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Comparative Haematology and Serum Biochemical Responses of Two Chicken Hybrids following Newcastle Disease Virus Infection

Gabriel Odenigbo[1]* and Prof. Shoyinka S.V.O [2]
[1]Department of Veterinary Pathology, Faculty of Veterinary Medicine,
University of Benin, Benin City, Edo State, Nigeria.
[2]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine,
University of Nigeria, Nsukka, Enugu State, Nigeria.

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ABSTRACT

Newcastle disease (ND) remains one of the most significant viral threats to poultry production worldwide. This study evaluated the haematological and serum biochemical responses of two commercial breeds: Dominant Black (DB) and Isa Brown (IB) pullets, following experimental infection with a velogenic Newcastle disease virus (KUDU 113 strain). A total of 120 ten-week-old pullets were randomly assigned into four groups: DB-infected, DB-control, IB-infected, and IB-control. Birds in infected groups received 0.1 mL of 10^6.4 EID_{50} of the virus intramuscularly, while controls received phosphate-buffered saline. Blood samples were collected before inoculation and on day 4 post-infection for haematology and serum biochemistry. Results showed significant ($P < 0.05$) reductions in packed cell volume, haemoglobin concentration, erythrocyte count, and total leukocyte count in infected groups compared with controls. Differential counts also revealed decreases in heterophils, lymphocytes, and monocytes relative to the controls. Serum total protein, albumin, and globulin concentrations were also significantly ($P < 0.05$) reduced. The anaemia observed was normocytic and hypochromic, suggesting acute blood loss and intravascular haemolysis, while leucopenia and hypoproteinaemia may indicate immunosuppression or liver dysfunction. No significant differences were observed between DB and IB pullets, indicating similar in susceptibility. This aligned with the gross and histopathological lesions, previously reported for these same experimental groups, where absence of breed-related differences in susceptibility was consistent for the two breeds. These findings highlight the profound disruption of blood and biochemical parameters caused by KUDU 113 NDV and reinforced the need for integrated control strategies that go beyond vaccination to include improved nutrition, stress reduction, and strict biosecurity.

Keywords: Chickens, Haematology, Biochemistry, Newcastle Disease, Poultry Health

*Corresponding author:
email: gabriel.odenigbo@unben.edu
Tel: +234 (0)803 379 7826

INTRODUCTION

Poultry production plays a vital role in global agriculture, contributing significantly to food security, income generation, and the supply of high-quality animal protein (Mottet and Tempio, 2017). Despite its importance, the industry remains highly vulnerable to the effects of climatic change and infectious agents due to intensive farming systems and frequent interactions with the environment (Grace, 2018; Espinosa *et al*., 2020). Newcastle disease (ND), caused by Newcastle disease virus (NDV), is one of the most devastating poultry diseases globally (Dimitrov, 2023). Velogenic strains such as the KUDU 113 strain has been associated with high mortality and severe economic losses in Nigeria (Shittu *et al*., 2016).

Disease control strategies in poultry commonly rely on vaccination, strict biosecurity, and prudent antibiotic use. However, enhancing genetic resistance through selective breeding offers a sustainable alternative that could reduce disease burden and limit antimicrobial dependence (Ziebe et al., 2025). Anecdotal evidence from poultry farmers suggests that certain hybrids, such as the Dominant Black, may be more resilient to infections compared to hybrids like the Isa Brown. In a related study using the same experimental infection, we demonstrated that Dominant Black and Isa Brown pullets exhibited indistinguishable clinical outcomes, gross and histopathological lesions (Odenigbo et al., 2025). Velogenic NDV causes severe clinical disease, high mortality, and marked pathological lesions in susceptible chickens (Asok Kumar Mariappan *et al*., 2018; EL-Morshidy *et al*., 2021).

This study sought to compare the haematological and serum biochemical responses of Dominant black and Isa brown pullets experimentally infected with a velogenic strain of NDV (KUDU 113). The objective was to determine whether differences in physiological responses support the perceived variation in disease susceptibility between these two hybrids.

Materials and Methods

The study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (Approval Ref. No: FVM-UNN-IACUC-2024-12/200), and conducted in accordance with International Animal Welfare guidelines.

A total of 120 day-old chicks - 60 Dominant black (DB) and 60 Isa brown (IB) pullets were sourced from a reputable hatchery in Ibadan, Oyo State, Nigeria. All the test chicks were hatched on the same day and not vaccinated against NDV during the study. Brooding was done separately on a deep litter system in disinfected pens and under similar environmental conditions at the Faculty of Veterinary Medicine Farm, University of Nigeria, Nsukka. Birds received oral vaccination against infectious bursal disease (IBD) on day 10 of life (Aliyu *et al*., 2016). Amprolium was administered intermittently in drinking water to prevent coccidiosis (Page, 2008), but feed and water were provided *ad libitum*.

To ensure the absence of NDV antibodies, weekly haemagglutination-inhibition (HI) tests were conducted as described by Kaufman *et al*. (2017), and only seronegative birds were used for viral challenge. The velogenic NDV strain KUDU 113, with a mean embryo infective dose (EID_{50}) of $10^{6.4}$ per mL (Onyema *et al*., 2019; Okechukwu *et al*., 2020), was obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria. The virus was transported under cold chain and administered according to standard protocols (Ng *et al*., 2020). At 10 weeks of age, the experimental birds were randomly assigned to four groups (n = 30 per group):

- **Group 1 (DB):** DB pullets inoculated intramuscularly (IM) with 0.1 mL of NDV KUDU-113
- **Group 2 (IB):** IB pullets inoculated IM with 0.1 mL of NDV KUDU-113
- **Group 3 (CDB):** control DB pullets inoculated IM with 0.1 mL of phosphate-buffered saline (PBS)
- **Group 4 (CIB):** control IB pullets inoculated IM with 0.1 mL of PBS

Blood samples (2 mL) were collected from six randomly selected birds per group on days 0 and 4 post-inoculation via the jugular or wing vein using sterile 5 mL syringes and 18-gauge

needles (Kelly and Alworth, 2013). Samples for haematological analysis were transferred into EDTA bottles (EClinpath, 2021). The packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) count, total and differential white blood cell (WBC) counts of the experimental birds were compared with the control values. Serum from individual samples was analyzed for total protein, albumin, and globulin levels as described in standard procedures by Jain (1993) and Tietz (2006).

Data analysis

Data were expressed as mean ± standard error of mean (SEM). Differences between infected and uninfected groups were analyzed using Student's t-test for independent samples. Homogeneity of variances was assessed using Levene's test. Mean differences at 5% level of probability ($P < 0.05$) were accepted as significant (LibGuides, 2018).

Results

Haematological analyses

Comparison between the two breeds (DB versus IB) showed no significant ($P > 0.05$) differences in any of the haematological or biochemical parameters measured. Differential counts revealed significant ($P < 0.05$) decreases in heterophils, lymphocytes, and monocytes relative to their controls, while eosinophils and basophils were unaffected.

Packed cell volume: The PCV of velogenic Newcastle disease challenged DB birds which was 32.58±1.76% on day 0 was observed to have decreased significantly ($P<0.05$) to 26.92±1.20% at day 4; similarly, Isa brown birds with 34.75±3.24% on day 0 got reduced to 29.42±0.57% at day 4. Their respective control values however remained unchanged when the values at day 4 were compared to day 0. There was also no significant ($P > 0.05$) differences in the PCV values of Dominant black and Isa brown birds at day 4 of the study (Table 1). The normal PCV range for birds is 22.0 – 35.0 (Rao, 2000).

Table 1: Packed cell volume of birds challenged with velogenic NDV and controls

	Mean percentage (%) ± SEM			
Days post- infection	DB	CDB	IB	CIB
0	32.58±1.76	32.58±1.76	34.75±3.24	34.75±3.24
4	26.92±1.20*	33.50±2.03	29.42±0.57*	35.50±7.56

Key: DB=Dominant black; CDB=control Dominant black; IB= Isa brown; CIB=control Isa black ; SEM=standard error of the mean; n=6; NDV=Newcastle disease; * = significant differences at $p<0.05$.

Haemoglobin concentration (Hb): The haemoglobin concentration of velogenic Newcastle disease infected DB birds on day 0 (9.032±0.421 g/dL) was significantly ($P<0.05$) reduced to 7.405±0.558 g/dL on day 4 but the change in Hb value of the control from 9.032±0.421 to 8.150±0.500 g/dL within the same period was insignificant ($P > 0.05$). In the same manner, the haemoglobin concentration of NDV-infected IB birds decreased significantly ($P<0.05$) from 9.00±3.24 g/dL on day 0 to 7.53±0.274 g/dL at day 4. A change in the control between day 0 and day 4 (9.00±0.97 to 8.743±0.772 g/dL) was not found to be significant ($P > 0$). There were no observed significant ($P>0.05$) differences in the Hb values of Dominant black and Isa brown birds at day 4 of the study (Table 2). The normal range for haemoglobin concentration of birds is 8.7-11.0 g/dL (Holmes *et al.*, 1933).

Table 2: The Haemoglobin concentration of birds infected with Velogenic NDV and controls

	Mean Hb (g/dL) ± SEM			
Days post- infection	DB	CDB	IB	CIB
0	9.032±0.421	9.032±0.421	9.00±3.24	9.00±0.97
4	7.405±0.558*	8.150±0.500	7.53±0.274*	8.743±0.772

Key: Hb=haemoglobin concentration; DB=Dominant black; CDB=control Dominant black; IB= Isa brown; CIB=control Isa black ; SEM=standard error of the mean; n=6; NDV=Newcastle disease virus; * = significant differences at p<0.05.

Total erythrocyte (RBC) count: The erythrocyte count of velogenic Newcastle disease infected DB birds on day 0 (2.64±0.351 $x10^6/\mu L$) was significantly (P<0.05) reduced to 2.07±0.335 $x10^6/\mu L$ at day 4. The change in RBC count of control DB birds from day 0 to the value in day 4 was not significant (P<0.05). In the same manner, the RBC count of NDV-infected IB birds was not significantly (P<0.05) different from day 0 (2.54±0.56 $x10^6/\mu L$) to 2.64±0.445 $x10^6/\mu L$ at day 4 (Table 3). The normal range of total erythrocyte count for birds is 2.5-3.5 x $10^6/\mu L$ (Rao, 2000).

Table 3: Red Blood Cell count of birds infected with Velogenic NDV and controls

	Mean RBC ($x10^6/\mu L$) ± SEM			
Days post- infection	DB	CDB	IB	CIB
0	2.64±0.351	2.60±0.451	2.54±0.56	2.50±0.50
4	2.07±0.335*	2.51±0.575	2.64±0.445*	3.15±0.55

Key: RBC= Red blood cell count; DB=Dominant black; CDB=control Dominant black; IB= Isa brown; CIB=control Isa black; SEM=standard error of the mean; n=6; NDV=Newcastle disease virus; * = significant differences at p<0.05.

Mean Corpuscular Volume

At day 4, the mean corpuscular volume (MCV) of Dominant black (DB) was significantly (P <0.05) reduced compared to the control (CIB). MCV of DB also increased significantly (P <0.05) at day 4 compared to day 0. Conversely, day 4 MCV of control IB decreased significantly (P <0.05) compared to MCV of control IB at day 0. On day 0, MCV of both Isa brown and its control were significantly (P <0.05) elevated compared to MCV of both DB and control DB. However, MCV of both DB and its control were significantly (p<0.05) higher compared to IB and CIB values at day 4 (Table 4). The normal MCV range for birds is 90-140 fL (Rao, 2000).

Table 4: Mean corpuscular volume (MCV) of birds infected with Velogenic NDV and controls

	Mean MCV (fL) ± SEM			
Days post- infection	DB	CDB	IB	CIB
0	123.41	125.31	136.81	139.00
4	130.05*†	133.47	111.44↓	112.70

Key: MCV= Mean corpuscular volume ; DB=Dominant black; CDB=control Dominant black; IB= Isa brown; CIB=control Isa black; SEM=standard error of the mean; n=6; NDV=Newcastle disease virus; * = significantly ($p<0.05$) reduced compared to CDB value at day 4; ↓= significant ($p<0.05$) decrease compared to CIB value at day 4; †= significantly ($p<0.05$) elevated compared to IB or CIB values at day 4.

Mean corpuscular haemoglobin (MCH): At day 0, MCH of DB was not significantly ($P > 0$) from CDB but MCH of IB was significantly ($P < 0.05$) increased compared to that of CIB. At day 4, MCH of DB (35.77 pg) was significantly ($P < 0.05$) higher than that of CDB (32.47 pg). MCH values of IB (28.52 pg) was also observed to be elevated compared to that of control IB which was 27.76 pg (Table 5). The normal MCH range for birds is 33.0 – 47.0 (Rao, 2000)

Table 5: Mean corpuscular haemoglobin of birds infected with Velogenic NDV and controls

	Mean MCH (pg/) ± SEM			
Days post- infection	DB	CDB	IB	CIB
0	34.21	34.74	35.43	26.00
4	35.77*	32.47	28.52*	27.76

Key: MCH= Mean corpuscular haemoglobin; DB=Dominant black; CDB=control Dominant black; IB= Isa brown; CIB=control Isa black ; * = significant ($p<0.05$) increase compared to the control; SEM=standard error of the mean; n=6; NDV=Newcastle disease virus.

Mean corpuscular haemoglobin concentration (MCHC): At day 0, there was no significant ($P > 0$) difference in MCHC values of DB and CDB, neither is there any between IB and CIB. At day 4 however, MCHC values of both controls CDB and CIB were significantly ($P < 0$) reduced compared to their respective treatment groups (DB and IB) (Table 6). The normal MCHC range for birds is 26.0 – 35.0 (Rao, 2000).

Table 6: Mean corpuscular haemoglobin concentration of birds infected with Velogenic NDV and controls

	Mean MCHC (g/L) ± SEM			
Days post- infection	DB	CDB	IB	CIB
0	27.72	27.72	25.90	25.90
4	27.51	24.34*	25.59	24.63+

Key: MCHC= Mean corpuscular haemoglobin concentration ; DB=Dominant black; CDB=control Dominant black; IB= Isa brown; CIB=control Isa black; *= control value (CDB) significantly ($p<0.05$) reduced compared to CDB; + = control value (CIB) significantly ($p<0.05$) reduced compared to IB; SEM=standard error of the mean; n=6; NDV=Newcastle disease.

Total Leucocyte Count: At day 4 post-infection, the Total leukocyte count (WBC) in DB (16.69±1.033 x10^3/µL) was significantly ($P < 0.05$) reduced compared to the control (CDB) with 21.19±1.731 x10^3/µL. In the same way, WBC in IB value of 16.87±0.765 x10^3/µL was also significantly ($P < 0.05$) reduced compared to the control (CIB) with 18.22±0.671 x10^3/µL. There was however no significant ($P > 0.05$) difference in Total leucocyte counts of DB and IB (Table 7). The normal Total leucocyte count of birds is 20-40 x10^3/µL (Rao, 2000).

Table 7: Mean Total leucocyte count of birds infected with Velogenic NDV and controls

	Mean RBC (x10^3/µL) ± SEM			
Days post- infection	DB	CDB	IB	CIB
0	21.15±1.58	21.15±1.58	17.01±0.82	17.01±0.82
4	16.69±1.033*	21.19±1.731	16.87±0.765*	18.22±0.671

Key: RBC= Red blood cell count; DB=Dominant black; CDB=control Dominant black; IB= Isa brown; CIB=control Isa black ; SEM=standard error of the mean; n=6; NDV=Newcastle disease virus ; * = significant ($p<0.05$) compared to their controls.

Absolute Differential Leucocyte Count: The mean Absolute Differential counts revealed significant ($P < 0.05$) decreases in heterophils, lymphocytes, and monocytes relative to the controls, while eosinophils and basophils were unaffected (Table 8).

Table 8: Mean Absolute Differential Leucocyte Count of birds infected with Velogenic NDV and controls

Mean ± SEM					
Test day	**Cell type**	**DB**	**CDB**	**IB**	**CIB**
0	H	8565 ± 12.00	8565 ± 22.00	8635.55 ± 21.20	8635.55 ± 23.20
	L	11526.75 ± 17.30	11526.75 ± 19.30	11949.75 ± 20.12	11949.75 ± 21.12
	M	941.18 ± 53.00	1142.10 ± 54.00	719.10 ± 56.00	722.56 ± 42.00
	E	0	0	0	0
	B	0	0	0	0
4	H	5841.50±193.20*	8565.75±120.00	7059.15±164.20	7439.23±212.00
	L	10180.90±183.00*	11526.75 ± 173.00	9270.40 ± 121.00*	10312.52 ± 217.00
	M	575.50 ± 53.00*	1184.40±54.00	578.34±56.00*	637.70±42.00
	E	0	0	0	0
	B	0	0	0	0

Key: DB=Dominant black; CDB=control Dominant black; IB= Isa brown; CIB=control Isa black; H= Heterophil; L= Lymphocyte; M= Monocyte ; E= Eosinophil ; B= Basophil ; SEM=standard error of the mean; n=6; NDV=Newcastle disease virus; * = significant ($p<0.05$) decrease compared to their controls.

Total Serum Proteins: The concentration of total proteins significantly ($P < 0.05$) reduced in infected groups compared to the control at day 4 (Table 9). The normal protein levels in birds is 4.5-5.5 (Rao, 2000).

Table 9: Mean Total serum proteins of birds infected with Velogenic NDV and controls

	Mean (g/dL x 10) ± SEM			
Days post-infection	DB	CDB	IB	CIB
0	3.167±0.217	3.167±0.217	3.208±0.146	3.208±0.146
4	2.658±0.232*	3.167±0.217*	2.333±0.102*	3.208±0.146

Key: DB=Dominant black; CDB=control Dominant black; IB= Isa brown; CIB=control Isa black ; * = significant ly ($p<0.05$) lower compared to controls; SEM=standard error of the mean; n=6; NDV=Newcastle disease virus.

Serum globulin: The mean serum globulin concentration of infected DB birds (1.082±0.087 g/dL x 10) was significantly ($P < 0.05$) lower compared to the control CDB birds (1.235±0.141 g/dL x 10) at day 4. Similarly, serum globulin level of infected IB birds (0.870±0.072 g/dL x 10) was significantly ($P < 0.05$) lower compared to CIB value of 1.240±0.076 g/dL x 10 (Table 10).

Table 10: Mean Serum globulin of birds infected with Velogenic NDV and controls

	Mean (g/dL x 10) ± SEM			
Days post-infection	DB	CDB	IB	CIB
0	1.235±0.141	1.235±0.141	1.240±0.077	1.240±0.077
4	1.082±0.087*	1.235±0.141	0.870±0.072*	1.240±0.076

Key: DB=Dominant black; CDB=control Dominant black; IB= Isa brown; CIB=control Isa black ; * = significantly ($p<0.05$) lower compared to controls; SEM=standard error of the mean; n=6; NDV=Newcastle disease virus.

Serum albumin level: The mean serum albumin concentration of infected DB birds (1.577±0.203 g/dL x 10) was significantly ($P < 0.05$) lower compared to the control CDB birds (1.948±0.189 g/dL x 10) at day 4. Similarly, serum albumin level of infected IB birds (1.387±0.066 g/dL x 10) was significantly ($P < 0.05$) lower compared to CIB value of 1.968±0.142 g/dL x 10 at day 4 (Table 11).

Table 11: Mean Serum albumin level of birds infected with Velogenic NDV and controls

	Mean (g/dL x 10) ± SEM			
Days post-infection	DB	CDB	IB	CIB
0	1.948±0.189	1.948±0.189	1.948±0.142	1.948±0.142
4	1.577±0.203*	1.948±0.189	1.387±0.066*	1.948±0.142

Key: DB=Dominant black; CDB=control Dominant black; IB= Isa brown; CIB=control Isa black ; * = significantly ($p<0.05$) lower compared to controls; SEM=standard error of the mean; n=6; NDV=Newcastle disease virus.

Serum albumin-globulin ratio: The albumin/globulin ratio did not differ significantly (P > 0.05).

Table 12 : Mean Serum albumin-globulin ratio of birds infected with Velogenic NDV and controls

	Mean (g/dL x 10) ± SEM			
Days post- infection	DB	CDB	IB	CIB
0	1.730±0.365	1.730±0.365	1.628±0.179	1.628±0.179
4	1.507±0.241	1.730±0.365	1.692±0.257	1.628±0.179

Key: DB=Dominant black; CDB=control Dominant black; IB= Isa brown; CIB=control Isa black ; * = significantly (p<0.05) lower compared to controls; SEM=standard error of the mean; n=6; NDV=Newcastle disease virus.

Discussion

The present study demonstrated that infection with velogenic NDV (KUDU 113 strain) induced marked haematological and biochemical alterations in both Dominant Black and Isa Brown pullets. The anaemia observed, characterized by decreased PCV, Hb, and RBC values, was normocytic and hypochromic, suggesting acute blood loss and intravascular haemolysis. This is consistent with earlier reports that NDV damages blood vessels, resulting in haemorrhage and haemolysis (Okechukwu *et al*., 2020).

Leucopenia in infected birds was attributable to reductions in heterophils, lymphocytes, and monocytes. Such findings support the view that NDV infection leads to immunosuppression through destruction of circulating immune cells and lymphoid depletion (Dimitrov *et al*., 2017). The decline in lymphocytes particularly reflects impaired adaptive immunity, which may predispose infected birds to secondary infections. The haemo-biochemical responses observed in this study were comparable between Dominant Black and Isa Brown pullets, reinforcing previous findings that clinical signs, gross and histopathological lesions were also indistinguishable between the two breeds (Odenigbo et al., 2025). Taken together, these results indicated that breed-related factors play little or no role in determining susceptibility to velogenic NDV. Instead, the outcome appeared to be primarily determined by the virulence of the challenge strain, as evidenced by the uniform high mortality and severe pathological lesions (Zhang *et al*., 2023).

The significant reduction in serum total protein, albumin, and globulin concentrations is indicative of hypoproteinaemia. Possible mechanisms include impaired hepatic protein synthesis due to hepatocellular damage, dehydration, and protein-losing enteropathy resulting from severe intestinal lesions commonly associated with velogenic NDV (Shittu *et al*., 2016; Nagra and Dang, 2023). The unchanged albumin/globulin ratio suggests that both fractions were proportionately reduced (Total Protein and Albumin/Globulin (A/G) Ratio, 2024).

Contrary to anecdotal reports of enhanced resilience in Dominant Black chickens, no significant differences were observed between DB and IB pullets. This suggests that both breeds were equally susceptible to haematological and biochemical changes induced by NDV infection.

In a nutshell, these results proved that velogenic NDV did not only cause clinical disease and mortality but has the tendency to disrupt blood and biochemical homeostasis. Such physiological impairment could compromise productivity even in birds that survive infection (Zhang *et al*., 2023). Effective control strategy therefore requires integrated measures that may involve vaccination, optimized nutrition, stress management, and stringent biosecurity to improve flock resilience.

REFERENCES

Aliyu, H.B., Sa'idu, L., Jamilu, A., Andamin, A. D., and Akpavie, S.O. (2016). Outbreaks of infectious bursal disease in flocks of battery cage brooding system in Zaria, Nigeria. *Journal of Veterinary Medicine and Animal Health, 8*(9), 125–129. https://doi.org/10.5897/JVMAH2016.0487

Asok Kumar Mariappan, Palanivelu Munusamy, Kumar, D., Latheef, S. K., Singh, S. D., Singh, R., and Kuldeep D. (2018). Pathological and molecular investigation of velogenic viscerotropic Newcastle disease outbreak in a vaccinated chicken flocks. *Virus Disease*, 29(2), 180–191. https://doi.org/10.1007/s13337-018-0445-5

Dimitrov, K. (2023, May 3). *Newcastle Disease in Poultry*. MSD Veterinary Manual. https://www.msdvetmanual.com/poultry/newcastle-disease-and-other-paramyxovirus-infections/newcastle-disease-in-poultry

EL-Morshidy, Y., Abdo, W., Elmahallawy, E. K., Abd EL-Dayem, G. A., El-Sawak, A., El-Habashi, N., Mosad, S. M., Lokman, M. S., Albrakati, A., and Abou Asa, S. (2021). Pathogenesis of Velogenic Genotype VII.1.1 Newcastle Disease Virus Isolated from Chicken in Egypt via Different Inoculation Routes: Molecular, Histopathological, and Immunohistochemical Study. *Animals*, 11(12), 3567. https://doi.org/10.3390/ani11123567

Espinosa, R., Tago, D., and Treich, N. (2020). Infectious diseases and meat production. *Environmental and Resource Economics, 76* (4), 1019–1044. https://doi.org/10.1007/s10640-020-00439-1

Jain, N. C. (1993). *Essentials of Veterinary hematology*. Lea & Febiger, Philadelphia. pp. 3-455.

Kaufman, J., Walker, B. A., and Jacob, J.P. (2017). The influence of the MHC on resistance to infectious diseases in chickens. *Avian Diseases, 61*(2), 131–137. https://doi.org/10.1637/11519-031317-Review.1

Kelly, L.M., and Alworth, L.C. (2013). Techniques for collecting blood from the domestic chicken. *Lab Animal*, 42(10), 359–361. https://doi.org/10.1038/laban.394

LibGuides (2018). *SPSS Tutorials: Independent Samples t Test*. Kent.edu. https://libguides.library.kent.edu/spss/independentttest

Mottet, A. and Tempio, G. (2017). Global poultry production: current state and future outlook and challenges. *World S Poultry Science Journal*, *73*(2), 245–256. https://doi.org/10.1017/s0043933917000071

Nagra, N., and Dang, S. (2023). Protein-Losing Enteropathy. Nih.gov; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK542283/

Ng, C. Z., Lean, Y.L., Yeoh, S.F., Lean, Q.Y., Lee, K. S., Suleiman, A. K., Liew, K.B., Kassab, Y.W., Al Worafi, Y.M., and Long, C.M. (2020). Cold chain time- and temperature-controlled transport of vaccines: A simulated experimental study. *Clinical and Experimental Vaccine Research*, 9(1), 8–14. https://doi.org/10.7774/cevr.2020.9.1.8

Odenigbo, G.I., Onoja R.I., Shoyinka S.V.O, and Okoye J.A.O (2025). Comparison of Susceptibility to and Pathology of the

Velogenic Newcastle disease virus Infection in Chicken pullets of Dominant Black and Isa Brown breeds. *Journal of Veterinary and Applied Sciences*, 15(2): 1108-1118.

Okechukwu, H.N., Chukwuedo, A.A., Eze, D.C., Igwe, A.O., Ihedioha, J.I., and Okoye, J. O. A. (2020). Triple La Sota re-vaccinations can protect laying chickens for 3 months against drop in egg caused by velogenic viscerotropic Newcastle disease virus infection. *Veterinary Medicine and Science*, 6(3), 470–476. https://doi.org/10.1002/vms3.241](https://doi.org/10.1002/vms3.241)

Onyema, I., Eze, D.C., Abba, Y., Emennaa, P.E., Shoyinka, S.V.O., Okwor, E.C., Ezema, W. S., Ihedioha, J. I., and Okoye, J.O.A. (2019). Lesions of velogenic viscerotropic Newcastle disease virus infection were more severe in broilers than pullets. *Journal of Applied Animal Research*, 47(1), 189–194. https://doi.org/10.1080/09712119.2019.1598420](https://doi.org/10.1080/09712119.2019.1598420)

EClinpath. (2021). Sample collection. https://eclinpath.com/hematology/sample-collection-heme/

Shittu, I., Joannis, T.M., Odaibo, G.N., and Olaleye, O.D. (2016). Newcastle disease in Nigeria: epizootiology and current knowledge of circulating genotypes. *VirusDisease*, *27*(4), 329–339. https://doi.org/10.1007/s13337-016-0344-6

Tietz, N.W. (2006). *Textbook of clinical chemistry and molecular diagnostics* (4th ed.). Elsevier Saunders.

Total Protein and Albumin/Globulin (A/G) Ratio. (2024). Medlineplus.gov. https://medlineplus.gov/lab-tests/total-protein-and-albumin-globulin-a-g-ratio/

Zhang, D., Ding, Z., and Xu, X. (2023). Pathologic Mechanisms of the Newcastle Disease Virus. *Viruses*, 15(4), 864. https://doi.org/10.3390/v15040864

Ziebe, S.D., Ngom, R.V., Adonis, B.H.P., and Zangue, H.A. (2025). Impact of Biosecurity on Production Performance and Antimicrobial Usage in Broiler Farms in Cameroon. *Animals*, *15*(12), 1771–1771. https://doi.org/10.3390/ani15121771