

Journal of Poultry Sciences and Avian Diseases

Journal homepage: www.jpsad.com



Comparative Serological Effectiveness of Newcastle Disease Vaccines and Vaccination Regimens on Commercial Broilers: A Multisite Study in Iran



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Article Info

Article type:

Original Research

How to cite this article:

Yousefi, A. R., Abdoshah, M., Motamed, N., & Al-Masri, F. (2026). Comparative Serological Effectiveness of Newcastle Disease Vaccines and Vaccination Regimens on Commercial Broilers: A Multisite Study in Iran. *Journal of Poultry Sciences and Avian Diseases*, 4(2), 1-8.

<http://dx.doi.org/10.61838/kman.jpsad.174>



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ABSTRACT

Newcastle disease (ND) is a worldwide viral disease that imposes major economic losses on the poultry industry due to the vast vaccination and catastrophic death after infection, especially in broilers. This study aimed to compare the serological effectiveness of ND vaccines produced by the Razi Vaccine and Serum Research Institute with three imported vaccines and to evaluate the impact of two vaccination regimens on broilers. Two commercial farms with four houses in each in Alborz and East Azerbaijan provinces were randomly assigned to receive one of four ND vaccine brands (V1, V2, V3, and Razi). In Alborz, flocks were vaccinated with ND B1 at 1, 10, and 36 days of age (DOA), plus an inactivated bivalent ND+AI vaccine at 10 DOA (each house had the same regime but a different vaccine brand). In East Azerbaijan, the vaccination regimen included ND B1 combined with the ND+AI vaccine at 7 DOA, followed by ND LaSota and ND B1 at 20 and 29 DOA, respectively. Seroconversion against ND was measured at 1, 21, and 42 DOA by hemagglutination inhibition (HI) assay. In Alborz, the proportion of protected birds ($HI \geq 4$) varied significantly at 21 DOA (80%, 50%, 60%, and 75% for V1, V2, V3, and Razi, respectively; $P < 0.05$). By 42 DOA, neither mean titers nor protection rates differed significantly among groups ($P > 0.05$). In East Azerbaijan, at 21 DOA, the V1 and Razi groups had the highest proportion of birds with protective titers. By 42 DOA, groups V1 and Razi exhibited significantly higher titers compared to V2 ($P < 0.05$). The East Azerbaijan program yielded suboptimal antibody responses during the critical 3-week period, followed by higher titers at later ages, possibly due to stronger immune system booster by the LaSota strain. In contrast, the Alborz regimen stimulated an earlier serological response, but not significantly different from the Azerbaijan experiment ($P > 0.05$), providing more reliable early protection. In conclusion, in spite of some differences in antibody responses at 21 DOA, particularly with V1 and the domestic vaccine (Razi), with higher proportions of protected birds, these differences diminished by 42 DOA, indicating that all evaluated vaccines were ultimately capable of inducing protective immunity.

Keywords: Antibody response, Broilers, Immunity, Newcastle disease, Vaccination regimen

Article history:

Received 4 June 2025

Revised 13 July 2025

Accepted 29 July 2025

Published online 01 January 2026

1 Introduction

Newcastle disease (ND) is a significant threat to the global poultry industry, particularly in regions where the virus is endemic. Effective control of ND relies primarily on biosecurity and vaccination (Hassanzadeh et al., 2024). Both live and inactivated oil-adjuvanted ND vaccines are widely applied to induce a strong systemic antibody response (Rauw et al., 2009). Live vaccines, derived from avirulent or lentogenic strains, are often preferred because they stimulate both local and systemic immunity, thereby conferring broader protection (Ghafouri et al., 2024; Steensels et al., 2025).

It is well established that differences in vaccination programs and vaccine strains can significantly influence protection and be associated with higher HI titers by the chosen threshold (Dimitrov et al., 2017; Ghafouri et al., 2024). The selection of vaccine strain and vaccination schedule often varies depending on different epidemiological conditions (Rehmani, 1996). For example, in high-risk areas, frequent vaccinations at short intervals may be necessary to lower the mortality rate in broilers (Ghafouri et al., 2024; Steensels et al., 2025). Conversely, in regions with lower NDV prevalence, immunization programs are usually designed mainly to reduce airsacculitis in broilers or to prevent sudden drops in egg production in layers and breeders (Miller et al., 2010; Steensels et al., 2025). Therefore, an appropriately planned vaccination program, combined with effective ND vaccine administration, is crucial not only for protecting against virulent field strains but also for minimizing virus shedding.

The duration of vaccine-induced immunity varies depending on the vaccine formulation and host immune status at the time of vaccination (Abdoshah et al., 2012; Kapczynski et al., 2013). Typically, the primary immune response develops more slowly and is less persistent (approximately 2–3 weeks), whereas a booster vaccination enhances both the magnitude and provides long-lasting immunity. Importantly, once peak antibody levels are achieved, subsequent vaccinations mainly extend the duration of immunity rather than further elevating peak titers. For this reason, repeated administration of live or a combination of live and inactivated vaccines is commonly recommended to achieve reliable protection against virulent NDV challenge.

In Iran, ND has remained endemic and continues to threaten commercial broiler production, particularly in the Alborz and East Azerbaijan provinces (Abdoshah et al.,

2022; Abdoshah et al., 2012). Although numerous ND vaccines are widely used, few comparative studies have evaluated their relative efficacy or assessed different vaccination regimens under field conditions. Therefore, the present study was designed to investigate the immunogenicity of several commercially used live and inactivated ND vaccines in two broiler farms located in geographically distinct provinces of Iran.

2 Material and Methods

Experimental design and study area. This study consisted of two field experiments and study units conducted in Alborz Province (central Iran) and East Azerbaijan Province (northwest Iran); both recognized as high-risk regions for ND outbreaks (Abdoshah et al., 2012). Two commercial broiler farms were selected: one in Alborz with four houses of 6000 and 24,000 Ross 308 broilers in total, and a farm in East Azerbaijan with four houses of 14500 chicken and a total of 58,000 Ross 308 broilers. Each house received the same regimen but a different vaccine brand. All broilers were reared on the litter with free access to feed and water. Birds received the routine vaccination program for broilers (including infectious bursal disease, infectious bronchitis, and inactivated avian influenza vaccines), in addition to the ND vaccination program under investigation. The ND vaccines were obtained from different manufacturers and anonymized as V1, V2, V3, and Razi (Tables 1 and 2). Each house from the poultry site (either Alborz or Azerbaijan) received a vaccine package of a different Newcastle vaccine strain from a single vaccine brand

Blood sampling and Hemagglutination inhibition (HI) assay. On the first day of age (DOA), maternal-derived antibody (MDA) titers against NDV were determined from 20 randomly selected chicks per site. At 21 and 42 DOA, 20 blood samples were collected from each of the 4 experimental groups. Sera were separated, and specific antibody titers against NDV were measured using the HI assay according to the procedure described by the World Organization for Animal Health (World Organisation for Animal Health, 2021).

Performance indices. Throughout the trial, birds were observed daily for clinical signs of ND or other diseases. Mortality, final body weight, and feed conversion ratio (FCR) were recorded to compare production performance among experimental groups.

Statistical analysis. Data were analyzed using a completely randomized design. Serological data were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS software (version 9.4, SAS Institute, Cary, NC, USA). Results were expressed as mean \pm standard deviation (SD). The GENMOD procedure was used to compare the proportion of birds achieving protective HI titers ($HI \geq 4$). Tukey’s test was applied for multiple mean comparisons, and statistical significance was set at $P < 0.05$.

3 Results

Serum antibody titers

Experiment 1: Alborz farm

Serum HI antibody titers against NDV and the proportion of birds achieving protective titers are summarized in [Figure 1](#) and [Table 2](#), respectively. The average MDA titers were $5.58 \pm 0.95 \log_2$ titers. At 21 DOA, mean HI antibody titers ranged from 3.60 to 4.20 \log_2 and did not differ significantly between groups. However, the proportion of birds with protective titers was significantly higher in the Razi and V1 groups compared with the V2 group ($P < 0.05$). By 42 DOA, mean HI antibody titers (4.10 to 4.70 \log_2 titers) and the proportion of birds with protective titers (65 to 85%) did not differ significantly among groups ($P > 0.05$). ([Figure 1](#) And [Table 2](#))

Table 1. Vaccination program against Newcastle disease applied in broiler flock located in Alborz province

Vaccine	Vaccination method	Age of vaccination (d)
ND B1*	Eye drop	1
Inactivated ND + AI	Subcutaneous	10
ND B1	Eye drop	10
ND B1	Drinking water	36

Note: The birds were vaccinated against infectious busal disease virus (IBDV) and infectious bronchitis virus (IBV) according to the routine vaccination program of the farm.

*As the live bivalent vaccine (B1+H120).

Table 2. Proportion of birds with protective Newcastle disease antibody titers ($HI \geq 4$) inoculated by different commercial ND vaccines in broiler farm located in Alborz province

Vaccine group	Days of blood sampling	
	21	42
V1	80% (16/20) ^a	85% (17/20)
V2	50% (10/20) ^b	65% (13/20)
V3	60% (12/20) ^{ab}	70% (14/20)
Razi	75% (15/20) ^a	80% (16/20)

Note: The birds were vaccinated according to the vaccination program shown in Table 1.

^{a-b} values with different superscripts in each column are significantly different ($P < 0.05$).

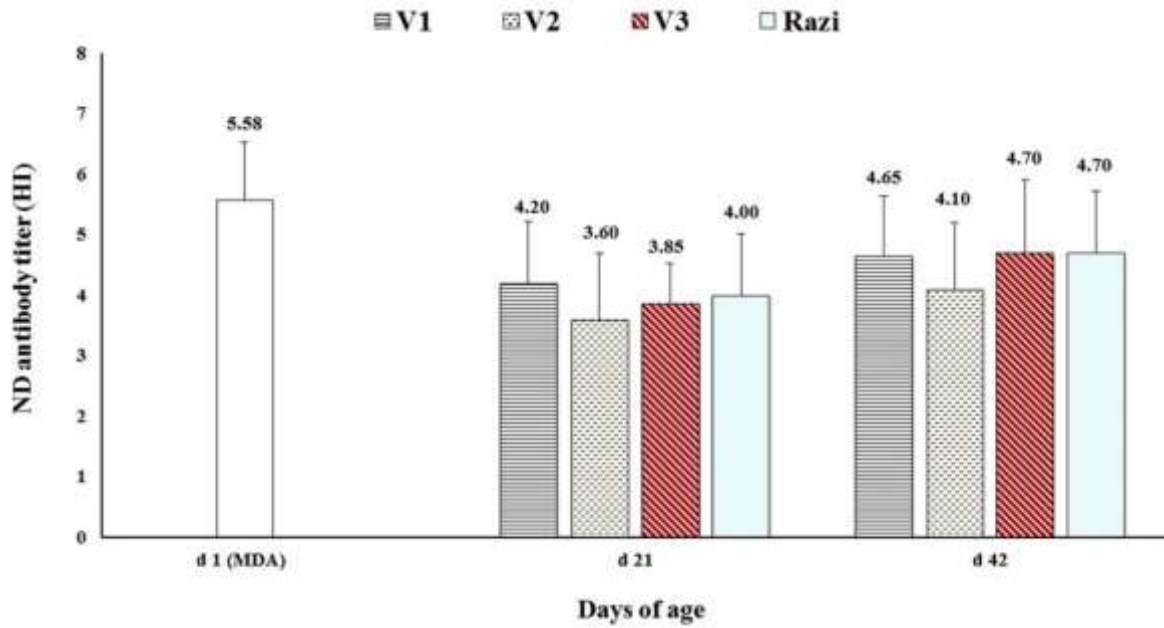


Figure 1. The HI antibody titers of vaccinated chickens with different commercial Newcastle disease vaccines (V1 to V3 and Razi) in broiler farms located in Alborz province. Note: The birds were inoculated according to the vaccination program reported in Table 1; Error bar= Standard deviation.

Experiment 2: East Azerbaijan farm

Mean MDA on the first DOA was 7.1 ± 0.96 log₂ titers. On 21 DOA, mean HI antibody titers did not differ significantly, although the highest values were observed in V1 and Razi groups, whereas V3 showed the lowest titers ($P > 0.05$). The proportion of birds with protective titers was higher in V1 and Razi groups compared with V3 on 21 DOA

(40% and 35% vs. 10%; $P < 0.05$), while differences involving V2 were not significant ($P > 0.05$). At 42 DOA, mean HI antibodies were higher in V1 and Razi compared to V3 groups ($P < 0.05$); however, no significant differences were detected among groups regarding the percentage of birds with protective titers (Figure 2 and Table 4).

Table 3. Vaccination program against Newcastle disease applied in broiler flock located in East Azerbaijan province

Age of vaccination (d)	Vaccination method	Vaccine
7	Eye drop	ND B1
7	Subcutaneous	Inactivated ND + AI
20	Drinking water	ND LaSota
29	Drinking water	ND B1

Note: The birds were vaccinated against infectious busral disease virus (IBDV) and infectious bronchitis virus (IBV) according to the routine vaccination program of the farm.

Table 4. Proportion of birds with protective Newcastle disease antibody titers ($HI \geq 4$) inoculated by different commercial ND vaccines in broiler farm located in in East-Azerbaijan province

Vaccine group	Days of blood sampling	
	21	42
V1	40% (8/20) ^a	95% (19/20)
V2	20% (4/20) ^{ab}	80% (16/20)
V3	10% (2/20) ^b	85% (17/20)
Razi	35% (7/20) ^a	90% (18/20)

Note: The birds were vaccinated according to the vaccination program shown in Table 3.

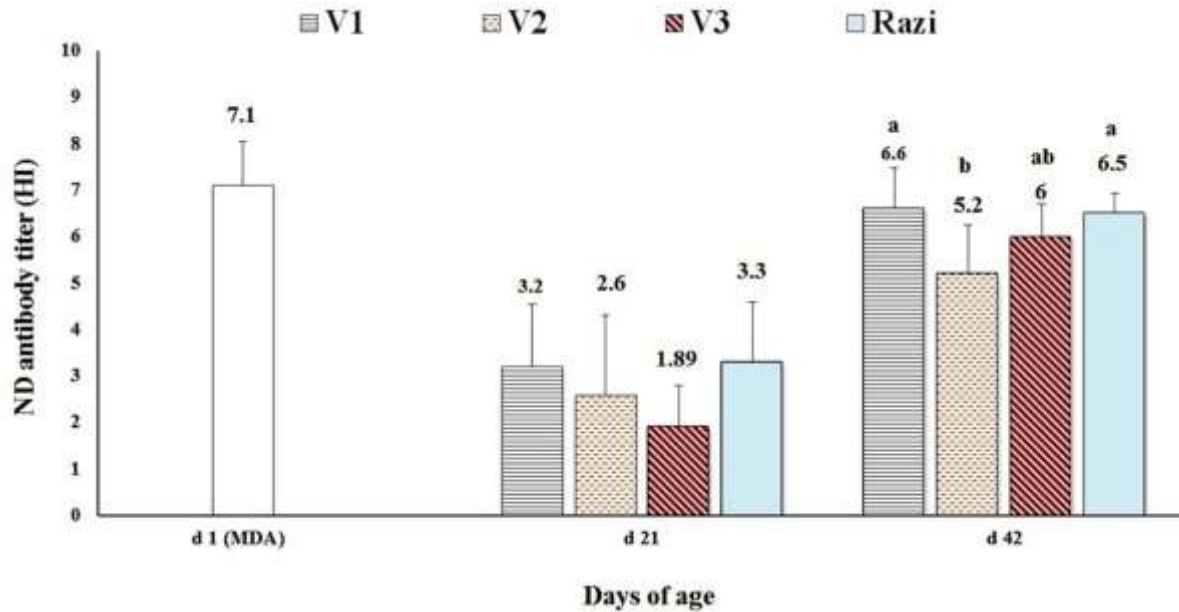


Figure 2. The HI antibody titers of vaccinated chickens with different commercial Newcastle disease vaccines (V1 to V3 and Razi) in broiler farms located in East Azerbaijan province. Note: The birds were inoculated according to the vaccination program reported in Table 3; Error bar= Standard deviation. Values with different superscripts (a,b) are significantly different ($P<0.05$).

Production Performance Indices

Production performance indices for broilers vaccinated with different ND vaccines in Alborz and East Azerbaijan are presented in Tables 5 and 6, respectively. In Alborz, mortality ranged from 6.24% to 9.68%, final body weight ranged from 2.880 to 2.910 kg, and FCR ranged from 1.92

to 1.99. Production indices tended to be higher in the Razi group (~296) compared with other groups (~267–280). In East Azerbaijan, mortality ranged from 2.80% to 4.30%, FCR ranged from 1.99 to 2.00, and production index ranged from ~293 to 335 across groups.

Table 5. Description of performance indices of broilers receiving different commercial Newcastle disease vaccines in Alborz province

Parameters	Flock No 1. V1 vaccine	Flock No. 2 V2 vaccine	Flock No. 3 V3 vaccine	Flock No. 4 Razi vaccine
No. Chicken	6250	6250	6250	6250
Mortality (%)	9.68	7.84	7.73	6.24
Average weight (kg)	2.880	2.910	2.905	2.910
FCR	1.99	1.94	1.95	1.92
Production index	267	281	280	296

Table 6. Description of performance indices of broilers receiving different commercial Newcastle disease vaccines in East-Azerbaijan province

Parameters	Flock No 1. V1 vaccine	Flock No. 2 V2 vaccine	Flock No. 3 V3 vaccine	Flock No. 4 Razi vaccine
No. Chicken	14,500	14,500	14,500	14,500
Mortality (%)	4.30	2.93	2.80	3.03
Average weight (kg)	3.00	3.219	3.156	3.236
FCR	2.00	1.96	1.99	1.91
Production index	293	325	315	335

4 Discussion

The control of ND in both commercial and backyard poultry remains essential to minimize economic losses (Hassanzadeh et al., 2024). Vaccination is the cornerstone of ND prevention, and in the endemic regions, it is scheduled by priming within the first week of life, followed by one or more boosters to ensure sustained protective antibody levels.

Live attenuated vaccines are widely used because they stimulate both humoral and cell-mediated immunity. Protection against ND largely depends on the magnitude and persistence of systemic antibodies, primarily IgY, which are routinely measured by serological assays. IgA also contributes importantly to mucosal immunity and can be accessed via tracheal washes or tears (Dimitrov et al., 2017). Lentogenic vaccinal strains such as LaSota and B1 Hitchner are effective on inducing both local and systemic responses. The ND vaccines manufactured from strains isolated during the 1940s–1960s have been administered for many years; however, their efficacy for suppressing viral shedding, reducing transmission, and controlling outbreaks under high challenge pressure remained controversial (Ambali et al., 2017; Hassanzadeh et al., 2024; Miller et al., 2007; Samakkhah et al., 2023). Biosecurity measures are still critical, but vaccine type (live vs. inactivated), timing, and manufacturer-specific characteristics strongly influence protective outcomes.

In this study, MDA titers were at satisfactory levels, indicating efficient transfer from breeder flocks. Since MDA can interfere with live vaccines (Steensels et al., 2025), early ocular administration is preferable to induce local immunity with negligible neutralization. Both experiments applied the first vaccination via eye drop, facilitating early mucosal priming.

Vaccine performance varied by location and schedule. In Alborz, V1 and Razi yielded higher protective antibody titers than V2, whereas in East Azerbaijan, V1 and Razi outperformed V3 at 21 DOA. Meanwhile, protective antibody titers were comparable across groups by 42 DOA. The higher early protection in Alborz likely reflects a more intensive vaccination schedule (B1 at the first DOA, boosted with B1 plus an inactivated ND+AI vaccine at 10 DOA). In contrast, in the East Azerbaijan experiment, vaccination was started by administration of B1 and inactivated ND+AI administration at 7 DOA. Because the primary responses typically take ~2 weeks, earlier priming is critical in broiler chicken. In the present study, early ND vaccine priming

most probably explains the higher and stronger antibody levels at third weeks of experiment in Alborz. The administration of LaSota at 20 DOA in East Azerbaijan subsequently resulted in more robust titers at 42 DOA, consistent with its higher immunogenicity.

According to WOA (World Organisation for Animal Health, 2021), effective ND protection will obtain when at least 85% of flock has an HI antibody titer $\geq 4 \log_2$. In our study, the Alborz flock almost reached this threshold ($\geq \log_2 4$ with 95% CI) earlier, which probably is associated with immunization in younger chickens. On the other hand, initiation of vaccine administration at the first DOA compared to 7 DOA did not result in satisfactory protection rate in some of the experimental groups, showing the influence of some other influential criteria such as vaccine quality and management measures. These findings demonstrated that in ND high-risk regions, early vaccination alongside biosecurity observation could help to acquisition earlier protective immunity. Given ND outbreaks usually occur during the 4th week of age in Iran, protective immunity induction is recommended as soon as possible (Abdoshah et al., 2022; Abdoshah et al., 2012). In the East Azerbaijan experiment higher immune coverage was noted and at least 80% of the chickens showed HI antibody titers ≥ 4 with CI=95%. Therefore, to acquisition a longer duration of immunity against ND, vaccination regimens including a sequential live vaccine administration with more virulent strain is recommended.

Variation in protection between flocks, even with the same vaccine strain, has been reported previously (Ambali et al., 2017; Samakkhah et al., 2023). In our experiments, differences between vaccine groups (e.g., superior performance of V1 and Razi compared to V2 and V3) most likely reflect differences in vaccinal seed properties, manufacturer-related quality, and formulation. Such vaccine intrinsic factors can strongly affect immunogenicity, particularly during the early stages of immune response (Kapczynski et al., 2013; Rauw et al., 2009). Moreover, the discrepancies between the two experimental sites (Alborz vs. East Azerbaijan) are more plausibly attributed to environmental and management-related factors, vaccination schedules, and interactions between vaccine type and field conditions. Variables such as flock management, stocking density, stressors, and MDA levels may modulate the effectiveness of otherwise identical vaccine strains. This highlights that vaccine effectiveness is related to its specification, as well as farm management and production conditions. Additionally, genetic background, stress, or

metabolic disorders can delay antibody responses in birds (Oberländer et al., 2020), but booster vaccinations help to synchronize immunity across the flock. These influencing factors may therefore explain why the same vaccine can perform differently across geographical or environmental attributes.

Multiple revaccinations enhance and prolong protective immunity: triple LaSota revaccination protected laying hens for 3 months against velogenic NDV (Okechukwu et al., 2020), Boasiako et al. reported stronger titers with at least three vaccine doses. Generally, two to three well-timed vaccinations suffice for broilers, while longer-lived flocks such as breeders or layers require extended programs (Boasiako et al., 2024; Steensels et al., 2025).

Regarding formulations, we used bivalent inactivated ND+AI vaccines alongside live vaccines. Previous studies have shown that bivalent inactivated IB+ND vaccines can induce higher antibody titers than monovalent ND vaccines (El-Dabae et al., 2023; Gough et al., 1977). However, viral interference should be considered, as IBV infection may impair Harderian gland function. Careful use of well-formulated vaccines can help overcome these limitations.

Beyond immunological outcomes, production indices are a practical measure of vaccination program success. In both experiments, growth performance and feed efficiency were within normal ranges in Iran, suggesting that productivity was not impaired. Although no significant differences in performance parameters were detected among groups, the East Azerbaijan flocks exhibited slightly lower mortality and higher production indices, likely reflecting better management practices or stronger MDA levels. These findings underscore that effective ND vaccination, while essential for disease control, should also be evaluated in relation to production performance to ensure both biological protection and economic viability.

Overall, our findings indicate that both imported and domestic ND vaccines used in the current study can elicit an effective humoral immune response, but vaccination schedule and management are critical determinants of success. The study showed that priming on the first day of chicken age could lead to a higher humoral antibody titer and proportion rate of positive responses, HI titer $\geq 4 \log_2$, (Alborz experiment) during first 3 weeks of age in comparison with priming on the 7th DOA (Azerbaijan experiment). Therefore, it can be concluded that in high-risk regions for ND outbreaks, earlier immune competency is needed, priming at the first DOA via the ocular-nasal route, even in the presence of maternal antibodies, would be

preferred. In addition, in case of priming at the 7th DOA in order to reach more than 85% positive HI titers and longer duration of immunity, using more immunogenic strains such as the LaSota vaccine might be more effective.

5 Limitations

It is worth mentioning several limitations of the study, which may affect the results, such as observational field setting, confounding by site/management and mixed vaccine components (live + inactivated ND+AI), lack of virology and challenge evaluation, and unit of analysis limitations.

6 Conclusion

This multisite study demonstrated that both imported and locally produced ND vaccines used in the current study, provided broadly comparable levels of serological protection and production performance in commercial broiler flocks. However, some differences were observed in antibody responses at 21 DOA, particularly with V1 and the domestic vaccine (Razi), yielding higher proportions of birds with protective titers; these differences diminished by 42 DOA, indicating that all evaluated vaccines were ultimately capable of inducing protective immunity. The vaccination program implemented in Alborz promoted earlier serological responses, while the East Azerbaijan regimen resulted in delayed but stronger antibody titers, likely due to the inclusion of LaSota at midcycle. Production indices remained within acceptable commercial ranges across all groups, with a slight advantage observed for the domestic vaccine (Razi). Overall, the findings suggest that ND vaccines from both foreign and domestic manufacturers, when applied under appropriate vaccination regimens, can provide effective immune response and sustain satisfactory flock performance in Iranian broiler production systems.

Acknowledgements

The authors would like to thank RVSRI for support this study.

Conflict of Interest

We declare that no conflict of interest.

Author Contributions

The manuscript was written collaboratively by all authors, with each author providing substantial input, revisions, and final approval of the submitted version.

Data Availability Statement

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

Ethical Considerations

This study was conducted in accordance with institutional and national guidelines for the care and use of animals. The study protocol was approved by the Research Ethics Committee of the Razi Vaccine and Serum Research Institute, Iran, under ethics approval code IR.RVSRI.REC.1400.003. All field and laboratory procedures, including bird handling, vaccination, and blood collection, were performed by trained personnel in accordance with accepted veterinary standards and animal welfare principles. Permission for access to birds and sampling procedures was obtained from the relevant commercial farm authorities. All necessary measures were taken to reduce distress and ensure biosafety and biosecurity throughout the study.

Funding

This study was financially supported by RVSRI (Grant no.: 527/250)

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