

The Different Time Administration of Cherry Leaf Teabag (*Muntingia calabura* L.) to Broiler Immunity

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Abstract : This study purpose was to analyze the different time administration of cherry leaf teabag (*Muntingia calabura* L.) to broiler immunity. This study used 2 treatment groups. The first group (T1) was given cherry leaf teabag (*Muntingia calabura* L.) every day in drinking water (T1), and the second group (T2) was given cherry leaf teabag (*Muntingia calabura* L.) every two days. The dosage of cherry leaf used in this study 420 mg/kg BW. Paired samples t-test was used to compare the results of the two treatment groups. To determine the effect of different time administration of cherry leaf teabag on the broiler immunity, the parameter observed was the antibody titer in response to Newcastle Disease vaccination, another parameter observed is the weight percentage of the lymphoid organs (Bursa of Fabricius and Spleen) to find out that the herbs with immunostimulatory activity do not cause lymphoid organs to work excessively does not cause the lymphoid organs to work excessively. Based on the results of this study, it was concluded that giving Cherry leaf teabag (*Muntingia calabura* L.) every two days had almost the same effect in increasing broiler immunity as giving every day, and giving Cherry leaf teabag (*Muntingia calabura* L.) every day also does not have a negative effect on the lymphoid organs which are responsible for immunity

Keywords: Broiler, Bursa of Fabricius, Different time, *Muntingia calabura* L.

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INTRODUCTION

On modern poultry farms, broiler are faced with stressful conditions including limited movement, long light periods, and poor air quality which can have a negative impact on the immune system (Hofmann *et al.*, 2020). With the ban on the use of AGP in the poultry industry (Abreu *et al.*, 2023, Attia *et al.*, 2023), it is necessary to study the role of herbs as an alternative ingredient to stimulate the broiler immune system (Haniarti *et al.*, 2019). In recent years, the use of herbs in the poultry industry has increased. Various research results have explored herbs such as rosella (*Hibiscus sabdariffa*) (Putri *et al.*, 2014), red ginger (*Zingiber Officinale*), Brotowali (*Tinospora crispa*) (Shaffira *et al.*, 2023), turmeric (*Curcuma longa*) (Nurhayati, *et al.* 2014), and temulawak (*Curcuma xanthorrhiza*) (Candra *et al.*, 2014), which contains substances as antioxidants, antiseptics, anti-inflammatories and immunoregulators which have the potential to increase the broiler productivity and immunity. Phytochemical compounds derived from herbs have been explored and widely used as alternative feed additives to replace growth-promoting antibiotics in broilers (Rafeeq, *et al.*, 2023).

Cherry (*Muntingia calabura* L.) is a plant that is easy to cultivate and is often found throughout the year, but its use has not been fully explored maximum (Fitri *et al.*, 2017). Sari *et al.* (2020) explained that cherry leaves contain various bioactive compounds such as flavonoid compounds, triterpenoids, saponins and polyphenols which show antimicrobial and antioxidant activity. Saponins are natural glycosides bound to steroidal alkaloids or triterpenes, which have pharmacological effects as immunomodulators (Panda *et al.*, 2023). The results of Pratiwi's study showed that administration of cherry leaves using the teabag method at



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a dose of 420 cmg/KgBB had an effect as an immunomodulator for broilers after the Newcastle Disease (ND) vaccination (Pratiwi *et al.* , 2020). In farm, administering vitamins or medicines that are not given through feed will be quite time consuming, and the limited costs for medicines in broiler rearing period must be a consideration in using herbs as alternative. The aim of this study was to analyze the different time administration of cherry leaf teabag (*Muntingia calabura L.*) to broiler immunity.

Materials and Methods

2.1 Research Procedure

Preparation of cherry leaf teabag

The cherry leaves used in this research are dark green cherry leaves. Cherry leaves that have been collected, selected, then dried for 2-4 days until the water content is <10%. The dried cherry leaves were ground using a blender then sieved using a 30 mesh sieve. Finally, the fine cherry leaves are put into a teabag according to the dosage given.

Research Design

Sixty broilers strain MB 202 unsexed were reared for 28 days and given feed and water ad libitum. The feed used was BR1 and BR2 commercial feed from PT. Japfa Comfeed. The broilers divided into two groups. The first group (T1) was given cherry leaves teabags (*Muntingia calabura L.*) every day in drinking water (T1), and the second group (T2) was given cherry leaves teabag (*Muntingia calabura L.*) every two days. Cherry leaf teabag given in the morning between 06:00 – 07:00 WIB from days 6th until 29th. The dosage of cherry leaves used in this study 420 mg/kg BW refers to previous studies by (Pratiwi *et al.*, 2020).

2.2 Observed parameters

The parameters observed in this study were Newcastle Disease antibody titers and the percentage of lymphoid organ weights (Bursa of Fabricius and Spleen).

2.2.1 Antibody titer

To determine the effect of different times administration of Cherry leaf teabag on the broiler immunity, the parameter observed was the ND antibody titer. The first ND vaccine was given at the hatchery using the Lasota ND vaccine. The second ND vaccine was given when the chicken was 18 days old using ND Hitchener B1. Broiler antibody titers were evaluated based on the Hemagglutination Inhibition (HI) test. Blood sampling was carried out on days 3rd, 9th, 13th, 17th, 21st, 25th, and 29th. Periodic serum collection can be seen in Figure 1.

Blood sampling was carried out through the vectoral vein located on the wing using a syringe of ± 2 cc . Blood is immediately put into an EDTA (Ethylene Diamine Tetraacetic Acid) tube to prevent the blood clotting process. The serum that has separated from the blood plasma is put into a micro tube. Then the micro tube containing the serum is put into the cooling box. The serum is then taken to the laboratory for analysis.

2.2.2 Lymphoid organ weight percentage

Another parameter observed in this study is the weight percentage of the lymphoid organs (Bursa of Fabricius and Spleen). This parameter observed to determine that the material does not cause the lymphoid organs to work excessively. The lymphoid organ weight percentage calculated using the formula:

$$\text{Lymphoid organ weight (\%)} = \frac{\text{Lymphoid organ weight}}{\text{live weight}} \times 100 \%$$

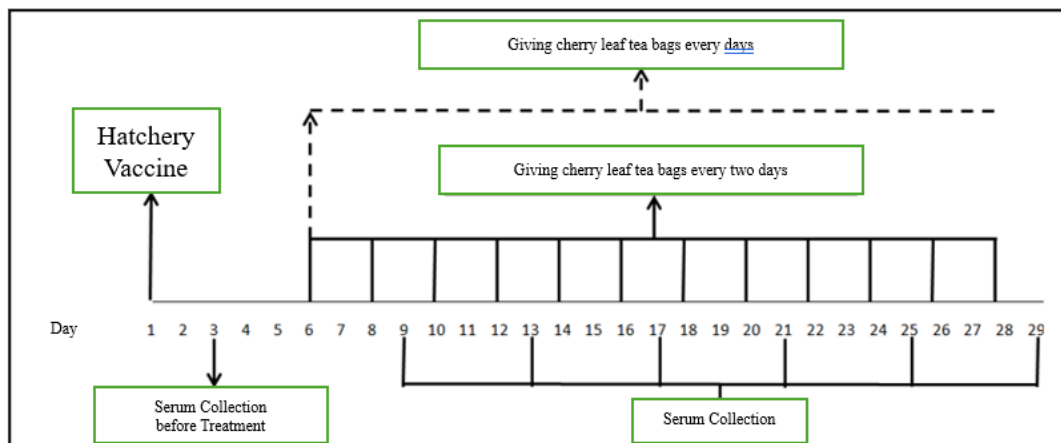


Figure 1. Treatment, sample collection and vaccine administration in broilers.

2.3 Analysis of Data

Paired samples t-test was used to compare the results of the two treatment groups (Stell and Torrie, 1995) with formula

$$t_{test} = \frac{x_1 - x_2}{\sqrt{\frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{n_1+n_2-2} \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

Results and Discussion

3.1 Antibody titer

This study was conducted to analyze the broiler immunity which given cherry leaf teabag (*Muntingia calabura L.*) at different times administration. This study used 60 broilers MB 202 strain which divided into 2 treatment groups. The first group (T1) was given cherry leaves teabags (*Muntingia calabura L.*) every day in drinking water. and the second group (T2) was given cherry leaf teabag (*Muntingia calabura L.*) every two days. Cherry leaf teabag start given on 6th day. To determine the effect of different time administration of cherry leaf teabag on the broiler immunity, the parameter observed was the antibody titer in response to ND vaccination. The first blood collection was carried out through the heart on day 3rd to determine the antibody titer of broiler before treatment. Next, blood samples were taken on days 9th, 13th, 17th, 21st, 25th via the branchial vein. The Hemagglutination Inhibition (HI) test is used to determine broiler antibody titers. The broiler antibody titers during study can be seen in Figure 2.

Based on Figure 2, it can be seen that the broiler antibody titer on day 3 (before administration of Cherry leaf teabag) in the both not different were 26.75 and 25.75 respectively. Cherry leaf teabag were given on the 6th day. The antibody titer decreased to 25,17 on the 13th day but still at the protective level. Based on the assessment standard of Geometry Mean Titre (GMT) in the Hemagglutination Inhibition test, the accumulated titer HI log 24 is defined as protective against ND (OIE, 2012). The antibody titer on 17th day in broiler given cherry leaf teabag was 23.33, while in another broiler it was 23.40. On the 17th day, the antibody titer against ND was already in a non-protective level. ND antibody titer < HI log 24 is defined as a condition that is nonprotective against ND (OIE, 2012) so revaccination is necessary (Oberländer *et al.*, 2020). In this study, ND revaccination was carried out on day 18. Administration second ND vaccine on day 18 significantly increased circulating antibody levels (Giambone *et al.*, 1986). On 21st and 25th day or 3rd – 7th day after vaccination, the broiler antibody titer continued to decrease with a titer of 22.5 (daily cherry leaf teabags) and 21.83 (cherry leaf teabags every two days) on 21st and the antibody titer was 22.83 and 22.20 in each group on 25th day. On 29th or 11th day postvaccination, the antibody titer for each group was

22.67 and 22.33. This shown that there was an increase in ND antibody titers in response to vaccination. Based on HI titer, ND antibodies were detected on 12th day after antigen injection, although the antibody titer in each group is still low. The antibody titer increased after second antigen (Putri *et al.*, 2018; Putri *et al.* 2022).

Furthermore, the HI test results from the two groups were followed by a paired sample t-test to know the treatment effect on the two groups. Based on statistical results, it is known that the antibody titers in the two groups were not significantly different. This indicate that giving cherry leaf teabag every day and every two day did not affect the antibody titer of broiler against ND until the 29th day.

In this study, based on the results of antibody titers from 3rd to 29th day, a regression analysis was carried out to predict the next development of antibody titers. Based on the results of the regression analysis, protective antibody titer will reach on 41st day for broiler giving Cherry leaf teabag every day and another broiler reached protective antibody titer on 45th day. It was found that daily administration reached the protective titer faster than the another. Based on these results, it is recommended that Cherry leaf teabag can be used to increase broiler immunity by administering it every two days.

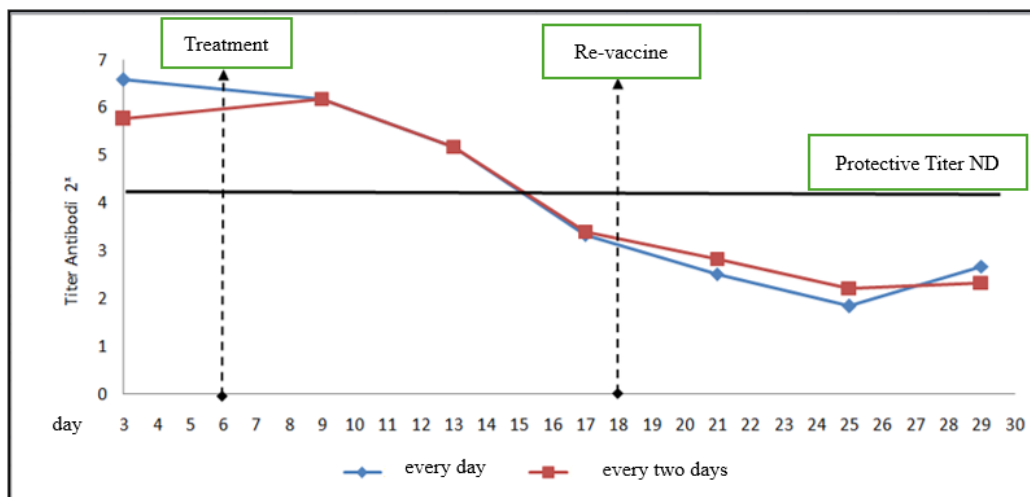


Figure 2. Broiler antibody titers

3.2 Percentage by Weight of Bursa of Fabricius

The Bursa of Fabricius is the primary lymphoid organ, and is responsible for the establishment and maintenance of B-cell compartment in avian species (Härtle and Ratcliffe, 2014). The normal percentage weight of Bursa of Fabricius in broiler ranges 0.12% - 0.29% (Ressang, 1998). The average weight percentage of the Bursa of Fabricius in this study can be seen in Figure 3.

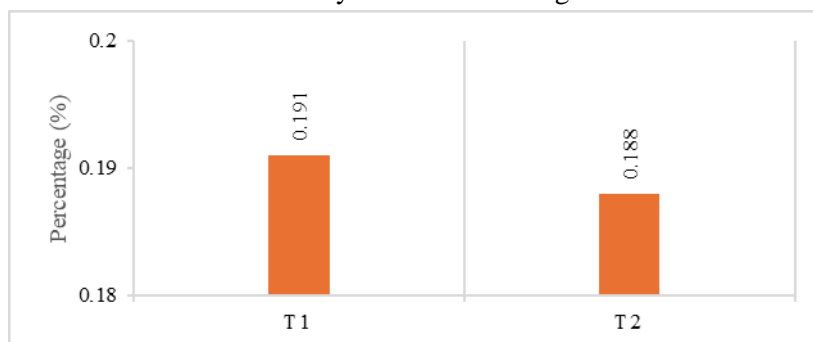


Figure 3. The average weight percentage of the Bursa of Fabricius

Based on the figure 3, it shown that cherry leaf teabags (*Muntingia calabura L.*) at different times (every day and every two days), did not affect the working mechanism of the Bursa of Fabricius organ which was created by presenting the weight of the Bursa of Fabricius which was still in a normal condition. normal range.

3.3 Spleen weight Percentage

The Spleen is the largest secondary lymphoid organ in the body and as such hosts a wide range of immunological functions alongside its roles in hematopoiesis and red blood cell clearance (Lewis *et al.*, 2019). The average weight percentage of Spleen can be seen in Figure 4.

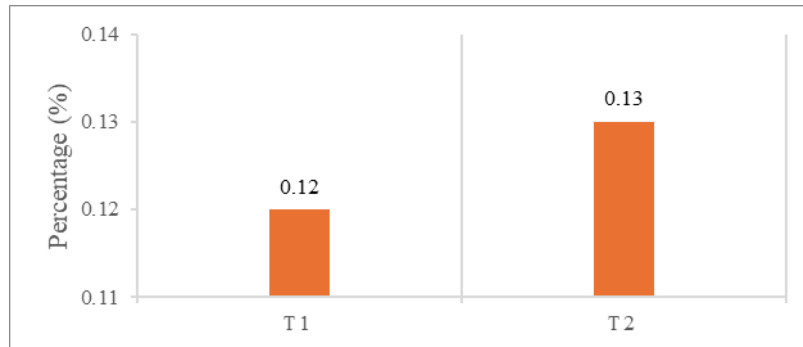


Figure 4. The average weight percentage of Spleen

Figure 4 shown the average weight percentage of Spleen in the broiler given Cherry leaf teabag every day 0.12% and another 0.13%. The weight percentage of Spleen is still in the normal range. The normal weight percentage of Spleen 0.11% - 0.23% (Ressang, 1998). This result indicated that Giving cherry leaf teabag (*Muntingia calabura L.*) in drinking water at different times (every day or every two days), did not affect the working mechanism of the Spleen. Based on the statistical analysis (paired sample t-test) showed that the different time of giving cherry leaf tea bags (*Muntingia calabura L.*) had no effect on the weight percentage of Spleen.

Conclusion

Based on the results of this study, it was concluded that giving Cherry leaf teabag (*Muntingia calabura L.*) every two days had almost the same effect in increasing broiler immunity as giving them every day and giving Cherry leaf teabag (*Muntingia calabura L.*) every day also does not have a negative effect on the lymphoid organs which are responsible for immunity.

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