Mycopath (2017) 15(2): 55-59 **Research Article**

Effect of levels of equisetin and fumonisin mycotoxins on blood parameters of broiler chicks

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Abstract

This study was carried out to assess the effect of equisetin and fumonisin mycotoxins on the blood parameters of broiler chicks. A total of 280 day old broiler chicks (Abor Acre) were acquired from a commercial hatchery in Ibadan, Oyo State, Nigeria for this study. The chicks were randomly assigned to 7 treatments combinations in a completely randomized design. Treatment A had equisetin mycotoxin in three levels of concentration (25, 50 and 100%), treatment B had fumonisin also with three levels of concentration (25, 50 and 100%), while treatment C was the control. Each treatment concentration was replicated twice and the mycotoxin was administered at 20 birds per replicate. One milliliter sterile syringes were used per treatment to inject 0.1 mL solution of the 2 toxins intravenously through the wing web of the broiler chicks on days 8, 10, 12 and 14. On day 15, 5 mL sterile syringes were used to collect 4 mL of blood samples from the birds for haematological and biochemical analysis. Data collected were subjected to analysis of variance and treatment means were separated using Duncan multiple range test. With the exception of white blood cell count (WBC) and platelet (PLT), all other parameters measured for haematological studies showed no significant variation. This could mean that the broiler chicks had slow response to the toxin effect. However, the higher WBC value (89.44-98.73 µL) in birds administered mycotoxin, with the exception of A50, relative to the control (85.47 µL) indicated the presence of toxin in the birds' blood stream. Results obtained on serum biochemical analyses showed a significant variation ($P<0.05$) on all the parameters measured. The ALT (4.10-8.30 IU L^{-1}) and AST (46.90-123.30 mg dL^{-1} values obtained from experimental chicks were significantly higher than that of the control (2.50 IU L^{-1}, 38.10 mg dL^{-1}. Chicks exposed to A50 mycotoxin concentration had the highest creatinine value which an indication of kidney impairment. It is concluded that the exposure of broiler birds to mycotoxin has a negative effect on blood parameters and the functionality of internal organs of the birds. Feed ingredients should, therefore, be properly dried and stored under conducive condition to prevent mold growth and feed quality control measures should be established at feed mills.

Keywords: Biochemical analysis, Broiler chicks, Equisetin, Fumonisin, Haematology, Mycotoxins.

Introduction

Mycotoxins are a group of metabolites produced by molds which grow on agricultural commodities under certain conditions. They are biologically active secondary fungal metabolites found as contaminants of feedstuffs that exert toxic effects on animals and people (Fink-Gremmels, 1999; Tiemann *et al.*, 2014). Agricultural commodities are vulnerable to mold damage during pre as well as post-harvest stages of production. Crops can be contaminated with mold in the field, during harvest, or during storage, processing, or feeding (Jacob, 2015). Mycotoxin contamination of foods and feeds is a world problem which has been attracting global attention in view of their health, economic and political considerations. Several animal diseases have been associated, either directly or indirectly with mycotoxins and there is increasing interest in the biological effects of these toxins in feedstuffs. Livestock feeds especially poultry are formulated and compounded using cereal grains as ingredients. Frequently, microbial contaminants of raw materials are never evaluated before purchase or compounding them into poultry feeds (Adejoro, 1991). Because of the susceptibility of these grains to mycotoxins, poultry birds after ingesting the grains are prone to mycotoxicosis.

Mycotoxicosis is one of the major causes of many unknown disease problems leading to economic losses in poultry as it affects body weight gain, egg production and causes mortality of birds (Mohiuddin, 2007). The effects of mycotoxins in poultry feed depend on the specific mycotoxin or mycotoxins present, the level of contamination, the length of time the animal has been consuming the mycotoxin(s), the animal's age, sex, and level of stress (Jacob, 2015). Hundreds of mycotoxins have been identified, and many are pathogenic. Mycotoxins may have additive or synergistic effects with other natural toxins, infectious agents, and nutritional deficiencies. Many are chemically stable

and maintain toxicity over time. The significance of mycotoxin problems in poultry is probably considerable but yet insidious (Frederic, 2016). Though the most obvious effect of mycotoxins on poultry production is mortality because this can be readily diagnosed and quantified, the chronic effects of mycotoxins which can pose the most problems to poultry producers include feed refusals, bruising, hemorrhaging and poor growth, residues in meat and eggs, degeneration of internal organs and reproductive failure (reduction in fertility and hatchability) (Scott, 2014; Frederic, 2016).

Although Nigeria does not have standard regulations on mycotoxins in poultry feed yet, the risk of mycotoxins exist in the Nigerian poultry sector since the common feed ingredients such as maize and groundnut cake are known to contain high levels of mycotoxins (Kpodo and Bankole, 2008). According to Ezekiel *et al*. (2013) about 50% of groundnut cake and stored maize in Nigeria had co-occurrence of more than one of 10 mycotoxins. The study of mycobiota in Nigeria is not yet robust, however literatures showed that lot of researches had been carried out in Brazil (Rosa *et al*., 2006), Argentina (Dalcero *et al*., 1997) and Spain (Accensi *et al*., 2004). Nigeria, a tropical country with attendant high temperature and humidity, harbors large number of heterogeneous filamentous fungi which exists heterotropically (Brandh *et al*., 2012). Some of these have been implicated in both animal and human illness and death due to the production of mycotoxins (Fapohunda *et al*., 2012).

Due to the impact of mycotoxins on the productivity and health status of animals, this research was carried out to study the effects of equisetin and fumonisin mycotoxins on the haematological and serum biochemical indices of broiler chicks.

Materials and Methods

Site of the study

The research was conducted at Babcock University, Ilishan-Remo, Ogun State, Nigeria. Ilishan is in the south west geo-political zone of Nigeria and falls on latitude of $6^0$54'N of the equator and longitude $3^0$42'E of Greenwich.

Chicks housing and management

A total of two hundred and eighty (280) day old broiler chicks (Abor Acre) were purchased from a commercial hatchery in Ibadan, Oyo State, Nigeria for this study. The broiler chicks were brooded in movable pens which had adequate air ventilation. Each pen was properly covered on all sides with polythene nylon to conserve heat generated by the 200 watts electric bulbs fixed in each pen. Vitalyte® anti-stress was mixed with the drinking water of the birds for the first three days of their arrival. The chicks were acclimatized for one week and then randomly assigned to 7 treatments combinations in a completely randomized design. Each treatment was replicated twice with 20 birds per replicate. The occasional management carried out was vaccination and supply of multivitamin supplement. Chicks were fed with commercial broiler starter and adequate quantity of water and feed was supplied *ad libitum* throughout the experiment.

Experimental treatments and mode of administration

Treatment A was Equisetin with three levels of concentration (25, 50 and 100%), treatment B was Fumonisin also with three levels of concentration (25, 50 and 100%) while treatment C was the control. One millilitre sterile syringes were used per treatment to inject 0.1 mL solution of the 2 toxins intravenously through the wing web of the broiler chicks on days 8, 10, 12 and 14.

Blood collection

On day 15^{th}, 5 mL sterile syringes were used to collect 4 mL blood samples from 5 birds per replicate through the jugular vein. The blood samples collected were transferred into EDTA bottles and non-heparinized bottles at 2 mL per bottle for haematological and biochemical analysis respectively. Analysis carried out on haematological studies were packed cell volume (PCV), hemoglobin (HGB), white blood counts (WBC), lymphocyte (LYM), Neutrophil (NEU) and platelets (PLT), while Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), urea and creatinine were analyzed for biochemical studies. ALT, ALP activities were determined with the use of spectrophotometric methods as described by Rej and Holder (1983).

Statistical analysis

Data collected for hematological and biochemical studies were subjected to analysis of variance (ANOVA) (SAS, 1999), and treatment means were separated using Duncan multiple range test (Steel and Torries, 1980).

Results and Discussion

Haematological studies

Results obtained on the haematological parameters are shown in Table 1. Significant variations ($P<0.05$) only occurred in the WBC and the PLT. All other parameters measured across the different treatments had values not significantly different ($P> 0.05$) from each other.

Packed Cell Volume (PCV) is the percentage of red blood cells in blood and it is an index of toxicity reduction in the blood. Presence of toxic factor has adverse effect on blood formation (Oyawoye and Ogunkunle, 1998) and a low PCV

suggests anaemia (Ashom *et al.*, 2016). The PCV values were within the normal range of 25-45% as reported by Mitruka and Rawnsley (1977). This indicates that fumonisin and equisetin had no significant effect (P>0.05) on the PCV of broiler chicks. This agrees with the findings of Sadagopan (2007) who reported that in quail chicks, the PCV and erythrocyte were not affected by dietary aflatoxin levels. Haemoglobin (HBG) is the tool for the transportation of oxygen and carbon dioxide in and out of the body respectively. HGB values were within normal range of 7.0-13.0% as reported by Ross *et al.* (1978). Results of this study also supported Sadagopan (2007) findings who reported that hemoglobin content, PCV, RBC count and serum protein content were not influenced by tested levels of aflatoxin in quail chicks.

White blood cells (WBC) or leukocytes are cells of the immune system involved in defending the body against both infection and foreign materials. The slightly higher levels of WBC, with the exception of A50, in chicks injected with aflatoxin to the control chicks are an indication of the presence of a toxin. This supports the findings of Mohiudddin *et al*. (1993) who reported that increase in total WBC counts suggests the effect of eliciting inflammatory response to mycotoxins. Lymphocytes (LYM) are made up of cells which destroy pathogens. They are largely responsible for the development of specific immunity against invading microbes. Compared to the control, there was no significant (P>0.05) difference between treatments in group A and treatments in group B. This indicates that mycotoxins did not significantly affect the immune system of the chicks. It could also mean that the immune response to the toxins was slow since blood collection was done a day after administration of the final dose of the toxins. According to Jacob (2015), the effects of mycotoxins in poultry feed depend on the specific mycotoxin or mycotoxins present, the level of contamination and the length of time the animal has been exposed to the mycotoxins.

Neutrophils (NEU) are responsible for providing the body with a defense against invading micro-organism (Frandson, 1981). Though the neutrophil levels in chicks exposed to mycotoxins were numerically higher than that of control, there was no significant difference among the treatments. This may also be an indication of a slow response to the toxins. Platelets (PLT) are small irregularly shaped clear cell fragments which function in blood coagulation. With the exception A50 and B100, the other values were slightly lower than the control. Low platelets indicate deficiency in clot retraction which is characterized by easy bruisability and multiple subcutaneous haemorrhage (Shareef, 2009).

Biochemical analysis

Results of serum biochemical analysis are presented in Table 2. Serum biochemical parameters such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities provide a sensitive and specific measure of hepatic function or injury (Abbes *et al*., 2006). Values reported for AST and ALT across the treatments were significantly higher (P<0.05) than the control. There was a direct relationship between the level of concentration of equisetin (A), fumonisin (B) and the ALP values obtained with the exception of A100. Though the urea and creatinine values obtained for all the treatment did not follow a particular trend, significant variations (P<0.05) also exists among them.

The increase in the concentration of AST and ALT in birds exposed to mycotoxins indicated a malfunctioning of liver probably to detoxify the mycotoxins. This supports the finding of Sharma *et al.* (2008) who reported an increase in serum AST activity in chicks fed with fumonisin B1 of moniliformin. Similar results were observed in other studies (Shi *et al*., 2006; Gowda *et al*., 2008) and this suggests that mycotoxins exert a direct toxic effect on birds' liver. Krishnamoorthy *et al.* (2006) also reported that increased AST and ALT values might be attributed to liver damage in toxin fed birds.

ALP-a part of the numerous liver enzymes-increases as flow of bile into the liver reduces (Kristiina, 2008). Significant differences were observed in values reported for ALP and treatments A100 and B25 had ALP values higher than the control. This indicated hepatic disorders and it agrees with the findings of Gelderblom *et al.* (1988) who reported that serum ALP activity increases in birds with hepatic disorders and biliary obstruction. Serum ALP activity has been described by Kubena *et al*. (1997) as a valuable parameter to detect hepatic injury and malfunction. Coles (1986) and Harrison *et al.* (1986) in their studies with rats, horses and pigs reported that serum ALP increased significantly following exposure to fumonisin in feeds.

According to Nworgu *et al.* (2007) kidney malfunction may raise the level of blood urea. The significantly high urea value in birds injected treatment B100 in relation to the control group, is an indication of renal malfunction as a result of high level of injected toxin. Creatinine is derived from creatine and creatine phosphate in muscle tissue and may be defined as a nitrogenous waste product. An elevation of plasma creatinine is an indication of under-excretion of the kidney which suggests kidney impairment. Results obtained in this study showed that effect of mycotoxins on creatinine was not consistent. This may be due to the time frame between blood collection and when creatinine was analyzed, as creatinine was the last parameter to be analyzed.

Conclusion

Fumonisin and equisetin were toxic to the chicks as indicated by PCV, WBC, serum ALT,

AST, ALP, urea and creatinine values obtained. It is, therefore, recommended that feed ingredients should be properly dried and stored under conducive conditions to prevent mold growth. Prevention of mycotoxins should be based mainly on the use of mycotoxin-free feeds and feed ingredients and on management practices that can prevent growth of molds and the formation of mycotoxins during feed transport and storage. Also, regular inspection of stored feeds and animal feeding systems could also be done. Furthermore, quality control measures should be in place at feed mills; and the various raw materials used in compounding feed should be analyzed for the presence of mycotoxins. Further studies involving longer period of observation of chicks administered with the mycotoxins is also recommended.

Table 1: Effect of fumonisin and equisetin on hematological parameters of broiler chicks.

TRTS	PCV (%)	HGB (g dL^{-1})	WBC × 10^3 (µL)	LYM (%)	NEU (%)	PLT (10^4 µL)
A_{100}	29.83±9.24	9.93±3.07	96.05±9.91^{c}	98.83±0.41	1.17±0.41	6.50±3.67^{a}
A_{50}	30.50±4.72	10.17±1.56	73.41±2.16^{a}	97.10±3.00	2.90±30	11.20±3.39^{b}
A_{25}	32.70±5.56	10.90±1.85	90.34±1.67^{c}	95.90±5.74	4.10±5.74	8.10±3.35^{ab}
B_{100}	31.80±2.39	10.60±0.80	98.73±3.39^{c}	97.30±2.98	2.70±2.98	9.10±3.64^{ab}
B_{50}	30.90±2.13	10.30±0.70	95.48±9.38^{c}	96.30±4.67	3.70±4.67	8.20±3.23^{ab}
B_{25}	31.80±2.70	10.59±0.91	89.44±1.66^{b}	98.60±0.52	1.40±0.52	7.70±2.00^{ab}
C	32.20±4.78	10.72±1.58	85.47±2.49^{b}	98.40±0.52	1.60±0.52	8.30±4.62^{ab}

Means in the same column with different superscripts are significantly (P<0.05) different. Treatments (TRTS), Packed cell volume (PCV), hemoglobin (HGB), white blood counts (WBC), lymphocyte (LYM), Neutrophil (NEU), platelets (PLT).

Table 2: Effect of fumonisin and equisetin on serum biochemical parameters of broiler chicks.

Treatments	ALT(IU/l)	AST(mg dL^{-1})	ALP (mg dL^{-1})	UREA (mg dL^{-1})	CREATININE
A_{100}	5.40±1.07^{c}	46.90±1.66^{b}	719.14±41.22^{a}	0.33±0.11^{a}	4.85±0.52^{e}
A_{50}	8.30±0.95^{f}	98.70±6.17^{f}	1319.85±23.95^{e}	0.44±0.06^{b}	3.10±0.28^{bc}
A_{25}	4.10±0.88^{b}	123.30±8.41^{g}	1077.24±27.37^{d}	0.74±0.08^{c}	3.47±0.47^{cd}
B_{100}	5.20±1.23^{c}	74.40±4.22^{e}	941.08±36.66^{c}	1.32±0.11^{e}	4.20±0.54^{de}
B_{50}	7.00±1.25^{e}	67.60±3.06^{d}	903.66±29.26^{bc}	0.70±0.09^{c}	2.98±0.35^{a}
B_{25}	6.10±0.74^{d}	54.50±8.55^{c}	745.02±35.07^{a}	0.95±0.11^{d}	3.15±0.30^{bc}
C	2.50±0.71^{a}	38.10±2.73^{a}	895.95±50.07^{b}	0.45±0.07^{b}	3.80±0.50^{d}

Means in the same column with different superscripts are significantly (P≤0.05) different. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphate (ALP).

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