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ASSESSMENT OF THE EFFICACY OF BENTONITE-MONTMORILLONITE BINDER OR YEAST TO ALLEVIATE EFFECT OF AFLATOXIN IN TURKEY POULTS

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ABSTRACT: In a 28-day feeding trial, the alleviation of the effect of aflatoxicosis in turkey was investigated by feeding 2 concentrations of yeast and bentonite-montmorillonite binders (BB) in diets contaminated with aflatoxin. One hundred and ninety two 21-day-old turkey poults were randomly allotted to six experimental diets in a completely randomized design (CRD) as follows: D1 (positive control without aflatoxin), D2 (negative control with 0.15mg/kg of aflatoxin), D3 (negative control + 3g/kg BB), D4 (negative control + 6g/kg BB), D5 (negative control + 1.5g yeast/kg diet), D6 (negative control + 3.0g yeast/kg diet). The experiment was conducted under a standard experimental conditions and all animal management procedures were followed Addition of BB or yeast in the aflatoxin contaminated diets significantly improved the feed intake and body weight gain of turkey poults. Feed conversion ratio also showed significant improvement in turkeys fed positive control diets (D1), and diets that were treated with yeast and bentonite-montmorillonite binder in D3, D4, D5 and D6 when compared with the negative control diet (D2) which was not treated with any toxin binder. No significant difference (P>0.05) was observed in the values of serum parameters in this study both for diets containing toxin binder and none. However, values of packed cell volume, haemoglobin and red blood cells were significantly improved with addition of yeast or BB. In conclusion, addition of yeast and bentonite-montmorillonite binder at the two levels in this study was able to bind the aflatoxin in the diet, thus making the aflatoxin in the diet ineffective.

Key words: Aflatoxin, alleviation, turkey poults, yeast, bentonite-montmorillonite

INTRODUCTION

Aflatoxin, a class of mycotoxin produced by 2 fungal species *Aspergillus flavus* and *Aspergillus parasiticus* is a secondary metabolite of these fungi species. Aflatoxin is known to interact with the basic metabolic pathways of the cell disrupting key enzyme processes including carbohydrate and lipid metabolism and protein synthesis (Cheeke and Shull, 1985). By virtue of the multiplicity of actions of aflatoxin on the liver and other cells containing mixed function oxidase systems, the effects of this toxin on animals are profound and far reaching.

Fig. 1: The infested ear of corn

The use of mycotoxin binders, which can trap the mycotoxin molecule by means of ion exchange and thereby hindering their absorption into the gastrointestinal tract, has gained much attention in prevention of mycotoxicosis. The efficacy of clay minerals depends on the geographical source and surface characteristics of the clay, with products having a larger surface area being more effective. Modified yeast cell wall has also been used to sequester aflatoxin and its effectiveness has been variable across species of animals (Diaz *et al*., 2004; Kutz *et al*., 2009). Yiannikouris *et al*. (2004) proposed that the glucan portion of the yeast cell wall interacts with the mycotoxin molecule and is the active component.

The effects of aflatoxin on domestic turkeys have been documented. Extensive mortality was produced in young domestic turkeys that were given 0.4mg/kg aflatoxin or more of dietary aflatoxin (Giambrone *et al*., 1985). In view of the extreme susceptibility of turkey to aflatoxin, the use of bentonite montmorillonite binder and yeast to alleviate the effect of aflatoxin in turkeys was investigated.

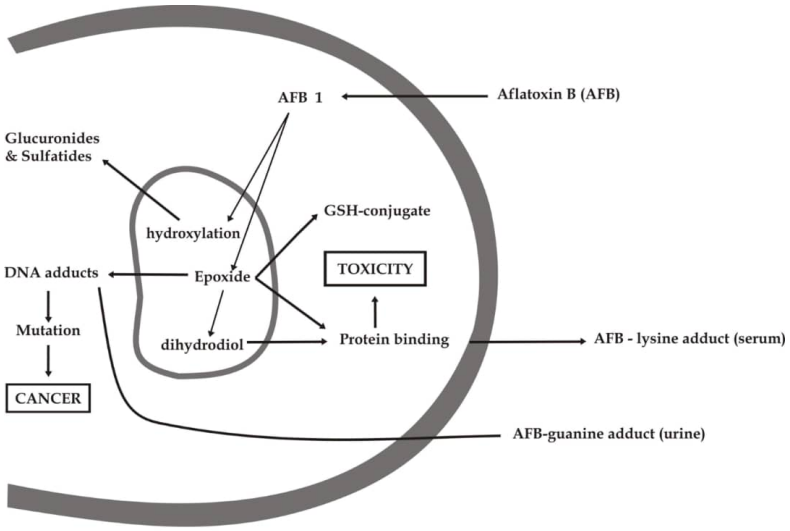

Fig. 2: Mechanism of Aflatoxin toxicity in the liver
Source: Eaton and Gallagher (1994)

MATERIALS AND METHODS

Birds and experimental design

A total of 192 twenty-one-day old turkey poults were used for this study. They were weighed at the third week and randomly allotted to six dietary treatments as follows: D1 (positive control with no aflatoxin), D2 (negative control with 150ppb aflatoxin), D3 (negative control + 3g/kg BB), D4 (negative control + 6g/kgBB), D5 (negative control + 1.5g yeast/kg diet) and D6 (negative control + 3.0g yeast/kg diet). Each dietary treatment was divided into 4 replicates with each replicate having 8 poults in a completely randomized design. The birds were reared in a well ventilated standard poultry house of the Teaching and Research Farm, University of Ibadan following all standard protocols of the institution for

animal experimentation. The feeding trial lasted for 28 days with routine management practices adhered to.

Feed 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 1.01mg/kg 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.*

Table 1: Gross Composition(%) of Turkey Poults Starter Diets

Ingredients	**PC (D1)**	**NC (D2)**	**NC+3g/kgBB (D3)**	**NC+6g/kgBB (D4)**	**NC+1.5g/kgY (D5)**	**NC+3.0g/kgY (D6)**
Pure maize	52.40	37.40	37.40	37.40	37.40	37.40
Contaminated Maize	-	15.00	15.00	15.00	15.00	15.00
Soybeanmeal	40.00	40.00	40.00	40.00	40.00	40.00
Fish Meal (72%)	5.00	5.00	5.00	5.00	5.00	5.00
Dicalcuim Phosphate	1.20	1.20	1.20	1.20	1.20	1.20
Limestone	1.00	1.00	1.00	1.00	1.00	1.00
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25	0.25	0.25
Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Nutrients						
Crude Protein (%)	28.44	28.44	28.44	28.44	28.44	28.44
ME (kcal/kg)	3021.37	3021.37	3021.37	3021.37	3021.37	3021.37
Crude Fibre (%)	3.90	3.90	3.90	3.90	3.90	3.90
Calcium (%)	0.92	0.92	0.92	0.92	0.92	0.92
Phosphorus (available)	0.55	0.55	0.55	0.55	0.55	0.55

[1]PC = positive control without aflatoxin or binders, NC = negative control with 150ppb aflatoxin,

[2]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 – 1.86mg; Cobalt – 0.268mg; Selenium – 0.108mg. ME – Metabolizable Energy, BB = bentonite-montmorillonite binder, Y = yeast.

RESULTS AND DISCUSSION

In this study, no clinical signs were observed in the positive control group (with no aflatoxin or supplementation) while in the negative control group, all signs associated with aflatoxicosis e.g. anorexia, mortality, severe depression, ruffling and stunting were seen but these signs were less pronounced in turkeys fed diets supplemented with BB and yeast (D3 to D6). Mortality was 56% and 12% in D2 and D5 respectively while other

groups recorded no mortality. Results on mortality in this study is in agreement with the work of Rauber *et al*. (2007) who reported that turkeys that received 200ppb and more of aflatoxin during 21 days of age recorded more than 37% mortalities. Two important factors may be responsible for this- During aflatoxicosis, aflatoxin B1 is hydrolysed to 8, 9-epoxide and then to 2, 3-dihydrodiol which is the most carcinogenic form of the toxin. This results in quick onset of necrosis of the liver and death of the animal (TDRI, 1984). Mortality could be high during acute aflatoxicosis because of the interference of the toxin with the immune system of the turkey poults thereby reducing their resistance to other infections.

Table 2: Performance of turkey poults fed aflatoxins contaminated diets supplemented with BB or Yeast at 28 days

Parameters	PC (D1)	NC (D2)	NC+3g/kgBB (D3)	NC+6g/kgBB (D4)	NC+1.5g/kgY (D5)	NC+3.0g/kgY (D6)	SEM
Initial Weight (g)	168.75	162.50	157.75	159.00	159.50	163.25	2.04
Feed Intake (g)	1493.13^{a}	1139.09^{c}	1432.94^{ab}	1360.63^{ab}	1334.38^{b}	1359.38^{ab}	23.04
Final Weight (g)	858.25^{a}	572.25^{c}	774.25^{ab}	739.50^{b}	747.75^{ab}	750.25^{ab}	17.67
Weight Gain (g)	689.50^{a}	409.75^{c}	571.00^{b}	580.50^{b}	588.25^{b}	522.50^{b}	13.90
Feed Conversion Ratio	2.17^{b}	2.75^{a}	2.55^{ab}	2.34^{b}	2.28^{b}	2.34^{b}	0.06
Mortality (%)	0.00	56.00	0.00	0.00	12.00	0.00	

D1 = positive control without aflatoxin or binders, D2 = negative control with 150ppb aflatoxin,
Means on the same row with different superscripts are significantly different (P<0.05). SEM = standard error of mean. BB = bentonite-montmorillonite binder, Y = yeast

The effects of yeast and BB additives on feed intake, body weight gain and feed conversion ratio are presented in Table 2. Feeding of aflatoxin-contaminated diet without supplementation with a binder caused a significant reduction ($p<0.05$) in feed intake and body weight gain. The feed intake of the turkey poults that were fed with aflatoxin and graded levels of yeast and BB showed significant increase ($p<0.05$) in their values compared with diet with aflatoxin alone. Turkeys fed diets containing no aflatoxin and binders recorded the highest feed intake (1493.13g) while turkeys fed with diets containing aflatoxin and no binders recorded the least intake of feed (1139.09g). The values of feed intake in diets D3, D4 and D6 with3g/kg yeast, 6g/kgyeast and 3g/kg BB respectively were not significantly different but their values were higher than that of the negative control (D2).

Supplementation with BB or yeast improved the performance parameters as seen in turkeys fed diets containing BB or yeast supplements, although with variations but the best performance among them was seen in turkeys fed 3g/kg BB supplement in terms of feed intake and weight gain. Similar results were obtained by Sehu *et al*. (2007) and Zhao *et al* (2010) who concluded that HSCAS at 5% concentration could

significantly and completely ameliorate the growth depressing effect of aflatoxin B1 as silica binders have been shown to bind the toxins in the digestive tract, making them unavailable for gut absorption and allowing them to pass harmlessly through the animal.

The mean values of packed cell volume, haemoglobin, red blood cells, white blood cells and platelets were significantly increased ($p<0.05$) with the addition of BB and yeast to the aflatoxin-contaminated diets (Table 3). The effect of aflatoxin on haematological parameters has been reported in earlier studies. Oguz *et al.* (2005) observed significant decreases in RBC, PCV, haemoglobin, thrombocyte and lymphocytes counts in broilers that were fed with aflatoxin contaminated diets during 21 days period. The suppressive effect of aflatoxin on haematopiesis and immune response was also reported by Huff *et al*. (1986). The values of haematological parameters in the present study were significantly improved by the supplementation of bentonite-montmorillonite binder (BB) and yeast (Table 3). Packed cell volume, haemoglobin and red blood cells also showed significant improvement in turkeys fed diets supplemented with BB or yeast compared with those that were fed with aflatoxin diet alone.

Table 3: Haematological values of poults fed aflatoxins contaminated diets supplemented with BB or yeast at 28 days

PARAMETERS	PC (D1)	NC (D2)	NC+3g/kgBB (D3)	NC+6g/kgBB (D4)	NC+1.5g/kgY (D5)	NC+3.0g/kgY (D6)	SEM
Packed Cell Volume (%)	37.50^{b}	39.80^{ab}	37.00^{b}	43.13^{a}	41.75^{ab}	41.75^{ab}	2.42
Haemoglobin (g/dl)	12.46^{b}	13.09^{ab}	12.36^{b}	14.44^{a}	14.05^{ab}	14.15^{ab}	0.21
Red Blood Cells ($x10^6/mm^3$)	3.74^{bc}	3.85^{abc}	3.63^{c}	4.23^{ab}	4.34^{a}	4.27^{ab}	0.06
White Blood Cells($x10^6/mm^3$)	19213^{bc}	22056^{ab}	21631^{ab}	20369^{ab}	17888^{c}	23775^{a}	369.06
Heterophil ($x10^6/mm^3$)	50.25	60.88	53.13	60.25	56.13	60.75	1.43
Lymphocytes ($x10^6/mm^3$)	46.13	34.25	43.63	34.75	40.13	35.50	1.43
Monocytes ($x10^6/mm^3$)	2.63	3.88	2.00	3.63	2.50	3.25	0.21
Eosinophil ($x10^6/mm^3$)	1.38	0.75	1.13	1.38	1.00	0.50	0.13
Basophil ($x10^6/mm^3$)	0.38	0.25	0.13	0.00	0.25	0.00	0.05
Platelets ($x10^3/l$)	168750^{ab}	192625^{ab}	176000^{ab}	154875^{b}	154625^{b}	204250^{a}	0.16

D1 = positive control without aflatoxin or binders, D2 = negative control with 150ppb aflatoxin,
Means on the same row with different superscripts are significantly different ($P<0.05$), SEM = standard error of mean, BB = bentonite-montmorillonite binder, Y = yeast

CONCLUSION AND OUTLOOK

It can be concluded that addition of bentonite-montmorillonite binder or yeast up to 6g/kg and 3.0g/kg, respectively reduced the toxicity of 0.15mg/kg aflatoxin in turkey, resulted in improved performance and reduced mortality.

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