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The immunomodulatory effects of ginger extract on hematological indices and humoral immune response in ostriches vaccinated against Newcastle disease

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Abstract Since current Newcastle disease vaccination programs are not able to completely control the virus, the resulting mortality seriously harms the economy of ostrich farming, so the development of methods to protect ostrich chicks against this virus is necessary. Among these methods, it is important to enrich the diet of ostrich chicks with compounds that can promote the humoral immunity of ostrich chicks. *Zingiber officinalis*, having biologically active compounds, has been to have modulating effects on the immune system. The aim of this study was to investigate the effect of ginger extract on the immunogenicity of the Newcastle vaccine in one-month-old ostrich chicks. Thus, a total of 40 one-month-old ostriches were randomly divided into four groups of 10 ostriches each (with five repetitions). They were kept under the same management, breeding, and nutritional conditions. The first group, as a control group, received only the Newcastle vaccine and did not receive the ginger extract. The second group received 1 gram of ginger extract daily for 3 consecutive days prior to receiving the Newcastle vaccine. The third group received ginger extract 3 days after receiving the ND vaccine. The fourth group received ginger extract continuously from 3 days before vaccination to 3 days after vaccination. One-way ANOVA analysis of the resulting data showed that the changes in HI antibody titer in ostrich chickens between groups 1 and 2 are not significant, but groups 3 and 4 had statistically significant HI antibody titers compared with control group. Therefore, it can be concluded that the consumption of ginger at the rate of 1 gram per day for 3 consecutive days after vaccination or 6 days (3 days before and 3 days after vaccination) can increase the immunogenicity of Newcastle vaccination in ostrich chicks.

Introduction

Newcastle disease (ND), caused by the Newcastle disease virus (NDV), is a highly contagious viral infection that affects a wide range of bird species, including ostriches. NDV belongs to the *Paramyxoviridae* family and can cause significant economic losses in the poultry industry [1]. In the ostrich, ND manifests as a severe respiratory and neurological disease. Affected birds may show clinical signs such as coughing, sneezing, facial swelling, nasal discharge, and reduced egg production. In addition, NDV can cause neurologic manifestations, including paralysis and tremors [2]. Transmission of NDV in ostriches occurs mainly through direct contact with infected birds, as well as through contaminated feed, water, and equipment. The virus can spread rapidly in a herd and lead to high mortality (3). Control measures

for ND in ostriches include strict biosafety protocols, rapid detection, and quarantine measures to prevent the spread of the virus [1]. Vaccination is a main tool in the prevention of ND. Vaccines, including live attenuated and inactivated strains, induce protective immunity in ostriches and reduce disease severity. However, choosing the right vaccine and administering it correctly is essential to ensure its effectiveness [4]. Efforts to manage and prevent ND in ostriches require a comprehensive understanding of virus biology, transmission dynamics, and host immune responses. Regular surveillance, early detection, and rapid response are very important in containing outbreaks and minimizing economic losses [5]. Research to develop more effective vaccines and antiviral treatments continues to help reduce ND in ostriches and other avian species [1].

Many evidences show that ND is an epidemic disease in Iran [2]. Therefore, if proper hygiene and vaccination are not observed, the occurrence of this disease is inevitable [2]. If the disease is observed, drug treatment and help to recover the affected birds is very important. There is no effective combination for definitive treatment, but sometimes scientific and personal experiences help treat this disease [4]. In this regard, medicinal plants with biologically active compounds are suitable candidates for treatment. These compounds are not toxic and do not leave harmful substances in meat and eggs. Therefore, in order to produce poultry without using antibiotics and harmful chemical compounds, these compounds have been taken into consideration [6].

Ginger (*Zingiber officinalis*) is a flowering plant from the *Zingiberaceae* family. This plant is native to Southeast Asia, but it is currently cultivated in various tropical and subtropical regions around the world for its medicinal and aromatic properties [6]. Ginger has been used for centuries in traditional medicine systems such as Ayurveda and Traditional Chinese Medicine for its potential health benefits [7]. The underground rhizome of *Zingiber officinalis* is the most commonly used part of the plant. It contains bioactive compounds such as gingerol, shogaol, and zingerone, which are responsible for its characteristic flavor and potential medicinal properties [8]. Ginger is known for its anti-nausea effects and is often used to reduce nausea, morning sickness during pregnancy, and chemotherapy-induced nausea [6]. It has also anti-inflammatory and antioxidant properties, which may contribute to its potential role in supporting overall health [9]. Extensive studies have been conducted on the effects of ginger on growth indicators, antibacterial, antiviral and antioxidant properties in different poultry species [7, 8]. In the recent study, the immunogenic properties of this plant are evaluated after the administration of the ND vaccine in ostriches. Therefore, the aim of the recent study is to evaluate the use of ginger as an immune response enhancer in vaccination program.

Materials and Methods

This study was approved in ethical committee of Veterinary Medicine Faculty in Shahrekord branch, Islamic Azad University (IR.IAU.SHK.REC.1402.028). A total of 40 one-month-old ostriches were randomly divided into 4 groups with 5 repetitions (each repetition 2 ostriches) and kept under the same management, growing, and nutritional conditions. For evaluation of ginger effect on ND vaccine immunity, the inactivated ND vaccine was prepared from Avivac co., Russia. The first group, as a control group, received only the ND vaccine and did not receive the ginger extract. The second group received 1 gram of ginger extract daily for 3 consecutive days prior to receiving the ND vaccine. The third group received ginger extract 3 days after receiving the ND vaccine. The fourth group received ginger extract continuously from 3 days before vaccination to 3 days after vaccination. The groups receiving ginger extract received 1000 mg of dry standard ethanolic extract of ginger (Paradis Pharmaceutical Co., Iran) every day via daily drinking water, orally. The blood samples were prepared 10 days after vaccination with and without anticoagulant. Blood samples with anticoagulant were used to check hematology indicators comprising total cell blood count (CBC), Hemoglobin (HB) concentration, packed cell volume (PCV), Mean corpuscular

volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The samples without anticoagulant were used to prepare serum and measure the humoral immune response against ND vaccine. For evaluation of immune response, the HI (Hemagglutination Inhibition) titer was achieved based on 4 HA (Hemagglutination) unit with conventional method. Each hematological indices and HI titer against ND vaccine in the control group were compared with the intervention groups.

Statistical Analysis

Quantitative data were analyzed by SPSS statistical program (version 22.0) (SPSS Inc., USA) and one-way analysis of variance (ANOVA) method, and if there is a statistical difference, the P value is determined by Tukey's test. The level of significant difference was considered $P<0.05$.

Table 1. The hematological and HI titer in the studied ostriches

Parameters	Group 1	Group 2	Group 3	Group 4
NDV titer (Log_2)	4.13 ± 0.48^{c}	4.32 ± 0.28^{c}	5.12 ± 0.15^{b}	5.65 ± 0.11^{a}
WBC ($\times10^3/mm^3$)	15.05 ± 0.30^{b}	15.23 ± 0.45^{ab}	15.73 ± 0.30^{ab}	16.20 ± 0.40^{a}
Lymphocyte ($\times10^3/mm^3$)	2.93 ± 0.14^{b}	2.92 ± 0.12^{b}	3.16 ± 0.09^{b}	3.64 ± 0.04^{a}
Heterophil ($\times10^3/mm^3$)	11.24 ± 0.52^{a}	11.27 ± 0.50^{a}	11.59 ± 0.74^{a}	11.66 ± 0.94^{a}
Monocyte ($\times10^3/mm^3$)	0.87 ± 0.13^{a}	0.99 ± 0.14^{a}	0.94 ± 0.11^{a}	0.89 ± 0.24^{a}
Platelet ($\times10^3/mm^3$)	21.41 ± 0.40^{a}	21.49 ± 0.43^{a}	20.50 ± 0.70^{a}	20.13 ± 0.48^{a}
RBC ($\times10^6/mm^3$)	1.93 ± 0.028^{b}	1.90 ± 0.025^{b}	1.91 ± 0.018^{b}	2.01 ± 0.015^{a}
Hb (g/dl)	18.13 ± 0.32^{b}	18.70 ± 0.25^{ab}	18.73 ± 0.28^{ab}	19.11 ± 0.25^{a}
PCV (%)	37.43 ± 1.08^{a}	38.17 ± 0.78^{a}	38.33 ± 0.88^{a}	39.23 ± 0.80^{a}
MCV (fL)	194.93 ± 11.53^{a}	201.89 ± 10.42^{a}	200.89 ± 8.78^{a}	195.17 ± 8.23^{a}
MCH (pg)	94.37 ± 5.88^{a}	99.10 ± 5.88^{a}	98.06 ± 4.35^{a}	95.04 ± 4.60^{a}
MCHC (g/dl)	48.41 ± 2.37^{a}	49.06 ± 2.99^{a}	48.81 ± 3.41^{a}	48.68 ± 3.02^{a}

*The different superscript in each rows represents the significant difference between groups ($P<0.05$).

Results

The MCV, MCH, MCHC, number of platelets, PCV, number of heterophiles, and number of monocytes did not show any significant difference among the studied groups.

The number of white blood cells (WBC), the number of lymphocytes and the number of red blood cells (RBC) in the group receiving ginger extract before and after vaccination was significantly higher than the control group ($P<0.05$) (Table 1).

The results show that the HI titer against ND vaccine in ostriches receiving ginger extract before and after vaccination is significantly higher than the groups that received the extract before or after vaccination ($P<0.05$). In all experimental groups, the HI titer against ND vaccine is significantly higher than the control group ($P<0.05$).

Discussion

The results of this study demonstrated that the application of ginger extract along with ND vaccination can be effective in promoting the immune response against the vaccine. The immune response against ND vaccine when ginger extract is received before and after vaccination is significantly higher than when it is received only before or only after the vaccine. Considering the increase in the production of antibodies against the ND vaccine and on the other hand, the increase in the number of white blood cells and lymphocytes, we can emphasize the immunogenic role of ginger extract through the stimulation of humoral and cellular immunity. Although no similar report has been found regarding the role of ginger in promoting the immune response in ostriches, the results obtained from the present study are consistent with previous studies that investigated the immunogenic role of ginger extract in poultry [7, 8, 10]. In this regard, in a study, consumption of 5 and 10 g/kg ginger rhizome powder per day in broiler chickens for 35 days increased humoral immunity by increased response to ND vaccine (10). Also, in another study, the use of 100 and 200 mg/kg of ginger extract for 42 days in broilers stimulated innate immunity by increasing the phagocytic capacity of heterophils and stimulated humoral immunity by increasing antibody production [7]. In addition, in another study in quail, the consumption of 2.5 g/kg ginger powder in the diet for 28 days improved the immune response and microflora of the digestive tract [8]. Herbal compounds have a positive effect by releasing antioxidant substances and protecting immune cells against oxidant agents in the process of immunogenesis and prevent the destruction of immune cell walls against oxygen free radicals [6]. Furthermore, the antibacterial properties of this plant prevent the increase in the number of bacteria and the production of toxic metabolites of bacteria in the digestive tract environment, which minimizes the production of bacterial toxins and the effects of oxidative stress caused by it [7]. In addition, there are several studies that state that ginger directly affects immune cell lines and stimulates immune cells that produce immunoglobulins. In this regard, in a study, they showed that gingerol at a concentration of 10 mg/kg increased the host T helper and T helper-17, as well as the expression of IFN-γ and IL-17 and activated the p38 MAPK signaling pathway [9, 11].

Conclusion

In conclusion, considering the spread of NDV in different regions of Iran and in different species of birds, including ostriches, it seems that the use of ginger extract, as a compound to enhance the immune response, from 3 days before to 3 days after vaccination against ND can provide a favorable immune response and play an important role in controlling this disease.

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Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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