

Original Article

Effect of Oregano Essential Oil Combined with Live and Killed Newcastle Disease Vaccines on Immune Response in Broilers Chicks in Erbil, Iraq: A Comparative Study

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Abstract

This trial was conducted to evaluate the effects of oregano essential oil supplementation at the concentration of 50 mL/1000L of drinking water on immune response and growth performance in broiler chickens. A total of 390 one-day-old Rose chickens were housed together in six experimental boxes (n=65 each), including non-vaccinated groups and non-vaccinated oregano treated groups. Group A: vaccinated with LaSota vaccine via eye drop at 5-, 15-, and 28-day-old adding oregano essential oil in drinking water 1 day post-vaccination. Group B: vaccinated with LaSota vaccine via eye drop at 5-, 15-, 28-day-old only without oregano essential oil. Group C: vaccinated with inactivated Newcastle disease (ND) vaccine at 5-day-old via subcutaneous (SC) injection one dose adding oregano essential oil in drinking water 1 day post-vaccination for 3 days. Group D: vaccinated with inactivated ND vaccine at 5-day-old via SC injection one dose only without oregano essential oil. Group E: non-vaccinated as the control group. Group F: non-vaccinated and adding oregano essential oil for 3 days in drinking water as the control group. The effect of oregano essential oil in drinking water combined with two forms of live and killed ND virus vaccines on the immune responses and serum proteins was investigated in broiler chickens. The results showed the significant effect of oregano on the immune response of chicks in groups D and C that were vaccinated with the killed vaccine at 23- and 33-day-old. Based on the findings, a peak was recorded at 38-day-old with a significant difference ($P<0.05$) in vaccinated and non-vaccinated groups, as well as a significant increase in 38-day-old for group C ($P<0.05$), compared to other groups regarding the oregano effects on the immune response of groups. In addition, an increase in the serum protein was significantly different ($P>0.05$) in the vaccinated broiler chicks, and serum globulin showed a quantitative increase in the vaccinated broiler chicks, compared with non-vaccinated groups. However, the results showed that the effect of oregano was non-significantly different ($P>0.05$) and it had no beneficial effect on growth performance. The results of this study showed the effects of commercial Oregano supplementation (50 mL/1000L drinking water), which increased serum total protein, including globulin, and provoked the best immunological response to ND vaccinations in broiler chickens. More research is needed to develop a state that may beneficially affect the health and uniformity of chickens.

Keywords: Newcastle disease vaccine, Supplement oregano essential oil, Immune response, Serum proteins, Chicken

1. Introduction

Newcastle disease virus (NDV) is a worldwide spread disease and is caused by a virus of the genus *Avulovirus*, belonging to the family *Paramyxoviridae*, a

subfamily of *Paramyxovirinae* (1). Various vaccination programs have been implemented to mitigate the effect of ND on local chicken production in Iraq (2), including management practices that can reduce the

exposure of chickens to the disease; however, these measures have been ineffective mostly because of the production system used in the rural areas (3). Vaccination is one of the most effective ways of minimizing the outbreak of ND (4). Vaccination is meant to offer protection to an animal by triggering the production of antibodies to specific antigens. Several types of vaccines are available and used; however, the most successful and widely used ones are the mild live vaccines known as Hitchner B1 and LaSota (5).

The vaccines can be used by drops into the eye and through drinking water. In numerous viral infections, humoral and cell-mediated immune responses play a role in protecting against such diseases. Both humoral and cell-mediated immune responses are essential for complete protection (6).

Vaccines can be administered using either mass vaccination methods or individually. Eye-drop application is an individual method that produces more consistent protection in more birds than mass vaccination methods (7). Eye drop vaccination stimulates the Harderian gland to produce specific local antibodies and significantly increases plasma cells in the sections of the Harderian gland (8).

Essential oils and their main components have been investigated in the last decades, particularly in relation to their antimicrobial, anti-inflammatory, antioxidant, and anticancer activities that were recently reviewed (9, 10). Researchers use wild herbs, especially from the *Lamiaceae* family, such as oregano and lavender, in the treatment of various illnesses (11). *Origanum glandulosum* Desf. is an endemic plant in the African-Mediterranean countries (12, 13). This plant is used by local populations in traditional medicine to cure numerous diseases and is also commonly used as a skin powder in traditional bathrooms (12, 13). *Glandulosum* is rich in essential oils that have antibacterial, antifungal, insecticidal, antioxidant, antithrombin, antimutagenic, angiogenic, antiparasitic, and antihyperglycemic properties (12, 14, 15). In the literature, thymol, carvacrol, γ -terpinene, and p-cymene are reported as the main components of the oregano

essential oil (13, 15). These chemicals have received increasing interest, particularly carvacrol, due to their various biological activities, including antibacterial and antifungal, antispasmodic effects, and acetylcholinesterase inhibitory activity. As the uses of antibiotic feed additives and treatment have been discontinued or reduced in certain countries in the animal husbandry industry, the search for new alternatives for antibiotic growth promoters has been and will continue to be one of the interesting areas of research for several years. The current study aimed to investigate the effect of commercial oregano antibody titer in pre- and post-vaccination broiler chicks and establish the basis for understanding immunological changes in post-vaccination.

2. Materials and Methods

2.1. Chemicals

Oregano is a liquid solution manufactured by Olebiotec company, France. It is supplemented in drinking water at a dose of 50 mL per 1,000 L.

2.2. Animals and Study Design

A total of 390 one-day-old Rose chickens were obtained from Sardam hatchery in Erbil, Iraq. The chickens were housed together in six experimental boxes (n=65 each), located in Sydan Private Poultry farm in Erbil. The experimental procedure was performed from April to May 2021. The vaccination program was as follows: Newcastle Disease Vaccine, B1 Type, as an eye drop for vaccinating groups A, B, C, and D on the 1st day, followed by a booster dose with inactivated ND Vaccine by subcutaneous (SC) route in the neck at 5-day-old for groups C and D, and live ND vaccine by eye drop route at 15-day-old and 28-day-old for groups A and B in accordance with the instructions of the manufacturer. ND LaSota attenuated vaccine (when using 4HAu antigen titer 1/64 to 1/128) was administered through eye drop routes for the two groups A and B, while the inactivated ND vaccine (Pasoc Co., Iran) was used through SC injection for the two groups of C and D. The two groups of E and F were left as the control.

Group A: vaccinated with LaSota vaccine via eye drop at 5-day-, 15-day, and 28-day-old adding oregano essential oil in drinking water one day after the vaccination;

Group B: vaccinated with only LaSota vaccine via eye drop at 5-day-, 15-day-, and 28-day-old without oregano essential oil,

Group C: vaccinated with inactivated ND vaccine at 5-day-old via SC injection one dose adding oregano essential oil in drinking water one day postvaccination for three days;

Group D: vaccinated with inactivated ND vaccine at 5-day-old via SC injection one dose only without oregano essential oil;

Group E: non-vaccinated as the control group; and

Group F: non-vaccinated and adding oregano essential oil for 3 days in drinking water as the control group.

Dietary and environmental conditions were identical in each of the six groups. They were placed in separate boxes provided with litter in the form of wood shaving. The chicks were supplied with a basal scientific diet for commercial broiler chickens.

2.3. Blood Collection

In each group, 10% of chickens were subjected to blood sampling (1-2 mL) on the first 5 days, as well as blood samples obtained from the brachial vein on days 15, 28, and 39 (16). The collected blood samples were allowed to clot at room temperature; subsequently, they were labeled and transported immediately in a cool box to the laboratory. In the next stage, the samples were centrifuged at 2,500 rpm for 15 min, and the sera were collected in new tubes and stored at -20°C for further analysis.

2.4. ELISA

Antibody titers against NDV were determined in the study. The levels of antibody titers in chicken serum were determined by a diagnostic enzyme-linked immunosorbent assay kit (Biochek, ND kit, code: CK 116, Goudo, Holland) as described by the manufacturer's instruction.

2.5. Statistical Analysis

Statistical analysis of data was expressed as mean \pm standard error. Data were compared and determined by Duncan's Multiple Range Test. Data processing was performed using the SAS statistical software (2005), version 9.1 (SAS Institute Inc., Cary, N. C., USA) (17).

3. Results and Discussion

The preventive instruction on the use of antibiotics as feed additives has led to searching for alternative feed additives, such as phytogetic herbs and essential oils. Commercial oregano essential oil may have more than one mode of action, including the secretion stimulation of digestive enzymes, endocrine stimulation, antimicrobial activity, antiviral activity, coccidiostat activity, immune stimulation, and anti-oxidative activity (18). The efficacy of essential oils has been reported to be affected by the dose of essential oil, concentration of active components of essential oils, infection, diet composition, and environment (19, 20).

Table 1 tabulates the results of oregano (essential oil) on the immune response of broiler chicks after vaccination with killed and live attenuated LaSota vaccine against NDV. The recorded data showed that the maternal antibody titer at the age of 3-day-old was 8301.5 ± 1325.87 . The decline in antibody titer was obtained at 656.17 ± 46.32 recorded at 13-day-old of age. The reason for this decline was explained by Tizard (21) who mentioned that a decrease in antibody titers occurred due to the neutralization of the vaccine virus by circulating maternal antibodies. Moreover, this reason, along with a decrease in the level of circulating antibody response for a long duration in the control group, was reported earlier in a study by Waheed, Siddique (22). The immune response, as antibody titer, determined in this study showed a significant effect of oregano on the immune response of the cases in the C and D groups that were vaccinated with the killed vaccine at 23- and 33-day-old. The antibody titer started to increase on the 23rd day, inducing efficient and long-lasting immunity and recorded a peak on the

38th day with a significant difference in vaccinated and non-vaccinated groups, as well as on the 38th day for the group C, which significantly increased, compared with other groups. The induced immunity was maintained in broiler chickens up to the marketable age. The effects of oregano on immune response using live attenuated LaSota vaccine in group A was significantly increased, in comparison to the group B and control groups. To the best of our knowledge, few studies have reported the better protection of inactivated ND vaccine than live ND vaccine (23), as well as showing that the slow release of antigen provides high and long-term immunogenicity against ND (24). The results of a study conducted by Botsoglou, Christaki (25) showed an improvement in the immune responses of chicks because of the assured antioxidant, antibacterial, and

antiviral activities of carvacrol, which was consistent with the findings of numerous studies. According to the results of a study by Lillehoj, Kim (26), feeding birds with diets containing carvacrol and thymol significantly improved the immune response in chickens and lowered poultry infectious diseases. Likewise, Hashemipour, Kermanshahi (27) reported that feeding birds with diets containing carvacrol plus thymol linearly increased the primary and secondary immune responses. Moreover, Acamovic and Brooker (28) and Andrade and De Sousa (29) reported the immune-stimulating activity of thymol and oregano with respect to the system of mononuclear phagocyte system, cellular and humoral immunity, and the activity of thyme and carvacrol could improve the immune functions through acting as antioxidants and extending the activity of vitamin C.

Table 1. Mean±SE of post-vaccination ELISA antibody titer in the serum of chicks of different ages, compared to the control group and oregano essential oil supplement-administrated group

Antibody titer (mean±standard error)					
Factors	3 day	13 day	23 day	33 day	38 day
Overall mean	8301.5±521.88	905.94±62.42	3113.14±628.95	2822.19±511.45	3686.11±533.67
Levels	NS	**	**	**	**
Group A (Live vaccine+oregano)	8301.5±1325.87 ^a	1130±66.33 ^a	1253±204.8 ^b	2075.14±642.79 ^b	3739.5±801.45 ^c
Group B (Live vaccine)	8301.5±1325.87 ^a	974.5±86.76 ^a	1568.43±644.97 ^b	2845.43±1358.85 ^b	1177.33±53.88 ^d
Group C (Killed vaccine+oregano)	8301.5±1325.87 ^a	1178.17±134.48 ^a	5968±982.38 ^a	5791.57±1414.56 ^a	8336.17±360.88 ^a
Group D (Killed vaccine)	8301.5±1325.87 ^a	1160.5±123.69 ^a	8062.71±2124.62 ^a	5702.71±1115.78 ^a	6405±305.57 ^b
Group E (Control)	8301.5±1325.87 ^a	656.17±46.32 ^b	553.86±1451.45 ^b	124±22.41 ^b	687.83±253.16 ^d
Group F (Oregano)	8301.5±1325.87 ^a	336.33±6.38 ^c	272.86±98.43 ^b	394.29±277.92 ^b	1770.83±1197.88 ^d

Different letteres of each cells have a significant difference

3.1. Performance (Live Body Weights)

The effects of oregano essential oil supplement on the growth performance of broilers at different ages are presented in table 2. The findings of some studies have shown that the supplementation of oregano essential oil through drinking water at different doses improved broiler performance (27, 30-32). The results obtained from this experimental study showed that the supplementation of oregano essential oil to broiler had a non-significant difference compared with control group in case of live body weight ($P>0.05$) (Table 2) and had no beneficial effect on growth performance. These findings were consistent with those reported in

studies conducted by Botsoglou, Christaki (25) and Lee, Everts (20), indicating that no effect was observed on growth performance and body weight gain; the reason may be related to feeding composition and environmental conditions.

3.2. Blood Chemistry

The serum total blood protein, serum albumin, and serum globulins in control and vaccinated chicks are expressed as mean±standard error for each value (Table 3). The results showed an increase in the serum protein of the vaccinated broiler chicks; however, the serum protein findings revealed that there were significant differences ($P>0.05$) and increases in serum protein in

group D, compared to other groups. Moreover, an increase was observed in the serum albumin in the vaccinated broiler chickens in group D, which was significantly different ($P>0.05$), compared to other groups except for group C. However, the increase was not significantly different ($P>0.05$) in the broiler, and there was a non-significant increase in the oregano essential oil in the control group. The serum globulin showed a quantitative increase in the vaccinated broiler chicks, compared to the non-vaccinated groups (Table 3); similar results were observed in studies conducted by dos Santos Schmidt, Paulillo (33) reporting that the serum proteins elevation was more in chickens that presented higher antibody response, which may be associated with hyperglobulinemia due to conversion to immunoglobulin. In this respect, it is concluded that supplementation of oregano essential oil combined with the killed ND vaccine may give a better immune response and increase serum globulin. Albumin is the most abundant protein found in plasma, and therefore,

hypo-proteinemia is always due to concentration reduction in plasma albumin. The quantitative increase in globulin concentration observed in the present study was in agreement with the reports of studies carried out by Zachary and McGavin (34). Inflammation due to microorganisms leads to a rise in antibodies that are gamma globulins. Serum proteins have been known to play important roles in the invasion of the body by pathogens, such as viruses and bacteria (35), and as a vital substrate for antibody formation (36).

In conclusion, the results of this experimental study showed that commercial Oregano supplementation (50mL/1000L drinking water), increased serum total protein, including globulin, and provoked the best immunological response to ND vaccinations in broiler chickens. Since boosting productive performance and immune system in broiler chickens are highly beneficial, it is recommended that more research be conducted to develop a state that may favorably affect the health and uniformity of chickens.

Table 2. Total weight gain (g/bird) of broiler chickens fed by oregano essential oil supplemented diets

Factors	Performance (g/bird)	
	23 day	40 day
Overall mean	978±6.75 NS	2644.67±38.54 NS
Group A (Live vaccine+oregano)	980±14.14 ^a	2688±78.38 ^a
Group B (Live vaccine)	980±19.75 ^a	2666±41.90 ^a
Group C (Killed vaccine+oregano)	980±9.49 ^a	2570±122.07 ^a
Group D (Killed vaccine)	986±12.08 ^a	2650±92.20 ^a
Group E (Control)	970±23.66 ^a	2774±129.91 ^a
Group F (Oregano)	972±23.54 ^a	2520±80.00 ^a

Different letters of each cells have a significant difference

Table 3. Serum biochemical protein values and globulin level in broiler chickens receiving oregano essential oil supplement in drinking water

Factors	Total serum protein	Serum albumin	Serum globulin	Albumin/Globulin ratio ≈
Overall mean	3.26±0.12 NS	1.18±0.05 **	2.07±0.08 NS	0.57
Group A (Live vaccine+oregano)	3.28±0.20 ^{ab}	1.21±0.04 ^b	2.07±0.17 ^a	0.58
Group B (Live vaccine)	2.89±0.41 ^b	0.98±0.13 ^b	1.91±0.29 ^a	0.51
Group C (Killed vaccine+oregano)	3.52±0.20 ^{ab}	1.23±0.08 ^{ab}	2.29±0.16 ^a	0.53
Group D (Killed vaccine)	3.74±0.06 ^a	1.48±0.08 ^a	2.27±0.04 ^a	0.67
Group E (Control)	2.78±0.11 ^b	1.03±0.03 ^b	1.75±0.14 ^a	0.59
Group F (Oregano)	3.33±0.33 ^{ab}	1.17±0.09 ^b	2.16±0.25 ^a	0.54

Different letters of each cells have a significant difference

Authors' Contribution

Study concept and design: A. A. A.

Acquisition of data: A. A. A.

Analysis and interpretation of data: F. M. D.

Drafting of the manuscript: F. M. D.

Critical revision of the manuscript for important intellectual content: A. I. A.

Statistical analysis: A. I. A.

Administrative, technical, and material support: A. A. A.

Ethics

The study was approved by the Research Ethics Committee of the Kufa University, Kufa, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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