



Influence of Some Herbals on Immunostimulation of Cellular Immunity in Experimentally Ndv-Vaccinated Chicks



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AN EFFECTIVE substitute for the conventional virus control techniques used by the poultry business is the supplementation of herbal plants (cheap, naturally available and less toxic) in chicken diets. Application of them has been associated with notable improvements in the immunity and performance of birds. 180 Cobb chicks were split into six equal-sized groups for the present study. A group A was left as a negative group, two groups (groups B and C) were left as control positive non-vaccinated infected group and control positive vaccinated infected group, respectively. Groups (groups D, E, and F) received an injection of vNDV strain associated with the ND Clone 30 vaccine and live LaSota vaccine and treated with medicinal herbs. The performance of the birds is enhanced by oral administration of *Nigella sativa* (6%), commercial curcumin® (1%), and Oregano sol.®. All infected chicks secreted virus through trachea and cloaca, but the pattern differed among different groups. The chicks in group B showed more significant virus secretion. Similarly, increased expression of cytokine genes promotes immunostimulation action in different groups, leading to the reduction of NDV pathogenesis. When chicks were given Oregano sol.®, there was a greater evident reduction in viral shedding and an improvement in their immune responses.

Keywords: Chicks, Curcumin, NDV, *Nigella sativa* and Oregano sol.®.

Introduction

According to [1], chicken has emerged as a significant source of animal protein in Egypt. This massively profitable sector is threatened by a number of fatal illnesses. The *paramyxoviridae* family of viruses known as avian orthoavulavirus 1 (APMV-1) is the culprit that causes the fatal disease known as Newcastle disease (ND) in birds [2]. The spread of viruses among wild birds, however, poses a serious concern to both poultry and other wild bird populations [3]. It is known that over 250 species of domestic and wild birds exhibit different clinical signs due to viral infection [4]. In poorer nations where the virus is enzootic in chickens that have

received vaccinations as well as those who have not, the number of nations reporting their NDV incidence to OIE is low [5]. Based on clinical signs, it can be classified into three classes: mesogenic, neurotropic, and viscerotropic velogenic. Low mortality, neurological problems, and mostly respiratory indications are among the indications. Egypt experienced a significant velogenic Newcastle disease virus (vNDV) outbreak in commercial birds in 2005 [6]. The virus was the source of high death rates and unexpected deaths [7]. By direct contact with sick birds or materials, an avian avulavirus can infect humans, resulting in influenza-like symptoms like fever, headaches, and malaise or even conjunctivitis [8].

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When they infect domestic chicken species, avirulent avian avulavirus strains, usually velogens and mesogens, become enzootic over most of Africa, Asia, and the Middle East, as well as in some regions of South and Central America [5, 9]. However, virus adaptation may lead to the emergence of new host species that are more susceptible, which could pose a greater threat to both local and global populations [10]. Since the first infection in 1948, NDV has been widely distributed in Egypt, where genotype VIIId is the most common strain. It has been connected to multiple epidemics in Egyptian chicken farms that resulted in large financial losses [11, 12].

Egypt's unique combination of free-roaming, domesticated, and migratory birds may allow for both the potential for APMV-1 mutation development and direct transmission to backyard populations and commercial flocks [13]. Due to its three continents and importance as a stopover for migrating birds leaving Europe in the spring and autumn, Egypt is a great place to see migratory birds. Approximately two million birds migrate through it each year, making it the second-most significant bird migration site in the world [14]. Despite widespread immunization campaigns for both backyard and commercial chicken flocks, Egypt has had numerous catastrophic epidemics, which have caused significant socioeconomic losses [13]. Inadequate biosecurity protocols may potentially jeopardise the poultry sector by augmenting the likelihood of disease transmission via wildlife-poultry interaction or contaminated feed [15].

The current urgency of developing genotype-matched vaccinations is highlighted by the ability of the commercially available ND vaccines to only prevent clinical symptoms rather than virulent heterologous viral shedding since they were created using NDV isolates that were genetically different from field strains [16]. The type of vaccination program is determined by the level of field viral intensity and herd immunity. Reducing and eventually eliminating the clinical symptoms that result in poor viral shedding and death when paired with severe NDV infection is the primary goal of the ND vaccination. Since a variety of low and high pathogenicity NDV genotypes can be created, vaccination with the inactivated NDV vaccine may be beneficial [17]. Furthermore, it was discovered that the documented failure of the ND vaccination was partly due to genetic and antigenic variations between vaccine strains and wild infectious strains [18, 19].

New, reasonably priced medicines that could help in the treatment or prevention of particular illnesses are crucial for both human health and the chicken industry. In poultry farms, immunomodulation is a

major concern [20]. Plant-based products known as phytochemical feed additives (PFA) are added to animal and human feed with the goal of enhancing health and increasing the release of digestive enzymes [21]. PFA products are widely present in the form of oleoresin, spices, herbs, and essential oils [22]. PAF has been employed in numerous *in vivo* and *in vitro* studies to determine its antiviral, antibacterial, cocciostats, immune-potent, anti-inflammatory, antioxidant, and anthelmintic qualities in both its pure forms and various combinations [23]. According to [24], the ingestion of feed and the stimulation of digestive secretions may be the causes of PFA's beneficial effects on chickens.

Curcuma longa, also known as turmeric, is a PFA and an ancient spice used for colouring that has been used traditionally as a medicinal all throughout the world [25]. Curcumin, a noteworthy component of turmeric, possesses the potent antiviral action previously reported against a range of viruses, including NDV [26, 27]. Additionally, curcumin has been connected to an improved immunological response to infection [28]. Furthermore, as it has been used as a hepatoprotective [29], anti-inflammatory [30], antibacterial [31], antifungal [32], anticancer [33], and antivenom [34].

PFA also has the most valuable historical medicinal seeds: *Nigella sativa*, or black cumin seeds. All around the world, but particularly in South Asian and Middle Eastern nations, it is produced for medicinal uses [35]. Numerous research on *Nigella sativa* (*N. sativa*) have shown how important the plant is for the wide range treatment ailments due to its anticancer, antioxidant, antibacterial, anti-inflammatory, and immune system-stimulating capabilities [36–40]. The bronchodilator, antihypertensive, anthelmintic, antidiabetic, analgesic, and renal protective spasmolytic properties are other benefits [41]. The pulverised seeds of *N. sativa* also improve growth, metabolism, and meat quality in addition to improving layers performance and egg quality [42, 43].

Moreover, oregano possesses anti-inflammatory, antioxidant, antifungal, antiviral, and antiasthmatic properties and is utilized in folk medicine for unsettled stomach, colds, cardiovascular disorders, mental disorders, and general health difficulties [44]. *Origanum vulgare* plants are used to extract oregano essential oils (OEO) from their flowers and leaves. They contain a variety of constituents, the majority of which are carvacrol and thymol, which together account for 78–82% of OEO [45]. Strong antioxidant properties beside the raise of the T lymphocytes differentiation percentage that are CD4+ and CD8+ are potent properties of OEO [46, 47].

The purpose of the current research is to test the hypothesis that *Nigella sativa*, commercial curcumin®, and oregano sol.® could be used to enhance the host immunological response during NDV vaccination, decreasing the viral shedding and subsequently minimizing the mortality rate among birds especially during the rising outbreaks. This hypothesis is based on the benefits of herbal components that have been previously mentioned.

Material and Methods

Experimental chicks and Virus

A total of 180 one-day-old (Cobb) chicks were raised till the end of their fourth week of life, thanks to a local hatchery. Using proven methods for viral isolation (VI) in eggs and hemagglutination inhibition (HI), it was determined that each chicken was serologically naive and free of the Avian Newcastle virus [48]. Apart from that, the chicks were put into six groups, which were called A, B, C, D, E, and F. The Animal Health Institute's birds were kept in separate, wire-floored, high-efficiency particulate arresting (HEPA) isolators using conventional management techniques. The experiment was conducted in compliance with the committee's criteria after the Medical study Ethics committee of Egypt's National Research Centre approved the experimental study plan with number (12710112021). The virus was first identified as velogenic ND virus (vNDV) genotype VII (Chicken/Giza/Egypt/2020) during a field test, and it was assigned the accession number OM243951 [6]. Nine to ten-day-old chicken embryonated eggs were used to titrate viral stocks, and the method outlined

Experimental Design and treatments

As was previously explained by [6], the chicks were segregated into 6 groups (30 birds/group) as following:

- **Group A:** Negative control (without vaccination, supplementation and without challenge).
- **Group B:** Positive control (without vaccination, supplementation, but was infected with the vNDV strain).
- **Group C:** Positive vaccinated control (with vaccination, supplementation, but was infected with the vNDV strain).
- **Groups D, E, and F:** Were inoculated with the vNDV strain and treated with *N. sativa*, curcumin, and oregano solution, respectively.

Water and food were available to all experimental groups at all times during the trial. Groups B, C, D, E, and F received intra-nasal (IN) injections of the vNDV (10^6 EID 50/100 mL/bird) strain during the

in [49] was used to calculate the embryo infectious dose (EID₅₀). The virus was applied at 106 EID₅₀/ml titer. Commercial chick feed had a total protein content of 22% and was made without the use of antibiotics, vitamins, mineral supplements, or toxin binder. Before being employed, aflatoxin, ochratoxin, and zearalenone levels were checked in each batch of the basic feed [50].

Medicinal plants

***Nigella sativa* (NS) Seeds**

We bought the NS seeds from a nearby herbal shop. The National Research Centre in Dokki, Egypt, verified the plant seeds' taxonomic identity. According to [51], the seeds were first thoroughly cleansed with distilled water to get rid of any extra material, and then they were allowed to dry at room temperature in the shade. After being weighed using an analytical scale and processed into a coarse powder using an electronic grinder, they were then fed to the diets at a rate of 6% of the entire ration.

Oregano solution®

It was introduced to the chicks' drinking water (1.25 ml/L), containing glyceryl polyethylene (Glycol ricinoleate) 220000 mg/L water, oregano oil (carvacol) 40000 mg/L, and thymol (Thymus vulgaris) oil 2500 mg/L (CCPA International Company in France) [45].

Commercial curcumin®:

Bio-grade curcumin in vegan capsules (1%) containing 1300 mg of biocurcumin (with 1235 mg of curcuminoides) and 90 mg of biopepper (German company) were added as 1% to the ration.

third week of the birds' lives. During the first seven days following infection (DPI), the fatality rate was noted [52].

Determination of virus shedding

Every day, between 1 and 7 DPI, trachea and cloacal swabs were taken from each chick in order to assess the amount of virus shedding in 1 mL of PBS containing 1% gentamycin. After that, the samples were stored at -80°C until they were evaluated. The QIAamp Viral RNA Mini Kit (QIAGEN) was used for RNA extraction following the manufacturer's instructions. Using the vNDV F gene as the target, quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) was carried out to represent the virus titer in each sample [53]. The virus titer was determined using the mean \pm standard deviation of the virus titer per millilitre of sample.

Quantification of IFN γ gene expression

At 1 to 8 DPI, chicks' spleen samples were obtained and submerged in an RNA lysis solution. RNeasy Mini Ki was used to extract and calculate the total amount of RNA. IFN γ gene expression was quantified with qRT-PCR employing QuantiTect probe RT-PCR. The 28S rRNA probe was utilized as the endogenous control (Table 1). For qRT-PCR, the

Roche Diagnostics, Switzerland light cycler[®] 480, Real-Time PCR equipment was utilized. The operating parameters were as follows: one cycle at 50°C for 30 minutes, 94°C for 10 minutes, and 40 cycles of 94°C for 15 seconds and 60°C for 60 seconds. This ratio was used to analyze the data: CT stands for cycle threshold. ($2^{-\Delta\Delta ct}$) utilizing the Light cycler[®] 480 software, Version 1.5 SW.

TABLE 1. Oligonucleotides primers used in this study

Gene	Primer/ probe sequence (5'-3')	Ref.
NVD (F gene)	F+4839 TCCGGAGGATACAAGGGTCT	[53]
	F-4939 AGCTGTTGCAACCCCAAG	
	F+4894 [FAM]AAGCGTTTCTGTCTCCTTCTCCA[TAMRA]	
28S rRNA	GCGAAGCCAGAGGAAACT	[54]
	GACGACCGATTGTCACGTC	
	(FAM) AGGACCGCTACGGACCTCCACCA (TAMRA)	
IFN- γ	AAACAACCTTCTGATGGCGT	[55]
	CTGGATTCTCAAGTCGTTTCATCG	
	(FAM) TGAAAGATATCATGGACCTGGCCAAGCTC (TAMRA)	

Statistical analysis

The statistical software SPSS 20.0 for Microsoft Excel was utilized to evaluate the data. In order to compare the means across many groups, an ANOVA was utilized where P-values were less than 0.05, were regarded as significant.

Results

For seven days following infection, birds were observed for mortalities, at three days post infection (DPI), the mortality rates for groups B, C, and D were 43.3%, 33.3%, and 23.3%, respectively. However, groups A, E and F did not have any fatalities as previously represented in published data by [6].

Detection of viral shedding by real time RT-PCR:

Before being vaccinated, all chicks tested negative for vNDV by serology and virus isolation in eggs. The vNDV F gene was found in tracheal and cloacal swabs, indicating that all of the hens in the inoculation groups contracted vNDV. Table (2) provides an overview of the NDV shedding results from tracheal and cloacal swabs taken on the third, fifth, and seventh day after the challenge. Nearly all of the tested birds in group B (whose virus shedding ranged from 66.7% to 100%) and group C (whose virus shedding ranged from 55.5% to 77.7%), demonstrated virus shedding. Viral shedding was minimal in Group D, ranging from 11.1% to 66.6%. Only in the fifth DPI, groups D and E displayed the

least amount of viral shedding (ranging from 11.1% to 55.5%).

As can be shown in Figure 3, after infection, tracheal swabs from chicks in groups B, C, D, E, and F showed increased levels of viral shedding ($P < 0.001$). Moreover, tracheal swabs from chicks in groups E and F revealed lower viral titers than those from groups B and C, and this difference was statistically significant ($P < 0.001$). Group B chicks exhibited elevated viral shedding, with a statistically significant difference at 3 DPI ($P < 0.001$).

With regard to cloacal swabs, chicks in group B exhibited a significantly higher correlation ($P < 0.001$) at 5 DPI and 7 DPI compared to those in groups C, D, E, and F following infection. Moreover, at 5 and 7 DPI, there was a substantial ($P < 0.001$) difference in the viral titre in cloacal swabs between chicks in groups D, E, and F and chicks in groups B and C. But compared to tracheal swabs taken after infection, cloacal swabs showed a distinct pattern of viral shedding, with reduced virus shedding in all groups.

The acquired results indicated that, in comparison to group B and C, the virulence of the vNDV virus was decreased in chicks treated with *N. sativa*, commercial curcumin[®], and orego sol.[®] based on RT-PCR data, different groups had different times to peak vNDV shedding in swabs (Figs. 1 and 2), vNDV shedding peaked in the tracheal and cloacal swabs of chicks in group B at 7 DPI. Furthermore, compared to groups B and C, the vNDV fed *N. sativa*, commercial curcumin[®], and orego sol.[®] had a shorter virus shedding duration.

TABLE 2. Results of viral shedding from tracheal and cloacal swabs at the 3rd, 5th and 7th DPI by RT-PCR:

Groups	3 DPI				5 DPI				7 DPI			
	Tracheal		Cloacal		Tracheal		Cloacal		Tracheal		Cloacal	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A	0/9	0	0/9	0	0/9	0	0/9	0	0/9	0	0/9	0
B	8/9	88.9	9/9	100	6/9	66.7	9/9	100	7/9	77.8	9/9	100
C	6/9	66.6	7/9	77.7	5/9	55.5	6/9	66.6	6/9	66.6	7/9	77.7
D	2/9	22.2	4/9	44.4	1/9	11.1	4/9	44.4	5/9	55.5	6/9	66.6
E	0/9	0	0/9	0	2/9	22.2	5/9	55.6	0/9	0	0/9	0
F	0/9	0	0/9	0	1/9	11.1	3/9	33.3	0/9	0	0/9	0

- Total no. of birds examined in each group was 9 birds.
- RT-PCR: Real time polymerase chain reaction with VDNV specific primers.

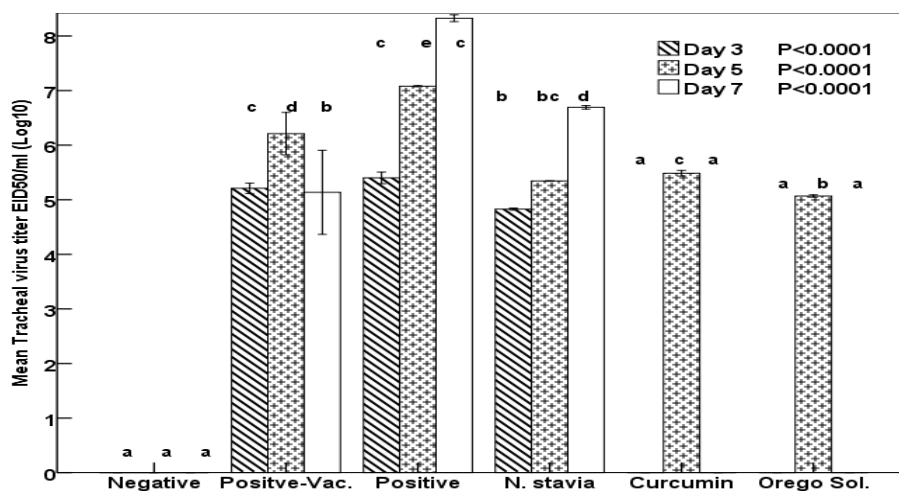


Fig. 1. Mean NDV virus titer values (log₁₀ EID₅₀/ml) found in tracheal swabs 3, 5, and 7 days after NDV inoculation in various groups. Using several groupings, the error bar displays the standard deviation.

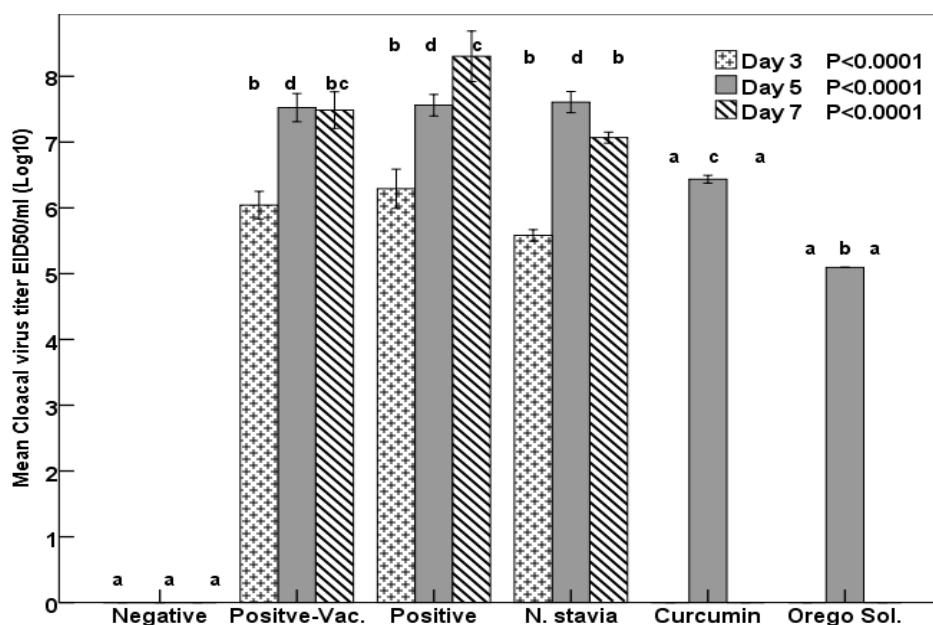


Fig. 2. The mean EID₅₀/ml virus titer levels of NDV were found in cloacal swabs at 3, 5, and 7 days after NDV inoculation in various groups. In several groupings, the standard deviation is displayed via the error bar.

Quantification of Cytokines mRNA:

IFN γ mRNA RT-PCR was used to evaluate the effects of *N. sativa*, commercial curcumin®, and orego sol.® treated groups on cytokine gene expression. Chicks from groups D, E, and F showed

a significant increase ($p < 0.0001$) in their IFN γ mRNA expression levels at 3, 5, and 7 DPI when compared to chicks from positive vaccinated group C and positive non-vaccinated control group B. IFN γ mRNA expression was highest in Group F (orego sol.® treated) (Fig. 3).

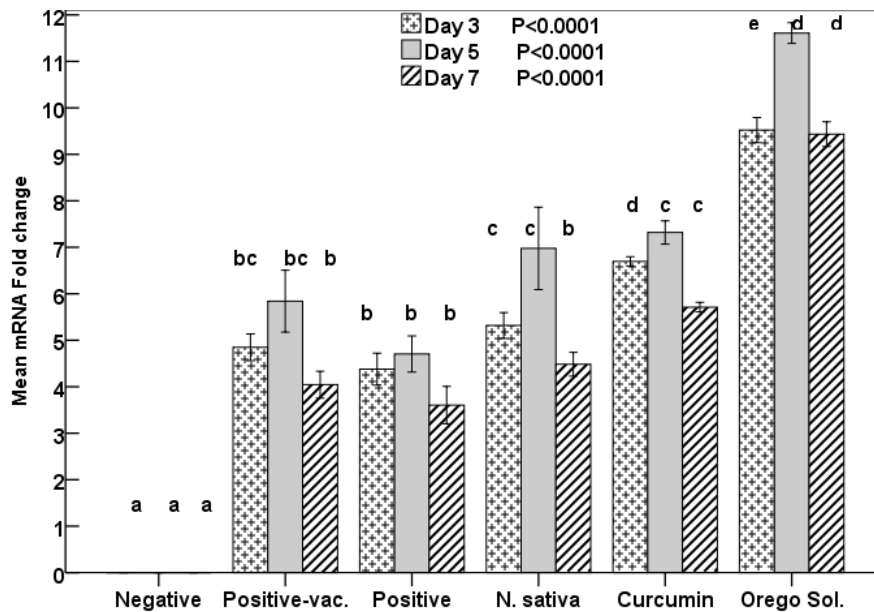


Fig. 3. Error bars indicate the amount of IFN γ mRNA that was extracted from the spleen of chickens in several groups following experimental infection with vNDV and treatment with *N. sativa*, commercial curcumin®, and Orego sol.®. There is a significant difference in superscripts amongst treatments (a, b, c, and d) at ($p < 0.05$).

Discussion

Newcastle disease (ND) is a globally prevalent virus that poses a significant threat to the poultry industry. Its effects on clinical practice and financial losses are both substantial. Additionally, it influences zoonotic behaviour. These results are supported by citations [56, 57]. It's the second most frequent endemic disease in several countries, according to the OIE list A [58]. Due to a multitude of factors, including high mortality rates (up to 100%), decreased productivity, higher costs for disease prevention and control, and trade restrictions in both developed and developing countries where chickens are raised in small household or commercial sectors, ND has a significant impact on the poultry industry [56]. The severity and fatal toll of the ND field challenge might be increased by concurrent diseases as well as by inappropriate immunization administration, storage, and transportation. It is debatable if heterogeneous genotype cross-protection takes place. Due to inadequate vaccination, immune suppression, and viral mutation, recurrent ND virus (NDV) infections can happen even in birds who have received vaccinations [59]. All NDV strains belong to a single serotype, notwithstanding the variety of

virulent genotypes that have been documented throughout the disease's recent history in Egypt. This suggests that a vaccination made from any strain or genotype can produce antibodies in a lab setting, protecting birds from viruses that are extremely deadly and reducing or preventing clinical symptoms and death [17]. Other factors, however, must also be taken into account because they are not determined by serotype and cannot be generated by utilizing inactivated vaccines, such as cellular immunity, which is one possibility available to genotype VII. It is challenging to mount an effective defense against the various NDVs that are in use in Egypt. According to [60], the vaccine may offer good protection (96–100%) against infection with genotype VII of the heterologous Newcastle virus strain and reduce viral shedding in experimental and field immunisation campaigns that comprise NDV genotype II. Nonetheless, in the field, a number of variables could greatly diminish the efficacy of immunization such as: the high rate of NDV infection, even in birds that have received vaccinations, as a result of vaccination errors, viral mutations or changes in the virus's genomic sequence that may result in the existence of multiple

serological variations, immune suppression, and malfunctioning programs that could lead to the loss of cell-mediated immunity. Other things to think about include the possibility of disease transfer from wild or migrating birds to domesticated birds and vice versa, as well as insufficient biosecurity measures. Stricter and stronger biosecurity measures need to be the cornerstone of the solution in order to decrease the amount of environmental viruses and stop their mutation [61].

Many tactics have been used to enhance animal health, productivity, and profitability since ancient times [62]. In the last 25 years, numerous extensive screening programs have been started worldwide to evaluate the antiviral activity of medicinal plants. This has led to the discovery of some traditional medicinal herbs with strong antiviral qualities; some of these herbs have even been used to treat humans and animals that are afflicted [63, 64]. This study examined the lymphatic tissue of chicks that were experimentally challenged with vNDV and given supplements of *N. sativa*, curcumin, and Orego sol. The goal of the experiment was to assess the immunomodulatory and protective qualities of herbals in birds in order to identify potential applications for treating infected humans and birds in the future.

The idea that commercial curcumin®, orego sol.®, and NDV vaccination regimens (LaSota vaccines and clone 30) are superior than *N. sativa* plus, NDV vaccination programs is supported by this finding. The pleotropic cytokine chicken IFN is comparable to its mammalian orthologue in many aspects. Its released molecular weight is roughly 16.8 kDa, owing to its 169 amino acids (aa), which includes a 19aa signaling region [65]. The primary producers are natural killer cells (NK) and tumor cells [66]. It boosts the production of Th2 cytokines (IL-4 and IL-10), improving antigen presentation, antigen and antigen processing, and intracellular pathogen elimination, and inhibits the Th1 response by boosting the expression of MHC I and MHC II. It also controls the activation of macrophages in birds with suppression of viral replication [67, 68]. It has been shown that T-cell release of IFN γ following challenge is a reliable predictor of cell-mediated immunity in chickens after vaccination. Every measurable time point following NDV vaccination showed a substantial rise in IFN γ levels across all treated groups. Increased cytotoxic T-cells of cell-mediated immunity for virus clearance may be linked to elevated IFN γ levels, protecting birds against NDV viral infection. Interestingly, P=0.0001 shows that hens in groups C, D, E, and F expressed more IFN γ mRNA than hens in group B. These findings suggest that the live LaSota strain NDV vaccination and the clone 30 vaccination may have partially or not fully activated cell-mediated immune responses.

N. sativa has been shown to reduce the replication of influenza viruses by boosting T-helper, cytotoxic T, and bone marrow cellularity [69, 70]. Moreover, *N. sativa* has been demonstrated to decrease proinflammatory cytokines, viral proteins (integrase, protease), and RNA polymerase II, all of which are essential for viral replication, and to raise splenocyte proliferation and interferon expression in a dose-dependent manner. These actions all contribute to a reduction in the deleterious effects of viral pathogens [69, 71]. These findings support previous research [51, 70, 72] which demonstrated that cytotoxic T lymphocytes can eliminate the H9N2 virus and stop viral shedding, shielding poultry from early-stage viral infections. It has been observed that vNDV genotype VII infection can cause epidemics in commercial poultry farms. These outbreaks are often acute but can also be sub-acute or chronic, leading to significant mortality [73].

Conclusion

Based on the previously reported results, we may conclude that *N. sativa*, commercial curcumin®, and orego sol.® feed additives have immune-boosting effects against vNDV. Additionally, tracheal and cloacal swabs showed low viral load and high viral clearance associated with stimulation and proliferation of T cells (IFN- γ) by *N. sativa*, commercial curcumin®, and orego sol.®. Moreover, Orego sol.® has a higher potency than feed additives containing *N. sativa* or commercial curcumin.

Conflict of Interest:

No conflict of interest.

Funding statement

No funding has been provided.

Consent to Publish

All the authors have given their consent for publish this manuscript.

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تأثير بعض الاعشاب على تحفيز المناعة الخلوية في كتاكيت محصنه مختبريا ضد فيروس النيوكاسل

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يعد استخدام نباتات الأعشاب (الرخصه، المتوفره طبيعيا، الآمنه صحيا) في علف الدواجن من البدائل الفعاله المستخدمه في صناعة الدواجن للوقايه المعتاده من الفيروسات حيث أن اضافتهم تعد مصحوبه بتحسنت ملحوظه في المناعه وأداء الطيور. في الدراسه الحاليه تم استخدام عدد 180 كتكوت من سلالة Cobb وقد تم توزيعهم على 6 مجموعات متساويه. في المجموعه A تم تركها كمجموعه سالبه، بينما تم حقن الفيروس في المجموعتين B و C هذا وقد تركت مجموعه B بدون تحصين بينما تلقت المجموعه C تحصيلي Clone 30 و live LaSota معا. في المجموعات E و F و D تم حقنهم بالفيروس (عتره شديده) والتحصينات بالاضافه الى علاجهم بالأعشاب الطبيه.

أداء الطيور قد تحسن بعد العلاج بالحبه السوداء تركيز 6% والكرم التجاري تركيز 1% وسائل الزعتر. كل الكتاكيت كانت تقوم باخراج الفيروس عن طريق القصبه الهوائيه وفتحة الاخراج ولكن بمعدلات مختلفه بين المجموعات المختلفه. الكتاكيت في المجموعه B أظهرت أكبر معدلات اخراج للفيروس. بالاضافه الى كل ما سبق زادت معدلات المناعه الخلويه والتحفيز المناعي في المجموعات المختلفه والتي كلما زادت ادت الى تقليل تطور المرض. هذا وقد أظهرت المجموعه التي تناولت سائل الزعتر اقل معدلات خروج للفيروس واعلى معدل تحسن في الاستجابات المناعيه.

الكلمات الداله: كتاكيت، كرم، فيروس النيوكاسل، عشبة حبة البركه، سائل الزعتر.