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Study of Synergistic Effects of Herbal Meniran (*Phyllanthus Niruri* L) and Sambiloto (*Andrographis Paniculata*) as A Substitute Commercial Additive Feed to Improve Immunity Response and Free Chicken Egg Production

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Abstract: This study aims to look at the immune response and egg production of free-range chickens after ND vaccination by administering meniran and Sambiloto extracts. The design used was a completely randomized design. Data on egg production were analyzed using analysis of variance and data on antibody and lymphocyte titers were analyzed descriptively. The results showed that on D+7 after vaccination, there was an increase in ND antibody and lymphocyte titers and in vaccinated chickens the ND titer was in a protective condition (≥log26), whereas in non-vaccinated chickens, the ND titer and lymphocyte levels were in a less protective condition. On H+28 after the vaccine only the vaccine treatment showed a protective condition, but the administration of meniran and Sambilotol extracts was able to maintain the titer rate longer than without giving Meniran and Sambiloto extracts. Drinking water consumption (Sig.941), feed consumption (Sig.890), egg production (Sig.480) and feed conversion (sig.558) showed no difference. Based on the results of the study, the treatment of vaccines and extracts of meniran and Sambiloto gave a better immune response than without being vaccinated. Administration of meniran and Sambiloto extracts was able to maintain the level of immunity compared to no control.

Keywords: Bitter; Immune response, Meniran; ND vaccine

Introduction

In the field of chicken farming, vaccination is very important, because it is not uncommon for a large population of chickens to have outbreaks or disease infections. Outbreak of a disease generally causes significant losses. Vaccination in a farm has an important meaning because by vaccinating the body of the chicken it will produce antibodies which are useful for neutralizing germs that enter the body of the chicken. Cases of Newcastle disease (ND) globally rank fourth (Rehan et al., 2019). Therefore vaccination against ND disease must still be carried out in chicken farms. In general, commercial vaccines on the market have a fairly good effectiveness. As carried out by Khoury et al. (2021), they found that several commercial vaccines can produce good antibody titers.

Even though vaccination has been done to prevent disease, many of the vaccinations have not produced satisfactory results, so it is necessary to look for alternative efforts to prevent and control disease. Apart from that, vaccinating can also pose several risks, namely if it is not done aseptically, an infection can occur at the injection site. Another effort is the administration of antibiotics which can also pose several risks, one of which is that it can cause resistance. For this reason, it is necessary to take preventive measures in other ways. One way to improve the health condition of chickens is to provide natural feed additives (Ali et al., 2021). Natural feed additives that have the potential to replace commercial feed additives include medicinal plants. Medicinal plants are expected to provide maximum results and affect the immune system, so as to increase production (Gopalakrishnan et al., 2016). Medicinal

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plants that meet the above criteria are meniran (*Phyllanthus niruri* L) and Sambiloto (*Andrographis paniculata*).

Meniran is indicated to have the ability to maintain body resistance because it contains flavonoids which are able to increase the work of immune cells thereby increasing the immunity of chickens after vaccination. Besides, meniran also contains lignans, alkaloids, triterpenoids, fatty acids, vitamin C, potassium, resin, tannins and geranins. These plants are reported to exhibit various modes of biological activity in vivo and in vitro namely, antibacterial, antiviral, anti-inflammatory, anti HIV (Human immune deficiency virus), immune modulating and anticancer (Zulhendri et al., 2021).

Sambiloto contains Andrographolid lactones (bitter substances), diterpenes, glucosides & flavonoids which can boost immunity. According Hossain et al. (2021), the Andrographolide content in Sambiloto can interfere with the transfer of viral and bacterial genetic material so that it is effective against infectious agents. Sambiloto extract is known to have the potential to increase the phagocytic index value and increase the number of leukocytes. Sambiloto contains Andrographolide and flavonoids can increase lymphocyte proliferation and increase antibodies (Hidayat & Patricia Wulandari, 2022).

The results of previous studies showed that giving meniran water extract 30% mixed in broiler drinking water suppressed feed consumption with body weight gain and feed conversion not different from without giving meniran water extract. Subsequent research (Atun et al., 2023) showed that giving meniran ethanol extract 50 mg per liter of drinking water, was able to increase the immune response (ND titers, gumboro titers and lymphocytes) of free-range chickens and egg production. Khandia et al. (2020) showed that the ethanol extract of Phyllanthus niruri leaves as much as 10-300 mg/kg BW of broiler chickens increased lymphocytes. Research on the use of meniran and Sambiloto (Astuti & Irawati, 2022), showed that ND titers and lymphocyte levels of broiler chickens vaccinated and given meniran extract were higher than those given Sambiloto extract.

The immune system functions to maintain the integrity of the body as protection against harmful substances, including viruses, bacteria and fungi (Gombart et al., 2020). Improving the immune response of chickens is very important to do (Astuti & Irawati, 2022), because the higher the chicken immunity, the less likely the chickens will become sick, so that they can produce both meat and eggs optimally.

The effectiveness of the vaccination program can be seen from the improvement in the overall health status and productivity of the vaccinated population. The indicators are the level of mortality and morbidity, other parameters such as the Feed Conversion Ratio (FCR), achievement of body weight and uniformity, egg production and quality of eggs produced (Ruesga-Gutiérrez et al., 2022). The immunity of livestock can be seen from the antibody titers and the number of white blood cells.

This study aims to look at the immune response and egg production of free-range chickens after ND vaccination by administering meniran and Sambiloto extracts.

Method

The design used was a completely randomized design. Data on egg production were analyzed using analysis of variance and data on antibody and lymphocyte titers were analyzed descriptively.

The treatment applied was ND vaccination and administration of meniran and Sambiloto ethanol extracts as follows: Control 1 (T0): chickens vaccinated without giving the extract, Control 2 (T1): vaccinated chickens were given 25 mg of bacitracin per liter of drinking water, Treatment 3 (T2): unvaccinated chickens were given 50 mg of meniran extract per liter of drinking water, Treatment 4 (T3): vaccinated chickens were given 50 mg of meniran extract per liter of drinking water, Treatment 5 (T4): unvaccinated chickens were given 50 mg of Sambiloto extract per liter of drinking water, Treatment 6 (T5): vaccinated chickens were given 50 mg of Sambiloto extract per liter of drinking water, Treatment 7 (T6): unvaccinated chickens were given a mixture of 25 mg meniran extract and 25 Sambiloto per liter of drinking water, Treatment 8 (T7): vaccinated chickens were given a mixture of 25 mg meniran extract and Sambiloto per liter of drinking water. Parameters observed were ND antibody titers, lymphocyte levels, consumption of drinking water, feed consumption, egg production, and feed conversion.

Result and Discussion

Immune Response

ND Antibody Titers

The immune system functions to defend the body as protection against harmful substances including viruses, bacteria and fungi. The immune response produced by livestock is obtained after an infection occurs or after being given a vaccine. ND antibody titers and lymphocyte values. ND titers data are in Table 1 and lymphocyte values are in Table 2.

Table 1. ND Antibody Titer Serological Test Results

Treatment	H-0	H+7	H+14	H+21	H+28
Control	3.33	6.00	5.50	5.00	4.00
Bacitracin	3.00	5.50	5.50	5.25	4.25
Meniran	3.00	3.50	4.00	3.50	3.25
Vaccines+Meniran	3.00	6.00	5.75	5.25	5.00
Sambiloto	3.33	3.50	3.75	3.50	3.00
Vaccine+Sambiloto	3.00	6.00	5.75	5.25	5.00
Meniran+whileoto	3.00	3.00	3.50	3.75	4.00
Vaccines+(Meniran+Sambiloto)	3.33	6.00	5.75	5.50	5.00

Table 1 explains that before the chickens got the ND vaccine, antibodies had formed, but were not yet protective against ND. All treatment groups had an average antibody titer that was not significantly different between groups. Detectable antibodies at 25 weeks of age have an ND titer of about 3 (log2). An increase in antibody titers began to be seen on the 7th day after the chickens were vaccinated with ND or given meniran and Sambiloto extracts. An increase in antibody titers was seen in all treatments and the most protective (100%) were vaccines and meniran and Sambiloto extracts. Until the 14th day after the vaccine is still protective. On the 21st day, the ND antibody titer value decreased, all vaccine treatments were still in a protective condition, namely the log 25 number.

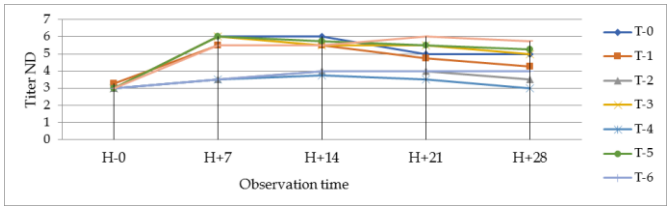

Figure 1. ND antibody titers of chickens that received vaccines and extracts of meniran and sambiloto

Meniran and Sambiloto extracts were able to maintain the titer until the 5th week (28th day after the vaccine). Whereas in the non-vaccine treatment, administration of meniran and Sambiloto extracts showed less protective ND titers (40% protection for titers <4). However, in the mixture of meniran and Sambiloto, there is an increase in titer, although not up to 100% protective. So even though the ND antibody titer is not protective, the chickens are still immune. Factors that affect the protective level of ND antibody titers include the quality of meniran and Sambiloto, and the process of making extracts. In addition, inaccurate blood sampling, the presence of other disease infections and inappropriate vaccination management can cause ND antibody titers in non-protective conditions. In this study, giving meniran and Sambiloto extracts was able to maintain immunity levels longer than without giving the extracts.

Chicken Blood Lymphocyte Value

The content of chicken blood lymphocytes is shown in Table 2. Lymphocytes are a type of white blood cell that can defend the body against infection. Lymphocytes are a superior type of leukocyte in the blood of poultry, including laying hens (Tsujii et al., 2017). Lymphocyte values in all treatments on H-0 showed non-protective numbers. The blood lymphocyte value of chickens at the age of one day was 15.9%, and at the age of 3 days was 38.7%. At the age of one week the lymphocyte value was 75, then decreased until the sixth week, the lymphocyte value showed 69. So that the lymphocyte value of chicken blood in all treatments was in accordance with the statement of (Saki et al., 2018).

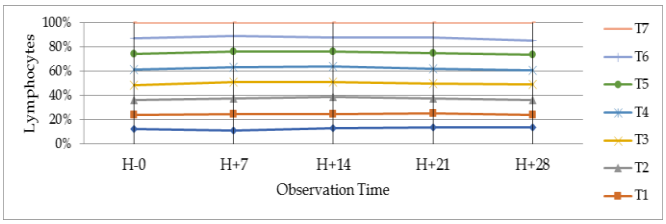

Figure 2. Chicken blood lymphocytes that were vaccinated and given meniran and sambiloto extracts conclusion

Table 2. The Value of Blood Lymphocytes in Free Range Chickens

Treatment	H-0	H+7	H+14	H+21	H+28
Control	63.50	74.00	82.00	85.33	82.00
Bacitracin	56.50	89.25	74.33	74.33	66.50
Meniran	63.00	87.33	86.00	77.50	72.33
Vaccines+Meniran	62.50	88.00	79.00	76.33	78.75
Sambiloto	65.00	81.67	82.00	75.50	74,00
Vaccine+Sambiloto	63.00	84.00	76.00	81.00	76.75
Meniran+whileoto	65.00	86.00	75.50	80.00	73.33
Vaccines+(Meniran+sambiloto)	64.50	71.50	73.50	76.67	88.00

After the 7th day of vaccination, the lymphocyte values increased in all vaccine treatments and entered the normal lymphocyte values, namely 45-70 with an average of 60 (Graeber et al., 2021). According to Pantaya et al. (2018) the blood lymphocyte content of normal laying hens is between 24-84%. The increase in lymphocytes occurs because of antigens that enter the body in order to form antibodies. The graph below shows that the treatment of the vaccine and Sambiloto meniran extract or a mixture of the two is almost the same range from H+7 to D+28.

Production of Domestic Chicken Eggs

Analysis of egg production including consumption of drinking water, feed consumption, egg production, and feed conversion can be seen in Table 3.

Table 3. Production of Domestic Chicken Laying Obtaining Meniran and Sambiloto Vaccines and Extracts

Parameter	T0	T1	T2	T3	T4	T5	T6	T7
Consumption of drinking water (ml/head/day)	251.50	243.80	243.70	239.67	251.57	252.73	238.30	242.87
Feed consumption (g/head/day)	76.13	76.63	75.33	74.34	76.87	76.20	71.70	76.45
Egg Production (%)	68.70	67.53	67.52	67.69	67.45	68.80	67.17	69.63
Feed conversion	2.55	2.72	2.62	2.69	2.69	2.68	2.83	2.62

Consumption of Drinking Water

Water is a nutrient whose level of availability is vital for free-range chickens. This is more due to the fact that most of the chicken's body consists of water in the body, water has a very important function for the process of metabolism of feed ingredients, absorption of nutrients, and transportation processes.

From Table 3 above, it is known that the average drinking water consumption of native chickens in this study was 245.52 ± 15.75 ml/head/day. The results of the analysis of variance showed that the drinking water consumption of native chickens treated with vaccines and meniran extract and Sambiloto extract was not significantly different (Sig .941). (McCrickerd & Forde, 2016) states that the taste of chicken plays a relatively small role in determining the amount of food/drink consumed. That chicken is less sensitive to taste, because it only has 24 taste buds, so consumption of drinking water is not affected by administration of meniran or bitter extracts.

Chickens should consume water in the range of 1.5-2 ml/gram of feed consumption. The water requirement for chickens at an ambient temperature of 25°C is twice the amount of feed, but at an ambient temperature of 30-32°C the water consumption can increase to 4 times the amount of feed consumption. In this study the consumption of drinking water reached 4 times the amount of feed consumption. The results of this study consume more drinking water. This is possible because of the temperature difference. Described by (Sudarman et al., 2012), in general, chickens consume drinking water twice the weight of the feed consumed. Meanwhile, according to (Orakpoghenor et al., 2021) at normal temperatures, drinking water consumption for chickens is 1.6-1.8 times that of feed consumption.

Feed Consumption

The average feed consumption is 75.30 ± 4.30 gram/head/day. The results of the analysis showed that feed consumption was not affected by administration of naksin and extracts of meniran and Sambiloto (Sig .890). Administering vaccines and extracts of meniran and Sambiloto increases ND titers. Meniran contains flavonoids which can increase the work of immune cells and Sambiloto contains andrographolid which has anti-bacterial properties, so the chickens become healthier (Rehman et al., 2021). Feed consumption is influenced by the genetic characteristics of chickens, temperature, feed quality, feeding system, feed frequency, and chicken health (Irwani et al., 2022). However, in this study the feed consumption was the same. Besides that, the quality of the feed in this study was the same for all treatments, so it did not affect feed consumption. This is in accordance with what was produced by (Roque et al., 2021).

Egg Production

Chicken egg production in the study was 68.06 ± 1.52% HDA. The results of the analysis showed that there was no difference between treatments (Sig.480). Giving vaccines and extracts of meniran and Sambiloto does not affect egg production. This explains that chickens that were vaccinated were given meniran and Sambiloto extracts and those that were not vaccinated did not affect egg production. Meniran contains terpenoids which can inhibit Escherichia coli and Staphylococcus aureus bacteria (Nahrul et al., 2022), while Sambiloto contains andrographolide as an anti-bacterial. In general, those who were vaccinated and given meniran and Sambiloto showed that chickens were more immune than those who were not vaccinated, chickens became healthier, digestion was better so that the feed consumed could be absorbed better. However, in this study the feed consumption was the same, so egg production was not different.

Feed Conversion

Feed conversion for chickens vaccinated and given meniran and Sambiloto extracts ranged from 2.55 to 2.83 with an average of 2.68 ± 0.15. Feed conversion for chickens vaccinated with meniran and Sambiloto extracts was no different from chickens that were not vaccinated (Sig.558). This feed conversion was in accordance with data on feed consumption and egg production. The results of research by Usturoi et al. (2023) on native chickens fed feed with a protein content of 18%, the feed conversion was 2.77. Feed conversion in this study was lower. This is possible due to differences in the amount of protein, in this study using feed with a protein content of 16%. Habiyah et al. (2015) on Arabic chickens with a ration containing 16% protein resulted in a feed conversion of 2.77. So that the feed conversion in this study is lower.

Conclusion

Based on the results of the study it can be concluded that: Chickens that were vaccinated and given both meniran and Sambiloto extracts gave a better immune response than those without the vaccine. Meniran and Sambiloto extracts were able to maintain their immunity level compared to those without the extracts. The egg production of chickens that were vaccinated and given Meniran and Sambiloto extracts was no different from chickens that were not vaccinat.

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Author Contribution

Conceptualization, Puji Astuti, Heru Suripta and Diwi Acita Irawati; methodology, Puji Astuti; validation, Heru Suripta, and Diwi Acita Irawati; formal analysis, Heru Suripta.; investigation, Puji Astuti and Diwi Acita Irawati; resources, Puji Astuti and Heru Suripta.; data curation, Heru Suripta: writing—original draft preparation, Diwi Acita Irawati and Heru Suripta; writing-review and editing, Diwi Acita Irawati: visualization, Heru Suripta and Diwi Acita Irawati; supervision, Heru Suripta; project administration, Puji Astuti.; funding acquisition, Diwi Acita Irawati and Puji Astuti. All authors have read and agreed to the published version of the manuscript.

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Conflicst of Interest

No Conflicts of interest.

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