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Research Article

Mitigation effect of aflatoxin on serum biochemical indices of broiler chickens

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Abstract: Aflatoxin has been known to be toxic to both crops and livestock. Several methods to mitigate aflatoxin effect have been employed. Knowledge on use of organic methods in aflatoxin mitigation has not been adequately documented. Therefore, effect of aflatoxin bio-control method (Aflasafe) on 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 experimental design was completely randomised with five replicates (n=255) per treatment for 8 weeks. Blood (5mL) was collected at 8th week and serum was separated and stored for further analysis. Total protein (TP) value of birds fed ACDTWB was significantly lower compared to those of AMBD and FF at the starter and finisher phases. The highest TP values were observed in birds fed AMBD at starter (3.04±0.25g/dl) and finisher (3.41±0.14g/dl) phases. Birds fed AMBD (T1) had highest albumin value (1.39±0.05g/dl).), while birds fed ACDWTB was least (1.26±0.00g/dl).Use of AMBD in poultry is recommended as it has no adverse effect on chickens.

Keyword: Aflasafe, Broiler Chickens, Haematology, Serum, Aflatoxin-Contaminated Diet

1. INTRODUCTION

Research from the past few decades has illustrated the negative effects of AF on poultry performance**.** It has been noted to contaminate food and animal feeds worldwide, resulting into serious health problems and livestock production losses. According to Leesons *et al*. (1991), aflactoxicosis in domesticated birds causes poor bird performance, decreased feed intake growth rate, poor feed utilization and mortality (Tedesco *et al*., 2004; Shi *et al*., 2006) metabolism failure, coccidiosis susceptibility (Hamilton, 1971), compromised immune competence (Coulumbe, 1994; Turner *et al*., 2000), reduction in the activity of enzymes that digest starch, proteins, lipids and nucleic acids, increase the activity of serum enzymes that indicate liver damage (Ellis *et al.,* 1991; Aravind *et al*., 2003), decrease blood proteins, total cholesterol and urea, as well as inhibition of lipid transport in chickens according to Osborne and Hamilton (1981). The most important effect of aflatoxin is the inhibition of protein synthesis, causing a marked reduction in the level of plasma protein, mainly albumin and globulin (Espada *et al*., 1997). Activities of serum or plasma enzymes such as aspartate amino-transferases (AST) has been used to measure aflatoxin toxicity in chickens (Tessari *et al*., 2010). The productive deterioration has been shown to be associated with changes in biochemical and hematological parameters (Denli *et al.,* 2004; Bintvihok & Kositcharoenkul, 2006). Several methods have been investigated to prevent or reduce the slivestock feed in order to bind the aflatoxins and prevent its absorption in animal gastrointestinal tract (Ramos and Hernadez, 1996). Some of these include the use of enteroabsorbants which are additives such as

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calcium aluminosilicate (HSCAS) that can selectively bind aflatoxin B1 without depleting micronutrients, and are widely used in animal feeds (Williams *et al.*, 2004). These limitations have resulted into the development of a recent biological control method involving the use of Aflasafe™ by International Institute of Tropical Agriculture (IITA), which is a mixture of four strains of *Aspergillusflavus* of Nigerian origin, allowed to internally colonize the test ingredient, sorghum grain. This study is designed to evaluate the influence of aflatoxin on the serum biochemical 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.

2. MATERIALS AND METHODS

This study was carried out at 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 x 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).

2.1 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 four (4) experimental pens at 255 birds per treatment, replicated five times with 51 birds per replicate. The experiment lasted for 56 days. The broiler chickens were housed in floor pen, in the broiler unit within the farm at an average temperature of 22^{0}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 *ad libitum,* throughout the period of the experiment.

Four (4) experimental diets were formulated based on the nutrient requirement of the broilers as shown in Tables 1 and 2. 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, and 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 in experimental diets was quantified using analytical planar chromatography. Concentration of aflatoxin obtained in the broiler starter and finisher diets for AMBD is 1.25µg/kg, FF+toxin binder 103.3µg/kg, ACDTB 306.3 µg/kg and ACDWTB 306.3 µg/kg.

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. Three (3) mls of blood was collected into anticoagulant-free bottles for serum biochemical analysis early in the morning. The tubes were kept in slanting position and allowed to clot. The blood samples were spun at 3000 rpm for 10 minutes.Serum samples were then separated into sterile tubes for analysis.

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.

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
MetabolizableEnergy (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.

2.3 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.

3. RESULTS

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
NFE	48.96	51.33	56.07	53.93

Met energy- Metabolizable energy, 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.

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
NFE	48.96	51.33	56.07	53.93

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

Serum Biochemical Indices of Broiler Chickens to Aflatoxin-Contaminated Diets

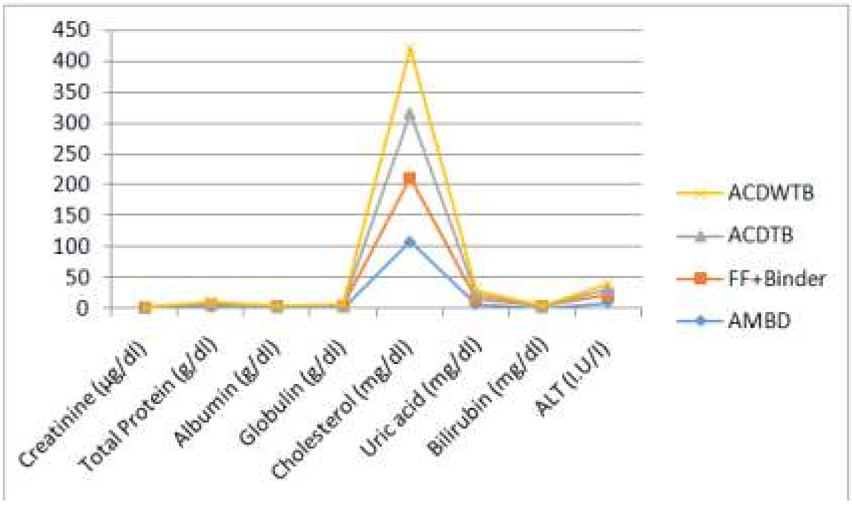

Figure1: Serum Biochemical Response of Broilers to Experimental Starter Diets

ab - means on the same rows but with different superscripts are significant (P<0.05). ALT =Alanine amino transferase. AMBD =Aflasafe maize-based diet, FF +Toxin binder= Farm feed + toxin binder, ACDTB= Aflatoxin contaminated diet + toxin binder, ACDWTB= Aflatoxin contaminated diet without toxin binder

Table 6: Serum Biochemical Response of Broilers to Experimental Finisher Diets

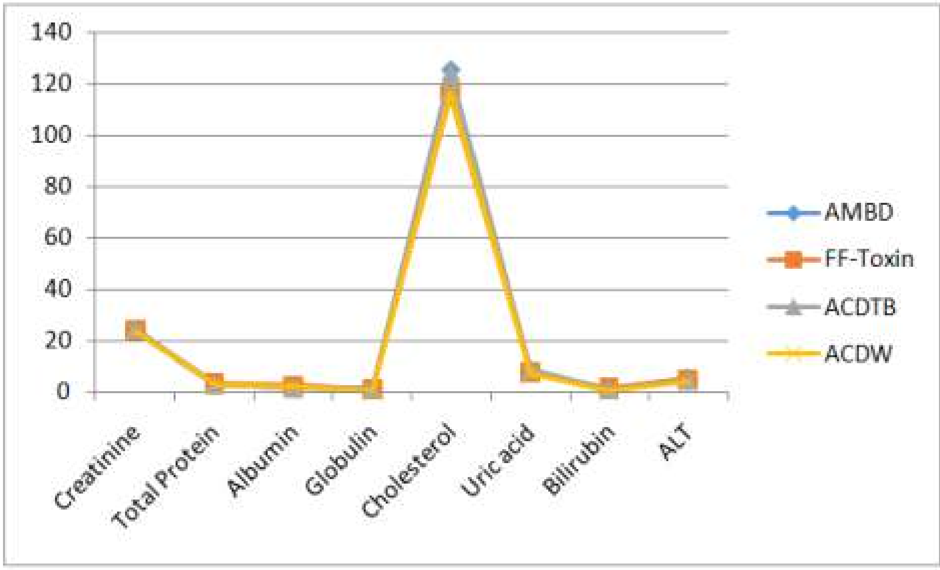

Figure 2: Serum biochemical response of broilers to experimental finisher diets

a, b, Means along the same row with different superscripts are significantly different (p<0.05)AMBD =Aflasafe maize-based diet, FF+toxin binder= Farm feed + toxin binder, ACDTB= Aflatoxin-contaminated diet + toxin binder, ACDWTB= Aflatoxin-contaminated diet without toxin binder.

3.1 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 significantly different (P<0.05) value observed 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 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.

3.2 Serum Biochemical Response of Broilers to Experimental Diets at Starter and Finisher Phases

The result of serum biochemical response of broilers fed the experimental diets at starter and the finisher phases are shown in figures 1 and 2. The purple, green, red and blue lines, which represented the ACDWTB, ACDTB, FF + Binder, and AMBD respectively peaked at the cholesterol values, both the starter and finisher phases. Result showed that there were significant effects (p<0.05) of aflatoxin (the test ingredient) on total protein and bilirubin of the chicken. However, there was no significant effect (p.0.05) of the aflatoxin on albumin, globulin creatinine, cholesterol, Alanine aminitransferase (ALT) and uric acid. At the starter phase, the total protein value (2.56±0.12g/dl) obtained in birds fed the aflatoxin-contaminated diet without toxin binder was observed to be significantly (P< 0.05) lower than the value recorded in birds fed the control diet (FF+toxin binder). However, the total protein of broilers on AMBD was higher than that of ACDTB (2.75±0.11g/dl) and ACDWTB (2.56±0.12g/dl). At the finisher phase, it was observed that the total protein value (2.94±0.09 g/dl) of the broiler chickens fed the aflatoxin-contaminated diet with toxin binder was significantly (P<0.05) lower than the control value (3.33±0.11g/dl). However, the total protein of birds fed AMBD (3.41±0.14) was higher than those of FF + toxin binder (3.33±0.11) and ACDWTB (3.02±0.11), although the mean values were not significantly (P<0.05) different. Serum albumin value recorded in the experimental birds ranged from 1.59±0.04g/dl to 2.50±0.49g/dl with the highest value obtained in birds fed control diet at the finisher phase of the experiment. However, it was observed that at the starter phase, the highest albumin value (1.39±0.05g/dl) was observed in birds fed AMBD and the least value was observed in birds fed ACDWTB (1.26±0.0g/dl). At the starter phase, the mean values recorded for globulin ranged from 1.30±0.12g/dl to 1.80±0.24g/dl with the highest value obtained in birds fed the FF+Toxin binder and the least value in birds fed ACDWTB. It was observed that broilers fed the Farm Feed + toxin binder had the highest level of bilirubin which was significantly (P< 0.05) different from that of birds fed AMBD, ACDTB and ACDWTB diets at both starter and finisher phases. The bilirubin values 1.08±0.08 and 0.84±0.19mg/dl obtained in birds fed aflatoxin-contaminated diet without toxin binder at the starter and finisher phases, were significantly (P<0.05) lower than those of the control values (FF+ toxin binder) 1.45± 0.06 and1.71±0.31mg/dl.

4. DISCUSSION

Total protein is an indication of protein digestion, absorption and metabolism. It is an estimation of the nutritive state of the birds and helps to monitor how the diet is being utilized, reflecting alterations in metabolism. The total protein value of birds fed AMBD and FF were observed to be significantly higher than that of ACDWTB. This could be as a result of the diet (AMBD) fed to birds in treatment 1 and FF (treatment 2), which had a higher crude protein values when compared to that of ACDTWB fed to the birds in treatment 4, containing a higher level of contamination of aflatoxin. This could have perhaps induced the inhibition of RNA and DNA synthesis. This could have consequently impaired protein synthesis and reduced serum total protein. The result of this study is in agreement with the results of Kubena *et al.* (1990) who observed a decrease in the serum total protein and albumin in broilers fed aflatoxin contaminated diet as well as Nazar *et al*. (2012), who also observed a significant reduction in serum total protein concentration and albumin in the plasma of Japanese quails exposed to aflatoxin contamination in their diet. However, the result of this total protein disagrees with that of Choi *et al*. (1995), who observed no direct influence of the total blood protein by the dietary treatment. According to Makki *et al.* (2014), addition of 500 ppb of AFB_1 to the diet of broilers significantly decreased direct bilirubin, while he noticed a significant increase in the total bilirubin of birds fed the experimental diet.

5. CONCLUSION AND RECOMMENDATION

Based on the results of this study, Aflatoxin influenced the total protein values across the treatments, although the values recorded were within the normal range of total protein for birds. The values of the serum biochemical parameters observed showed that the level of aflatoxin in the diets 3 and 4 did not significantly alter majority of the values analysed. Alfasafe maize-based diet can be used without causing any effects on poultry. It is therefore recommended that further studies can be conducted to test the ameliorative effect of bio-control methods on aflatoxin contaminated diets in poultry.

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