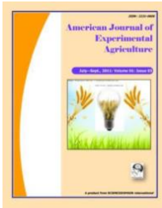

American Journal of Experimental Agriculture
7(3): 163-169, 2015, Article no.AJEA.2015.116
ISSN: 2231-0606

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Performance and Gut Morphometry of Broiler Fed Maize Based Diets Supplemented with Charcoal and Honey as Anti-aflatoxin

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OAA and UVO designed the study, authors UVO and CG carried out the feeding trial and handled literature searches. Author Adeniji wrote the first draft. Author OAA performed the statistical analyses. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJEA/2015/13012
Editor(s):
(1) Zhen-Yu Du, School of Life Science, East China Normal University, China.
(2) Daniele De Wrachien, Faculty of Irrigation and Drainage, State University of Milan, Italy.
Reviewers:
(1) Anonymous, Botswana.
(2) Anonymous, Egypt.
(3) Anonymous, Pakistan.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=917&id=2&aid=8133

Original Research Article

Received 29th July 2014
Accepted 4th October 2014
Published 16th February 2015

ABSTRACT

The study was conducted to assess 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 broilers 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 15 ppb AF); T2= Rejected maize diets (negative control, with 32 ppb 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

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of birds from duodenum was recorded on AFL (180 x 10^2 mm) an indication of effect of aflatoxin. On the ileum, AFL (138 x 10^2 mm), CTL-Ho (118 x 10^2 mm) and AFL-Ho (116 x 10^2 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.

1. INTRODUCTION

Recent trend in research for animal nutritionist has been directed to solving the issue of contamination of animal feeds with mycotoxins since they pose serious threat to both humans and animals (Hussein and Brasel [1]; Wu and Munkvold [2]; Zhang and Cauper [3]). Mycotoxins are secondary toxic metabolites of fungi growth (*Aspergillus flavus, A. parasiticus and A. nominus)* on grains, forages and even on dead woods under favourable environmental conditions Dutta and Das [4]. Mycotoxin contamination of poultry feeds have been the second major stumbling block in feed industry after increasing price of convectional feedstuffs Sundu et al. [5]. It has been reported that 25% world's grain was contaminated by mycotoxins as revealed by FAO survey Devegowda and Murthy [6,7]. Among over 300 of mycotoxins identified aflatoxins (B_1 B_2 G_1 and G_2) are the most studied and the main concern for animal nutritionist as well as its residue in animal products like milk, muscle i.e. AFM_1 and AFM_2 Yiannikouris and Jouany [8].

Several approaches have been employed in the research of aflatoxin decontamination. Out of the chemical, physical and biological means of detoxification of aflatoxin Lopez-Garcia and Park [9]; Sinha [10] studies carried out, adsorbent-based studies have been reported to be effective in removing aflatoxin from contaminated feed and minimise the toxicity of aflatoxin in poultry Ibrahim et al. [11]. Of all the commercially available adsorbing agent in the market, zeolite Mazzo et al. [12], bentonites Rosa et al. [13]; Pasha et al. [14] and Clinoptilolite (CLI) Oguz and Kurtoglu; Oguz et al. [15,16] were the most preferred as a result of their capacity to bind effectively with AF as well as the reduction in the effect of AF-absorption from the gastrointestinal tract. However, the use of clays have been reported to be toxisorbent (mycotoxin specific) and binds to certain essential nutrients needed for animal growths Chestnuts, [17]. The use of charcoal has been reported to be enterosorbent i.e. has the capacity to binds to several mycotoxins Whitlow [18] due to its large surface area with negatively charged site. Edrington et al. [19] have shown that charcoal was used as a binder in broiler. Wellford et al. [20] reported that honey had an antifungal effect against *A. flavus* and *A. parasiticus* and an even stronger anti-aflatoxigenic effect. Honey contains some unknown substances that make it serve as a therapeutic agent against sores or lesions. This makes it a good ameliorating agent against the effects of AF on the organs of the animals

2. MATERIALS AND METHODS

2.1 Aflatoxin Quantification Analysis

High performance liquid chromatography (HPLC) was used to identify and quantify the presence of aflatoxins {B_1 B_2 G_1 and G_2} and other mycotoxins if presents. This analysis was carried out in the pathology unit of the International Institute of Tropical Agriculture (IITA), Ibadan. After extraction, the sample was made up to 1 ml and 4ul spotted on thin layer chromatography (TLC) plate. The plate was developed in (96% diethyl ether, 3% methanol and 1% water) and spot visualized at 365 um wavelength before scanning on TLC.

2.2 Experimental Design and Birds

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

2.3 Diets Preparation

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

2.4 Sample Collection

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

2.5 Histopathology

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

2.6 Statistical Analysis

Data were analysed using analysis of variance (ANOVA) of the General Linear Model procedure of SAS software, [21]. Differences among treatments means were tested for significance by using Multiple Range Duncan Test.

3. RESULTS AND DISCUSSION

3.1 Growth Performance

Table 2 shows performance characteristics of broiler birds fed aflatoxin contaminated feed supplemented with charcoal and honey as anti-aflatoxin additives. Although it has been believed that contamination of the diet with aflatoxin could detrimentally affect feed quality and thus bird performance, 0.032 ppm or 32 ppb 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. [22], 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 – 100 ppb AF) within a period of 46 days (Oguz et al. [20,21]; Ortatatli et al. [23]; Magnoli et al. [24]. Miazzo et al. [12] however reported that BW gains were lower ($P < 0.05$) for broilers that were fed AF in their diets.

Table 1. Gross composition of experimental diets (kg)

Composition (%)	CTL	AFL	CTL-Ch	AFL-Ch	CLT-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 Quantified (ppb)	15	15	15	15	15	15

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

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=.05)
ILW: initial live weight; FLW: final live weight; 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

The lowered growth rate experienced upon feeding AF was due to reduction in body utilization of protein and energy Smith and Hamilton, [25]; Ianza et al. [26] vis-à-vis impaired nutrient absorption and reduced pancreatic enzymes for digestive purposes Osborne and Hamilton, [27] and subsequently appetite Sharline et al. [28]. 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 [6,7].

3.2 Intestinal Morphology

According to Bouhet et al. [29], 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 Lecoeur [30] and Agence Française de Sécurité [31] 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 nutrients. The villus height to crypt depth ratio according to Caspary [32] reflects differences in the digestion and absorption of the small intestines. Applegate et al. [33] reported that crypt depth of gut increases linearly with aflatoxin concentrations vis-à-vis influencing the villus crypt ratio.

A previous study by Girish and Smith [34] reported that grains naturally contaminated with DON significantly reduced the height, width, and surface of villus in the duodenum and jejunum of broilers. The present are in agreement with Yang et al. [35] statement that there was a decrease significantly in villus height and the ratio of villus height to crypt depth when broilers birds were fed daily with diets contaminated with AFB_1 and AFB_2. Long-term exposure of AFB_1 and AFB_2 mainly would affect the morphology of the duodenum as a result of stimulating proximal gastrointestinal tract Yang et al. [35]; the characteristics of the gut morphometric attributes vis-à-vis altering nutrients absorption. Girgris et al. [36] observed an increase in the villus height of jejunum and ileum of birds fed a contaminated diet with Fusarium mycotoxins which is contrary to the present result. The authors suggested a compensation for the reduced surface area of the 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=.05)
CTL-normal diet (positive control i.e. diet containing 15 ppb Aflatoxin), AFL-Rejected maize diet (negative control i.e. diet containing 32 ppb 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

4. CONCLUSION

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 sours generated by mycotoxins absorption on the surface of the organs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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