Vol. 10(1): 114-123, March 2022 DOI: https://dx.doi.org/10.23960/jipt. v10i1.p114-123

Jurnal Ilmiah Peternakan Terpadu

Journal homepage: https://jurnal.fp.unila.ac.id/index.php/JIPT

p-ISSN: 2303-1956
e-ISSN: 2614-0497

Suplementasi Tepung Daun Sambiloto (*Andrographis paniculata*) Melalui Air Minum Ayam Broiler Terhadap Titer Antibodi *Newcastle Disease, Avian Influenza,* dan *Infectious Bursal Disease*

Supplementation of Sambiloto (Andrographis paniculata) Leaf Flour through Broiler Drinking Water against Antibody Titers of Newcastle Disease, Avian Influenza, and Infectious Bursal Disease

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ARTICLE HISTORY:
Submitted:4 February 2022
Accepted: 15 March 2022

KATA KUNCI:
Avian Influenza
Broiler
Infectious Bursal Disease
Newcastke Disease
Sambiloto (*Andrographis paniculata*)

ABSTRAK

Tujuan penelitian ini 1) mengetahui pengaruh suplementasi tepung daun Sambiloto (*Andrographis paniculata*) terhadap peningkatan titer antibodi *Newcastle Disease*, *Avian Influenza*, *Infectious Bursal Disease*; dan 2) mengetahui dosis terbaik suplementasi tepung daun Sambiloto terbaik terhadap peningkatan ketiga titer antibodi tersebut. Penelitian dilaksanakan di unit kandang broiler Laboratorium Lapang Terpadu Fakultas Pertanian Universitas Lampung menggunakan metode percobaan Rancangan Acak Lengkap 5 perlakuan 5 ulangan dan tiap ulangan 5 ekor broiler Cobb CP 707 strain, total 125 ekor broiler. Lima perlakuan dengan dosis berbeda yaitu air minum tanpa suplementasi tepung daun Sambiloto (P0); air minum dengan tepung daun Sambiloto 3 mg/kg BB (P1); 6 mg/kg BB (P2); 12 mg/kg BB (P3); 24 mg/kg BB (P4). Serum darah broiler umur 26 hari diperoleh dari vena brachialis menggunakan *disposable syringe* 3 mL. Pengujian titer antibodi ND dan AI dengan uji HI dan titer antibodi IBD dengan uji *indirect* ELISA di Laboratorium Vaksindo Agri Lab. Data dianalisis dengan analisis sidik ragam satu arah dengan uji lanjut Tukey menggunakan perangkat lunak SPSS 24. Hasil penelitian bahwa suplementasi tepung daun Sambiloto dalam air minum broiler tidak berpengaruh secara signifikan (P>0,05) terhadap titer antibodi ND dan AI, sedangkan terhadap titer antibodi IBD memberikan pengaruh signifikan (P≤0,05). Kesimpulan penelitian ini 1) suplementasi tepung daun Sambiloto pada air minum broiler mampu meningkatkan titer antibodi *Infectious Bursal Disease*; dan 2) dosis terbaik suplementasi tepung daun Sambiloto pada air minum broiler adalah 12 mg/kg BB broiler.

ABSTRACT

The aims of this study were 1) to determine the effect of Sambiloto (Andrographis paniculata) leaf flour supplementation to increase

KEYWORDS:
Avian Influenza
Broiler
Infectious Bursal Disease
Newcastke Disease
Sambiloto (*Andrographis paniculata*)

the antibody titer for Newcastle Disease, Avian Influenza, Infectious Bursal Disease; and 2) to determine the best dose of Sambiloto leaf flour supplementation to increase the three antibody titers. The research was carried out in the broiler cage unit of the Integrated Field Laboratory, Faculty of Agriculture, University of Lampung using a completely randomized design experiment with 5 treatments 5 replications and each replication 5 broilers Cobb CP 707 strain, a total of 125 broilers. All broilers were treated with ND, AI, IBD vaccination. Five treatments with different doses, namely drinking water without supplementation with Sambiloto leaf flour (P0); drinking water with Sambiloto leaf flour 3 mg/kg BW (P1); 6 mg/kg BW (P2); 12 mg/kg BW (P3); 24 mg/kg BW (P4). Broiler blood serum aged 26 days was obtained from the brachial vein using a 3-mL disposable syringe. Testing for ND and AI antibody titers with HI test and IBD antibody titer with indirect ELISA test at Vaksindo Agri Lab Laboratory. Data analysis with one-way analysis of variance with Tukey's further test using SPSS 24 software. The results showed that the supplementation of Sambiloto leaf flour in broiler drinking water had no significant effect (P>0.05) on ND and AI antibody titers, while on antibody titers. IBD had a significant effect (P≤0.05). The conclusions of this study were 1) the supplementation of Sambiloto leaf flour in broiler drinking water was able to increase the Infectious Bursal Disease antibody titer; and 2) the best dose of Sambiloto leaf flour supplementation in broiler drinking water is 12 mg/kg broiler body weight.

1. Introduction

The gross income per capita of the Indonesian population in the 2015-2019 period has increased. In 2015 the gross per capita income was Rp. 45,000,000.12 and experienced an increase in 2019 of 31.1% with a gross per capita income of Rp. 59,000,000.10 (BPS, 2020). This also affects the consumption of animal protein in Indonesian society. Broiler chicken is a source of protein originating from poultry, which plays an important role in meeting the needs of animal protein for the people of Indonesia.

Broilers have a weakness that is susceptible to disease, especially viral diseases such as Newcastle Disease (ND), Avian Influenza (AI), and Infectious Bursal Disease (IBD). These three diseases are diseases that are very dangerous for poultry because they cause repeated outbreaks and can cause the same clinical symptoms and pathological lesions in poultry with high morbidity and mortality rates and cause substantial economic losses for the poultry industry.

Newcaste disease (ND) is a disease whose main cause comes from a virus that belongs to the virulent strain of Avian Paramyxovirus type 1 (APMV-1) of the Avulavirus genus, subfamily Paramyxovirinae, family Paramyxoviridae. The Paramyxoviruses were

separated from avian species and classified serologically and then analyzed for phylogenetic analysis into ten subtypes, namely APMV-1 to APMV-10. Clinical symptoms caused in chickens infected with ND virus are grouped into five pathotypes, namely viscerotropic velogenic, highly pathogenic with a high mortality rate and hemorrhagic lesions in the intestines; neurotropic velogenic, can cause a high mortality rate, respiratory and neurological symptoms; mesogenic, with low mortality, respiratory and neurological symptoms; lentogenic, with mild clinical signs from the respiratory tract; and asymptomatic enteritic, with a subclinical form of enteric infection (OIE, 2012).

Avian Influenza is an infectious disease from poultry to other birds. In addition, this disease can also be transmitted to humans or zoonoses. Generally, cases of AI infection that are transmitted to humans are caused by contact between poultry farms and objects infected with AI. The origin that causes the AI virus is thought to come from migratory birds and the transfer of infected birds. The Avian influenza virus strain that attacks poultry originating from Southeast Asia is thought to have grown in Southern China before early 1997 and is a new strain of H5N1 that poses a pathogenic risk, thus gaining worldwide attention and unexpectedly spreading across class lines (Perkins and Swayne, 2003), thereby causing transmission from birds to mammals (pigs, cats, and humans). The AI virus spread to Indonesia since August 2003 and then the government officially announced it in January 2004. Poultry infected with the AI virus include broilers, ducks, laying hens, and quail. Basically, type A of AI virus is not transmitted to humans, but certain subtypes such as H5N1, H7N7 which are very pathogenic so that they can be transmitted to humans which can cause death. The transmission system that occurs in AI viruses originating from poultry commodities to humans can be through direct or indirect contact from sick birds through media such as air, fistula, saliva, and livestock equipment that has been contaminated with the AI virus.

Infectious Bursal Disease (IBD) or Gumboro disease is a disease that first occurred in Gumboro Deaware Village, the United States of America in 1957. Infectious Bursal Disease is caused by a virus from the Birnaviridae family, which is a double RNA virus that forms 2 segments and attacks the bursa of Fabricius so that chickens susceptible to disease. Infectious Bursal Disease is contagious and acute so it can cause high rates of motility and morbidity and have an impact on economic losses. Chickens that have been infected by the virus will be very susceptible to infection by other secondary diseases and

result in the failure of the vaccination process (Lukert and Saif, 2003). This disease was first discovered in Indonesia in 1983 which attacked poultry in the Sawangan Bogor area (Partadiredja et al., 1983).

Disease cases in poultry caused by viruses can be prevented by increasing antibody titers in broilers. The antibody titer can be increased by giving additional ingredients to poultry as immune system stimulants or known as immunomodulators. The author aims to determine the effect of Sambiloto leaf flour supplementation (*Andrographis paniculata*) as an immunomodulator against antibody titers of Avian Influenza, Infectious Bursal Disease and Newcastle Disease in broilers to assist farmers in preventing these three diseases and can be used as a reference in giving the best dose of Sambiloto leaf flour supplementation (*Andrographis paniculata*) so that it can help improve the welfare of farmers through improving broiler health.

2. Materials and Methods

2.1. Materials

The study was carried out in broiler cages at the Integrated Field Laboratory, Faculty of Agriculture, University of Lampung using the experimental method Completely Randomized Design (CRD) using 5 treatments which were repeated 5 times, each replication consisting of 5 broilers of the Cobb CP 707 strain, for a total of 125 broilers. The equipment used in this study were cages, bamboo (as a plot of 25 cages), newspapers and husks (as litter), plastic tarpaulin (as curtains), 15 watt lamp (heat source), hanging feeder and chick feeder tray, manual drinking holder, hand sprayer, digital scale, analytical scale, thermohygrometer, raffiaa rope, sack, plastic, disposable syringe, eppendorf tube, scissors, knife, micromixer, microplate, stationery and paper. While the materials used for this study included day-old chicks Cobb strain CP 707, commercial rations, water, Sambiloto (*Andrographis paniculata*) leaf flour, ND and AI killed vaccine (Medivac ND-AI®), live ND vaccine (Medivac ND Clone®), IBD vaccine (Medivac Gumboro A®), chorion allantois fluid, antisera (AI, ND, RBC 1%), isotonic PBS.

2.2. Methods

Sambiloto (*Andrographis paniculata)* leaf in flour form. Sambiloto leaf flour was produced by drying the bitter leaves under the sun to dry, then the dried leaves were ground into a mortar and crushed using a blender to flour form. Sambiloto leaf flour was dissolved in broiler drinking water by mixing the flour and drinking water using a blender. Supplementation of Sambiloto (*Andrographis paniculata*) was added to drinking water as an immunomodulator with a dose according to body weight with the following treatment:

P0 : drinking water without Sambiloto (*Andrographis paniculata*)
P1 : drinking water + 3 mg Sambiloto (*Andrographis paniculata*)/kg BW
P2 : drinking water + 6 mg Sambiloto (*Andrographis paniculata*)/kg BW
P3 : drinking water + 12 mg Sambiloto (*Andrographis paniculata*)/kg BW
P4 : drinking water + 24 mg Sambiloto (*Andrographis paniculata*)/kg BW

A Day Old Chick (DOC) were included in the brooding area within 1 day, DOC is given a drink with a mixture of sugar as an electrolyte. At 07.00, the chickens were weighed in order to obtain data in calculating the dosage of Sambiloto (*Andrographis paniculata*) supplementation according to the treatment. Before giving treatment, broilers were fasted with drinking water for 1 hour. Provision of rations ad libitum, supplementation was done by dissolving Sambiloto (*Andrographis paniculata*) leaf flour into broiler daily drinking water. The lights are turned on from 05.30 PM to 06.00 AM, the humidity of the cage is measured three times a day, namely at 07.00 AM, 12.00 AM, and 05.00 AM measured by a thermohygrometer.

The vaccines given consisted of AI, IBD, and ND vaccines. The ND and AI vaccines were given subcutaneously, live ND vaccine was given by eye drops when broilers are 7 days old, IBD vaccine was given when broilers are 14 days old by mouth drops, live ND repeat vaccine was given when broilers are 21 days old by eye drops.

Blood samples were taken when the broilers were 26 days old by taking 1 sample per experimental plot, the sample was taken from the brachial vein using a 3 ml disposable syringe, the blood sample was placed at room temperature for 1-2 hours and then placed in a container at a temperature of 40°C for 18-24 hours until a yellow blood serum is formed, then the blood serum was sent to the Agri Lab Vaccination Laboratory to be

analyzed for ND, AI and IBD antibody titers using the Hemagglutination Inhibition test. The ND, AI, and IBD antibody titer profile data were compiled using a tabulation system so that the available data was then processed by statistical analysis using one-way analysis of variance (One Way ANOVA) at 5% level using software the SPSS (Statistical Package for Social Science) version 24 and further tested using the Tukey test.

3. Results and Discussion

The results of the analysis test and standard deviation of antibody titers based on the study of Sambiloto leaf flour supplementation (*Andrographis paniculata*) through drinking water of broilers against antibody titers of Newcastle Disease, Avian Influenza, Infectious Bursal Disease were presented in Table 1.

Table 1. Test results of antibody titer supplementation of Sambiloto (*Andrographis paniculata*) in broiler drinking water against Avian Influenza, Infectious Bursal Disease, Newcastle Disease

Treatment	Antibody Titer (Log X)		
	AI	IBD	ND
P0	18.40 ± 13.16	117.00 ± 84.45 [a]	41.60 ± 21.47
P1	16.80 ± 9.96	340.20 ± 136.17 [ab]	33.60 ± 27.94
P2	14.40 ± 11.52	204.20 ± 144.22 [ab]	20.80 ± 24.40
P3	20.80 ± 10.73	505.40 ± 277.20 [b]	25.60 ± 8.76
P4	11.20 ± 11.80	196.80 ± 204.93 [ab]	24.00 ± 22.63

Information :
P0 = drinking water without Sambiloto (*Andrographis paniculata*) leaf flour, P1 = drinking water + 3 mg Sambiloto leaf flour/kg BW, P2 = drinking water + 6 mg Sambiloto leaf flour/kg BW, P3 = drinking water + 12 mg Sambiloto leaf flour/kg BW, P4 = drinking water + 24 mg Sambiloto leaf flour/kg BW. AI = Avian Influenza, IBD = Infectious Bursal Disease, ND = Newcastle Disease. [a, b, ab] Differences in superscripts with letters in the same column showed significant differences in each antibody titer test ($P<0.05$). Result of analysis : Vaksindo Agri Lab (2021)

Based on the results of the one way analysis of variance and then the data processed using the statistical application of SPSS (Statistical Package for Social Science) version 24, it was found that the supplementation of Sambiloto (*Andrographis paniculata*) leaf flour in broiler drinking water in various treatments (Table 1) had no significant effect on ND and AI antibody titers, while the IBD antibody titer had a significant effect ($P\leq0.05$) then Tukey's further test showed that the best dose of Sambiloto (*Andrographis paniculata*) leaf flour supplementation through broiler drinking water at a dose of 12 mg/kg broiler body weight.

According to the Office International des Epizootic (2008), if the antibody titer HI test value shows a minimum value of log 24 or log 16, then the antibody titer is considered to have a protective effect against ND. The antibody titer value can indicate the ability of antibodies to protect the body against virus attacks, so if the antibody titer is low, the antibody has not been able to protect the body. Antibody titer is considered protective against AI virus if it shows an antibody titer value of at least log 16. According to the Agri Lab Vaccination Laboratory standard (2021) that antibody titer is considered to have a protective effect against AI at least log 32, against ND at minimum log 32 and against IBD at least log 400. According to Tizard (1987), the bursa fabricius in the lymphoid organ acts as a site for maturation and differentiation of antibody-forming cells in broilers. If the development of the bursa fabricius is inhibited due to infection with IBD, it can reduce broiler antibody titers.

Sambiloto is a type of medicinal plant that can be used as an immunomodulator in addition to noni, *Echinacea purpurea*, meniran and ginger. Compounds belonging to the flavonoid group, curcumin, catechins, limonoids, vitamin C and vitamin E have good enough potential to increase the activity of the immune system in the body (Sylviatullatviya, 2015). Sambiloto also contains andrographolide, eoxyandrographolide, 14-deoxy-11, neoandrographolide, 12-didehydroandrographolide, diterpenoids, flavonoids, and homoandrographolide compounds (Muhlisah, 2006).

One of the active compounds in Sambiloto (*Andrographis paniculata*) is andrographolide. Andrographolide belongs to the trihydroxylactone group with the molecular formula $C_{20}H_{30}O_5$ and is the main component of the Sambiloto plant which is easily soluble in methanol, pyridine, ethanol, acetone and acetic acid, but slightly soluble in water and ether. The physical properties of andrographolide are having a melting point of 228-230°C, ultraviolet cell in ethanol a maximum of 223nm (Kumoro, 2007). In accordance with research Sumaryono (2002) stated that Sambiloto plants contain andrographolide compounds that can improve the performance of the body's defense system which triggers white blood cells to fight bacteria and other antigens, flavonoids which act as anti-inflammatory, and tannins which act as antidiarrheal. Muhlisah (2006) stated that andrographolide can act as an immunomodulator that acts as an immunostimulant by increasing the production of peripheral blood mononuclear cells, tumor necrosis factor (TNF)-α, interferon (IFN)-α, and (IFN)-γ, and phagocytic activity.

Macrophages that are able to increase the activity of the immune system in the body. In Alkandahri's research (2018) that andrographolide can act as an immunostimulant on specific and non-specific immune functions through NK cells, macrophages, and cell cytokine induction. Kapil et al. (2003) added that Sambiloto is able to act as a strong antioxidant because it contains glucoside compounds derived from andrographoside and neoandrographolide.

Research conducted by Wiedosari (2007) that the administration of a Sambiloto herbal extract formula combined with red ginger and temu ireng in broilers infected with *Eimeria tenella* which also uses water as a solvent can increase the immune system by producing leukocytes. The administration of Sambiloto at different doses using a coccidiostat (sulfa preparation) was able to increase the heterophile in broiler blood (Cahyaningsih et al., 2003). Heterophiles are part of the immune system that acts to destroy foreign objects that enter the body (Tizard, 2017). Sambiloto supplementation can increase the value of heterophile, which is thought to be closely related to the dual function in Sambiloto which can be an immunosuppressant as well as an immunostimulant (Puri et al., 2013). The increase in the percentage of blood heterophile values after giving Sambiloto was due to the performance of Sambiloto which acts as an immunostimulant (Puri et al., 1993). Sambiloto can stimulate the formation of phagocytic cells derived from the formation of non-specific and specific immune systems. The formation of phagocytic cells comes from the interaction of specific antigens that are able to produce B lymphocytes on a large scale and then produce antibodies in the form of plasma glycoproteins that are able to bind to antigens and stimulate the phagocytosis process on foreign objects (Decker, 2000).

Sambiloto functions as an immunostimulator that will increase the immune response to the body's resistance (Wurlina, 2017), namely the presence of flavonoids that are able to send intracellular signals to cell receptors to increase their activity when the activity of the immune system decreases (Khumairoh et al., 2013). Previous studies have shown that the content of alkanoid compounds found in Sambiloto has a substitute role for IFNγ which maintains immunity by increasing non-specific immune responses in leukocyte cells and specific immune responses in macrophage cells in carrying out their function as phagocytic cells that attack infectious agents in the body (Mayer, 2009; Wurlina, 2017; Xu, 2009).

The flavonoid content in Sambiloto can increase the proliferation of lymphocyte cells and can increase the production of IL-2 (Jiao et al., 1999). Middleton et al. (2000) suggested that the proliferation of lymphocytes, especially T lymphocytes, which are responded to by antigens, is regulated by the effect of IL-2 on receptors on the surface of IL-2. The differentiation of B and NK cells and their proliferation are also stimulated by IL-2. Flavonoids besides being used as immunostimulants also have immunosuppressant effects. The cytotoxic effect and immunosuppressant effect allowed there to be no difference between the effect of the treatment and the control group.

4. Conclusion

Conclusions of this research were :

1) Supplementation of Sambiloto (*Andrographis paniculata*) leaf flour in broiler drinking water could increase antibody titers against Infectious Bursal Disease.
2) The best dose of Sambiloto (*Andrographis paniculata*) leaf flour supplementation in broiler drinking water was 12 mg/kg broiler body weight.

Acknowledgments

The authors would like to thank the Directorate of Learning and Student Affairs Directorate General of Higher Education Ministry of Education and Culture of the Republic of Indonesia, for funding the Exact Research Student Creativity Program (PKM-RE) 2021 as stated in the announcement of the 2021 PKM Funding Number: 1949/E2/KM.05.01 /2021.

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