

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 morphometric of broiler birds fed naturally aflatoxins contaminated maize based diets in a comparative study was carried out. In a completely randomised design, two hundred and forty one-week old Arbor acre broiler birds 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. Normal routine management exercises were carried out including feeding and provision of water 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 birds in the experiment were not different ($P<0.05$) significantly from all the treatments despite having different feed conversion ratio. Results from the gut morphology shows that the least villus height of birds from duodenum was recorded on AFL (180 x 10²mm) an indication of effect of aflatoxins. On the ileum, AFL (138 x 10²mm), CTL-Ho (118 x 10²mm) and AFL-Ho (116 x 10²mm) 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 shows 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 effective while 2% honey did not prevent absorption of aflatoxins across the GIT but heals any lesions as a results of toxicity.

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

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33 **Introduction**

34 Recent trend in research for animal nutritionist has been directed to solving the issue of
 35 contamination of animal feeds with mycotoxins since they pose serious threat to both humans
 36 and animals (Hussein and Brasel 2001; Wu and Munkvold 2008; Zhang and Cauper 2012).
 37 Mycotoxins are secondary toxic metabolites of fungi growth (*Aspergillus flavus*, *A.*
 38 *parasiticus* and *A. nomenus*) on grains, forages and even on dead woods under favourable
 39 environmental conditions (Dutta and Das 2001). Mycotoxin contamination of poultry feeds
 40 have been the second major stumbling block in feed industry after increasing price of
 41 convectional feedstuffs (Sundu et al 2012). In 2005, Devegowda and Murthy reported that
 42 according to survey by FAO that about 25% world's grain was contaminated by mycotoxins.
 43 Among over 300 of mycotoxins identified (Yiannikouris and Jouany 2002), aflatoxins (B₁ B₂
 44 G₁ and G₂) are the most studied and the main concern for animal nutritionist as well as its
 45 residue in animal products like milk, muscle i.e. AFM₁ and AFM₂

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

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65 **Material and methods**

66 **Aflatoxin quantification analysis**

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

73 **Experimental design and birds**

74 Two hundred and forty one- week old Abhor acre broiler birds used were randomly
75 distributed into six dietary treatments using the simple completely randomised design. The
76 birds were fed basal diet for one week before allotting them into their pens with four replicate
77 for each treatments containing ten birds per replicate. Normal routine management exercise
78 was carried out and signs of disease and mortality were recorded.

79 **Diets preparation**

80 Dietary treatments consisted of the basal diet (15ppb aflatoxin) (Diet formulated with normal
81 maize, the aflatoxin in the diet was as a result of other ingredients added), other diets were
82 formulated with rejected maize (the maize contain 32ppb aflatoxin). These two diets
83 contained either 2% honey or charcoal to form experimental other diets as shown in the table
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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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95 **Sample collection**

96 Data on intake, final weight gain, body weight gain were collected during the experiments
97 while feed conversion ratio was calculated. At the end of the experiment, Fifty four (54) birds
98 were slaughtered for gut morphology (Duodenum, Jejunum and Ileum) and histopathology on
99 the organs

100 **Histopathology**

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

106 **Statistical analysis**

107 Data were analysed using analysis of variance (ANOVA) of the general linear model
108 procedure of SAS software (2009). Differences among treatments were tested for significance
109 by using Multiple Range Duncan Test.

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111 **Results and Discussion**

112 **Growth performance**

113 Although it has been believed that contamination of the diet with aflatoxin could deteriorate
114 feed quality and thus bird performance, 0.032ppm or 32ppb aflatoxins in broiler diets did not
115 impair broiler performance in this study. This lack of difference in growth performance might
116 be due to low levels of AF (32 ppb) within a period of 42 days. This statement is in agreement
117 with the report by Mahmoud *et al.* (2011), which claims that there were no changes in
118 performance of broiler birds fed feed with lower level of AF (70 ppb AF) for a shorter period
119 of 21 – 42 days of age. This is also similar to reports claiming that the reason for no changes in
120 production parameters (Oguz et al 2000; Ortatatli et al 2005; Magnoli et al, 2011), is because
121 the birds were fed feed low in AF level (50 – 100ppb AF) within a period of 46days. The
122 results from the present study are not in an agreement with other studies where significantly
123 reduced body weights were observed when birds were exposed to higher dietary AF (400 -
124 600ppb AF). The lowered growth rate experienced upon feeding AF was as a result of
125 reduction in body utilization of protein and energy (Smith and Hamilton, 1970; Ianza et al
126 2002) vis-à-vis impaired nutrient absorption and reduced pancreatic enzymes for digestive
127 purposes (Osborne and Hamilton, 1981) and subsequently appetite (Sharline et al 1980). The
128 more promising figure in this present study is that there is improvement in the feed conversion
129 ratio of birds fed diets supplemented with the mycotoxin binder charcoal at 2% (AFL-Ch).
130 This finding clearly supports previous reports of Murthy and Devegowda (2005).

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141 Table 2: Performance characteristics of broiler birds fed aflatoxin contaminated feed
142 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

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

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

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161 **Intestinal morphology**

162 According to Bouhet et al (2004), the GIT is the first organ to come in contact with chemicals,
 163 natural toxins and foods and such should be affected with greater potency as compared to other
 164 organs. Table 3 shows the results of intestinal morphology. There is a decrease in the villus
 165 height of birds duodenum and ileum but increase in the jejunum ($P<0.05$) fed rejected maize
 166 diets (negative control with 32 ppb Aflatoxin, AF). The reason for the decrease in the villus
 167 height was explained by Cavret and Lecoer (2006) and Agence Française de Sécurité (2009)
 168 that about $>80\%$ of aflatoxins are absorbed at the duodenum part of the intestines. As such
 169 mycotoxins always compromise intestinal epithelium either before or throughout the entire
 170 intestines by non-absorbed toxins. The ratio of villus height to crypt depth in the duodenum
 171 decreased but experienced increase in the jejunum and ileum section. However, the
 172 supplementation of 2% charcoal to the diets contaminated with 32 ppb AF i.e. AFL-Ch diet
 173 showed a significant ($P<0.05$) increase in the villus height of duodenum and ileum which it is
 174 not significantly difference from the normal diets (CTL). Although, 2% honey
 175 supplementation showed an increase in the villus height of birds duodenum but no effects on
 176 jejunum and ileum. The ratio of villus height to crypt depth of the three intestinal segments
 177 follows the same trend. It is evidenced that increasing of the villus height suggested increased
 178 surface area vis-a-vis greater absorption of available nutrients. The villus height to crypt depth
 179 ratio according to Caspary (1992) reflects differences in the digestion and absorption of the
 180 small intestines. Applegate et al (2009) reported that crypt depth of gut increases linearly with
 181 aflatoxins concentrations vis-à-vis influencing the villus crypt ratio.

182 A previous study by Girish and Smith (2008) reported that grains naturally contaminated with
 183 DON significantly reduced the height, width, and surface of villus in the duodenum and
 184 jejunum of broilers This presents study is in agreement with Yang et al (2012) statement that
 185 there was a decrease significantly in villus height and the ratio of villus height to crypt depth
 186 when broilers birds were fed daily with diets contaminated with AFB₁ and AFB₂. Further,
 187 Yang et al (2012) concluded that long-term exposure of AFB₁ and AFB₂ mainly would affect
 188 the morphology of the duodenum as a result of stimulating proximal gastrointestinal tract; the
 189 characteristics of the gut morphometric attributes vis-à-vis altering nutrients absorption. Report
 190 by Girgris et al (2010) claims that they observed an increase in the villus height of jejunum and
 191 ileum of birds fed a contaminated diet with Fusarium mycotoxins which is contrary to the
 192 present study. The authors suggested a compensation for the reduced surface area of the
 193 duodenum villi resulting from reduced villi heights in these birds.

Table 3: Gut parameters 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
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 width	16 ^{ab}	13 ^b	17 ^a	16 ^{ab}	16 ^{ab}	16 ^{ab}	1.1	0.3
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 ($P \leq .05$)

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.

204 **Conclusions**

205 It can be concluded from the study that:

- 206 • Both charcoal and honey are good in ameliorating the effect of mycotoxins. Charcoal
207 was able to prevent the absorption of the toxins into the enterocyte of the animal vis-
208 à-vis giving better bird performance and gastrointestinal tracts attributes.
- 209 • However, honey was therapeutic in nature as it tries to heal sores generated by
210 mycotoxins absorption on the surface of the organs.

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