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Mycotoxin Binders and Monosodium Glutamate Influence on Growth, Intestine, Blood Urea and Organoleptic Characteristics of Broilers Fed Contaminated Diet

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Abstract – **The study assessed the effects of two mycotoxin binders and monosodium glutamate (MSG) on the growth performance, intestine, meat organoleptic properties and urea of broilers fed contaminated diets. There were 5 experimental diets that were formulated for the study. Contamination of maize was induced by addition of 50% water to a unit weight of maize (w/w) and stored for 2 weeks for fungal growth and mycotoxin formation. The contaminated maizewas included in the basal diet at 2%and it served as the control. The inclusion of the test supplements in the contaminated diets was a 2x2 factorial arrangement. The 2 factors were mycotoxin binder (with 2 types) and MSG (at 2 levels of supplementation of 0 and 3g/Kg). The contaminated basal diets were supplemented with Detoxizyme® and AlquerFeedAntitox®forT1 and T2, respectively. Furthermore, T3 and T4 were contaminated diets supplemented with Detoxizyme® and AlquerFeedAntitox® respectively, as well as MSG supplementation. Detoxizyme®, AlquerFeedAntitox® and MSG were added to these contaminated diets at 1, 0.5 and 3 g/Kg, respectively.**

The results showed that contaminated diet and maize contained 65 and 157 ppb aflatoxins, respectively. Diet did not significantly affect the growth performance of broilers in the starter, finisher and total period of the study. However, significant effects of the supplements added to contaminated diets were observed. Broiler starters fed contaminated diet supplemented with Detoxizyme® only (T1) had the highest final body weight FBW (P<0.0021) and heaviest weight gain WG (P<0.018). In the total period of the study (1-42 days), chickens fed contaminated diet containing Detoxizyme® and MSG (T3) had maximum FBW(P<0.049) and best WG (P<0.046). Significantly (P<0.001) higher gastrointestinal weights were displayed by broiler chickens fed contaminated diets with the supplements except for those fed AlquerFeedAntitox® only (T2) .Meats from broiler chickens fed contaminated diet with added MSG were more acceptable by the consumers.

Keywords – **Aflatoxin, Contaminated Diet, Detoxifying Enzymes, Binder, Broilers.**

I. INTRODUCTION

Fungi contamination of animal feeds with the consequent mycotoxin production is one of the major threats to human and animal health [1]. Cereals, concentrate, hay and other animal feeds have been reported as substrate for fungi growth and mycotoxin production [2]. Maize is a food and feed crop that is commonly contaminated with mycotoxins which are chemical products of moulds such as aflatoxins, fumonisins and zearalenone [3]. Some authors noted that in developing countries of the world, tropical conditions like high temperatures and moisture, monsoons, unseasonal rains during harvest and flash floods can lead to fungal proliferation and production of mycotoxin [4]. In Africa and other developing countries, the World Health Organization [5] noted that fumonisins and aflatoxins are likely to be of significance.

Aflatoxin intake in broilers is associated with liver damage, poor performance, immune-suppression and mortality [6]. Aflatoxin toxicity in poultry is also associated with biochemical, haematological, reproductive and pathological changes [7]. Cases of concurrent aspergillosis and aflatoxicosis confirmed that Aspergillus species threaten poultry production in the feed, litter and environment [8]. The ill effect of mycotoxicosis is prevented by the addition of non-nutritive and natural adsorbent material to contaminated feed in order to selectively bind the mycotoxins during the digestive process and make it harmless to the birds [9].

Monosodium glutamate MSG, the sodium salt of amino acid glutamate, is a food additive popularly used over the world as flavour enhancer [10]. The safety of MSG usage has generated much controversy locally and globally [11]. It has been widely used for many years in human and livestock diets to improve consumption rates of a particular feed item [12]. The availability of many commercial mycotxin binders in the market and testing efficacy of these products served as the impetus for the study. Furthermore, researches that investigated MSG and contaminated feedstuffs are not common. The study investigated the effects of two commercial mycotoxin binders and MSG supplemented to contaminated diet on the growth performance, intestinal weight, organoleptic characteristics of meat and blood urea of broiler chickens.

II. Materials and Methods

Site of the experiment: The study was carried out in the Broiler Unit of Teaching and Research Farm, LadokeAkintola University of Technology, Ogbomoso, Nigeria. It was a 6 week study.

Source of commercial mycotoxin binders: Commercial mycotoxin binder, Detoxizyme® was used for the study to counteract the negative effect of the mycotoxins in the experimental diets. Detoxizyme® was manufactured by Polchem Hygiene Laboratories PVT limited India and it was obtained from Animal Care Services Konsult Limited, Lagos, Nigeria. Detoxizyme® composed of a blend of specific natural detoxifying enzymes such as epoxide reductase, esterase, peptidase, afltoxin B1carbonyl reductase and hydrated sodium calcium aluminosilicate, HSCAS (carrier). Rate of inclusion in the contaminated diet was 1g⁄Kg.

AlquerFeedAntitox® was manufactured by Biovet S.A. Laboratories, Constanti Spain. It contained 100% HSCAS (E-552 and E-554). It was supplemented in the contaminated diet at 0.5g⁄Kg.

Contamination of maize: Fifty percent water was added to a unit weight of maize (w⁄w)and thereafter stored for 2 weeks to induce contamination. Contaminated maize at 2% was then included in the experimental diets.

Formulation of experimental diets: Five contaminated diets were formulated for the study. Contaminated maize was included in these five diets at 2%. Control contained contaminated maize without any mycotoxin binder supplement. Diets in T1and T2 contained contaminated maize supplemented with Detoxizyme® and AlquerFeedAntitox®, respectively. Contaminated diets in T3 and T4 were supplemented with Detoxizyme® and AlquerFeedAntitox®, respectively and MSG was also added to the last two diets.

Data collection: Data were collected on growth performance and weight of gastrointestinal tract (GIT). On the 42nd day of the study, six broiler chickens per treatment were randomly separated, fasted for 18 hours and slaughtered by decapitation to estimate the organs of broilers fed contaminated diets. The GIT weights were expressed based on the final body weight.

Chemical Analysis: Samples of contaminated maize and diet containing contaminated maize were analyzed for aflatoxin concentration by enzyme linked immunoassay (ELISA) method[13]. Six blood samples per treatment were collected into EDTA bottles through brachial veins in the wings of the broiler chickens on the 36th day of the study to assess the urea concentration and blood samples collected into bottles without anticoagulant were used for serum enzymes estimation. The determination of serum alanine and aspartate aminotransferases were carried out according to the methods of [14] and [15] respectively using Sigma enzymatic kits. The serum alkaline phosphatase was analyzed by the method of [16]. Urea was analysed by the method of [17].

Statistical Analysis: All data collected were subjected to factorial analysis of variance within the completely randomised design using [18]. Significant means were separated using Duncan option of the same software. A probability of 5 percent was considered significant.

III. Results

Aflatoxin (AF) concentration in the contaminated maize was higher the AF level in the diet containing contaminated maize (Table 2). The results of growth performance of broiler chickens fed diets containing contaminated maize supplemented with two commercial mycotoxin binders and MSG are shown in Table 3. Dietary treatment did not significantly influence growth performance of broilers at the starter, finisher and total period of the study. Notable significant effects were exhibited by the type of mycotoxin binder used to counteract the adverse effects of contamination on the performance of the birds. Broiler starters fed contaminated diet supplemented with Detoxizyme® (T1) had the highest final body weight and weight gain whereas those fed AlquerFeedAntitox® plus MSG (T4) were the lowest. In the finisher phase, broilers fed contaminated diet with added Detoxizyme® and MSG (T4) had the heaviest final body weight (FBW, P= 0.049) and the best weight gain WG (P = 0.046,) in the total period of the study. MSG did not display significant effect on FBW and WG in all growth phases. Numerical increases (P>0.050) were observed in broilers fed MSG when compared to those fed without MSG. The organoleptic properties of the breast meat samples, intestinal weight and blood urea were displayed in Table 3. Diet significantly (P< 0.036) affected only the flavour of the breast meat, with those fed AlquerFeedAntitox® plus MSG (T4) had the highest flavour concentration, indicating 'slightly strong'. The interaction effect of binder type and MSG played crucial and significant (P<0.019) role on the flavour of the meat. Furthermore, MSG had significant effects on the overall acceptability (P<0.014) with broilers fed AlquerFeedAntitox® plus MSG (T4) being more accepted by the consumers than others. This showed that the consumers showed 'like moderately' acceptability. Experimental diets significantly (P<0.007) affected the development of the gastrointestinal tract (GIT) of broiler chickens. The interaction effect of binder type and MSG also significantly influenced the GIT weight as broilers fed contaminated diet supplemented with the binders and MSG had significantly improved GIT than those fed contaminated diet without the binders and MSG. The blood urea concentration of broiler chickens was also significantly reduced by MSG when compared to those fed contaminated diet without MSG (Table 3).

IV. Discussion

The AF concentrations in these contaminated feedstuffs were higher than acceptable maximum level in the natural feedstuffs (20ppb) [19], hence its negative effect on the growth performance of broilers fed the contaminated diet without the supplements. The enhanced FBW and the heaviest WG noticed in broilers fed contaminated diet

containing Detoxizyme® with or without MSG revealed that the synergistic effects of detoxifying enzymes (such as epoxide reductase, esterase, peptidase, Aflatoxin B1 carbonyl reductase) and the HSCAS may be responsible for the observation. Also, MSG did not showed significant influence on the parameters of the growth performance. Micro-organisms such as bacteria and fungi contain enzymes that degrade mycotoxins into non-toxic compound [20]. Bacterial enzymes can act as mycotoxin detoxifiers, although they act as mycotoxin bio-transforming agents [20 and 21]. Prior to mycotoxin absorption in the animals intestinal tract, the enzymes secreted from bacterial micro-organisms can work to transform mycotoxins into nontoxic metabolites which can be absorbed by the animal with no toxic effect and thus, improving performance [22]. Deoxynivalenol can be enzymatically transformed to the nontoxic metabolite deepoxydeoxynivalenol DOM-1by anepoxidase of Eu bacterium BBSH797, a gram positive, anaerobic bacterium [20 and 22]. Other enzymes which can transform mycotoxins include proteases, carboxypeptidases and lactonohydrolases[20]. Several fungi species contain enzymes whichcould also degrade mycotoxins. One fungus, *Gliocladiumroseum*can open structural lactone rings to detoxify zearalenone ZEA by 80 to 90% [20 and 22]. The synergistic effect of component enzymes and HSCAS in the Detoxizyme® assisted the birds to cope well with the contaminated diet than those fed pure HSCAS only (AlquerFeedAntitox®).

The improvement in the GIT weight may due to role of glutamate (supplied via MSG) and probably the interconversion of glutamate and glutamine in the intestine. Hence, it could be suggested that the improvement in the FBW and WG for broilers fed contaminated diet supplemented with Detoxizyme® and MSG could be related to the enhanced development of GIT to metabolize efficiently the nutrients contained in the contaminated diet. The beneficial roles of glutamine have been noted in human medicine [23], poultry gastrointestinal development [24] and [25]). Glutamine and glutamate may be converted to each other in different organs such as intestine, liver and kidney, and both are related to the development of gastrointestinal tract of broiler chickens [23, 26 and 27]. Glutamine is the most abundant amino acid in plasma under healthy conditions, however circulating concentration fall precipitously after injury, surgery, infection or other stress [28]. Dietary broilers glutamine at 5 and 10 g/Kg improved performance and carcass characteristics of broilers [29].

In the adult rat, the small intestine is able to extract 25-30% of arterial glutamine in a single pass, but absorption of arterial glutamate is insignificant [30]. Glutamine and glutamate are two closely related amino acids but have functionally different roles in the intestine [30]. The extent of glutamate metabolism in the lumen of the small intestine exceeds that of arterial glutamate metabolism [31]. Dietary glutamine may be the most important fuel for small intestine mucosa [32] connected the formation of glutamate [23 and 31]. Different studies have shown that addition of glutamine to the diet of broilers could increase the relative weights of duodenum and jejunum [33 and 34]. There appear to be an important difference in the utilization of glutamine and glutamate for lumen replication in the small intestine, glutamine being absorbed and utilized arterially in connection with the formation of glutamate whereas glutamate is absorbed and utilized directly in the lumen [23 and 31]. Hence, supplementation of MSG provided glutamate for intestinal development of broilers fed contaminated diet in this study. Also note that the level of common salt in the diet (0.2% equivalent to 2g per Kg feed) with the addition of 0.66 gram of sodium per kilogramme diet coming from MSG did not exceed the expected salt concentration in the broiler diet. The enhancement of breast meat flavour by MSG may be due to the characteristic property of MSG as a flavour enhancer and subsequent acceptability by the consumers. It has been reported that MSG through its stimulation of the orosensory receptors improved the palatability of meals and influenced the appetite positively which induced weight gain [35]. MSG is one the most intensely studied food ingredients in the food supply and has been found safe, the Joint Expert Committee on Food Additives of the United Nations Food and Agricultural Organization and World Health Organization placed it in the safest category for food additives [36] and[37].

V. Conclusion

In conclusion, the detoxifying enzymes with HSCAS improved the final body weight and weight gain of broilers through synergistic effect, whereas MSG enhanced development of gastrointestinal tract with probable efficient utilization of the protein in the contaminated diet leading to reduce level of blood urea.

Table 1: Ingredient composition of broiler basal starter and finisher diets containing contaminated maize

Ingredients	Starter	Finisher
Maize	57.75	40.10
Soybean meal	31.85	28.00
Corn bran	2.65	26.17
Fish meal (72%)	4.40	2.50
Bone meal	2.00	2.00
Limestone	0.50	0.50
Methionine	0.15	0.13
Salt	0.20	0.20
Vitamin Premix*	0.50	0.50
Calculated Analysis		
Energy (ME Kcal/Kg)	3034.90	2856.73
Crude Protein (%)	22.03	20.04
Calcium (%)	1.04	0.99
Available Phosphorus (%)	0.56	0.51
Crude Fibre (%)	3.57	5.72
Methionine (%)	0.52	0.46
Lysine (%)	1.24	1.06

*Vitamin premix supplied the following vitamins and trace elements per Kg diet: 25,000IU Vit. A; 5,000IU Vit D_3; 80mgVit E; 4mg,Vit K3; 60mg, Vit B1; 110mg Vit B2; 1100mg Niacin; 230mg Calcium Pantothenate; 100mg Vit B6; 0.5mg Vit B12; 1000mg Choline Chloride; 20mg Folic Acid; 0.16 Biotin; 240mg Manganese; 2000mg Fe; 160mg Zn; 17mg Cu; 3mg Iodine; 0.6mgCo; 0.24mg Se and 240mg Antioxidant.

Table 2: Aflatoxin concentration in contaminated maize and diet containing contaminated maize

Ingredients	Aflatoxin concentration (ppb)
Contaminated Maize	157
Contaminated diet	65

Table 3: Growth performance of broilers fed diets containing contaminated maize supplemented with mycotoxinbinders and monosodium glutamate at different growth phases (g/bird).

Parameters	Control	T1 Detoxy	T2 Anti	T3 Detoxy MSG	T4 Anti MSG	P-value	SEM	Binder Type	MSG Level	Interaction
Initial Body Weight at day old	45.00	44.55	45.25	43.71	44.56	0.818	0.95	0.485	0.489	0.945
Final Body Weight										
Starter 1-21 days	640.75	652.93	616.67	646.34	607.82	0.126	12.81	0.021*	0.572	0.934
Finisher 22-42 days	1610.32	1698.15	1666.20	1744.53	1592.86	0.226	47.87	0.049*	0.743	0.170
Weight Gain										
Starter	595.75	608.38	571.42	602.63	563.27	0.111	12.60	0.018*	0.604	0.928
Finisher	969.57	1045.22	1049.53	1098.19	985.04	0.433	51.25	0.243	0.897	0.210
Total Period 1-42 days	1565.32	1653.60	1620.95	1700.82	1548.30	0.218	47.61	0.046*	0.755	0.166
Feed Intake										
Starter	1039.38	1050.73	1028.57	1084.52	1062.27	0.521	23.33	0.406	0.219	0.999
Finisher	2773.60	2851.40	2847.70	2867.20	2702.70	0.922	148.24	0.587	0.675	0.603
Total Period 1-42 days	3813.00	3902.10	3876.30	3951.70	3764.90	0.910	150.86	0.501	0.843	0.608
Feed Conversion										
Starter	1.75	1.73	1.80	1.80	1.89	0.244	0.05	0.154	0.164	0.901
Finisher	2.90	2.73	2.70	2.63	2.77	0.939	0.23	0.763	0.891	0.731
Total Period 1-42 days	2.45	2.36	2.40	2.33	2.44	0.956	0.13	0.575	0.972	0.775

*Significant at specified probability levels

Table 4: Organoleptic characteristics, selected serum biochemistry and gastrointestinal weight of broiler chickens fed diets containing contaminated maize supplemented with binder and MSG

Parameters	Control	T1 Detoxy	T2 Anti	T3 Detoxy MSG	T4 Anti MSG	P-value	SEM	Binder Type	MSG Level	Interaction
Colour	6.70	6.20	5.60	6.00	6.00	0.685	0.53	0.606	0.864	0.607
Flavour	4.40^{ab}	4.20^{ab}	3.10^{b}	4.00^{ab}	5.60^{a}	0.036	0.54	0.652	0.044	0.019
Tenderness	5.40	5.00	3.50	5.50	5.00	0.172	0.62	0.124	0.124	0.436
Juiciness	5.40	5.20	4.30	5.10	6.00	0.339	0.57	1.000	0.168	0.123
Texture	3.90	4.60	4.60	5.40	5.70	0.118	0.51	0.765	0.065	0.765
Overall Acceptability	6.30	5.10	5.00	6.00	7.20	0.069	0.59	0.367	0.014	0.288
Blood Indices										
ALP (IU/L)	47.83	34.33	51.00	59.17	41.17	0.413	9.35	0.948	0.466	0.101
ALT (IU/L)	4.83	5.00	3.83	4.33	2.83	0.659	1.12	0.272	0.488	0.889
AST (IU/L)	77.00	80.17	90.83	76.67	67.00	0.464	8.88	0.956	0.146	0.274
Blood Urea (mMol/L)	2.29^{a}	1.38^{ab}	1.85^{a}	0.78^{b}	0.53^{b}	0.002	0.30	0.699	0.002	0.209
Organ										
GIT (%)	4.95^{bc}	6.27^{a}	4.40^{c}	5.18^{bc}	5.51^{ab}	0.007	0.33	0.015	0.964	0.001

[ab] Means along the same row with different superscripts are significantly different ($P < 0.05$)

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