

HAEMATOLOGICAL RESPONSE OF BROILER CHICKENS TO BIO-CONTROL METHOD OF AFLATOXIN MITIGATION

*¹Ojo Olayinka Abosede , ²Ewuola Emmanuel Olubisi and ³Bitto I. Immanuel

¹Department of Animal Production, Fisheries and Aquaculture, Kwara State University, Nigeria; ²Department of Animal Science, University of Ibadan, Nigeria; ³Department of Animal Production, University of Agriculture, Makurdi, Nigeria.

Corresponding author:- ykmelodya1@yahoo.com

Aflatoxin is toxic, carcinogenic and ubiquitous in nature affecting both crops and livestock. Mitigating aflatoxin effect using toxin binders has not been very effective. Information on the use of biological methods in aflatoxin mitigation has not been adequately documented. Therefore, effect of aflatoxin bio-control method (Aflasafe) on growth indices, haematology and serum biochemistry of broiler chickens (BC) was investigated. One-day old Marshal BC (n=1020) were allotted to four treatments: Aflasafe maize-based diet (AMBD), farm feed (FF), aflatoxin-contaminated diet with toxin binder (ACDTB) and aflatoxin-contaminated diet without toxin binder (ACDWTB). The contaminated diets contained 306.3ppb aflatoxin and the experimental design was completely randomised with five replicates (n=255) per treatment for 8 weeks. Blood (5mL) was collected at 8th week to determine the erythrocytes, packed cell volume (PCV), Haemoglobin (Hb), eosinophil, basophil, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH). Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$. The highest value (28.08±0.61%) for PCV at the starter phase was obtained in birds on treatment 3 fed ACDTB, while the least value (27.16±0.34%) was recorded in birds on treatment 1 fed AMBD. Birds fed the FF+ toxin binder (T2) had the highest Hb value (9.11±0.17g/dl), while the birds fed AMBD had the least value (8.67±0.11g/dl). However, at the finisher phase, PCV of birds fed (T3) was significantly (P<0.05) higher (29.06±0.72%) than that of birds fed the AMBD (26.88±0.79%). The erythrocyte count (3.90±0.12x10⁶) of broiler chickens

fed the ACDWTB (T3) was observed to be significantly (P<0.05) higher than the values obtained in birds fed the AMBD (3.30±0.34x10⁶/l). The mean value recorded for eosinophils in birds fed the ACDWTB (2.00±1.2%) was significantly (P<0.05) lower than that of the birds fed the FF (3.16±1.72%) which contained toxin binder (Control diet). Basophil values (0.20±0.5%, 0.83±1.4% and 0.32±0.6%) of birds fed AMBD (T1), ACDTB (T3) and ACDWTB (T4) respectively were not significantly different from that of the control value (0.32±0.8%). Birds fed ACDTB (7.92±0.85%) had a significantly (P<0.05) lower MCV value compared with the value (8.95±0.98%) obtained from the control diet (FF+ Toxin binder).

Keywords: Aflatoxin-contaminated diet, Aflasafe, Aflatoxicosis, Broiler and chickens

Aflatoxin (AF), produced by *Aspergillus* species, has been established as been the most toxic, mutagenic and carcinogenic mycotoxins so far known (Williams *et al.*, 2009; Khan *et al.*, 2010). It has been noted to contaminate food and animal feeds worldwide, resulting into serious health problems and livestock production losses. The detrimental effect of aflatoxin on various animals has been well documented (Keyl and Booth, 1971; Zain, 2010). Aflatoxins affects poultry metabolism through reduction of the activities of enzymes that digest starch, proteins, lipids and decrease blood protein, total cholesterol, urea, also increases the activity of serum enzymes indicating liver damage (Aravind *et al.*, 2003). Different bio-control methods have been used to ameliorate the effect of

aflatoxin contamination in livestock, one of it involve the use of micro organisms. Many microorganisms have been documented to successfully degrade aflatoxins (Liu *et al.*, 2001 and Kasmani *et al.*, 2012) such as *Mycobacterium*, *Stenotrophomonas maltophilia*, *Trichoderma viride* etc. However, most of them were not allowed to be included in food or feed as a result of their ability to pose further health risks. This has prompted more attempts to develop novel bio-control method with biological mechanism which involves displacing toxigenic strains of *Aspergillus flavus* from agricultural fields with strains of *Aspergillus flavus* that do not produce aflatoxin (atoxigenic strains). Statistics have shown that the incidence of terminal diseases such as cancer, is on the increase in the world and especially in Nigeria. This might have resulted partly from the consumption of aflatoxin-contaminated products, which is one of the major world challenges accompanying food security as stated by the United Nations. Animal product contaminated with aflatoxins has been proven to suppress immune system and undermines human and animal health. According to Olafedehan *et al.* (2010) and Etim *et al.* (2014), the haematological evaluation of birds act as a pathological reflector of the health status of poultry exposed to various toxicants such as aflatoxin. This is because birds having good blood composition are likely to show good performance (Isaac *et al.*, 2013). The purpose of this study is to evaluate the influence of aflatoxin on the haematological parameters of broiler chickens exposed to aflatoxin contaminated diet and to investigate the efficacy of Aflasafe™ as a biological control of AF in broiler chickens.

MATERIALS AND METHODS

This study was carried out at the Obasanjo commercial broiler farm, located at Alomaja Town, off Lagos-Ibadan express way in the South-western part of Nigeria. Aflatoxin-contaminated maize grain and aflasafe maize grains used for this experiment were obtained from the plant pathology unit, International Institute of Tropical Agriculture, (IITA), Ibadan, Nigeria. Other

ingredients used for the feed formulated were purchased from God's Grace feed Mill located at Lagun Town, along Ibadan- Iwo road, where the study was conducted. Maize grain which was used as the aflatoxin carrier was inoculated with toxigenic strain of *Aspergillus flavus* of Nigerian origin. The culturing and inoculation was done at the plant pathology unit, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria using 5% V8 juice and 2% agar, having a PH 5.2 and a spore load of 2.475×10^6 per ml. Aflatoxins were quantified using scanning densitometer, CAMAG TLC scanner 3 with -CATS 1,4,2 software (Camag AG, Muttenz, Switzerland), (Suhagia *et al.*, 2006).

Experimental birds and management

A total number of 1020 one-day-old broiler chickens of Marshall breed were used for this study. The broiler chickens were randomly assigned to experimental pen /units at 255 birds per treatment, replicated five times with 51 birds per replicate. The experiment lasted for 8 weeks. The broiler chickens were housed in floor pen. The birds were housed in the broiler unit within the farm at an average temperature of 22°C, under 16 hours lightning. The vaccination and medication program for broiler and laying chickens were strictly adhered to. Birds were fed basal diets for 2 weeks after which they were fed the experimental diets and given fresh water provided *ad libitum* throughout the period of the experiment.

Experimental diets and aflatoxin concentration determination

Four (4) experimental diets were formulated based on the nutrient requirement of the broilers (Tables 1 and 2). The diets are as outlined below;

Treatment 1 (T1) – Aflasafe maize-based diet

Treatment 2 (T2) – Farm feed with toxin binders (Control diet)

Treatment 3 (T3) – Aflatoxin contaminated diet with toxin binder

Treatment 4 (T4) – Aflatoxin- contaminated diet without toxin binder.

Each diet was subjected to chemical analysis to obtain the proximate compositions (Tables 3 and 4) and aflatoxin concentration

TABLE 1: Composition of Broiler Starter Diet experimental diet (%)

INGREDIENTS	DIETS			
	AMBD	FF+Toxin binder	ACDTB	ACDWTB
Aflasafe Maize	56.100	0.000	0.000	0.000
Contaminated Maize	0.000	0.000	56.100	56.100
Normal Maize	0.000	56.100	0.000	0.000
Soyabean Meal	39.900	39.900	39.900	39.900
Bone Meal	2.190	2.190	2.190	2.190
Lime Stone	0.590	0.590	0.590	0.590
Lysine	0.059	0.059	0.059	0.059
Methionine	0.240	0.240	0.240	0.240
Salt	0.340	0.340	0.340	0.340
Mycofix	0.000	0.100	0.100	0.000
Enzymes	0.034	0.034	0.034	0.034
Starter Premix	0.240	0.240	0.240	0.240
Aivlosin	0.099	0.099	0.099	0.099
Selcon Forte	0.024	0.024	0.024	0.024
Vitamin C	0.019	0.019	0.019	0.019
Acidifier	0.099	0.099	0.099	0.099
Zinc bacitracin	0.005	0.005	0.005	0.005
Total	100.000	100.000	100.000	100.000
Calculated Nutrient				
Crude Protein (%):	23.74	23.74	23.74	23.74
Met. Energy (kcal/kg):	3005.48	3005.48	3005.48	3005.48
Crude Fibre (%):	3.76	3.76	3.76	3.76
Ether Extract (%):	2.85	2.85	2.85	2.85

Premix supplied per kg diet Vitamin A (15,000 IU), Vitamin D3 (3,000 IU), Vitamin E (30 IU), Vitamin K (2.5mg), thiamine (2.0mg), Riboflavin (6mg), Pyridoxine (4mg), Niacin (40mg), Cobalamin (0.02mg), Panthotenic acid (910mg), Folic acid (1.0mg), Biotin (0.08mg), Choline chloride (0.05mg), Manganese (0.096g), Zinc (0.6g), Iron (0.024g), Copper (0.006g), Iodine (0.0014g), Selenium (0.24mg), cobalt (0.006g) Iodine (0.0014g), Selenium (0.24mg), Cobalt (0.024mg), Antioxidant (0.125g). AMBD= Aflasafe maize-based diet, FF=Farm feed + Toxin binder, ACDTB =Aflatoxin-contaminated diet with toxin binder and ACDWTB = Aflatoxin-contaminated diet without toxin binder.

in experimental diets were quantified using analytical planar chromatography. Concentration of aflatoxin obtained in the broiler starter and finisher diets are; for AMBD 1.25µg/kg, FF+toxin binder 103.3µg/kg, ACDTB 306.3 µg/kg and ACDWTB 306.3 µg/kg.

Experimental design

All data obtained from the studies were subjected to descriptive statistics and one-way analysis of variance (ANOVA) in a completely randomized design using

statistical analysis software (SAS, 2008). Means were separated using Duncan multiple range test.

Blood collection

At the end of each phase of the experiment, (4th and 8th week), 20 birds were randomly selected from each treatment and blood sample was collected through the jugular vein. Blood collection was done early in the morning. Two (2) mls of blood was collected into heparinized bottles for laboratory analysis.

TABLE 2: Composition of the Broiler Finisher Diet experimental diet (%)

Ingredient	AMBD	FF+Toxin binder	ACDTB	ACDWTB
Aflasafe Maize	62.300	0.000	0.000	0.000
Contaminated Maize	0.000	0.000	62.300	62.300
Normal Maize	0.000	62.300	0.000	0.000
Soyabean Meal	33.700	33.700	33.700	33.700
Bone Meal	2.190	2.190	2.190	2.190
Lime Stone	0.600	0.600	0.600	0.600
Lysine	0.060	0.060	0.060	0.060
Methionine	0.250	0.250	0.250	0.250
Salt	0.350	0.350	0.350	0.350
Mycofix	0.000	0.100	0.100	0.000
Enzymes	0.035	0.035	0.035	0.035
Finisher premix	0.249	0.249	0.249	0.249
Selcon Forte	0.025	0.025	0.025	0.025
Vitamin C	0.025	0.025	0.025	0.025
Acidifier	0.099	0.099	0.099	0.099
Zinc bacitracin	0.005	0.005	0.005	0.005
Total	100.000	100.000	100.000	100.000
Calculated Nutrient				
Crude Protein (%):	21.72	21.72	21.72	21.72
Metabolizable Energy (kcal/kg):	3137.28	3137.28	3137.28	3137.28
Crude Fibre (%):	3.53	3.53	3.53	3.53
Ether Extract (%):	3.11	3.11	3.11	3.10

Premix supplied per kg diet Vitamin A (15,000 IU), Vitamin D3 (3,000 IU), Vitamin E (30 IU), Vitamin K (2.5mg), thiamine (2.0mg), Riboflavin (6mg), Pyridoxine (4mg), Niacin (40mg), Cobalamin (0.02mg), Panthotenic acid (910mg), Folic acid (1.0mg), Biotin (0.08mg), Choline chloride (0.05mg), Manganese (0.096g), Zinc (0.6g), Iron (0.024g), Copper (0.006g), Iodine (0.0014g), Selenium (0.24mg), cobalt (0.006g) Iodine (0.0014g), Selenium (0.24mg), Cobalt (0.024mg), Antioxidant (0.125g). AMBD= Aflasafe maize-based diet, FF=Farm feed + Toxin binder, ACDTB =Aflatoxin-contaminated diet with toxin binder and ACDWTB = Aflatoxin-contaminated diet without toxin binder.

RESULTS AND DISCUSSION

Proximate composition of broiler chicken starter and finisher experimental diets.

The proximate composition of broiler chicken starter and finisher diets are shown in tables 3 and 4. The result of the proximate composition indicated that the metabolizable energy (kcal/kg) of the AMBD (3221.12 kcal/kg) in the starter diet, containing no toxin binder is higher than that of the control (FF+ toxin binder) (3188.60 kcal/kg). Metabolizable energy of the finisher diet followed the same trend with that of starter diet. The crude protein content (20.51%) of

AMBD evidently has the highest value across the treatments in both the starter and finisher diets. This is an indication that Aflasafe maize-based diet (AMBD) containing no toxin binder was higher in quality compared to other feeds. There was a difference in the Ash content of all the feeds with the Farm feed + toxin binder (9.5%) having the higher Ash content. The Crude fibre content observed in ACDTB (4.75%) of starter diet was the highest while the least value was recorded in ACDWTB as observed in both starter and finisher diets. The highest ether extract value (7.5%) was recorded in AMBD and ACDWTB, while

Table 3: PROXIMATE COMPOSITION OF BROILER STARTER DIET

Composition	AMBD	FF+Toxin binder	ACDTB	ACDWTB
Met energy(kcal/kg)	3221.12	3188.6	3369.38	3243.42
Crude protein	20.51	18.38	16.97	17.68
Ash (%)	8.5	9.5	4.5	7.1
Crude fibre	4.5	4.25	4.75	4.0
Ether extract	7.5	7.0	7.5	7.5
Dry matter	89.97	90.46	89.79	90.21

Table 4: PROXIMATE COMPOSITION OF BROILER FINISHER DIET

Composition	AMBD	FF+Toxin binder	ACDTB	ACDWTB
Met energy(kcal/kg)	3466.52	3413.55	3470.08	3529.72
Crude protein	20.51	18.38	16.97	17.68
Ash (%)	8.50	9.50	4.50	7.10
Crude fibre	4.5	4.25	4.75	4.00
Ether extract	7.50	7.00	7.50	7.50
Dry matter	89.97	90.48	89.79	90.21

Met energy- Metabolizable energy AMBD= Aflatoxin- free diet FF+toxin binder =Farm feed +toxin binder ACDTB= Aflatoxin contaminated diet with toxin binder ACDWTB=Aflatoxin contaminated diet without toxin binder.

the lowest value was observed in FF+ Toxin binder (7.0 %). The ether extract values obtained for the broiler finisher diet was observed to follow the same trend as those in the starter diet. The proximate composition indicated that the four feeds contained slightly high dry matter with the control diet (FF+Toxin binder) having the higher dry matter content (table 3) in both starter and finisher diets.

Haematological response of Broilers to experimental diets at starter phase

The result on haematological response of broilers to experimental diets at starter phase is shown in Table 5. All the haematological parameters evaluated at the starter phase of broiler chickens were not significantly different across the treatments. The highest value ($28.08 \pm 0.61\%$) for PCV was obtained in birds on treatment 3 fed ACDTB, while the least value ($27.16 \pm 0.34\%$) was recorded in birds on treatment 1 fed AMBD. It was also observed that the birds fed the FF+ toxin binder (T2) had the highest Hb value ($9.11 \pm 0.17\text{g/dl}$), while the birds fed AMBD

had the least value ($8.67 \pm 0.11\text{g/dl}$). Mean values obtained for erythrocyte count were similar among the treatment with the higher value recorded in birds fed the ACDTB (T3). Haematological picture of an animal act as a pathological reflector of the status of exposed animals to various toxicants (Olafedehan *et al.*, 2010; Etim *et al.*, 2014). Haematological value is a measured of the ability of an animal to withstand some level of respiratory stress (Sainsbury, 1983; Kana *et al.*, 2014). Animals having good blood composition are likely to show good performance (Isaac *et al.*, 2013). An observed increase in the red blood cell count is usually associated with low quality of consumed feeds. At the starter phase of this present study, all haematological parameters evaluated were not significantly influenced by the dietary treatments. This result corroborate the findings of Bara (2008) who observed no significant difference in hemoglobin content and PCV between the coturnix fed different levels of aflatoxin. Also Alo *et al.* (2009) observed no significant difference in the PCV, RBC and

Table 5: Haematological response of Broilers to experimental diets at starter phase

Parameters	T1	T2	T3	T4
Packed cell volume (%)	27.16±0.34	27.92±0.45	28.08±0.61	28.04±0.43
Haemoglobin (g/dl)	8.67±0.11	9.11±0.17	8.96±0.21	8.99±0.15
Erythrocytes(x10 ⁶ /l)	3.35±0.10	3.52±0.12	3.62±0.10	3.60±0.14
Leukocytes(x10 ³ /l)	21.04±0.86	21.20±0.86	20.64±0.87	20.66±0.71
Platelets (x 10 ³ /l)	148.88±10.86	146.24±9.01	161.48±12.08	163.04±10.51
Lymphocytes (%)	68.12±1.95	64.60±2.04	64.72±1.98	63.44±1.20
Heterophils (%)	26.16±1.71	28.96±1.82	28.72±1.90	31.56±2.04
Monocytes (%)	3.24±0.30	3.20±0.37	3.20±0.29	3.12±0.28
Eosinophils (%)	2.44±0.37	3.36±0.31	2.96±0.29	3.00±0.30
Basophils (%)	0.28±0.11	0.28±0.12	0.20±0.10	0.32±0.15
MCV (fL)	8.24±0.20	8.07±0.19	7.86±0.23	8.01±0.24
MCH (pg/cell)	2.63±0.07	2.64±0.07	2.51±0.05	2.60±0.11
MCHC (g/dL)	0.32±0.02	0.34±0.02	0.32±0.01	0.32±0.01

AMBD= Aflatoxin- free diet FF+toxin binder = Farm feed +toxin binder ACDTB= Aflatoxin contaminated diet with toxin binder ACDWTB=Aflatoxin contaminated diet without toxin binder. MCV=Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration.

thrombocyte values of chickens exposed to dietary aflatoxin. According to Rawal *et al.* (2010), fish and poultry are extremely sensitive to AFB1 but among poultry species, chickens are considered relatively resistant (Lazano and Diaz, 2006). Perhaps, since broiler chickens grow fast and the aflatoxin exposure period was very short (4 weeks), the insidious effects of dietary aflatoxin could not be elicited on blood the parameters. The degree of aflatoxicosis depend on the doses of aflatoxin ingested, duration of exposure, physiological status of the animal disease factor that impact on the uptake, biotransformation, deposition and excretion of aflatoxin (Bryden, 2012).

Haematological response of Broilers to experimental diets at finisher phase

The result on haematological response of broilers fed experimental diets at finisher phase is shown in Table 6. Among the haematological parameters assessed, it was observed that, the Packed Cell Volume (PCV), Haemoglobin (HB), erythrocyte (RBC), eosinophil, basophil and Mean

Corpuscular Volume (MCV), values were significant among the treatments. The value obtained for Packed Cell Volume (PCV) in birds fed aflatoxin-contaminated diet with toxin binder (T3) was significantly ($P<0.05$) higher ($29.06\pm 0.72\%$) than that of birds fed the AMBD ($26.88\pm 0.79\%$), although, all PCV values recorded across the treatment are within the normal range (22.0-35.0%) for poultry. Mean Corpuscular Volume (MCV) were significantly ($P<0.05$) different among the treatment. A similar trend was also observed in the value obtained for haemoglobin. The erythrocyte count ($3.90\pm 0.12 \times 10^6$) of broiler chickens fed the aflatoxin-contaminated diet with toxin binder (T3) was observed to be significantly ($P<0.05$) higher than the values obtained in birds fed the aflasafe maize-based diet ($3.30\pm 0.34 \times 10^6/l$) and fell within the normal physiological range of 2.20-4.50 $\times 10^6$ reported for poultry. The level of oxygen in the tissues of an animal has been documented to govern the hemopoietic activity of the bone marrow and aflatoxin has been shown to cause mild to marked

Table 6: Haematological response of Broilers to experimental diets at finisher phase

Parameters	AMBD	FF+Toxin Binder	ACDTB	ACDWTB
Packed cell vol. (%)	26.88±0.79 ^b	27.72±0.57 ^{ab}	29.04±0.72 ^a	27.92±1.02 ^{ab}
Haemoglobin (g/dl)	8.70±0.31 ^b	8.99±0.31 ^{ab}	9.60±0.12 ^a	8.96±0.65 ^{ab}
Erythrocytes (x10 ⁶)	3.30±0.34 ^b	3.22±0.17 ^b	3.90±0.12 ^a	3.53±0.24 ^{ab}
Leukocytes (x10 ³ /1mm ³)	20.00±6.82	20.00±6.45	20.53±6.52	20.60±0.79
Platelets (x 10 ³ /L)	136.96±30.55	139.24±33.01	144.83±47.32	153.76±50.50
Lymphocytes (%)	62.36±9.61	63.32±12.35	66.25±10.50	65.84±8.91
Heterophils (%)	32.72±9.38	30.12±13.12	29.13±10.67	29.52±8.91
Monocytes (%)	2.40±1.26	2.88±1.66	2.86±1.54	2.72±1.28
Eosinophils (%)	2.40±1.55 ^{ab}	3.16±1.72 ^a	2.95±1.9 ^a	2.00±1.2 ^b
Basophils (%)	0.20±0.5 ^b	0.32±0.8 ^{ab}	0.83±1.4 ^a	0.32±0.6 ^{ab}
MCV (fL)	8.35±0.98 ^{ab}	8.95±0.98 ^a	7.92±0.85 ^b	8.12±1.9 ^{ab}
MCH(pg/cell)	2.70±0.33	2.90±0.35	2.59±0.26	2.60±0.57
MCHC (g/dL)	0.32±0.02	0.33±0.01	0.32±0.02	0.32±0.04

abc: Means along the same row but with different superscripts are significantly (P<0.05) different. AMBD= Aflatoxin- free diet FF+toxin binder =Farm feed +toxin binder ACDTB= Aflatoxin contaminated diet with toxin binder ACDWTB=Aflatoxin contaminated diet without toxin binder. MCV =Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration.

congestion in lungs of broilers (Rathod et al., 2013) causing hypoxia. In response to this, the erythropoietic stimulating factor from the plasma might have resulted into the stimulation of the bone marrow to produce an increased amount of red blood cells into the blood which continued since the cause of the hypoxic state was not eliminated throughout the experimental period. This might have enhanced significant increase in the PCV as well as a concomitant increase observed in the values of hemoglobin and RBC values of birds fed ACDTB. Platelets functions in reducing loss of blood from injuries by adhering to the wall of the blood vessels and to other cells in the area of the injury. In this study, it was observed that platelet value which was higher in broilers fed ACDWTB may be attributed to possible injurious effect of dietary aflatoxin on the walls of the blood vessels which might have resulted into an increase in the platelete count. Leucocytes are important component

of cellular defence in the body. An increase in total leukocyte count usually indicates that infection or cancer of the leukocytes-producing-tissues is present. In this study, leucocyte counts are observed to be similar across the treatment. The basophil count of birds fed AMBD was observed to be low and birds fed ACDTB was recorded to be higher than the FF+ toxin binder, however, the basophil function in clearing the fat from plasma. Its cytoplasmic granules contain heparin which is an anticoagulant that moderates inflammatory micro environment by inhibiting the formation of fibrin in clotting. Perhaps, the increase in the basophil value of birds fed ACDTB indicated that there was an inflammatory micro environment in the birds fed the diet, resulting into the necessity of heparin release to effect moderation while the low basophil count in birds fed AMBD indicated a normal micro environment without a need for moderation. It has been documented that

basophilia occurs only in conjunction with eosinophilia which was observed in broiler chickens fed FF+toxin binder and ACDTB diets. The mean value recorded for eosinophils in birds fed the aflatoxin-contaminated diet without toxin binder ($2.00 \pm 1.2\%$) was observed to be significantly ($P < 0.05$) lower than that of the birds fed the farm feed ($3.16 \pm 1.72\%$) contained toxin binder (Control diet). However, the eosinophil value of broiler chickens on treatments 1, 2 and 3 were not significantly different from one another. The low eosinophil value of birds fed AMBD and FF+toxin binder was within normal physiological limits of eosinophil, while the mean value obtained for birds fed ACDTBW was observed to be higher compared to that of control (FF+toxin binder) and exceeds the normal physiological limit of eosinophils in poultry (0.78-2.48%) known as eosinophilia. Eosinophils is a fraction of leukocyte which functions in detoxification and are mobilized at the antigen-antibody reaction site in an animal. A decrease in its value is an indication of a stressful physiological condition of the broiler chickens under study. The Mean corpuscular volume (MCV) value of birds fed the AMBD was observed to be significantly higher compared to that of ACDTB. The result of this study agrees with the findings of Jiang et al. (2014) who reported a numerical increase in the MCV value of broiler chickens fed naturally aflatoxin-contaminated diet.

CONCLUSION

Aflatoxin adversely affects the haematology of birds and can result into anaemia another blood related diseases. Alfasafe maize-based diet can be used without causing adverse effects on poultry, it is therefore recommended as a bio-control method for ameliorating aflatoxin.

REFERENCES

1. Alo O. S., Oyeboji O. and Abatan O. M. (2009). Hematological and immunological effect on chicken exposed to aflatoxin. *Veterinary World*, Vol.2 (1): 5-7.
2. AOAC (1990): Official methods of analysis, 15th ed. Association of official Analytical chemist, Washington D.C. pp. 69-88.
3. Aravind K. L., Patil, V.S., Devegowda G., Umakantha B., and Ganpule S. P. (2003). Efficacy of modified glucamannan to counteract mycotoxicosis in naturally contaminated feed on performance, serum biochemistry and hematological parameters in broilers. *Poultry Science* 2003; 82: 570-6.
4. Bara B. (2005). The effects of aflatoxin appearance in the feed stuffs upon the poultry production. *Ecotoxicologie, zootehnie S I tehnologh de industrie alimentara*, Vol. V1, Anul 6, 2008.
5. Bryden W.L. 2012. Mycotoxin contamination of the feed supply chain: I mplications for animal productivity and feed security. *Anim. Feed Sci. Technol.*, 173(1-2): 134-158.
6. Etim N. N., Williams M. E., Akpabio, U. and Offiong E. E. A (2014). Haematological parameters and factors affecting their values. *Agric. Sci.*, 2(1): 37-47.
7. Isaac L. J., Abah G, Akpan B and Ekaette I. U. (2013). Haematological properties of different breeds and sexes of rabbits. *Proceedings of the 18th Annual conference of Anim.Sci. Assoc. Nig.*, p. 24-27.
8. Kasmani F. B., Krimi M. A. T., Allameh A. and Shariatmadari F., (2012). A novel aflatoxin-binding Bacillus probiotic: Performance, serum biochemistry and immunological parameters in Japanese quail. *Poultry Science* (2012) 91 (8): 1846-1853. Doi:10.3382/ps.2011-01830.
9. Khan W. A., Khan, M. Z., Khan, A. and Hussain, I. (2010). Pathological effects of aflatoxin and their amelioration by vitamin E in White Leghorn layers. *Pak. Vet. J.*30 (3):155-162.

10. Keyl, A. C. and Booth A. N. (1971). Aflatoxin effects in livestock. *J. Amer. Oil Chem. Soc.* 48 :599-604.
11. Liu, D.L., Yao, D.S., Liang, Y.Q., T.H., Zhou, Y.P., Song, Zhao, L. and Ma, L. (2001). Production, purification and characterization of an intracellular aflatoxin-detoxification from *Armillarella tuberseens* (E-20). *Food Chem. Toxicol* 39:161-166.
12. Olafedehan C.O., Obun A. M., Yusuf M. K., Adewumi O. O, Olafedehan A.O., Awofolaji, A. O. and Adeniji, A. A. (2010). Effects of residual cyanide in processed cassava peel meals on haematological and biochemical indices of growing rabbits. *Proceedings of 35th Annu. Conf. Nig. Soc. Anim. Prod.*, p. 212.
13. Rathod P. R., Kulkarni, G.B and Gangane, G. (2013). Pathological effect of low grade aflatoxicity in Broilers. *The bioscan* 8(3): 1115-1118, 2013 (supplement on Toxicology).
14. Rawal S., Kim J. E. and Coulombe J. R. (2010). Aflatoxin B1 in poultry: Toxicology, metabolism and prevention. *Res.Vet. Sci.*, 89:325-331.
15. Sainsbury D. (1983). *Animal health*. 1st Edition. Granada Publishers Ltd. New York.
16. SAS/STAT.2008. SAS user's guide:Statistics released version 9.2. Statistical Analysis System Institute. Inc: Cary, North Carolina USA, 133.
17. Suhagia B. N., Shah, S. A., Rathod I. S., Patel H. M., Shah, D. R, and Marolia B. P (2006). Determination of gatifloxacin and ornidazole in tablet dosage forms by high-performance thin-layer chromatography. *Anal Sci.* 2006;22 (5):743–745. doi: 10.2116/analsci.22.743.
18. Williams D. E. G. Orner K. D. Williard S. Tilton and J. D. Hendricks. (2009). Ranibow trout (*Oncorhynchus mykiss*) and ultra-low dose cancer studies. *Comp.Biochem. Physiol. C*, 149: 175-181. PMID : 19135172
19. Zain M. E. (2010). Impact of mycotoxins on humans and animals. Original Article *Journal of Saudi Chemical Society* (2011) 15, 129-144.