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Serological, Biochemical, and Histopathological Responses of Broiler Chickens to Different Commercial Newcastle Disease Vaccination Programs

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A B S T R A C T

Newcastle disease virus is a globally dispersed virus that infects nearly every bird species, both wild and domestic. It belongs to the genus Orthoavulavirus, subfamily Avulavirinae, family Paramyxoviridae. In chickens, respiratory infection symptoms can range from mild to severe. The disease causes problems with the respiratory system, the digestive tract, and the neurological system, and it is known to cause a high rate of death. Vaccination, along with biosecurity measures and monitoring systems, is recommended as a comprehensive strategy for managing avian diseases. This experiment examined serological, biochemical, and histopathological changes in different commercial Newcastle disease vaccination programs in broiler chickens. The average titers of maternal-derived antibodies targeting Newcastle disease virus were 15315.6±498.64 and showed a consistent reduction across all groups. Post-vaccination ELISA antibody titers differed considerably among groups (p<0.05). Program-1 showed the strongest antibody response (10076±420.36), and Program-3 a close second (9603±489.26); both differed significantly (p<0.001) from the PBS control group (576.8±142.45). Program-2 caused a slight but substantial antibody rise (1428.6±247.80; p<0.05). Serum biochemical examination showed no significant changes (p>0.05) in total protein and globulin levels among groups. Albumin levels differed significantly (p<0.05), with Program-2 showing the highest values (1.24 g/dL) and Program-3 the lowest (1.002 g/dL) compared with the PBS control. At day 35, Program-1 and Program-2 demonstrated normal hepatic portal regions, cords, sinusoids, tracheal mucosa, and cartilage, indicating superior safety. In contrast, Program-3 caused hepatic central vein congestion, sinusoidal congestion, vacuolar abnormalities, mild tracheitis with leukocytic infiltration, and mucosal thickening. Among the tested programs, Program 1 induced the highest ELISA antibody titers and was not associated with evident liver or tracheal lesions in the sections examined. Further challenge and field studies are required to confirm protective efficacy and practical superiority.

Keywords: *Newcastle Disease Virus, Chicken, Antibody, Biochemical Examination, Pathological.*

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1 Introduction

Newcastle disease (ND) causes considerable financial losses in the global poultry sector. Avian orthoavulavirus 1 (AAvV-1) causes the illness; it is a negative-sense, single-stranded, non-segmented RNA virus with an envelope. It is classified within the genus *Orthoavulavirus*, subfamily *Avulavirinae*, family *Paramyxoviridae*, and order *Mononegavirales* (Amarasinghe et al., 2018; International Committee On Taxonomy Of Viruses, 2026; Sultan et al., 2020). The viral genome contains six open reading frames (ORFs) encoding nucleoprotein (NP), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN), and large polymerase (L) proteins. The HN protein is an immunogenic and virulence factor for Newcastle disease virus (NDV). In contrast, the F protein cleavage site is considered the primary determinant of NDV pathogenicity (Hassan et al., 2020). Genetically, Newcastle Disease Viruses (NDVs) are classified into class I and class II, which together encompass 21 genotypes (I–XXI); most are virulent, although a few are avirulent NDVs (Amarasinghe et al., 2018). Among these, genotype VII is the predominant class II genotype and has been linked to numerous epidemics (Amarasinghe et al., 2018; Yi et al., 2011). It is an exceedingly transmissible viral illness that presents a considerable worldwide risk to avian agriculture (Dimitrov et al., 2017; Rima et al., 2019). Clinical signs vary widely and include respiratory distress, neurological signs, and gastrointestinal disorders (Mao et al., 2022). Velogenic strains, the most pathogenic variants, can lead to significant epidemics and result in up to 100% illness and death rates in unvaccinated poultry populations. The virus spreads via direct interaction with infected birds and indirect exposure to contaminated objects (Ola et al., 2026).

Immunization remains a fundamental approach to managing ND and works alongside rigorous biosecurity and sanitation measures (Elbestawy et al., 2024; Hu et al., 2022). Thorough control strategies include mandatory preventive vaccination with both live and inactivated NDV vaccines, multi-phase vaccination plans, eradication of infected populations, and stringent biosecurity measures (Ahmed et al., 2024). Genotype VII is one of the most widespread and virulent strains among the several viral genotypes, extensively circulating in Asia and Africa (Hu et al., 2009). To alleviate this risk, existing vaccination approaches depend on three main types: live attenuated, inactivated, and recombinant vector vaccines (Andey et al., 2024). Conventional lentogenic strains, such as LaSota, B1, and F, are commonly employed in live vaccination preparations (Sarker et al., 2021). These vaccines are delivered via ocular drops, aerosols, or potable water, enabling swift, effective large-scale distribution (Guérin et al., 2024). Nonetheless, a major challenge in breeder immunization strategies is that maternal antibodies may neutralize live vaccine viruses, undermining vaccine effectiveness (Manar et al., 2020). Inactivated vaccines are extensively used to elicit strong humoral immune responses (Alsakini et al., 2024). In these formulations, chemical inactivators such as formalin, β-propiolactone, or binary ethylenimine render NDV particles non-infectious. Adjuvants are commonly used in injectable inactivated vaccines to increase immunogenicity (Sedeik et al., 2019). When given as supplementary doses after initial vaccination with live vaccines, they confer robust and enduring protective immunity (Haque et al., 2024). In Iraq, many vaccine formulations are widely used, but NDV remains highly endemic in regional poultry populations, where velogenic genotype VII lineages often cause severe outbreaks in broiler flocks (Al-Zuhariy et al., 2017; Faraj et al., 2024, 2025). In the Iraqi poultry industry, field veterinarians and farmers apply several vaccination programs, but little data exist on their protective efficacy and physiological tolerance (Al-Mansouri & Almremdhy, 2025; Al-Zuhariy, 2023). This study provides a comprehensive assessment of three experimentally optimized, field-relevant immunization strategies to resolve field uncertainty. Also provides guidelines for field-representative and experimental programs to optimize immunogenic blueprints for regional avian health.

Assessing cellular alterations caused by organopathies in birds requires blood biochemistry determination because, even when critically unwell, birds show little or no clinical indications of illness (Harr, 2009; Okorie-Kanu et al., 2016). Avian medicine uses blood tests far less than large-animal veterinary care. Serum biochemical characteristics can provide valuable information about a bird's health status. Animal illnesses are one of many potential influences on the biochemical composition of chicken serum. Infectious bronchitis, infectious bursal disease, and ND are among the poultry illnesses that affect serum levels of some biochemical parameters in hens (El-Mandrawy & Ismail, 2017; Talebi, 2006). In different organs, ND causes several systemic pathogenic alterations (Etriwati et al., 2017). Concurrently, robust viral replication triggers intense oxidative stress, culminating in severe hepatocellular damage and subsequent liver dysfunction. The most prominent histopathological hallmarks include multifocal

necrosis with congestion, fibrin exudates, and lymphocyte buildup in the perivascular area of the spleen (Susta et al., 2014; Venkata Subbaiah et al., 2015). This study compared NDV antibody titers, selected serum biochemical parameters, and liver and tracheal histopathology in broiler chickens receiving three commercial vaccination programs.

2 Materials and Methods

2.1 Animals

A total of 105 one-day-old chicks of the Ross 308 broiler strain, both sexes, weighing 35-45 g, were obtained from Shukr Poultry Hatchery. The chicks were vaccinated against Avian Influenza (AI), infectious bronchitis (IB), and ND by standard maternal methods. At arrival, five chicks were randomly selected to establish baseline maternal immunity. The remaining birds (100) were randomly assigned to experimental groups and housed in high-containment chicken isolators fitted with HEPA-filtered equipment and positive pressure. House temperature was tightly controlled at 32–33°C during the first 3 days, then decreased by 2–3°C weekly. For the first week, the lighting program was 23 hours of light and 1 hour of darkness to stimulate early activity. It was then placed on an 18-hour light schedule. The birds had plenty of space to move around, nipple drinkers with water, and a balanced feed; the ration was antibiotic-free and consisted of corn and soybean meal.

2.2 Vaccines and Vaccination Programs

A multifactorial experimental design, as outlined in Table 1, was used to systematically evaluate and compare the biological performance of the selected immunization programs. Before vaccine administration, all chickens were thriving and showed no clinical abnormalities. The experiment included 100 one-day-old broiler chicks, equally distributed among four experimental groups of 25 chicks each. Each program tested a specific commercial method reflecting regional field practices in Iraq. We documented and standardized the chronological schedule, exact antigenic components, live or inactivated formulation status, specific viral strains, manufacturers, administration routes, and precise dosages per bird across all groups to ensure full experimental reproducibility. Moreover, parallel administration routes established a negative control group that provided a strict baseline physiological reference for all subsequent humoral analysis.

Table 1. Vaccination programs for the experimental groups.

Programs	Number of chicks	vaccines	Dose/bird
Program 1	25	Inactivated vaccine of NDV by the S/C route and live vaccine of NDV by the intraocular route on day 1	0.25 mL for the inactivated vaccine 0.1 mL for the live vaccine
		Repeat the live vaccine of NDV by the intraocular route on day 8	
Program 2	25	Inactivated vaccine of mixed AI and ND by the S/C route on day 1	0.25 mL for the inactivated vaccine
		Repeat the inactivated vaccine on day 8	
Program 3	25	Live vaccine of mixed IB and ND by the intraocular route on day 1	0.1 mL for the live vaccine
		Repeat the live vaccine on day 8	
		Repeat the live vaccine on day 18	
PBS Control	25	Control injected with PBS at 1-, 8-, and 18-day by the S/C route	0.25 ml

2.3 Serological analysis

Blood specimens were collected from the brachial (wing) vein of the birds. Birds were euthanized only on day 35; only once, five chicks were randomly chosen from each experimental group for serum collection. In summary, whole blood was gathered in sterile tubes and permitted to coagulate at 37°C for one hour in a sloping position. The tubes were then stored overnight at 4°C, after which they underwent centrifugation at 3,000 rpm for 15 minutes to isolate the sera. The collected sera were promptly divided into sterile Eppendorf tubes and preserved at −20°C for further examination. Serum antibody levels against NDV were measured with a commercial indirect ELISA kit (ID Screen® Newcastle Disease Indirect Conventional Vaccines, IDvet, Grabels, France) in precise adherence to the manufacturer's instructions. All serum samples and

manufacturer-supplied positive and negative controls were analyzed in double technical replicates (duplicates) to ensure technical reproducibility and minimize intra-assay error. The test is validated if the mean optical density (OD) of the positive control (ODpc) is greater than 0.25 and/or the ratio of the mean OD values of the ODpc and the negative control (ODnc) is greater than 3. We used a two-step standardized algebraic calculation model to calculate the sample-to-positive (S/P) ratio and the corresponding antibody titer for each serum sample. The first calculation of the S/P ratio was performed using formula 1:

$$S/P\ ratio = \frac{ODsample - ODnc}{ODpc - ODnc}$$

Then the ELISA antibody titer was calculated by converting the calculated S/P ratio using the specific logarithmic regression equation provided by the manufacturer, as shown in formula 2:

$$\mathbf{Log_{10}\ (titer) = 1.00 \times log_{10}(S/P) + 3.520}$$

which is mathematically and explicitly solved as:

$$\mathbf{Titer = 10^{log10(titer)}}$$

The final numbers were interpreted according to the diagnostic threshold criteria listed in Table 2.

Table 2. Means of antibody levels against NDV in the serum of chickens in the experimental groups.

Groups	MDI	Program-1	Program-2	Program-3	Control (PBS)
Average	15315.6	10076	1428.6	9603	576.8
SD	498.64	420.36	247.80	489.26	142.45

2.4 Measurement of Serum Biochemical Parameters

For biochemical assessment, commercial kits (ELITechGroup, Holland) and spectrophotometry (Selectra XS Pro, Holland) were used to measure total blood protein levels as per Henry (1974) (Henry et al., 1974), while serum albumin levels were evaluated using the technique established by Doumas et al. (1981) (Doumas et al., 1981). Serum globulin concentration was determined by subtracting albumin from total protein (Doumas & Biggs, 1972).

2.5 Histopathological examination

On Day 35, one bird from each experimental group was randomly selected, and liver and tracheal tissue samples were collected for histopathological evaluation. For each organ, three independent tissue sections were processed and promptly preserved in 10% neutral buffered formalin. We then prepared standard paraffin wax blocks for each specimen and cut 5 µm-thick tissue sections. We stained the sections with hematoxylin and eosin (H&E) and examined them by light microscopy (Kuiken et al., 1999). To ensure objectivity, a veterinary pathologist blinded to group allocation evaluated the slides. Tissue changes were scored on a semi-quantitative scale of 0-3 depending on severity and distribution: 0 (normal/no lesions), 1 (mild, <25% area affected), 2 (moderate, 25-50% area affected) and 3 (severe, >50% area affected).

2.6 Statistical analysis

We used GraphPad Prism version 8 (GraphPad Software, Inc., La Jolla, CA, USA) to analyze the results of the present study. Before parametric statistical analysis, we assessed the normality of the data distribution using the Shapiro–Wilk test. When the normality criteria were met, we performed a one-way ANOVA followed by Dunnett's multiple-comparisons test to determine significant differences between groups. Results are expressed as mean ± standard deviation. Significance was defined as $p < 0.05$.

3 Results

3.1 NDV Antibody Titer

Immunological responses, as shown by antibody titers, varied considerably between vaccine groups ($p<0.05$); see Figure 1. The baseline analysis of maternal-derived immunity (MDI), evaluated exclusively on Day 1 (at hatch), showed a mean titer of 15315.6±498.64 across the shared initial population. Chickens vaccinated in program-1 showed an increase in ELISA antibody titers to 10076±420.36, compared with the control group, which recorded 576.8±142.45 ($p<0.001$). Program 2 induced a statistically significant ($p<0.05$) but comparatively modest increase in NDV ELISA antibody titers (1428.6±247.80) compared with the PBS control group. Additionally, ELISA antibody titers differed considerably between the Program-3 group and the PBS unvaccinated group ($p<0.001$), with a value of 9603±489.26 (Table 3 and Figure 1).

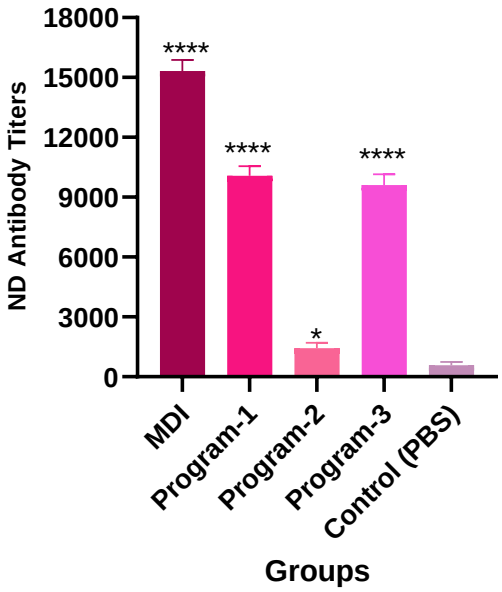

Figure 1. Mean ELISA antibody titers of NDV in the vaccinated and unvaccinated experimental groups.

Table 3. Biochemical Parameters of Broiler Chickens in different groups (n=25, Mean±SE)

	Groups				
Parameters	Program-1	Program-2	Program-3	Control (PBS)	Reference Values
Total Protein	3.26±0.08	3.28±0.15	3.22±0.07	3.2±0.03	3.00-4.00[M]
Albumin	1.12±0.07	1.24±0.05	1.002±0.04	1.07±0.01	1.17-2.74[M]
Globulin	2.14±0.08	2.04±0.08	2.218±0.03	2.13±0.02	-

[M]=(Meluzzi et al., 1992). Values are expressed as Mean±SE.

3.2 Serum Biochemical Analysis

The current study's findings showed no discernible difference (p>0.05) in total protein and globulin levels between all vaccinated groups (Program- 1, Program- 2, and Program- 3) when compared with the control group. For albumin levels, Program-2 and Program-3 showed a statistically significant difference (p<0.05) compared with the PBS control group. Among the vaccinated populations, Program-2 showed the highest total protein and albumin (3.28±0.15 and 1.24±0.05, respectively), remaining within the expected reference range (1.17–2.74) (Meluzzi et al., 1992). Conversely, Program-3 exhibited the lowest albumin levels (1.002±0.04). For globulin fractions, Program-3 showed the highest value (2.218±0.03), while Program-2 showed a lower value (2.04±0.08) (Table 4, Figure 2).

Table 4. Biochemical Parameters of Broiler Chickens in different groups (n = 5, mean ± SD).

	Groups				
Parameters	Program-1	Program-2	Program-3	Control (PBS)	Reference Values
Total Protein g/dL	3.26±0.08	3.28±0.15	3.22±0.07	3.2±0.03	3.00-4.00[M]
Albumin g/dL	1.12±0.07	1.24±0.05	1.002±0.04	1.07±0.01	1.17-2.74[M]
Globulin g/dL	2.14±0.08	2.04±0.08	2.218±0.03	2.13±0.02	-

[M]= Meluzzi D., Primiceri G., Giordani R., Fabris G. (1992) . Determination of Blood Constituents Reference Values in Broilers1. Poultry Science, 71(2): 337–345. Values are expressed as means ± SD.

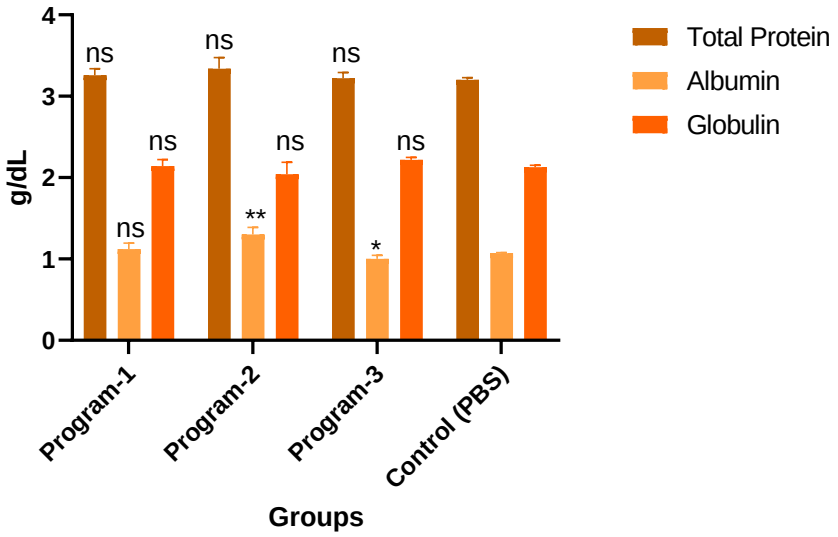

Figure 2. Biochemical Parameters of Broiler Chickens Vaccinated with Different Commercial Vaccination Programs.

3.3 Histopathology results

Histopathological evaluation 35 days post-vaccination showed descriptive structural observations between experimental groups. Microscopic examination of the liver and trachea in program-1 and program-2 groups showed well-preserved tissue architecture with no pathological changes. The examined liver sections exhibited preserved portal areas, hepatic cords, and sinusoids (Figures 3 and 4). Liver sections from the Program-3 group showed histopathological changes, including central vein congestion, sinusoidal congestion, and hepatocellular vacuolation (Figure 5). Conversely, in the PBS group (untreated control), the liver showed mild congestion of the central vein and sinusoids, and the hepatic cords were structurally intact (Figure 6). Likewise, tracheal sections showed no changes in mucosal glands; the subepithelial tissue was preserved, and tracheal cartilage was intact (Figures 7A and 7B). The tracheal structure was also affected, with moderate tracheitis characterized by mucosal thickening and leukocyte infiltration (Figure 7C). The tracheal mucosa showed mild thickening with leukocyte infiltration and congestion (Figure 7D). We performed a semi-quantitative lesion-scoring analysis on three separate sections per organ to assess the severity and distribution of tissue changes (Table 5). Microscopic evaluation showed different lesion-scoring profiles among the experimental groups. The overall tissue score was 0 in the Program-1 and Program-2 groups.

Table 5. Histopathological score of examined organs.

Organ	Histopathological Parameters	Program-1 (n=1, 3 sections)	Program-2 (n=1, 3 sections)	Program-3 (n=1, 3 sections)	Control PBS (n=1, 3 sections)
	Central vein/Sinusoidal congestion	0	0	3 (Severe)	1 (Mild)
Liver	Vacuolar degeneration	0	0	2 (Moderate)	0
	Vacuolar changes of hepatic cords	0	0	1 (Mild)	0
	Mucosal thickening	0	0	2 (Moderate)	1 (Mild)
Trachea	Leukocytic infiltration/ Tracheitis	0	0	3 (Severe)	1 (Mild)
	Subepithelial / Cartilage degradation	0	0	0	0
Summary	Overall Score (Total / 18)	0 (Normal)	0 (Normal)	11 (Severe Alterations)	3 (Mild Alterations)

Note: The scoring was based on the mean evaluation of three independent sections per organ per bird: (0) Normal/No lesions; (1) Mild changes affecting <25% of the fields; (2) Moderate changes affecting 25–50% of the fields; (3) Severe changes affecting >50% of the fields examined. Overall Score is the sum of all evaluated lesion parameters (maximum potential score of 18). N: Numbers.

In contrast, the Program-3 group showed the highest overall lesion score (11/18). The untreated control (PBS)

group, meanwhile, showed a low baseline overall score of 3. These results are based on a single representative bird per group (n=1).

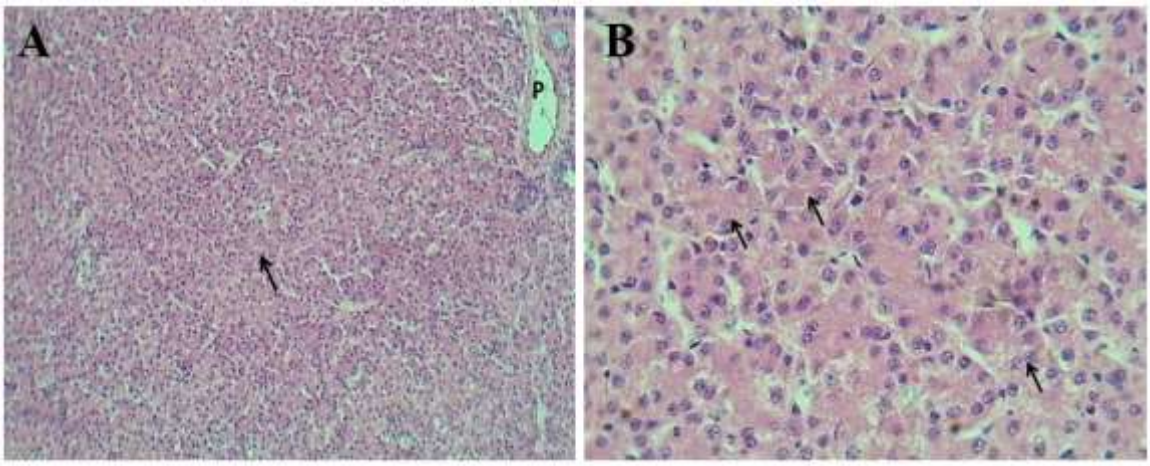

Figure 3. Histopathological changes of a section of Liver in the program-1 group. A) shows a normal portal area (p) with normal hepatic cords and sinusoids (arrow). H&E. 100x. B) shows normal hepatic cords and sinusoids (arrow).H&E.400x.

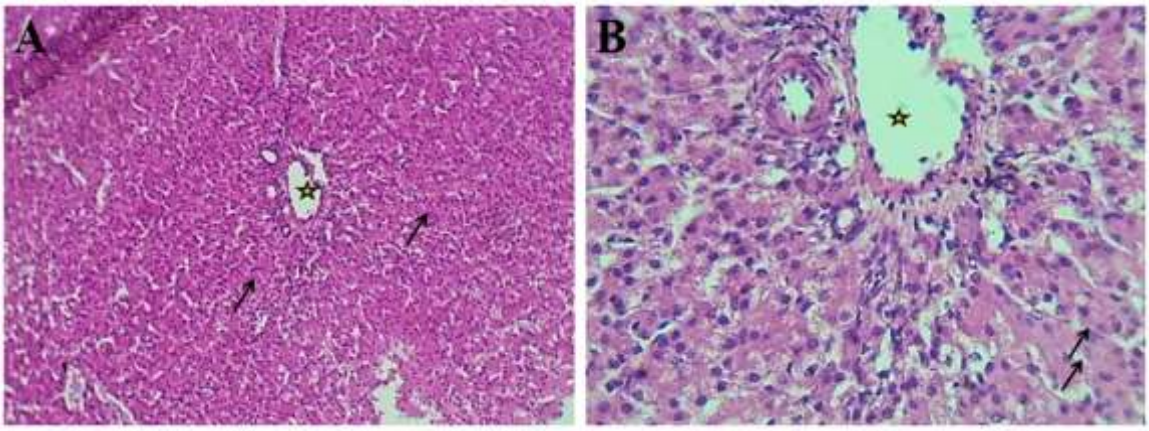

Figure 4. Histopathological changes of a section of Liver in the program-2 group. A) shows a normal portal area (Asterisk) with normal hepatic cords and sinusoids (arrow). H&E. 100x. B) shows a normal portal area (Asterisk) and hepatic cords and sinusoids (arrow).H&E.400x.

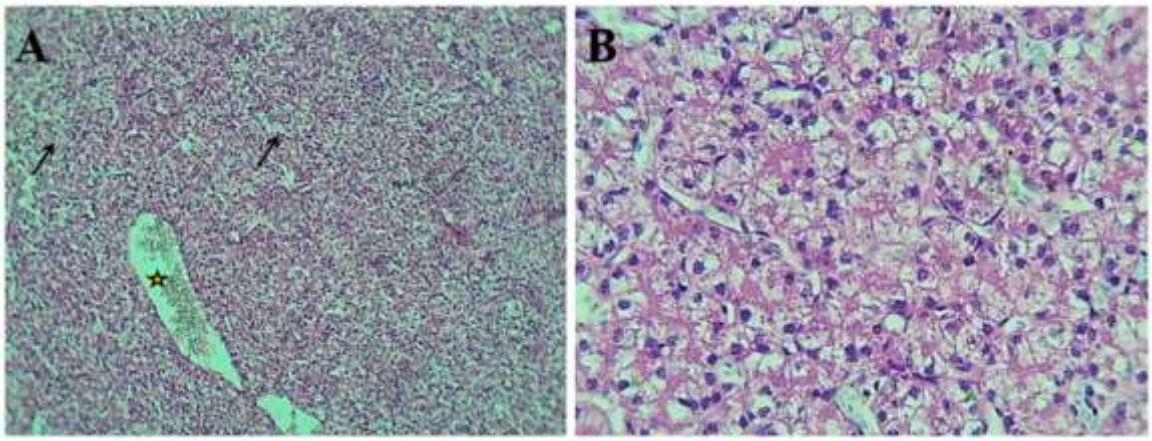

Figure 5. Histopathological changes of a section of Liver in the program-3 group. A) shows congestion with dilation of the central vein (Asterisk) & vacuolar changes of hepatic cords and sinusoidal congestion (arrows). H&E. 100x. B) shows vacuolar degeneration of hepatic cords with sinusoidal congestion .H&E.400x.

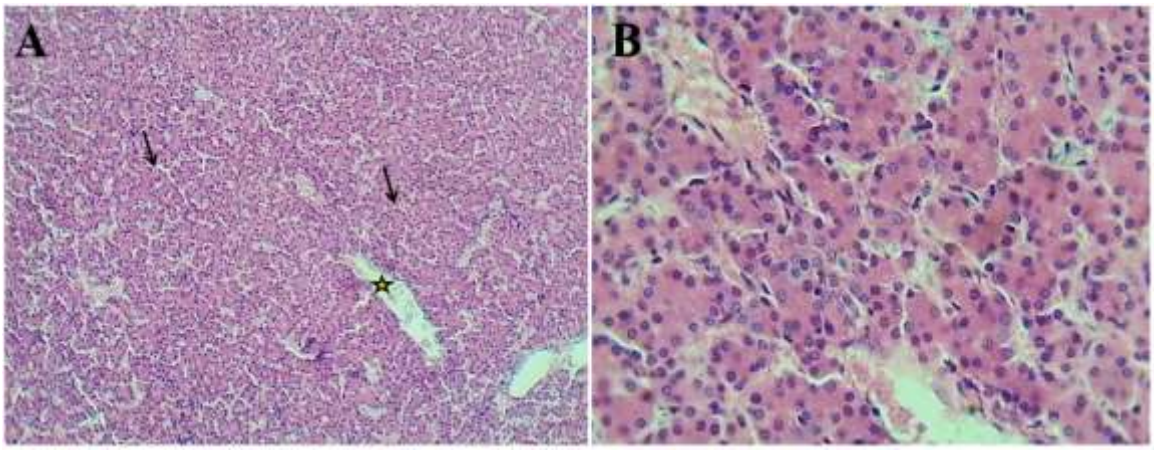

Figure 6. Histopathological changes of a section of Liver in the control PBS group. A) shows mild congestion of the central vein (Asterisks) & sinusoids with normal hepatic cords (arrows). H&E. 100x. B) shows mild congestion of the central vein & sinusoids with normal hepatocytes.H&E.400x.

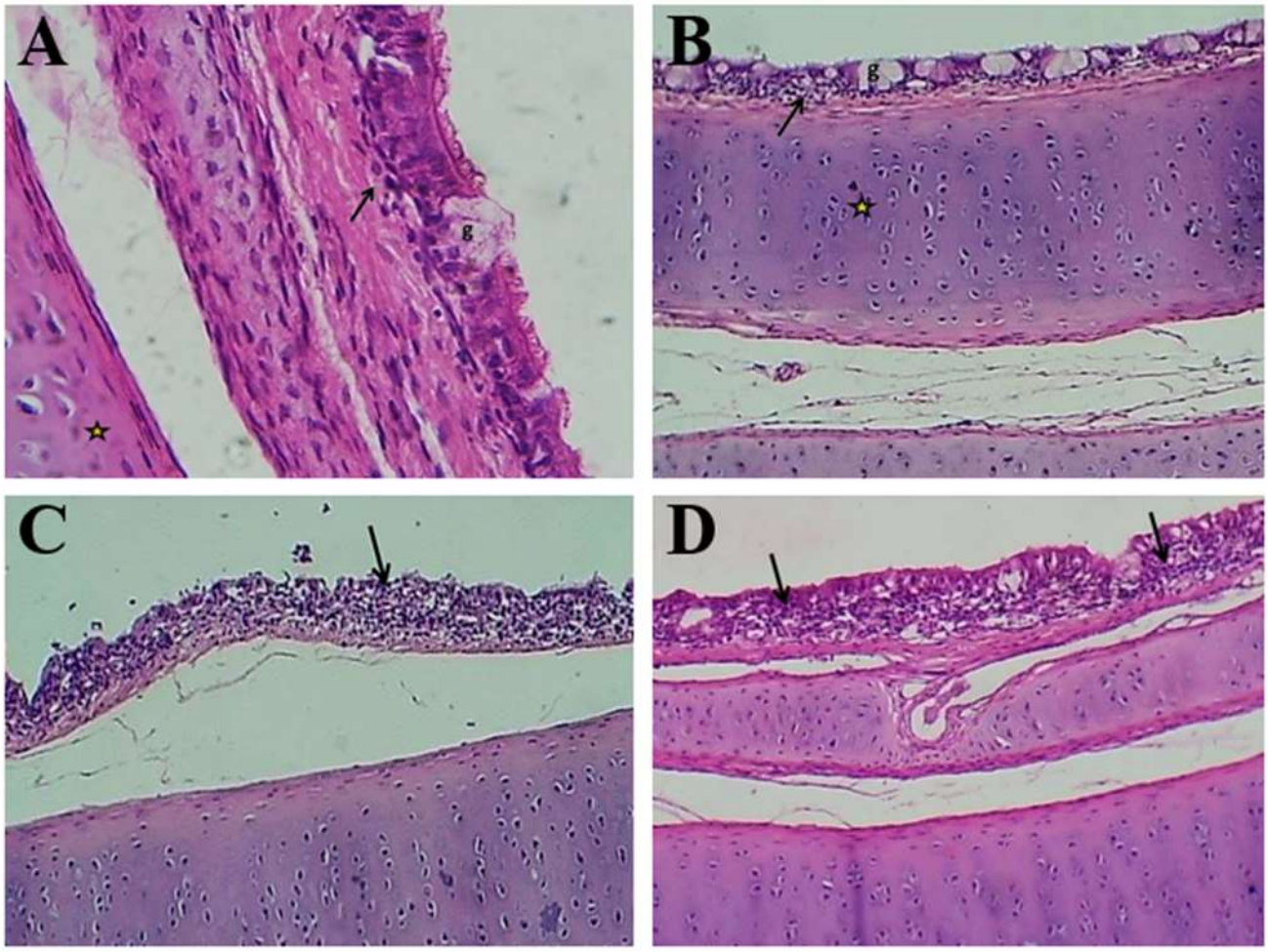

Figure 7. Histopathological changes of section of trachea experimental groups **A)** In program-1 group, shows normal mucosal glands (g), subepithelial tissue (Arrow) & tracheal cartilage (arrow). H&E. 200x. **B)** In program-2 group, shows normal mucosal glands (g), subepithelial tissue (Arrow) & tracheal cartilage (arrow).H&E.100x **C)** In program-3 group, shows moderate tracheitis with marked mucosal thickening related to infiltration of leukocytes (arrow).H&E.100x **D)** In control PBS group, shows mild mucosal thickening with leukocytes infiltration with congestion (Arrow).H&E.100x.

4 Discussion

Newcastle disease, avian influenza, infectious bronchitis, and infectious bursal disease are endemic in Iraqi poultry (Al-Shareef & Abawi, 2024; Faraj et al., 2024). The majority of commercial flocks are lost to these diseases. Mass immunization, surveillance, and bird segregation or culling can prevent viral pathogen infection (Chung et al., 2021). Vaccinating chickens against local viral infections is vital. Therefore, vaccination programs aim to drastically minimize the transmission of illnesses against which animals have been vaccinated, enhance flock health, and reduce economic losses from these diseases (Al-Mansouri & Almremdhy, 2025; Saif et al., 2020). According to Table 1, this study examined the humoral immune response and biochemical and histopathological alterations caused by three vaccination programs, encompassing all vaccines given to chicks on day one of their hatchery lives.

The baseline analysis of MDI was exclusively evaluated on day 1 across the shared initial population. By the final sampling on day 35, these maternal antibodies showed a clear physiological decline, with titers dropping in the unvaccinated PBS control group. Serum sampling was restricted to two discrete chronological events (day 1 baseline and day 35 endpoint), with no intermediate monitoring. Chickens' immune systems produce antibodies to fight foreign invaders. A booster immunization boosts the chicken's immune system. Booster vaccinations activate memory cells, multiply rapidly, and stimulate specific antibodies, increasing antibody titers faster (Ramlah et al., 2023). In this study, the humoral immune response (Figure 1) showed significant post-vaccination increases in serum antibody titers in program-1 and program-3 groups compared to the PBS control group ($p<0.001$). This substantial upregulation highlights the greater immunogenicity of these programs. The higher antibody titers in Programs 1 and 3 may reflect combined effects of live and inactivated vaccines or repeated exposure. Live vaccines efficiently prime cellular and local mucosal defense mechanisms. Still, oil-adjuvanted inactivated components have a depot effect that prolongs systemic antibody synthesis, explaining the increased statistical significance in Program 1 and Program 3 (Alian Samakkhah et al., 2023; Chai & Hu, 2025; Kim et al., 2024; Luo et al., 2026). Results from the program-2 group differ significantly ($p<0.05$) from the PBS control group. This supports Stone (1991)'s discovery that inactivated ND vaccinations increased antibody levels (Stone, 1991). This also aligns with recent literature showing that inactivated oil-emulsion formulations prolong antigen release, resulting in stronger and longer-lasting humoral antibody responses than in unvaccinated birds (Khair et al., 2026).

Serum protein profiles are important in birds for monitoring systemic homeostasis and identifying gastrointestinal, hepatic, renal, and infectious diseases (Arzour-Lakehal & Boudebza, 2021). These circulating protein fractions depend heavily on host factors, particularly chronological age. The higher total protein baseline in older birds reflects a physiological response to an increased immunoglobulin pool (Arzour-Lakehal & Boudebza, 2021; Vergneau-Grosset et al., 2016; Zheng et al., 2020). The present study showed no significant difference in total protein and globulin levels between vaccinated and unvaccinated populations, indicating that the candidate vaccination programs did not cause severe systemic metabolic dysregulations. However, albumin levels in the Program-1, Program-3, and PBS control groups were below the reference range (1.17-2.74 g/dL). The negative control group (PBS) also showed this decrease, suggesting it likely reflects baseline physiological variation, environmental conditions, or age/strain-specific differences rather than vaccine-induced toxicity. Conversely, Program-2 resulted in a small but significant increase in serum albumin compared with the PBS control group (Figure 2), which remained within the expected reference range. Talebi (2006) reported a significant decrease in total protein and albumin indices of broilers after NDV vaccination (Talebi, 2006). Low total protein and albumin synthesis commonly indicate poor nutrient utilization or severe liver damage (Augustine et al., 2020). Although the observed albumin profiles do not conclusively prove absolute hepatic safety because baseline deviations from the reference range were observed, this study did not show any severe suppression in the vaccinated groups (programs 2 and 3), which is in accord with a clinical study that reported no hepatotoxic discomfort or pathological abnormalities in the hepatic parenchyma after administration of modern lentogenic or oil-emulsion NDV combinations (Faluyi et al., 2015). The measured serum protein fractions therefore did not provide strong evidence of systemic protein imbalance. These results provide a baseline assessment of functional stability across the tested schedules, rather than a conclusive assessment of physiological advantage or overall safety profile for one program over another. Broader biochemical and clinical endpoints are needed to establish vaccine safety more conclusively.

Histopathology of liver and trachea revealed descriptive structural observations among the experimental groups. Liver sections from the Programs 1 and 2 groups showed normal portal regions, well-organized hepatic cords, and sinusoidal spaces, indicating structural integrity, with an overall tissue score of 0. However, liver sections from Program-3 revealed enlarged central veins, congested sinusoids (score 3), and vacuolar degeneration of hepatic cords (score 2) (Figure 5). The PBS control group showed less congestion (score 1), likely due to euthanasia or physiologic changes (Figure 6). The vacuolar changes observed in Program 3 may indicate hepatocellular stress, but the underlying mechanism cannot be determined from the present data. These results are in agreement with recent literature that emphasized that, although typical lentogenic NDV vaccine formulations preserve structural homeostasis, aggressive systemic antigen processing with intense or high-titer vaccine sequences may result in hepatic parenchymal alterations such as inflammation, congestion, and vacuolation (Al-Murshedy et al., 2023; Ebrahimi et al., 2023; Rasheed et al., 2022). All experimental groups showed respiratory and local tissue responses, as shown by tracheal histology (Figure 7). Program-1 and program-2 exhibited intact tracheal architecture, normal mucosal glands, and healthy subepithelial tissues, with an overall tissue score of 0 (Figures 7A & 7B). The PBS control group showed mild mucosal thickening (score 1) and congestion (Figure 7D), while program-3 showed severe tracheitis and leukocyte infiltration (score 3). The mucosal hypertrophy (score 2) and pronounced leukocyte infiltration (score 3) in program-3 are possibly associated with a post-vaccination respiratory tract reaction. Still, confirmation would require direct detection of vaccine virus replication or additional respiratory lesion scoring. Recent histomorphometry studies confirm that multiple live vaccination strain exposures can rapidly induce mucosal inflammation, deciliation, and thickening (Rocha et al., 2022; Wilson et al., 2021). Overall, these objective scoring data confirm the safety profiles of the first two candidate programs and emphasize the structural risks associated with Program-3. Vaccination can induce local tracheitis, but robust immune cell recruitment protects the mucosa. The better tissue preservation seen in Programs 1 and 2 suggests that a more balanced formulation that achieves protective humoral antibody titers while minimizing hepatic and respiratory inflammatory tissue damage is superior.

In the present study, we interpreted differences in humoral titers, serum protein profiles, and descriptive tissue characteristics in light of the experimental design. The three vaccination programs studied differed in their overall antigen composition, vaccine formulation, and booster schedules over time. Still, they followed comparable routes of administration to avoid procedural bias in the negative control group (PBS control). Program-2 included an inactivated ND+FLU (H9N2) combination, and Program-3 was a live mixed H120 (IBV)+CLONE formulation, administered at different time points. Therefore, this study acknowledges that the differences in antibody responses, protein profiles, and histopathological scores reflect the combined, cumulative biological effects of each holistic commercial vaccination program, not the isolated effect of NDV antigen exposure alone. These parameters are inseparable field variables in commercial poultry systems. This approach limits the ability to dissect the mechanics of individual antigens. Still, it provides a highly realistic assessment of the synergistic performance of these multi-valent programs as encountered in production environments.

5 Conclusion

In Programs 1 and 2, serum protein levels remained at baseline and projected physiological levels, and the liver and tracheal sections showed structural maintenance on Day 35. However, Program-3 exhibited a higher scheduling intensity. This program induced significant localized tissue changes, such as vacuolar degeneration and leukocytic infiltration. Program-1 demonstrated the highest NDV ELISA antibody titers, and no obvious hepatic or tracheal lesions were observed in the sections examined. Further controlled challenge and field studies are required before recommending this program as superior for commercial broiler production. Limitations of this study include a narrow biochemical panel and a descriptive, limited sample size for tissue evaluation, which preclude generalization of these data to establish clear safety profiles or systemic superiority. Future large-scale trials are needed to confirm and optimize the long-term safety of these commercial programs.

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Use of Artificial Intelligence

During the preparation of this work, the author(s) used Perplexity AI in order to identify and correct potential grammatical errors and improve the overall flow and readability of the manuscript. After using this tool, the author reviewed and edited the content as needed and take full responsibility for the content of the published article.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

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

Ethical Considerations

All experimental protocols were carried out in accordance with the ethical standards for animal care and were approved by the College of Veterinary Medicine, University of Baghdad (Approval No. P.G 1448, 30.06.2026). Birds were euthanized using cervical dislocation by trained personnel in strict accordance with international animal welfare guidelines.

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