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Research Article

The Effects of Mycotoxins, Toxin Binder and Deactivator on the Hematology, Serum Chemistry and Immune Response of Broiler Chickens Vaccinated Against Newcastle Disease

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Abstract

Objectives: This study was designed to investigate the effects of mycotoxins and toxin binder and deactivator on the hematology, serum chemistry and immune response of broiler chickens vaccinated against Newcastle disease. **Materials and Methods:** The finisher feed was moistened with water, and stored in bags for 21 days. It was divided into three parts: A, B, and C. Part A and part B was treated with toxin binder (Toxorid®) and deactivator (Zerotox®) respectively. Part C was not treated with toxin binder or deactivator. The contaminated feeds were stored at 25-30°C for 2 weeks before use. Fresh uncontaminated and untreated feed, 'D' served as the positive control and all evaluated for fungal growth and mycotoxins. A total of 224 broilers were used in this study. Broilers of 5-weeks old were divided into eight groups and fed experimental finisher feeds (A, B, C, D). Two weeks Post Exposure (PE), groups AK, BK, CK and DK were revaccinated with ND vaccine (Komarov®). The hematocrit, erythrocyte counts, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration of groups A, B, C and D were ascertained. The total and differential leukogram of groups A, B, C and D were determined. The Aspartate Aminotransferase (AST) activity, uric acid, total serum protein, serum albumin and serum globulin concentrations of groups A, B, C and D were determined. The HI titers of all the groups were obtained. The data obtained were subjected to Analysis of Variance (ANOVA). **Results:** The phenotypic characterization, DNA extraction and Polymerase Chain Reaction (PCR) amplification of the feed samples revealed that the isolates recovered from groups A, B, C and D were mycotoxigenic fungi. The mean total aflatoxins detected via competitive ELISA were 1.25 ppb for A, 0.97 ppb for B, 2.14 ppb for C and 0.73 ppb for D. The hemoglobin and mean corpuscular hemoglobin concentration of group D, at week 9 of age, was significantly ($p<0.05$) higher than C, but not ($p>0.05$) significant with A and B. There was significant increase in ($p<0.05$) leukocyte counts in groups C, A and B when compared with the control group (D) between weeks 2-4 PE. In the differential leukogram, ($p<0.05$) marked heterophils, eosinophils, monocytes and lymphocytes were seen in groups C, A and B when compared with D between weeks 2-4 PE. Uric acid concentrations of group C at week 2 PE (2.35 ± 0.38) was significantly lower ($p<0.05$) than those of D (3.58 ± 0.60), B (7.01 ± 2.24) and A (4.17 ± 0.47). The total serum protein and the serum albumin concentrations of group C was significantly ($p<0.05$) lower than, A and B, at week 4 PE. The serum globulin and serum albumin globulin ratio concentrations presented the same pattern as seen above. The HI titres of the revaccinated groups, (AK, BK, CK and DK) increased ($p<0.05$) significantly when compared with groups A, B, C and D between weeks 3-5 PE. **Conclusion:** The emanating hematological, HI titers, and biochemical lesions observed among the exposed groups indicated the extent of systemic damage by mycotoxins and ameliorating effects of binders in salvaging contaminated feed. This research outcome is a useful guide to policy formulators, farmers, nutritionists and researchers.

Key words: Antibody response, binders, blood chemistry, chickens, contaminated feed, hematology, mycotoxins

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Worldwide, production of poultry meat and eggs has increased consistently over the years, and this trend is expected to continue[1,2]. Unfortunately, this productivity is negatively affected by inadequate nutrition[1] and diseases, including Newcastle Disease (ND) which is one of the most important diseases in Nigeria[3-6]. ND is a highly contagious and infectious disease that affects almost all avian species including poultry, caged and wild life birds[7-9]. It causes enormous economic losses, not only due to high flock mortality and egg deformities, but also due to trade restrictions and embargoes[9-12]. Based on the form or pathogenicity of the virus[9], among other factors, mortality due to ND ranges from negligible to 100%. Infection occurs through oral, ocular, and respiratory routes, and following invasion of intestinal or tracheal mucosa, it is spread systemically through the bloodstream and lymphatic system to organs with reticuloendothelial tissues.[9,12]. In Nigeria, vaccination is used to increase herd immunity threshold to withstand establishment of ND in flocks[4,9]. The Komarov vaccine is a live attenuated Newcastle disease virus of mesogenic strain that is usually used to elicit stronger immunogenicity[13,14], as booster dose after initial vaccination with LaSota derived of lentogenic strain[4]. This Komarov is readily available in Veterinary Research Institutes in Nigeria, and confers stronger immunity against Newcastle disease[4,14]. Mycotoxicosis, on the others hand is a disease condition caused by mycotoxins, which is produced by fungi in feed and feed raw materials[15-18]. In poultry, they are usually seen when fungi grow in feed and feed raw materials[19]. Many mycotoxins cause disease and are classified as hepatotoxic, nephrotoxic, enterotoxic and neuro-musculotoxic, depending on organs affected[20,21]. All avian species are susceptible but chickens, turkeys and water fowls are mostly affected[22-25]. Mycotoxins of economic importance are aflatoxins, fumonisins, ochratoxins, vomitoxin (DON), trichothecenes (T-2-toxin), and zearalenone[16,19,24,26]. They are most commonly produced by plant pathogenic fungi like *Fusarium* spp *(F. moniliforme*, *F. roseus*, *F. trincictum*, *F. nivale)*, storage fungi like *Aspergillus* and *Penicillium* spp (*A. flavus* and *A. Parasiticus*) and advanced deteriorating fungi like *A. clavatus*, *A. fumigatus*, Rhizopus, Mucor etc, and found on feed raw materials and feed[9,19,22,23,27-29]. Generally, mycotoxins affect all production and profitability indices in poultry management including feeding, immune response to vaccination, weight gain, feed conversion efficiency and ratio and increase cost of medication[30]. Factors like warmth and high humidity during maturation, harvest, transport or storage of grains promote *Aspergillus* and mycotoxigenic fungal growths[24]. A Temperature near 30°C and high humidity provide ideal conditions for mycotoxin biosynthesis, although substrate, time, carbon dioxide levels, and other environmental factors also play a role[25]. It is best to prevent and control diseases before harvest by managing the crop, harvesting, and storing it properly[30]. No matter what preventive measures are taken, 100% absence of mycotoxins may not be possible in Nigeria right now, due to lack of holistic mechanization of agriculture. However, in practice, mycotoxins deactivators and binders, for example, organic (Zerotox®) or inorganic (Toxorid®), have been used to deactivate and eliminate a wide range of mycotoxins from feed. Hematological and biochemical parameter assessments give insight to homeostatic condition and are used to assess the nutritional and clinical health status of animal[31]. These profiles provide enormous data for the analysis of varying conditions of the bird[32,33].

The aim of this study was to investigate the effects of mycotoxins and toxin binder and deactivator on the hematology, serum chemistry and immune response of broiler chickens vaccinated against Newcastle disease. The specific objectives were to; evaluate the effect of Toxorid® and Zerotox® on the hematology and blood chemistry of broiler vaccinated against ND fed mycotoxin contaminated feed, assess the antibody response of ND vaccinated broiler exposed to mycotoxin contaminated feed, and determine the effect of Toxorid® and Zerotox® on the immune response of ND vaccinated broiler fed mycotoxin contaminated feed.

MATERIALS AND METHODS

This study was conducted in 2022 and used experimental design modified by Mgbeahuruike *et al.*[24]. Fungal growth was induced in commercial broiler finisher feed by moistening with clean water at the rate of 8.75 Kg/L. The moistened feed was stored in leak proof bags for 21 days. The feed was divided into three parts: A, B, and C. Part A and B were treated with toxin binder (Toxorid®) at dose rate of 0.5 kg/g and deactivator (Zerotox®) at dose rate of 4.0 kg/g, respectively as recommended by the manufacturers. Part C was not treated with toxin binder or deactivator. Contaminated feeds were stored at 25-30°C for 2 weeks before use. Fresh uncontaminated and untreated feed, 'D' served as the positive control. Each of the four feed samples was processed for fungal phenotypic and molecular identification and total aflatoxin concentration detection. A total of 224 day-old broiler chicks (n = 224) were brooded for 4 weeks and

administered ND (LaSota) and infectious bursal disease (Gumboro) vaccines. During the first four weeks of broiler rearing, commercial broiler super starter feed and water were given *ad libitum*.

Experimental site and ethical statement: Experimental animal house of the Faculty of Vocational and Technical Education (VTE) University of Nigeria, Nsukka was used. Approval was obtained from the Faculty of Veterinary Medicine Institutional Animal Care and Use Committee (IACUC) with the certificate No: FVM-UNN-IACUC-2021-0877.

Experimental birds and Design: The birds were randomly divided into eight groups at the age of five weeks as follows: A (contaminated feed+Toxorid®, unrevaccinated), B (contaminated feed+Zerotox®, unrevaccinated), C (contaminated feed only, unrevaccinated), D (fresh feed, unrevaccinated), AK (contaminated feed+Toxorid®+Komarov), BK (contaminated feed+Zerotox®+Komarov), CK (contaminated feed only, +Komarov), and DK (Fresh feed+Komarov) (Table 1). From the beginning of the study until the end, the birds were fed the same quantity of each feed category (10 weeks). At seven weeks of age, birds in groups AK, BK, CK and DK were administered with NDV Komarov vaccine. The hematology (hematocrit, erythrocyte count, total leukocyte counts, differential leukocyte counts, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration), serum chemistry (total serum proteins, serum albumin, serum globulin and albumin-globulin ratio, aspartate aminotransferase and uric acid levels) and hemagglutination inhibition (HI) tests were conducted. All the values were subjected to statistical analysis using analysis of variance (ANOVA) and Post Hoc test employed to determine the level of significance at $p \leq 0.05$.

RESULTS

Fungi isolates from feed samples (A, B and C) were *Aspergillus niger*, *Aspergillus flavus*, *Curvularia lunata* and *Millerozyma farinosa* based on the morphology, appearance, elevation, and colony color of each isolate, as seen in Table 2. Table 3 shows the results of BLAST search on the DNA data base and Fig. 1 shows the result of the phylogenetic tree of the feed sample isolates.

The total aflatoxins detected using ELISA kit in feed A was 1.25 ppm while 0.97, 2.14 and 0.73 ppm were detected in B, C and D, respectively as shown in Table 4. The average value of the batches was obtained as a representative of each group. The hematocrit, erythrocyte counts, mean corpuscular volume, and mean corpuscular hemoglobin of groups A, B, C and D did not vary ($p>0.05$) significantly at the age of 5, 7 and 9 weeks when compared. Similar results were seen in the hemoglobin

Table 1: Experimental Designs

Groups	Treatments
A	Contaminated feed+Toxorid®, unrevaccinated with Komarov vaccine
B	Contaminated feed+Zerotox®, unrevaccinated with Komarov vaccine
C	Contaminated feed only, unrevaccinated with Komarov vaccine
D	Fresh feed, unrevaccinated with Komarov vaccine
AK	Contaminated feed+Toxorid®+revaccinated with Komarov vaccine
BK	Contaminated feed+Zerotox®+revaccinated with Komarov vaccine
CK	Contaminated feed only, + revaccinated with Komarov vaccine
DK	Fresh feed + revaccinated with Komarov vaccine

Table 2: Colony morphology of the isolates from all the feed samples

Feed sample ID	Isolate	Shape	Texture	Colour	Elevation
A (Contaminated+Toxorid®)	Isolate 9	Irregular	Hairy	Cream	Flat
	Isolate 10	Irregular	Hairy	Red	Flat
	Isolate 12	Filamentous	Shiny	Whitish-cream	Flat
	Isolate 13	Round	Hairy	Red	Flat
	Isolate 14	Irregular	Hairy	Cream	Flat
B (Contaminated+Zerotox®)	Isolate 4	Covered	Dull	Cream	Flat
	Isolate 5	Round	Hairy	Black	Raised
	Isolate 6	Irregular	Hairy	Cream	Flat
	Isolate 7	Irregular	Hairy	Cream	Flat
	Isolate 8	Filamentous	Shiny	Cream	Flat
C (Contaminated only)	Isolate 1	Round	Hairy	Red	Flat
	Isolate 2	Irregular	Thick	Cream	Raised
	Isolate 3	Irregular	Shiny	Cream	Flat
D (Uncontaminated)	Isolate 15	Irregular	Dull	Cream	Flat
	Isolate 16	Irregular	Hairy	Cream	Raised
	Isolate 17	Round/Spotted	Dull	Cream	Flat
	Isolate 18	Round/Spotted	Dull	Cream	Flat
	Isolate 19	Irregular	Spotted	Black	Flat
	Isolate 20	Irregular	Spotted	Black	Flat

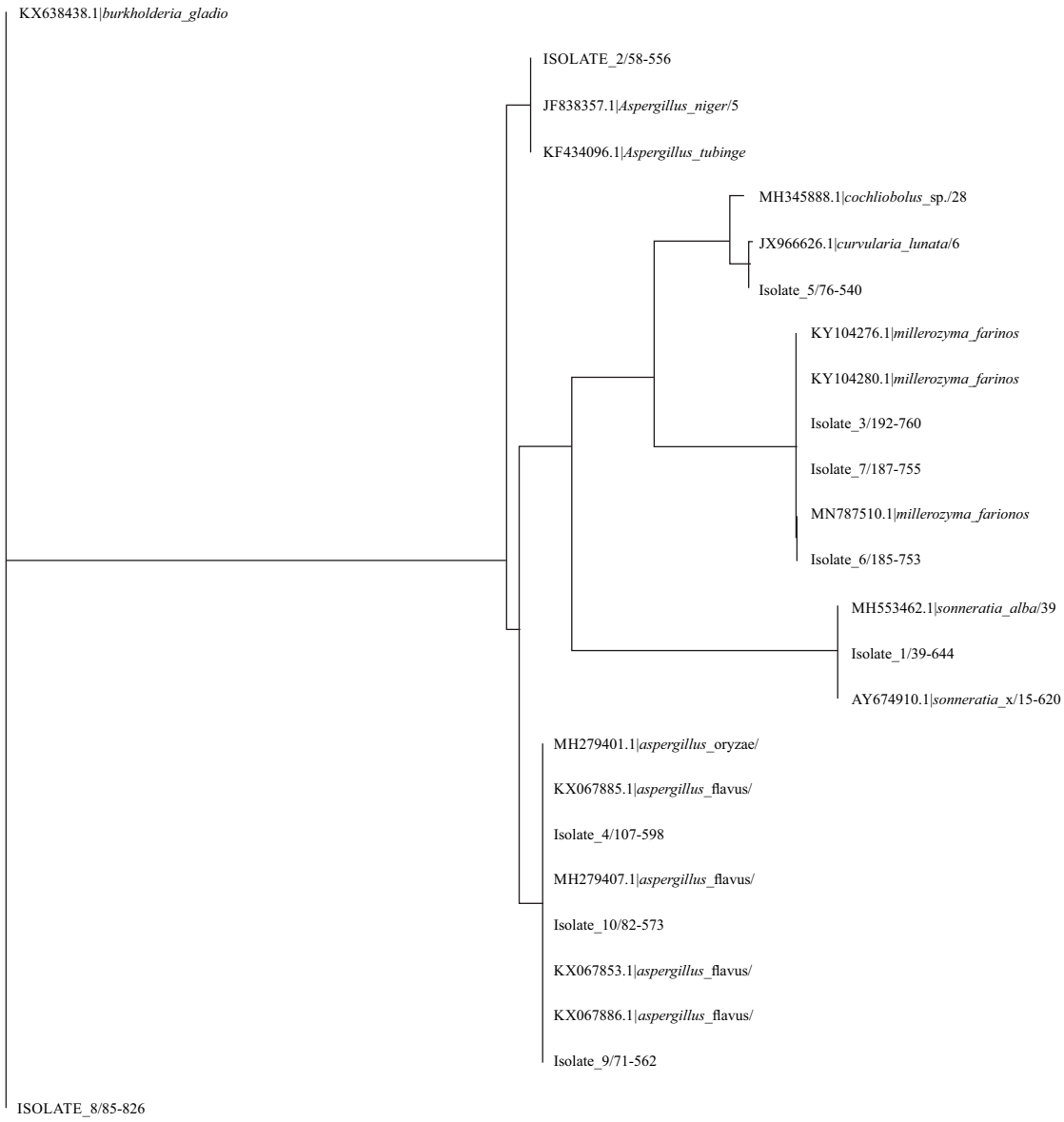

Fig 1: Phylogenetic tree of fungal isolates from the feed samples

and mean corpuscular hemoglobin concentration, except that at week 9 of age, group C birds had significantly ($p<0.05$) lower values when compared to group D birds (Table 5 and 6). There was significantly ($p<0.05$) marked leukocyte counts in groups A, B and C when compared with the control group (D). In the differential leukogram, significantly ($p<0.05$) marked heterophil, eosinophil, monocyte and lymphocyte counts were seen in A, B, and C when compared with D at week 7 and 9 of age (Table 7). The Aspartate aminotransferase (AST) did not vary ($p>0.05$) significantly among groups A, B, C and D at week 5, 7 and 9 of age. The uric acid did not vary ($p>0.05$) significantly among the groups A, B, C and D at the age of 5 and 9 weeks, however, at week 7 of age, group C (2.35 ± 0.38) was significantly lower than those of A (7.01 ± 2.24) B (4.17 ± 0.47) and D (3.58 ± 0.60), when compared. The total serum protein and the serum albumin of groups A, B, C and D did not vary ($p>0.05$) significantly at the age of 5 and 7 weeks, while group B was significantly ($p<0.05$) lower than, C, and D, but did not vary ($p>0.05$) significantly when compared with A at week 9 of age. The serum globulin and serum albumin globulin ratio presented the same pattern as seen above at week 5 and 7 of age, but group C was significantly ($p<0.05$) lower than group D, but not ($p>0.05$) with A and B when compared (Table 8). The HI titres of groups A, B, C and D did

Table 3: DNA sequences BLAST result on DNA subway database of the feed samples

Sample ID	Accession No:	Details	Aln. Length	Bit Score	E Val.	Mis-matches
Isolate 1	AY674910.1	*Sonneratia*×hainanensis -5.8S ribosomal RNA gene, complete sequence	628	1128	0.0	2
Isolate 2	JF838357.1	*Aspergillus niger*-internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	601	1079	0.0	2
Isolate 3	KY104276.1	*Millerozyma farinosa*-internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	672	1204	0.0	0
Isolate 4	KX067885.1	*Aspergillus flavus*-internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	628	1124	0.0	0
Isolate 5	JX966626.1	*Curvularia lunata*-internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	533	940	0.0	1
Isolate 6	MN787510.1	*Millerozyma farinosa*-internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	637	1150	0.0	0
Isolate 7	KY104276.1	*Millerozy mafarinosa* -internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	672	1213	0.0	0
Isolate 8	KX638438.1	*Burkholderia gladioli*	863	1532	0.0	6
Isolate 9	HQ285572.1	*Aspergillus flavus*-internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	598	1079	0.0	0
Isolate 10	KX067886.1	*Aspergillus flavus*-internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	619	1108	0.0	0

Table 4: Total aflatoxin values of the feed samples

	Groups			
Batches	A (ppb)	B (ppb)	C (ppb)	D (ppb)
1	0.85	2.38	2.74	0.55
2	2.52	0.17	1.77	1.38
3	0.38	0.35	1.91	0.27
Mean	1.25	0.97	2.14	0.73

not show significant ($p \geq 0.05$) difference at week 5 and 7 of age. However, after Komarov vaccination at week 7 of age, the titres of vaccinated groups (AK, BK, CK and DK) increased ($p<0.05$) significantly when compared with the unrevaccinated (A, B, C and D) (Table 9).

DISCUSSION

Mgbeahuruike *et al.*[24] reported that mycotic agents grew more readily in moist experimental finisher feed samples. *Aspergillus niger*, *A. flavus*, *Curvularia lunata* and *Millerozyma farinosa* were among the mycotic agents isolated from the moisture-treated feed samples. They are reported to elaborate mycotoxins[9,29,33-35]. *Aspergillus niger* produces fumonisin B and Ochratoxin A due to corn storage deterioration[36]. *Curvularia lunata* produces curvularin which is an airborne contaminant found in soybeans and cotton mills, which can cause hepatic necrosis[37,38]. A yeast known as *Millerozyma farinosa* produces a toxin known as a killer toxin that inhibits the growth of other yeasts and fungi in the same medium and causes catheter-related infections in humans. There are a number of mycotoxins produced by *Aspergillus flavus*, such as Aflatoxins, Ochratoxin A, and Patulin, among others[39]. These mycotoxins are reported to cause a wide range of clinical signs and pathologic lesions such as weight losses, hepatotoxicity, nephrotoxicity, enterotoxicity, carcinogenesis and depressed immune response in livestock as previously reported by CAST[20] and Pierron *et al.*[40], Pereira *et al.*[16], Nazhand *et al.*[17] and Awuchi *et al.*[18]. The detection of the mycotoxins in feed raw materials is very important before production of finished feed, since it will be a guide to the right counteraction solution for protecting livestock on the field[41].

The erythrocyte response in all the unrevaccinated groups had similar pattern in this study and there were no significant ($p \geq 0.05$) changes in the PCV and EC throughout the study, but there was significant ($p \leq 0.05$) decrease in HbC and MCHC in group C birds compared with the control (group D) birds. Meanwhile, the values were within the normal range for broilers[42]. The Mean Corpuscular Volume (MCV) and the Mean Corpuscular Hemoglobin (MCH) responses in all the chicken groups were normocytic and Mean Corpuscular Hemoglobin Concentration (MCHC) was hypo-chromic, indicating that circulating RBC at those points were mainly reticulocytes, with less hemoglobin[43-47]. There was significant increase in the leukocyte and differential WBC of the chickens throughout the period of exposure, far higher than the reference value[42]. Monocytosis, a form of leukocytosis associated with increases in monocytes, was observed. This may be caused by presence of tissue insults, infections, and other numerous disease syndromes[47]. The monocytes in the blood are in transit

Table 5: Hematological Parameters of unrevaccinated broiler chickens exposed to mycotoxin contaminated feed and binder or deactivator

	Groups											
	A			B			C			D		
Age (weeks)	PCV	EC	HbC	PCV	EC	HbC	PCV	EC	HbC	PCV	EC	HbC
5	29.30±0.34	3.16±0.02	8.13±0.21	31.70±1.66	3.32±0.10	8.71±0.71	29.44±1.31	3.05±0.16	8.28±0.32	30.10±1.07	3.16±0.10	8.47±0.42
7	27.70±0.64	2.97±0.10	9.19±0.74	27.60±1.03	2.91±0.12	8.56±0.49	26.96±0.66	2.85±0.10	8.93±0.80	28.02±1.47	2.93±0.17	9.27±0.63
9	25.90±1.10	2.75±0.17	6.52±0.27^{AB}	24.90±1.16	2.60±0.16	6.69±0.36^{AB}	25.00±1.37	2.61±0.17	5.95±0.18^{A}	25.50±0.35	2.64±0.04	7.06±0.30^{B}

Values on the same row with different superscript vary significantly at P≤0.05
PCV = Packed Cell Volume %,
EC = Erythrocyte Counts ×10^{6}/µL,
HbC = Heamoglobin Concentrations g/dL

Table 6: Calculated Hematology Parameters of unrevaccinated broiler chickens exposed to mycotoxin contaminated feed and binder or deactivator

	Groups											
	A			B			C			D		
Age (weeks(	MCV	MCH	MCHC	MCV	MCH	MCHC	MCV	MCH	MCHC	MCV	MCH	MCHC
5	92.71±0.58	25.75±0.73	27.78±0.79	95.18±2.48	26.07±1.37	27.35±0.90	96.82±1.70	27.27±0.67	28.20±0.80	95.11±0.88	26.90±1.72	28.29±1.84
7	93.28±1.13	31.08±2.83	33.28±2.86	94.99±0.89	29.59±1.94	31.10±1.80	94.88±1.07	31.43±2.88	33.11±2.93	95.90±1.29	31.88±2.19	33.24±2.24
9	94.75±1.78	24.01±1.41	25.31±1.23^{AB}	96.16±1.45	25.78±0.25	26.82±0.25^{AB}	95.96±1.16	23.03±1.02	23.98±0.89^{A}	97.01±0.78	24.92±1.38	27.70±1.31^{B}

Values on the same row with different superscript vary significantly at P≤0.05
MCV = Mean Corpuscular Volume fl,
MCH = Mean Corpuscular Hemoglobin pg
MCHC = Mean Corpuscular Hemoglobin Concentrations g/dl

Table 7: Total Leukocyte Counts (TLC) and Differential Leukocyte Counts (DLC) of unrevaccinated broiler chickens exposed to mycotoxin contaminated feed and binder or deactivator $\times 10^3/\mu L$

Age (Weeks)	Groups T	H	L	M	E
A					
5	35.50±3.01	12.58±3.06	28.47±6.66	4.97±0.53	2.40±0.37
7	71.50±3.92^{C}	12.76±0.72^{C}	42.50±1.97^{B}	10.49±2.35^{B}	5.26±0.40^{BC}
9	101.90±2.64^{B}	17.15±1.02^{B}	61.50±1.94^{B}	7.59±0.67^{C}	4.64±1.37^{A}
B					
5	50.00±6.89	9.93±1.25	35.56±6.19	4.83±0.72	1.83±0.47
7	57.80±2.00^{B}	8.94±1.01^{B}	40.05±2.11^{AB}	4.77±0.70^{A}	5.95±0.96^{C}
9	60.20±7.63^{A}	9.42±1.35^{A}	40.15±6.32^{A}	5.06±0.72^{B}	15.91±0.56^{B}
C					
5	45.76±4.53	10.79±1.94	28.00±4.14	6.87±1.25	2.58±0.65
7	63.50±5.37^{BC}	8.61±1.19^{A}	41.67±5.69^{B}	3.96±0.75^{A}	4.05±0.56^{AB}
9	56.80±4.03^{A}	5.57±0.47^{C}	42.87±2.83^{A}	3.12±0.36^{A}	5.54±1.09^{A}
D					
5	41.40±1.83	9.03±1.51	25.59±2.07	5.24±0.40	1.95±0.29
7	43.00±1.41^{A}	6.00±0.28^{A}	30.62±1.24^{A}	4.09±0.68^{A}	2.29±0.23^{AB}
9	57.10±6.53^{A}	14.66±2.04^{D}	35.33±4.54^{A}	2.92±0.20^{A}	6.21±1.44^{A}

Values on the same row with different superscript vary significantly at P≤0.05, T: Total leukocyte count, H: Heterophil, L: Lymphocyte, M: Monocyte and E: Eosinophil

Table 8: Serum biochemistry of unrevaccinated broiler chickens exposed to mycotoxin contaminated feed and binder or deactivator

Age (Weeks)	Groups AST	UA	TSP	SA	SG
A					
5	82.56±6.46	9.66±1.85	2.95±0.09	2.15±0.05	0.80±0.08
7	66.74±3.69	7.01±2.24^{B}	3.37±0.12	2.21±0.02	1.17±0.13
9	81.32±3.86	4.98±0.56	4.52±0.43^{B}	2.21±0.05^{B}	2.15±0.28^{B}
B					
5	90.62±4.02	10.62±1.53	3.51±0.34	2.45±0.09	1.06±0.27
7	75.62±6.12	4.17±0.47^{AB}	3.40±0.16	2.26±0.07	1.14±0.16
9	86.75±5.19	4.60±0.34	4.21±0.22^{B}	2.35±0.05^{C}	1.85±0.22^{AB}
C					
5	78.39±5.87	8.20±1.34	2.88±0.03	2.05±0.02	0.82±0.03
7	63.28±4.12	2.35±0.38^{A}	3.25±0.16	2.18±0.06	1.08±0.14
9	74.46±2.55	3.60±0.72	3.30±0.18^{A}	2.08±0.04^{A}	1.23±0.15^{A}
D					
5	83.82±2.59	7.47±0.59	3.20±0.13	2.34±0.07	0.86±0.10
7	65.05±4.95	3.58±0.60^{AB}	3.48±0.21	2.33±0.08	1.15±0.15
9	79.67±3.86	5.22±0.52	3.86±0.25^{AB}	2.14±0.02^{AB}	1.72±0.24^{AB}

Values on the same row with different superscript vary significantly at p≤0.05, AST: Aspartate aminotransferase, UA: Uric Acid, TSP: Total serum proteins, SA: Serum Albumin and SG: Serum globulin

Table 9: Hemagglutination Inhibition (HI)Titre of unrevaccinated and revaccinated broiler chickens exposed to mycotoxin contaminated feed and binders or deactivator (GMT)

Age (weeks)	Groups A	AK	B	BK	C	CK	D	DK
5	1.99±0.47	1.99±0.47	1.16±0.17	1.16±0.17	1.99±0.42	1.99±0.42	1.71±0.26	1.71±0.26
6	1.16±0.17	1.16±0.17	2.13±0.40	2.13±0.40	1.16±0.35	1.16±0.35	2.13±0.40	2.13±0.40
7	0.74±0.34^{A}	0.74±0.34^{A}	1.99±0.35^{B}	1.99±0.35^{B}	1.16±0.17^{AB}	1.16±0.17^{AB}	2.13±0.40^{B}	2.13±0.40^{B}
8	0.78±0.42^{A}	0.60±0.17^{A}	1.99±0.35^{B}	1.57±0.31^{B}	1.02±0.14^{B}	1.02±0.26^{AB}	1.57±0.31^{B}	1.30±0.35^{AB}
9	0.78±0.28^{A}	4.20±0.34^{B}	1.36±0.44^{A}	4.76±0.35^{B}	0.32±0.51^{A}	5.17±0.40^{B}	3.51±0.40^{C}	5.87±0.34^{B}
10	3.23±0.84^{B}	6.15±0.42^{C}	2.96±1.16^{B}	6.98±0.14^{C}	4.76±0.28^{B}	5.73±0.31^{B}	5.18±0.63^{B}	4.76±0.35^{B}

Values on the same row with different superscript vary significantly at p≤0.05, GMT: Geometric mean titre

between the marrow and tissues, where they are transformed into tissue macrophages[48,49]. They participate virtually in all inflammatory and immune disorders such as severe fungal, viral or bacterial infections for mopping up of the pathogens and necrotic debris[50,51]. A marked increase in circulating leukocytes was observed in exposed groups of birds following

exposure to the fungi contaminants isolated from the feed. The lymphoid tissues may have responded with leukocytosis (heterophilia, monocytosis, eosinophilia and lymphocytosis) due to tissue insults and hypersensitivity reactions caused by fungi infections and mycotoxins. In a disease condition, immune cells and humoral antibodies are heavily mobilized in the complex mechanisms of immune response where many are consumed in the process[52]. A sequential study in chickens after VNDV infection was made by Lam[53] who detected that the virus induced programmed cell death principally in mononuclear cells-monocytes and lymphocytes.

At the end of the study, exposed birds had significantly higher levels of serum albumin, serum globulin, and total serum proteins than controls. These effects still reflected in the overall performance of the birds. Previous authors reported decrease in total serum proteins (alpha, beta and gamma globulin) with IgG being more sensitive than IgM[11,54]. Some effects of mycotoxin exposure are direct, while others are indirect, such as reduced feed consumption[21,30].

The exposed chickens fed contaminated feed with mycotoxin binders displayed poor HI antibody titers by weeks 7-9 of age. This was below the protective threshold, 2^3 of antibody titer while higher antibody titers were observed in the chickens fed fresh feed at the same time, indicating that the immune system in chickens may have had rapid antibody decay in the presence of mycotoxins[11,55,56]. After revaccination at week 7, some groups (AK, BK, CK and DK) gained antibodies far above protective levels between weeks 8 and 9 of age[55]. Through the elevation of antibody titers, vaccination was beneficial for birds, especially those exposed to the virus. As a result of the field experience, antibody titers develop rapidly after vaccination, and they decay rapidly, short-term after vaccination. This is usually observed in flocks exposed to feed and milled without mycotoxin testing. There was a higher antigenic stimulation and response after vaccination and vaccinal virus, followed by a faster consumption of antibody substrates. Antibodies are developed in germinal centers containing memory cells specifically sensitized by antigen as suggested by Thomson[50], Nester *et al.*[57], Talaro[58] and Mehrzad *et al.*[49].

CONCLUSION

In this study, fungi infections caused elevated leukocyte counts and their series. Mycotoxins formed in the feed may have depleted iron reserves, especially after prolonged exposure. The study demonstrated that, looking at the overall results, antibody responses were not significantly boosted by Toxorid® and Zerotox®. Based on the results of the study, possible answers were found to the causes of incessant vaccine failures and poor antibody responses against Newcastle disease in Nigeria. A public health warning is issued regarding the danger of mycotoxins in both human and animal diets and the possibility of hidden losses due to poor feed quality and poor feed management in poultry production. The results exposed the effect of mycotoxins on hematology, blood chemistry and antibody responses of birds vaccinated against ND. Researchers, nutritionists, and farmers can use these results to determine the best approach to feed handling and feed detoxification.

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