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Prevalence of aflatoxin b1 in some common poultry feed ingredients and optimum inclusion levels of mycofix® binder as feed additive on performance of broiler chickens

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Abstract

A preliminary study was carried out to determine the prevalence of Aflatoxin B1 (AfB1) contamination and common moulds growing in some selected poultry feed raw materials in Zaria. Five different feed raw materials:Maize (MZ), soybean cake (SBC), groundnut cake (GNC), brewers dried grain (BDG) and maize offal (M/O), were collected from four feed mills and the open market. The common moulds isolated from the samples were Mucor spp., Aspergillus spp., Fusarium spp., Penicillum spp., Curvularia spp. and Rhizopus spp. AfB1 contamination showed that maize and soya bean cake were less than the 20 parts per billion (ppb) permissible limits for AfB1 in poultry feed ingredients, while BDG, M/O and GNC were 40, 60 and 80 % respectively above 20 ppb permissible limits. A feeding trial was conducted to evaluate the response of broiler chickens to Mycofix® a toxin binder. Three hundred and thirty Ross, 1-day old broiler chicks were assigned to five dietary treatments, each with three replicates of 22 chicks per replicate in a completely randomized design (CRD). Treatment 1 was the control diet without Mycofix® while treatments 2, 3, 4 and 5 had Mycofix® included at 100, 200, 300 and 400 g /100kg diet, respectively at both starter and finisher phases. Experimental diets and water were given to the birds ad libitum during eight weeks of the feeding trial. Data were subjected to Analysis of Variance (ANOVA) using General Linear Model procedure of SAS and significant differences among treatment means were compared using Dunnette test of significance. Results of the starter phase showed that birds fed 400 g/100kg Mycofix® diet had the best final body weight (902.67g), weight gain (839.67g) and average daily weight gain (29.98g). Birds fed 200 g /100 kg Mycofix® had the lowest feed conversion ratio of 1.60 and the least cost per kilogram gain (₦138.52). At the finisher phase, birds on 400 g/100kg Mycofix® diet had the best final weight (2345.3g), weight gain (1403.3g), average daily gain (50.1g), feed conversion ratio (2.1) and least feed cost per kilogram gain (₦167.20). It may be concluded that addition of Mycofix® to diets of broiler chickens at 400g/ 100kg at both starter and finisher phases of the study improved performance significantly and gave the least cost of production.

Keywords: Broiler chickens, Mycotoxins, Toxin binders, Mycofix® performance, Aflatoxins

Introduction

Feed represents the greatest single expenditure associated with poultry production. Nutritional research in poultry has therefore centered on issues related to identifying barriers to effective digestion and utilization of nutrients, and on approaches for improving feed *utilization* (Ravindran, 2010). The quality of feed ingredients is very important as this will determine the feed end-product quality, as such a more precise evaluation of the quality of feed raw materials is needed (Kersten *et al.*, 2005). Feed materials may be contaminated at any time during growing, harvesting, processing, storage and distribution of the feed. Feeds may contain diverse microflora that are acquired from multiple environmental sources, including dust, soil, water, and insects (Maciorowski *et al.*, 2006).

According to the Food and Agricultural Organization (FAO), 25 % of the world's crop harvests are contaminated with mycotoxins (FAO, 2012). Surveys of mycotoxin levels in poultry feeds often reveal the presence of a number of different toxins with most samples in a recent survey containing at least 10 contaminants. Contamination of feeds with mycotoxins is a worldwide problem, with the most important in poultry being those produced by the genera *Fusarium, Aspergillus and Penicillium* (Siska, 2013).

Nutritional researchers' have therefore implored the use of toxin binders as an approach to salvaging feed contamination with mycotoxins and protecting animals from disease problems and losses in performance.

The study was therefore; designed to alleviate mycotoxin incidences in feed and to evaluate the effect of Mycofix® (a mycotoxin binder) on performance of broiler chickens. The Mycofix®, is a product line from Biomin® an animal nutrition company in Austria, represents a specially developed feed additives that protect animal health by deactivating mycotoxins found in contaminated feed. Mycofix® is suitable for use in poultry, pig and ruminant feed as well as fish and shrimp diets (The Poultry Site, 2014).

Materials and methods

Feed ingredient sample collection

Samples of Maize (MZ), soybean cake (SBC), groundnut cake (GNC), brewers dried grain (BDG) and maize offal (M/O) were purposively collected from four major feed mills in Zaria and Giwa grain market in Giwa, for Aflatoxin B1 contamination and mycobiota analysis. Samples of maize, soya bean cake, groundnut cake, maize offal and brewer's dried grain were collected. About 500 grams of each ingredient were collected from the designated feed millers and put into medium size transparent polythene bags and labeled. Thereafter, samples were taken to the Mycotoxin laboratory of Department of Crop Protection, Faculty of Agriculture, Ahmadu Bello University, Zaria for Aflatoxin B1(AfB1) analysis while fungi isolation and identification was done in the Plant Pathology laboratory.

Aflatoxin determination

AfB1was analysed using the Enzyme Linked Immune Sorbent Assay (ELISA) method. An indirect competitive ELISA protocol was used for the quantitative analysis of aflatoxin. Aflatoxin extraction and determination were performed according to the manufacturer's procedure following the AOAC (2000) laboratory procedures for aflatoxin determination. All reagents and standards were from Trilogy analytical laboratory Washington. Samples were incubated in Techmel and Techmel USA, model; TT – 9082 incubator and an ELISA READER model; Bio – rad (404-750nm) was used to take the readings.

Categorization of level of contamination of Aflatoxin B1

Collected samples were categorized into low (< 20 ppb), medium (20-50 ppb) and high (>50 ppb) levels of contamination of AfB1 following the general guidelines of United States (FDA) Food and Drug Administration (Hanif *et al.*, 2006).

Fungal cultivation and isolation

The growth media used for the study was potato dextrose agar (PDA) amended by streptomycin. The media were prepared and thereafter sterilized by autoclaving at a temperature of 121 °C for 15 minutes at 6.5 kg per square inch. They were then allowed to cool to 45 °C on the workbench before plating out into petri dishes. The dishes were inoculated with feed ingredient samples and incubated at room temperature for 5 days at the end of which they were examined for fungal growth. Fungi were isolated and cultured according to the method described by Pitt and Hocking (2009). Growths were further sub-cultured onto fresh media for another 5 days to obtain pure cultures. After isolation, identification of the pure isolates shown on the plates was confirmed using macroscopic and microscopic morphology (light microscope) observation and the interpretative key of some common genera of moulds (Samson *et al.*, 2004; Pitt and Hocking, 2009).

Experimental site and location

The experiment was carried out at the poultry unit of Department of Animal Science Teaching and Research farm, Faculty of Agriculture, Ahmadu Bello University, Samaru, Zaria. The site is located in the guinea savannah zone of Nigeria, Latitude 11° 9' 46" N and Longitude 7°37'45"E at an altitude of 610m above sea level. The temperature ranges between 26-40°C depending on the season while the relative humidity during the dry and wet seasons are 21 and 72% respectively. The wet period in Zaria is between May and October with annual rainfall of about 1500mm (Institute for Agricultural Research, Meteorological Unit, 2016).

Experimental design and management of birds

Three hundred and thirty (330) Ross strain, 1 day-old broiler chicks were weighed, and randomly assigned into five dietary treatments with three replications of 22 chicks per pen, in a completely randomized design (CRD). The birds were reared in deep litter pens and managed with all the necessary routine management practices. Feed and water were provided *ad libitum* during the 8 weeks period of the experiment. Five diets were formulated at both the starter (0 -28days) and finisher (28-56days) phases of the feeding trials. The diets were formulated to meet the NRC (1994) recommended nutrient levels for broiler chickens. Diet 1(without Mycofix®) was control, while diets 2. 3, 4 and 5 had 100, 200, 300 and 400g Mycofix® / 100kg feed respectively. Composition of ingredients and estimated nutrient contents of diets are shown in Table 1 and 2, respectively.

Table 1: Ingredient composition of broiler starter diets (0 – 4 weeks)

	Mycofix® Levels of Inclusion (g / 100kg)				
Ingredients	**0**	**100**	**200**	**300**	**400**
Maize	56.00	56.00	56.00	56.00	56.00
Soya bean cake	29.70	29.70	29.70	29.70	29.70
Groundnut cake	10.00	10.00	10.00	10.00	10.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50
Common salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10
Vitamin premix[A]	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis					
ME Kcal /kg	2902	2902	2902	2902	2902
Crude protein (%)	23.19	23.19	23.19	23.19	23.19
Crude fibre (%)	3.59	3.59	3.59	3.59	3.59
Ether extract (%)	3.16	3.16	3.16	3.16	3.16
Calcium (%)	1.32	1.32	1.32	1.32	1.32
Phosphorous (%)	0.87	0.87	0.87	0.87	0.87
Lysine (%)	1.46	1.46	1.46	1.46	1.46
Methionine (%)	0.56	0.56	0.56	0.56	0.56
Feed cost (₦/kg)	83.82	83.88	83.94	84.00	84.06
Total AfB1 ppb	75.14	75.14	75.14	75.14	75.14

[A]Biomix Broiler starter premix provide per kg diet Vit. A, 10,000 I.U; Vit D_3, 2000 I.U; Vit E 23mg; Vit. K, 2mg; Calcium Pantothenate, 7.5mg B12, 0.015mg; Folicacid, 0.75mg; Choline Chloride, 300mg; Vit B_1 1,8mg, Vit. B_2, 5mg; Vit B_6, 3mg; Manganese, 40mg; Iron, 20mg; Copper, m3g; Iodine, 1mg; Cobalt, 0.2mg; Selenium, 0.2mg' Zinc, 30mg

Table 2: Ingredient composition of broiler finisher diet (5 – 8 weeks)

	Mycofix® Levels of Inclusion (g / 100kg)				
Ingredients	**0**	**100**	**200**	**300**	**400**
Maize	58.00	58.00	58.00	58.00	58.00
Soya bean cake	20.00	20.00	20.00	20.00	20.00
Groundnut cake	13.00	13.00	13.00	13.00	13.00
Maize offal	4.70	4.70	4.70	4.70	4.70
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50
Common salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10
Vitamin premix[A]	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis					
ME Kcal/kg	2929	2929	2929	2929	2929
Crude protein (%)	20.54	20.54	20.54	20.54	20.54
Crude fibre (%)	4.11	4.11	4.11	4.11	4.11
Ether extract (%)	3.35	3.35	3.35	3.35	3.35
Calcium (%)	1.32	1.32	1.32	1.32	1.32
Phosphorous (%)	0.85	0.85	0.85	0.85	0.85
Lysine (%)	1.27	1.27	1.27	1.27	1.27
Methionine (%)	0.50	0.50	0.50	0.50	0.50
Feed cost (₦/kg)	79.44	79.50	79.56	79.62	79.68
Total AfB1	99.04	99.04	99.04	99.04	99.04

[A]Biomix Broiler Finisher premix provide per kg f diet Vit. A, 10,000 I.U; Vit D_3, 2000 I.U; Vit E 23mg; Vit. K, 2mg; Calcium, Pantothenate, 7.5mg B12, 0.015mg; Folic acid, 0.75mg; Choline Chloride, 300mg; Vit B_1 1,8mg, Vit. B_2, 5mg; Vit B_6, 3mg; Manganese, 40mg; Iron, 20mg; Copper, m3g; Iodine, 1mg; Cobalt, 0.2mg; Selenium, 0.2mg' Zinc, 50mg

Growth study

The initial average body weights of the day old chicks were taken, and then subsequently body weights of the birds and feed intake were recorded weekly at both starter and finisher phases. While feed to gain ratio and feed cost per kg gain were calculated from the values obtained from weight gain and feed intake at both phases. Mortality was recorded as it occurred.

Carcass analysis

At the end of the 8th week, six chickens were randomly selected from each treatment, which represented the average weights of the group for carcass evaluation. The birds were fasted of feed overnight, weighed and slaughtered by severing the jugular vein to bleed. The birds were then scalded in hot water to remove their feathers. Live weight was taken before slaughtering, the dressed weight, cut parts (breast, thigh, drum stick, back, wings, neck and head) were measured and expressed as percent dressed weight while dressing percentage was calculated as live weight divided by dressed weight multiplied by 100. The organs were measured and expressed as percent of their live weights.

Digestibility study

At the end of the finisher phase, six (6) birds were selected from each treatment, which had representative weights of the group and kept in individual metal cages for total faecal collection. The birds were allowed a period of three days to adjust to the cage environment. Thereafter, a known amount of the experimental feed was weighed and given daily for 5 days with water. Trays were placed under each cage to enable daily feacal collection. Total faecal droppings were collected for five consecutive days, weighed and oven-dried at 65 ^{0}C for 24 hours. The dried samples were then assayed for their nutrient contents using methods described by AOAC (2000) at the

Biochemistry Laboratory of the Department of Animal Science, Ahmadu Bello University Zaria. Nutrient retention was determined for crude fibre, crude protein, ether extract, ash and nitrogen free extract using the formula below:

% Retention of Nutrient =

$$\frac{\text{Nutrient in feed} - \text{Nutrient in faeces}}{\text{Nutrient in feed}} \times 100$$

Statistical analysis

All data generated from the feeding trial were subjected to Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS) package. Significant differences between treatment means were separated using Dunnetts Test (SAS, 2003).

Results and discussion

Feed Ingredient survey for Aflatoxin B1

Prevalence of Aflatoxin B1 (AfB1) in some selected ingredients used as poultry finished feed is shown in Table 3. Results obtained from feed mill A showed 0.1 ppb in Maize (MZ) and 1.8 ppb in Soya bean cake (SBC) which were less that 20 ppb AfB1 permissible level in feed ingredients and therefore categorised as low, while 736.5 ppb in Groundnut cake (GNC), 129.3 ppb in Brewers dried grain (BDG) and 61.7 ppb in Maize offal (M/O) were above 20 ppb and categorized as high concentration. Aflatoxin B1 prevalence in feed ingredients obtained from feed mill B were 5.0 ppb MZ, 3.3 ppb SBC, 7.8 ppb M/O, were categorised as low in concentration, while 15320.9 ppb in GNC and 160 ppb in BDG were above 20ppb. Feed mill C ingredients contained 1.7ppb in MZ, 3.8 ppb in SBC, 0.1 ppb in M/O and 1.2 ppb BDG which were below 20 ppb while 6539.9 ppb in GNC was above 20ppb. Feed mill D ingredients contained, 0.1 ppb in MZ 2.2 ppb in SBC, 3.4 ppb in GNC, 0.1 in M/O and 0.8 ppb in BDG which were all below 20 ppb and the random samples E ingredients contained 0.1ppb in MZ, 0.3 ppb in SBC, 6.0 ppb in BDG were below 20ppb and 80ppb in M/O and 111.5 ppb in GNC which were above 20ppb.

Contamination of feed ingredients with aflatoxins is not an uncommon phenomenon. Several workers (Okoli *et al.*, 2007; Nemati *et al.*, 2014) in different parts of the world have reported incidence of aflatoxins. The result of the present study showed the presence of AfB1 in the sampled ingredients. The general guidelines of USFDA (United States Food and Drug Administration) recommended the permissible limits of 20 ppb contamination level of aflatoxins in poultry ingredients and feed (Ghahri, 2010).

Table 3: Mycotxin analysis of some feed ingredient samples

	Aflatoxin B1 parts per billion (ppb)					
Ingredients	**A**	**B**	**C**	**D**	**E**	**Codex Limit**
Maize	0.1	5.0	1.7	0.1	0.1	20 ppb
Soya bean cake	1.8	3.3	3.8	2.2	0.3	20 ppb
Groundnut cake	736.5	1532.9	6539.9	3.4	111.5	20 ppb
Brewers dried grain	129.3	160.0	1.2	0.8	6.0	20 ppb
Maize Offal	61.7	7.8	0.1	0.1	80.0	20 ppb

This high incidence of AfB1 in GNC is not only hazardous to poultry alone because of direct exposure, but also suggests a high risk to human health with the possibility of indirect exposure through contaminated meat, eggs and other poultry products and by products (Maqbool *et al.*, 2004; Bintvihok and Kositcharoenkul, 2006).

Fungi isolation and identification

The overall frequency of isolation of

different fungal species from feed raw materials used in feed mills / Toll millers in Zaria is shown in Table 4. Six fungal species were identified with *Aspergillus spp* having the highest prevalence rate (30.77 %), followed by *Rhizopus spp* (23.08 %) and *Fusarium spp* (20.0 %) while *Mucor, Penicilliun* and *Curvularia* spp were 12.31, 7.69 and 6.15 %, respectively.

Table 4: Overall frequency of fungal species from feed raw materials sold in feed mills in Zaria

Fungi species	Frequency	Percentage
Aspergillus	18	30.77
Rhizopus	15	23.08
Fusarium	13	20.0
Mucor	8	12.31
Penicillium	5	7.69
Curvularia	4	6.15
Total	**63**	**100**

The present study revealed that *Aspergillus spp, Rhizopus spp* and *Fusarium spp* were the common moulds growing in poultry commercial feed raw materials sold in the feed mills sampled in Zaria. The high prevalence rate of fungal species seen in this study supports the findings of Atanda *et al.* (2013), Bastinaelli and Le Bas (2002) and Cheesbrough (2006), which stated that tropical climates such as exist in Nigeria are more conducive for fungal and microbial contaminations of poultry feed raw materials. Certain agricultural produce have been observed to permit the growth of some moulds over others, maize for example, allows the growth of aflatoxins and fumonisins producing moulds above others, while groundnuts have been found to be excellent substrate for aflatoxin contamination (Bankole and Adebanjo, 2003). Several factors such as storage conditions (Moss, 2002), climatic conditions (Dersjant-Li *et al.*, 2003) like high temperature and humidity have been found to provide optimal growth conditions for moulds and affect fungal colonization in grains and compound feeds (Rawal *et al.*, 2010).

The result of the growth performance of broiler chicks fed different levels of Mycofix® is shown in Table 5. Final body weight, weight gain, feed intake, feed conversion ratio and feed cost per kilogram gain were significantly (P < 0.05) affected by dietary treatments.

The final body weight (902.67g), weight gain (839.67g) and daily gain (29.98g) were significantly (P<0.05) higher in diets containing 400g Mycofix®. Feed intake was higher (P<0.05) in treatments containing 300, 400g Mycofix® and control compared to 100 and 200g Mycofix® inclusions

Table 5: Growth performance of broiler chicks fed diets with Mycofix® (0 – 4 weeks)

	Levels of Mycofix inclusion (g/100 Kg diet)					
Parameters	**0**	**100**	**200**	**300**	**400**	**SEM**
Initial wt (g/bird)	63.13	63.06	63.01	63.05	63.03	0.03
Final weight (g/bird)	833.33[c]	818.33[c]	827.00[c]	874.33[b]	902.67[a]	13.65i
Wt gain (g/bird)	770.33[c]	755.27[c]	763.99[c]	811.33[b]	839.67[a]	14.07
Av. daily Wt. gain (g/bird)	27.51[c]	26.97[c]	26.80[c]	28.97[b]	29.98[a]	0.50
Total FI (g/bird)	1454.7[a]	1264.33[b]	1260.67[b]	1469.33[a]	1401.33[a]	26.98
Av daily feed intake (g/bird	51.95[a]	45.15[b]	45.02[b]	52.04[a]	50.04[a]	0.96
Feed conversion ratio	1.87[b]	1.63[a]	1.60[a]	1.73[a]	1.67[a]	0.03
Feed cost/kg gain (₦/kg)	158.46	140.23	138.52	152.20	140.23	
Mortality	0.00	1.50	0.09	0.00	3.00	0.01

abc, Means with different superscripts along same rows show significant differences (P < 0.05) SEM: Standard error mean; Av: Average , FI: Feed intake, %: percentage, wt; weight, kg; kilogram

All the treatments containing Mycofix® had a significantly (P< 0.05) lower feed conversion ratio value than in the control treatment. Feed cost per kilogram gain were significantly (P < 0.05) better for all the Mycofix® treatments compared to the control and T_3 (200g Mycofix®) had the least cost (₦138.52). Mycofix® inclusion at 100, 200, 300 and 400 g/100kg diet reduced the cost of production by 11.51% (₦18.23), 12.54% (₦19.94), 3.95% (₦6.26) and 11.51 % (₦18.23) respective per bird at the starter phase. The highest percentage mortality (3.0) was recorded in the 400g Mycofix® diet.

The increase in body weight gain with increasing Mycofix® may be attributed to the detoxifying effect on mycotoxins present in the feed ingredients which improves the quality of the diet. Liu *et al*. (2011) mentioned that several grain sources were the constituents of usual poultry diets which might be contaminated by different mycotoxins. It was observed that higher inclusion levels of Mycofix® gave better results. The use of Mycofix® binders in the feed decontaminated the toxins to a level that enhanced better nutrient utilization with increased body weight gain and improved growth. The result obtained showed that Mycofix® was able to mitigate to a reasonable level the problem mycotoxins in broiler feed through better availability and utilization of nutrients that manifested in enhanced growth performance of the birds. Birds fed 400g/100kg Mycofix® in their diet had 8.97% gain in body weight than those on the control feed. They also had 11.2% gain above broilers fed diets with 300g/100kg Mycofix® inclusion. There appear to be an improved gain in weight as the binder level increased in the diets. This result agreed with that of Hedayati *et al.* (2014), where birds fed toxin binder in their feed had the best and highest body weight among all the treatments groups.

Agboola *et al*. (2015) reported that supplementation with mycotoxin binder and probiotics resulted in improved body weight gain over the control diet, an indication of the positive effect of mycotoxin binder on broiler performance through the control of the gut microbiota. The results obtained showed that Mycofix® at 300 and 400 g/100kg levels of inclusion was able to ease the problem of mycotoxins in feed by enhanced performance of the birds. In other studies conducted where toxins were introduced into the basal diet and toxin binders was used to evaluate the efficacy of handling the toxins, similar results were obtained. The marked increase in feed intake observed at higher inclusion levels also showed the positive effect of the binder on feed consumption by the birds. Shareef (2010) reported that there were 20.03, 23.29, 19.91, 18.27 and 20.60 % reduction in treatments as compared with the control group when mycofix+3 was added to contaminated diet to give significantly (P < 0.05) improved body weight gain by 33.26, 28.66, 17.62, 15.37 and 18.65 This agreed with results obtained by Abdelaziz *et al.* (2015), who reported a profound increase in feed consumption during the growing period between 4-5week to be 9.9%, in treatments given different natural toxin binder such as Pepper mint *Mentha piperita* oil and biological toxin binder which includes Mycofix plus and rice hulls.

The use of Mycofix® at the starter phase significantly (P<0.05) gave a lower feed conversion ratio, for birds on the Mycofix® treatments groups. The inclusion of Mycofix® showed the ability of the binder to effect a good measure of interaction between growth and consumption. This contradicted the findings of Kamalzadeh *et al*. (2009), that feed conversion ratio decreased significantly with the concentration of Mycosorb in the diets. Inclusion of Mycosorb reduced feed conversion by 2.09, 5.24 and 5.47% in treatments with 0.5, 1.0 and 1.5 g Mycosob kg-1 diet, respectively compared with the control.

The best feed cost per kilogram gain was observed in the treatment groups with the highest inclusion level (400g/100kg) of Mycofix®. This showed that it was profitable and cost effective to use Mycofix® in broiler starter diets at this inclusion level.

Mortality percentage recorded for birds on 400g Mycofix® diet was not attributed to the treatment effect, as post mortem results showed that the birds that died did not show signs of any disease in particular and might have just be a natural occurrence. Their hearts were however enlarged which could be attributed to the rapid growth rate observed in the group compared with the others.

Growth performance of broiler finisher chickens (5 -8 weeks)

The growth parameters of broiler finisher chickens fed diets containing four levels of Mycofix® is shown in Table 6. Significant ($P < 0.05$) differences were observed across the treatments for final weight, weight gain, total feed intake, FCR and feed cost per kilogram gain. Birds on 400g/100kg Mycofix® diet had the highest ($P < 0.05$) final body weight (2350.3g), weight gain (1403.3g), average daily weight gain (50.1g) in all the treatment groups compared with the control. Total feed intake was significantly ($P < 0.05$) higher in the control, 100, 300 and 400g Mycofix® compared to 200g Mycofix®. The feed conversion ratio was better (2.10) in birds fed diets containing 400g Mycofix®. The feed cost per kilogram gain of ₦167.20 was the least in 400g/100kg Mycofix® diet. Mycofix® at 400 g/100kg inclusion level reduced the cost of production per bird by 12.31% or ₦23.47. Mortality percentage was highest (3.5%) in 200g Mycofix® diet.

Table 6: Effect of Mycofix® on growth performance of broiler finisher chickens

	Levels of Mycofix® inclusion (g/100 Kg diet)					
Parameters	**0**	**100**	**200**	**300**	**400**	**SEM**
Initial weight (g/bird)	947.04	947.06	947.10	947.07	947.04	0.03
Final weight (g/bird)	2186.7^b	2060.3^b	1936.7^b	2105.3^b	2350.34^a	79.94
Weight gain (g/bird)	1239.7^b	1113.3^b	989.6^b	1158.2^b	1403.30^a	79.95
Av. daily weight gain (g/bird)	44.3^{ab}	39.8^b	35.3^b	41.4^b	50.10^a	2.86
Total Feed intake (g/bird)	3039.0^a	2844.3^a	2579.7^b	2852.3^a	2997.30^a	87.53
Av. daily feed intake (g/bird)	108.5^a	101.6^a	92.1^b	101.9^a	107.0^a	3.13
Feed conversion ratio	2.40^b	2.50^b	2.50^b	2.40^b	2.10^a	0.12
Feed cost (₦/kg)	79.44	79.50	79.56	79.62	79.60	
Feed cost/kg gain (₦/kg gain)	190.67	198.75	198.90	191.09	167.20	
Mortality (%)	1.70	1.70	3.50	0.00	3.00	

ab: Means with different superscripts along same rows show significant differences ($P < 0.05$); SEM : Standard Error of Means , %, percentage AV; average; %; percentage

The improved performance recorded for birds fed 400g Mycofix® per 100kg diet for all the parameters measured were better in all their treatments, this may be attributed to the fact that a high concentration of the specific binder (Mycofix®) maybe needed to counteract the effect of the toxins present in the diet which resulted in an improved performance. The same trend was observed at the starter phase, which may be due to early introduction of the binder in the chicks diet and been more effective as a form of protection in the gut.

At the finisher phase however, 400g /100kg inclusion clearly performed best in all the parameters measured, which is indicative of the need for a higher level at this phase of the birds to improve growth rate. Lan *et al.* (2007) reported that gut dominant contaminants becomes more complex as broilers grow older. The result showed that the binder improved the basal diet and suppressed the effect of contamination. The diets containing Mycofix® positively influenced the feed conversion ability of the birds on the treatments because they showed a better FCR than the control. This suggested that Mycofix® was able to aid conversion of feeds appropriately that resulted in production of meat and reduced the cost of production per bird. Ogunwole *et al.* (2013) reported that Mycofix® inclusion at 0.3% gave the lowest feed conversion ratio value compared with the control and four other types of binders namely charcoal, Toxiroak, Toxynil and A- Tox E.

The mortality recorded was not as a result of the Mycofix® as post mortem results showed signs of chronic respiratory tract disease which was treated appropriately with the drugs prescribed at the Veterinary clinic of the Ahmadu Bello University, Zaria.

The results obtained from the carcass evaluation of broiler chickens fed four levels of Mycofix® are shown in Table 7. The result showed that treatment with 400g Mycofix® inclusion had a higher (P<0.05) values for live weight and carcass weight. Dressing percentage, cut parts and organ weights showed no significant (P>0.05) differences across all the treatments.

The better carcass weight of the birds fed with diets that contained 400g Mycofix® treatment could be attributed to the final weight compared with the other treatments. The improved final weight will afford the farmer a better market value when Mycofix® 400g /100kg of diets were fed although the effect was not significant in the other parameters measured. The result contrasts that of Abdelaziz *et al*. (2015) thatno significant differences for carcass characteristics when Mycofix® and three other organic binders were used.

Table 7: Effect of Mycofix® on the carcass of broiler finisher chickens (5 – 8 weeks)

	Inclusion levels of Mycofix® g/100Kg diet					
Parameters	**0**	**100**	**200**	**300**	**400**	**SEM**
Live weight g)	2138.67^{b}	1928.33^{c}	1949.17^{c}	2072.00^{b}	2240.83^{a}	23.96
Carcass weight (g)	1343.83^{b}	1387.50^{b}	1273.33^{c}	1344.67^{b}	1457.50^{a}	48.61
Dressing percentage %	62.72	64.31	63.67	64.71	65.01	1.87
Cut parts expressed as percentage of carcass weight (%)						
Back (%)	18.23	17.12	20.11	22.04	19.49	1.36
Breast muscle (%)	27.27	28.04	30.65	30.79	32.34	1.65
Thigh (%)	16.06	15.48	17.71	18.15	17.68	0.85
Drum stick (%)	13.35	14.17	15.64	16.54	16.13	1.02
Wings (%)	11.04	13.26	12.88	12.79	12.68	0.68
Organs weights expressed as percentage of live weight (%)						
Heart (%)	0.39	0.39	0.40	0.44	0.40	0.04
Full gizzard (%)	2.71	3.08	3.44	3.28	3.07	0.20
Empty gizzard (%)	2.08	2.13	2.41	2.44	2.09	0.16
Liver (%)	1.68	2.13	1.91	1.91	1.62	0.15
Lungs (%)	0.61	0.63	0.72	0.68	0.60	0.06
Kidneys (%)	0.61	0.63	0.59	0.58	0.60	0.06
Spleen (%)	0.13	0.15	0.15	0.15	0.16	0.02
Abdominal fat (%)	1.62	0.80	1.49	1.95	1.47	0.29
Intestinal weight (%)	4.55	4.99	5.07	5.37	4.40	0.39
Intestinal length (cm)	251.67	289.33	274.50	251.17	271.33	11.05

[abc:] Means with different superscripts along same rows show significant differences, SEM: Standard Error Means.

The nutrient digestibility results of broiler finisher chickens fed four levels of Mycofix® are presented in Table 8. There were no significant (P>0.05) differences in the digestibility of crude fibre, ether extract and nitrogen free extract. Dry matter for T_5 (400g Mycofix®) was significantly (P <0.05) higher than remaining treatments. The crude protein was similar (P>0.05) for control, 300 and 400g Mycofix® treatments, but significantly (P<0.05) higher than 100 and 200g Mycofix® which were similar. The ash content also followed the same trend as crude protein.

The measure of apparent digestibility reflects the net effect of all digestive and absorptive processes along the digestive tract (Grenier and **Applegate**, 2013). The improved nutrient digestibility of dry matter, crude protein and ash observed in broiler chickens fed 300 and 400g Mycofix® treatments, was an indication that Mycofix® positively affected digestion of nutrients. The increased apparent digestibility of nutrients could be attributed to the ability of Mycofix® to protect the gut from direct contact with the possible myotoxins present in the feed.

Table 8: Effect of Mycofix® in broiler finisher diet on apparent nutrient digestibility

	Levels of Mycofix® inclusion (g/100kg) diet					
Parameters	**0**	**100**	**200**	**300**	**400**	**SEM**
Dry matter (%)	92.71[b]	89.12[b]	90.33[b]	92.29[b]	94.74[a]	1.05
Crude protein (%)	95.60[a]	93.80[b]	94.12[b]	95.36[a]	96.35[a]	0.49
Crude fibre (%)	95.60	93.10	94.53	95.06	95.87	0.79
Ether extract (%)	97.39	95.04	96.06	94.31	98.23	0.84
Ash (%)	94.88[a]	92.01[b]	92.51[b]	94.38[a]	94.47[a]	0.95
NFE (%)	89.34	84.64	86.10	89.00	89.12	1.96

[abc]: Means with different superscripts along same rows show significant differences ,SEM: Standard Error of Means, NFE: Nitrogen free extract

Galvano *et al*. (2001) reported that toxin binders prevent toxic interactions with the consuming animal and prevent mycotoxin absorption across the digestive tract. The known dietary factors that interact with mycotoxins include nutrients such as fat, protein, fiber, vitamins and minerals. The presence of mycotoxins in diets of poultry feeds have been reported in literatures (Applegate *et al*., 2009; Dietrich *et al*., 2012) to have effect on protein, energy and nutrients usage in the gastro intestinal tract of poultry.

Conclusion

There was an occurrence of multiple fungi species in the all feed ingredients sampled, even though the levels varied. This may indicate a potential health hazard to both animals and humans in terms of direct consumption of fungal contaminated feed or their toxins by farm animal and subsequent public health problem.

AfB1contamination of maize grain and soya bean cake were less than 20 ppb while, groundnut cake, maize offal and brewers dried grains were 80, 60 and 40 % respectively above 20 ppb permissible limits by United States Food and Drug Administration (USFDA).

The highest inclusion level of Mycofix® (400g/100kg feed) gave the best performance (final body weight, weight gain and feed conversion efficiency) at both starter and finisher phases of the birds. This was above the 2-3 kg / tonne recommended by the manufacturers.

Mycofix® at 100, 200, 300 and 400 g/100kg diet reduced the cost of production by 11.51% (₦18.23), 12.54% (₦19.94), 3.95% (₦6.26) and 11.51 % (₦18.23) respective per bird at the starter phase and Mycofix® at 400 g/100kg inclusion level reduced the cost of production per bird by 12.31% or ₦23.47 at the finisher phase.

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