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Original Article

Potency of green chiretta *(Andrographis paniculata)* leaf extract on antibody titers of laying chickens in the starter phase

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Abstract

Objective: Andrographolide, biological component, is present in Green chiretta (*Andrographis paniculata*), as an immunomodulator and feed additive for laying hens, has been potential to be explored. The objective of this study is to investigate the impact of green chiretta (*Andrographis paniculata*) leaf extract on the levels of antibodies against Avian Influenza (AI) and Newcastle Disease (ND) in laying hens during the initial phase of egg production.
Methods: A total of 100 Isa Brown strain laying hens were were used ini this study consisted of 20 box cages with 60x50x50 cm. The study employed a completely randomised design (CRD) with 5 treatments, i.e. T0 = Drinking water without EEGC, T1 = Drinking water + 0.5% EEGC, T3 = Drinking water + 1% EEGC, T4 = Drinking water + 1.5% EEGC, T5 = Drinking water + 2% EEGC. The study was replicated four times for each treatment.
Results: The analysis of variance revealed that the treatment with green chiretta leaf extract did not show a significant impact on AI and ND antibody titers at 3, 4, and 5 weeks of age. However, this treatment significant affect on AI and ND antibody titers at 6 weeks of age.
Conclusions: The addition of green chiretta (*Andrographis paniculata*) leaf extract to drinking water boosts up the development of AI and ND antibody levels. It exhibited superior antibody levels in comparison to the control treatment. To clarify, applying green chiretta (*Andrographis paniculata*) leaf extract to drinking water can maintain AI and ND antibody levels.

Keywords: Antibody titers; Bird flu; Laying hens; Newcastle disease; Green chiretta leaf extract

INTRIDUCTION

The laying hen farming business is a potential business to be developed because laying hens can produce high egg production and increase their genetic capabilities [1,2]. The enhanced genetic capacity of chickens renders them more susceptible to environmental factors and prone to infections from viral or bacterial sources. The prevalence of diseases in laying hens poses a significant challenge for the global farming industry, resulting in elevated mortality rates and substantial financial losses [3,4]. The most damaging diseases in poultry production are viral infections especially avian diseases such as Avian Influenza (AI) and Newcastle Disease (ND), as they have the potential to result in mortality rates approaching 100%.

The maintenance of laying hens is divided into 3 phases: the starter, grower, and layer. The starter phase starts from 1-day-old chickens (DOC) until 6-8 weeks of age [5,6]. The starter phase holds significant importance in terms of its influence on the subsequent phase. Inadequate management during this phase will have repercussions on the subsequent phase. During the starter phase, hens are subjected to extensive preparation in order to meet performance standards. However, this period also involves a rigors vaccination schedule and causes them to become susceptible to stress as a result of the vaccinations [7,8].

Stress that arises due to post-vaccination reactions has an impact on the non-optimal formation of antibody titers in chickens. Non-optimal antibodies make chickens vulnerable to disease and affect their growth. Therefore, immunomodulators are needed to boost the formation of chicken's antibody titers, natural herbal that have immunomodulatory activity, one of which is Green chiretta (*Andrographis paniculata*). Natural extracts have shown

positive effects on poultry production as prospective alternatives derived from natural sources do not pose toxicity risks and do not leave behind any residues [9,10].

Green chiretta (*Andrographis paniculata*) leaves contain bioactive substances such as tannins, saponins, flavonoids, andrographolide [11,12]. Andrographolide is an immunomodulator that enhances the activity of the immune system, particularly white blood cells, responsible for targeting and neutralising foreign substances entering the body [13]. Andrographolide compounds can increase the phagocytic activity of macrophage cells and become a natural anti-inflammatory agent that can stimulate specific and non-specific immune functions [14,15]. The bioactive content of andrographolide in Green chiretta (*Andrographis paniculata*) has the potential to be used as an immunomodulator and feed additive for laying hens. This study aimed to see the response of AI and ND antibody titers in starter phase laying hens fed with Green chiretta (*Andrographis paniculata*) leaf extract at 3, 4, 5, and 6 weeks.

MATERIAL AND METHODS

Preparation and extraction of green chiretta leaves

The process of producing Green chiretta leaf extract is performed by maceration. A total of 100 g of Green chiretta leaf powder was put into Erlenmeyer, and 100 ml of 96% ethanol (1:10) was added and allowed to stand for 24 hours [15]. The extract was then filtered to separate the pulp and filtrate. Furthermore, the filtrate was evaporated at 40°C to obtain Ethanol Extract of Green Chiretta (EEGC).

Experimental cage preparation

The tools used were box-type experimental cages measuring 60x50x50 cm, as many as 20 units. The cages were cleaned and sprayed with disinfectant before use. Each experimental cage plot was filled with 5 chickens. The heater used is a 25-watt bulb lamp placed in each treatment plot.

Treatment, vaccination, and research ration

One hundred Isa Brown strain laying hens aged 1-42 days were divided into 5 Green chiretta extract dosage treatments, each repeated 4 times. The treatment EEGC was conducted from 1 to 24 days old (3 days after the last post-vaccination) in the morning and evening. AI and ND vaccinations were conducted at 7 days of age, AI vaccination via subcutaneous and ND vaccination via eye drops. ND-IB vaccination was repeated at 21 days of age using the eye drop method. The ration used was commercial. The nutritional content of the starter phase laying hen ration can be seen in Table 1.

Table 1. Nutrient content of starter phase laying hens ration

Nutrition Contents	0-6 (starter)
Moisture	Max. 12%
Crude protein	Min. 18%
Crude fat	Min. 3%
Crude fiber	Max. 6%
Ash	Max. 8%
Calcium (Ca)	0.9% (0.9-1.2%)
Phosporus (P)	0.4% (>0.35)
Lysin	0.85% (>0.90%)
Metionin	0.30% (>0.40%)
Metabolism Energy	>2700 kcal/kg

Source : *NRC [29]

Sampling

At 3, 4, 5, and 6 weeks of age, blood samples were collected to test for AI and ND antibody titers. One blood sample was collected from a single experimental animal in each experimental plot. Specimens were collected using a 3 ml syringe via the brachial vein, extracting around 1-1.5 ml. The blood sample was allowed to equilibrate at ambient temperature until the blood cells and blood serum were isolated. Subsequently, the blood serum is placed into a microtube and appropriately labelled based on the specific treatment. Subsequently, the serum was examined to determine the quantity of antibody titers by the Hemagglutination Inhibition (HI) test, following the standard of World Organisation for Animal Health (OIE) [16,17].

Research design

The research employed a Completely Randomised Design (CRD). This design encompassed 5 distinct drinking water treatments, each containing varying amounts of EEGC. Additionally, each treatment was replicated 4 times. The drinking water treatments include: T1: Drinking water + 0% EEGC, T2: Drinking water + 0.5% EEGC, T3: Drinking water + 1% EEGC, T4: Drinking water + 1.5% EEGC, T5: Drinking water + 2% EEGC. ANOVA analyzed the data, and the significance of the treatment was tested by Duncan Test (DMRT).

RESULTS

Effect of EEGC on AI antibody titer

The antibody titer results of starter phase laying hens were expressed in geometric mean

titer (GMT) with HI log 2 unit. The variance analysis results indicate that the application of EEGC treatment in drinking water did not significantly affected (p>0.05) on the antibody titer of AI in laying hens in weeks 3, 4, and 5 of age. At week 3, the AI antibody titers of EEGC-treated hens (T2, T3, T4, and T5) had higher GMT compared to the control treatment (T1), which means that the formation of AI titers in treatments such as T2, T3, and T4 and T5 were faster than the control treatment (T1). Further tests with DMRT showed that treatment T1 was not different (P>0.05) from treatments T2, T3, and T4 but significantly different (P<0.05) from treatment T5. Treatment T2 is not different (P>0.05) from treatments T1, T3, and T4. Treatments T3 and T4 did not differ (P>0.05) with all treatments, while treatment T5 did not differ (P>0.05) with treatments T3 and T4 but differed (P<0.05) with treatments T1 and T2. GMT of AI on each treatment and the graph of the AI antibody titer of laying hens are presented in Table 2 and Figure 1, respectively.

Table 2. Geometric titer value (GMT) of on each treatment

Treatments	Ages (Weeks)			
	3	**4**	**5**	**6**
T1	2.5	32	136	96[a]
T2	8.5	34	144	136[a]
T3	7	78	352	224[ab]
T4	23	204	384	320[ab]
T5	10	72	672	448[b]

T0 = Drinking water without EEGC, T1 = Drinking water + 0.5% EEGC, T3 = Drinking water + 1% EEGC, T4 = Drinking water + 1.5% EEGC, T5 = Drinking water + 2% EEGC. Different lowercase superscripts indicate significantly different treatment effects (P<0.05).

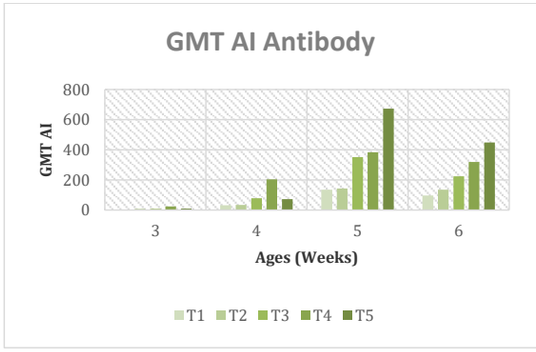

Figure 1. Geometric titer value (GMT) Chart of AI antibody

Drinking water without EEGC, T1 = Drinking water + 0.5% EEGC, T3 = Drinking water + 1% EEGC, T4 = Drinking water + 1.5% EEGC, T5 = Drinking water + 2% EEGC. Different lowercase superscripts indicate significantly different treatment effects (P<0.05).

Effect of EEGC on ND antibody titer

The geometric mean titer (GMT) value of ND laying hens for all treatments can be seen in Table 3. The analysis of variance results indicate that the application of EEGC treatment in drinking water did not yield a statistically significant impact (P>0.05) on the ND antibody titers of laying hens during weeks 3, 4, and 5 of age. The analysis of variance revealed that the application of EEGC treatment in drinking water had a statistically significant impact (P<0.05) on the ND antibody titers of laying hens at week 6 of the week. Table 3 indicates that the GMT (geometric mean titer) of ND (Newcastle disease) at week 6 demonstrated an increase compared to the previous week. This happens because at the age of 20 days, ND revaccination is carried out on chickens. The GMT of ND in each treatment and the ND antibody titer graph for laying hens are presented in Table 3 and Figure 2, respectively.

Table 3. Geometric titer value (GMT) of ND on each treatment

Treatments	Ages (Weeks)			
	3	**4**	**5**	**6**
T1	44	64	128	208[a]
T2	76	168	160	192[a]
T3	68	152	224	320[ab]
T4	88	168	224	896[b]
T5	96	224	288	768[ab]

T0 = Drinking water without EEGC, T1 = Drinking water + 0.5% EEGC, T3 = Drinking water + 1% EEGC, T4 = Drinking water + 1.5% EEGC, T5 = Drinking water + 2% EEGC. Different lowercase superscripts indicate significantly different treatment effects (P<0.05).

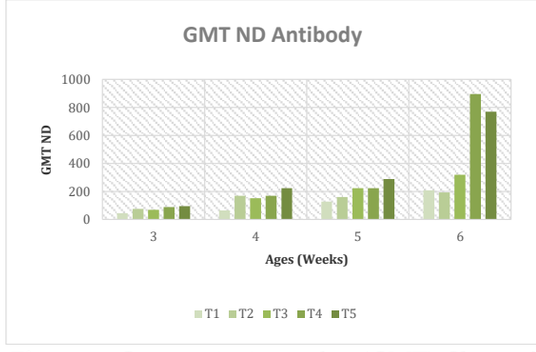

Figure 2. Geometric titer value (GMT) Chart of ND antibody

T0 = Drinking water without EEGC, T1 = Drinking water + 0.5% EEGC, T3 = Drinking water + 1% EEGC, T4 = Drinking water + 1.5% EEGC, T5 = Drinking water + 2% EEGC. Different lowercase superscripts indicate significantly different treatment effects (P<0.05).

DISCUSSION

The enhanced formation of antibody titers following vaccination can be attributed to the presence of andrographolide compounds within EEGC. These compounds enhance the phagocytic activity of macrophage cells, enabling macrophages to effectively eliminate invading pathogens or foreign entities within the body [18]. As reported by Yu *et al*. [19, 20] and Vetvicka & Vannucci [21], andrographolide compounds have been shown to enhance macrophage cell phagocytic activity in response to exogenous agents within the organism. The GMT value of AI antibodies in weeks 4 and 5 also showed that the treatments given EEGC (T2, T3, T4, and T5) had higher GMT values compared to the control treatment (T1). This is because the AI antibody titer formation response in the EEGC-treated treatments was faster, so the antibodies formed in the following week were also higher. AI antibody titers for all treatments in weeks 4 and 5 were above the minimum protective AI antibody titers, where the standard GMT of protective AI antibodies is at least GMT 16 [11,22].

The analysis of variance test on AI antibody titers at week 6 revealed that the application of EEGC treatment in drinking water had a statistically significant impact ($P<0.05$) on the AI antibody titers of starter phase laying hens. Research reported by Widowati *et al*. [23] also indicates that the application of Green chiretta (*Andrographis paniculata*) leaf powder at a dosage of 24 mg/kg body weight to broiler chickens can enhance the antibody titer of Infectious Bursal Disease (IBD) in broiler chickens aged 26 days, in comparison to broiler chickens of with the same phase without the application Green chiretta (*Andrographis paniculata*) leaf powder supplementation. According to Ibrahimu *et al*. [24], The powdered leaves of *Andrographis paniculata*, often known as green chiretta, have been observed to enhance the antibody titer against Infectious Bursal Disease (IBD) in broiler chickens. Based on Table 2, it can be seen that at week 6, there was a decrease in AI antibody titer compared to the previous week. Several factors, such as field virus challenge, infected chickens, stress, and time interval from the last vaccination, can cause the decrease in antibody titer.

At week 3, it was seen that the ND antibody titer of EEGC-treated chickens (T2, T3, T4, and T5) showed a protective GMT value, where the value of protective GMT in laying hens was a minimum of 64 [17]. At the same time, the T1 treatment that was not given EEGC had a GMT value of 44 and was below the protective standard. Additionally, another study found that supplementation of 50 mg/liter EEGC in drinking water promoted the ND antibody titer in broiler chicks on the 7th day following vaccination. [25]. The research findings indicate that the use of Origanum vulgare extract combined with *Andrographis paniculata* can enhance the performance of grill chickens and decrease the presence of intestinal bacteria [26]. A further investigation revealed that the use of phytobiotics, encompassing plant extracts such as *Andrographis paniculata*, exhibited a positive impact on the enhancement of antibody titers in chickens when supplied alongside avian influenza virus vaccines [27].

In Table 3, it can also be seen that the supplementation of EEGC gave a higher antibody formation response compared to the control (T1). Compared with AI antibody titers, it can be seen that the formation of ND antibody titers is faster. This is due to the form of vaccine given, where the AI vaccine is inactive while the ND vaccine uses an active vaccine. Active vaccination is formulated by attenuating harmful bacteria, resulting in a prompt generation of antibodies; nevertheless, the duration of this immune response is limited. In contrast, the inactive vaccine is a type of vaccination that is produced by deliberately inactivating the pathogenic microbe before supplementation. This inactivation process results in a slower antibody production response, although the antibodies generated have a longer duration compared to those induced by the active vaccine [28].

CONCLUSION

In summary, the supplementation of Green chiretta leaf extract (EEGC) to starter phase laying hens at a dosage of 2% through drinking water leads to a faster immune response in the production of Avian Influenza and Newcastle Disease antibodies. Additionally, it results in a higher Geometric Mean Titer (GMT) value for Avian Influenza and Newcastle Disease. Additional investigation is required to validate and measure extracts' chemical makeup that enhance poultry's performance.

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