

## EFFECT OF DIFFERENT SCHEDULES OF VACCINES ON GROWTH AND PERFORMANCE OF BROILERS

## RAUF U<sup>1</sup>, HAQ SU<sup>2</sup>, ARSHAD Z<sup>3</sup>, USMAN M<sup>4</sup>, SAJID M<sup>5</sup>, WAHEED SF<sup>6</sup>, HUSSAIN T<sup>7</sup>, HUSSAIN A<sup>6</sup>, AMEER S<sup>8</sup>, NAZEER HAA<sup>9</sup>, ULLAH A<sup>1</sup>, \*HASSAN A<sup>10</sup>, SABIR A<sup>11</sup>

<sup>1</sup>Veterinary Research Institute, Zarar Shaheed Road, Lahore Cantt, Pakistan

<sup>2</sup>Department of Pharmacology, Shantou University Medical College, Shantou, Guangdong 51504, China

<sup>3</sup>Department of Pathology, University of Agriculture Faisalabad, Pakistan

<sup>4</sup>Department of Poultry Science, UAF Sub Campus Toba Tek Singh, Pakistan

<sup>5</sup>Riphah College of Veterinary Sciences, Riphah International University, Lahore, Pakistan

<sup>6</sup>Department of Veterinary Sciences, Faculty of Veterinary & Animal Sciences, Azad Jammu & Kashmir University

of Bhimber-Bhimber AJK-10040

<sup>7</sup>Institute of Animal and Dairy Sciences, Toba Tek Singh, University of Agriculture Faisalabad, Pakistan

<sup>8</sup>Animal Science Division, Nuclear Institute for Agriculture and Biology NIAB-C (PIEAS), Faisalabad, Pakistan

<sup>9</sup>College of Veterinary Sciences, University of Agriculture Peshawar, Pakistan

<sup>10</sup>Department of Microbiology, University of Agriculture Faisalabad, Pakistan

<sup>11</sup>Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences Lahore Sub-Campus Jhang,

Pakistan

\*Corresponding author email address: <u>aasimahassan73@gmail.com</u>

(Received, 12<sup>th</sup> March 2024, Revised 7<sup>th</sup> June 2024, Published 17<sup>th</sup> June 2024)

Abstract A vaccination program is a schedule of vaccinations that varies by nation or property. This study set out to assess the productivity and overall health of broiler chickens with different immunization schedules. From a total of 110 Cobb 500 chickens, three treatment groups—each with six duplicates—were chosen at random. For every treatment group, there were different immunization schedules for Newcastle disease, infectious bronchitis, and infectious bursal disease. On the other hand, broilers in Treatment 2 (T2) received vaccinations against ND+IB on days 3 and 7, whereas broilers in Treatment 3 (T3) received vaccinations against ND+IB on days 7 and 21 as well as IBD on day 14. The grill control group in treatment 1 (T1) was vaccinated against ND+IB and IBD on days 7 and 14, respectively. During the 42-day study period, samples and data were gathered to evaluate the animals' development performance, immunological status, carcass characteristics, and meat quality. T1 broilers fared better than T2 and T3 broilers in terms of growth rate, white blood cell count, carcase features, and meat quality. The results of this study show that immunizing broilers against ND+IB and IBD on days 7 and 14 is the most successful vaccination schedule for broiler production, as demonstrated by the animals' improved health and productivity. **Keywords:** *broiler*; *vaccination*; *meat quality*; *health*; *immunization* 

#### Introduction

The chicken business is significantly decreasing Pakistan's protein supply-demand gap. Commercial chicken raising in Pakistan began in the early 1970s and grew dramatically over the next several decades. The business's early success was largely due to the government's promotional measures and the resilience of the chicken farming sector. For a lot of years, the chicken business was free from import charges, sales taxes, and income taxes. This resulted from the government's development of special incentives for this business and its identification of the chicken production chain as a crucial component of the food processing industry. As a result, the industry grew at a rate of 18-32% per year in the early 1980s and then another 15-20% per year in the 1990s. The domestic market was the main driver of this increase, with poultry meat consumption rising by more than 4% a year. In Pakistan, the production of chicken accounts for 27% of all livestock output, 5.8% of the country's agricultural sector, and 1.3% of its GDP. It is a highly structured and dynamic industry. Over 1.6 million people are currently employed in the poultry business, which has seen significant growth in recent years (Sadiq 2004). Although the grill business has grown, there are still many barriers standing in the way of it, which could lead to unstable economies and decreased food security as a result of disease outbreaks (Chung et al.





Broiler farms necessitate effective 2019a). management and immunization, as flocks with a higher bird count are more susceptible to disease transmission. Newcastle disease (ND), infectious bronchitis (IB), and infected bursal disease (IBD) are the most common infections. Aini (2006) posits that ND is the disease that induces the most substantial pecuniary loss. Certain viruses have the potential to multiply in particular organs before disseminating to other regions of the body. ND and IB are the consequences of respiratory tract infections caused by paramyxoviruses and coronaviruses. On the other hand, juvenile birds infected with ND may develop neurological symptoms, including torticollis and wing or limb paralysis, whereas IB-infected birds may develop systemic disease in the reproductive and renal systems (Jackwood 2012). Fabricius' bursa is malfunctioning due to the immune system suppression caused by the IBD virus. Enlarged cloaca bursa and distributed hemorrhages in the thigh and breast muscles are common, even in the absence of specific clinical indicators (Dey et al., 2019). IBD is transmitted through the fecal-oral route, whereas ND and IB can be transmitted through the air. However, contaminated clothing, footwear, wild animals, and equipment can all mechanically spread all of these illnesses (Aini 2006; Jackwood). 2012). Vaccination is a useful method of preventing these illnesses, even if they are linked to several hazards in the chicken business (Muller et al., 2012). As a result, immunization is an essential part of managing the grill industry.

Vaccination protects birds from endemic diseases and helps farms avoid incurring losses due to infections or decreased productivity (Sharma, 1999). business, In the poultry recombinant, killed/inactivated, and live-attenuated vaccines are the three most commonly used forms. Liveattenuated vaccinations are mostly used to develop active immunity in chickens, which offers durable defence against certain illnesses (Sharma 1999). They contain weaker or more temperate pathogen strains. Inactivated or destroyed vaccines contain microorganisms that are unable to proliferate in vaccinated animals after they are administered. Supplemental doses need to be given often to maintain optimal immunity levels since inactivated vaccines do not produce strong enough immune responses (Marangon and Busani, 2006). In comparison to live-attenuated and inactivated vaccines, recombinant vaccines are relatively new. Modern gene technology is employed to combine live, attenuated strains of hazardous organisms to produce recombinant vaccines. To serve as a vector, a virus that has been genetically modified contains the genetic coding for an alien antigen. Recombinant immunizations safeguard birds from both the vector organism and the foreign antigen produced by the

vector (Sharma, 1999). In birds, vaccines frequently induce humoral and cell-mediated immunity. Macrophages, T lymphocytes, and B lymphocytes are among the immune cells that are stimulated by the vaccine's antigens (Chung et al. 2019b).

Because broilers have a shorter production time than layers and breeders, their vaccination schedules are less complicated. Live vaccines are usually used to actively immunize poultry, especially broilers, against illnesses such ND, IB, and IBD (Sharma, 1999). Immunization against ND and IB is normally given on the first day of life or between the ages of 3 and 21, but immunization against IBD is normally given between the ages of 14 and 21. There are several vaccination schedules in place, and some of these might stress out birds more than others (Yang et al., 2011). The health and growth performance of broilers may be hampered by these demands manifesting as incorrect handling procedures or vaccination administration (Liu et al., 2015).

As such, constant observation of broilers is necessary to avoid the development of any physical problems after vaccination. After vaccination, antibody titre is determined by serological testing to determine the immunization's effectiveness (Yang et al., 2011). This research is important because it helps determine a suitable vaccination schedule, which lowers costs for broiler farms, avoids over-immunization, and keeps broilers from going through needless stress during the rearing stage. There hasn't been much research done on how various vaccination schedules affect the health and production of commercial broilers. Therefore, this experiment's main goal was to find out how different vaccination schedules affected the immune system, carcase features, meat quality, and growth performance of commercial broilers.

#### Materials and methods Experimental material

Following the one-day-old weight of 108 male Cobb 500 broiler chicks from a nearby hatchery, three treatment groups were assigned at random. Each of the six replications of each treatment contained six broilers. The broilers were housed in an open-sided building and raised in battery cages with wired floors. Commercial starter and finisher feeds were given to the broilers from days 0 to 21 and 22 to 42, respectively. Water and food are available to broilers at all times. There were differences in the immunization schedules for every treatment group (see Table 1). The broilers were given three different regimens: As part of Treatment 1 (T1), the most widely used approach in the field, the broilers received live ND+IB vaccinations on days 7 and 14. (control). On days 21 and 7, they then received live IBD treatment. On days 3 and 7, the broilers received live ND+IB immunizations. On day 14, they received live IBD treatment. The second

treatment was this one (T2). Third treatment (T3). On days 3 and 7, the ND+IB vaccination was given intraocularly; on days 14 and 21, the IBD and ND+IB immunizations were given by drinking water. To keep the broilers from going through too much stress, all vaccinations were done as soon as possible in the morning.

## Experimental design

The feed conversion ratio (FCR) and body weight gain were calculated by recording the weekly body weight and feed ingestion of each replicate during the 42-day study period. These values were subsequently employed to evaluate the growth performance. Six broilers were randomly selected from each treatment group on days 21 and 42, and their brachial vein blood was collected into anticoagulant (EDTA) blood tubes for white blood cell analysis (Chung et al., 2020). Following a blood sample, the same broilers were killed on day 42 to assess the carcass features and meat quality.

### Analysis of white blood cells

Wright Stain was manually added to peripheral blood smears to determine the total and differential white blood cell (WBC) counts. As part of the TWBC procedure, WBCs were counted in ten different slide fields using a 40x objective to obtain the average WBC number. To find the expected TWBC count per microliter, multiply the average value by 2000. Carisch et al. (2019) calculation method was followed.

For differential WBC counts, 210 WBC were manually focused at 100x magnification using emulsion oil. After that, the data were used to calculate percentages (Lilliehook et al. 2008).

## **Carcase parameters**

The following weights were recorded after the corpses were methodically dissected: dressing percentage, final live weight, kill-out weight, defeathered weight, drumsticks, wings, neck, head, shank, gastrointestinal tract, heart, and liver (Chung et al. 2019a).

# Meat quality investigation

Chung et al. (2019a) provided a detailed description of the methods used to assess pH, colour, drip loss, and culinary losses. To assess heating losses, 30 g of breast flesh from each treatment group's samples were weighed and recorded to establish the starting weight represented as (W1) and W2 as the final weight.

## Statistical analysis

The means of the treatment group were compared by using ANOVA and Tukey test.

#### Results

#### Impact on Growth

The impact of various vaccination schedules on the growth performance of broilers is displayed in Table 2. Significant variations in body weight and weight gain were seen in the first phase across the treatment groups. The T2 broilers grew the least amount of body weight in comparison to the T1 and T3 broilers. The T1 broilers showed numerically larger values, but there were no significant changes in the other parameters. There was a significant difference in the cumulative feed intake and FCR during the finisher phase. The growth efficacy of T1 broilers was superior to that of T2 and T3 broilers, as evidenced by a lower feed intake and a higher cumulative FCR.

## White blood cell count

Table 3 illustrates the impact of various immunization schedules on the fluctuations in white blood cell counts of broilers over a six-week growth period. During the first phase, the heterophils to lymphocytes (H/L) ratio was the sole significant variation that was found. The H/L ratio was lower in T2 broilers than in other regimens. Significant alterations were observed in the percentages of lymphocytes, heterophils, and H/L ratios during the finisher stage. T1 broilers outperformed T2 and T3 broilers in terms of health; they had the lowest percentage of heterophils, the highest percentage of lymphocytes, and the lowest H/L ratio.

### **Carcase characteristics**

Table 4 shows the effects of various vaccination schedules on the traits of broiler carcasses over a sixweek maturation period. Drumsticks varied considerably among treatment groups as did gastrointestinal tract weight, dressing percentage, kill-out and de-feathered weight, and drumsticks. Not much varied in the end live weight, carcass weight, breast, wings, neck, head, shanks, adipose, heart, liver, and full or empty gizzard. The T1 grill routinely had the largest offal, body section, and carcase weights among the treatments, suggesting that the carcass had better qualities.

A mean  $\pm$  standard error was used to represent all data. Columns a and b with superscripts show statistically significant variations in the data.

#### Meat quality

Different vaccination schedules during a six-week maturation period affected the quality of grilled meat, as Table 5 shows. Among the interventions, there were significant differences in drip loss and cooking on days 1 and 7 of storage. Meat colour measures showed no statistically significant variations in muscle pH, brightness, redness, or yellowness. The meat quality of T1 broilers was found to be superior to that of T2 and T3, as shown by their lower measured values.

#### Discussion

The immunization schedule for a flock of chickens needs to take several things into account. Since immunizations might be expensive overall, the schedule should only cover those that are necessary (Marangon and Busani, 2006). When assessing whether a vaccine is acceptable, consider the flock's past, its closeness to nearby farms, endemic

infectious agents, and the degree of biosecurity implemented on the property. According to Marangon and Busani (2006), all regimens contain immunization against a few major epidemic disorders that plague broilers, such as ND, IB, and IBD. Improper administration can harm the quality of meat, health, growth performance, and carcass features, even though vaccination is an essential part of managing the chicken industry. This investigation aimed to determine the immunological status and production performance of broilers given different vaccination schedules. performance. Growth especially more immunological stress, is one indicator that can be used to gauge the degree of stress. Cook (1999) found that vaccinations slowed the growth rate. Research has demonstrated that regularly giving birds high doses of immunizations may cause immunological stress (Yang et al., 2011). Rather than synthesizing body fat and protein to satisfy dietary requirements and generate immunological effector molecules, immunological stress induces the disintegration of body food stores (Liu et al., 2015; Wang et al., 2015). The additional immunizations administered to each treatment group on days 3 and 21 of this trial had an impact on T2 and T3 broilers. In an attempt to offset the increased stress the birds endured during handling and administration, dietary nutrients may have been redirected from growth to immune-boosting metabolic responses, which is why T2 broilers gained less weight during the starting period (Liu et al., 2015).

Broiler chicks go through several significant physiological changes in the first seven days after hatching, including the development of their immune system, gastrointestinal tract, and thermoregulatory system (Christensen, 2009). At this point, the chick starts to metabolize the leftover yolk, which serves as its main food source. The T2 chicks' lower body weight gain during the early stage suggests that the immune system may need calories for growth and development in reaction to the vaccination. However, following a period of restricted experience development, birds usually complementary growth, marked by a growth spurt aimed at reaching a weight similar to other birds growing at a normal rate on a free-choice diet (Hornick et al., 2000). During the finisher phase, broilers in the T2 and T3 groups showed a significant increase in feed consumption compared to the T1 broilers. As a result, broilers receiving vaccinations more frequently saw a decline in feed efficiency, which harmed their performance.

A flock's health performance can be evaluated by looking at its mortality rate and certain blood indicators. The H/L ratio, an efficient stress metric, can also be used to evaluate the health of vaccinated broilers (Chung et al., 2020). According to Scanes

(2016), heterophils are phagocytic lymphocytes that control the avian acute inflammatory response. T and B lymphocytes are two more subtypes of lymphocytes. T cells initiate cell-mediated immunity, but B lymphocytes have a major impact on humoral immunity (Chung et al., 2019b). Stress affects the number of lymphocytes in the bloodstream. In addition to immunological stress, poor vaccination management or distribution can cause stress during immunizations. According to Krams et al. (2012), heat stress can make this kind of stress worse by quickly reducing the amount of lymphocytes in the bloodstream, which results in immunosuppression. Therefore, the timing and length of the vaccination should be carefully planned to avoid putting broilers through needless stress, as was the case in the current study.

According to the research, broilers from T2 and T3 had a far greater H/L ratio than broilers from T1, indicating that during the starter and finisher stages, the broilers were under more stress and were not as healthy. The stress of the immunization in this experiment may have caused corticosterone to be released into the bloodstream. Because corticosterone can decrease the amount of lymphocytes in circulation, the H/L ratio rises (Virden and Kidd, 2009). This result was consistent with another study's (Cook, 1999; Scanes, 2016) findings, which showed a negative relationship between stress and the H/L ratio in chickens.

Several variables can affect the quality of meat and the quality of the carcass, including vaccinations, age at slaughter, breed, health, and environment. To determine carcase features, one can weigh the complete body, offal, abdominal fat, and chopped parts (Bansal et al., 2011). Increased circulation corticosteroid levels may cause immunological stress, which may be harmful to carcass features (Nelson et al., 2018). This has to do with matters of growth and health. It may also result in less feed being consumed and a reduction in the grill's effectiveness (Sahin and Forbes, 1999). The decreased carcase yield will eventually result in production losses as well as monetary losses (Yang et al., 2011). Abdullah and Matarneh (2010) found a significant relationship between dressing percentage and carcass weight in addition to a significant link between body and carcass weight. Similar outcomes were seen in this investigation, where T2 and T3 broilers showed lower carcase characteristic values than T1 broilers. These results were in line with those of Wang et al. (2015), who noted that broilers given larger doses of the ND vaccination showed a decline in carcase features, such as a decrease in body weight rise and FCR. Stress can hasten the development of rigor mortis, alter muscle biochemistry, and trigger post-mortem glycolysis synthesis, all of which can result in unfavorable

changes in meat quality. Nevertheless, stress has a significant impact on grilled meat (Lu et al. 2017). The accumulation of lactic acid in the muscles may be induced by the tension induced by vaccination, which can lead to a decrease in pH. Protein denaturation is induced by low muscle pH, which exacerbates muscle fiber degradation and reduces their ability to retain water and texture (Mir et al., 2017). The external appearance of meat is pallid as a result of the increased denaturation of proteins, which restricts the transmission of light across the muscle surface (Fletcher et al., 2000). The roasting rates and flow losses of the broilers in the T2 and T3 groups were marginally lower than those of the other broilers due to the muscle pH levels. Nevertheless, the pH levels of the muscles did not exhibit any substantial fluctuations during the current investigation. The meat appeared paler than that from T1 broilers, which may have contributed to a decrease in consumer approval, as per Petracci et al. (2004).

# Conclusions

The present study examined the impact of different immunization schedules on the health and production performance of grills. The increased growth performance. immunological state, carcass characteristics, and meat quality of T1 broilers that got two vaccinations, ND+IB on day 7 and IBD on day 14, demonstrated the optimal vaccination schedule for broiler farms. On the other hand, T2 and T3 broilers demonstrated increased health but lower production performance after receiving an additional dose of ND+IB vaccination on days 3 and 21, respectively. These results may be attributed to immunological and handling stress. Consequently, future research on antibody titres and hormone alterations will contribute to a more comprehensive comprehension of the correlation between broiler performance and vaccination stress.

# References

- Abdullah, A., & Matarneh, S. (2010). Broiler performance and the effects of carcass weight, broiler sex, and postchill carcass aging duration on breast fillet quality characteristics. *Journal* of Applied Poultry Research **19**, 46-58.
- Aini, I. (2006). "Newcastle Disease." In Diseases of Poultry in Southeast Asia, edited by M. Zamri-Saad, 225–233. 1st ed. Selangor: UPM Press.
- Bansal, G., Singh, V., & Sachan, N. (2011). Carcass quality characteristics of broilers as affected by Dried Poultry Excreta supplementation.
- Carisch, L., Stirn, M., Hatt, J. M., Federer, K., Hofmann-Lehmann, R., & Riond, B. (2019). White blood cell count in birds: evaluation of a commercially available method. *BMC veterinary research* **15**, 1-7.

- Christensen, V. L. (2009). Development during the first seven days post-hatching. *Avian Biology Research* 2, 27-33.
- Chung, E. L. T., Jesse, F. F. A., Marza, A. D., Ibrahim, H. H., Abba, Y., Zamri-Saad, M., Haron, A. W., Lila, M. A. M., Saharee, A. A., & Omar, A. R. (2019). Responses of proinflammatory cytokines, acute phase proteins and cytological analysis in serum and cerebrospinal fluid during haemorrhagic septicaemia infection in buffaloes. *Tropical animal health and production* 51, 1773-1782.
- Chung, E. L. T., Kamalludin, M. H., Jesse, F. F. A., Reduan, M. F. H., Ling, L. W., Mahzan, N. M., Henipah, N. N. M. M. A., Loh, T. C., & Idrus, Z. (2020). Health performance and blood profile changes in commercial broilers supplemented with dietary monocalcium phosphate. *Comparative Clinical Pathology* 29, 573-579.
- Cook, M. E. (1999). Nutritional effects on vaccination. *Veterinary vaccines and diagnostics* 47, 53-58.
- Dey, S., Pathak, D. C., Ramamurthy, N., Maity, H. K., & Chellappa, M. M. (2019). Infectious bursal disease virus in chickens: prevalence, impact, and management strategies. *Veterinary Medicine: Research and Reports*, 85-97.
- Fletcher, D., Qiao, M., & Smith, D. (2000). The relationship of raw broiler breast meat color and pH to cooked meat color and pH. *Poultry science* **79**, 784-788.
- Hornick, J.-L., Van Eenaeme, C., Gérard, O., Dufrasne, I., & Istasse, L. (2000). Mechanisms of reduced and compensatory growth. *Domestic animal endocrinology* 19, 121-132.
- Jackwood, M. W. (2012). Review of infectious bronchitis virus around the world. Avian diseases 56, 634-641.
- Krams, I., Vrublevska, J., Cirule, D., Kivleniece, I., Krama, T., Rantala, M. J., Sild, E., & Hõrak, P. (2012). Heterophil/lymphocyte ratios predict the magnitude of humoral immune response to a novel antigen in great tits (Parus major). *Comparative Biochemistry and Physiology Part* A: Molecular & Integrative Physiology 161, 422-428.
- Lilliehöök, I., Wall, H., Tauson, R., & Tvedten, H. (2004). Differential leukocyte counts determined in chicken blood using the Cell-Dyn 3500. *Veterinary clinical pathology* **33**, 133-138.
- Lim Teik Chung, E., Kamalludin, M. H., Jesse, F. F. A., Reduan, M. F. H., Loh, T. C., & Idrus, Z. (2019). Effect of Monocalcium Phosphate Supplementation on the Growth Performance, Carcass Characteristic, Gut Histomorphology, Meat and Bone Quality of Broiler Chickens.

*Pertanika Journal of Tropical Agricultural Science* **42**.

- Liu, L., Qin, D., Wang, X., Feng, Y., Yang, X., & Yao, J. (2015). Effect of immune stress on growth performance and energy metabolism in broiler chickens. *Food and agricultural immunology* 26, 194-203.
- Lu, Z., He, X., Ma, B., Zhang, L., Li, J., Jiang, Y., Zhou, G., & Gao, F. (2017). Chronic heat stress impairs the quality of breast-muscle meat in broilers by affecting redox status and energysubstance metabolism. *Journal of agricultural* and food chemistry 65, 11251-11258.
- Marangon, S., & Busani, L. (2007). The use of vaccination in poultry production. *Revue Scientifique et Technique-Office International des Epizooties* 26, 265.
- Müller, H., Mundt, E., Eterradossi, N., & Islam, M. R. (2012). Current status of vaccines against infectious bursal disease. *Avian pathology* 41, 133-139.
- Nelson, J. R., McIntyre, D. R., Pavlidis, H. O., & Archer, G. S. (2018). Reducing stress susceptibility of broiler chickens by supplementing a yeast fermentation product in the feed or drinking water. *Animals* 8, 173.
- Petracci, M., Betti, M., Bianchi, M., & Cavani, C. (2004). Color variation and characterization of broiler breast meat during processing in Italy. *Poultry science* 83, 2086-2092.

- SADIQ M. Pakistan poultry sector still. World Poultry. 20, 10–11.
- Sahin, A., & Forbes, J. (1999). Corticosterone, dietary fat source and diet selection in relation to body composition. *British Poultry Science* 40, 52-54.
- Scanes, C. G. (2016). Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. *Poultry science* **95**, 2208-2215.
- Sharma, J. (1999). Introduction to poultry vaccines and immunity. *Advances in veterinary medicine 41*, 481-494.
- Virden, W., & Kidd, M. (2009). Physiological stress in broilers: Ramifications on nutrient digestibility and responses. *Journal of Applied Poultry Research* 18, 338-347.
- Wang, X., Zhou, Q., Shen, J., Yao, J., & Yang, X. (2015). Effect of difference doses of Newcastle disease vaccine immunization on growth performance, plasma variables and immune response of broilers. *Journal of animal science* and biotechnology 6, 1-5.
- Yang, X., Li, W., Feng, Y., & Yao, J. (2011). Effects of immune stress on growth performance, immunity, and cecal microflora in chickens. *Poultry science* **90**, 2740-2746.

Table 1:	vaccination	treatment	group

vaccination Regime					
Treatments	Day 3 (IO)	Day 7 (IO)	Day 14 (DW)	Day 21 (DW)	
T1 (Control)	-	ND+IB	IBD		
T2	ND+IB	ND+IB	IBD		
Т3		ND+IB	IBD	ND+IB	

Parameters	Treatments			
	T1	T2	Т3	P-value
1–21 days (Starter phase)				
Initial body weight (kg)	$0.05 \pm 0.01$	$0.05\pm0.01$	$0.05 \pm 0.01$	0.61
Body weight (kg)	0.99 ± 0.01a	$0.94\pm0.01b$	$0.93\pm0.01b$	0.04
Body weight gain (kg)	0.95 ± 0.01a	$0.90\pm0.01b$	0.93 ± 0.01a	0.04
Feed intake (kg)	$1.27\pm0.03$	$1.25\pm0.01$	$1.27\pm0.02$	0.31
Cumulative FCR	$1.34 \pm 0.05$	$1.35 \pm 0.01$	$1.37\pm0.02$	0.88
22–42 days (Finisher phase)				
Body weight (kg)	$2.45\pm0.08$	$2.45\pm0.03$	$2.44\pm0.07$	0.39
Body weight gain (kg)	$2.40\pm0.09$	$2.39\pm0.03$	$2.37\pm0.07$	0.63
Feed intake (kg)	$4.42\pm0.08b$	$4.74\pm0.05a$	$4.67\pm0.09a$	0.02
Cumulative FCR	$1.84\pm0.02b$	$1.98\pm0.02a$	$1.97\pm0.03a$	0.04

Treatments					
Parameters	T1	T2	T3	P-value	
1-21 days (Starter phase)					
Total WBC count (10-310-3L)	$28.80 \pm 1.25$	$31.33 \pm 2.76$	33.13 ± 0.89	0.27	
Heterophils (%)	$35.00\pm0.91$	$36.83\pm0.28$	$35.00 \pm 0.48$	0.08	
Lymphocytes (%)	$59.83 \pm 0.82$	$58.00\pm0.66$	59.67 ± 0.59	0.15	
H/L Ratio (Unit)	$0.59 \pm 0.02b$	0.64 ± 0.01a	$0.59 \pm 0.01b$	0.01	
Monocytes (%)	$2.17 \pm 0.11$	$2.00 \pm 0.18$	$2.00 \pm 0.37$	0.86	
Eosinophil (%)	$1.43 \pm 0.11$	$1.40\pm0.12$	$1.33 \pm 0.11$	0.33	
Basophil (%)	$1.17 \pm 0.17$	$1.17 \pm 0.12$	$1.07 \pm 0.21$	0.86	
22–42 days (Finisher phase)					
Total WBC count (10-310-3L)	$25.73 \pm 1.38$	$21.77\pm2.96$	$27.17 \pm 4.51$	0.49	
Heterophils (%)	$31.42\pm0.91b$	$37.75 \pm 1.14a$	$38.58\pm0.83a$	0.01	
Lymphocytes (%)	62.67 ± 1.06a	57.08 ± 1.36b	56.75 ± 0.91b	0.01	
H/L Ratio (Unit)	$0.50\pm0.02b$	$0.67\pm0.03a$	$0.68 \pm 0.03a$	0.01	
Monocytes (%)	$2.50 \pm 0.18$	$2.17 \pm 0.25$	$2.00 \pm 0.18$	0.25	
Eosinophil (%)	$1.75 \pm 0.25$	$1.58 \pm 0.30$	$1.42 \pm 0.24$	0.68	
Basophil (%)	$1.67 \pm 0.17$	$1.42 \pm 0.15$	1.25 ± 0.21	0.29	

#### Table 3: Effect of vaccination on white blood cells

Table 4: Effect of vaccination on the carcase characteristics

Treatments					
Parameters	T1	T2	T3	P-value	
Final live weight (g)	$2484.33 \pm 40.55$	$2404.33 \pm 14.91$	$2463.33 \pm 10.24$	0.54	
Kill-out weight (g)	$2385.67 \pm 28.90a$	$2319.00 \pm 22.67b$	2351.00 ± 19.81a,b	0.03	
De-feathered weight (g)	$2298.87 \pm 13.80a$	$2221.00 \pm 10.45b$	2276.67 ± 17.76a,b	0.04	
Carcase weight (g)	$1826.00 \pm 22.42$	$1801.00 \pm 22.24$	$1820.67 \pm 20.30$	0.09	
Dressing percentage (%)	$73.00 \pm 0.63a$	$70.00 \pm 1.32b$	$71.67 \pm 0.56b$	0.04	
Breast (g)	$569.00 \pm 17.77$	$556.33 \pm 16.90$	$556.67 \pm 17.58$	0.15	
Drumstick (g)	254.00 ± 6.34a,b	$235.67 \pm 3.04b$	$252.33 \pm 7.38a$	0.04	
Wings (g)	247.33 ± 9.17	$243.33 \pm 7.21$	$245.33 \pm 15.73$	0.09	
Neck (g)	$52.33 \pm 2.74$	$50.33\pm2.14$	$50.67\pm3.55$	0.58	
Head (g)	$65.00 \pm 2.90$	$63.33 \pm 1.52$	$62.00 \pm 3.16$	0.73	
Shanks (g)	$108.67 \pm 1.48$	$107.00 \pm 1.32$	$108.33 \pm 4.23$	0.17	
Full gizzard (g)	$36.00\pm1.32$	$34.33\pm0.92$	$34.00 \pm 1.32$	0.13	
Empty gizzard (g)	$27.33 \pm 0.92$	$27.33 \pm 0.56$	$28.33 \pm 1.38$	0.14	
Fat (g)	$77.00 \pm 2.56$	$71.33 \pm 7.25$	$73.67 \pm 7.61$	0.19	
GIT (g)	158.33 ± 7.52a	130.67 ± 5.25b	152.00 ± 6.23a	0.02	
Heart (g)	$12.67 \pm 0.21$	$11.33 \pm 0.73$	$11.67 \pm 0.21$	0.60	
Liver (g)	43.33 ± 4.18	$45.00\pm0.97$	$44.67 \pm 2.01$	0.13	

### Table 5: Effect of vaccinations on meat quality parameters

Treatments					
Parameters	T1	T2	T3	P-value	
Cooking Loss (%)	25.67 ± 1.52b	28.33 ± 1.48a	26.33 ± 1.38b	0.04	
Muscle pH	6.01 ± 0.03	6.00 ± 0.02	6.00 ± 0.02	0.95	
Drip Loss at Day 1 (%)	$0.81\pm0.09b$	$1.10\pm0.07a$	0.88 ± 0.10a,b	0.04	
Drip Loss at Day 7 (%)	$5.68\pm0.62b$	$7.71\pm0.48a$	6.18 ± 0.72a,b	0.04	
Colour L* (lightness)	$59.02\pm0.55$	$61.08\pm0.98$	60.33 ± 1.22	0.33	
Colour a* (redness)	3.55 ± 0.42	3.47 ± 0.26	3.14 ± 0.28	0.30	
Colour b* (yellowness)	15.98 ± 0.23	15.14 ± 0.03	$15.58 \pm 0.43$	0.10	

Declaration Ethics Approval and Consent to Participate Not applicable. Consent for Publication The study was approved by authors. Funding Statement Not applicable Conflict of Interest There is no conflict of interest among the authors regarding this case study. Authors Contribution All authors contributed equally.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licen ses/by/4.0/. © The Author(s) 2024