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# Effects of Mycotoxin Detoxifier Supplementation to Contaminated Groundnut Cake on the Growth Performance, Organs and Serum Enzymes of Two Broiler Strains

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***Abstract*** **– The study assessed the effects of supplementation of mycotoxin Detoxifier to contaminated groundnut cake (GNC) diet on the growth performance, selected organs and serum enzyme concentrations of two strains of broilers. A total of 198 unsexed day old broilers were randomly distributed to 6 dietary treatment groups. Each treatment group was replicated thrice of 33 birds. Corn-groundnut cake-based diet was used for all treatments. Diet that contained non-contaminated GNC was the positive control (PC) whereas diet containing contaminated GNC served as the negative control (NC). Next diet contained contaminated GNC supplemented with mycotoxin Detoxifier. The contamination was achieved by adding 25% water to a unit weight of GNC (w/w), thereafter, stored for one week for fungi growth and mycotoxin formation. Two percent contaminated GNC was then included in the contaminated diets. These three diets were to the two Arbor Acre and Marshal broilerstrains leading to 6 dietary groups.The results showed that highest total aflatoxin concentration was present in contaminated GNC. Also non-contaminated and contaminated diets had AF concentration greater 20ppb. Marshal broiler starters fed contaminated GNC diet supplemented with mycotoxin Detoxifier had the heaviest final body weight (FBW, P<0.008) and weight gain (WG, P<0.009). Feeding contaminated GNC to broiler starters slightly reduced the FBW and WG for the two strains. Diet imposed did not significantly affect the growth performance of broiler finishers. The main effect of the mycotoxin Detoxifier had been to restore the liver weight (P<0.023), comparatively to those in the positive control group. Length of the gastrointestinal tract of Marshal broiler chickens was significantly (P<0.026) longer than those of Arbor Acre fed on contaminated GNC diets with or without Detoxifier. Marshal broilers fed contaminated GNC diet with or without Detoxifier died (P<0.054) more than Arbor Acre broiler fed these two diets.**

***Keywords*** **– Broilers, Groundnut Cake, Contamination, Serum Enzymes, Detoxifier, Mortality.**

## I. INTRODUCTION

Groundnut (Arachic hypogea) is a species in the legume family Fabaceae native to South America and Central America [1]. Groundnut can be processed into several agricultural products such as peanut cake (kulikuli, a groundnut based snack indigenous to West African coast) [2], groundnut cake, produced by expelling the oil from the groundnut kernels [3]. Groundnut cake (GNC) has a protein content of 38-47% depending on the extraction process [4].Very few data are currently available on GNC in terms of its safety and nutritional status [2]. Available information on groundnuthas focused on the microbiological quality, nutritive attributes and functional characteristics of "kulikuli" [5 and6].The main limitation to the utilization of GNC has been contamination with fungi and subsequent aflatoxin formation which could be detrimental to the health of animals consuming the contaminated GNC. The contamination of feeds with mycotoxins poses a serious risk for animal health [7]. Aflatoxins cause substantial economic losses to poultry industry, impeding the proper growth of birds, increasing feed conversion ratio and mortality, as well as reducing feed utilization and egg production [8 and 9].

Several nutritional, physical, chemical and biological approaches have been proposed to detoxify mycotoxin contaminated feed and feedstuffs [10].Although GNC is a good substrate for the formation of aflatoxins, minimising its deleterious effects in animals have not been given much attention in studies whereas serious efforts have been directed to mitigate theadverse effects of contaminated maize. GNC is a common feed ingredient in poultry feeding in most developing countries of the world. Hence, concerted efforts must be directed towards minimising the adverse effect of feeding GNC containing mycotoxin contaminants.The study therefore assessed the growth performance, organs and serum biochemical indices of broilers fed contaminated groundnut cake.

## II. MATERIALS AND METHODS

### *Site of Experiment*

The experiment was carried out at the Poultry unit of Teaching and Research Farm, LadokeAkintola University of Technology, Ogbomoso, Nigeria. A period of 6 weeks was observed for the study with 3 weeks each for starter and finisher phase.

*Source of Mycotoxin Detoxifier and two broiler strains*

The mycotoxin detoxifier was manufactured by PolchemHyiegene Laboratories PVT Limited, an ISO 9001:2000 Certified Company, Pune 411 004, India. This product is distributed in Nigeria by Animal Care Services Consult Iperu road, Lagos Express way, Ogere- Remo, Ogun State, Nigeria. The recommended dosage of 1g/Kg was used in the study. The detoxifier is a blend of specific natural detoxifying enzymes such as epoxide reductase, esterase, peptidase, aflatoxin B1 carboxyl reductase and hydrated sodium calcium aluminosilicate HSCAS (carrier). The two broiler strains were procured from reputable hatcheries; Farm House Nigeria Limited and Zartech Farm Nigeria Limited, Ibadan, Oyo State, Nigeria,

*Experimental Design and Animal Management*

Completely randomised design was adopted for the study. A 2x2 factorial experiment for the test ingredients was also used for the study. The two factors were mycotoxin detoxifier (at 0 and 1g/Kg) and broiler strain (Arbor Acre and Marshal). A total of 198 day old unsexed broiler chicks (99 chicks for each ofArbor Acre and Marshalstrains) were randomly distributed to 6 dietary treatment groups. A treatment group contained 33 birds of 3 replicates. Feed and water were supplied to the birds *ad libitum*. Medication and vaccination were carried out as at when due. The rate of mortality of the experimental birds was also monitored during the study.

*Formulation of Experimental Diets*

There were six dietary groups were used for the study. The first dietary group contained non-contaminated GNC and it served as the positive control. The second contained contaminated GNC which served as negative control, whereas the third diet contained contaminated GNC supplemented with mycotoxin detoxifier. These diets were fed to both strains [Arbor Acre (AA) and Marshal broilers] consequently leading to 6 dietary groups (Tables 1 and 3).

*Organs Measurement of broiler chickens*

On the 42nd day of the study, 6 birds per treatment were fasted for 18 hours but they have accessed to water during the fasting period. These birds were slaughtered by decapitation and defeathered. Organs were separated and measured with the aid of a sensitive weighing scale. The weights of the organs were expressed on final body weight as a percentage. Gastrointestinal tract length (cm) was measured from the end of crop to the cloaca and it was expressed on the final body weight.

*Chemical Analysis*

Aflatoxins were extracted from the feed samples with a mixture of methanol and water (7:3) using enzyme linked immunosorbent assay according to the method of [11].On the 36thday of the study, six blood samples per treatment were collected into bottles without anticoagulant to estimate the enzymes activities of broiler chickens fed experimental diets.The following methods[12, 13 and 14] were used to estimate the serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, respectively.

*Statistical Analysis*

All collected data were analysed using factorial analysis of variance within the completely randomised design of SAS [15]. Significant means were separated using Duncan option of the same statistical software. A probability of 5 percent was considered significant.

Table 1: Gross composition of broiler starter and finisher diets

| Ingredients | Starter diet | Finisher diet |
|---|---|---|
| Corn | 57.70 | 42.00 |
| Groundnut cake | 32.00 | 27.25 |
| Fish meal (72%) | 4.25 | 3.00 |
| Fixed ingredients[a] | 6.05 | 27.75 |
| **Total** | **100.00** | **100.00** |
| Calculated Analysis | | |
| Energy (Kcal ME/Kg) | 3032.91 | 2872.34 |
| Crude Protein (%) | 21.98 | 20.06 |
| Crude Fibre (%) | 3.57 | 5.51 |
| Methionine (%) | 0.52 | 0.48 |
| Lysine (%) | 1.39 | 1.21 |
| Available Phosphorus (%) | 0.56 | 0.51 |
| Calcium (%) | 1.08 | 1.04 |

[a]Fixed ingredients for starter diets contained 2.65% corn bran, 0.6% limestone, 2% bone meal, 0.15% methionine, 0.15% lysine, 0.25% salt and 0.25% vitamin premix[b]. The finisher diets contained 24.35% corn bran, 0.6% limestone, 2% bone meal, 0.15% methionine, 0.15 % lysine, 0.25% salt and 0.25% vitamin premix[b].

[b]The vitamin premix supplied the following vitamins and trace elements per Kg diet as follows:7812.50 IU Vit. A; 1562.50IU Vit D3; 25mg Vit.E; 1.25mg Vit.K3; 1.88mg Vit.B1; 3.44mg Vit.B2; 34.38mg Niacin; 7.19mg Panthotenate, 3.13mg Vit.B6; 0.02mg Vit. B12; 312.50mg Choline Chloride; 0.63mg Folic Acid; 0.05mg Biotin; 75mg Mn; 62.50mg Fe; 50mg Zn; 5.31mg Cu; 0.94mg I; 0.19 mg Co; 0.08mg Se; and 75mg Antioxidant

Table 2: Total aflatoxin concentration (ppb) in the experimental diets and contaminated groundnut cake

| Parameters | Aflatoxin concentration |
|---|---|
| Non-contaminated diet | 45 |
| Contaminated diet | 95 |
| Contaminated groundnut cake | 105 |

Table 3: Growth performance and rate of mortality of two strains of broiler starters and finishers fed contaminated groundnut cake with or without mycotoxin Detoxifier

| Parameters (g/bird) | T1 | T2 | T3 | T4 | T5 | T6 | P-value | SEM | Strain | Detoxifier | INT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ← ArborAcre strain → | | | ← Marshal strain → | | | | | | | |
| | PC | NC | Detox | PC | NC | Detox | | | | | |
| Day old body weight | 42.76 | 43.58 | 43.51 | 44.85 | 44.39 | 44.44 | 0.667 | 0.96 | 0.208 | 0.779 | 0.838 |
| **Final body weight** | | | | | | | | | | | |
| Starter phase | 426.26[a] | 388.21[ab] | 344.44[b] | 417.27[a] | 383.64[ab] | 430.30[a] | 0.008 | 14.15 | 0.004 | 0.313 | 0.082 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Finisher phase | 799.45 | 792.42 | 731.85 | 839.15 | 643.24 | 787.21 | 0.177 | 50.75 | 0.418 | 0.469 | 0.099 |
| **Weight gain (g/bird/day)** | | | | | | | | | | | |
| Starter phase | 18.26 [a] | 16.40[ab] | 14.33[b] | 17.73 [a] | 16.15[ab] | 18.37 [a] | 0.009 | 0.70 | 0.005 | 0.337 | 0.089 |
| Finisher phase | 17.77 | 19.26 | 18.45 | 21.69 | 10.76 | 17.00 | 0.124 | 2.48 | 0.119 | 0.370 | 0.252 |
| **Feed intake (g/bird/day)** | | | | | | | | | | | |
| Starter phase | 58.14 | 49.21 | 49.34 | 56.08 | 51.92 | 55.66 | 0.183 | 2.79 | 0.012 | 0.947 | 0.897 |
| Finisher phase | 68.78 | 73.07 | 74.77 | 71.70 | 60.60 | 65.38 | 0.385 | 4.94 | 0.103 | 0.601 | 0.801 |
| **Feed conversion** | | | | | | | | | | | |
| Starter phase | 3.19 | 3.01 | 3.45 | 3.17 | 3.23 | 3.06 | 0.771 | 0.22 | 0.575 | 0.415 | 0.180 |
| Finisher phase | 3.87 | 3.79 | 4.17 | 3.38 | 8.42 | 3.85 | 0.154 | 1.35 | 0.225 | 0.237 | 0.169 |
| **Mortality (%)** | 7.58 | 3.03 | 1.52 | 9.09 | 10.61 | 8.33 | 0.426 | 3.35 | 0.054 | 0.516 | 0.894 |

[ab]Means with uncommon superscripts along the same row are significantly different (P<0.05). PC= Positive control, NC= Negative control, INT= Interaction effect of strain and Detoxifier. Detox= Detoxifier

## III. RESULTS

The total aflatoxin (AF) concentrations in the diets and contaminated groundnut cake are shown in Table 2. The AF concentration in the non-contaminated diet was the lowest whereas the contaminated GNC had the highest. In addition, the AF concentration in the diet that contained contaminated GNC was intermediate (45, 95 and 105 ppb). Diet imposed had significant effects on the final body weight (FBW, P<0.008) and weight gain (WG, P<0.008) of 21 day old broiler starters (Table 3).Marshal starters fed contaminated GNC supplemented with the mycotoxin Detoxifier had the heaviest FBW and WG. Furthermore, broiler starters fed non-contaminated GNC had the highest FBW and WG for the two strains when compared along their strain groups. Marshal broiler starters fed contaminated GNC supplemented with mycotoxin Detoxifier had significantly higher FBW (P<0.004) and WG (P<0.005) than Arbor Acre counterparts fed the same diet. The interaction effects of strain and mycotoxin Detoxifier tend to significantly affect the FBW (P<0.082), WG (P<0.089) of the starters and FBW (P< 0.099) of the finishers. The rate of mortality was higher for Marshal broilers fed non-contaminated and contaminated GNC with or without Detoxifier than Arbor Acre broilers in this study (Table 3). Serum enzyme activities and selected organs of broiler chickens fed experimental diets are displayed in Table 4. Serum AST and ALP of broilers fed diet contaminated GNC were significantly affected by the contamination. Arbor Acre broilers fed diet containing contaminated GNC diet had significantly higher serum AST (P<0.028) than their counterparts fed supplemental contaminated GNC diets. Serum ALP concentration of Marshal broiler chickens fedunsupplemented contaminated GNC diet was significantly (P<0.011) higher than their Marshal counterparts fed contaminated GNC diet supplemented with mycotoxin Detoxifier. Dietary treatment did not significantly affect the selected organs except for the length of gastrointestinal tract (GIT). However, mycotoxin Detoxifier significantly (P<0.023) affected the liver weight. Broiler chickens fed contaminated GNC diet for the two strains broilers had the lowest liver weight, comparatively along each of the strain groups. Addition of Detoxifier to contaminated GNC diet fed to broilers restored the weight of the liver (when compare to the positive control). The GIT lengths of Marshal broilers fedunsupplemented contaminated GNC diet and Marshal fed contaminated GNC diet supplemented with Detoxifier were significantly (P<0.026) longer than Arbor Acre broilers fed these two diets. Mycotoxin Detoxifier tends to significantly (P<0.059) affect the lungs weight, restoring the weight of the lungs of broilers fed contaminated NC (when compare to the positive control).

Table 4: Serum enzymes and selected organs oftwo strains of broiler chickens fed contaminated groundnutcake with or without mycotoxin detoxifier.

| Parameters | T1 | T2 | T3 | T4 | T5 | T6 | P-value | SEM | Strain | Detoxifier | INT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ←ArborAcre strain → | | | ←Marshal strain→ | | | | | | | |
| | PC | NC | Detox | PC | NC | Detox | | | | | |
| Serum AST (IU/L) | 79.67[ab] | 132.00[a] | 55.33[b] | 70.83 [b] | 96.00[ab] | 69.67[b] | 0.028 | 15.75 | 0.282 | 0.943 | 0.083 |
| Serum ALT (IU/L) | 11.00 | 19.50 | 2.00 | 11.83 | 18.83 | 3.83 | 0.169 | 5.62 | 0.776 | 0.080 | 0.915 |
| Serum ALP (IU/L) | 45.83[b] | 55.83[b] | 58.67[b] | 46.17 [b] | 113.67[a] | 55.67b | 0.011 | 13.41 | 0.534 | 0.544 | 0.742 |
| Liver (%) | 3.25 | 2.85 | 3.06 | 3.48 | 2.75 | 3.60 | 0.129 | 0.25 | 0.330 | 0.023 | 0.155 |
| Kidney (%) | 0.21 | 0.25 | 0.38 | 0.46 | 0.27 | 0.22 | 0.087 | 0.07 | 0.345 | 0.598 | 0.181 |
| Lungs (%) | 0.57 | 0.49 | 0.59 | 0.52 | 0.47 | 0.57 | 0.427 | 0.05 | 0.664 | 0.059 | 0.986 |
| GIT (%) | 6.64 | 7.52 | 8.03 | 7.52 | 7.18 | 7.47 | 0.510 | 0.49 | 0.363 | 0.411 | 0.823 |
| GIT (cm/Kg) | 181.72[bc] | 170.77[c] | 188.83[bc] | 208.89[ab] | 197.91[ab] | 219.15[a] | 0.037 | 10.75 | 0.026 | 0.116 | 0.823 |

[abc]Means with uncommon superscripts along the same row are significantly different (P<0.05).PC= Positive control, NC= Negative control, INT= Interaction effect of strain and Detoxifier. Detox= Detoxifier, AST= Aspartate aminotransferase, ALT= Alanine aminotransferase, ALP= Alkaline phosphatase.

## IV. DISCUSSION

The AF concentration in the experimental diets and contaminated GNC were higher the recommendation of EEC [16] of 20ppb indicating that the AF levels in these contaminated diets and non-contaminated diets were toxic for the birds. Marshal strain broilers fed contaminated GNC supplemented with Detoxifier responded positively better thanArbor Acre broilers fed the same diet. It had been reported that the negative effects of these toxins in animals could be influenced by a range of factors including concentration of aflatoxins, duration of exposure, species, gender, age and general health status of the animals [17 and 18]. Broilers fed non-contaminated diet had slightly better FBW than those fed contaminated GNC. This observation concurred with a number of studies involving aflatoxin contaminated feedstuffs. Aflatoxins cause losses to poultry industry, impeding the proper growth of birds, increasing feed conversion ratio, egg production and mortality [8 and 9]. Aflatoxin is a common contaminant in a wide variety of tropical and subtropical food/feed stuffs particularly GNC. At levels of even less than 1ppm, they damage cells within the organism and depressed growth performance in animals [19]. The FBW of broiler strains fed GNC whether contaminated or non-contaminated GNC was lower than the observation of many authors who had worked GNC. The FBW of broilers fed 22.03% GNC at the finisher phase were 712 and 1293 gram for 5 week old broilers for Experiments 1 and 2 [20]. Low levels of lysine and methionine in GNC as reported by [21] may account for low FBW in the present study.

The increased serum AST and ALP activities of broilers fed contaminated GNC without the Detoxifier indicated that damages to the liver and kidney for Arbor Acre and Marshal broilers. Aflatoxin toxicity in broiler may be manifested by increased hepatic enzyme activities such as AST and ALT; and if AST and ALP are found together in elevated amount of the blood, liver damage is most likely present [20]. Aflatoxin increase lipid peroxidation in liver and kidney tissues and induce cellular damage causing impaired morphology of the organs [23 and24]. These two organs are critical in detoxification and excretion of mycotoxin contamination in animals hence, their morphological changes leading to high concentrations of the hepatic and nephritic enzymes in the blood.

Literature regarding effects of the mycotoxin on the gastrointestinal tract wasreported to be scanty and non-conclusive [25]. Significantly higher gastrointestinal weights were observed in broiler chickens fed diet containing contaminated maize supplemented with Detoxizyme® [26]. The present study revealed that Detoxifier improved the length of the GIT which was in agreement with the observation of [26]on the improved GIT weight. Gastrointestinal tract is the first organ coming into contact with mycotoxin of dietary origin and should be expected to be affected by AF B1 with greater potency as compared with other organs [25]. In this study, mycotoxin impaired the development of the GIT leading to shorter length whereas the Detoxifier alleviated the harmful effect on the development of the GIT yielding longer length. Consequently, it improved nutrients absorption and enhanced the rate of growth for broilers fed contaminated GNC with Detoxifier.Detoxifying enzyme is an attractive alternative to the use of live microbes to counteract mycotoxins in animal feed. It is the application of enzymes responsible for the degradation of mycotoxins [27]. Enzymatic reactions offer a specific often irreversible efficient and environmental friendly way of detoxification that leaves neither toxic residue nor undesired by products [28].

## V. CONCLUSION

Conclusively, feeding contaminated GNC to the two broiler starter strains slightly reduced the final body weight and weight gain. Marshal broiler starters fed contaminated GNC diet supplemented with mycotoxin Detoxifier responded better in terms offinal body weight andweight gain than the Arbor Acre strain. Shorter GIT length were observed in the two broiler strains fed contaminated GNC compared to birds fed contaminated GNC supplemented with Detoxifier. Arbor Acre broilers fed contaminated GNC diet had less mortality than the Marshal strain broilers.

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