Effects of a Garlic–Ginger–Turmeric–Lempuyang phytogenic formula on ND and AI humoral immunity in broilers

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Abstract. The excessive use of antibiotics in poultry production raises the risk of residues and antimicrobial resistance. This study evaluated the effectiveness of a phytogenic blend composed of garlic (*Allium sativum*), ginger (*Zingiber officinale* var. officinale), turmeric (*Curcuma longa*), and lempuyang (*Zingiber zerumbet*) as an alternative to antibiotics for enhancing the immune response in broiler chickens. Two formulations were tested: Formula A=50% garlic+25% ginger+12.5% turmeric+12.5% lempuyang; Formula B=75% garlic+15% ginger+5% turmeric+5% lempuyang. A total of 600 Cobb strain day-old chicks were divided into six groups, including positive and negative controls, and herbal treatments (Formulas A and B) administered through drinking water at 50 mL/L. Birds were vaccinated against Newcastle Disease (ND) and Avian Influenza (AI), and antibody titers were measured using hemagglutination inhibition (HI) at days 14, 28, and 35. The results showed that Formula B significantly increased ND antibody titers compared to controls ($p<0.05$), while Formula A yielded the highest AI antibody titers ($p<0.05$). Both herbal treatments demonstrated immune-boosting effects that were comparable to antibiotic controls. These findings suggest that phytogenic supplementation can serve as a sustainable strategy to enhance vaccine-induced immunity and reduce antibiotic dependence in broiler production.

1 Introduction

Modern poultry production is undergoing a significant paradigm shift as growing scientific and public health concerns continue to highlight the unintended consequences of frequent reliance on conventional chemotherapeutic agents. Therefore, growing concerns regarding antimicrobial resistance, drug residues in food products, and environmental contamination have prompted global regulatory restrictions on antibiotic use in livestock production [1]. This paradigm shift has created an urgent need for natural, sustainable alternatives that can

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maintain poultry health without compromising food safety or contributing to resistance development. Rising levels of antimicrobial resistance, persistent drug residues in edible poultry products, and the ecological risks posed by pharmaceutical contamination have collectively intensified calls for more responsible and sustainable production practices worldwide [2], [3]. In response, considerable research attention has turned toward naturally derived alternatives capable of maintaining flock health, supporting productivity, and safeguarding food safety without contributing to the growing burden of antimicrobial resistance.

Phytogenic feed additives represent a promising solution, offering diverse bioactive compounds with antimicrobial, antioxidant, and immunomodulatory properties [4]. Herbs possess antimicrobial, anti-inflammatory, and immunomodulatory properties. A number of herbs containing medicinal compounds, including garlic, anise, oregano, thyme, and pepper, have been applied due to their satisfactory effects on performance, antioxidative properties, and immune response of boilers [5]. These plant-derived substances have been utilized in traditional medicine for centuries and are increasingly recognized for their potential in modern animal agriculture. The complex phytochemical profiles of herbs provide multiple mechanisms of action, including pathogen inhibition, immune system modulation, and oxidative stress reduction [6].

In recent years, herbal supplements have been getting high priority in livestock and poultry productions, because of their wide continuum of advantageous effects, like supporting growth, production, and immunocompetence, as well as balancing the level of biochemical compounds in the circulatory system [7], [8]. Among the most studied phytogenic compounds, garlic (*Allium sativum*) contains organosulfur compounds such as allicin, which exhibit broad-spectrum antimicrobial activity and immunostimulatory effects [9], [10]. Ginger (*Zingiber officinale*) provides gingerols and shogaols that possess anti-inflammatory properties and enhance cellular immunity [11]. Turmeric (*Curcuma longa*) is rich in curcuminoids, particularly curcumin, which demonstrates potent antioxidant and immunomodulatory activities while serving as a natural vaccine adjuvant [12]. Lempuyang (*Zingiber zerumbet*), a traditional Indonesian medicinal plant, contains zerumbone and other bioactive sesquiterpenes with demonstrated antibacterial and immunoenhancing properties [13].

In addition to their individual bioactive properties, the combined use of these herbs has been reported to exert synergistic effects on livestock health and productivity. Phytogenic formulations can enhance nutrient absorption by stimulating digestive secretions, improving gut morphology, and modulating the intestinal microbiota toward beneficial bacterial populations. Several studies have shown that multi-herbal blends containing *A. sativum*, *Z. officinale*, or *C. longa* improve feed efficiency, promote weight gain, and enhance antioxidant capacity in broilers by reducing oxidative stress markers and supporting metabolic stability [14]. Moreover, phytogenic supplementation has been associated with increased lymphoid organ development and elevated antibody titers following vaccination, indicating its potential role in strengthening vaccine-induced immunity [15], [2]. These findings collectively highlight the relevance of evaluating herbal combinations as functional feed additives that enhance both physiological resilience and immune performance in broiler production systems.

Newcastle Disease (ND) and Avian Influenza (AI) represent two of the most economically significant viral diseases in poultry production worldwide. Effective vaccination programs are essential for disease prevention, but vaccine efficacy can be influenced by various factors, including immune status, stress, and nutritional factors. Phytogenic supplementation may enhance vaccine-induced immunity by supporting immune organ development, promoting antibody production, and reducing immunosuppressive stress factors.

Despite extensive research on individual herbs, limited information exists regarding the combined immunomodulatory effects of multi-component phytogenic blends on vaccine responses in broiler chickens. Therefore, this study aimed to evaluate the efficacy of two distinct herbal formulations containing garlic, ginger, turmeric, and lempuyang in enhancing humoral immune responses to ND and AI vaccines in commercial broiler production.

2 Materials and Methods

2.1 Ethical approval

This study received approval from the Local Ethics Committee for Animal Experiments at the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (Approval No. 50/EC/FKH/Int/2024).

2.2 Experimental Design and Animals

This experiment was conducted using a completely randomized design with a factorial arrangement to evaluate the effect of herbal supplementation on vaccine-induced humoral immunity in broiler chickens. A total of 600 day-old Cobb chicks were sourced from a commercial hatchery and randomly assigned to six treatment groups, each consisting of 100 chickens (Table 1). Two phytogenic formulations were evaluated: Formula A, consisting of 50% garlic, 25% ginger, 12.5% turmeric, and 12.5% galangal; and Formula B, consisting of 75% garlic, 15% ginger, 5% turmeric, and 5% galangal. The negative control (NC) group received a basal diet without antibiotic inclusion, while the positive control (PC) group was provided with the same basal diet supplemented with Bacitracin Zinc (QILU) at the recommended dose of 300 g/ton of feed (0.0003%), following standard guidelines for medicated feeding. All birds were managed under uniform rearing conditions to minimize environmental variation and ensure that observed responses could be attributed solely to the dietary treatment.

Table 1. Experimental treatment groups and design.

Group	**Infection Chalance *E. coli***	**Antibiotic**	**Herbal Treatment**
NC	0	v	0
PC	v	v	0
1	0	0	Formula A
2	0	0	Formula B
3	v	0	Formula A
4	v	0	Formula B

NC: Negative control
PC: Positive control
1: Formula A (non-challenged)
2: Formula B (non-challenged)
3: Formula A (challenged)
4: Formula B (challenged)

2.3 Housing and Management

The birds were reared in a well-managed, environmentally controlled facility equipped with mechanical ventilation and an automated lighting system. Each treatment group was housed in an individual pen to prevent cross-contamination, and strict biosecurity protocols were implemented and maintained throughout the entire experimental period. Ambient temperature and relative humidity were monitored and adjusted daily in accordance with the recommended management guidelines for Cobb broilers. All birds were provided with a commercial broiler diet (CP-11; PT Charoen Pokphand Tbk), which was supplied uniformly to the herbal treatment groups (1, 2, 3, and 4) as well as the negative control (NC) group. The positive control (PC) group received the same basal diet supplemented with antibiotics. The housing arrangement, environmental control, and biosecurity measures were designed to ensure that any observed differences in immune response or performance could be attributed exclusively to the dietary treatments rather than environmental variation.

2.4 Nutrition and Feeding

The diets used in this study were supplemented with antibiotics and non-antibiotic feed. Routine health management practices, including vaccination against Newcastle Disease (ND) and Avian Influenza (AI), were carried out according to commercial farm standards. Strict sanitary measures were implemented to prevent infectious disease exposure during the study.

2.5 Vaccination and Challenge

Birds were raised for 35 days under standard management and vaccinated against ND and AI. All birds were vaccinated according to standard commercial protocols. ND vaccination was administered on day 7 using live attenuated La Sota strain vaccine via drinking water, followed by a booster vaccination on day 21. AI vaccination was performed on day 14 using inactivated H5N1 vaccine via subcutaneous injection. For challenged groups, *Escherichia coli* suspension (10^6 cfu/ml) was administered orally on day 21 to simulate field stress conditions and evaluate the protective effects of herbal supplementation under immune challenge

2.6 Administration of Herbal Formula

All birds were fed a standard commercial broiler diet formulated to meet the nutrient requirements recommended by the National Research Council (NRC). The amount of feed provided was adjusted according to the daily nutrient requirements of broiler chickens based on age and body weight, following the management standards for the Cobb strain. Feed and water were supplied ad libitum throughout the experiment. All treatment groups received the same type of basal diet to ensure that any observed differences in outcomes could be specifically attributed to the additional variable, i.e., herbal supplementation. Herbal supplementation was administered primarily through incorporation into drinking water, with compositions and dosages based on previous reports. The ingredients included garlic (*Allium sativum*), turmeric (*Curcuma longa*), ginger (*Zingiber officinale* var. officinale), and lempuyang (*Zingiber zerumbet*), in combination. Supplementation was provided daily for the entire 35-day rearing period.Two fermented herbal formulas with different composition percentages, Formula A and B were administered through the drinking water. Both formulas were standardized at a concentration of 14 g/L and administered daily at a dose of 50 mL/L of drinking water for 12 days from the start of the chicken farming during 35-day rearing.

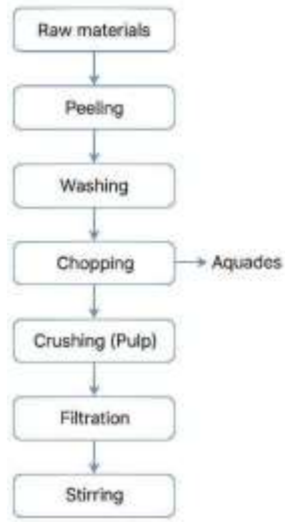

Fig. 1. Diagram of herbal formula preparation.

The preparation of the herbal formula A and B was carried out through a standardized multistep extraction protocol to ensure the preservation and activation of bioactive phytochemicals. Fresh botanical materials were first subjected to a primary sorting and peeling process to remove extraneous tissues and reduce microbial load. The cleaned materials then underwent thorough washing under running water to eliminate debris and potential surface contaminants. Subsequently, the herbs were finely chopped to increase the surface area and enhance cell wall disruption efficiency. The chopped materials were mechanically macerated to obtain a homogeneous herbal slurry, after which distilled water (aquades) was incorporated as an extraction medium to facilitate solubilization and diffusion of active compounds. The resulting mixture was pressed and filtered to separate the liquid extract (filtrate) from solid residues (marc).

The filtrate was subsequently enriched with EM4, a consortium of beneficial microbial cultures, to initiate controlled biofermentation. The mixture was homogenized through continuous stirring to ensure even microbial distribution and substrate availability. Fermentation was performed for one week under aerobic conditions, allowing microbial enzymatic activity to modify, enhance, and bioactivate key phytochemical constituents. This fermentation step is critical for improving the stability, potency, and functional properties of the herbal formulation. The complete workflow of the preparation process is illustrated in Figure 1.

2.7 Sample Collection and Laboratory Analysis

To evaluate the immune response, blood samples were taken from the brachial vein as much as 1-2 ml from 10 birds randomly selected birds per group three times on days 14, 28, and 35. Serum was separated by centrifugation at 3,000 rpm for 10 minutes and stored at –20 °C until analysis. Antibody titers against Newcastle Disease (ND) and Avian Influenza (AI) viruses were determined using the hemagglutination inhibition (HI) assay, following OIE, (2018) standard procedures and the Food and Agriculture Organization (FAO). HI titers were expressed as as $\log_2$ values of the reciprocal of the highest serum dilution that completely inhibited hemagglutination. Titers $\geq 4 \log_2$ were considered protective for ND, while titers $\geq 6 \log_2$ were considered protective for AI.

2.8 Statistical Analysis

All data were analyzed using a completely randomized design (CRD) with a factorial arrangement to evaluate the effects of infection status (infected vs. non-infected), treatment type (antibiotic and herbal supplementation), and their interactions on broiler performance and immune response. Antibody titers variables against ND and AI were expressed as mean ± standard error (SE). Data were tested for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) before analysis. A two-way analysis of variance (ANOVA) was performed to assess main effects and interactions among treatments. Where significant differences were detected ($p < 0.05$), mean comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test as a post hoc analysis. All statistical procedures were carried out using SPSS version XX (IBM Corp., Armonk, NY, USA) or equivalent software.

3 Results and Discussion

3.1 Post-Vaccination Antibody Titer

Analysis of ND antibody titers revealed a more complex and biologically meaningful pattern in response to herbal supplementation. Broilers receiving Formula B (Groups 2 and 4) exhibited higher hemagglutination inhibition (HI) titers compared to the negative control (NC), with the difference reaching statistical significance ($p < 0.05$) (Figure 2). This increase suggests that the phytogenic composition of Formula B—dominated by a higher proportion of garlic and ginger—may provide stronger immunostimulatory activity than Formula A, likely due to the higher concentrations of organosulfur compounds, gingerols, and shogaols, which are known to enhance B cell differentiation and antibody secretion [15].

The persistent increase in titers observed in Groups 2 and 4 up to day 35 further suggests that the herbal constituents may have supported the primary and secondary phases of the humoral immune response. This trend is consistent with previous findings showing that garlic (Allium sativum) and ginger (Zingiber officinale) can potentiate vaccine-induced immunity by enhancing antigen presentation, increasing macrophage activity, and reducing oxidative stress that typically suppresses lymphocyte function [14]. In contrast, the NC group showed lower titers, indicating the dependence of optimal vaccine performance on adequate immunonutritional support.

Despite these limitations, the significantly higher ND antibody titers in broilers fed Formula B provide strong evidence that this phytogenic blend enhances the immunological effectiveness of ND vaccination. This finding is consistent with a report that turmeric supplementation improved both innate and adaptive immune responses in broilers [12]. These data underscore the potential value of targeted herbal formulations as practical immunonutritional tools to enhance disease resistance and reduce reliance on synthetic drugs in broiler production systems, further supporting the growing body of literature advocating phytogenic feed additives as a sustainable alternative in poultry health management [4]. The mechanism underlying enhanced ND immunity likely involves multiple pathways, Garlic-derived organosulfur compounds, particularly allicin are known to stimulate lymphocyte proliferation and antibody production, which may explain the elevated ND titers in birds receiving Formula B. The immunopotentiating effect of garlic supplementation has also been confirmed in a comprehensive review by Abd El-Ghany, which highlighted its role in strengthening immune organ development and serum immunoglobulin levels [6].

The mechanism by which these herbs enhance ND antibody titers likely involves multiple pathways (a) Adjuvant-like effects, Curcuminoids in turmeric, and phenolic compounds in ginger may facilitate antigen presentation and improve vaccine responsiveness. (b)

Antioxidant protection, Phytochemicals neutralize reactive oxygen species (ROS), thereby protecting immune cells from oxidative stress that otherwise suppresses antibody production [13]. (c) Antimicrobial activity: Garlic, ginger, turmeric, and lempuyang reduce the intestinal pathogen load [6], [9], which decreases competition for nutrients and indirectly supports the immune system.

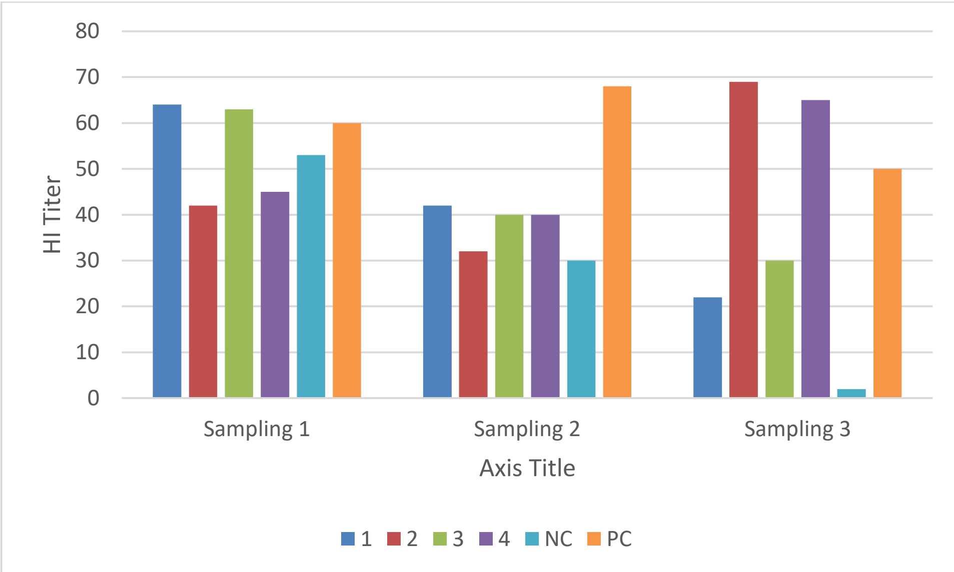

Fig. 2. Newcastle Disease **(**ND) antibody titers across sampling times.

Administration of formula A to groups 1 and 3 resulted in a significant increase in antibody titers against Avian Influenza (AI) compared to the negative control (NC) ($p<0.05$). At the final sampling, Formula A consistently produced the highest AI antibody titers among the treatments from sampling 2 to sampling 3 (Figure 3).

The results in this study may be due to the specific phytochemical profile of Formula A, which includes garlic and turmeric. Garlic has been documented to exhibit antiviral activity by modulating interferon production and stimulating macrophage function [1], [6]. Turmeric contains curcumin, a polyphenolic compound with potent immunomodulatory and antiviral effects, capable of enhancing humoral and cellular immunity [12]. A recent review by El-Zahar et al. emphasized that curcumin supplementation enhances vaccine efficacy in poultry by increasing antibody titers and reducing viral replication [6].

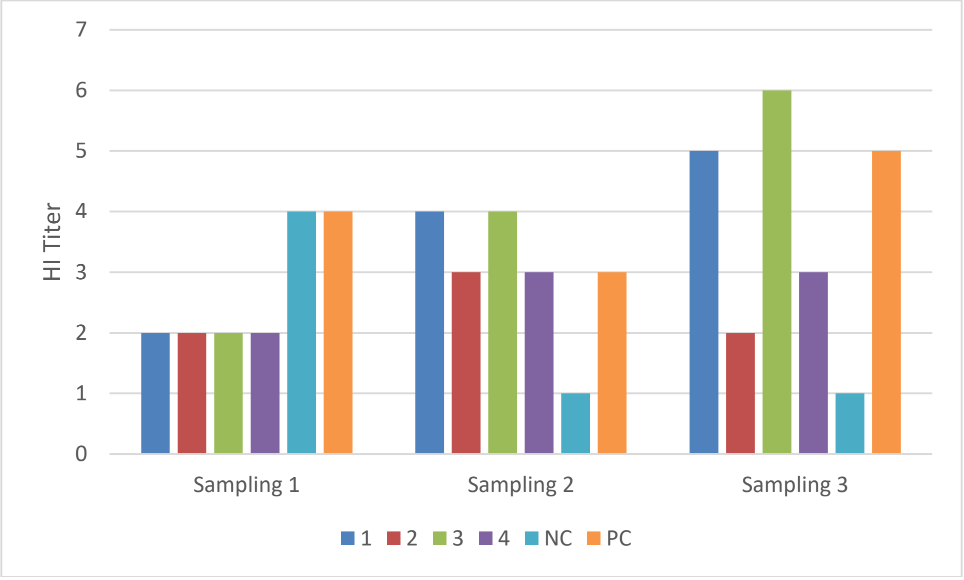

Fig. 3. Avian Influenza (AI) Antibody Response.

The observed differential effects between Formula A on AI titers and Formula B on ND titers highlight the importance of herbal composition in determining immunological outcomes. Although both formulas contain potent immunostimulants, their varying ratios and specific combinations likely influence the type and magnitude of the immune response elicited.

3.2 Comparison with Antibiotics

Our findings align with and further reinforce the expanding body of evidence demonstrating that phytogenic feed additives can function as effective natural alternatives to conventional antibiotic-based performance enhancers in poultry production. Numerous studies have reported that phytobiotics improve digestive efficiency, antioxidant capacity, and immune modulation without contributing to the escalation of antimicrobial resistance [9], [4]. Phytochemicals from Zingiberaceae plants—including gingerols, shogaols, curcuminoids, and zerumbone—exert strong antimicrobial, anti-inflammatory, and antioxidant effects, thereby supporting both local gut integrity and systemic immune function [13]. These bioactive compounds have been shown to enhance macrophage activation, upregulate cytokine expression, and stimulate lymphocyte proliferation, facilitating stronger vaccine-induced immune responses in poultry [15]. Similar synergistic effects have been reported in studies evaluating multi-herbal blends, where combinations of garlic, ginger, and turmeric significantly enhanced antibody titers, vaccine responsiveness, and resistance to respiratory pathogens [14]. Collectively, these findings provide mechanistic support for the superior immunological outcomes observed in broilers receiving the herbal formulations tested in the present study.

A deeper examination of the antibody kinetics in the present study suggests that the immunostimulatory effects of the herbal formulations emerged progressively as the influence of maternal antibodies dissipated. The limited differences in early HI titers are consistent with reports indicating that maternal antibodies can persist for up to two weeks and attenuate early vaccine responses in broilers. As maternal antibodies declined, the differences between treatments became more pronounced: both herbal groups showed higher ND and AI titers than the positive control (PC), suggesting that the phytogenic compounds may have promoted more efficient antigen presentation, increased B cell activation, and improved humoral memory development. The substantial elevation in antibody titers by the third sampling suggests that standardized herbal formulations may not only match but potentially surpass the immune-supportive functions of antibiotics once the birds' adaptive immune system becomes fully active. This supports broader recommendations advocating phytogenic feed additives as viable immunonutritional tools for disease control and productivity enhancement in broiler systems.

3.3 Implications for Poultry Production

The ability of Formula B to enhance ND titers and Formula A to elevate AI titers underscores the potential of tailoring phytogenic formulations to specific immunological targets. This flexibility provides a practical strategy for poultry producers to improve vaccine responses against major viral diseases. The broader implications include reduced antibiotic dependence, supporting global initiatives for antimicrobial stewardship, improved vaccine efficacy, leading to lower morbidity and mortality during outbreaks of ND or AI, and sustainable poultry production, as herbal supplements are environmentally friendly and consumer-preferred.

The results of this study are consistent with numerous reports on the immunomodulatory potential of herbs, which show that garlic promotes antibody production and enhances resistance to viral infections [6]. Ginger improves immune responses in vaccinated broilers by stimulating cytokine activity. Turmeric enhances both humoral and cell-mediated immunity and acts as a natural vaccine adjuvant [12]. Lempuyang (*Z. zerumbet*), though less studied in broilers, has demonstrated antibacterial and antioxidant activity that may indirectly support immune function [13]. Collectively, these findings emphasize that phytogenic feed additives act through multiple mechanisms, including direct immunostimulation, antioxidant protection, modulation of gut microbiota, and antiviral activity.

4 Conclusion

This study demonstrated that phytogenic supplementation enhanced vaccine-induced immunity in broilers. Formula B significantly increased antibody titers against Newcastle Disease virus, while Formula A elevated antibody titers against Avian Influenza virus. Herbal treatments were comparable or superior to antibiotic controls, indicating their potential as sustainable alternatives to antibiotics in broiler production. These findings strengthen the evidence for integrating herbal feed additives into poultry health management to improve vaccine responses and flock resilience.

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