

Original Research Article

Comparative evaluation of charcoal and honey as anti-aflatoxin feed additives in a broiler maize based diets

Abstract

The effect of charcoal and honey on the zoo technical performance and gut morphometry of broiler birds fed naturally aflatoxins contaminated maize based diets in a comparative study was carried out. In a completely randomised design, 240 one-week old Arbor Acre broiler were distributed randomly to six dietary treatments with four replicates of ten birds per replicate. The treatments were as follows: T1= Normal diets (diet formulated with normal maize) (positive control, with 15ppb AF); T2= Rejected maize diets (negative control, with 32ppb AF); T3=Positive control plus 2% charcoal; T4=Rejected maize diets plus 2% charcoal; T5=Positive control plus 2% honey; T6=Rejected maize diet plus 2% honey. Feeding and provision of water were supplied ad-libitum. On the 42th day, nine birds per treatments were slaughtered for gut morphometric attributes (villus height, crypt depth, villus width and villus height to crypt depth ratio) of duodenum, jejunum and ileum part of the gut. The zoo technical performance of broiler in the experiment were not significantly different ($P<0.05$) from all the treatments despite having different feed conversion ratio. Results from the gut morphology showed that the least villus height of birds from duodenum was recorded on AFL ($180 \times 10^2\text{mm}$) an indication of effect of aflatoxin. On the ileum, AFL ($138 \times 10^2\text{mm}$), CTL-Ho ($118 \times 10^2\text{mm}$) and AFL-Ho ($116 \times 10^2\text{mm}$) of villus height of birds were not significantly ($P<0.05$) different from each other. However, the histopathology of liver, kidney and bursa of fabricius showed healing power of honey as no visual lesions was seen on the slides of the organs prepared. In conclusion, 2% charcoal-to-aflatoxins-contaminated feed was more effective than 2% honey.

Keywords: Growth performance, Gut morphology, Organ histopathology, Adsorbent

31 Introduction

32 Recent trend in research for animal nutritionist has been directed to solving the issue of
33 contamination of animal feeds with mycotoxins since they pose serious threat to both humans
34 and animals (Hussein and Brasel 2001; Wu and Munkvold 2008; Zhang and Cauper 2012).
35 Mycotoxins are secondary toxic metabolites of fungi growth (*Aspergillus flavus*, *A.*
36 *parasiticus* and *A. nominus*) on grains, forages and even on dead woods under favourable
37 environmental conditions (Dutta and Das 2001). Mycotoxin contamination of poultry feeds
38 have been the second major stumbling block in feed industry after increasing price of
39 convectional feedstuffs (Sundu et al 2012). It has been reported that 25% world's grain was
40 contaminated by mycotoxins as revealed by FAO survey (Devegowda and Murthy 2005).
41 Among over 300 of mycotoxins identified aflatoxins (B_1 B_2 G_1 and G_2) are the most studied
42 and the main concern for animal nutritionist as well as its residue in animal products like
43 milk, muscle i.e. AFM_1 and AFM_2 (Yiannikouris and Jouany 2002).

44 Several approaches have been employed in the research of aflatoxin decontamination. Out of
45 the chemical, physical and biological means of detoxification of aflatoxin (Lopez-Garcia and
46 Park 1998; Sinha 1998) studies carried out, adsorbent-based studies have been reported to be
47 effective in removing aflatoxin from contaminated feed and minimise the toxicity of aflatoxin
48 in poultry (Ibrahim et al 2000). Of all the commercially available adsorbing agent in the
49 market, zeolite (Mazzo et al 2000), bentonites (Rosa et al 2001; Pasha et al 2007) and
50 Clinoptilolite (CLI) (Oguz and Kurtoglu 2000; Oguz et al 2000) were the most preferred as a
51 result of their capacity to bind effectively with AF as well as the reduction in the effect of AF-
52 absorption from the gastrointestinal tract. However, the use of clays have been reported to be
53 toxisorbent (mycotoxin specific) and binds to certain essential nutrients needed for animal
54 growths (Chestnuts 1992). The use of charcoal has been reported to be enterosorbent i.e. has
55 the capacity to binds to several mycotoxins (Whitlow 2006) due to its large surface area with
56 negatively charged site. Edrington et al (1997) have shown that charcoal was used as a binder
57 in broiler. Wellford et al (1978) reported that honey had an antifungal effect against *A. flavus*
58 and *A. parasiticus* and an even stronger anti-aflatoxigenic effect. Honey contains some
59 unknown substances that make it serve as a therapeutic agent against sores or lesions. This
60 makes it a good ameliorating agent against the effects of AF on the organs of the animals

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62 Material and methods

63 **Aflatoxin quantification analysis**

64 High performance liquid chromatography (HPLC) was used to identify and quantify the
65 presence of aflatoxins {B₁ B₂ G₁ and G₂} and other mycotoxins if presents. This analysis was
66 carried out in the pathology unit of the International Institute of Tropical Agriculture (IITA),
67 Ibadan. After extraction, the sample was made up to 1 ml and 4ul spotted on thin layer
68 chromatography (TLC) plate. The plate was developed in (96% diethyl ether, 3% methanol
69 and 1% water) and spot visualized at 365 um wavelength before scanning on TLC.

70 **Experimental design and birds**

71 Two hundred and forty one- week old Arbor Acre broilers used were randomly distributed
72 into six dietary treatments using the simple completely randomised design. The birds were fed
73 basal diet for one week before allotting them into their pens with four replicates for each
74 treatments containing 10 birds per replicate. Normal routine management exercise was carried
75 out and signs of disease and mortality were recorded.

76 **Diets preparation**

77 Dietary treatments consisted of the basal diet (15ppb aflatoxin) (diet formulated with normal
78 maize, the aflatoxin in the diet was as a result of other ingredients added), other diets were
79 formulated with rejected maize (the maize contain 32ppb aflatoxin). These two diets
80 contained either 2% honey or charcoal to form experimental other diets as shown in table 1.

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Table 1: Gross Composition of Experimental diets (kg)

Composition (%)	CTL	AFL	CTL-Ch	AFL-Ch	CTL-Ho	AFL-Ho
Normal maize	60	-	60	-	60	-
Rejected maize	-	60	-	60	-	60
Groundnut cake	18	18	18	18	18	18
Soyabean meal	15	15	15	15	15	15
Fish meal	2	2	2	2	2	2
Dicalcium Phosphate	4	4	4	4	4	4
Vitamin-mineral Premix	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.26	0.26	0.26	0.26	0.26	0.26
Methionine	0.24	0.24	0.24	0.24	0.24	0.24
Calculated composition values						
M. E (kcal/kg)	3013	3013	3013	3013	3013	3013
C.P (%)	23	23	23	23	23	23
Calcium (%)	1	1	1	1	1	1
Av. P (%)	0.98	0.98	0.98	0.98	0.98	0.98
Aflatoxin	15	15	15	15	15	15
Quantified (ppb)						
CTL= Normal diets (positive control with 15 ppb AF); AFL=Rejected maize diets (negative control with 32ppb Aflatoxin, AF); CTL-Ch=Positive control with 15 ppb AF plus 2% charcoal; AFL-Ch=Rejected maize diets with 32ppb Aflatoxin, AF plus 2% charcoal; CTL-Ho= Positive control with 15 ppb AF plus 2% honey; AFL-Ho= Rejected maize diet (32ppb AF) plus 2% honey M.E=Metabolisable energy, C.P.= Crude Protein, Av.P= Available Phosphorus						

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93 **Sample collection**

94 Data on feed intake, final weight gain and body weight gain were collected during the
95 experiments. At the end of the experiment, 54 birds were slaughtered for gut morphology
96 (duodenum, jejunum and ileum) and histopathology on the organs

97 **Histopathology**

98 Post-mortem examinations were performed on fifty four birds from all the treatments, three
99 from each replicate. After being slaughtered, samples of liver, kidney and bursa of fabricius
100 were collected and fixed in 10% neutral buffered formalin. Fixed tissues were trimmed,
101 embedded in paraffin, sectioned at 4µm, and stained with hematoxylin and eosin stain. Tissue
102 samples from all treatments groups were examined microscopically.

103 **Statistical analysis**

104 Data were analysed using analysis of variance (ANOVA) of the General Linear Model
105 procedure of SAS software (2009). Differences among treatments means were tested for
106 significance by using Multiple Range Duncan Test.

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Results and Discussion

Growth performance

Although it has been believed that contamination of the diet with aflatoxin could detrimentally affect feed quality and thus bird performance, 0.032ppm or 32ppb aflatoxins in broiler diets did not impair broiler performance in this study. This lack of difference in growth performance might be due to low levels of AF (32 ppb) within a period of 42 days. This statement is in agreement with the report by Mahmoud *et al.* (2011), who found that there were no changes in performance of broilers fed diet with lower level of AF (70 ppb AF) for a shorter period of 21 – 42 days of age. The reason for no changes in production parameters is because the birds were fed diet low in AF level (50 – 100ppb AF) within a period of 46 days (Oguz *et al* 2000; Ortatatli *et al* 2005; Magnoli *et al*, 2011). The lowered growth rate experienced upon feeding AF was due to reduction in body utilization of protein and energy (Smith and Hamilton, 1970; lanza *et al* 2002) vis-à-vis impaired nutrient absorption and reduced pancreatic enzymes for digestive purposes (Osborne and Hamilton, 1981) and subsequently appetite (Sharline *et al* 1980). There was an improvement in the feed conversion ratio of birds fed diets supplemented with the mycotoxin binder charcoal at 2% (AFL-Ch). This finding clearly supports previous reports of Murthy and Devegowda (2005).

Table 2: Performance characteristics of broiler birds fed aflatoxin contaminated feed supplemented with charcoal and honey as anti-aflatoxin additives

Treatment	CTL	AFL	CTL-Ch	AFL-Ch	CTL-Ho	AFL-Ho	SEM	P-Value
ILW (g/bird)	173 ^b	187 ^a	178 ^{ab}	178 ^{ab}	158 ^c	167 ^{bc}	3.6	0.1
FLW (g/bird)	1475	1566	1533	1485	1481	1488	59.0	0.3
BWG (g/bird)	1297	1331	1369	1324	1323	1320	58.6	0.4
TFC (g/bird)	2591	2591	2709	2818	2637	2497	93.7	0.1
CFCR	2.7 ^a	2.0 ^{ab}	1.8 ^b	2.1 ^{ab}	2.2 ^{ab}	1.9 ^{ab}	0.2	0.2

abc means with different superscript on the same row are significantly different ($P=0.05$)

ILW: initial liveweight; FLW: final liveweight; BWG: body weight gain; TFC: total feed consumed; CFCR: cumulative feed conversion ratio. CTL-normal diet (positive control i.e. diet containing 15ppb Aflatoxin), AFL-Rejected maize diet (negative control i.e. diet containing 32ppb Aflatoxin), CTL-Ch: normal diet (positive control)+2% charcoal, AFL-Ch: rejected maize diet (negative control) +2% charcoal, CTL-Ho: normal diet (positive control) + 2% honey, AFL-Ho: rejected maize (negative control) +2% honey

Intestinal morphology

According to Bouhet et al (2004), the GIT is the first organ to come in contact with chemicals, natural toxins and foods and such should be affected with greater potency compared to other organs. Table 3 shows the results of intestinal morphology. There is a decrease in the villus height of birds duodenum and ileum but an increase in the jejunum ($P<0.05$) of birds fed rejected maize diets (negative control with 32 ppb Aflatoxin, AF). Cavret and Lecoœur (2006) and Agence Française de Sécurité (2009) explained that about >80% of aflatoxins are absorbed at the duodenum part of the intestines. As such mycotoxins always compromise intestinal epithelium either before or throughout the entire intestines by non-absorbed toxins. The ratio of villus height to crypt depth in the duodenum decreased but experienced increase in the jejunum and ileum section. However, the supplementation of 2% charcoal to the diets contaminated with 32 ppb AF i.e. AFL-Ch diet showed a significant ($P<0.05$) increase in the villus height of duodenum and ileum. Although, 2% honey supplementation showed an increase in the villus height of birds duodenum no effects on jejunum and ileum. The ratio of villus height to crypt depth of the three intestinal segments follows the same trend. It is clear that increasing the villus height increased surface area vis-a-vis greater absorption of available

163 nutrients. The villus height to crypt depth ratio according to Caspary (1992) reflects
 164 differences in the digestion and absorption of the small intestines. Applegate et al (2009)
 165 reported that crypt depth of gut increases linearly with aflatoxin concentrations vis-à-vis
 166 influencing the villus crypt ratio.

167 A previous study by Girish and Smith (2008) reported that grains naturally contaminated with
 168 DON significantly reduced the height, width, and surface of villus in the duodenum and
 169 jejunum of broilers. The present are in agreement with Yang et al (2012) statement that there
 170 was a decrease significantly in villus height and the ratio of villus height to crypt depth when
 171 broilers birds were fed daily with diets contaminated with AFB₁ and AFB₂. Long-term
 172 exposure of AFB₁ and AFB₂ mainly would affect the morphology of the duodenum as a result
 173 of stimulating proximal gastrointestinal tract (Yang et al 2012); the characteristics of the gut
 174 morphometric attributes vis-à-vis altering nutrients absorption. Girgris et al (2010) observed an
 175 increase in the villus height of jejunum and ileum of birds fed a contaminated diet with
 176 Fusarium mycotoxins which is contrary to the present result. The authors suggested a
 177 compensation for the reduced surface area of the duodenum villi resulting from reduced villi
 178 heights in these birds.

179 Table 3: Gut parameters of broiler birds fed aflatoxin contaminated feed supplemented with
 180 charcoal and honey as anti aflatoxin additives

Treatment	CTL	AFL	CTL-Ch	AFL-Ch	CTL-Ho	AFL-Ho	SEM	P-Value
Duodenum (mmX10 ²)								
Villus height	270 ^{ab}	181 ^d	280 ^a	235 ^c	243 ^{bc}	214 ^c	9.9	0.4
Crypt depth	31 ^b	45 ^a	24 ^c	45 ^a	30 ^{bc}	45 ^a	2.4	0.6
Villus width	18 ^b	21 ^b	19 ^b	40 ^a	20 ^b	21 ^b	2.2	0.7
VH/CD	8 ^a	4 ^b	8 ^a	5 ^b	8 ^a	5 ^b	0.6	0.3
Ileum (mmX10 ²)								
Villus height	147 ^b	138 ^{bc}	174 ^a	182 ^a	118 ^c	116 ^c	7.8	0.3
Crypt depth	25 ^{ab}	19 ^b	26 ^a	20 ^b	22 ^{ab}	26 ^{ab}	2.0	0.1
Villus	16 ^{ab}	13 ^b	17 ^a	16 ^{ab}	16 ^{ab}	16 ^{ab}	1.1	0.3

width								
VH/CD	6 ^{bc}	7 ^b	6 ^{bc}	12 ^a	6 ^{bc}	5 ^c	0.7	0.5
Jejunum	(mmX10 ²)							
Villus height	162 ^d	205 ^a	157 ^d	167 ^{cd}	184 ^{bc}	203 ^{ab}	5.9	0.4
Crypt depth	24 ^{bc}	25 ^{bc}	30 ^{ab}	28 ^{ab}	32 ^a	20 ^c	1.9	0.1
Villus width	19 ^b	18 ^b	18 ^b	17 ^b	25 ^a	17 ^b	0.7	0.5
VH/CD	6 ^b	8 ^a	5 ^b	6 ^b	6 ^b	7 ^{ab}	0.5	0.1

abc means with different superscripts on the same column are significantly different ($P=.05$)

CTL-normal diet (positive control i.e. diet containing 15ppb Aflatoxin), AFL-Rejected maize diet (negative control i.e. diet containing 32ppb Aflatoxin), CTL-Ch: normal diet (positive control)+2% charcoal, AFL-Ch: rejected maize diet(negative control) +2% charcoal, CTL-Ho: normal diet (positive control) + 2% honey, AFL-Ho: rejected maize (negative control) +2% honey, VH/CD: Villus height to crypt depth ratio.

Conclusions

It can be concluded from the study that charcoal was able to prevent the absorption of the toxins into the enterocyte of the animal vis-à-vis giving better bird performance and gastrointestinal tracts attributes. However, honey was therapeutic in nature as it healed sores generated by mycotoxins absorption on the surface of the organs.

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