\%$. Based on numerical, the average from the highest to the lowest in sequence, T3, T1, T4 with T5, and T2. The difference of heterophil levels in each treatment compared to control decreased by 25.09% (T2), 12.73% (T4 and T5), and increased by 12.34% (T3). Based on the results of the analysis of variance, there was no significant difference. On day 21 and 35, T2 with supplementation of 100 ppm bamboo leaf extract showed minimum level of heterophil. Heterophils are part of the non-specific immune response with the earliest in defense against infection. So, when the number of heterophil is too low, broilers will be more susceptible to disease.

3.2. Lymphocyte Levels in Broiler Chickens

The lymphocyte levels data of broiler chickens supplemented with bamboo leaf extract are presented in Table 3. The average percentage of lymphocyte levels in broiler chickens on day 21, ranged from 45.33% to 82.33%. The average from the highest to the lowest in sequence, T5, T4, T3, T1, and T2. Highest percentage of lymphocyte levels showed by T5) $82.33 \pm 6.02\%$ and the lowest by T2) $45.33 \pm 29.32\%$. The difference of lymphocyte levels in each treatment compared to control increased by 1.3% (T5) and decreased by 44.26% (T2). Based on the results of Duncan's test, the supplementation of bamboo leaf extract showed that T5 had higher lymphocyte levels ($p < 0.05$) than T2. Supplementation of 700 ppm bamboo leaf extract (T5) can increase the lymphocyte levels. Lymphocytes are an important element in the immune system by forming antibodies [19]. The main function of lymphocytes is to respond against antigen and stress by increasing circulated antibodies as immune system development [20].

Table 3. Effect of Bamboo Leaf Extract on Heterophil and Lymphocyte of Broiler Chickens

Variable	Day	T1	T2	T3	T4	T5	Average
		----- % -----					
Heterophil	21	4.00 ± 1.63	2.00 ± 0.82	3.00 ± 0.82	2.33 ± 0.47	2.33 ± 0.47	2.73 ± 0.71
	35	2.67 ± 0.40	2.00 ± 0.82	3.00 ± 0.82	2.33 ± 0.47	2.33 ± 0.47	2.47 ± 0.34
Lymphocyte	21	81.33 ^b ± 2.49	45.33 ^a ± 29.32	81.33 ^b ± 1.70	81.33 ^b ± 4.11	82.33 ^b ± 6.02	74.33 ± 14.51
	35	84.33 ^a ± 2.05	85.67 ^a ± 2.87	82.00 ^a ± 2.45	87.00 ^a ± 0.82	82.67 ^a ± 3.09	84.33 ± 1.85

* Different superscripts in the same column showed significant differences $p < 0.05$. T1: bamboo leaf extract-free control diet (control), T2: control + 100 ppm bamboo leaf extract, T3: control + 300 ppm bamboo leaf extract, T4: control + 500 ppm bamboo leaf extract, T5: control + 700 ppm bamboo leaf extract

Table 4. Effect of Bamboo Leaf Extract on H/L Ratio of Broiler Chickens

Variable	Day	T1	T2	
H/L Ratio	21	0.05 ± 0.02	0.16 ± 0.13	0.
	35	0.03 ± 0.01	0.02 ± 0.01	0.

* Different superscripts in the same column showed significant differences $p < 0.05$. T1: bamboo leaf extract-free control diet (control) leaf extract, T5: control + 700 ppm bamboo leaf extract

Flavonoids in bamboo leaf extract can increase the lymphocyte levels, it is potentially possible to fill the gap as an immunity booster. Flavonoids in bamboo leaf extract can help the immune response by inducing cells to increase lymphocyte production [9]. While the percentage of lymphocyte levels on day 35 ranged from 82.00% to 87.00%. The highest percentage of lymphocyte levels was showed by T4) $87.00 \pm 0.82\%$ and the lowest by T3) $82.00 \pm 2.45\%$.

The average from the highest to the lowest in sequence, T4, T2, T1, T5, and T3. The difference of lymphocytes levels in each treatment compared to control decreased by 2.76% (T3) along with 1.97% (T5), and increased by 1.58% (T2) along with 3.16% (T4). Based on the analysis of variance, there was no significant difference. The decreasing number of lymphocytes will increase the H/L ratio and stress conditions will increase [21].

3.3. Heterophil to Lymphocyte Ratio in Broiler Chickens

The heterophils to lymphocyte ratio data of broiler chickens supplemented with bamboo leaf extract are presented in Table 4. The average heterophil to lymphocyte ratio of broiler chickens on day 21 ranged from 0.05 to 0.16. Based on numerical, the highest heterophil to lymphocyte ratio showed by T2) 0.16 ± 0.13 . While the other treatments, T3, T4, T5, and T1 showed the same results respectively. The difference of

the heterophil to lymphocyte ratio compared to control increased by up to two times (T2). Based on the analysis of variance, there was no significant difference ($P > 0.05$). T2 showed higher heterophil to lymphocyte ratio than other treatments, indicates that T2 produced more heterophil caused by early detect of higher infections or stress level. The higher the ratio, the higher the stress level as a form of adaptation to the environment. An increase in the heterophil to lymphocyte ratio can occur if the decrease in lymphocyte levels is greater than the decrease in heterophil levels [22].

While, the heterophil to lymphocytes ratio on the day 35 ranged from 0.02 to 0.04. Based on numerical, the highest lymphocyte heterophile ratio was showed by T3) 0.04 ± 0.01 and the lowest by T2) 0.02 ± 0.01 . The average from the highest to the lowest in sequence, T3, T4, T5, T1, and T2. The difference in heterophil to lymphocyte ratio in each treatment compared to the control increased by of 33.33% (T3) and a decrease of 33.33% (T2), while T4 and T5 had no difference. Based on the results of the analysis of variance, there was no significant difference.

4. CONCLUSION

The supplementation of 500 ppm bamboo leaf extract (T4) in diet showed that bamboo improved health status by reducing the H:L ratio. Flavonoids and vitamin C in bamboo leaf extract improved the lymphocyte levels and decreased the heterophil levels which affected the H:L ratio. Supplementation of bamboo leaf extract reduced

the H:L ratio, as an indicator of stress reduction and immune competence.

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