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Experimental trials to assess the immune modulatory influence of thyme and ginseng oil on NDV-vaccinated broiler chickens

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Abstract

Background: The use of traditional medicine against viral diseases in animal production has been practiced worldwide. Herbal extracts possess organic substances that would improve chicken body performance.

Aim: The current study was designed to evaluate the effect of either thyme or ginseng oil in regard to their immune-modulatory, antiviral, and growth promoter properties.

Methods: Two hundred and forty-one-day-old broiler chicks were allocated into eight equal groups as the following: group 1; nonvaccinated and nontreated and group 2; Newcastle disease virus (NDV) vaccinated and nontreated. Birds of groups 3 and 4 were treated with thyme oil (200 mg/l of drinking water for 12 hours/day) without or with NDV vaccination. Birds of groups 5 and 6 were treated with ginseng oil (200 mg/l of drinking water for 12 hours/day) without or with NDV vaccination. Birds of groups 7 and 8 were treated with a combination of ginseng oil (100 mg/l of drinking water) and thyme oil (100 mg/l of drinking water) for 12 hours/day. On the 35th day of life, birds in all the experimental groups were given 0.1 ml of a virulent genotype VIId NDV strain suspension containing 106.3 EID50/ml intramuscularly.

Results: Administration of ginseng and thyme oils each alone or simultaneously to birds either vaccinated or nonvaccinated elicited a significant improvement in body performance parameters. Administration of thyme and ginseng each alone or concurrently to vaccinated birds (Gp 4, 6, and 8) induced a higher hemagglutination inhibition (HI) titer of 6, 7.3, and 6.3 log2 at 21 days of age, 6.7, 7.6, and 7 log2, at 28 days of age and 7, 8, and 6.8 log2 at 35 days of age, respectively. Challenge with vNDV genotype VII led to an increase in the NDV-specific HI-Ab titers 10 days post challenge in all the experimental groups. In addition, thyme, ginseng oils, or a combination of them improved the protection from mortality in vaccinated birds; by 100%, 100%, and 90%, respectively, compared with 80% protection from mortality in vaccinated-only birds post-NDV challenge. Moreover, NDV-vaccinated birds treated either with thyme; ginseng or their combination showed negative detection of the virus in both tracheal and cloacal swabs and nonvaccinated groups that received oils showed improvement in vNDV shedding in tracheal and cloacal swabs.

Conclusion: It could be concluded that the administration of thyme and ginseng essential oils to broilers can improve productive performance parameters, stimulate humoral immunity against, and protect from vNDV infection.

Keywords: NDV, Ginseng, Thyme, Chicken, Genotype VII.

Introduction

Strict control measures must be taken to limit viral, bacterial, and parasitic diseases that threaten the poultry industry and to avoid economic losses. Inadequate management, nutritional conditions, and stress factors can lead to the breakdown of the bird's immune system which becomes susceptible to many diseases, particularly in areas where intensive poultry breeding exists. Immunostimulants have a long history; they can be synthetic or natural substances that boost the immune system's defenses against infections. Almost all facets of animal husbandry, including production and welfare, can benefit from the use of immunostimulants (Hamill *et al.*, 2008).

A number of serious challenges and significant financial losses are caused by avian viral infections, including Newcastle disease (ND), infectious bronchitis, infectious bursal disease, and avian influenza. The disorders indicated above may cause immunological suppression, stunted growth, increased mortality

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rates, and/or respiratory discomfort. Moreover, they have the ability to cause illnesses on their own or in conjunction with one another (Roussan *et al.*, 2008). The Newcastle disease virus (NDV), which is a member of the Paramyxoviridae family's genus Avulavirus, is the cause of ND. RNA genomes of ND viruses are encased in one of the two envelopes (Hines and Miller, 2012). To prevent the disease, several immunization programs are implemented and a number of commercially accessible live, inactivated, or recombinant vaccines are available (Swayne and King, 2003). NDV outbreaks, however, continue to occur, and novel genotypes have appeared in a number of global locations. Moreover, vaccination is not enough to battle endemic viruses because it is hard to eradicate them from a certain area. Instead, additional strategies are needed. The use of essential oils (EOs) combinations and other natural antimicrobial agents as a means of enhancing the immune system and overall health of birds has garnered attention as a means of managing various infections (Gutierrez *et al.*, 2008). Medicinal plants yield EOs, and depending on what is in them, these oils can have antiviral or antibacterial properties (Soković *et al.*, 2010; Qorbanpour *et al.*, 2018). It has been demonstrated by recent studies (Zhai *et al.*, 2014; Zhu *et al.*, 2014; El-Shall *et al.*, 2020; Hassanin *et al.*, 2020) that EOs can enhance the humoral and cellular immune responses of chickens.

A member of the Lamiaceae family, thyme (*Thymus vulgaris L.*) is a flavorful and aromatic herb that is frequently used in cooking. One of the most common plants utilized in the culinary and medicinal sectors is thyme. There is 1% to 2.5% EO in dried thyme. A combination of monoterpenes, primarily thymol and its phenol isomer carvacrol, make up *T. vulgaris* EO. Furthermore, it has been shown that flavonoid compounds such as flavonoid aglycones and flavonoid glycosides, as well as phenolics such as caffeic acid, p-cymene-2,3-diol, and certain biphenyls, exhibit antioxidative activities and other favorable effects for chickens (Miura and Nakatani, 1989; Fachini-Queiroz *et al.*, 2012). Moreover, these extracellular oils have the ability to disrupt the viral envelope and conceal viral elements necessary for adhesion, infiltration, or virus penetration into host cells. Reducing NDV replication, mortality, and clinical symptoms against virulent NDV challenge is all possible with thymol oils (Rezatofighi *et al.*, 2014; El-Shall *et al.*, 2020).

China has been using ginseng as a natural remedy for thousands of years (Soković *et al.*, 2010). Ginsenosides, are among the components of ginseng that have been thoroughly studied for their biological activities and are primarily responsible for the herb's pharmacological effects (Kiefer and Pantuso, 2003). Vaccination is one of the best intervention options for the control of diseases associated with infectious agents, according to Song and Hu (2009). Adjuvants are typically added to vaccine formulations to increase immunization efficacy. However, scientific literature has described more than 100 adjuvants. Among these are the saponins that were extracted from *Panax ginseng* C.A. Meyer (GS) roots. According to Kong *et al.* (2004), GS saponin enhanced the cellular and humoral immune responses of hens to the ND vaccine.

We can conclude, from the evidence so far, that extracts from ginseng and *T. vulgaris* show promise as immune stimulants, adjuvants for vaccines, and antiviral therapeutics to enhance the health of chickens and the financial status of poultry breeders. Thus, the immune modulatory, antiviral, and growth-promoting qualities of the EOs extracted from either ginseng or thyme were assessed in the current study.

Material and Methods

Essential oils

Ginseng and thyme oils were obtained from Now Company (USA). They were administered on drinking water in the form of emulsion (20%) by using gum acacia as an emulsifying agent (10%). Thyme and ginseng oils were applied at 200 mg/l of drinking water for 12 hours (Asmaa *et al.*, 2020), in the instance of single administration. The mixed administration was 100 mg/l of drinking water for 12 hours with equivalent oils from each of them (Farag *et al.*, 2018).

Experimental birds and housing

Two hundred and forty-one-day-old broiler chicks were raised at the Animal Research Unit in the Faculty of Veterinary Medicine at Zagazig University in Egypt. The chicks were purchased from El-Dakahlia Company. A continuous lighting program was offered for the duration of the trial. The temperature was 33°C at the beginning, and then progressively dropped by 2°C every week until it reached 21°C in the fifth week. As a litter, clean wood shavings were utilized. The birds were fed a commercial ration that included El-Eman Company's 21% protein. The birds received different treatments based on the experimental design and were grouped in a completely randomized manner.

Vaccines

Jovac NDV B1 (Jovac) is a live freeze-dried vaccine that was administered as an eye drop to birds on their third day of life. It contains live NDV (Hitchner B1 strain).

LaSota NDV is a component of the oil-emulsion-killing vaccination Volvac® ND KV (Boehringer Ingelheim). The vaccination was administered subcutaneously (0.5 ml/bird) in the middle portion of the neck at 10 days of age.

At 13 days old, one eye drop of Volvac® ND LaSota MLV (Boehringer Ingelheim), a live freeze-dried vaccine containing live NDV (LaSota strain), was given to each bird (0.03 ml/bird).

Experimental design

To assess the immunomodulatory, prophylactic, and virucidal effects of ginseng and thyme, 240-day-old broiler chicks were divided into eight equal groups

and treated differently (Table 1). Group 1 received no NDV vaccine or treatment with ginseng and thyme oils (control negative). Group 2 received NDV vaccines (0.03 ml of Hitchner B1 vaccine via eye drop, 0.5 ml of inactivated NDV vaccine subcutaneously, and 0.03 ml of LaSota vaccine via eye drop) on the 3rd, 7th, and 14th days of life, but was not given ginseng or thyme oil. Group 3 was not vaccinated and was only treated with thyme oil (200 mg/l of drinking water for 12 hours/day). Group 4 was vaccinated with NDV and treated with thyme oil. Group 5 was not vaccinated with NDV and was only treated with ginseng oil (200 mg/l of drinking water for 12 hours/day). Group 6 was vaccinated with NDV and treated with ginseng oil. Group 7 was not vaccinated with NDV and was treated with ginseng oil (100 mg/l of drinking water) and thyme oil (100 mg/l of drinking water) for 12 hours/day. Group 8 was vaccinated with NDV and treated with ginseng and thyme oil. Ginseng and thyme were orally administered intermittently for 3 days weekly to treated groups. Birds in each group were weighed on the 1st, 7th, 14th, 21st, 28th, and 35th days of life to determine growth performance parameters. Ten birds were tagged and bled once a week until day 35. After that, they were bled again for serum separation to be used in the hemagglutination inhibition (HI) test on days 39 and 45 of the experiment. Birds were injected intramuscularly with 0.1 ml of a virulent genotype VIId NDV strain solution with 106.3 EID50/ml on the 35th day of life. The inoculum's titer was then verified in chicken embryonated eggs using reverse titration. Five cloacal and oropharyngeal swabs were taken from each group on the fourth post-challenge day to measure the amount of viral shedding using reverse transcription-quantitative polymerase chain reaction.

HI assay

Eight HA units of the LaSota vaccine NDV strain (genotype II) were used as an antigen in the standard HI test, along with 0.5% washed chicken red blood cells. According to the OIE approach, the titers demonstrated HI when expressed as Log2 of the highest dilutions.

Statistical analysis

Significant differences between groups' serological assay data were evaluated statistically using a One-Way assay of Variance and SPSS (ver. 21.0; IBM, USA).

Ethical approval

Zagazig University's Institutional Animal Care and Use Committee (ZU-IACUC/2/F/413/2023) approved all the animal work that was done for this study under biosafety guidelines.

Results

Protective effect of thyme, ginseng oils, or their combination on virulent NDV strain (genotype VIId) challenged broiler chickens

The protective and immune stimulant effects were evaluated based on mortality prevention and viral shedding reduction.

Table 1. Experimental design of thyme and ginseng herbal oil extracts differentially treated groups.

Days	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7	Gp8
	--------	-------	Thyme oil	Thyme oil	Ginseng oil	Ginseng oil	Thyme and ginseng oil	Thyme and ginseng oil
	Non-vaccinated	Vaccinated	Non-vaccinated	Vaccinated	Non-vaccinated	Vaccinated	Non-vaccinated	Vaccinated
	Groups treated for 3 days weekly							
3	HB1 vaccine							
7	Blood collection (serum) + body weight							
10	Inactivated ND vaccine							
13	Clone vaccine							
14	Blood collection (serum) + body weight							
18								
21	Blood collection (serum) + body weight							
22								
28	Blood collection (serum) + body weight							
35	Blood collection (serum) + body weight							
36	Challenge							
38	oropharyngeal and fecal swabs							
39	Blood collection (serum)							
40	oropharyngeal and fecal swabs							
46	Blood collection (serum)							

Mortality rate

As illustrated in Figure 1, the non-NDV vaccinated and untreated group (Gp1) was challenged with a virulent NDV strain (genotype VIId) and experienced 80% mortality between days 5 and 8. A group of birds given NDV vaccines (Gp2) died between the fifth and eighth day after the challenge, with 80% of the birds surviving. Birds given thyme or ginseng oils orally alongside NDV vaccination (Gp4 and 6) demonstrated 100% mortality protection. However, simultaneous oral administration of thyme and ginseng oils to NDV-vaccinated birds (Gp8) demonstrated 90% protection from mortality, with mortality occurring between days 5 and 8 post challenge. In addition, the administration of thyme and ginseng oils each alone or concurrently without NDV vaccination (Gp3, 5, and 7) led to 40% protection in the vNDV-challenged birds.

Virus shedding

The virus replicated and was efficiently excreted from the oropharyngeal and cloacal routes on the fourth day after the NDV challenge, as evidenced by the fact that the non-NDV vaccinated and non-EOs-treated group (Gp1) had 5/5 vNDV positive tracheal swabs and 4/5 positive cloacal swabs. In the instance of nonvaccinated groups that orally administrated either thyme or ginseng oil had 4/5 positive vNDV tracheal swabs and 2/5 positive cloacal swabs. In addition, administration of a mix of thyme and ginseng oil led to 4/5 tracheal and cloacal swabs positive for vNDV. However, vaccination with NDV vaccines either alone or combined with herbal oils leads to negative detection from either route.

Effect of either thyme, ginseng oil, or their combination on humoral immune responses to NDV vaccines

Broiler chickens were administered thyme, ginseng, or a combination of the two for three consecutive days at weekly intervals, in conjunction with NDV vaccination regimens, so as to evaluate the immunomodulatory effects of the oils. All non-NDV vaccinated groups (Gp1, 3, 5, and 7) have lacked HI antibody titers below 2 log2 since the 21st day of age, according to the monitoring of NDV-HI Ab responses. Conversely, detectable antibody titers of σ4 log2 were present in all the assessing time points (Fig. 2) for all the birds in the NDV-vaccinated groups (Gp2, 4, 6, and 8). Comparing the NDV-vaccinated only group (Gp2) to the three vaccinated groups administered herbal oil extracts (Gp4, 6, 8), the NDV-HI-Ab titers increased consistently at 21, 28, and 35 days of age in all three groups. Therefore, at 21 days of age, the average HI-Ab titers were 6 log2, 7.3 log2, and 6.3 log2, at 28 days of age, they were 6.6 log2, 7.3 log2, and 7 log2, and at 35 days of age, they were 7 log2, 8 log2, and 6.6 log2, respectively, in contrast to the vaccinated-only group, which had average HI-Ab titers of 4.6, 5.6, and 5.6 log2 at the same ages.

The HI antibody titer in the NDV-vaccinated group (Gp2) averaged 8 log2 10 days after the challenge (Fig. 2). Increased HI titers of 9.3, 10, and 9.3 log2 were observed in the vaccinated groups that received ginseng, thyme, or a combination thereof.

Effect of either thyme, ginseng oil, or their combination on body performance parameters

To assess the impact of thyme and ginseng oil administration on the growth performance of birds, we monitored weekly variations in their body weights

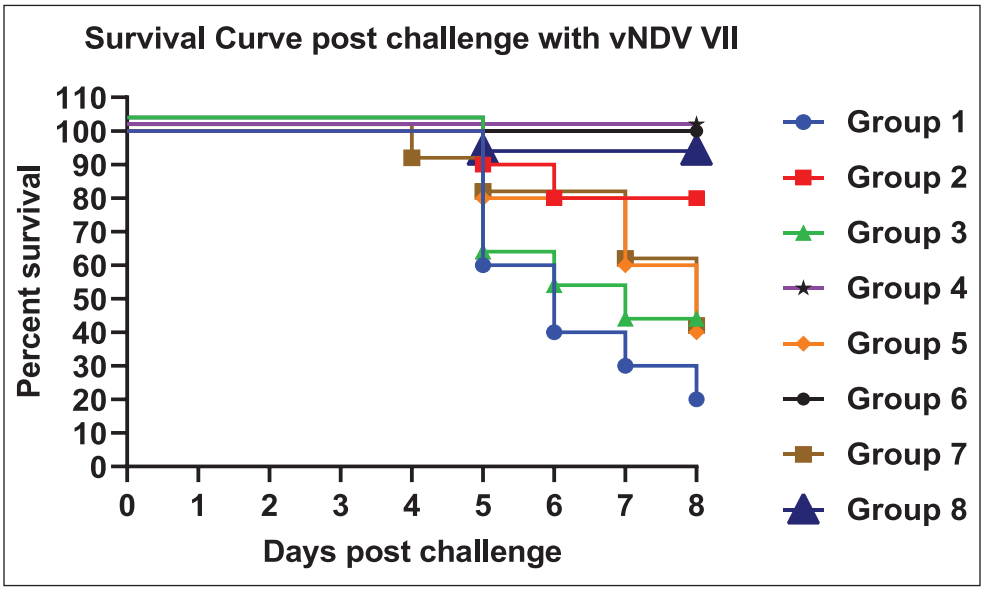

Fig. 1. Survival curve post challenge with $10^{6.5}/EID_{50}$/100 ul per bird of virulent NDV genotype VII strain (NDV/chicken/Egypt/1/2015) at 35 days old via oculonasal route.

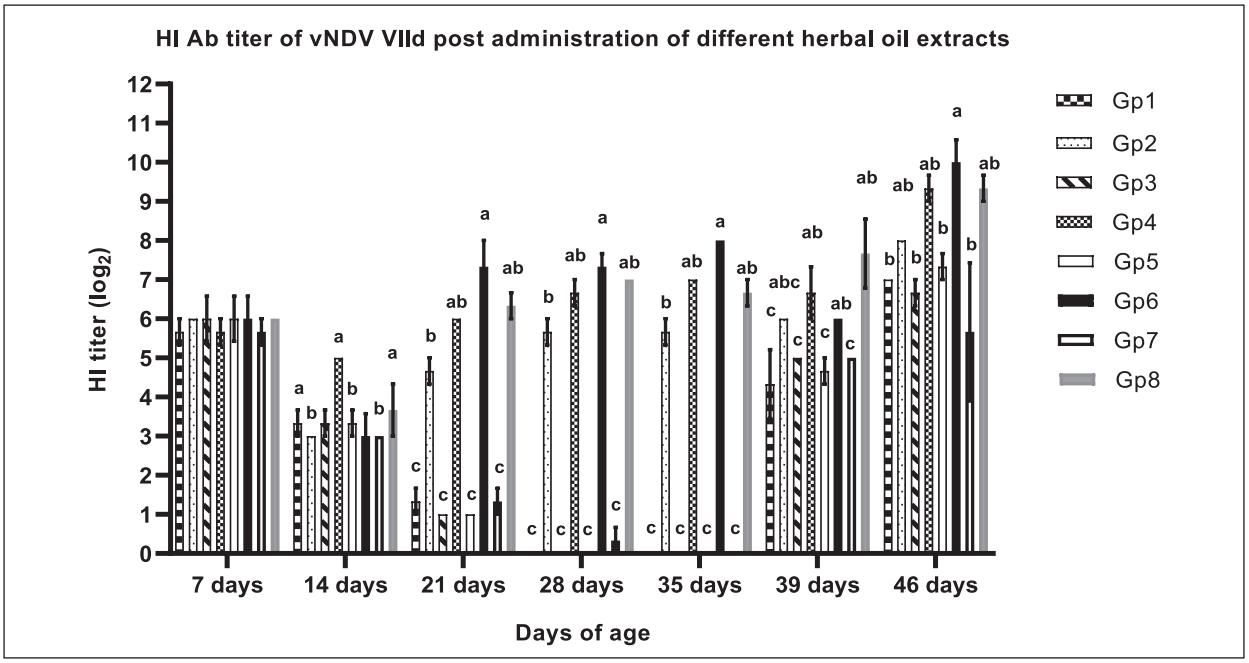

Fig. 2. Pre-challenge and post-challenge serological responses of broiler chickens after NDV vaccination and bird treatment with either ginseng oil extracts, thyme oil extracts, or a combination of them.

throughout the experimental period (Table 2). The administration of thyme oil to vaccinated groups resulted in a statistically significant increase (p < 0.05) in body weights and gains of body weight on the 14th, 21st, 28th, and 35th days of age, respectively, when compared to the control vaccinated group. The body weight gain was determined by subtracting the initial body weight in the same week (W1) from the ultimate body weight at W2 during the intended week (W2). The mean weight gain per bird per group was determined by calculating the weekly body weight gain and averaging the results to derive the average weight gain per bird per group. Gain in body mass = W2−W1. In comparison to the control vaccinated group, ginseng oil extracts significantly increased the body weight and weight gains of birds at the 21st, 28th, and 35th days of age (p 0.05). The concurrent application of thyme and ginseng oils to NDV-vaccinated birds resulted in a statistically significant increase (p < 0.05) in body weight at the 21st, 28th, and 35th days of age, as well as body weight gains at the 21st and 35th days of age, in comparison to the control NDV-vaccinated group.

Furthermore, among the nonvaccinated groups, administration of thyme oil had a significant increment (p < 0. 05) in the bird's body weight and body weight gains at the 14th, 21st, 28th, and 35th days of age in comparison to the control nonvaccinated groups. When administered ginseng either alone or in combination with thyme, the body weight and body weight gains of birds at the 21st, 28th, and 35th days of age improved significantly (p < 0.05) in comparison to the control group that did not receive vaccinations.

At the fifth week of age, all experimental groups that received treatment with thyme, ginseng, or a combination of the two exhibited greater body weight gain than the control groups (Table 3).

Ten days subsequent to the challenge (Fig. 2), the NDV vaccinated group (Gp2) exhibited a mean HI antibody titer of 8 log2. The HI titers of the vaccinated groups that were given ginseng, thyme, or a combination of the two increased to 9.3, 10, and 9.3 log2, respectively. Administration of thyme, ginseng, or their combination in vaccinated or nonvaccinated birds significantly improved body performance parameters in comparison with control birds.

Discussion

Traditional medicine has been used all throughout the world to treat viral diseases that affect animal production. According to Ghazalah and Ali (2008), organic compounds like phytobiotics found in herbal extracts enhance the physical performance of chickens. The current study unequivocally shows that giving ginseng and thyme oils to vaccinated or nonvaccinated birds, either alone or concurrently, resulted in a marked improvement in body performance metrics as compared to the control group of birds. These findings are consistent with the findings of (Herawati, 2010), who reported that when ginseng oil extracts were administered to birds, there was a significant improvement in body performance indicators when compared to the control group. Amaefule *et al.* (2019) reported that broiler mortality, feed intake, feed conversion ratio, and body weight increase were all

Table 2. Effect of thyme and ginseng on body weights of commercial broiler chickens.

Groups	7 days	14 days	21 days	28 days	35 days
Gp1	283 ± 8.72^{ab}	430 ± 9.86^{b}	749.5 ± 15.37^{b}	1,299.37 ± 49.36^{b}	2,170.8 ± 146.66^{c}
Gp2	274.8 ± 8.49^{ab}	395.7 ± 7.92^{c}	728 ± 18.80^{b}	1,289.8 ± 30.05^{b}	2,181 ± 162.63^{c}
Gp3	301 ± 8.97^{a}	486.5 ± 15.14^{a}	841.37 ± 28.48^{a}	1,358 ± 44.68^{a}	2,300 ± 91.61^{a}
Gp4	295 ± 2.95^{ab}	476 ± 9.30^{a}	815.5 ± 17.79^{a}	1,391 ± 50.80^{a}	2,350 ± 100.01^{a}
Gp5	270.5 ± 8.69^{b}	440.7 ± 15.78^{b}	795.75 ± 22.25^{a}	1,394.7 ± 40.43^{a}	2,341 ± 132.5^{a}
Gp6	299 ± 15.67^{a}	425.5 ± 6.34^{bc}	823.8 ± 28.23^{a}	1,406.5 ± 34.62^{a}	2,331 ± 64.73^{a}
Gp7	287 ± 4.38^{ab}	445.5 ± 5.94^{b}	810 ± 12.21^{a}	1,355.6 ± 20.61^{a}	2,280 ± 27.06 ^{b}
Gp8	274 ± 5.94^{ab}	429.7 ± 12.14^{b}	795.8 ± 13.62^{a}	1,345.8 ± 27.61^{a}	2,290 ± 127.26^{b}

Data represented as Mean ± SEM. The same column not followed by the same letter differs significantly ($p < 0.05$).

Table 3. Effect of thyme and ginseng on body weight gains of commercial broiler chickens.

Groups	7 days	14 days	21 days	28 days	35 days
Gp1	238.83 ± 8.98^{ab}	148.16 ± 11.99^{bc}	320.25 ± 19.43^{c}	539.88 ± 51.14^{b}	870.50 ± 16.43^{b}
Gp2	230.67 ± 8.56^{ab}	122.20 ± 6.35^{c}	332.25 ± 16.65^{c}	530.87 ± 39.62^{b}	891.38 ± 17.69^{b}
Gp3	257.33 ± 8.76^{a}	184.17 ± 11.30^{a}	354.87 ± 34.69^{b}	517.75 ± 49.23^{b}	941.88 ± 84.28^{a}
Gp4	251.33 ± 3.18^{ab}	180.00 ± 10.61^{a}	340.13 ± 17.93^{bc}	575.88 ± 58.78^{a}	960.63 ± 86.16^{a}
Gp5	226.67 ± 9.05^{ab}	170.67 ± 25.39^{b}	354.00 ± 28.68^{b}	598.00 ± 36.02^{a}	945.13 ± 14.22^{a}
Gp6	255.00 ± 15.71^{a}	125.00 ± 12.76^{c}	398.38 ± 29.58^{a}	582.63 ± 35.99^{a}	925.75 ± 57.52^{a}
Gp7	242.83 ± 4.08^{ab}	159.17 ± 7.97^{b}	364.50 ± 14.17^{b}	545.63 ± 23.60^{ab}	925.13 ± 34.00^{a}
Gp8	231.33 ± 5.49^{ab}	153.17 ± 16.34^{b}	365.13 ± 12.21^{b}	550.00 ± 36.14^{ab}	940.63 ± 139.87^{a}

Data represented as Mean ± SEM. The same row not followed by the same letter differs significantly ($p < 0.05$).

considerably improved when ginseng (4 g and 6 g per 5 kg feed) was added to the diet in comparison to the control group. Similar findings were reported when quails fed a basal diet supplemented with varying amounts of ginseng (treatment groups) were compared to the control group and showed a substantial increase in body weight, body weight gain, and feed conversion ratio (Elnaggar *et al.*, 2022). This growth performance enhancement can be explained by the bioactive components of ginseng, specifically the ginsenosides or saponins that enhance the gut flora. The aforementioned may enhance the release of enzymes and safeguard the mucosa of the gastrointestinal tract, improving feed digestion and utilization (Yildirim *et al.*, 2013; Xie *et al.*, 2020).

Our findings support the administration of thyme oil (Lü *et al.*, 2009; Noruzi *et al.*, 2022). As a result, they claimed that when thyme oil was administered to the birds, the birds' body weight and weight gain increased significantly when compared to the control group. Wade *et al.* (2018) reported a noteworthy enhancement in the body weight and weight gain of birds that were fed a diet that included 100 mg/kg of thyme oil in comparison to the control group. Furthermore, research revealed that broilers fed a food supplemented with 150 and 200 mg/kg of thyme EO under heat stress conditions had considerably higher body weight and weight gain than the control group (Rafat Khafar *et al.*, 2019). Furthermore, it was discovered that adding 100 mg/kg of thyme EO to the broiler meal considerably increased the birds' body weights and weight growth when compared to the control group (Moustafa *et al.*, 2020). The active ingredients thymol and carvacrol of thyme, which are thought to stimulate digestion in addition to their antimicrobial and antiprotozoal activities that enhance feed intake and food conversion ratio, may be responsible for the increased body weight and weight gain in birds (Alçiçek *et al.*, 2003; Naidoo *et al.*, 2008; Hashemipour *et al.*, 2013; Attia *et al.*, 2017).

NDV-antibody titers increased in this research when broiler chickens were given thyme or ginseng oil extracts, or both, for 3 days at weekly intervals in addition to NDV vaccination regimens. In addition, 10 days after the challenge, the NDV-vaccinated control group (Gp2) had an average HI antibody titer of 8 log2, but the vaccinated groups treated with ginseng, thyme, or a combination of both had an increase to 9.5, 10, and 9.4 log2, respectively. In addition to NDV-HI Ab titers, these results are consistent with those of (Zhai *et al.*, 2011), who discovered that hens administered ginseng stem-and-leaf saponin (GSLS)

exhibited substantially increased levels of intestinal intraepithelial lymphocytes, lymphocyte proliferation test, and IgA+ cells. Consequently, when GSLS was administered orally via drinking water at a rate of 5 mg/kg for a duration of 7 days, systemic and intestinal mucosal immunity in poultry that had received the ND vaccine was significantly improved. Likewise, improvements were observed in several biomarkers when gensenoid-Rg1 was consumed orally at a rate of 300 mg/kg body weight (Song *et al.*, 2021). These biomarkers included the immunological organ index, serum IgG, IgM, and IgA, complement C3 and C4, and anti-inflammatory cytokines (IL-1, IL-2, and IL-10; IgA secretion from the intestinal mucosa). Furthermore, GSLS restored humoral Ab responses to the bivalent vaccine, stimulated splenocyte proliferation, and increased the number of IgA+ cells in cyclophosphamide-immunocompromised chickens 7 days before inactivated bivalent NDV and AIV vaccine vaccination (Yu *et al.*, 2015). An upregulation of IgM+, IgG+, and IgA+ plasma cells in the harderian glands, along with NDV-specific HI and IgA, was detected when selenium was added to the GSLS (GSLS-S) in conjunction with live NDV vaccination (Ma *et al.*, 2020). The immunomodulatory effects of GSLS-Se can be enhanced by activating the signaling pathways for toll-like receptors and mitogen-activated protein kinase. Heo *et al.* (2016) found that ginseng enhances the immunological response of T helper (Th) cells by increasing the production of cytokines by Th1 (including IFN, TNF, and IL-2) and Th2 (including IL-4, IL-10, and IL-13) cells. Furthermore, ginseng has been shown to possess potent immune-stimulating, anti-inflammatory, and antioxidant properties in numerous studies (Yu *et al.*, 2015). These findings of thyme oil are consistent with those of El-Shall *et al.* (2020), who found that applying the ND vaccine in conjunction with the Eos blend (thymol, carvacrol, and eucalyptol) boosted the HI antibody titers in comparison to the group that received NDV vaccination alone. According to Hashemipour *et al.* (2013), thyme oil contained two main active components namely, thymol and carvacrol, which enhanced the hypersensitive reaction and reduced the ratio of heterophil to lymphocyte in a dose-dependent way. Thyme can improve the antioxidant status by increasing serum SOD activity, lowering glutathione concentration, and lowering malondialdehyde concentration. It can also boost the immune system by increasing immunoglobulin concentrations (Abd El-Hack and Alagawany, 2015). The recommended dosage of thyme for basal layer diet is 3, 6, or 9 g/kg. Furthermore, in chickens offered a supplement to their basal diet, the spleen lymphocyte count was considerably increased by either adding formic acid (5 g/kg) or thyme alone (1 g/kg) (Ragaa *et al.*, 2016).

However, since the age of 21 days, neither of the non-NDV vaccinated groups has had an H.I. antibody titer greater than 2 log2. In this study, birds that were given either ginseng oil or thyme orally along with NDV vaccination (Gp 4 and 6) showed 100% protection from mortality, while the group of birds that received both ginseng and thyme at the same time as NDV vaccination (Gp 8) showed 90% protection from mortality, with mortalities starting between days 5 and 8 after the challenge. In addition, in NDV-challenged birds, administration of thyme and ginseng oils, or a combination of them, without NDV vaccination (Gp 3, 5, 7) resulted in 40% protection. It is thought that ginseng has antiviral properties via a variety of mechanisms. By inhibiting the inflammatory mediators IL-6, IL-1, IL-8, TNF, and ROS, it exhibits anti-inflammatory properties. In addition, it blocks the entry of viruses into cells by binding to the HA receptor. activates CD4+ and CD8+ T lymphocytes, which in turn stimulates the production of IFN and causes the infected cells to undergo apoptosis (Nguyen and Nguyen, 2019). Ginseng is able to demonstrate greater resistance against virulent challenges thanks to these activities. In addition, (Zhu *et al.*, 2014) reported that thyme EOs, at dosages of 0.1 and 0.25 mg/kg, could enhance protein metabolism, increase lipolysis, and strengthen the immune system in broiler chickens. The antiviral properties of thyme EO are said to arise from its interference with the virus envelope and its ability to conceal viral components necessary for attachment, penetration, or entry into host cells. Thyme EO was used to treat NDV in Ovo, which reduced NDV replication by more than 56 times (Rezatofighi *et al.*, 2014). The aforementioned studies provided a clear explanation for thyme oil's superior protection against virulent NDV challenges. Four days after the vNDV challenge, the antiviral properties of ginseng or thyme oils were amply established by the reduction of virus shedding via the respiratory or tracheal routes. The latter amply demonstrated how the antiviral, immunostimulant, antioxidant, and anti-inflammatory properties of thyme and ginseng EOs might protect against virulent NDV challenges. These findings concur with those of El-Shall *et al.* (2020), who reported that giving thymol and carvacrol to birds resulted in a considerable reduction in the titer of viral shedding, an increase in clinical protection, and a decrease in the pathological image.

Conclusion

According to the study's findings, giving thyme and ginseng oils to broiler animals improves production performance parameters, such as body weight and weight gain, boosts humoral immunity as evidenced by an elevated NDV HI antibody titer, and significantly protects against virulent NDV challenge. Specifically, the vaccinated groups show 100% protection from mortality, while the nonvaccinated groups show 40% protection. The swabs from the nonvaccinated groups show decreased virus shedding, while the vaccinated groups show complete disappearance of virus shedding.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Author contributions

OH titrated the vaccines, supervised sample collection, and performed PCR and HI tests. HK and SA were involved in the preparation of thyme and ginseng emulsion and supervised sample collection. AE was responsible for experimental animal care and performed PCR and HI tests. All Authors: Participated in the writing and revision processes.

Date availability

The data supporting the conclusions of this study are available upon reasonable request from the corresponding author.

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