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Immunogenetic Impact of Infectious Bronchitis Vaccines Live on Newcastle Disease Immunity and Glandular/Tracheal Pathology

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Abstract

Respiratory viral infections, particularly Infectious Bronchitis Virus (IBV) and Newcastle Disease Virus (NDV), remain major threats to poultry health and productivity worldwide. Despite widespread vaccination, co-infection and immunological interference between these live attenuated RNA viruses continue to cause economic losses. This study aimed to evaluate the effects of different NDV–IBV vaccination schedules on immune response, tissue pathology, and growth performance in broiler chickens. A total of 200 Ross 308 chicks were randomly assigned to four groups (ND-IB-1, ND-IB-5, ND-IB-8, and ND-IB2-1) and vaccinated with combinations of NDV Clone 30 and IBV 4/91 (and Ma5) at different ages via the ocular route. Growth performance was monitored up to day 35, and samples were collected for serological (IgY ELISA), cytokine (IFN-γ ELISA), histopathological, and molecular (RT-qPCR) analyses. Results showed that delaying IBV vaccination to day 8 (Group ND-IB-8) significantly improved body weight gain (2107.8 ± 45.2 g), enhanced NDV-specific IgY titers (18115 ± 275 U/ml), and reduced IFN-γ levels (45.6 ± 1.9 pg/ml) compared with earlier schedules. Histopathology revealed minimal tracheal and Harderian gland lesions (score = 1.5 ± 0.3–0.4) in ND-IB-8 birds, while early co-vaccination induced severe epithelial damage. RT-qPCR confirmed the absence of viral RNA at day 35, indicating full vaccine clearance. In conclusion, optimal scheduling, specifically delaying IBV 4/91 vaccination until day 8, minimizes immunological interference, reduces inflammation, preserves respiratory tissue integrity, and enhances overall growth and immune performance. The findings provide a practical framework for synchronizing NDV–IBV vaccination to improve health and productivity in commercial broiler production.

Keywords:

Cytokines, harderian gland, immune response, live vaccines, sialic acid receptor.

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Introduction

Poultry production forms a part of the world's food security, but it is constantly endangered by respiratory viral infections, especially Infectious Bronchitis Virus (IBV) and Newcastle Disease Virus (NDV) (Bhuiyan et al., 2021; Kouakou et al., 2015; Mohamed Suhail, 2025). Both of these RNA viruses are among the most economically harmful avian diseases because of their quick spread, high mutation levels, and devastating effect on productivity and the immune system (Rehan et al., 2019; Suhail & Sufna, 2025; Chan et al., 2021). IBV and NDV result in significant financial losses in the form of lowered growth rate, lower egg yield, lower efficiency of feed conversion, and higher medical and management costs (Rafique, 2018; Shrivastav & Malakar, 2024). The continued use of vaccination programs has not helped the poultry producers, as they still grapple with frequent problems related to these infections in different production areas (Pillai & Panigrahi, 2024; Shimizu et al., 2020).

The viral variants, which have antigenically different characteristics, are emerging incessantly, compromising the effectiveness of existing vaccines. Mutations in the spike (S1) glycoprotein cause widespread antigenic variation in IBV that makes designing cross-protective vaccines difficult (Niewiesk, 2014). Likewise, NDV genetic changes also affect the performance of vaccines and pathogenicity of the virus (Kim & Samal, 2016; Jackwood, 2012). All these, along with the fact that RNA viruses have a high mutation rate, lead to vaccine evasion and partial immunity (Abozeid, 2023; Ike et al., 2021). It then leads to a high number of vaccine failures and high costs due to production losses and the costs of curbing the disease in the global poultry industry (Zandi & Pourtaghi, 2023; Yuan et al., 2011; Abd El-Ghany, 2024). However, to the contrary, as vaccination is the most practical and effective control measure to prevent disease conditions, subsequent or simultaneous infection of live-attenuated NDV and IBV vaccines causes complex immunological systems in the host (Ayyappan, 2025; Bhatia & Ramesh Iyer, 2025). Such interactions are competition over sialic acid receptors, interference between vaccine strains, and regulation of innate and adaptive immune response (Saleem et al., 2024; Lazzaro & Tate, 2022). Research shows that primary vaccination with IBV 4/91 strain causes severe histopathological changes in the trachea, including desquamation of the epithelium and ciliary paralysis that reduce mucociliary clearance and expose birds to secondary infections (Wang et al., 2024). These pathological tissue damages undermine the mucosal immunity and the protective effects of vaccination.

Immunologically, the issue that needs to be addressed to establish an optimal vaccination strategy is the need to balance between adequate protection and immune overactivation (Sanders et al., 2011). The timing of vaccination is an important aspect of this balance because it affects the match between innate and adaptive reactions (Moharam et al., 2019; You et al., 2023; Vaziry, 2010). Premature vaccination at the time of residual maternal antibody interference can lead to neutralization of live vaccine antigens, and hence, lower their immunogenicity (Abd El-Ghany, 2024). As a result, inefficient scheduling will be associated with reduced vaccine efficacy and high economic costs, such as reduced productivity, high mortality rates, high treatment expenses, and trade restrictions following outbreaks (Ali et al., 2018). In view of these issues, the current study aims to examine the immunogenetic, pathogenic, and performance effects of various vaccine regimens of live NDV (Clone 30) and IBV (4/91 variant) in broiler chickens. The experiment evaluates the effect of vaccination timing on humoral (IgY antibody) and cellular (IFN-γ) immunity, tracheal and Harderian gland histology, and growth performance parameters (weight gain and feed ratio) (Malhotra & Iyer, 2024). This is proposed to hypothesize that NDV would be immunologically interfered with, and that delaying the live IBV vaccination

past the first week of life would cause decreased immunological interference with NDV, reduced inflammatory cytokine response, epithelial integrity preservation, and improved immune efficacy and growth performance.

This study contributes to the existing knowledge in the field of immunogenetic balance between NDV and IBV through the timing of vaccination. The results indicate that the NDV-specific immunity is improved by delaying the administration of the IBV vaccine (day 8), which results in less competition at the receptor level of sialic acid binding, less expression of IFN-γ to avoid excessive inflammation, and less tracheal and Harderian gland epithelial lesions. These findings reveal that immunogenetic outcomes of vaccine schedules go beyond protective titers to encompass basic host-pathogen interactions on the health and productivity of tissues.

The article provides a comprehensive evaluation of the immunological, pathological, and economic dimensions of vaccine scheduling. It shows that an optimized vaccination schedule is able to enhance both immune balance and production efficiency at the same time, reduce tissue pathology, and inflammatory stress. On the whole, this research paper helps in developing poultry immunology through offering a scientifically proven vaccination model to synchronize the live NDV and IBV (4/91) immunizations to optimize disease prevention, maintain respiratory integrity, and productivity of poultry systems.

Materials and Methods

Experimental design and housing

A total of 200 one-day-old Ross 308 broiler chicks were obtained from a commercial hatchery and randomly divided into four experimental groups, each comprising 50 chicks. The birds were housed in environmentally controlled floor pens (2.5 m × 3.0 m) at the Poultry Research Unit, College of Veterinary Medicine, University of Baghdad. The pens were kept in a state of cross-contamination, non-contact, and a litter of clean wood shavings was introduced to each pen, 5 cm deep.

The following is how the environmental conditions were maintained: at the beginning of the first week, the temperature was 32°C, and then it was lowered by 2-3°C per week until the final 24°C was reached. The relative humidity was kept at 50-60%. The first week was a period of 23 hours a day of lighting, and later the lighting was increased to 20 hours a day and 4 hours of darkness. A negative pressure ventilation system supplied approximately ten air exchanges per hour.

Chicks were provided with ad libitum access to fresh water and food. There were two commercial diets, a starter feed (21% crude protein) and a grower feed (19% crude protein), and both were created to match the nutrient needs of the NRC (1994). Birds were checked twice per day and feed replenished twice per day to check health, behaviour, and feed consumption during the experiment.

Vaccination Protocol

The vaccination schedules are summarized in Table 1. All vaccines were supplied by Merial and Intervet (France and the Netherlands, respectively) and stored at 4°C prior to use. A calibrated micropipette was used to administer 0.03 mL of vaccine solution via the ocular route for each chick.

Vaccine characteristics

NDV Clone 30 (LaSota genotype II) - a lentogenic live attenuated strain of the LaSota lineage, and is a common primary NDV vaccine. It is characterized by great immunogenicity and a safety profile in young chicks, which gives strong humoral and mucosal immunity.

IBV 4/91 (variant 4/91, or CR88 or 793B) - a live attenuated variant strain of lineage GI-13. It exhibits a high level of antigenic variation over classical strains and has been shown to result in cross-protection in a number of field isolates 793B-like, but has been reported to cause transient tracheal lesions when inoculated at a young age.

IBV Ma5 (Massachusetts-type) - a classical IBV strain (lineage GI-1) that is widely used as a universal vaccination. When used together with variant strains like 4/91, it offers heterologous protection, which leads to wider immune coverage.

This combination of NDV and IBV vaccines was chosen to assess the influence of vaccination timing and strain diversity on immune balance, tissue health, and growth performance.

Table 1. Vaccination schedule outlining the combination, timing, and administration route of live Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) vaccines in experimental broiler groups

Group	Vaccines administrated	Day(s) of Vaccination	Route
ND-IB-1	Clone 30 (NDV) + 4/91 (IBV)	Day 1, Day 14	Ocular
ND-IB-5	Clone 30 (NDV) (Day 1, 14) + 4/91 (IBV) (Day 5, 14)	Specified	Ocular
ND-IB-8	Clone 30 (NDV) (Day 1, 14) + 4/91 (IBV) (Day 8, 14)	Specified	Ocular
ND-IB2-1	Clone 30 (NDV) + 4/91 (IBV) + Ma5 (IBV)	Day 1, Day 14	Ocular

The vaccines came from established pharmaceutical companies, Merial and Intervet, and needed proper refrigeration at 4°C before use. A micropipette with a calibrated volume delivered 0.03 ml of vaccine solution for each chick through its eye (Table 1).

Sample collection

Blood samples were collected from the brachial vein on days 1, 14, and 35 using sterile 1 mL syringes. The samples were spun at 3,000 rpm for 10 minutes to separate the serum stored at −20°C until analysis.

At day 35, five birds per group were euthanized by cervical dislocation following humane procedures. Tissues of the Trachea and Harderian glands (upper third area) were taken and washed with phosphate-buffered saline (PBS), then fixed in 10% neutral-buffered formalin to have histological samples. The electronic balance (with an accuracy of ±1 g) was used to record the individual body weights on days 1, 14, and 35. The mass per pen was used to compute the ratio of feed consumed to pen (feed conversion ratio).

Serological analysis

ELISA IgY: The serum antibody concentration of NDV and IBV was determined with commercial ELISA Ab Tests (IDEXX NDV and IBV Ab test, IDEXX Laboratories, USA). This was done in accordance with the instructions given by the manufacturer, and optical density (OD) was determined at 450 nm under a BioTek ELx800 microplate reader.

IFN-γ ELISA: Chicken interferon-gamma (IFN-γ) concentrations were determined using a commercial sandwich ELISA kit (Cusabio Biotech, China). All standards and samples were analyzed in duplicate, and absorbance values were recorded at 450 nm.

Histopathological examination

One of the common procedures used in tissue processing was to fix the specimens using formalin and then paraffin. Sections were placed on glass slides at 5 μm and were to be stained by means of H&E. The observation was done under light microscopy (Olympus CX41) with 40x and 100x magnification levels.

Lesions were graded semi-quantitatively on a scale of 0–3:

0 = No lesion, 1 = Mild epithelial/ciliary alteration, 2 = Moderate epithelial sloughing and inflammatory infiltration, 3 = Severe tissue damage with epithelial necrosis and lymphoid depletion.

A blinded veterinary pathologist performed all histopathological evaluations.

Molecular detection by RT-qPCR

The extraction procedure for tracheal tissue RNA followed Qiagen RNeasy Mini Kit protocols in Germany (commercial viral RNA kit). The process of equivalent DNA production occurred through the utilization of Super Script III Reverse Transcriptase from Thermo-Fisher Scientific. RT-qPCR analysis featuring matrix (M) gene primers and TaqMan probes against NDV and S1 gene primers and TaqMan probes against IBV was performed using methods originally reported by (Mohamed Suhail, 2025). The Bio-Rad CFX96 Real-Time PCR Detection System performed thermal cycling per the given protocol: Reverse transcription: 50°C for 30 minutes; and Amplification: 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Samples with Ct values up to 35 were considered positive. The reverse transcription temperature and time were 50°C for 30 min; when the PCR cycles reached 40 times and the Ct value was lower than or equal to 35, the samples were then considered positive. The PCR followed these conditions: Reverse transcription at 50°C for 30 min and an initial denaturation step at 95°C for 10 min, followed by amplification through 40 cycles at 95°C for 15 sec and 60°C for 1 min.

Growth performance

Growth performance parameters were calculated for each group. In (1), Average daily gain (ADG) was determined as:

$$ADG=\frac{Final\ body\ weight-Initial\ body\ weight}{Number\ of\ days} \quad (1)$$

In (2), Feed conversion ratio (FCR) was calculated as:

$$FCR=\frac{Feed\ intake\ (g)}{Body\ weight\ gain\ (g)} \quad (2)$$

Standard necropsy examinations were performed on any birds that died during the study to identify causes of mortality.

Statistical analysis

Data were analyzed using SPSS Statistics software (Version 22; IBM Corp., Armonk, NY, USA). The Shapiro–Wilk test was applied to assess data normality. Parametric data were analyzed using two-way ANOVA (factors:

group × time) followed by Fisher's Least Significant Difference (LSD) post hoc test. Non-parametric data were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparisons test. All results are expressed as mean ± standard deviation (SD), and statistical significance was set at $p < 0.05$.

Results

Growth Performance

Table 2 and Figure 1 show the results of broiler body weight (BW) measurements of the experimental groups during days 1, 14, and 35. The initial uniformity of the chicks was proven by the fact that on day 1, all the experimental groups showed similar mean BW values. Group ND-IB-8 had the highest final BW (2107.8 ± 45.2 g) at Day 35, which was significantly higher ($p < 0.05$) compared to all others. Conversely, Group ND-IB-1, which was vaccinated against IBV on day 1, had the lowest final BW (1785.6 ± 52.4 g).

The increase in Group ND-IB-8 is indicative that the early immunological interference was avoided by administering the IBV vaccine a day later (day 8), hence enabling the maximum development of the immune system and the utilization of the nutrients. These results are in line with the vaccinating broilers against IBV after the first week, which positively influenced their weight gain, and (Bhuiyan et al., 2021), who reported that early vaccination against IBV impacted feed efficiency negatively because of transient respiratory distress and epithelial damage. Likewise, (Chhabra et al., 2015) emphasized how the timing of a vaccine is affected by the virulence of the virus and the use of management factors to affect the growth outcomes.

In general, the current evidence supports the fact that the IBV vaccination regimen has a significant impact on broiler growth performance, and timely vaccination can increase feed conversion ratio and reduce immunological stress (Bhuiyan et al., 2021; Rafique, 2018; Chhabra et al., 2015).

Table 2. Effect of vaccination schedule on broiler body weight at days 1, 14, and 35

(Means with different superscript letters in the same column differ significantly, $p \leq 0.05$.)

Group	Day 1	Day 14	Day 35
ND-IB-1	43.2 ± 1.5	420.5 ± 15.2 c	1785.6 ± 52.4 °C
ND-IB-5	42.9 ± 1.6	435.3 ± 12.8 b	1903.5 ± 48.7 b
ND-IB-8	43.5 ± 1.4	448.7 ± 14.3 a	2107.8 ± 45.2 a
ND-IB2-1	43.1 ± 1.3	440.1 ± 13.9 b	1935.2 ± 50.1 b

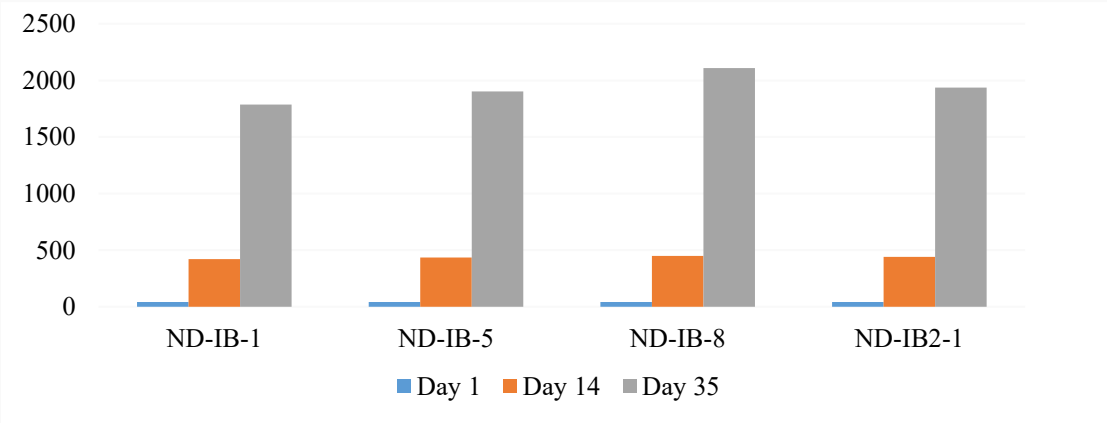

Figure 1. Effect of vaccination schedule on broiler body weight at days 1, 14, and 35

Humoral Immune Response (IgY Levels)

Serum IgY titers against NDV and IBV at day 35 are presented in Table 3 and Figure 2. The highest NDV-specific IgY titer was recorded in Group ND-IB-8 (18115 ± 275 U/ml), which was significantly greater compared to all the other groups ($p < 0.05$). This growth would probably occur due to the decreased competition of sialic-acid receptors in the case of the IBV vaccine at day 8, which will allow better recognition and antigen-producing antibodies (Tan et al., 2020).

Conversely, Group ND-IB2-1, which received both Ma5 and 4/91 IBV vaccines, showed the highest IBV-specific IgY titer (16000 ± 285 U/ml), consistent with (Bande et al., 2015), who reported synergistic immune enhancement when combining classic and variant IBV strains. The data indicate that both vaccine strain selection and administration timing strongly determine antibody responses.

Table 3. The measured value of serum IgY (U/ml) existed on day 35

Group	NDV IgY	IBV IgY
ND-IB-1	12681 ± 320	13599 ± 300
ND-IB-5	15195 ± 310	13276 ± 290
ND-IB-8	18115 ± 275	13599 ± 285
ND-IB2-1	14000 ± 295	16000 ± 285

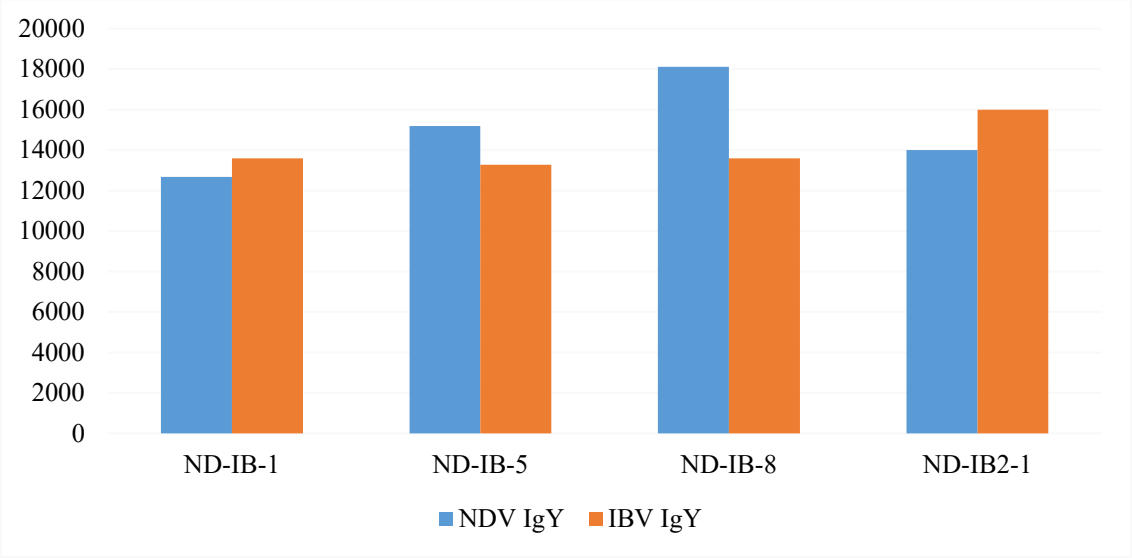

Figure 2. IgY titers against NDV and IBV in different groups

Cellular Immune Response (IFN-γ Concentration)

Table 4 and Figure 3 summarize the serum IFN-3 concentrations on day 35. Groups ND-IB-1 (52.7 ± 2.1 pg/ml) and ND-IB2-1 (51.4 ± 2.3 pg/ml) had the highest levels of IFN-7, which indicated a greater pro-inflammatory response. Group ND-IB-8, in turn, demonstrated the lowest level (45.6 ± 1.9 pg/ml), pointing to the occurrence of moderate cellular activation with a slight inflammation.

These results align with (Ingrao et al., 2018; Drury et al., 2019), who reported that early IBV vaccination enhances IFN-γ expression and leukocyte infiltration, while delayed vaccination reduces cytokine overproduction. Elevated IFN-γ levels are often linked to greater tissue inflammation and pathology (Abozeid, 2023; Low et al., 2022).

Table 4. The concentrations of IFN-γ were measured in serum samples at day 35 (pg/ml) (mean ± SD)

Group	IFN-γ (pg/ml)
ND-IB-1	52.7 ± 2.1
ND-IB-5	47.2 ± 2.4
ND-IB-8	45.6 ± 1.9
ND-IB2-1	51.4 ± 2.3

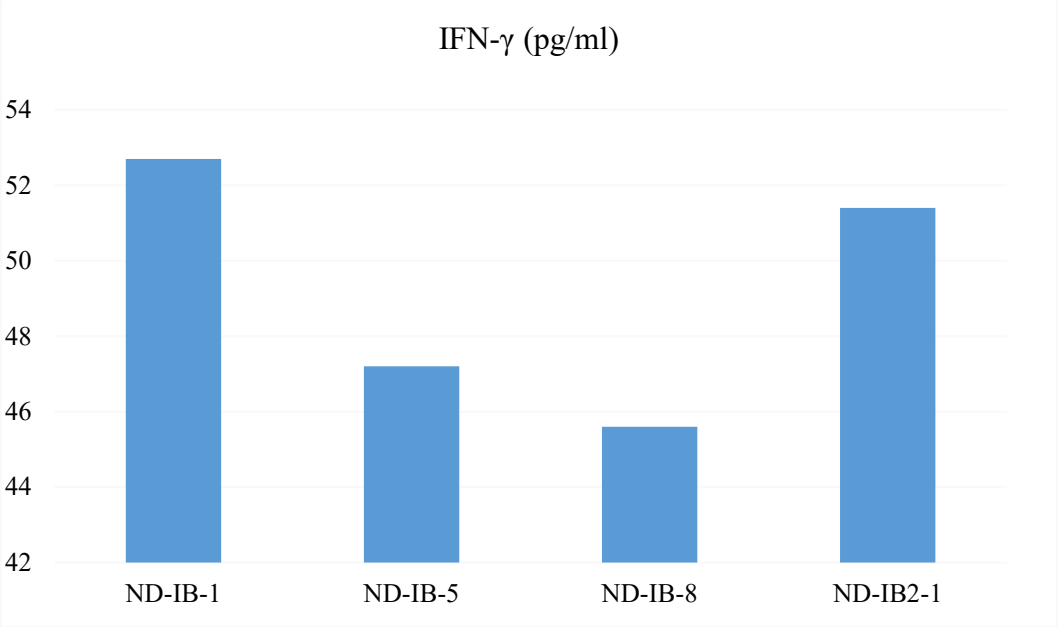

Figure 3. IFN-γ concentrations among groups at day 35

Histopathological Findings

Semi-quantitative lesion scores for trachea and Harderian gland tissues at day 35 are shown in Table 5 and Figure 4. The lesions of group ND-IB-5 (score = 3.0 ± 0.0) were the most pronounced, including epithelial death, loss of cilia, and destruction of lymphoid tissue. Lesions were of moderate degree in the ND-IB-1 group (2.0 ± 0.4), and the least changes were found in the ND-IB-8 group (1.5 ± 0.3 – 1.5 ± 0.4).

These results support previous reports that early IBV vaccination causes greater tracheal and Harderian gland damage (Bande et al., 2015; Hassan et al., 2016; Eid et al., 2024). The vaccination that was delayed for 8 days kept the epithelium intact and also reduced the microscopic lesions, a finding which is in agreement with the results of (Eid et al., 2024). It is possible that the genetic structure of the 4/91 variant, which is characterized by mutations in nsp3 and nsp14, is the reason for its strong epithelial attraction and pro-inflammatory capability (Beltrán et al., 2019; Jordan, 2017).

Table 5. Mean ± standard deviation of lesion scores in tracheal and Harderian gland tissues of broiler chickens subjected to different NDV–IBV vaccination schedules

Tissue	ND-IB-1	ND-IB-5	ND-IB-8	ND-IB2-1
Trachea	2.0 ± 0.4	3.0 ± 0.0	1.5 ± 0.3	2.0 ± 0.5
Harderian gland	2.0 ± 0.3	3.0 ± 0.0	1.5 ± 0.4	2.0 ± 0.4

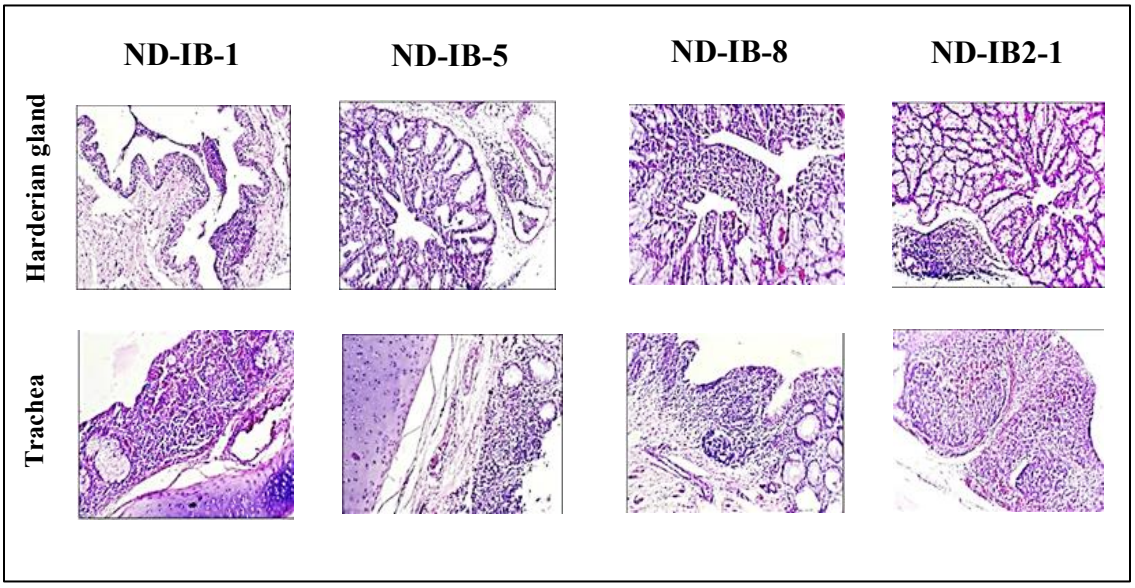

Figure 4. Histopathological comparison of trachea and harderian gland tissues in broiler chickens under different NDV–IBV vaccination schedules (H&E, 100×)

Molecular Detection (RT-qPCR)

RT-qPCR results for tracheal tissues collected on day 35 are displayed in Table 6. Even the highest Ct values among the samples did not indicate the presence of any viral RNA from either NDV or IBV. Thus, it is safe to say that by the end of the experimental period, the vaccine strains had been totally wiped out. With the help of (Shosha et al., 2024; Nochi et al., 2018; Al-Rasheed et al., 2021), the same conclusion can be drawn, which states that under controlled conditions, live NDV and IBV vaccine strains do not last more than 28-35 days in their duration. This serves as a confirmation of the biosafety and transient nature of live vaccines administered.

Table 6. RT-qPCR detection of NDV and IBV RNA in tracheal tissues at day 35

Sample ID	Tissue	Ct Value	Interpretation
01NT	Trachea	No Ct	Negative
02NT	Trachea	No Ct	Negative
03NT	Trachea	No Ct	Negative
04NT	Trachea	No Ct	Negative
05NT	Trachea	No Ct	Negative

Discussion

The present observations taken together indicate that the timing of vaccination is a major determinant of the interplay between growth, immune response, and tissue integrity in broiler chickens vaccinated with live NDV and IBV (4/91 variant) strains. It was found that postponing IBV vaccination to day 8 significantly enhanced growth performance and immune efficiency, while at the same time, inflammation and tissue damage were reduced to a minimum. One possible explanation for the above-mentioned effects is that NDV and IBV compete at the receptor level for α2-3 sialic acid binding sites found on the tracheal epithelial cells (Isham et al., 2023). Early co-administration leads to interference, resulting in diminished NDV replication and antibody production, while delayed IBV vaccination partially lifts this effect, thus allowing the NDV-specific immunity to be more effective.

The increased NDV IgY titers and the decreased IFN-γ levels in Group ND-IB-8 indicate that humoral and cellular immunity were optimally balanced, thus avoiding the overactivation of the inflammatory pathways. In contrast to the delayed vaccination, the early IBV vaccination (Groups ND-IB-1 and ND-IB-5) resulted in the huge production of IFN-γ, which eventually led to the death of the respiratory epithelium and a decrease in growth, supporting the findings of (Abozeid, 2023; Low et al., 2022). Histopathological evaluations lend support to this mechanism, giving it strong backing indeed. The vaccinations given to the early groups were associated with severe lesions, and this is completely in line with the findings of (Bande et al., 2015; Hassan et al., 2016), who connected the lack of development of mucosal immunity with the increased injury from the vaccine. Genetic analysis of the IBV 4/91 variant also shows mutations that increase the ability to target epithelial cells and interfere with the signal of host interferon (Beltran et al., 2019; Jordan, 2017).

Finally, the postponement of IBV vaccination to day 8 after hatch led to an optimal immune coordination, reduced inflammatory pathology, and increased body weight gain without suppressing the viral clearance. The findings provide a basis to improve interdisciplinary NDV and IBV vaccination methods to boost productivity without interfering with mucosal health in broilers.

Conclusion

The results of this research paper indicate that the time when live Newcastle Disease Virus (NDV) and Infectious Bronchitis Virus (IBV, 4/91 variant) vaccination is administered is a significant factor in determining immune dynamics, tissue integrity, and growth performance that exist in broiler chicks. IBV vaccination at day 8 post-hatch resulted in significant differences in terms of improvement in body weight gain and feed efficiency due to the reduction of early immunological interference with NDV replication. This first-better schedule enhanced NDV-specific IgY antibody levels, decreased IFN-γ-induced inflammation, and preserved the structural integrity of tracheal and Harderian gland epithelia. The findings imply that premature co-infection with NDV and IBV (4/91) involves an excessive activation of cytokines and injury of the epithelium, which is probably caused by the competition at the receptor level of 2, 3 sialic acid binding sites and the increased epithelial affinity of the 4/91 variant. The spike glycoprotein of the 4/91 IBV mutations can also augment immunomodulatory activities, and this could also be the cause of transient tissue pathology in case of premature administration. On the whole, this paper has determined that the timing of vaccination should take into consideration not only the age of the birds but also the genetic and immunological composition of the vaccination strains applied. The vaccination of IBV with a delay of 8 days has the effect of making the immune response more balanced, reducing inflammation in the tissues, and finally, increasing the production. All these benefits set the ground rules for the whole process of trying to implement NDV–IBV vaccination programs, which will result in stronger immune protection, healthier respiratory systems, and sustained productivity in modern poultry farming.

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Author Contribution

Each of the authors played an equal part in conceiving the study, designing, carrying out the study, data analysis, and writing of the manuscript. The final version of the manuscript has been read and approved by both authors, and they have concurred to be responsible for all areas of the work.

Conflicts of Interest

The authors declare that they have no conflicts of interest related to this study.

Ethical approval

All animal procedures were conducted in accordance with the guidelines established by the Animal Care and Use Committee of the College of Veterinary Medicine, University of Baghdad. The study protocol (Approval No. P.G.9, dated 17 March 2025) received ethical clearance to ensure the humane handling and treatment of all experimental birds.

Data Availability Statement

The data that confirm the results of this study are not publicly available at the moment because they are still under research and additional analysis. The datasets are, however, available on reasonable request by the respective author.

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