Hematological and immunological effect on chicken exposed to aflatoxin

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Abstract

Chickens were exposed to aflatoxin sourced from rice substrate.Enzyme immunosorbent assay was used to evaluate the immune response to newcastle disease vaccinations vis a vis the hematological pictures. Results shows a dose dependent reduction in immune titer to newcastle disease.Hematological findings confirms leucopenia,throbocytopenia, with increased PCV and hemoglobin.Also increased is the heterophils which was masked by other lucocytes reduction. This hematological picture and immunosuppression is being linked to increased glucocorticoid in the subjects.

Keywords: Aflatoxin, Dose, Immunity, Newcastle, Titre.

Introduction

Studies on mycotoxin dates back to turkey poults deaths recorded in England due to consumption of contaminated groundnut meal imported from Brazil {Blout 1961}. Aflatoxin is acutely toxic mutagenic, carcinogenic.{Oguz hand kortoglu 2000}in addition aflatoxin has been implicated in immunosuppression.{Bondy 2000,Omigen 2004}.

The West African countries have tropical climates with all year round high ambient temperature and relative humidity that provides optimal condition for the growth of toxigenic moulds. The sub region has poorly developed infrastructures such as processing and storage facilities,transportation and skilled human resources.[Bankole and Adebayo 2003].

Aflatoxin has been fingered as a strong immunosuppuressive agent.[Omnigent research update 2004]. The complexity of the immune system implies that there are several methods by which immune function may be assayed.Simultaenous studies on heamotological and antibody profile of chicken subjected to aflatoxin exposure will reveal the interpay of cellualar and humoral factors in aflatoxin immunosuppression.

Materials and Methods

Aspergilus flavus La32g38,a toxigenic strain was procured from International Institute for Tropical Agriculture [IITA,pathology department].It was innoculated with rice substrate to produce crude aflatoxin according to the method of shotwell 1966. Quantification of aflatoxin was done with the aid of ROSA® Aflatoxin kit [quatitative]. The feed was supplemented with the rice substrate to give 50-100 ppb in the treatment diet.

Ninety day old chicks were used for the study. They were divided into three groups of thirty birds with three replicates, making ten birds per experimental unit.

They received experimental diets and water adlibitum for 42 days.The experimental groups include Control

2 basal diet plus 50 ppb afflatoxin ,in feed.

3 basal diet plus 100 ppb aflatoxin in feed.

Heamatology

Differential WBC counts were made on mono layer blood films and stained with Giemsa – wright's stain. Total red blood cell [TRBC] , Total white blood cell count were determined by manual method using hemacytometer [campbell 1995].Packed Cell Volume [PCV]was measured by a Capilllary tube technique using microhematocit capillary tubes centrifuged at 2500 rpm for 5min .Hemoglobin concentration was measured by cyanomethenoglobin method. [Ganti 2000] Erythrocyte indices, mean corpuscular volume [MCV) mean corpuscular heamoglobin.[MCH] and mean corpuscular hemoglobin concentration [MCHC] were calculated from TRBC, MCV and Hb. [Ritchie et al 1944].

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Table-1.

Group	PCV %	cell × 10^{12}/L RBC±SE	Cell×10^{6}/L plats ±SE	Cell×10^{6}/L TWBC±SE	Cell×10^{6}/L Cell×LYMP ±SE	Cell×10^{6}/L mono±SE	Cell×10^{6}/L MCHC±SE	Cell×10^{6}/L Eosin±SE	Cell×10^{6}/L Hetero±SE	G/gl HB ±SE
Control	24.7±1.0	2.0±0.23	102857±1463	16964±971.4	11778±714	391.29±56.0	32.7±0.42	392.86±51.3	4401.9±328.9	8.0±0.3
50ppb	23.1±1.08	2.0±0.23	138000±1463	15943±971.4	10.327±714	373.86±56.0	32.14±0.42	296.86±51.3	4934.9±328.9	7.6±0.3
100ppb	23.4±1.08	2.6±0.23	135000±14633	15557±971	10.09±714	204.14±56.0	32.14±0.42	276.71±51.3	5157.4±328.9	7.5±0.5

Serology.

Flockcheck Newcastel disease antibody test kit [Idexx Animal health] was used to provide a sensitive and specific method of antibody quantification. Statistical analysis.

All data generated were subjected analysis of variance [steel and toorie 1980] and means were separated by Duncan's multiple ranjge test. [Duncan 1995].

Results

Table-1 shows a dose dependent decrease in wbc, lymphocytes,monocytes,eosinophils,only decrease in wbc and lymphocytes is statistically significant.($p<0.05$).

Discussion

The blood picture of chicken subjected to low doses of aflatoxin 50ppb and 100ppb is sugestive of lymphopenia,monocytopenia,eosinopenia,and heterophilia.

The total white blood cells count suggested leucopenia.These observations were based on comparison with control which were statistically significant.[$p<0.05$]

The immunosuppressive effect of aflatoxin is well documented.This supports the reduced immune titre(newcastle disease) of chicken exposed to aflatoxin in a dose dependent pattern.It gives basis to the hypothesis that aflatoxins effect on immunity is both cell-mediated and humoral. Of significant scientific importance is the heterophila despite generalised lymphopenia.This work suggests that heterophils [neutrophils in other animals] are peculiarly sensiticed to aflatoxin intoxication as distinct from other granulocytes and lymphocytes. Dannis et al(2003) reported increased total WBC after aflatoxin dosing based on his work on rats which has higher number of neutrophils which thus probably masked reduced lymphocytes .

The increased heterophil in this work has been effectively masked by reduced lymphocytes with resultant genaralised leucopenia.In his works on wild turkeys poults,Quist et al(2000) observed increased PCV,WBC and lymphocyte.He claimed the increases in these parameters are either a direct or indirect effects of aflatoxin.

The numerical increase though not staistically significant in PCV,RBC and thrombocytes suggest an interplay of factors sequel to aflatoxin dosing. It is hypothesised sequel to this work, that the hematological picture following aflatoxin intoxication may not be a primary lesson but a secondary sequela to the general 'stressor' effects of a noxious chemical on the physiology of the animal. This becomes more evident, based on the fact that this hematology typifies glucocorticoid exposure.Glucocorticoids lowers the number of basophils in the circulation and increase the number of neutrophils,platlets and red blood cells.It also decreases the circulating lymphocyte count and the size of the lymph nodes and thymus by inhibiting lymphocyte mitotic activity.They also reduce secretion of cytokines by inhibiting the effect of NF-B on the nucleus.The reduced secretion of the cytokines K-2 leads to reduced proliferation of lymphocytes and these cells undergo apoptosis.(William 1990).The increased RBC,Hb and MCHC also correlate with stimulatory action of steriod on the renal erythropoietin secretion.(Bertram 1998).

It could be deduced from this study that hematological profile following aflatoxin intoxication may not be a primary lession but a secondary sequel a to the general "stressor" effect of noxious chemical on the physiology of the animal.We hypothesize that such continuous stressor stimuli of aflatoxin resulting

Table-2. Effect of 50ppb and 100 ppb aflatoxin [AF] on newscastel disease antibody titre for cockrels on day 1 to 42 days of age.

Control	50	100	Titer at day old	Titre at day 42
+	-	-	599±74	531±51.2
-	+	-	599±74	437±29.2
-	-	+	599±74	403.7±22.0

in immunosepression may form additional basis for aflatoxin carcinogenicity.

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