

PATHOLOGICAL EFFECT OF LOW GRADE AFLATOXICITY IN BROILERS

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ABSTRACT

Experimental mycotoxicoses was induced into broiler chickens by feeding 100 and 150 ppb aflatoxin from 0 to 45 days of age to evaluate the gross and histopathological changes. Grossly, the liver was enlarged, icteric, mottled soft and friable. Histopathologically, the liver showed hepatomegaly, focal necrosis, degenerative changes of hepatic cell, icteric mucous membranes, petechial haemorrhages, diffuse centrilobular necrosis, fatty change, chronic toxicity resulted in cirrhosis and bile duct hyperplasia. Renal parenchyma showed degeneration and necrosis of tubules with focal lymphocytic infiltration in kidneys. In lungs shows congestion and oedema. Brain was showed occasional congestion & vacuolation. Spleen was congested and slightly increased in size. Bursa of fabricius was showed slight congestion throughout the study. Thus from above observations it was concluded that aflatoxin fed at 100 and 150 ppb have adverse effect on general health of broilers.

INTRODUCTION

Aflatoxins are produced by the strains of *Aspergillus flavus* and *Aspergillus parasiticus* and can be present as contaminants in a variety of food and feedstuffs (Beg *et al.*, 2006; Richard, 2007; Shah *et al.*, 2010 and Starling *et al.*, 2012). The mechanisms of action of aflatoxins involve their metabolism to reactive intermediates, which bind to macromolecules with consequent disruption of transcriptional and translational processes (DIAZ, 2005). Aflatoxin B1 is the most prevalent form and has potent hepatotoxic, carcinogenic, genotoxic, immunotoxic and other adverse effects in many animal species including poultry (Miller and Wilson, 1994; Richard, 2007; Fink-Gremmels, 2008; Rawal *et al.*, 2010). Since definitive ways for complete detoxification of mycotoxin contaminated food and feed do not exist, new approaches to mitigate mycotoxicosis are being investigated (Bintvihok and Kositcharoenkul, 2006; Madrigal-Santillan *et al.*, 2007; Ozen *et al.*, 2009; Sirajudeen *et al.*, 2011). Aflatoxin B1 can occur as natural contaminant with poultry feed. Since aflatoxin B1 has been shown to exert an inhibitory effect on hepatic cytochrome P450 monooxygenases (Guerre *et al.*, 1996a, b, 1999, 2000; Meissonnier *et al.*, 2007). It has been suggested that a different doses of aflatoxins at low concentration may have negative effects. The above facts and the limited literature on this subject prompted the authors to study the pathological changes in various organs of broiler chickens fed with low grade aflatoxin.

MATERIALS AND METHODS

Broilers and diet

Ninety one-day-old, Vencobb 400 broiler chicks were obtained from a commercial hatchery. Individually weighed chicks were divided at random into three groups. The chicks were housed in electrically heated compartments with continuous lighting and were fed a commercial feed starter (maize and soybean based, 230 g protein, 13.80 MJ ME kg⁻¹) up to 21 days and thereafter a grower diet (215 g protein, 13.60 MJ ME kg⁻¹) up to 45 days. Broiler chickens were allowed access to the diets and water *ad libitum*. The basal diets were tested for possible residual aflatoxin before feeding and there were no detectable levels present.

Experimental design

The experimental design consisted of three dietary treatments: (1) Control: Basal diet; (2) Treatment Basal diet + 100 ppb aflatoxin in diet; (3) Treatment Basal diet + 150 ppb aflatoxin in diet. All the results were analysed statistically by analysis of variance to determine the means and standard error, as per the method described by (Snedecor and Cochran, 1967).

Aflatoxin

The Aflatoxin was produced from *A. parasiticus* NRRL 2999 culture (USDA, Agricultural Research Service, Peoria, IL) via fermentation of rice by the method of Shotwell *et al.* (1966). Successfully fermented rice was then steamed to kill the fungus, dried and ground to a fine powder. The aflatoxin content in rice powder was analyzed by the method of Shotwell *et al.* (1966) and measured by sending feed sample to Nammakkal.

Pathological examination

When the chicks reached 15th, 30th & 45th days of age, the feeding trial was terminated and 10 broilers from each treatment were selected at random and sacrificed for pathological examination. Selected animals were weighed before being sacrificed. A detailed necropsy was then conducted. The liver, kidney, lung, brain, spleen and bursa of fabricius were removed. Tissue samples from these organs were collected in 10% neutral buffered formalin. After fixation, samples were dehydrated in alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut at 5 μ m and stained with haematoxylin and eosin (Culling, 1974). Microscopically, hepatocellular degeneration in livers showed changes as follows; Slight mild hepatocellular swelling due to hydropic degeneration and fatty changes only in centrilobular areas. Moderate clear hepatocellular swelling in both centrilobular and midzonal areas. Severe diffuse and severe hepatocellular swelling, cytoplasm paleness and rupture.

RESULTS

Grossly, the livers from the 100 & 150 aflatoxin groups fed birds showed mild to moderate enlargement, pallor, occasional mottling and gall bladder distension respectively. Kidneys were enlarged, pale or congested with a few petechiae. Microscopically, the livers of the 100 & 150 aflatoxin groups fed birds revealed vacuolar degeneration, fatty degeneration (Fig. 3 b), lymphoid aggregation (Fig. 2) and degeneration of the hepatocytes. The hepatocytes showing fatty changes coalesced to form fatty cysts. The architecture of the liver was completely altered and the regenerating hepatocytes were arranged in acinar patterns (Fig. 3 a). The acinar hepatocytes also showed fatty degeneration and fatty cysts in some areas. Congestion, bile duct hyperplasia, focal infiltration of heterophils and mononuclear cells, perivascular infiltration of mononuclear cells and heterophils and fibrosis were also noticed in the livers. Kidneys revealed congestion, focal haemorrhages, increased glomerular cellularity (Fig. 1 a) and vacuolar degeneration of tubular epithelium (Fig. 1 b) in all toxin fed groups. In addition, occasional thickening of basement membrane in the 150 ppb group aflatoxin fed birds. Granular degeneration of sarcoplasm, necrosis and collection of mononuclear cells in the heart and hyaline degeneration, necrosis and mononuclear cell infiltration of the pectoral muscle were also observed. The spleens showed lymphoid depletion, an increase in the number of germinal centres and reticulum cell hyperplasia in all toxin treated birds. The bursa of the Fabricius of the 100 & 150 ppb aflatoxin groups fed revealed a lack of cortico-medullary differentiation, generalized lymphoid depletion and heterophilic infiltration. Mild to marked congestion in lungs was seen from birds. There was minimal vacuolation and congestion in the brain of birds aflatoxin fed at 150 ppb. The histopathological organs of birds belonging to control group did not show any change throughout the study period.

DISCUSSIONS

The gross and microscopic investigations in the previous

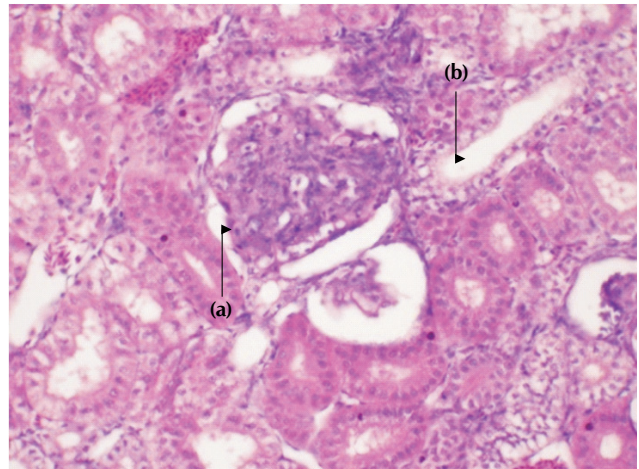


Figure 1: (a) Increase glomerular cellularity and (b) vacuolar degeneration in tubules of kidney of aflatoxicated group (X400 H and E)

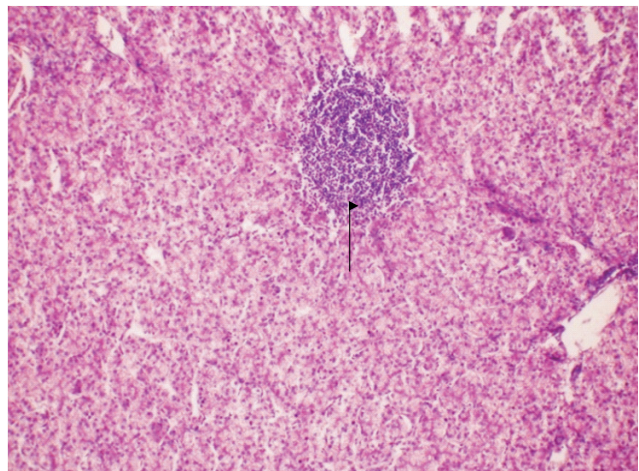


Figure 2: Liver section showing focal lymphoid aggregation with degenerative changes in aflatoxicated group (X100 H and F)

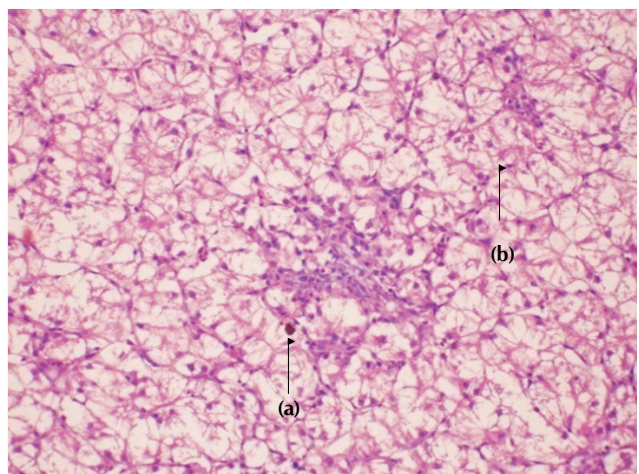


Figure 3: Liver section showing acinar pattern with mononuclear cell infiltration and (b) fatty degeneration in hepatic parenchyma in aflatoxicate group (X200 H and E)

studies showed that aflatoxin affected the organs belonging to the haemopoietic, immune and reticulo- endothelial systems (Ortatatli and Oguz, 2001, Kumar and Chidambaram, 2009; Khan *et al.*, 2010; Agha *et al.*, 2011 and Starling *et al.*, 2012). In this study, the detrimental effects of aflatoxin were investigated in the point of pathological changes. Liver, kidney and the immune system organs are considered to be target organs for aflatoxin and these are primarily affected in aflatoxicosis groups. The particular aim of this study was to induce chronic aflatoxicosis in broilers during one broiler production period (45 days) with lower aflatoxin levels (100 and 150 ppb), which may naturally occur under field conditions. Many studies have been performed to observe the toxic effects of aflatoxin on the target organs with higher levels of aflatoxin (1-5 ppm). It is also important to determine the minimal aflatoxin levels that affect broilers in terms of histopathology. In this study, the toxic effects of aflatoxin on liver, kidney and bursa of Fabricius have been clearly observed by feeding 100 and 150 ppb aflatoxin fed for 45 days. However, it cannot be concluded from the present investigation that whether 100 ppb aflatoxin level causes aflatoxicosis in broilers as no significant difference was observed compared to the control group. This critical aflatoxin level (150 ppb) has been supported by other studies that reported the clinical, haematological, biochemical and histopathological changes began at 150 ppb onwards in feed in broilers (Giambrone *et al.*, 1985; Marquez and Hernandez, 1995; Khajarearn and Khajarearn, 1999, Ortatatli *et al.*, 2005 & Sawarkar *et al.*, 2011). The published report on performance of the same broilers used in this study showed that 150 ppb aflatoxin in feed significantly reduced the performance of chicks (Ghosh *et al.*, 1989; Bakshi *et al.*, 1995; Oguz *et al.*, 2000 & Denli, M., 2009) in agreement with the pathological results in the present study. However, the humoral immunity of these chicks was affected in the lower AF dose (100 ppb) in feed (Oguz *et al.*, 2003, Ortatatli *et al.*, 2005 & Bedre *et al.*, 2010). That no macroscopic changes were found even in target organs in this study could be due to the lower levels of aflatoxin used. However, even if mild, some histopathological changes were seen in liver, kidneys and bursa Fabricius from the chicks receiving the 150 ppb aflatoxin alone diet. The moderate histopathological changes observed in this study are in agreement with the results of previous studies performed by various lower levels of aflatoxin (100 – 500 ppb) in broilers (Giambrone *et al.*, 1985; Balachandran, C. and R. Ramkrishnan 1987, Ghosh *et al.*, 1989; Marquez and Hernandez, 1995 and Bedre *et al.*, 2010), laying hens (Dafalla *et al.*, 1987), ducks (Sell *et al.*, 1998; Khajarearn and Khajarearn, 1999), wild turkeys (Quist *et al.*, 2000). Previous studies have stated that the periportal fibrosis and bile-duct hyperplasia findings, in particular, may constitute chronic aflatoxicosis cases and indicate the regenerative changes in the liver. (Espada *et al.*, 1992, Raja Kumar and Chidambaram Balachandran 2009, and Bedre *et al.*, 2010) have also reported that vacuolation of liver cells and cellular depletion in the follicle medulla of the bursa Fabricius appeared first and persisted during the recovery phase in experimental aflatoxicosis. These findings support our results, indicating that chronic aflatoxicosis can be produced by feeding lower levels of aflatoxin (100 and 150 ppb) over a long-term period (45 day). These results clearly

demonstrated that slight to moderate histopathological lesions were observed in broilers fed a diet containing 100 & 150 ppb aflatoxin toxicity respectively.

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