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The Performance and Heamatological Characters in Broiler Chicks Fed Ammonia-Treated Aflatoxin Contaminated Feed

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Abstract: Aflatoxins (AF), natural contaminants of foodstuffs are toxic metabolites produced by aspergillus flavus and parasiticus. Experiments were conducted to evaluate the ammoniation process on aflatoxin-contaminated corn in chicks Male broiler chicks (n = 320) divided into 4-experimental groups (n = 80) and fed on four different Diets (group A (control), basal diet containing uncontaminated corn; group B, basal diet containing ammonia treated uncontaminated corn; group C, basal diet containing aflatoxin B1 (AFB, 1 ppm); group D, basal diet having ammonia treated aflatoxin contaminated (1 ppm) corn). Detoxification of aflatoxin contaminated corn grains was done in a pilot plant with aqueous ammonia (1%, v/w). Chickens were monitored daily and then body weight and feed consumption were recorded. Every week and at end of the 21 and 42 days of age blood was collected and processed. The ammonia-treated detoxified 98.8% of aflatoxins. The AF treatment (group c) significantly decreased food consumption and body-weight gain and increased food conversion ratio ($p<0.05$). The results showed that ammonia-treatment improved efficiency of feed utilization as well as body weight ($p<0.05$). The relative weight of liver, kidney proventriculus and pancreas are decreased by ammonia-treated contaminated corn in comparision with diet containing aflatoxin ($p<0.05$). There was a significant increase in White blood cells counts mainly consisting of heterophi ($p<0.05$) in chicks given AF. There were no significant differences in percentage Monocyte and Eosinophil counts. The percentage of haematocrit in a group that fed forth diet increased compared with those fed aflatoxin-contaminated diet ($p<0.05$). There was alleviation in the alteration hematological parameters in chicks fed with detoxification diet (ammonia-treated). This priminarly study showed that ammoniation of aflatoxin-contaminated corn in a pilot plant (~200Kg) can efficiently modulate the toxic effects of aflatoxin B_1 on hematological characters.

Key words: Aflatoxin, ammonia, heamatological, broilers

INTRODUCTION

Corn is an important cereal world-wide, serving as seed for growers, food for human and livestock as well as an industrial raw material (FAO, 1981; Merounck, 1987). Unfortunately, it is also cosuitable substrate for growth, development and activity of spoilage fungi (Cuero *et al*., 1987; Lacey, 1990). The discovery that many spoilage fungi of grains are capable of producing mycotoxing has increased the evaluation of the importance of fungal attack of grains (Lillehoj and Zuber, 1988). Of the mycotoxins, aflatoxins (B_1, B_2 and G_1, G_2) are the most important in terms of occurrence especially in the tropics and B_1 occurs in the highest concentration and is the most toxic (Hesseltine *et al*., 1981; Lacey, 1990). Aflotoxins are mainly produced by the fungi Aspergillus flavus and Aspergillus parasiticus and strong hepatotoxins and are internationally classified as carcinogens (Stroka and Anklam, 2002). The carry-over of aflatoxin B1 and its metabolic transformation product, the aflatoxin M1, into tissues suitable for human cosumption, eggs and milk of food-producing animals, has been described for different animal species (Veldman *et al*., 1992). Determination of haematological toxic effects of AF is important for diagnosis of toxicosis in poultry (ledoux *et al*., 1999). Broiler chicks given 2.5-3.5 mg AF kg^{-1} diet have shown not only decreased amount of haemoglobin, haematocrit values, thrombocyte counts, percentage of lymphocyte and basophil counts (Huff *et al*., 1986), but also an increased percentage of heterophils (Kececi *et al*., 1998).

Anhydrous ammonia, NH_3 (gas), or aqua-ammonia, NH_4OH (liquid), can be used for removal of mycotoxins. Despite the bulk of information published mostly in 1970-1980s on aflatoxin inactivation by ammonia (Park *et al*., 1988), ammoniated products are not widely used. This is probably due to the safety precautions observed by the farmers. Ammoniation has been

approved of several producer nationals. The FDA has approved the ammonia treatment of cottonseed meal for use as a feed additive for specified quantities and uses. However, some questions still exist as to what are the possible side effects of ammoniation on livestock, although no problems have yet emerged in feeding trials.

The impact of aflatoxins in feed on intensive animal production has been illustrated by studies in the poultry. Because of market competition and relatively large volumes of broilers and egg production in most industrialized countries, a high standard of efficiency is required to maintain the hygienic and production parameters in the poultry. There are a few published data on the nutritive value of ammoniated agricultural products added to the broiler chicks and hens. Most of these studies were carried out with limited feeding of ammoniated products (Pyane *et al*., 1972; Waldroup *et al*., 1976). Galvano *et al*. (2001) have demonstrated that application of aqueous or gaseous ammonia effectively reduces aflatoxin levels on corn below the current food and Drug Administration (FDA) guideline level of 20 ppb. Elimination of aflatoxin by treating grains with ammonia can be confirmed by assessing the performance of farm animals fed on diets containing ammonia treated grains.

The objective of this study were to determine the effects of ammoniation method in reducing the aflatoxin toxicity in broiler chicks by observing their effects on performance and haematological features.

MATERIALS AND METHODS

A total of 320 1-day-old male commercial broiler (Ross 308) were divided at random into 16 replicate groups of 20 chickens. The experimental design was a completely random design with 4 treatment. The husbandry was similar to that practised in commercial flocks. Birds were fed a balanced diet, based on corn and soybean meal, formulated according to the recommendations of the National Research Council (1994). This consisted of: metabolizable energy (ME), 3000 kcal kg^{-1}; crude protein (CP), 18.75%; Ca, 0.84%; available phosphorus, 0.32%; methionine + cysteine, 0.67%; lysine, 0.96%. In treated groups, the rations were prepared by replacing corn containing known levels of aflatoxin. The final concentration of aflatoxin in diet was adjusted according to aflatoxin B1 (AFB) levels as representative mycotoxin. There were four experimental diets:

A (control) : Basal diet prepared with uncontaminated corn.

B : Basal diet containing ammonia (1%, v/w) treated uncontaminated corn.

C : Basal diet containing contaminated corn (1 ppm AFB).

D : Basal diet containing ammonia treated contaminated corn (1 ppm AFB).

High levels of aflatoxins were produced on rice as a natural substrate by toxigenic A. parasiticus (isolate #14) isolated from Pistachio nuts in our laboratory (Allameh *et al*., 2001). One-liter capacity flasks, each containing 150 g of rice, were inoculated with fungal spores (6.5×106-7.0×106) and then incubated at 28°C for 5 days. Further processing was done as per the procedure described by Shotwell *et al*. (1966). Successfully fermented rice was then steam heated to kill the fungi, the rice was then dried and ground to a fine powder. The rice powder was added to corn and aflatoxin was extracted and measured in rice powder using high performance liquid chromatography (HPLC) based on the procedure described by Wilson and Romer, using Mycosep multifunctional cleanup column. Aflatoxin containing rice powder was mixed with uncontaminated corn to obtain desired levels of aflatoxins, i.e., approximately 1.0 ppm. Grains were moistened with water prior to contamination with aflatoxin containing rice powder mixed thoroughly and left at room temperature for 7 days. Contaminated grains were divided into 2 parts, 1 part was subjected to ammoniation process and other part was incorporated into ration containing known level of toxin and considered as positive control (group C). Ammoniation process was carried out in a pilot plan designed and built based on the industrial plants available (Bagley, 1979; Brekke *et al*., 1978; Coker *et al*., 1985). The unit consisted of three parts, a 200-kg capacity stainless steel tank, a 5-l capacity ammonia vapor-generating tank and an electronic digital controlling box displaying pressure, temperature and moisture in the tank. Contaminated corn was added to the tank, moistened and by water prior to ammoniation process by starting ammonia-generation device. Ammonia vapors were generated from the generator beneath the main tank and were allowed to saturate the corn bed. The concentration of aqueous ammonia was adjusted to 1% (v/v) based on our recent experiment carried out directly on A. parasiticus, a potential aflatoxin-producing fungal strain (Namazi *et al*., 2002). The moisture in the tank was approximately 18% under a temperature ranging from 40-50°C. At the end of the process (48 h), corn grains were removed from the tank and spread at room temperature for 6-7 days to allow drying and to minimize unpleasant smell.

The chicks were weighed every week. Data on weekly food intake, food conversion ratio (FCR = food intake/weight gain) were recorded in each replicate group. At 42 days of age, 8 birds in each treatment were killed by decapitation. Liver, kidney, proventriculus and pancreas were collected and weighed. The weights expressed as percent of live weight.

On 21 and 42 days of age, blood was collected from 12 birds in each treatment into vials containing EDTA (1.0 mg mL^{-1}). The white blood cell (WBC) was determined by a haemocytometer method using Natt-Herrick Solution; hematocrit values was measured by microhaematocrit and differential leukocyte counts were determined as described by Konuk. Data for haematological values were grouped and expressed as mean±pooled standard errors of the means. Analysis of variance and treatment means were ranked by Duncan's multiple range test (SAS Institute Inc., 1994).

RESULTS AND DISCUSSION

The feed rations used for poultry feeding are summarized in Table 1. These data show that pretreatment of corn with 1% aqueous ammonia in the inactivation unit for 48 h almost completely removed aflatoxins, based on AFB level as the representative mycotoxin. Treatment of the corn contaminated with 1000 ppb aflatoxin B1 (AFB) by this procedure resulted in destruction of>98% of the aflatoxins. The result of mortality (Table 1) of chicken among different experimental groups showed that in control group (group A) at the end of 6 weeks the rate of mortality was 5.5%. The mortality rate was found to be higher in group C (22.5%) having chickens fed rations containing approximately 650 ppb AFB. Ammoniation process was found to be effective in reduction of mortality rate in chicken fed contaminated-ammoniated corn.

Aflatoxins may cause significant losses to the poultry industry due to reduced performance and health problems in the exposed birds. Data presented in Table 2 showed the effect of AFB and ammonia treated corn on feed intake, body weight gain and feed conversion ratio of broilers. Feed intake and feed body weights of broilers receiving AFB for the 3 and 6 week were significantly decreased in treatment 3 and increased in treatment 4 ($p<0.05$).

Several researchers have reported that aflatoxin decreases body weights and feed intake and increases relative organ weights in broilers (Raju and Devegowda., 2000; Kubena *et al*., 1990). The depression in body weight (BWG), feed intake(FI) and feed conversion ratio (FCR) (Table 2) upon aflatoxin has been attributed to reduced protein synthesis, impaired nutrient absorbtion and reduce pancreatic digestive enzyme productions (Swamy and Devegowda., 1998). These detrimental effects of AF on FI, BWG and FCR are due to anorexia, listlessness and the inhibitory of AF on protein synthesis and lipogenesis (Oguz and Kurtoglu., 2000; Kiran *et al*., 1998). Impaired livers functions and protein/lipid unilization mechanisms may also have affected growth and general health (Kececi *et al*.,1998). Dersjant-Li *et al*. (2003) have reviewed the impact of dietary aflatoxins on the performance and growth rate of pigs and broilers suggesting a relationship between aflatoxin in diet with the growth rate. Treatment of aflatoxin contaminated corn in pilot-plant scale inactivated aflatoxins and feeding of this diet improved the feed intake, body weight gain and feed efficiency values (Table 2). Chelkowski *et al*. (1982) reported results where day-old male and female broiler chicks were fed ammoniated corn from 4-7 weeks. Body weights of chickens fed ammoniated corn were essentially the same as those for birds on untreated products. Bolden and Jensen (1985) stated that the nutritional deficiency induced by the aflatoxin could have disrupted that

Table 1: Dietary rations used and the mortality rate in chickens maintained for 6 week

Exeperimental groups	Initial AFB_1 in corn (pbb)	Aqueous ammonia (%)	AFB_1 (ppb) in feed	Mortality rate (%)
A	0	0	0	5.8[a]
B	0	1	0	6.5[a]
C	1000	0	650	22.5[b]
D	1000	1	3.5	8.7[a]

Initial level of aflatoxins in corn was adjusted by mixing contaminated rice that was used as natural substrate for growth and production of aflatoxins by toxigenic A. parasiticus. The mortality rate was calculated at the end of breeding (after 6 weeks). Corn grains were ammoniated for 40-48 h. Means in the same column without common letters (a-c) differ significantly ($p<0.05$)

Table 2: Effects of corn treatment on feed intake and body weight and feed efficiency in broiler chickens*

Exeperimental	Feed intake (g)		Body weight (g)		Feed conversion ratio	
groups	0-3 week	0-6 week	0-3 week	0-6 week	0-3 week	0-6 week
A	38.1±1.9[a]	76.4±2.1[a]	21.3±0.54[a]	39.1±0.67[a]	1.8±0.82[a]	1.95±0.65[a]
B	37.8±1.3[a]	80.8±3.9[a]	21.8±0.85[a]	40.5±2.1[a]	1.73±0.79[a]	1.99±0.34[a]
C	33±0.5[b]	71.2±4.1[b]	17.1±0.38[b]	31.2±1.8[b]	1.93±0.35[b]	2.30±0.21[b]
D	35.9±0.9[a]	73.8±2.1[a]	19.7±0.64[a]	36.5±1.3[a]	1.82±0.5[a]	2.02±0.57[a]

*Values are expressed as group mean±S.D. means within columns with no common superscripts differ significantly ($p<0.05$). Experimental groups are as shown in Table 1. Feeding started from the first day of housing and parameters calculated on days 21 and 42 days of age. Data presented as mean±S.D. Mean values in same column without common letters (a-c) differ significantly ($p<0.05$)

activity of the digestive enzymes and the absorption of essential nutrients. Likewise the effect of feeding ammoniated agricultural products on nutritional and safety parameters have been documented and their usage are suggested in different animals (Chelkowski *et al*., 1982; Hoogenboom *et al*., 2001; Pyane *et al*., 1972; Waldroup *et al*., 1976). The production parameters together with the mortality rate were found to fairly recover when contaminated corn was replaced by ammonia treated grains containing <10 ppb aflatoxins (Table 1). Differences in the mortality rate shown in Table 1 may not be considered as the direct effects of ammonia, but probably the mortality rate is reduced as a consequence of improvements in the health and productions parameters. This was further substantiated by showing that uncontaminated corn grains treated with ammonia failed to change the mortality rate in case of group B (Table 1). Using this procedure the degraded products of aflatoxin B1, particularly, aflatoxin D1 (Grove *et al*., 1984; Piva *et al*., 1995), were not detected when analyzing aflatoxins in the diet suggesting that probably only small amount of the original AFB (0.1-1%) may remain in corn grains as shown earlier by Lee *et al*. (1974) and Schroeder *et al*. (1985).

Liver and kidney weights were increased by AF. Aflatoxin B1 being highly hepatotoxic and nephrotoxic, bring about appreciable changes in the general functioning and appearance of liver and kidney (Tung *et al*., 1975). Similar increase in size of liver and kidney with AF was reported (Kubena *et al*., 1997). Livers of the intoxicated chicks were larger, yellowish, fatty and more friable than those of control chick's (diet 1). The increase in relative weight of liver could be attributed to increases lipid deposition due to impaired fat metabolism (Tung *et al*., 1972). An extensive accumulation of lipid droplets (fatty liver) after 21 or 42 days of treatment and degenerative cell bodies were found to be dependent on the duration of exposure to aflatoxins. Prolonged exposure to low concentration of the toxin may produce merely reduced growth rates and significant hepatic signs. The enlargement may be partly due to hypertrophy of hepatocellular smooth endoplasmic reticulum and some degree of fatty change (Jones *et al*., 1993).

The effects of aflatoxin on proventriculus are believed to be a result of severe inflammation and the resultant thickening of the gastric Mucosa (Kubena *et al*., 1997). Aflatoxin induced greater kidney, proventriculus and pancreas weights in the present study and this is in agreement with the effects of aflatoxin on organ weights of poultry (Huff *et al*., 1986). The improvement in the relative weights of liver, kidneys and proventriculus (Table 3) observed when ammonia-treated corn was added to diet (group 4) suggested that the severity of aflatoxicosis was suppressed, that aflatoxins, in corn inactivated by ammonia-treated.

Aflatoxin might have affected the tissues of haemopoietic and immune systems and thereby the production of cells might have been affected. Various studies have reported that the haemotocrit, RBC counts and thrombocyte counts were decreased by AFB and aflatoxicosis caused lymphocytopenia and heterophilia in broiler chickens. In this study, the decreases in the mean value of haematocrit in AFB-fed chicks indicate the depressing effect of AF on haemopoietic tissue. The decrease in haematocrit in the chicks given aflatoxins may be related to the inhibition of protein synthesis by aflatoxins (Kubena *et al*., 1993). The results showing that aflatoxicosis caused lymphocytopenia in chicks given AF alone ($p<0.05$). There was a significant increase in WBC counts mainly consisting of heterophils in chicks given AFB ($p<0.05$). These increases in WBC and percentage of heterophil counts suggest that the toxin is eliciting an inflammatory response in the chicks. These data agree with the report by Mohiuddin *et al*. (1986). There was an alleviation in the alterations of heamatological parameters by feeding ammonia-treated corn, when compared to

Table 3: Effect of corn treatment on relative organ weight* of broiler chickens at 42 day of age**

Exeperimental groups	Liver	Pancreas	Kidney	Proventriculus
A	3.4±0.32^{a}	0.374±0.044^{a}	0.81±0.02^{a}	0.624±0.045^{a}
B	3.48±0.23^{a}	0.446±0.045^{ab}	0.84±0.029^{a}	0.729±0.044^{a}
C	5.24±0.36^{b}	0.545±0.043^{b}	1.24±0.035^{b}	0.838±0.045^{b}
D	3.7±0.42^{a}	0.496±0.056^{ab}	0.94±0.28^{a}	0.664±0.059^{a}

*values are expressed as grams/100 gr body weights. ** Values are expressed as group mean±S.D.means within columns with no common superscripts differ significantly ($p<0.05$). Experimental groups are as shown in Table 1

Table 4: Effect of corn treatment on haematocrit, total leukocyte (WBC) and differential leukocyte counts (hetrophil,lymphocyte) of broiler chickens at 21 and 42 days of age*

Exeperimental	WBC(x10^{3}/mm^{3})		PCV(%)		Hetrophil(%)		Lymphocyte(%)	
groups	21	42	21	42	21	42	21	42
A	2.83±0.09^{b}	6.75±45^{a}	34.6±0.65^{ab}	37±1.11^{a}	24±5.12^{a}	29.5±4.17^{a}	72.66±5.01^{a}	66.5±4.7^{a}
B	4.25±0.07^{b}	6.875±0.3^{a}	34±0.56^{ab}	34.5±0.8^{ab}	27±4.43^{ab}	30±3.2^{a}	71±4.34^{ab}	67±3.6^{ab}
C	13.12±.0.2^{a}	10.75±0.7^{b}	28.5±0.54^{c}	31±1.8^{d}	44.5±4.47^{bc}	42.5±2.8^{bc}	54±3.5^{bc}	54.5±4.25^{bc}
D	5.87±15^{b}	8.225±0.38^{a}	33.5±0.66^{ab}	35.5±1.3^{ab}	28.25±7.87^{ab}	32±3.5^{abc}	69.5±3.85^{ab}	64±4.5^{ab}

* Values are expressed as group mean±S.D.means within columns with no common superscripts differ significantly ($p<0.05$). Experimental groups are as shown in Table 1

Table 5: Effect of corn treatment on differential leukocyte counts (Eosinophil, Monocyte) of broiler chickens at 21 and 42 days of age*

Exeperimental groups	Eosinophil (%)		Monocyte (%)	
	21	42	21	42
A	1.33±0.24	3±0.16	1±0.25	1
B	1.75±0.17	2.5±0.2	0.25±0.1	0.5
C	1.25±0.21	3±0.16	0.25±0.11	1
D	1.72±0.11	2.5±0.28	0.25±0.12	1.5

*Experimental groups are as shown in Table 1

control values (Table 4). There are no significant differences in percentage of monocyte, eosinophil counts with these treatments (Table 5).

CONCLUSION

In conclusion, it seems that ammoniation strategy is potentially feasible for the reduction of aflatoxins in corn, leading to improvement of production parameters in broilers.

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