ASSESSMENT OF THE EFFICACY OF BENTONITE-MONTMORILLONITE BINDER AS BIOSYSTEMIC SEQUESTERING AGENT AT HIGH DIETARY AFLATOXIN LOAD IN TURKEYS POULTS.

A. S. Oyegunwa[1], E. O. Ewuola[2], A. F. Agboola[2], E. A. Iyayi[2]

[1]*Department of Agricultural Science, Tai Solarin University of Education, Ijagun Ijebu Ode Nigeria.*
[2]*Department of Animal Science, University of Ibadan, Ibadan Nigeria*
Author for correspondence: oyegunwaas@tasued.edu.ng; Tel:+2348066484845

ABSTRACT:

Aflatoxin has been implicated in the aetiology of many diseases in poultry and turkey is considered one of the most susceptible. The use of clay mineral has been reported to improve performance of broiler chickens during aflatoxicosis. However, information on the mitigation of aflatoxicosis in turkey using clay has not been adequately documented. Therefore, the use of Bentonite-montmorillonite binder (BB) to mitigate aflatoxicosis in turkey was investigated. Eighty 21-d-old Nicholas turkey poults were randomly assigned to five treatments of four replicates with each replicate having four poults. The experimental diets were: positive control (PC) with no aflatoxin or BB, negative control (NC) with 200ppb of aflatoxin, NC + 0.2% BB, NC + 0.4% BB and NC + 0.6% BB. The experiment was observed for 21 days during which necessary poultry management practices were observed. Data on feed intake, body weight gain, feed conversion ratio and mortality were collected as performance indicators while 0.5ml blood samples were collected via jugular venopuncture for serum biochemical analysis. Aflatoxin significantly reduced feed intake and body weight gain in poults that were treated with aflatoxin and BB. Mortalities were 0%, 56%, 44%, 56% and 38% for diets 1, 2, 3, 4 and 5 respectively. Serum protein and albumin were also reduced significantly in poults that received aflatoxin alone and with binder. In conclusion, there was no significant effect of inclusion of bentonite-montmorillonite binder up to 0.6% on the response criteria measured in this study.

Key words: Aflatoxicosis, turkey poults, bentonite-montmorillonite

INTRODUCTION

Aflatoxin is a class of mycotoxin produced by 2 fungi species, *Aspergillus flavus* and *Aspergillus parasiticus*. This mycotoxin has been implicated in the aetiology of many diseases in poultry like listlessness, anorexia, low growth rate, poor feed utilization, decreased egg production and increased mortality (Miazzo *et al*., 2000). In addition to these, anaemia (Oguz *et al*., 2000), reduction of immune function (Oguz *et al*., 2003), hepatotoxicosis, haemorrhage (Ortatatli and Oguz, 2001), are associated with aflatoxicosis.

The use of mycotoxin binders to ameliorate the effect of aflatoxicosis in turkey and other poultry species has become very important owing to the fact that it is practically impossible to have diets that is completely free from aflatoxin contamination (Oyegunwa *et al*., (2015). Bentonite is a natural clay that comes from volcanic ash (Walz *et al*., 1998). Because of its properties and accessibility, bentonite is widely used as a feed additive. It helps to eliminate aflatoxins, cadmium and radiocaesium, and ameliorate food allergies, mucus colitis, spastic colitis, viral infections, such as stomach flu, and parasites that are unable to reproduce in the presence of the clay (Walz *et al*., 1998; Santurio *et al*., 1999;). The basis for the use of bentonite refers to earlier information that bentonite has strong adsorptive powers and its role in binding aflatoxin in the gastrointestinal tract. Studies have shown that the use of sodium bentonite in broiler chickens diet would improve their weight gain (Tauquir *et al*., 2001; Prvulovic *et al*., 2008). Salari *et al*. (2006) indicated that chickens fed diets containing 1 and 2% sodium bentonite consumed more feed, had more weight gain and less feed conversion ratio. Pasha et al. (2008) reported birds fed diets containing sodium bentonite treated with either 0.5% or 1.0% acetic acid significantly increased protein efficiency ratio and protein digestibility, as compared to the control.

The main objective of this study is to assess the efficacy of graded levels of bentonite-montmorillonite binder to ameliorate the effect of dietary aflatoxin in turkey poults.

MATERIALS AND METHODS

Birds and experimental design

Eighty 21-d-old turkey poults were tagged, weighed and randomly allotted to five dietary treatments. Each treatment had 4 replicate and 4 birds per replicate. Treatment 1 was the positive control diet without aflatoxin or BB, treatment 2 was the negative control diet with 200ppb of aflatoxin but with no binder, treatments 3, 4 and 5 were the negative control diets with 0.2, 0.4 and 0.6%BB respectively.

Table 1: composition (g/100g) of turkey poults starter diets

Ingredients	Diet 1 (PC)	Diet 2 (NC)	Diet 3 (NC+0.2%BB)	Diet 4 (NC+0.4%BB)	Diet 5 (NC+0.6%BB)
maize	52.40	32.40	32.40	32.40	32.40
Contaminated Maize	-	20.00	20.00	20.00	20.00
Soybean meal	40.00	40.00	40.00	40.00	40.00
Fish Meal (72%)	5.00	5.00	5.00	5.00	5.00
Dicalcuim Phosphate	1.20	1.20	1.20	1.20	1.20
Limestone	1.00	1.00	1.00	1.00	1.00
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25	0.25
*Vit/mineral premix	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis					
Crude Protein (%)	28.44	28.44	28.44	28.44	28.44
*ME (kcal/kg)	3021.37	3021.37	3021.37	3021.37	3021.37
Crude Fibre (%)	3.90	3.90	3.90	3.90	3.90
Calcium (%)	0.92	0.92	0.92	0.92	0.92
Phosphorus	**0.55**	**0.55**	**0.55**	**0.55**	**0.55**

PC = positive control without aflatoxin or binder , NC = negative control with 200ppb aflatoxin *1kg premix contains: Vitamin A – 13340 I.U; Vitamin D3 – 2680 I.U; Vitamin E – 10 I.U.; Vitamin K – 2.68mg; Calcium pantothenate – 10.68mg; Vitamin B12 – 0.022mg; Folic acid – 0.668mg; Choline chloride – 400mg; Chlorotetracycline – 26.68mg; Manganese – 13mg; Iron – 66.68mg; Zinc – 53.34mg; Copper – 3.2mg; Iodine –

Production of aflatoxin and quantification

Aflatoxin was produced from the pure culture of *Aspergillus flavus* N3228. The culture was grown on maize by the method of Shotwell *et al*. (1966). The maize grains were subsequently sundried for 1 week to stop further growth of the mould. The toxin produced in the maize was quantified using the thin layer chromatographic method (Krska *et al*., 2007) to determine its concentration before mixing with the finished feed.

Diet preparation

The corn-based diets used in this study was formulated based on the nutritional requirements recommended by the NRC (1994) with CP adjusted to 28.44% and ME at 3021.37kcal/kg. The culture material with 1000ppb of total aflatoxin was added to each ration to reach the desired aflatoxin concentration in the diet (Table 1). Ration and water were provided *ad libitum*.

Data collection and statistical analysis

Feed intake was obtained by subtracting the leftover feed from the quantity servedand body weight gain for each replicate group was estimated at day 21 using standard procedure while feed conversion ratio was calculated as the ratio of feed intake to weight gain. On day 21 of the experiment, blood samples were obtained from the jugular vein of two poults per replicate at day 21. Blood samples were collected in bottles and allowed to stand for 30 minutes while serum is separated for biochemical analyses. The serum total protein of the turkey poults was determined following the method described by Kohn and Allen (1995) using Randox^{R} kits. The serum albumin was determined using Bromocresol Green (BCG) method as described by Peters et al. (1982).

Data obtained were analyzed using ANOVA in SAS (SAS, 1999) as completely randomized and means were separated using Duncan multiple range test of the same software.

Results and discussion

In this study, values obtained for feed intake and body weight were statistically similar in birds that were fed with diets 2 to 5 compared with birds on the positive control diet which was higher (Table 2). Mortality was also high in diets 2 to 5 (56, 44, 56 and 38% respectively). Results herein may indicate the failure of bentonite-montmorillonite to ameliorate the toxic effect of 200ppb aflatoxin on the turkeys.

Table 2: Performance of turkey poults fed dietary aflatoxin and bentonite-montmorillonite binder.

Parameters	Diet 1 PC	Diet 2 NC	Diet 3 (NC+0.2% BB)	Diet 4 (NC+0.4% BB)	Diet 5 (NC+0.6% BB)	SEM
Initial weight (g/poult)	232.25	231.50	206.75	230.75	231.00	0.01
Final weight (g/poult)	699.75^{a}	470.50^{b}	353.25^{b}	441.75^{b}	329.25^{b}	38.78
Weight gain (g/poult)	467.50^{a}	239.00^{b}	146.50^{b}	211.00^{b}	98.25^{b}	39.75
Feed intake (g/poult)	1187.80^{a}	734.50^{bc}	681.50^{bc}	528.30^{c}	769.00^{b}	64.45
Feed conversion ratio	2.58	6.35	5.83	2.58	9.10	1.83
Mortality (%)	0.00	56.00	44.00	56.00	38.00	

^{a,b,c} means on the same row with different superscript are significantly different (P<0.05)

PC = positive control without aflatoxin or binder, NC = negative control with 200ppb aflatoxin

Reduced feed intake as seen in turkeys fed with aflatoxin may be associated with injury in the gastrointestinal tract caused by the aflatoxin. Another factor that may have caused reduced feed intake in turkeys fed with aflatoxin-treated diets is low palatability as a result of the presence of the toxin. Feed consumption and body weight gain were significantly reduced in diets 2, 3, 4 and 5. This results contrast to the findings of (Rosa *et al*., 2001; Aravind *et al*., 2003; Miazzo et al., 2005; Kermanshahi *et al*., 2009) which reported that adding Sodium bentonite and yeast glucomannan to the diet is effective in reducing aflatoxin toxicity in terms of growth performance, haematological, serum biochemical and microscopic-histopathological analyses. Similarly, Aidin *et al*. (2011) also found that addition of 0.15% Na-bentonite to diets containing 250ppb of aflatoxin was effective in improving the feed intake and body weight gain of broiler chicks compared to broilers that were fed with aflatoxin without binders.

The effect of dietary supplementation of aflatoxin B1 on serum biochemical parameters in this study shows that serum total protein and albumin were significantly reduced in turkey poults fed with diets 2, 3 4 and 5 (Table 3). This again may indicate failure of bentonite-montmorillonite to counteract the effect of aflatoxin. Serum total protein and albumin in positive control diet were significantly higher than other diets that were treated with aflatoxin with or without binder The reduced levels of total protein and albumin in aflatoxin treated birds were indicative of the toxic effect of aflatoxin B1 on hepatic and renal tissues and are consistent with previous literature reporting aflatoxicosis (Kubena *et al*., 1993; Tejeda-Castaneda *et al*., 2008). The addition of BB was not significantly effective in the protection against aflatoxin B1 in this study as reflected in the decreased serum protein and albumin observed in diets supplemented with bentonite-montmorillonite. The result obtain in this study contrasts with previous reports by Kubena *et al*. (1990), Ledoux *et al*. (1999) and Gowda *et al*. (2008) who recorded improved serum parameters in broilers fed with Aflatoxin diets supplemented with binders. Wafaa *et al*. (2013) also reported that the addition of HSCAS and tumeric powder as supplement in turkey diet improved the serum protein and general performance of turkeys that were fed with 2.5ppm of aflatoxin.

Table 3: Serum parameters of turkey poults fed dietary aflatoxin and bentonite-montmorillonite binder.

Parameters	Diet 1 PC	Diet 2 NC	Diet 3 (NC+0.2% BB)	Diet 4 (NC+0.4% BB)	Diet 5 (NC+0.6% BB)	SEM
Total protein (g/dl)	5.34^{a}	3.75^{bc}	4.10^{b}	3.10^{c}	3.50b^{c}	0.17
Albumin (g/dl)	3.42^{a}	1.95^{b}	2.67^{ab}	1.40^{b}	1.86^{b}	0.24
Globulin (mg/dl)	1.92	1.80	1.43	1.70	1.64	0.14
AST (iu/l)	178.40	191.50	189.33	185.00	194.60	15.34
ALT (iu/l)	42.80^{a}	40.00^{ab}	31.67^{bc}	43.00^{a}	25.80^{c}	1.95

PC = positive control without aflatoxin or binder, NC = negative control with 200ppb aflatoxin
^{a,b,c} means on the same row with different superscript are significantly different.
AST = aspertate aminotransferase, ALT = alanine aminotransferase
The values of alanine aminotransferase (ALT) in diets 1, 2 and 4 were similar (42.80, 40.00 & 43.000) but significantly higher than the value in diet 3 and 5 (31.67 & 25.80). Elevated serum ALT in diets 1, 2 and 4 may not suggestive of liver damage since diet 1 which is the control diet was free of aflatoxin.

Although the ameliorative effect of bentonite has been studied in broilers (Bailey e*t al*., 2006) and rats (Abdel-Wahhab *et al*., 1998) with remarkable improvement in performance, information on the use of bentonite montmorillonite in turkey is still scanty (Oyegunwa et al., 2015). It has been reported that not all binders will bind aflatoxin because it may take multiple electrical sites to hold the aflatoxin molecule or because the dosage of binder necessary for it to work is very high. It could also be that the level of inclusion of the aflatoxin is too high to be sequestered by the binder. This may be the reason for the failure of the BB to mitigate the effect of aflatoxin.

CONCLUSION

It can be concluded from this study based on our findings that bentonite-montmorillonite binder at levels of 0.2, 0.4 and 0.6% could not ameliorate the negative effect of aflatoxin on turkey poults.

REFERENCES

Abdel-Wahhab, M. A., Hassan, A.M., Aly, S.E., Mahrous, K.F. 2003. Adsorption of sterigmatocystin by montmorillonite and inhibition of its genotoxicity in the Nile tilapia fish (Oreachromis niloticus). Mutation Research, 582, 20-27.

Aravind K.L., V.S. Patil, G Devegowda, B. Umakantha, and S.P. Ganpule (2003) Efficacy of esterified glucomannan to counteract mycotoxicosis in naturally contaminated feed on performance and serum biochemical and haematological parameters in broiler. Poultry science 82:571-576.

Bailey, C. A. Latimer, G.W., Barr, A.C., Wigle, W.L., Haq, A.U., Balthrop, J.E., Kubena, L. F., 2006. Efficacy of montmorillonite clay (Nova Sil Plus) for protecting full term broiler from aflatoxicosis. J. Appl. Poult. Res. 15:198-206. Abstract/Free full text.

Gowda, N.K.S. Ledoux, D.R. Rottinghaus, G.E., Bermudez, A.J., Chen, Y.C., 2008. Efficacy of tumeric (curcuma longa), containing a known level of curcumin, and a hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of Aflatoxin in broiler chicks. Poult. Sci. 87:1125-1130.

Kubena, L. F., Harvey, R. B., Huff, W. E., Corrier, D. E., Phillips, T. D. and Rottinghaus, G. E. 1990. Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. Poult. Sci. 69:1078–1086.

Kubena, L. F., Harvey, R. B., Phillips, T.D., Clement, B. A.1993. Effects of hydrated sodium calcium aluminosilicate on aflatoxicosis in broiler chicks. Poultry Science, 72, 651-657.

Kermanshahi, H., A.R. Hazegh and N. Afzali, 2009. Effect of sodium bentonite in broiler chickens fed diets contaminated with Aflatoxin B1 J.Anim. Vet. Adv.8:1631 – 1636.

Krska, R. Welzig, E. and Boudra, H (2007). Analysis of fusarium toxins in feed. Animal Feed Sci. Technol. 137:241-264.

Ledoux, D. R., Rottinghaus, G. E., Bermudez, A. J., Alonso-Debolt, M. 1999. Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poult. Sci 78:204–210.

Miazzo, R., Peralta, M.F., Magnoli, C., Salvano, M., Ferrero, S, Chiacchiera, S.M., Carvalho, E. C. Q., Rosa, C. A. Dalcero, A. 2005. Efficacy of sodium bentonite as a detoxifier of broiler feed contaminated with aflatoxin and fumonisin. Poult. Sci. 84:1–8.

Miazzo, R., Rosa, R. C. A. Carvalho De Queiroz, E. C., Magnoli, C., Chiacchiera, S., Palacio, M. G., Saenz, M., Kikot, A., Basaldella, E, Dalcero, A. 2000. Efficacy of synthetic zeolite to reduce the toxicity of aflatoxin in broiler chicks. Poult. Sci. 79:1–6.

NRC, 1994. National Research Council: Nutrient Requirement of Poultry, 9th Edition. National Academy Press, Washington DC.

Oguz, H., Kecceci, T., Birdane, Y. O., Onder, F., Kurtoglu, V., 2000 . Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during experimental aflatoxicosis. Res. Vet. Science, 69; 89-93.

Oguz, H., Hadimli, H. H., Kurtoglu, V., Erganis, O. 2003. Evaluation of humoral immunity of broilers during chronic aflatoxin (50 and 100ppb) and clinoptilolite exposure. Rev. Med. Vet., 154, 483-486.

Ortatatli, M. and Oguz, H. 2001. Ameliorative effects of dietary clinoptilolite on pathological changes in broiler chickens during aflatoxicosis. Res. Vet. Sci., 71, 59-66.

Oyegunwa, A. S. 2015. Alleviation of the effect of dietary aflatoxin with yeast or bentonite-montmorillonite binder. Ph.D Thesis, Department of Animal Science, University of Ibadan

Pasha T.N. Mahmood A, Khattak F.M., Jabbar M. nad Khan A.D., 2008. The effect of feed supplemented with different sodium bentonite treatmsnts on broiler performance. Turkish Journal of Veterinary and Animal Sciences, 32, 245-248.

Prvulovic D., Kojic D. Grubor-Lajsic G. and Kosarcic, S. 2008. The effect of dietary inclusion of hydrated aluminosilicate on performance and biochemical parameters of broiler chickens. Turkish Journal of Veterinary and Animal Sciences, 32:183-189

Rosa, C.A., Miazzo, R., Magnoli, C., Salvano, M., Chiac, S.M., Ferrero, S., Saenz, M., Carvalho, E.C., Dalcero, A. 2001. Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of Aflatoxin in broilers. Poultry Sci.., 80, 139-144.

Salari, S.H, Kermanshahi and M.H. Nasiri, 2006. Effect of sodium bentonite and comparison of pellet vs mash on performance of broiler chickens. Int. J. Poult Sci., 5:31-34.

SAS Institute, 2009. SAS/STAT User's guide statistics, version 9.1, edition. SAS Institute Inc. Cary, North Caroilina.

Santurio J.M., Mallmann C.A., Rosa A.P., Appel G., Heer A., Dageforde S. and Bottcher M., 1999. Effect of sodium bentonite on the performance and blood variables of broiler chickens intoxicated with aflatoxins. Bristish Poultry Science. 40:115-119.

Shotwell, O., Hesseltine, L. C. W., Stubblefield, R. D. 1966. Production of aflatoxin on rice. Appl. Microbiol. 15425.

Tanqir, N.A. G.I. Sultan and H. Nawaz, 2001. Effect of different levels of bentonite with varying energy levels on the performance of broilers. Int. J. Agric Biol., 3:85-88.

Tejada-Castaneda, Z.I., Avila-Gonzalez, E., Casaubon-Huguenin, M.T., Cervantes-Olivares, R.A., Vasquez-Pelaez, C., Hernandez-Baumgarten, E.M., Moreno-Martinez, E. 2008. Biodetoxification of aflatoxin-contaminated chick feed. Poultry Science, 87, 1569-1576.

Walz L.S., T.W. White, J.M. Fernandez, L.R. Gentry, D.C. Blouin, M.A. Froetche, T.F. Brown, C.J. Lupton and A. M. Chapa, 1998. Effects of fish-meal and sodium bentonite on daily gain, wool growth, carcass characteristics of lambs fed concentrate diets. J.Anim. sci., 76:2025-2031.

Wafaa .A.E., Mahmoud Essam E.H., 2013 Evaluation of the efficacy of feed additives to counteract the toxic effects of aflatoxicosis in broiler chickens. Research Journal of Poultry Sciences 6(1): 1-12, 2013.