Pathology of oviduct in spontaneous cases of Aflatoxicosis in commercial layers

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ABSTRACT


The present study was conducted to investigate the pathology of oviduct in spontaneous cases of aflatoxicosis in commercial layer chicken. Among the 85 commercial layer feed samples analyzed for aflatoxin, 40 were found positive. Within the positive samples two showed aflatoxin level of 100 and 260 ppb and the flocks received this ration exhibited the signs of clinical aflatoxicosis. In affected flocks, production drop, morbidity and mortality were 0 to 15, 0.5 to 20 and 0.5 to 4.0 per cent respectively. Grossly, congested and degenerated ovaries with distorted and pedunculated ovarian follicles along with mature ones and no pathological changes in oviduct except congestion of serosal blood vessels were observed. Liver was enlarged, pale and friable in consistency. Histopathologically, oviduct showed focal to diffuse degeneration, necrosis of surface epithelium, infiltration of mononuclear cells and atrophy of tubular glands. Liver showed fatty change, individual cell necrosis, congestion, hemorrhage and bile duct hyperplasia. Presence of aflatoxin was detected only in liver (4 to 6ppb) of the birds which received 260 ppb aflatoxin in feed.

Keywords: Aflatoxin, layer, oviduct, pathology.

INTRODUCTION

Presence of fungi and their toxic metabolites (mycotoxin) in poultry ration is virtually inevitable because they are naturally occurring compounds particularly in tropical countries like India. They contaminate crops before harvest or invade feedstuffs of laying hen during processing, transport or storage. Aflatoxins are toxic, carcinogenic secondary metabolites of Aspergillus flavus and A. parasiticus. Aflatoxin B1 is the most toxic and frequently produced metabolites and cause drastic reduction in egg production, increased susceptibility to other diseases and even leading to mortality in layer farms. Apart from this, it was also detected in eggs collected on day one after consuming contaminated feed. This indicates that the toxin was transmitted through egg white as egg yolk was fully developed before day 1. The egg white is formed in magnum region of oviduct and wherever the toxin or its metabolites get absorbed, it produces pathological changes there. However, the information available in the literature regarding the pathological effects of aflatoxin on oviduct in layer chicken is scanty. Hence the present paper describes the gross, histopathology of oviduct and tissue residues of aflatoxin in spontaneous cases of aflatoxicosis in commercial layer chicken.

MATERIALS AND METHODS

The study was carried out over a period of three years (2005 to 2008). A total of 6572 carcasses of commercial layer chicken above 20 weeks of age belonging to white leghorn breed from poultry farms situated in and around Namakkal district, Tamil Nadu, India were investigated. During postmortem examination, farms in which the birds showed oviduct abnormalities were selected for feed analysis. The feed samples collected from 85 farms constituting birds oviduct abnormalities were analyzed for the presence of aflatoxin according to Romer. The information regarding breed and strain of chicken, flock strength, age, method of rearing, vaccination schedule, production performance, symptoms manifested and mortality were collected from farms with aflatoxicosis and oviduct abnormalities. All the flocks were vaccinated according to a standard vaccinations schedule including Marek’s disease, Newcastle disease, infectious Coryza, infectious bronchitis, infectious bursal disease and fowl pox.

During post mortem examination of dead birds, the oviducts were examined for pathological changes. For microbiological analysis, heart blood and oviduct swabs were collected and placed in Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours. Oviduct, spleen, caecal tonsil, kidney and trachea were subjected to haemagglutination (HA) and haemagglutination inhibition (HI) test for detection of Newcastle disease virus (NDV) and infectious bronchitis virus (IBV). Materials for histopathology were collected from different parts of oviduct and fixed in 10% neutral buffered formalin for 24 hours. After fixation, samples were dehydrated and processed by following the routine
histological procedures. Histological sections after being stained with hematoxylin and eosin were examined by light microscopy. Liver and oviduct samples were collected in saturated sodium chloride solution for estimation of tissue residue levels of aflatoxin. The tissue samples were quantified for aflatoxin by using thin layer chromatography at the Central Animal Feed and Food Residues Analytical Laboratory, Centre for Animal Health Studies, TANUVAS, Chennai - 600 051.

RESULTS

Analysis of 85 feed samples collected from farms affected with oviduct abnormalities revealed the presence of aflatoxin ranging from 0 to 260 ppb. Out of the 85 feed samples subjected for analysis, no aflatoxin was detected in 45 samples. The presence of aflatoxin ranging from 0-9 ppb, 10-19 ppb, 20-99 ppb and 100 ppb and above was detected in 20, 18, 0 and 2 samples respectively. Among the 85 feed samples tested for aflatoxin, 83 were within the maximum permissible category (20 ppb) and two feed samples exceeded the maximum, permissible limit of aflatoxin. The two flocks which received the feed having 100 ppb and 260 ppb showed a production drop, morbidity and mortality of 9 and 15, 13 and 20 and 2.5 and 4 per cent respectively.

In flocks which received feed with dietary aflatoxin up to 20 ppb, the birds were healthy and no apparent effect on feed intake but fluctuation in egg production and slight increase in mortality were noticed, where as in farms which received 100 and 260 ppb showed a dose related decrease in feed intake, egg production, variation in egg size and increased in mortality. Heart blood and

Fig. 1. Ovary showing areas of haemorrhages and congestion on the degenerated ovarian follicles (Black arrow – congestion and Red arrow – haemorrhage), Fig. 2. Oviduct showing congestion of serosal blood vessels A- Normal, B- affected, Fig. 3. Magnum showing moderate atrophy of tubular glands and congestion of vessels. H&Ex400, Fig. 4. Isthmus showing atrophied mucosal folds with infiltration of lymphocytes in interglandular spaces. H&Ex400.
Histologically, flocks which received up to 20 ppb revealed intact cilia on surface epithelium, tubular glands with acidophilic cytoplasm and infiltration of few mononuclear cells in between the glands in different parts of oviduct. In flocks which received 100 ppb and above, the lining epithelium of infundibulum showed focal degenerative and necrotic changes. Congestion of blood vessels was noticed in the lamina propria and serosa. Mild infiltration of lymphocytes and plasma cells were observed in the lamina propria. Perivascular infiltration of mononuclear cells was observed in the muscular coat. The Magnum revealed degeneration, necrosis and desquamation of lining epithelial cells and accumulation of cellular debris within the lumen. The tubular glands showed moderate atrophy and the vessels of intertubular glands revealed congestion (Fig. 3). In isthmus, the surface epithelium and tubular glands showed degenerative changes and infiltration of inflammatory cells predominantly lymphocytes in the inter glandular spaces (Fig. 4). Uterus revealed vacuolar degeneration, mild necrotic changes and desquamation of the lining epithelial cells and tubular glands. Vagina revealed no appreciable microscopic changes except moderate congestion of stromal and serosal vessels. The liver showed fatty change characterized by round vacuoles in the cytoplasm of hepatocytes, individual cell necrosis, congestion, hemorrhages, dilated sinusoidal spaces along with mononuclear and heterophilic cellular infiltration around the triads as well as blood vessels and hyperplasia of bile duct.

In the present study, birds fed with feed having 260 ppb revealed the presence of aflatoxins residue in liver at the level of 4 to 6 ppb and the remaining liver samples were negative. Analysis of oviduct samples, did not reveal any appreciable level of aflatoxin residues in all the samples tested.

**DISCUSSION**

Eighty five samples of layer feed analyzed during 2005 to 2008 revealed the presence of aflatoxins in 40 samples. Flocks with toxin levels of 100 and 260 ppb showed clinical aflatoxicosis. Laying hens generally can tolerate higher levels than young birds, but levels should still be less than 50 ppb. The two flocks which received the feed having 100 ppb and 260 ppb showed a production drop, morbidity and mortality of 9 and 15, 13 and 20 and 2.5 and 4 per cent respectively. Aflatoxin contamination can reduce the bird's ability to withstand stress and cause morbidity and mortality in the affected flocks. Liver is considered to be the target organ for Aflatoxin B1 (AFB1) where most toxins are bioactivated to the reactive 8,9 eioxide form, which binds to DNA and protein, resulting in hepatic damage. This hepatic damage in turn interfere the egg formation through an impairment of normal mobilization of fat from the liver to the ovary. In this study no viral or bacterial agents could be detected in the two flocks affected with clinical aflatoxicosis and marked improvement in egg production was observed after withdrawal of the contaminated feeds. Hence, the drop in production in these farms was directly correlated with AFB1 contamination in the feed. Similar findings were reported earlier.

The affected birds revealed congested and shrivelled ovarian follicles with a few matured ones, distorted developed follicles pedunculated and in few birds it was ruptured and the contents were spilled into the abdominal cavity. These changes were in agreement with earlier report. These changes might be due to the individual variation in the sensitivity of the birds to toxin and concomitant nutritional deficiencies caused by the aflatoxin, by affecting the metabolism of lipid, carbohydrate and proteins. The oviducts were normal in size and the serosal vessels were severely congested. The mucosal folds were normal in appearance; however, in few birds mild congestion and dryness were observed. Swollen, pale and friable liver, mild enlargement of kidneys and pale pectoral muscles has been reported aflatoxicosis of chickens.

Histologically, the oviduct showed focal to diffuse degeneration, necrosis of surface epithelium, infiltration of mononuclear cells in the inter glandular spaces, congestion of vessels and marked infiltration of lymphocytes and plasma cells in the lamina propria, atrophy of tubular glands in the magnum and isthmus. These changes were in consistent with findings of...
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Vijayalinga Korandi. These changes might be due to potent damaging effect of aflatoxin on the cellular DNA. Histopathological changes in liver exposed to AFB1 are comparable to those reported in the literature on avian aflatoxicosis.

However, the most critical aspect of aflatoxins in poultry production is the presence of its residues in poultry products especially in eggs and tissues. In the present study, we observed 4 to 6 ppb of AFB1 in liver of the birds fed on the contaminated diets (260 ppb). These findings were in accordance with earlier worker, who also observed high level of AFB1 and its metabolites in the liver than in other tissues. This indicates the important role of liver in the metabolism and elimination of AFB1.

To conclude, the chickens fed with aflatoxin contaminated ration (100 ppb & above) might cause damages in ovary and oviduct due to toxicity; where as birds fed with dietary aflatoxin level up to 20 ppb showed appreciable pathological changes in reproductive organs. Detection of aflatoxin in liver tissue of birds which received 260 ppb pose public health significance on human consumption. Therefore, the control of aflatoxins contamination in rations of laying hen is essential in order to avoid the occurrence of aflatoxins residues in tissues and eggs intended for human consumption.

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