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ORIGINAL ARTICLE

Blood Biochemistry Responses of Chickens Experimentally Infected with a Velogenic Newcastle Disease Virus (Kudu 113)

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SUMMARY

This study investigated the blood biochemistry responses of cockerels experimentally infected with a velogenic Newcastle disease virus (NDV) strain, KUDU 113. One hundred Isa white cockerels were used for the study. The cockerels were obtained at day-old and randomly divided into groups A- vaccinated and infected, B - unvaccinated and infected and C- unvaccinated and uninfected (control) consisting of 30, 30 and 40 birds respectively. Group A was vaccinated against NDV with *La Sota* vaccine at three weeks of age while Groups B and C were not vaccinated. After six weeks, each bird in groups A and B were inoculated intramuscularly (im) with the velogenic NDV while the control group was not inoculated. Blood samples were randomly collected through the jugular vein from five birds in each group, allowed to clot at room temperature, centrifuged and serum harvested. Blood biochemistry determinations were carried out on days 0, 3, 6, 9, 12, 15 and 21 post-inoculation (pi). Parameters determined included serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activities, total serum proteins, albumin, globulin, total bilirubin, blood glucose level, total cholesterol, total calcium, blood urea nitrogen and uric acid. Data generated were analyzed using ANOVA and Student's t-test. Results showed decreased ($p<0.05$) total protein, albumin, globulin and total calcium levels and increased ($p<0.05$) plasma glucose and total cholesterol levels in groups A and B when compared with the unvaccinated uninfected control. Hypoproteinemia, hypoalbuminemia and hypocalcemia together with increased globulin, blood glucose and total cholesterol levels may be early signs of velogenic NDV infection in chickens. The absence of any negative effects on total proteins and calcium concentration in vaccinated infected when compared to the unvaccinated infected birds underscores the importance of vaccination not only in prevention of mortality due to velogenic NDV but also reduction of pathologic effects on vaccinated infected birds.

Key words: Chickens, Isa white, Velogenic NDV, KUDU 113, Blood biochemistry.

INTRODUCTION

Newcastle disease (ND) is a contagious viral disease of birds with a wide range of clinical signs (Alexander and Senne, 2008; CFSPH, 2008). It is caused by *Avian Paramyxovirus* type 1 in the *Paramyxoviride* family (Lamb *et al*., 2005). The clinical signs and lesions are dependent on the strain and pathotype of the virus, consequently, no lesions are considered pathognomonic in all forms of the disease. Pathotypes including velogenic viscerotropic ND, velogenic neurotropic ND, mesogenic ND and lentogenic ND have been described in chickens (Alexander and Senne, 2008). The velogenic form of ND is the most devastating in the poultry industry with up to 100% morbidity and mortality rates in susceptible birds (Wakamatsu *et al*., 2006; CFSPH, 2008; Bogoyavlenskiy *et al*., 2009; Aldous *et al*., 2010; Alexander *et al*., 2012). Newcastle Disease is endemic in many countries of Central and South America, the Middle East, Africa and Asia (USDA, 1992; Alexander, 2001; Wakamatsu *et al*., 2006). It is a list A disease when it meets the established criteria of virulence (OIE, 2012). Outbreaks of ND have tremendous economic impact due to the high mortality of commercial and village chickens and trade embargoes placed on affected countries (CEC, 1992; Alexander *et al*., 2008; CFSPH, 2008; Alexander, 2011). Also, the major source of meat and income for rural women in the developing countries is negatively affected as backyard poultry is the major occupation with the attendant negative effects on dietary protein and wellbeing (Aboe *et al*., 2006; Saidu *et al*., 2006; Olabode *et al*., 2008; Chaka *et al*., 2012; Solomon *et al*., 2012).

The history of ND is full of research activities and efforts towards the control and possible eradication, however, problems and losses due to outbreaks of the disease remain unabated worldwide.

Blood biochemistry determination is vital in evaluating cellular changes due to organopathies in birds as little or no clinical signs of disease are observed even when seriously ill (Hochleithner, 2004; Harr, 2009). Therefore, proper diagnosis of avian diseases requires in addition to knowledge of diagnostic sensitivities and specificities of tests and correct intervals for a specific test in a given species, a list of diseases with associated blood biochemistry changes. Information on the changes due to velogenic NDVs (To the knowledge of the authors) is scarce. The study was therefore designed to evaluate some blood biochemistry changes associated with a velogenic NDV infection to help in early detection of the disease and subsequently help in its eradication and control.

MATERIALS AND METHODS

One hundred day - old - cockerels (*Isa white*) were obtained from the hatchery section of Ajanla Farms, CHI Limited, Ibadan, Oyo state, Nigeria. The cockerels were vaccinated against infectious bursal disease appropriately. The cockerels were randomly divided into three groups as follows: Group A - Vaccinated and inoculated with NDV, Group B - Unvaccinated and inoculated with NDV, Group C - Unvaccinated and uninoculated cockerels (control). Group A cockerels were vaccinated with *La Sota* vaccine obtained from National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria via drinking water at three weeks of age. The vaccinated and unvaccinated groups were kept in different locations in fly-proof research animal houses of the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka. Feed and water were provided *ad libitum*. They were raised on deep litter for 6 weeks before challenge.

The study was approved by the University Committee on medical and scientific Research Ethics, University of Nigeria,

Nsukka and guidelines for the care and humane handling of animals were strictly adhered to all through the study (FASS, 2010).

The velogenic NDV strain, KUDU 113 (Echeonwu *et al*., 1993) was used for the challenge experiment. The ampoule was reconstituted to give a median embrayo lethal dose (ELD_{50}) of $10^{6.46}$ per ml. Each bird in infected groups was inoculated intramuscularly with 0.1ml of the inoculum while the uninfected group received 0.1ml of PBS im. The uninfected group (group C) was kept in a separate location.

The birds were observed for clinical signs from days 0 to 21 post inoculation (pi) and clinical signs recorded. Three birds in each group were sacrificed on days 0, 3, 6, 10, 15 and 21 pi for post mortem examination and samples of the thymus, spleen, bursa, kidney, liver, trachea, lung and brain were collected for histopathology.

Blood samples (3ml) were collected randomly from five birds in each group through the jugular vein on days 0, 3, 6, 9, 12, 15 and 21 pi. Blood was not taken from the same birds within a week to avoid inducing anaemia. Samples were put into clean test tubes and allowed for 30 minutes at room temperature to clot. It was centrifuged for 10 minutes at 3000g and serum harvested for blood biochemistry determinations. All the biochemical parameters except blood glucose level were determined using serum sample and Quimica Clinica Aplicada test kits (Quimica Clinica Aplicada, Spain) and a spectrum-Lab 21A spectrophotometer (SpectrumLab, England). The serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activites were determined by the Reitman-Frankel method (Reitman and Frankel, 1957). The serum alkaline phosphatase (ALP) was determined by the phenolphthalein monophosphate method (Klein *et al*., 1960; Babson *et al*., 1966). The total serum protein was determined by the direct Biuret method (Lubran, 1978) while the serum albumin was determined by the bromocresol green method (Doumas *et al*., 1971; Doumas and Peters, 1997). The serum globulin was calculated as the difference between the serum total protein and serum albumin (Colville, 2002). The total bilirubin was determined by the Jendrassik-Grof method (Doumas *et al*., 1973). The serum cholesterol was determined by enzymatic colorimetric method (Allain *et al*., 1974) while the serum calcium was determined by the o-cresolphthalein direct method (Kessler and Wolfman, 1964; Biggs, 1964). The blood urea nitrogen (BUN) was determined by the modified Berthlot-Searcy method (Fawcett and Scott, 1960; Searcy *et al*., 1967) while the serum uric acid was determined by the enzymatic colorimetric method (Fossati *et al*., 1980). The serum creatinine was determined by the modified Jaffe method (Blass *et al*., 1974) while the blood glucose level was determined using ACCU-CHEK® Glucometer (Roche Diagnostics GmbH, Nannheim, Germany) based on the glucose oxidase method (D'Orazio *et al*., 2005).

Data generated were analyzed using One-way Analysis of variance (ANOVA) and Students t-test using Statistical Package for Social Sciences (SPSS) version 16.0 for Windows (SPSS Inc, Chicago, IL). Variant means were separated using the Least Significant Difference (LSD) method. Significance was accepted at 5% probability level.

RESULTS

Clinical signs

The vaccinated and unvaccinated infected cockerels showed signs of weakness and greenish diarrhoea from day 2 pi but signs were more on the unvaccinated infected cockerels. By day 3 pi, all the birds in unvaccinated infected group were depressed and had greenish diarrhoea while the signs

Figure 1: Vaccinated infected cockerels 5 days pi showing torticollis (T), droppy wings (D), sick and live birds

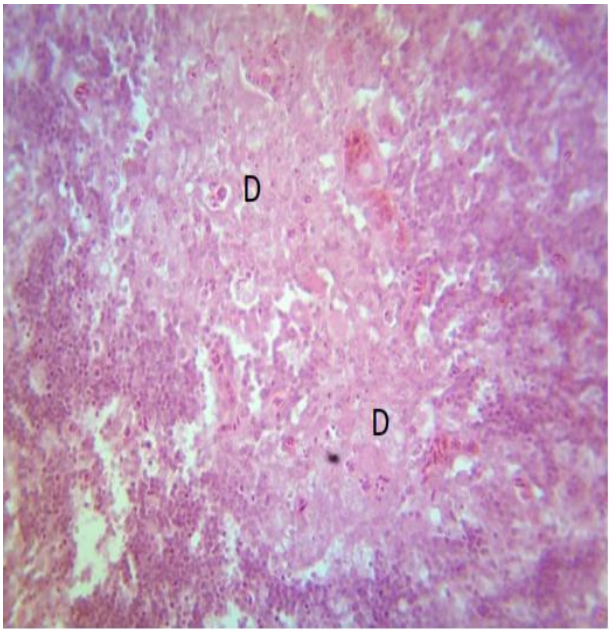

Figure 2: Depletion (D) of lymphocytes in the thymus of infected chicken on day 3 pi, H&E x 400

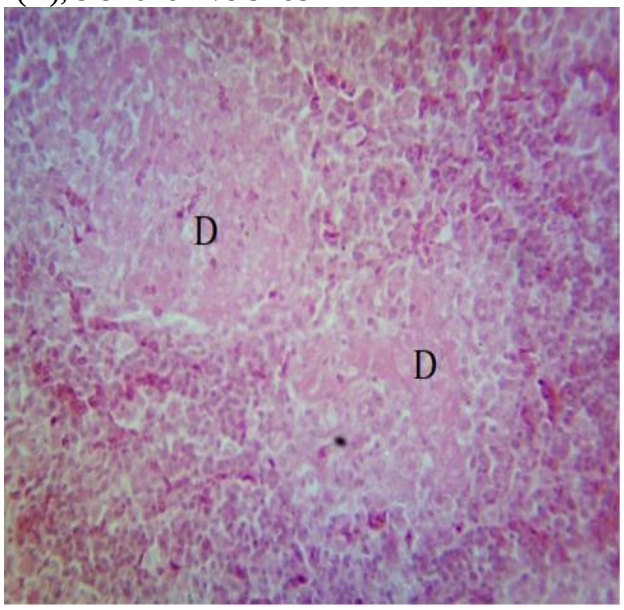

Figure 3: Depletion (D) of lymphocytes in the spleen of infected chickens on day 3 pi, H&E x 400

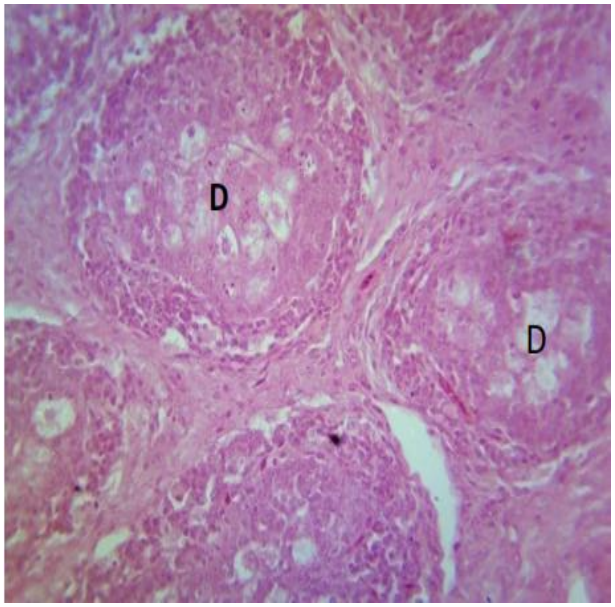

Figure 4: Depletion (D) of lymphocytes in the bursa of infected chicken on day 3pi, H&E x 400

seen in the vaccinated infected group included greenish diarrhea, torticollis and droopy wings in few birds (Figure 1). Mortality was first recorded on day 3 pi in both the vaccinated infected and unvaccinated infected cockerels with 2 and 3 birds respectively. On day 4 pi, 2 and 22 cockerels died in the vaccinated infected and unvaccinated infected groups respectively while five in the unvaccinated infected group died on day 5 pi with overall 13.33% and 100% mortality for vaccinated infected and unvaccinated infected groups respectively.

Gross and Microscopic lesions

Lesions recorded included atrophy of the thymus, bursa of Fabricius and the spleens of the unvaccinated infected and enlargement of the thymus of the vaccinated infected cockerels. There were congestion of the thigh and breast muscles, haemorrhages in the proventriculus and haemorrhagic ulcers in the caecal tonsils from day 3 pi. Microscopic lesions included lymphocytic depletion of the thymus (Figure 2), spleen (Figure 3) and bursa of Fabricius (Figure 4) on day 3 pi.

Total Proteins

The mean serum total proteins of the vaccinated infected and unvaccinated infected cockerels were not different ($P>0.05$) when compared with their unvaccinated uninfected controls on day 0 (TABLE I). On day 3 pi, the mean total protein of infected cockerels were lower when compared with their control but the unvaccinated infected group was significant ($p<0.05$, TABLE II). On day 9 pi, the mean value obtained for the vaccinated infected cockerels was lower ($p<0.05$) when compared with that recorded for the controls (TABLE IV). The mean values recorded for the vaccinated infected cockerels did not vary ($p>0.05$) when compared with the values obtained for the control on days 6, 12, 15 and 21 pi (TABLES III, V-VII).

TABLE I: Clinical chemistry profile of the cockerels on day 0.

Parameters	Means ± Standard error		
	Group A	Group B	Group C
ALT (IU/L)	36.42 ± 0.13	36.15 ± 0.28	36.11 ± 0.14
AST (IU/L)	53.19 ± 0.09	53.13 ± 0.07	53.15 ± 0.08
ALP (IU/L)	216.35 ± 13.41	211.70 ± 9.71	205.89 ± 9.00
T. Proteins (g/dl)	2.68 ± 0.05	2.68 ± 0.06	2.72 ± 0.10
Albumin (g/dl)	1.40 ± 0.10	1.46 ± 0.05	1.38 ± 0.08
Globulin (g/dl)	1.27 ± 0.13	1.34 ± 0.08	1.29 ± 0.04
Blood Glucose (mg/dl)	242.80 ± 4.02	242.60 ± 2.91	240.40 ± 4.58
T. Cholesterol (mg/dl)	100.00 ± 4.26	98.09 ± 5.13	93.33 ± 5.76
Calcium (mg/dl)	8.71 ± 0.21	8.81 ± 0.17	8.90 ± 0.25
Total Bilirubin (mg/dl)	1.51 ± 0.10	1.73 ± 0.14	1.77 ±0.11
BUN (mg/dl)	1.20 ± 0.26	1.02 ± 0.21	1.20 ± 0.26
Creatinine (mg/dl)	0.53 ± 0.08	0.53 ± 0.08	0.60 ± 0.07
Uric Acid (mg/dl)	5.67 ± 0.45	6.02 ± 0.43	5.84 ± 0.45

No difference between the groups ($p>0.05$).

ALT - Alanine aminotransferase; AST - Aspartate aminotransferase; ALP - Alkaline phosphatase;

BUN - Blood urea nitrogen

TABLE II: Clinical chemistry profile of the cockerels three days pi with the velogenic NDV

Parameters	Means ± Standard error		
	Group A	Group B	Group C
ALT (IU/L)	36.94 ± 0.09	37.03 ± 0.09	37.03 ± 0.17
AST (IU/L)	53.36 ± 0.06	52.91 ± 0.21	53.42 ± 0.17
ALP (IU/L)	214.12 ± 11.18	175.21 ± 4.94	203.83 ± 12.69
T. Proteins (g/dl)	2.67 ± 0.09[ab]	2.50 ± 0.17[a]	2.95 ± 0.07[b]
Albumin (g/dl)	1.17 ± 0.07	1.04 ± 0.02	1.13 ± 0.11
Globulin (g/dl)	1.52 ± 0.07	1.46 ± 0.19	1.50 ± 0.16
Blood Glucose (mg/dl)	253.25 ± 5.68[a]	261.25 ± 9.02[a]	226.75 ± 6.30[b]
T. Cholesterol (mg/dl)	142.86 ± 8.69[a]	101.19 ± 14.73[b]	85.71 ± 5.50[b]
Calcium (mg/dl)	9.88 ± 0.07[a]	7.74 ± 0.63[b]	8.49 ± 0.35[ab]
Total Bilirubin (mg/dl)	1.69 ± 0.14	1.94 ± 0.10	1.64 ± 0.05
BUN (mg/dl)	1.02 ± 0.26	1.23 ± 0.31	1.16 ± 0.25
Creatinine (mg/dl)	0.75 ± 0.08	0.59 ± 0.09	0.59 ± 0.09
Uric Acid (mg/dl)	6.50 ± 0.81	5.24 ± 0.49	5.43 ± 0.49

[ab]Different superscripts in a row indicate significant difference between the groups ($p<0.05$).
ALT - Alanine aminotransferase; AST - Aspartate aminotransferase; ALP - Alkaline phosphatase;
BUN - Blood urea nitrogen

TABLE III: Clinical Chemistry profile of the Cockerels six days pi with the velogenic NDV

Parameters	Means ± Standard error	
	Group A	Group C
ALT (IU/L)	36.44 ± 0.18	37.09 ± 0.17
AST (IU/L)	52.09 ± 0.18	51.37 ± 0.12
ALP (IU/L)	174.35 ± 5.48	180.71 ± 13.46
T. Proteins (g/dl)	2.60 ± 0.09	2.28 ± 0.21
Albumin (g/dl)	1.11 ± 0.06	0.92 ± 0.07
Globulin (g/dl)	1.49 ± 0.05	1.20 ± 0.10
Blood Glucose (mg/dl)	225.20 ± 7.94	224.40 ± 10.04
T. Cholesterol (mg/dl)	105.36 ± 5.89	97.56 ± 7.40
Calcium (mg/dl)	8.88 ± 0.30	8.93 ± 0.37
Total Bilirubin (mg/dl)	1.77 ± 0.11	1.73 ± 0.12
BUN (mg/dl)	1.39 ± 0.21	1.04 ± 0.17
Creatinine (mg/dl)	0.53 ± 0.08	0.47 ± 0.08
Uric Acid (mg/dl)	6.57 ± 0.47	6.11 ± 0.49

No difference between the groups ($p>0.05$).
ALT - Alanine aminotransferase; AST - Aspartate aminotransferase; ALP - Alkaline phosphatase;
BUN - Blood urea nitrogen

Albumin

The mean serum albumin of the vaccinated infected and unvaccinated infected cockerels were not ($p>0.05$) different when compared with the unvaccinated uninfected cockerels on day 0 and 3 pi (TABLES I and II). The mean serum albumin concentration recorded in the vaccinated infected cockerel from day 6 to 12 pi were not found to be different ($p>0.05$) from the control but was lower ($p<0.05$) when compared with the unvaccinated uninfected group from day 15 to 21 pi (TABLES VI and VII).

Globulin

The mean serum globulin of the vaccinated infected and unvaccinated infected cockerels were not different ($p>0.05$) when compared with the unvaccinated uninfected cockerels on day 0 and 3 pi (TABLES I and II). The mean globulin value of the vaccinated infected cockerels did not vary ($p>0.05$) when compared with the unvaccinated uninfected group from day 6 to 21 pi (TABLES III- VII) except on day 9 and 12 pi (TABLES IV and V) when the vaccinated infected group was lower ($p<0.05$) and higher ($p<0.05$) than the control group respectively.

Blood glucose

The mean blood glucose level of the vaccinated infected and unvaccinated infected cockerels did not vary ($p>0.05$) from the unvaccinated uninfected cockerels on day 0 (TABLE I) however, the two infected cockerel groups were higher ($p<0.05$) when compared with their unvaccinated uninfected control group on day 3 pi (TABLE II). The mean blood glucose value of the vaccinated infected cockerels was not ($p>0.05$) different from the value recorded for the unvaccinated uninfected cockerels from day 6 to 21 pi (TABLES III-VII).

TABLE IV: Clinical Chemistry profile of the Cockerels nine days pi with the velogenic NDV

Parameters	Means ± Standard error	
	Group A	Group C
ALT (IU/L)	36.43 ± 0.10	36.24 ± 0.18
AST (IU/L)	50.65 ± 0.10	50.67 ± 0.12
ALP (IU/L)	208.94 ± 9.41	205.76 ± 6.26
T. Proteins (g/dl)	2.20 ± 0.18[a]	2.58 ± 0.15[b]
Albumin (g/dl)	1.04 ± 0.05	1.15 ± 0.09
Globulin (g/dl)	1.16 ± 0.14[a]	1.43 ± 0.06[b]
Blood Glucose (mg/dl)	239.00 ± 2.55	226.60 ± 5.60
T. Cholesterol (mg/dl)	125.72 ± 15.47[a]	82.29 ± 2.29[b]
Calcium (mg/dl)	7.72 ± 0.22	7.61 ± 0.21
Total Bilirubin (mg/dl)	1.73 ± 0.07	1.68 ± 0.16
BUN (mg/dl)	1.39 ± 0.21	1.57 ± 0.33
Creatinine (mg/dl)	0.47 ± 0.08	0.47 ± 0.08
Uric Acid (mg/dl)	6.66 ± 0.45	6.57 ± 0.47

[ab]Different superscripts In a row indicate significant difference between the groups ($p<0.05$).
ALT - Alanine aminotransferase; AST - Aspartate aminotransferase; ALP - Alkaline phosphatase; BUN - Blood urea nitrogen

TABLE V: Clinical Chemistry profile of the Cockerels twelve days pi with the velogenic NDV

Parameters	Means ± Standard error	
	Group A	Group C
ALT (IU/L)	36.65 ± 0.24	36.76 ± 0.26
AST (IU/L)	50.92 ± 0.07	50.86 ± 0.09
ALP (IU/L)	203.65 ± 8.14	204.88 ± 8.52
T. Proteins (g/dl)	3.12 ± 0.05	2.80 ± 0.14
Albumin (g/dl)	1.14 ± 0.09	1.42 ± 0.06
Globulin (g/dl)	1.99 ± 0.08[a]	1.48 ± 0.16[b]
Blood Glucose (mg/dl)	211.00 ± 7.94	217.60 ± 18.49
T. Cholesterol (mg/dl)	86.15 ± 7.68	92.31 ± 17.47
Calcium (mg/dl)	7.47 ± 0.35	6.95 ± 0.26
Total Bilirubin (mg/dl)	1.52 ± 0.10	1.52 ± 0.10
BUN (mg/dl)	1.57 ± 0.51	1.39 ± 0.21
Creatinine (mg/dl)	0.60 ± 0.07	0.53 ± 0.08
Uric Acid (mg/dl)	6.94 ± 0.22	7.95 ± 0.73

No difference between the groups ($p>0.05$).

ALT - Alanine aminotransferase; AST - Aspartate aminotransferase; ALP - Alkaline phosphatase;

BUN - Blood urea nitrogen

TABLE V: Clinical Chemistry profile of the Cockerels fifteen days pi with the velogenic NDV

Parameters	Means ± Standard error	
	Group A	Group C
ALT (IU/L)	37.59 ± 0.10	37.64 ± 0.10
AST (IU/L)	51.62 ± 0.19	51.34 ± 0.06
ALP (IU/L)	186.35 ± 7.60	183.71 ± 5.28
T. Proteins (g/dl)	2.18 ± 0.10	2.48 ± 0.10
Albumin (g/dl)	1.13 ± 0.08[a]	1.38 ± 0.06[b]
Globulin (g/dl)	1.05 ± 0.05	1.10 ± 0.07
Blood Glucose (mg/dl)	234.20 ± 1.83	225.60 ± 12.93
T. Cholesterol (mg/dl)	86.67 ± 6.80	92.38 ± 10.39
Calcium (mg/dl)	8.93 ± 0.28	9.11 ± 0.40
Total Bilirubin (mg/dl)	1.73 ± 0.12	1.77 ± 0.11
BUN (mg/dl)	1.39 ± 0.21	1.22 ± 0.21
Creatinine (mg/dl)	0.53 ± 0.08	0.53 ± 0.08
Uric Acid (mg/dl)	6.01 ± 0.57	6.01 ± 0.45

[ab]Different superscripts in a row indicate significant difference between the groups ($p<0.05$).

ALT - Alanine aminotransferase; AST - Aspartate aminotransferase; ALP - Alkaline phosphatase;

BUN - Blood urea nitrogen

Total cholesterol

The mean serum total cholesterol of the vaccinated infected and unvaccinated uninfected cockerels were not different ($p>0.05$) when compared with the unvaccinated uninfected cockerels (control) on day 0 (TABLE I) On day 3 pi, the values obtained for the infected cockerels were higher when compared with the controls but only the vaccinated infected group was significant ($p<0.05$, TABLE II). The mean values obtained for the vaccinated infected cockerels were not different ($p>0.05$) from that obtained for the control from day 6 to 21 pi except on day 9 pi (TABLE IV) when the mean value obtained for the vaccinated infected group was higher ($p<0.05$) when compared with the control group.

Total calcium

The mean serum calcium values of the vaccinated infected and unvaccinated infected groups did not vary ($p>0.05$) from their control group from day 0 to 21 except on day 3 pi when the value obtained for the unvaccinated infected group was found to be lower ($p<0.05$) when compared with the vaccinated infected and unvaccinated uninfected groups (TABLE II).

The mean values of other parameters obtained did not vary ($p>0.05$) between the vaccinated infected and unvaccinated uninfected groups throughout the duration of the study.

DISCUSSION

The clinical signs, gross and microscopic lesions observed are in agreement with the findings of Hamid *et al*. (1991), Brown (1999), Okoye *et al*. (2000), Okwor *et al*. (2007) and Ezema *et al*. (2009).

There were no significant variations between vaccinated and unvaccinated birds in this study (TABLE I). This is in contrast with the reports of Talebi (2006) who reported significant reduction in the total proteins and albumin values in broilers vaccinated against NDV and that of El-Toukhy *et al*. (1989) and Kudair and Al-Hussary (2010) who also reported significant increases in the AST activity when compared with the unvaccinated

TABLE VII: Clinical Chemistry profile of the Cockerels twenty one days pi with the velogenic NDV

Parameters	Means ± Standard error	
	Group A	Group C
ALT (IU/L)	33.11 ± 0.26	32.68 ± 0.17
AST (IU/L)	52.85 ± 0.07	52.72 ± 0.09
ALP (IU/L)	180.53 ± 3.08	172.94 ± 6.88
T. Proteins (g/dl)	3.24 ± 0.05	3.44 ± 0.13
Albumin (g/dl)	1.23 ± 0.10[a]	1.55 ± 0.03[b]
Globulin (g/dl)	2.01 ± 0.09	1.69 ± 0.23
Blood Glucose (mg/dl)	233.80 ± 5.45	222.60 ± 12.25
T. Cholesterol (mg/dl)	87.37 ± 8.09	103.16 ± 12.18
Calcium (mg/dl)	8.23 ± 0.17[a]	9.49 ± 0.35[b]
Total Bilirubin (mg/dl)	1.69 ± 0.14	1.60 ± 0.09
BUN (mg/dl)	1.22 ± 0.21	1.22 ± 0.21
Creatinine (mg/dl)	0.53 ± 0.08	0.60 ± 0.07
Uric Acid (mg/dl)	6.11 ± 0.32	6.11 ± 0.56

[ab]Different superscripts in a row indicate significant difference between the groups ($p<0.05$).

ALT - Alanine aminotransferase; AST - Aspartate aminotransferase; ALP - Alkaline phosphatase;

BUN - Blood urea nitrogen

controls.

Hypoproteinaemia occurs mainly due to advanced liver disorder or malabsorption due to enteritis and AST activity increases with damage to organs, mainly liver or tissues (skeletal muscles) (Coles, 1986). The relationship between vaccination and injury to these organs and tissues is not well understood.

The reduced total protein and albumin levels recorded in the infected groups could be due to starvation and enteritis as the birds were anorexic from the onset of clinical signs. Ezema *et al*. (2009) reported anorexia among other clinical signs in vaccinated chickens challenged with a velogenic NDV. Intestinal ulcers and haemorrhages associated with ND also could have resulted to malabsorption and protein loosing enteropathy. The reduction in total protein in the unvaccinated infected when compared with the vaccinated infected could be due to the severity of enteritis which resulted in increased malabsorption and loss of protein.

Hypoproteinemia can be due to reduced synthesis as a result of chronic hepatopathies; malabsorption caused by enteritis, tumors and parasitism; increased loss due to glomerular disorder, starvation and malnutrition (Hochleithner, 1994; Harr, 2002; Campbell, 2004; Harr, 2009).

Albumin is the most abundant protein found in plasma (Harr, 2009) and therefore causes of hypoproteinemia are always due to reduction in plasma albumin concentration. The subsequent significant increase in globulin concentration is in agreement with reports of Snyder (2012). Inflammation due to microorganisms leads to rise in antibodies which are gamma globulins.

There was no significant variation in the AST activity in all the cockerels in this study. This is in variance with results of Rivetz and Bogin (1974) who reported an increase in AST activity in serum and intestines of fowl infected with a mesogenic strain of NDV.

There was also no effect on the serum ALP activity in the cockerels in this study which contrasts with the reports of Lust and Squibb (1967) and Rivetz *et al*. (1975) who reported reduction in the ALP activity in chickens infected with velogenic and mesogenic strains of NDV. They attributed the decrease to damage to the intestines as the predominant isoenzyme in plasma originates in the gut (Bide, 1970) and poor feeding and starvation due to reduced food consumption and intestinal activity (Bide, 1972). However, there were also reports of significant increases in ALP activity following velogenic NDV (Rivetz and Bogin, 1974) which was attributed to use of high doses of NDV. The differences in the responses of these birds to different strains of NDV may be due to differences in organ tropism of each strain.

The results of the ALT activity, blood glucose level, calcium and total cholesterol in both vaccinated and unvaccinated birds are in agreement with the reports of Talebi (2006) and Kudair and Al-Hussary (2010) who reported no significant variation between broilers vaccinated against NDV and unvaccinated ones (Table I).

The significant increase in the blood glucose level in the infected groups could be due to stress from the NDV infection. Hyperglycemia is caused by increased glucose production or release and due to excess glucocorticoid (Campbell, 2004; Hochleithner, 1994). For instance, it occurs after meals, following excitement or stress or due to decreased glucose usage as seen in diabetes mellitus (Amond, 1986; Hochleithner, 1994).

The increased mean total cholesterol levels in the infected groups may be attributed to reduced feed intake that was observed in the challenged groups as no signs of liver disease and bile duct obstruction were observed and the birds were not given high fat diets different from others. Reduced feed

intake also led to significant increase in total cholesterol level in ducks (Okorie-Kanu *et al*., 2016) Pathologic increase in total cholesterol level had been attributed to hypothyroidism, liver disease, bile duct obstruction and starvation or high fat diets (Hochleithner, 1994).

The reduced mean calcium level of the unvaccinated infected group may be associated with the reduced albumin concentration. Some calcium constituents are bound to albumin, therefore, hypoalbuminemia will reduce the quantity of bound calcium and consequently leads to decreased total calcium concentration without reducing biologically active or ionized calcium (Lumeji, 1990; Hochleithner, 1994).

Reduced albumin concentration occasioned by reduced feed intake and enteritis and the consequent reduction in total calcium level could be the reason behind decreased egg lay and other egg abnormalities including misshapen, abnormally coloured, rough or thin-shelled eggs observed in layers infected with NDV. The significant reduction in calcium level in the unvaccinated infected when compared to the vaccinated infected birds may also be due to severe enteritis.

Hypoproteinemia, hypoalbuminemia and hypocalcemia together with increased globulin, blood glucose and total cholesterol levels may be early signs of velogenic NDV infection in chickens.

The absence of any negative effect in total protein and calcium levels in the vaccinated infected birds may be attributable to the protection given by vaccination which led to the mild enteritis with resultant mild diarrhea and little effects on absorption and losses. Therefore, vaccination is vital not only in preventing mortality due to velogenic NDV but also reduces pathologic effects on vaccinated infected birds.

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