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Comparative Antibody Responses of Four Turkey Strains to Attenuated Salmonella Vaccine: A Path to Enhanced Poultry Production

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ABSTRACT

The increasing demand for turkey as a protein source necessitates effective management practices, particularly in combating Salmonella infections. This study evaluated the immune response of four turkey strains (black, white, exotic, and lavender) to a Salmonella gallinarum-9R strain vaccine. A total of 180 samples of day-old poults (45 from each strain) were used for the experiment. Following initial blood sampling at four weeks, all birds were vaccinated orally with the Salmonella vaccine. Monthly blood samples were collected over four months to assess antibody titres using a serial dilution method. Results revealed significant differences in the mean antibody responses among the strains. The black strain exhibited the highest mean antibody titre (1.38), followed closely by the exotic (1.32) and white (1.30) strains, while the lavender strain showed the lowest titre (1.17). The antibody titres significantly increased post-vaccination compared to pre-vaccination values ($p < 0.05$). These findings highlight the effectiveness of vaccination in enhancing immunity in turkeys, suggesting that selective breeding of more responsive strains could improve flock health and reduce the risk of Salmonella infection.

INTRODUCTION

Over recent decades, egg and poultry meat production and consumption have witnessed significant increases worldwide. Notably, in recent years, the consumption of eggs and meats from poultry, such as chicken and turkey, has upsurged (Can & Can, 2022; Connolly *et al.*, 2022). This surge connotes the growing importance of poultry products, particularly in Africa, where they serve as a critical source of income and play a vital role in the protein supply chain (FAO, 2010; Mottet & Tempio, 2017; Grace *et al.*, 2024). Nigeria has one of the lowest levels of animal protein consumption, averaging 45.4g which falls below the minimum benchmark of 53.8 g per capita daily protein intake recommended by the Food and Agriculture Organization (FAO) (Challenge *et al.*, 2020). The majority of animal protein from the poultry industry in Nigeria stems from chicken (Akhigbe & Akaeze, 2023), but there has been a recent decline in production owing to factors such as environmental changes (Wasti *et al.*, 2020; Osuji *et al.*, 2024) and production costs (Sesay, 2022). In this context, turkeys provide a complementary option to chickens, as they adapt well to various environmental conditions and offer superior meat quality characterized by lower fat content (Pinto e Silva *et al.*, 2008).

Despite their nutritional and economic potential, turkey breeding remains relatively underdeveloped. Turkeys, which belong to the genus Meleagris, include two primary species: Meleagris gallopavo (the domesticated strain) and Meleagris ocellata (the wild strain) (David, 2018). Unfortunately, these birds are particularly vulnerable to infections from Salmonella, a pathogen that can not only affect their health and productivity but also serve as a vector for transmission to humans. In Nigeria, outbreaks of food poisoning have been linked to Salmonella infections (Ojeniyi & Montefiore, 2009). Worldwide, approximately 1.3 billion cases of gastroenteritis each year are associated with Salmonella (CDC, 2019). While the increasing demand for protein drives the production of turkeys, the prevalence of Salmonella infections significantly undermines these efforts, resulting in reduced productivity and diminished commercial viability compared to more commonly produced poultry species (Lamichhane *et al.*, 2024). Nonetheless, turkey meat remains a popular dietary choice in Nigeria, as it is not subject to the same cultural or religious restrictions as other poultry sources. Thus, there is a pressing need to focus on producing healthy turkeys that are free from these harmful bacteria to improve economic stability and ensure the safety of consumers (Lamichhane *et al.*, 2024). However, diseases continue to pose substantial threats to Nigeria's burgeoning poultry economy. Among the various ailments that affect poultry, Newcastle disease, Salmonella infections, and blackhead disease are particularly prevalent. The presence of Salmonella is a significant concern, as it is responsible for causing salmonellosis, a disease that has far-reaching impacts on both commercial and local turkey populations due to their heightened susceptibility to this pathogen (Lamichhane *et al.*, 2024). Estimates suggest that salmonella infections may represent a greater economic burden than any other bacterial infection affecting livestock (Baptista *et al.*, 2023). Despite the widespread use of antibiotics and antiseptic treatments intended to prevent outbreaks, Salmonella infections persist, affecting even those birds that receive such interventions (Lamichhane *et al.*, 2024). In many developed nations, effective control measures

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have led to significant reduction but the most prevalent is in Sub-Saharan Africa (Stanaway *et al.*, 2019). The majority of Salmonella transmission to humans has been through food, including meat (Kirk *et al.*, 2015). However, in Nigeria, a multitude of factors complicates effective management, as numerous potential sources of Salmonella infection and product contamination exist within integrated poultry enterprises.

In light of these challenges, there is an increasing interest in developing effective prevention strategies, particularly through vaccination, to combat the impact of salmonellosis and improve turkey breeding, which has suffered due to the disease (Oyeagu *et al.*, 2022). This situation calls for a comprehensive investigation into the resistance of various turkey strains to Salmonella infection, especially following vaccination with the Salmonella gallinarum-9R strain. The findings from this research could contribute significantly to improving strategies for managing Salmonella infections, thereby enhancing turkey production and ensuring public health safety for consumers.

MATERIALS AND METHODS

Experimental Birds and Management Procedures

This study utilized day-old poults from four different strains: 45 black, 45 white, 45 exotic, and 45 Lavender. The poults were sourced from Francisco Turkelings, Calabar, Nigeria, and transported to the experimental site set up at the Department of Science Laboratory, Delta State Polytechnic, Delta State. The birds were kept in standard cages with natural ventilation. Before their arrival, the cages were thoroughly fumigated and disinfected using an organophosphate insecticide and Dettol to minimize the risk of infection. The poults were acclimatized for two weeks and provided with Turkey Starter feed and water ad libitum. After eight weeks, the feed was switched to grower feed.

Experimental Design

Following the acclimatization period, the poults were randomized into four groups using a Randomized Complete Design, with each group corresponding to a strain. There were 45 replicates per strain.

Vaccination and Blood Sampling

At four weeks of age, 1 ml of blood was collected from each strain through the wing veins using a sterile needle and syringe. This initial blood sample, collected into EDTA bottles, was used for antibody assessment (control). Subsequently, the birds were vaccinated with the Salmonella gallinarum-9R strain. The Salmonella gallinarum-9R strain vaccine was dissolved in 100 ml of water and 1 ml was given to each bird orally. Monthly blood sampling and antibody assessments were conducted for four months.

Preparation of Buffer Solution and Measurement of Antibody Titre

The buffer solution used for measuring antibody titre was phosphate-buffered saline (PBS), prepared as follows: sodium chloride (8 g/L), potassium chloride (0.2 g/L), disodium hydrogen phosphate (1.15 g/L), and potassium dihydrogen phosphate (0.2 g/L) were dissolved in 10 litres of distilled water. The buffer was maintained at a pH of 7.3. Antibodies present in the serum were detected using a serial dilution method. A constant dilution factor of 2 was applied, with serial dilutions ranging from 10^2 to 10^{256}. The butter solution was poured into a test tube in different volumes: 2 ml, 4 ml, 8 ml, 16 ml, 32 ml, 64 ml, 128 ml and 256 ml. Afterwards, 1 ml of antigen was added across all the buffer volumes followed by 1 ml of serum. The mixture was incubated for 1 hour, at room temperature before recording the result. Antibody titre was recorded as the highest dilution showing a positive reaction for each strain.

Statistical Analysis

All data obtained from the experiment were subjected to analysis of variance (ANOVA) and the means were separated using the least significant difference (LSD) test at a 5% level of probability.

RESULTS AND DISCUSSION

Response of Turkey Strains to Salmonella Vaccine

The antibody responses of the four turkey strains to the Salmonella vaccine are presented in Figure 1. The results reveal significant differences among the strains, indicating that the black, white, and exotic strains produced higher antibody titres compared to the lavender strain. Specifically, the black strain exhibited the highest antibody titre, measuring 1.38. This was closely followed by the exotic strain, which recorded an antibody titre of 1.32, and the white strain, with a titre of 1.30. In stark contrast, the lavender strain showed the lowest antibody titre value at 1.17.

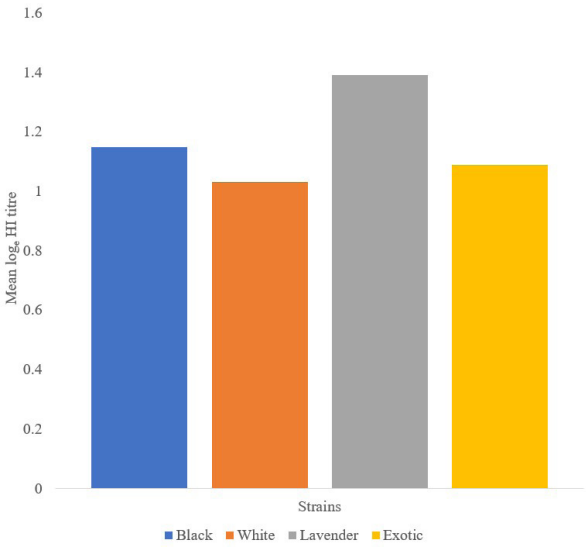

Figure 1: Mean titre response of four turkey strains to Salmonella vaccine

Antibody Titre Response before and after Vaccination

Table 1 presents the antibody titre values for the four turkey strains before and after vaccination with the Salmonella vaccine. A statistical analysis of the data indicated significant differences ($p < 0.05$) in the antibody titre values across the various turkey strains both before vaccination and at different time points following vaccination. In the case of the black strain, the initial antibody titre was the lowest recorded before vaccination. However, after vaccination, antibody titres increased, and by months 2, 3, and 4, the black strain showed consistent titres. For the white strain, the results were particularly noteworthy, revealing a marked increase in antibody titres, peaking at month 4 with a value of 1.78. This was accompanied by titres of 1.44 at month 3, 0.59 at month 2, and a control titre of 0.33 before vaccination. The lavender strain's response was characterized by some variability, with antibody titre values at months 2, 3, and 4 being statistically similar yet significantly higher ($p < 0.05$) than the initial response before vaccination. The exotic strain demonstrated the most pronounced response, achieving the highest mean titre value at month 4, which was measured at 1.84. This was followed by a strong response at month 3, with a titre of 1.44.

Table 1: Mean titre (loge HI) response of turkey strains before and after Salmonella vaccination

Duration	Black	White	Lavender	Exotic
Month 1(Initial)	0.45 ± 0.06^{b}	0.33 ± 0.06^{d}	0.63 ± 0.08^{b}	0.48 ± 0.07^{c}
Month 2	1.38 ± 0.09^{a}	0.59 ± 0.07^{c}	1.08 ± 0.13^{a}	0.59 ± 0.07^{c}
Month 3	1.38 ± 0.09^{a}	1.44 ± 0.06^{b}	1.33 ± 0.12^{a}	1.44 ± 0.06^{b}
Month 4	1.38 ± 0.09^{a}	1.78 ± 0.08^{a}	1.13 ± 0.10^{a}	1.84 ± 0.68^{a}

Mean values with different superscripts along the same vertical lines are significantly different ($p<0.05$). Haemoglobin inhibition (HI)

Discussion

Salmonella infection remains one of the most significant bacterial diseases affecting poultry, leading to considerable economic losses and public health concerns due to its zoonotic potential (Baptista *et al.*, 2023). In response to this challenge, various control strategies have been employed, including the administration of antibiotics and probiotics, as well as the implementation of selective breeding programmes aimed at enhancing the resistance of turkey stocks to these infections. Despite these efforts, the production of turkey meat continues to be constrained by the limited attention given to turkey research, particularly regarding their health management and immune responses (Oyeagu *et al.*, 2022). This neglect is troubling, especially considering the significant protein supply potential that turkeys offer, which is important for food security.

The findings from this study revealed significant differences in the antibody responses among the turkey strains, particularly highlighting that the black, white, and exotic strains produced notably higher antibody titres compared to the lavender strain, both before and after vaccination. This differential response indicates that the immunological capacity of these strains was significantly influenced by the vaccine, demonstrating varying levels of effectiveness in stimulating an immune response. The marked increase in antibody levels post-vaccination suggests a robust immunological response, critical for establishing protection against Salmonella. A similar trend was observed by Ikpeme (2021) following the vaccination of turkey strains with Newcastle vaccine. The observed increase in antibody levels post-vaccination signifies that the immune systems of these strains were effectively stimulated by the vaccine. The findings suggest that the administration of the Salmonella vaccine elicited a strong immune response, characterized by the production of antibodies that could help combat infection. This aligns with the principle that a greater quantity and diversity of antibodies correlate with the strength of the immune response (Zimmermann and Curtis, 2019, Cosgon *et al.*, 2023). The consistent titres recorded across the black, white, and exotic strains in the months following vaccination further support the notion that a higher antibody response is indicative of enhanced resistance to disease.

Interestingly, the white strain demonstrated the highest antibody response at four months post-vaccination, with a mean titre of 1.78, indicating an effective and sustained immune response. The exotic strain also exhibited strong titres, with 1.84 at month four, suggesting that these strains could be prioritized in breeding programmes aimed at improving overall flock health and productivity. In contrast, the lavender strain's response was less pronounced, though it did show a statistically significant increase in antibody levels at months 2, 3, and 4 compared to pre-vaccination titres. This accentuates the importance of evaluating breed-specific responses when developing vaccination strategies which has been noted in many reports (Ravikumar *et al.*, 2022; Miyumo *et al.*, 2024; Abdelaziz *et al.*, 2024; Geletu *et al.*, 2024). Moreover, the temporal dynamics of antibody levels observed in this study indicate a natural progression in the immune response. The decline in antibody levels following the peak response period is consistent with findings from earlier research (LeBaron *et al.*, 2009; Klein *et al.*, 2023; Vashishtha & Kumar, 2024), which indicated that while vaccines are effective in priming the immune system, antibody levels naturally decrease over time. This suggests the necessity for ongoing monitoring of antibody levels in the turkey population, as well as the potential need for booster vaccinations to sustain an adequate level of immunity over time. According to Chen *et al.* (2022),

antibody levels peak around one month after vaccination and gradually decline, stabilizing at a plateau significantly higher than pre-vaccination levels due to continuous production from long-lived plasma cells (LLPCs) in the bone marrow. This was the observation made across the different strains where the antibody titres were generally higher than levels before vaccination despite the variations between months. Maintaining optimal immunity is particularly important in environments where the risk of Salmonella exposure is heightened, as it directly impacts flock health and productivity. In addition, the implications of this research extend beyond individual strain performance. Understanding the differences in immune responses among various turkey breeds can inform breeding programmes and management practices aimed at enhancing disease resistance. Selecting strains with superior antibody responses could lead to a more resilient poultry population, ultimately contributing to better animal health, reduced transmission to humans, and reduced economic losses due to Salmonella.

CONCLUSION

This study demonstrates that vaccination with the Salmonella gallinarum-9R strain significantly enhanced the immune response of various turkey strains, with the black, white, and exotic strains showing notably higher antibody titres compared to the lavender strain. The results indicate that effective vaccination is essential for improving the health and productivity of turkeys, particularly in the context of Salmonella prevention. Given the significant differences observed in immune responses among the strains, it is evident that selective breeding for higher vaccine responsiveness could further enhance flock resilience against Salmonella infections. The present findings demonstrate the importance of implementing robust vaccination strategies in turkey management to ensure both animal health and food safety.

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