



## ORIGINAL RESEARCH ARTICLE

**Response of laying birds to mouldy corn based diets and sequestering effect of three toxin adsorbents**Akande T.O<sup>1</sup>, Alade R.A.<sup>2</sup>, Fatoki F.L.<sup>2</sup> and Osademe N.<sup>2</sup><sup>1</sup>Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria<sup>2</sup>Department of Animal Nutrition and Biotechnology, Ladoké Akintola University of Technology  
P.M.B. 4000, Ogbomoso, Nigeria.<sup>1</sup>Corresponding author: yakandetaiwo@yahoo.com; Tel.: +2318134543258**ABSTRACT**

*A study was conducted to determine the fungi profile and proximate composition of mouldy corn; response of laying birds fed such mouldy corn and the sequestering effect of three toxin adsorbents. There were eight dietary treatments; diets 1 to 4 contained clean corn supplemented with: no adsorbent, activated charcoal (CA<sup>1</sup>) or either of the two other commercial adsorbents (CA<sup>2</sup> and CA<sup>3</sup>). Diets 5 to 8 contained mouldy corn diets with supplementation as stated for diets 1 to 4 and arranged in a 2x4 factorial layout; two corn types and four supplementations. The results showed that the activities of the fungi depleted the organic components - protein, lipid and the calories of the grains by 23%, 51% and 4.5% respectively while compensating in terms of total ash. The mould-contaminated diets significantly ( $P<0.05$ ) decreased body weight gain (BWG) and Hen day production (HDP) while egg qualities and feed intake were not significantly ( $P>0.05$ ) affected. There were depression ( $P<0.05$ ) in haematological indices (erythrocytes, Haemoglobins and mean corpuscular volume) in birds subjected to contaminated diets. Higher ( $P<0.05$ ) retention of urea and creatinine and high concentration of alanine and aspartate transaminases were observed in blood of birds on contaminated diets. The addition of the three adsorbents in mouldy diets improved BWG and HDP but birds on CA<sup>1</sup> compared more closely ( $P>0.05$ ) with birds on clean corn diets. All the treatments were similar ( $P>0.05$ ) in terms of egg quality measurements. The results indicated that mould in poultry feeds retard growth, egg production and impair feed conversion ratio, whereas the addition of 0.4% CA<sup>1</sup>, CA<sup>2</sup> or CA<sup>3</sup> reduced the adverse effects of mould contamination to varying extents, with CA<sup>1</sup> being the most effective adsorbent in this study*

**Keywords:** Mouldy Maize, layer chickens, blood profile, performance**INTRODUCTION**

Mould refers to multiple types of fungi that grow in filaments and reproduce by forming spores which are very hardy and can survive under dry and harsh environments for continuity in different media. They are ubiquitous plant pathogens that are major spoilage agents of foods and feedstuffs. Moulds like other microorganisms will assimilate and utilize the most readily available nutrients in the materials they grow upon and spoilage may result in the loss of 5% and up to 100% of the nutrients in the feed (Okoli *et al.*, 2006). The infection by various fungi not only results in nutrient depletion but also contamination of grains with poisonous fungal secondary metabolites called mycotoxins (Shareef, 2010; Atanda *et al.*, 2013). Presently, mould contamination appears to

have a higher health risk than other contaminants, (Kuiper-Goodman, 1998). Report has also indicated performance losses of 5 – 10% are typical with consumption of mouldy feeds even in the absence of mycotoxins and on the other hand, mycotoxins contaminations increase production losses even when the mould is not readily visible (Atanda *et al.*, 2013). Although there are geographic and climatic variations in the production and occurrence of mould and its toxins, exposure to these substances occurs all over the world and much of the world's food supply is contaminated to some extent. Mould growth and mycotoxins production are promoted by humid and warm environment, a prevailing weather condition in the tropics. Water activity is undoubtedly the most important factor in

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determining whether or not mould growth is initiated on feed ingredient and when this condition becomes favourable for the fungi, it is very difficult to avoid the production of mycotoxins in animal feed. Owing to prevailing humid and warm conditions in this part of the world, grains are essentially prone to mould contamination on the field and during storage such that mouldy grains usually corn are found in open markets and some feed mills used them in making animal feed. As such animals and man are exposed to numerous health risks which essentially have huge economic implications. Some economic costs of mycotoxins include the cost of preventative and mitigation practices, reduced value of contaminated feeds, contamination of foods of animal origin and reduction in animal performance and health. Several studies have shown that economic losses due to mycotoxins occur at all levels of food and feed production, including crop and animal production, processing, storage and distribution (Weaver *et al.*, 2013, Katole *et al.*, 2013). Despite the most strenuous prevention efforts, contamination of agricultural products with mycotoxins still occurs. In 2010, regulatory agencies in Nigeria destroyed mycotoxin-contaminated foods worth more than #40,000,000. However, destruction or diversion of contaminated grains from animal uses is not always practicable in many developing nations of the world. The need for a comprehensive approach to eliminate the possible effect of mycotoxins has led to the use of various adsorbents to bind the toxins. The strategy is to decrease the bioavailability of the toxins in the compounded feed, which leads to a reduction of mycotoxin uptake as well as distribution to the blood and target organs in animals. However, this approach is not without some drawbacks. The economics, accessibility and potential to bind certain nutrients still constitute sources of worry. In this study attempt was made to compare the potency and economic costs of three adsorbents in the diets of laying hens.

### MATERIALS AND METHODS

#### Sample Preparation and experimental diets.

The experiment was carried out at the University Teaching and Research Farm, located at the rain forest zone south-western part of the country on Latitude 7.47°N and Longitude 4.5°E with

temperature range of between 22.1 – 33.0°C, annual rainfall of about 1000mm and relative humidity 90-95%. Two batches of clean corn grains were purchased from University Teaching and Research Farm. The corn grains were crushed to reduce the particle size and increased the surface area. One batch was subjected to conditions that promoted mould growth by raising the moisture content to 20% according to procedure of Akande *et al.* (2006). The Moistened corn was packed into sacks, stacked and kept for 12 days at 32°C. Thereafter, the mouldy corn was sun dried 2 days before milling and incorporation into the diets as shown in Table 1. Other feed ingredients were procured from the same Teaching and Research Farm while activated charcoal and two other commercial toxin adsorbents (tagged CA<sup>1</sup> CA<sup>2</sup> and CA<sup>3</sup>) were procured from a retail outlet and were used in diet preparation as indicated in Table 1.

#### Birds and Management

Eighty actively laying brown shavers (40 weeks of age) were purchased from a reputable farm in Ogbomosho, Oyo State, Nigeria for the experiment. The birds were kept in a galvanized battery cage system and randomly distributed into eight groups. Each group was replicated five times with two birds per replicate. Each replicate was housed in a cell in the cage. The birds were fed with a commercial feed (17% CP, 2650 kcal ME/kg) for two weeks to stabilize them before introduced to experimental diets and fed for another eight weeks. Each group was allotted a diet and feeding was carried out *ad libitum* with free access to water.

#### Data collection and Analysis

Isolation, macro and micro-morphological identification of the fungus were carried out in accordance with procedure of Cheesbrough (2000). The infected samples were cultured on Potato Dextrose Agar (PDA). Chloramphenicol (30mg/l) was added to the medium to discourage bacterial contamination (Adamu *et al.*, 2009) and incubated at room temperature for 5 days. After incubation, colonies of different shape and colours were observed on the plates. A pure culture of each colony type on each plate was obtained and maintained.

**Table 1: Composition of experimental diets**

Ingredients, %	Clean corn diets				Mouldy corn diets			
	No adsorbent	Comm. Adsorbent <sup>1</sup>	Comm. Adsorbent <sup>2</sup>	Comm. Adsorbent <sup>3</sup>	No adsorbent	Comm. Adsorbent <sup>1</sup>	Comm. Adsorbent <sup>2</sup>	Comm. Adsorbent <sup>3</sup>
Corn	44	44	44	44	44	44	44	44
Corn bran	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Wheat offal	15.0	15	15	15	15	15	15	15
Soybean meal	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Groundnut cake	10	10	10	10	10	10	10	10
Other ingredients	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5
Activated charcoal	0.0	0.4	0	0	0	0.4	0.0	0.0
Commercial Adsorbent 2	0.0	0.0	0.4	0.0	0.0	0.0	0.4	0.0
Commercial Adsorbent 3	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.4
<b>Total</b>	<b>100</b>	<b>100.4</b>	<b>100.4</b>	<b>100.4</b>	<b>100</b>	<b>100.4</b>	<b>100.4</b>	<b>100.4</b>
<b>Calculated Analysis</b>								
M E, kcal/kg	2715	2715	2715	2715	2715	2715	2715	2715
CP, %	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5
CF, %	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
EE, %	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25
Calcium, %	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55
Phosphorus %	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52

Other ingredients: Fish meal =2.0; Bone meal =2.5; Oyster shell =6.0; salt=0.3; premix=0.25; lysine=0.20; methionine=0.25.

The maintenance was done by sub-culturing each of the different colonies onto the SDA plates and incubated at room temperature again for 5 days. The technique of James and Natalie (2001) was adopted for identification of the unknown isolated fungi using cotton blue in lactophenol stain. The identification was achieved by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the mycelium from the fungal cultures was removed and placed in a drop of lactophenol. The mycelium was spread very well on the slide with the aid of the needle; covered with slip gently with little pressure to eliminate air bubbles. The slide was then mounted and observed with x10 and x40 objective lenses respectively. The species encountered were identified in accordance with procedure of Cheesbrough (2000). Data on proximate composition of test ingredient, performance characteristics of birds such as feed intake, body weight changes, hen day production, feed conversion ratio, egg quality characteristics (egg weight, yolk weight, albumen weight, shell thickness, yolk index) and blood indices (haematology and serum components) were determined after the 8-week feeding trials as

shown in Tables 2, 3 and 4. The data were subjected to Analysis of variance using SAS 2006 statistical package while the means were separated with Duncan multiple option of the same software package. All of the statistical procedures were performed using 0.05 as the critical level of probability of a type I error.

## RESULTS AND DISCUSSION

### Fungi in Mouldy Corn

Seven different fungi comprising five genera were isolated from infected corn grains. The fungi were identified as *C. gloeosporioides*, *F. oxysporum*, *Mucor* sp., *R. stolonifer*, *Aspergillus flavus*, *A. fumigatus* and *A. parasiticus*. *Aspergillus* species were more prevalent than any other species isolated in this study with the presence of the toxigenic strains. This was in consonance with earlier report of Reddy and Salley (2011) on co-occurrence of moulds in corn. While several fungal species cause spoilage of stored grains, the presence and prevalence of *Aspergillus* sp. particularly the toxigenic strains *A. flavus* and *A. parasiticus* in this study show that there is the production of aflatoxins in the grains that has a serious health implication, as they are highly toxic

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and carcinogenic thus rendered unfit for both human and animal consumption (Shenasi *et al.*, 2002). Hence, the moisture content of stored corn should be checked not to exceed 16% to prevent mould growth as earlier reported that moulds grow on grain under field conditions or during storage at moisture levels above 16% and at temperatures above freezing (Shamsudeen *et al.*, 2013). However, when contamination occurred, such grains should be sorted and eliminated from feed. Similarly, stored grains should be prevented from rodent infestation as their faeces and urine may promote grain mouldiness. More so, presence of other fungi species apart from *Aspergillus* species, capable of producing other mycotoxins suggest that single approach towards detoxification or preventing mycotoxicosis in animal may not be appropriate, a multiple approach will appear more feasible in decontamination of different mycotoxins as earlier indicated by Robens and Cardwell (2003) that any prevention strategy for fungal and mycotoxin contamination must be carried out at an integrative level due to the multiple possible origins of fungal infection.

### Proximate composition of Mouldy Corn

The nutritive value of mouldy corn declined appreciably as shown in Table 2. The caloric, protein and lipid contents fell by 4.5%, 23% and 51% respectively. Crude fibre content was higher by 20% in the mouldy corn than the clean corn. This increase in crude fibre portion may be assumed that these fungi tend to utilize the soluble portion in the grains than the insoluble fibre. This result was similar to earlier findings of Akande *et al.* (2006). The ash and mineral contents however had an upward trend. The activities of the fungi

appear to have depleted the organic components of the corn grains perhaps for their metabolic activities while compensating in terms of the inorganic element as shown in Table 2. The actual reason for the increase in mineral content in contaminated corn however, is not clear but obviously attributable to fungus activities in the infected corn as earlier described in the report of Chuck-Hernandez *et al* (2012). The outcome of this study has shown that, apart from the risk of mycotoxicosis in animals fed mouldy corn, the nutritional contents of the diets are compromised because corn alone form about 50% of the diets for many poultry species. The caloric loss implies that the energy value is one important factor that should be considered while formulating feed for farm animals especially poultry.

### Performance and egg quality characteristics of experimental layers

The results for performance and egg quality characteristics of experimental birds are as shown in Table 3. The experimental birds responded to dietary treatments in varying manners. There was significant ( $P < 0.05$ ) change in terms of feed intake between birds fed clean corn and mouldy corn whereas the addition of adsorbents did not affect ( $P > 0.05$ ) feed consumption. Following similar reports of Jindal *et al.* (1994) that stated that mould and mycotoxins have negative effect on feed consumption; it appears that laying birds are sensitive to feed mouldiness at intake while the use of adsorbents did not appear to exert effect in suppressing factors responsible for repulsiveness of mouldy feed to laying chickens. Both weight gained and hen day production were significantly ( $P > 0.05$ ) affected by dietary treatments.

Table 2: Effect of mould contamination on chemical constituents of corn grain.

Chemical constituents	Clean corn	Mouldy corn	Net change $\pm$	Per cent change
Moisture (%)	10.3	14.1	3.8	26.95
Crude protein (%)	11.65	8.4	-2.75	23.61
Ether extract (%)	2.4	1.4	-1.5	51.72
Crude fibre (%)	5.5	6.3	1.1	20
Ash (%)	1.6	2.25	0.65	40.63
NFE (%)	64.25	70.85	6.6	10.27
Gross energy(kcal/kg)	3944.6	3766.4	178.2	4.52
Calcium (%)	1.1	2.41	1.31	54.36
Magnesium (%)	21.04	37.02	15.98	43.19
Potassium (%)	77.48	142.05	64.57	45.46

Mould contamination significantly reduced the weight gain and hen day production. The reduction in hen day production in the non-supplemented groups is probably due to the presence of aflatoxins which have been reported to cause a drop in egg production (Patil *et al.*, 2014). Although, the use of adsorbents did not show a significant change in weight and egg production, a closer look revealed that the adsorbents are more appropriate for mouldy feed but should be cautiously used on clean diets or diet free of mycotoxin. Also, the numerical rise in production could make a lot of difference in terms of economic returns in birds fed mouldy diets with the addition of adsorbents. Hence, the three adsorbents slightly influenced performance of birds positively in terms of body weight and Hen day production with activated charcoal showing superiority over other two adsorbents in sequestering the effect of mould and mycotoxin in the experimental birds. This result corroborates the assertion of Manal *et al.* (2012) who indicated that the activated carbon is one of the most potent adsorbents for removal of mycotoxins. The egg quality characteristics measured in this study were not significantly ( $P>0.05$ ) affected by mould contamination or addition of adsorbents. The mycotoxin concentration in this study is probably below toxic level because Cortyl (2008) reported that consumption of aflatoxins reduces egg weight in chickens. It is a general view that the use of binders offers an approach to salvaging feeds with low levels of mycotoxins and to protecting animals from the background levels of mycotoxins that, although low in concentration, routinely occur and may cause chronic disease problems and losses in performance. Since most of the commercial toxin adsorbents are selective in their binding capacity, no single binder product could meet all the desirable characteristics, but the potential currently exists for practical use of mycotoxins adsorbents for reducing mycotoxin exposure to animals.

### **Blood profile**

The adverse effects of mould and mycotoxins on animal health are expressed in a diverse range of symptoms including homeostasis, blood system damage, immunosuppression, hepatotoxicity,

nephrotoxicity, neurotoxicity, and even death according to Adebajo *et al.* (1994). There were changes ( $P<0.05$ ) in Red blood cell (RBC), Haemoglobin concentration (Hb), pack cell volume (PCV) and Mean Cell Volume (MCV) in this study (Table 4). Their depletion in birds fed contaminated diets invariably indicated the immunosuppressive potential of these mould and their metabolites due to possible cytotoxic effect on the lymphoid cells. Lower RBC and PCV are indication of inhibition of erythropoiesis of the experimental birds fed contaminated diets. It is well known that a reduced quantity and quality of erythrocytes and a decreased haemoglobin level as observed in birds fed mouldy diets, led to a deteriorated oxygen supply and predisposed the animal to anaemia. This is similar to earlier report of Anjorin and Catherine (2014) who indicated the negative effect of *Aspergillus* metabolites on haematology of broiler chicks. Other haematological indices such as WBC, corpuscular measurements, MCH and MCHC were not significantly affected ( $P>0.05$ ). The use of adsorbents showed some ameliorative tendencies in these blood indices and a pronounced effect ( $P<0.05$ ) manifested in the quantity of erythrocytes and haematocrit with activated charcoal showing superiority over other adsorbents in its binding capacity of the fungi metabolites. It may also be that the two commercial adsorbents were selective in their capacities to bind toxins. Serum total protein level was reduced ( $p<0.05$ ) significantly in birds fed mouldy corn compared with those fed clean corn. However, it was restored by addition of adsorbents used in this study. These data suggested that serum protein may be a sensitive index for mycotoxin or mould contaminant investigations. This result agrees with report of Wang *et al.* (2006) that indicate a fall in serum protein and albumin but restored by addition of activated charcoal. The findings also suggests that prolonged use of adsorbents might affect protein metabolism significantly in broilers fed clean corn, though the mode is not clear. The blood concentration of excretory and electrolyte constituents is an important tool in assessing the functional capacity of the kidney (Dyer *et al.*, 2000).

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**Table 3: Effect of three toxin adsorbents on performance and egg quality characteristics of layers fed mouldy corn in diets**

Parameters	Treatments								Mean Separation					P-values				
	NA	CA <sup>1</sup>	CA <sup>2</sup>	CA <sup>3</sup>	NA	CA <sup>1</sup>	CA <sup>2</sup>	CA <sup>3</sup>	Maize (M)		Adsorbent (A)			Pooled SEM	M	A	M×A	
	Clean Maize				Mouldy Maize				Clean	Mouldy	NA	CA <sup>1</sup>	CA <sup>2</sup>					CA <sup>3</sup>
Feed intake, g	123.6	125.9	121.6	129.9	113.1	120.6	118.0	118.0	124.3 <sup>a</sup>	117.5 <sup>b</sup>	118.5	123.3	119.84	122.01	3.76	0.03	0.63	0.84
Body wt,g	0.42	0.38	0.26	0.19	0.12	0.16	0.14	0.12	0.32 <sup>a</sup>	0.14 <sup>b</sup>	0.29	0.32	0.20	0.16	0.53	0.03	0.48	0.86
HDP, %	61.08	61.18	59.70	58.34	51.86	54.10	52.66	52.84	60.08 <sup>a</sup>	52.83 <sup>b</sup>	56.50	57.64	56.18	55.59	2.47	0.04	0.41	0.95
Egg wt, g	62.70	62.20	62.28	60.50	60.20	60.90	61.70	59.90	61.92	60.70	61.45	61.55	61.99	60.20	1.64	0.32	0.74	0.94
FC ratio	3.27	3.35	3.26	3.37	3.68	3.67	3.65	3.81	3.46	3.59	3.27	3.52	3.56	3.79	0.22	0.42	0.20	0.88
Shell (mm)	1.70	1.93	1.46	1.46	1.46	1.46	1.30	1.56	1.63	1.45	1.58	1.70	1.38	1.51	0.006	0.31	0.42	0.75
Alb. wt(g)	4.43	4.86	4.23	4.53	4.20	4.36	4.23	4.06	4.51	4.21	4.31	4.61	4.23	4.15	0.35	0.56	0.35	0.97
Yolk index (mm)	1.40	1.36	1.40	1.33	1.40	1.36	1.43	1.43	1.37	1.41	1.40	1.36	1.42	1.38	0.11	0.07	0.10	0.41
Yolk wt (g)	14.59	16.3	15.2	17.10	16.23	15.43	16.20	16.20	15.80	16.01	15.41	15.96	15.7	16.67	1.56	0.59	0.08	0.64

<sup>abc</sup>means within each row with different superscripts are significantly different (P<0.05). NA- no adsorbent; CA<sup>1</sup>-Activated charcoal, CA<sup>2</sup>- commercial adsorbent 2, CA<sup>3</sup> – commercial adsorbent 3, M- maize, A-adsorbent, SEM- standard error of mean

**Table 4: Some blood constituents of laying chickens fed mouldy diet supplemented with three toxin adsorbents**

Parameters	Treatments								Mean Separation						P-values			
	NA	CA <sup>1</sup>	CA <sup>2</sup>	CA <sup>3</sup>	NA	CA <sup>1</sup>	CA <sup>2</sup>	CA <sup>3</sup>	Maize (M)		Adsorbent (A)			Pooled SEM	M	A	M×A	
	Clean Maize				Mouldy Maize				Clean	Mouldy	NA	CA <sup>1</sup>	CA <sup>2</sup>					CA <sup>3</sup>
PCV, %	33.00	32.50	30.00	27.00	24.50	27.60	25.50	24.00	30.63 <sup>a</sup>	25.40 <sup>b</sup>	28.75 <sup>ab</sup>	30.05 <sup>a</sup>	27.75 <sup>ab</sup>	25.50 <sup>b</sup>	1.22	0.05	0.04	0.59
Hb. conc., %	10.85	8.99	9.98	9.77	7.95	8.43	8.46	8.71	9.89 <sup>a</sup>	8.39 <sup>b</sup>	9.40	8.71	9.22	9.35	0.24	0.04	0.46	0.98
RBCx10 <sup>9</sup> /L	4.37	4.53	4.91	5.40	3.80	4.10	4.24	4.28	4.80 <sup>a</sup>	4.11 <sup>b</sup>	4.09 <sup>b</sup>	4.31 <sup>b</sup>	4.58 <sup>ab</sup>	4.84 <sup>a</sup>	0.05	0.05	0.04	0.78
WBC x 10 <sup>6</sup> /L	49.80	52.00	56.00	57.00	58.66	59.66	63.67	55.66	53.70	59.40	54.23	55.83	59.84	56.33	2.30	0.26	0.97	0.50
MCV, fl	75.51	71.74	61.10	55.00	64.47	62.20	60.14	56.07	65.84	60.70	69.99 <sup>a</sup>	69.97 <sup>a</sup>	60.75 <sup>ab</sup>	55.53 <sup>b</sup>	9.01	0.32	0.79	0.57
MCHC,%	32.90	27.70	33.33	36.20	32.40	33.10	33.20	36.40	32.53	33.78	32.65	30.40	33.30	36.30	0.50	0.58	0.56	0.94
MCH, pg	24.83	19.85	20.33	18.09	20.92	20.56	19.95	20.35	20.78	20.45	22.88	20.21	20.14	19.22	2.30	0.66	0.92	0.90
Urea, mg/dl	8.10	7.7	7.2	7.2	13.25	11.55	9.35	12.95	7.55 <sup>b</sup>	12.26 <sup>a</sup>	10.65	9.63	8.28	10.08	1.33	0.01	0.45	0.18
Creatinine, mg/dl	0.75	0.70	0.70	0.75	0.90	0.95	0.75	0.90	0.73 <sup>b</sup>	0.86 <sup>a</sup>	0.83	0.83	0.73	0.83	0.04	0.03	0.25	0.42
ALP, iu/l	70.5	82.5	80.5	93.5	92.5	91.5	84.5	99.0	84.13 <sup>b</sup>	89.5 <sup>a</sup>	81.5 <sup>b</sup>	87.25 <sup>b</sup>	82.5 <sup>b</sup>	96.25 <sup>a</sup>	10.71	0.04	0.03	0.03
AST, iu/l	75.0	93.2	84.5	105.5	132.5	106.5	111.0	105.5	89.55 <sup>b</sup>	113.8 <sup>a</sup>	103.5	99.85	97.75	105.5	8.37	0.01	0.44	0.07
ALT, iu/l	11.5	11.5	13.0	14.0	17.5	14.5	13.0	14.5	12.5 <sup>b</sup>	15.0 <sup>a</sup>	13.0	14.5	13.0	14.25	0.98	0.02	0.56	0.14
Total Protein, %	7.65	7.00	7.15	7.10	6.05	6.55	6.45	6.45	7.18 <sup>a</sup>	6.03 <sup>b</sup>	6.25	6.75	6.80	6.78	0.22	0.03	0.32	0.01
Albumin,%	2.65	2.55	2.05	2.75	2.55	2.45	2.83	2.1	2.50	2.35	2.63	2.50	2.44	2.43	0.09	0.09	0.07	0.05
Cholesterol, g/dl	118.5	125	121.5	114.5	129.5	120	131.5	118.5	119.88	124.88	124.0	122.5	126.5	116.5	7.80	0.21	0.15	0.12

<sup>abc</sup>means within each row with different superscripts are significantly (P<0.05) different. NA- no adsorbent; CA<sup>1</sup>-Activated charcoal, CA<sup>2</sup>- commercial adsorbent 2, CA<sup>3</sup> – commercial adsorbent 3, M- maize, A-adsorbent, SEM- standard error of mean, AST-aspartate transferase, ALT-alanine transferase

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Urea, creatinine and electrolytes are the most sensitive biochemical markers employed in the diagnosis of renal damage because urea and creatinine are excreted through the kidney. So in cellular damage, retention of urea and creatinine in the blood are expected (Flanigen, 1984). Marked increase in serum urea and creatinine as noticed in this study confirms an indication of functional changes in the kidney. The result was similar to report of Dyer *et al.* (2000). Similarly, Smith *et al.* (2001) reported that broiler serum uric acid was increased significantly by high level of DON (5-10 mg/kg) and fusaric acid (17 mg/kg), and was restored by addition of 0.2% esterified glucomannan (Swamy *et al.*, 2004). Therefore, the significant increase ( $P < 0.05$ ) in urea and creatinine levels in contaminated treatments is a classical sign that the kidney might have been affected by the exposure arising from intake of mouldy diets. Damage to the kidney may result in reduced erythropoietin production, resulting in high urea which may in turn be associated with low blood volume as noticed with low PCV. Pimpukdee *et al.* (2004) further observed that the rise in these excretory products may lead to an elevation in inflammatory cell types in the blood. However, the different adsorbents used were able to restore these blood excretory products differently ( $P > 0.05$ ). Increased serum uric acid level by DON (12 mg/kg) and fusaric acid (17 mg/kg) contamination, which was restored to normal level by 0.2% esterified glucomannan, was also reported in laying hens (Smith *et al.*, 2004).

Activity of the enzymes measured Alanine transferase (ALT) and Aspartate transferase (AST) in the serum of the chickens differ significantly across the treatments. The levels of the enzymes were higher ( $P < 0.05$ ) in birds fed mouldy corn which indicate a pathological change in these experimental animals. ALT is a key enzyme in the biotransformation and detoxification of various toxicants, reactive oxygen species, and endo- and xenobiotics. Rise in these enzymes are suggestive of presence of toxic compounds such as aflatoxin (not determined). The use of the adsorbent probably reduced the activities of the enzymes by lowering amount of mycotoxins available to the animals. Some studies have suggested that the detoxification pathways of some toxicants may be

connected to antioxidative defense mechanisms (Nordberg and Arner, 2001; Abdel-Wahab *et al.*, 2003).

### CONCLUSION

This study has indicated that mould contamination of corn in the study location, western part of Nigeria has high tendency of aflatoxin contamination because of prevalence of toxigenic strains of *Aspergillus* species (*A. flavus* and *A. parasiticus*). The reduced nutritive values in terms of crude protein, ether extract and energy may compromise nutrient requirement of animals fed such contaminated corn. Laying birds fed mould contaminated corn-based diets showed retarded growth, reduced egg production, poor feed conversion; depletion in some haematological parameters (haematocrit, haemoglobin concentration and erythrocyte count); reduced serum total protein and increased in serum excretory products (urea and creatinine) and increased activities of some liver enzymes which are indicative of a pathological shift. However, the addition of three adsorbents (activated charcoal and two other commercial adsorbents at 0.4%, prevents the adverse effects of mould contamination to varying extents, with activated charcoal showing some superiority in its adsorbing tendency over others adsorbents used in this study. It was also observed that addition of adsorbents in diets of birds on clean corn appeared to have capacity to impact negatively on all parameters measured in this study. It is therefore recommended that regulation of feed ingredients should be intensify to prevent use and entry of mould contaminated grain in poultry feed while feed millers and poultry farmers should avoid indiscriminate use of toxin adsorbents in layers' feed.

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### REFERENCES

- Abdel-Wahab, M.H., E-Mahdy, M.A., Abd-Ellah, M.F., Helal, G.K., Khalifa, F. and Hamada, F.M.A. 2003. Influence of p-coumaric acid on doxorubicin-induced oxidative stress in rat's heart. *Pharmacol. Res.*, 48: 461-465.



- Adamu, S., Bukar, A. and Mukhtar, M.D. 2009. Isolation and identification of postharvest spoilage fungi associated with sweet oranges (*Citrus sinensis*) traded in Kano Metropolis. *J. Pure & Appl. Sc.* 2(1):122 – 124.
- Adebanjo, L.O., Bamgbelu, O. A. and Olowu, R.A., 1994. Mould contamination and the influence of water activity and temperature on mycotoxin production by two *Aspergilli* in melon seed. *Natruing*, 38: 209-217.
- Akande, T.O., Odunsi, A.A. and Olayeni, T.B. 2006. Influence of activated charcoal on performance of laying hens fed fungus infected based diets. *Trop. J. Anim. Sci.*, 56(2):11-20
- Anjorin Samuel Toba and Catherine Oluebube Cyriacus 2014. Haematological effect of *Aspergillus* species metabolites on broiler chicks. *Am. J. Res. Com.* 2(1): 172.
- Atanda O, Makun H. A. and Ogara, I. M. 2013. Fungal and Mycotoxin Contamination of Nigerian Foods and Feeds In: Mycotoxin and Food Safety in Developing Countries 1<sup>st</sup> Ed. by Makun HA Published by InTech Janeza Trdine 9, 51000 Rijeka, Croatia
- Cheesbrough, M. 2000. District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press, Cambridge, U.K.
- Chuck-Hernandez, Garcia- Lara, C. S. and Serna-Saldivar, S.O. 2012. Conversion into bioethanol of insect (*Sitophilus zeamais* Motschulsky), mold (*Aspergillus flavus* Link) and sprout-damaged maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench). *J. Cereal Sc.* 55(3): 285-292.
- Cortyl, M. 2008. Mycotoxins in animal nutrition-problems and solutions. <http://www.aquafeed.com/docs/fiaap2008/Cortyl.pdf>.
- Dyer, A., Morgan, S., Wells, P., Williams, C. 2000. The use of zeolites as slow release anthelmintic carriers. *J. Helmit.*, 74: 137-141.
- Flanigen, E.M. 1984. Adsorption properties of molecular sieve zeolites. In: Pond, W.G., Mumpton, F.A., Eds. *Zeo-Agriculture*. International Community for Natural Zeolites, Brockport, pp 55-68.
- James, G. C. and Natalie, S. (2001). Microbiology. A laboratory Manual (ed.). Pp. 211-223.
- Jindal N., Mahipal S.K. and Mahajan N.K. 1994. Toxicity of aflatoxin B1 in broiler chicks and its reduction by activated charcoal. *Res. Vet. Sci.*, 56: 37-40
- Katole S.B., Kumar P and Patil R.D. (2013). Environmental pollutants and livestock health: a review *Vet. Res. Intl* 1: 1-13.
- Kuiper-Goodman, T. 1998. Food safety: mycotoxins and phycotoxins in perspective. In M. Miraglia, H. Van, Egmond, C. Brera & Gilbert J., eds. *Mycotoxins and phycotoxins - developments in chemistry, toxicology and food safety*. Oxford, United Kingdom, International Union of Pure and Applied Chemistry (IUPAC)
- Manal M. Z., El-Midany S. A., Shaheen H. M. and Laura Rizzi, 2012. Mycotoxins in animals: Occurrence, effects, prevention and management . *J. Toxicol. Environ. Health Sc.* 4(1), pp. 13-28
- Nordberg, J and Arner, E.S.J. 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic. Biol. Med.*, 31: 1287-1312
- Okoli I. C., Nweke C. U., Okoli C. G., and Opara M. N. 2006. Assessment of the mycoflora of commercial poultry feeds sold in the humid tropical environment of Imo State, Nigeria. *Intl J. Environ. Sci. Tech.* 3(1):9–14.
- Patil, R.D, Rinku S. and Rajesh K.A. 2014. Mycotoxicosis and its control in poultry: A review. *J. Poult. Sc. Tech.* 2(1):1-10
- Pimpukdee, K., Kubena, L. F., Bailey C. A., Huebner H. J., Afriyie-Gyawu, E. and Phillips T. D. 2004. Aflatoxin-Induced Toxicity and Depletion of Hepatic Vitamin A in Young Broiler Chicks: Protection of Chicks in the presence of low levels of NovaSil PLUS in the diet. *Poult. Sc.* 83: 737-744
- Reddy K.R.N and Salley B. 2011. Co-occurrence of mould and mycotoxins in corn grains used for Animal feed in Malaysia. *J. Anim. Vet. Adv.* 10(5): 668-673
- Robens J and Cardwell K 2003. The Costs of Mycotoxin Management to the USA: Management of Aflatoxins in the United States. *Toxin Rev.* 22(2-3): 139-152.
- S.A.S. 2006. Statistical Analysis System User's Guide. Version 9 edition. SAS Institute, Inc. Cary, North Carolina, USA.
- Shamsudeen, P., Shrivastava, H. Singh R and Chandra D. 2013. Effect of Chelated and Inorganic Trace Minerals on Aflatoxin

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- Synthesis in Maize. *J. Poult. Sc. Tech. 1(1)*: 13-16.
- Shareef, A. M. 2010. Molds and mycotoxins in poultry feeds from farms of potential mycotoxicosis *Iraq. J. Vet. Sc. 24(1)*:17-25.
- Shenasi, M., Aidoo, K. E. and Candlish, A. A. G. 2002. Microflora of Date Fruits and Production of Aflatoxin at Various Stages of Maturation. *Intl J. Food Microbiol. 79* : 113-119.
- Smith, T. K., E. J. MacDonald and S. Haladi. 2001. Current concepts in feed-borne mycotoxins and the potential for dietary prevention of mycotoxicoses. In: Science and Technology in the feed industry, Proceedings of Alltech's 17th Annual Symposium. Nottingham University Press (Ed. T. P. Lyons and K. A. Jacques), UK, pp.183-190.
- Smith, T.K., S.R. Chowdhury and H. V. L. N. Swamy. 2004. Comparative aspects of fusarium mycotoxicosis in broiler chickens, laying hens and turkeys and the efficacy of a polymeric glucomannan mycotoxin adsorbent: Mycosorb. In: Nutritional Biotechnology in the Feed and Food Industries, Proceedings of Alltech's 20th Annual Symposium. Nottingham University Press (Ed. T. P. Lyons and K. A. Jacques), UK, pp. 103-109.
- Swamy, H. V. L. N., T. K. Smith, N. A. Karrow and H. J. Boermans. 2004. Effects of feeding blends of grains naturally contaminated with Fusarium mycotoxins on growth and immunological parameters of broilers. *Poult. Sci. 83*:533-543
- Wang R. J., Fui1 S. X., Miao C. H. and Feng1 D. Y. 2006. Effects of Different Mycotoxin Adsorbents on Performance, Meat Characteristics and Blood Profiles of Avian Broilers Fed Mold Contaminated Corn . *Asian-Aust. J. Anim. Sci. 19 (1)* : 72-79.
- Weaver, A.C., See, M.T., Hansen, J.A., Kim, Y.B., Souza, A., Middleton, T.F. and Kim S.W. 2013 The Use Of Feed Additives To Reduce The Effects Of Aflatoxin And Deoxynivalenol On Pig Growth, Organ Health And Immune Status During Chronic Exposure. *Toxins, 5*:1261-1281