

## Evaluation of aflatoxicosis in hens fed with commercial poultry feed

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**Abstract:** The effect of aflatoxin in the growth of hens was histopathologically analyzed. Mycotoxigenic fungi were isolated and characterized as *Aspergillus flavus* and *Aspergillus niger*. The aflatoxin was extracted from *Aspergillus flavus* and their impact on the growth pattern of hens was evaluated. The histopathological analysis reveals that more lesions were found in the vital organs of hens in comparison with the control chick group. In the present study, it is concluded that the quality of poultry feed plays the most important role in a poultry farm. It is suggested that the use of chicks resistant to aflatoxicosis will help in minimizing the problem of poor growth rate and poor feed conversion, which perhaps are the 2 most important factors in poultry management.

**Key words:** Mycotoxigenic fungus, aflatoxin, aflatoxicosis, histopathology

### Introduction

Mycotoxins are chemically diversified low molecular weight compounds produced by secondary metabolism of fungal genera, such as *Aspergillus*, *Penicillium*, and *Claviceps* over a variety of food stuffs. These mycotoxins exhibit a wide array of biological effects and individual mycotoxins can be mutagenic, carcinogenic, embryotoxic, nephrotoxic, estrogenic, and immunosuppressive. Mycotoxin contamination of various feeds is continuous to be a serious quality and safety problem worldwide; considerable global attention is being focused on mycotoxins because contamination of feeds has adverse effects on animal

health. The occurrence of toxin production by strains isolated from foods and animal feed does not necessarily imply the presence of mycotoxins. However, it indicates a potential risk for a possible mycotoxin contamination. Furthermore, if these feeds represent a good substratum for mycotoxin production and if appropriate factors, especially moisture and temperature, exist, the contamination hazard tends to increase.

Aflatoxin affects all poultry species. Although it generally takes relatively high levels to cause mortality, low levels can be detrimental if continually fed. Young poultry, especially ducks and turkeys, are

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very susceptible. As a general rule, growing poultry should not receive more than 20 ppb aflatoxin in the diet. Laying hens generally can tolerate higher levels than young birds, but levels should still be less than 50 ppb. Aflatoxin contamination can reduce the bird's ability to withstand stress by inhibiting the immune system. This malfunction can reduce the egg size and possibly lower the egg production since the effects of mycotoxins on poultry are dependant upon age, physiological state, and nutritional status of the animals at the time of exposure and since mold growth can occur at various points within the feed production and distribution.

Aflatoxins are secondary metabolites of certain strains of *Aspergillus flavus* and *A. parasiticus*. Aflatoxin ingestion by chickens result in many different symptoms, such as reduced growth and increased susceptibility to infectious agents (1,2). The liver tissue is considered the aflatoxins' target organ due to the protein production inhibition pathway of aflatoxin elicited in the hepatocytes (2). The periportal fibrosis and bile duct hyperplasia observed in this study are in agreement with several reports of liver tissue damage due to aflatoxin exposure (3). Long term consumption of contaminated feed with relatively low aflatoxin content causes immunosuppression in broilers by impairment of humoral and cellular immune response (4). Heavy loss in poultry industry due to interaction of infectious bursal disease and aflatoxicosis was reported by Otim et al. (5). The symptoms observed in aflatoxicosis were anorexia and unthriftiness and the mortality rate was 0.03%. The clinical symptoms were depression and ruffled feathers. Some birds showed prostration before death, severe lymphocytolysis, hepatomegaly, splenomegaly, and hemorrhage in thigh, leg, and pectoral muscles were observed in almost all the cases. The interaction of infectious bursal disease (IBD) and aflatoxicosis (AF) led to increased mortality of 35.6% when compared to 3% - 21% mortality in IBD and 0.03% mortality in aflatoxicosis. A perusal of the literature reveals that there is no detailed report on mycotoxigenic fungi from hens fed from poultry farms in Vellore district, Tamilnadu, India, hence in the present study an attempt was made to isolate and identify the mycotoxigenic fungi from poultry feeds and also the

effect of aflatoxigenic fungi on the growth of hens with reference to histopathological analysis of hens fed with commercial poultry feed.

## Materials and methods

### Sample collection

Poultry feed samples were collected from the feed storage rooms of different poultry farms in Vellore district, Tamil Nadu such as Chittor, Ambur, and Gudiyattam during July 2006.

### Isolation of mycotoxigenic fungi

The potato dextrose agar plates were prepared and the collected poultry feed samples were serially diluted in saline. The samples were inoculated onto agar plates. The plates were incubated at  $28 \pm 2$  °C for 3 to 5 days. After incubation the total number of fungal population per gram of feed were estimated and fungal species were identified.

### Characterization and identification of fungi

Suspected colonies were taken and emulsified in lactophenol cotton blue and observed under microscopic examination, then each colony was inoculated onto potato dextrose agar separately to obtain isolated culture. Based on this morphological and cultural characteristic the fungal isolates were identified (6).

### Aflatoxin analysis

#### Mass production of *Aspergillus flavus*

The potato dextrose broth was prepared to culture the fungi for aflatoxin production. The pH was adjusted to 6.0 and the medium was distributed in a 2 L conical flask and sterilized at 121 °C, 6.804 kg pressure for 15 min. The flask was cooled and then inoculated with a spore suspension of *Aspergillus flavus* and incubated at  $28 \pm 2$  °C for 2 to 3 weeks.

### Extraction of aflatoxin

The potato dextrose broth was cooled and then inoculated with spore suspension of *Aspergillus flavus*. After 10 days, the mycelia were removed from the medium and the liquid culture was filtered through a Whatmann No.1 filter paper. The culture filtrate was concentrated under reduced pressure in an evaporator in a water bath. The concentrated culture filtrate was

shaken repeatedly with 100 mL volumes of chloroform and the extraction repeated 2 or 3 times; the chloroform extracts were combined and filtered through Whatmann No.1 filter paper. From the filtered chloroform extract the toxin was extracted with sodium bicarbonate solution by shaking the chloroform extract several times with 0.5 M sodium bicarbonate solution. All lipid materials were removed by filtration after keeping the sodium bicarbonate extract overnight in a separating funnel. Finally, the pH of the solution was brought down to 2.0 and the toxin was extracted from the concentrate into chloroform by repeated extraction with aliquots of chloroform. The extracts were pooled and concentrated and the crude toxin isolated.

Detection of aflatoxin by thin layer chromatography

Silica gel was coated on thin layer chromatographic plates and dried at 60 °C for 1 h. One milliliter concentration of chloroform extracts was spotted in the form of a thin line on chromatographic plates and developed with chloroform: ethyl acetate: formic acid: toluene (50: 40: 10: 2, V/V) solvent system in a closed chamber. After drying, the plate portion of the plate was sprayed with 1% p-di methyl amino benzaldehyde in n-butanol, dried with warm air and placed in a tank containing hydrochloric acid vapor for 15 min; a bright blue color reaction indicated the presence of aflatoxin B1. The mobility of extracted aflatoxin B1 and authentic aflatoxin was compared.

Effect of aflatoxin on hens

Preparation of aflatoxin mixed diet

The commercial poultry feed was obtained from poultry farms, Tamilnadu. They were powdered and then mixed with 100 ppm concentration of aflatoxin. Aflatoxin mixed feed was again palletized and dried at 37 °C for 5 days to evaporate the chloroform.

Evaluation of aflatoxin on the growth of hens

Healthy unvaccinated hens were obtained; they were separated in 2 groups as group 'A' (control) group 'B' (test). Each group consisted of 25 hens. Each group of hens was labeled for identification. The control hens were fed with a normal diet feed obtained from a commercial poultry farm. The test hens were fed

with an aflatoxin mixed diet. The hens were analyzed on the 25<sup>th</sup> day.

Histopathological analysis

Lung, intestine, kidney, and liver tissues were taken for histopathological studies. After sacrifice, each animal was necropsied and organ lesions were described, with special attention focused on gizzards. Samples of lungs, liver, intestinal tissue, and kidney tissue were taken, fixed in 10% buffered formalin, embedded in paraffin and cut on a microtome in slices of 4-5 mm and stained with hematoxylin-eosin (7).

## Results

The poultry industry probably suffers greater economic loss than any of the livestock industries because of the greater susceptibility of their species to aflatoxin than other species. Aflatoxicosis of animals is usually manifested by pathologic changes in liver but they have been found to be carcinogenic and teratogenic as well as causing impaired protein formation, coagulation, weight gains, and immunity.

Serological, hematological, and pathological effects and mortality have previously been observed in broiler chicks fed with aflatoxins. Results suggested that mycotoxin may affect immune function by suppressing proliferation and inducing apoptosis of lymphocytes. The poultry feeds were more susceptible to *Aspergillus niger* and *Aspergillus flavus* contamination. Aflatoxin affects all poultry species. Although it generally takes relatively high levels to cause mortality, low levels can be detrimental if continually fed. Young poultry, especially ducks, chicks, and turkeys are very susceptible. This study revealed the evaluation of aflatoxicosis in hens fed with commercial poultry feed.

In the present study a total of 3 samples were collected from a poultry farm in Vellore district, in Tamilnadu, India. From the feed, total fungal populations were studied. Totally 40 fungal isolates were observed in all 3 samples. The maximum fungal populations were 15 CFU (sample I), 0 CFU (sample II), and 25 CFU (sample III). Among the total of 40 fungal isolates, the dominant 2 fungal isolates were selected for characterization. The isolate I showed

velvety, yellow to green (or) brown colonies. Their conidiophores were of variable length, rough pitted and spiny characteristic sporing head, conidia were globose and echinulate. The hyphae were hyaline, septate, branch dichotomously the sterigmata only cover half of the conidia. Sterigmata were single.

The characteristics of isolate II were initially white to yellow, then turning dark brown to black colonies and their conidiophores were of variable length, sterigmata were double, covering entire vesicle form radiate head. Hyaline, septate hyphae were present. Conidial head was large in size with black to brownish black. The sterigmata were double, the primary sterigmata were long, and secondary sterigmata were short

Based on cultural and morphological features the fungal isolates were identified as *Aspergillus niger* and *Aspergillus flavus*. *Aspergillus flavus* was cultured in a production medium. The mass cultures were extracted for aflatoxin. The extracted material were separated by thin layer chromatography (TLC) and confirmed that the isolate I was producer of aflatoxin.

#### Histopathology analysis

No hens perished during the experiments. Gross lesions, except for the gizzards, were minimal and mainly involved mild degenerative changes and congestion of the liver and kidneys. More prominent lesions were noted in gizzard of aflatoxin treated animals, compared to control groups.

In the liver, degenerative reversible lesions were present, from mildest to severest degree with various distributions in test groups of mild parenchymatous degeneration characterized by granular appearance of the hepatocyte cytoplasm, observed, severe hydropic and vacuolar degeneration. The vast majority of hepatocytes had significant cytoplasmic vacuolization; disseminated necrotic cells were observed in the experimental groups.

In kidney, moderate parenchymatous tubular degeneration, predominantly of the distal tubules, manifested by epithelial swelling and fine granular appearance of cytoplasm was most prominent in aflatoxin treated hens. In the experimental animals, hydropic and vacuolar degeneration was also noted, but the severe degree characterized by desquamation

of epithelial tubular cells was present in almost all animals in contrast to the control group.

In lungs, congestion and mild perivascular edema were noted in all animals except for the control group. However, thickening and hyalinization of the blood vessel walls were present in all groups but the control group. Peribronchial and perivascular lymphocytic infiltration was noted in individual animals in each group

In the intestine, there were no changes at all, but the mildest form of inflammation, catarrhal inflammation, was observed in the untreated animal group (control group). An important finding was vacuolization of the mesenchymal cells of the lamina propria, which was found exclusively in test animals. (Figure).

#### Discussion

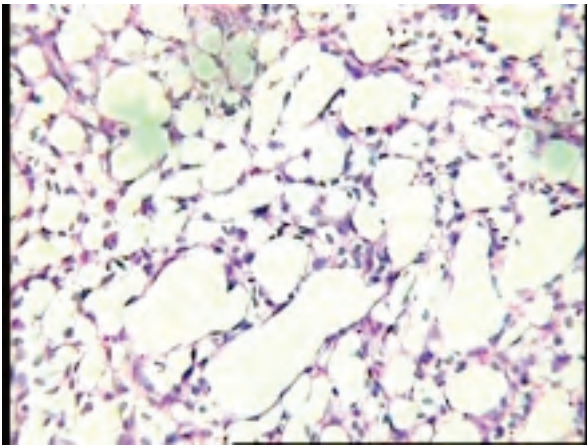
Aflatoxins are natural contaminants of feedstuffs (8). Hens are most sensitive to these toxins (9). Although chicks are claimed to be the most sensitive poultry animals. (10), sensitivity tests carried out on quails revealed that these animals may be easily affected by aflatoxin present in feed (11).

The aflatoxin producing fungi were isolated from 3 different poultry feed samples. Among these samples, sample III revealed the maximum fungal population (25 CFU/g), and the absence of fungal population was observed in sample II. This result reveals that feed samples III and I are from unhygienic storage condition. This finding is similar to the finding of Theophilus et al. (12) who reported mycotoxigenic fungi in maize.

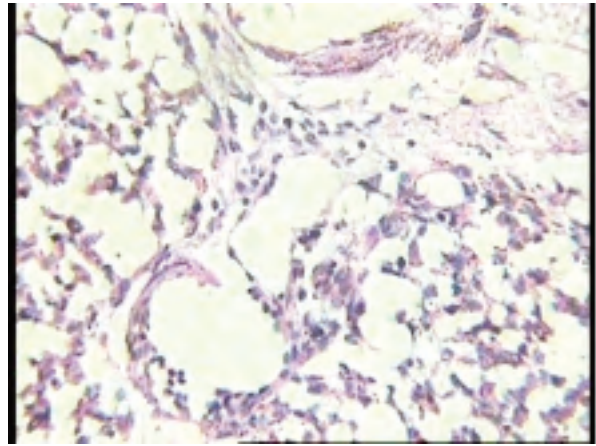
Totally 40 mycotoxigenic fungi were observed in all 3 different poultry feed samples. Among the 40 isolates, 2 dominant fungal isolates were selected, characterized and identified as *Aspergillus flavus* and *Aspergillus niger*. This finding has already been reported by Richard et al. (13).

Histopathological analysis revealed that lesions were observed in tissues of liver, kidney, intestine, and lungs. This result indicates the significant damage of vital organs in hens. These findings coincide with findings of Huff et al. (14) and Reddy et al. (15).

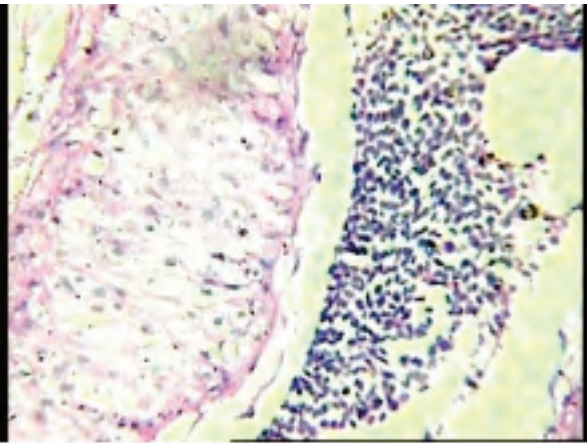




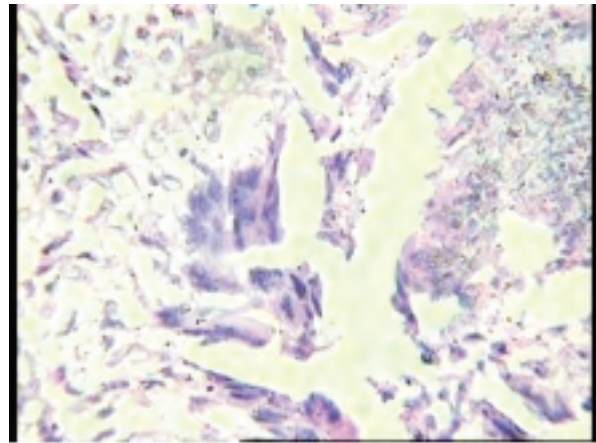
Normal lung cells (Control)



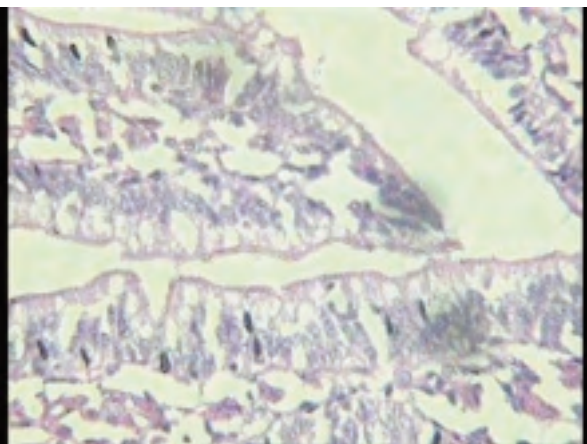
Mild infiltration by L and P (Test)



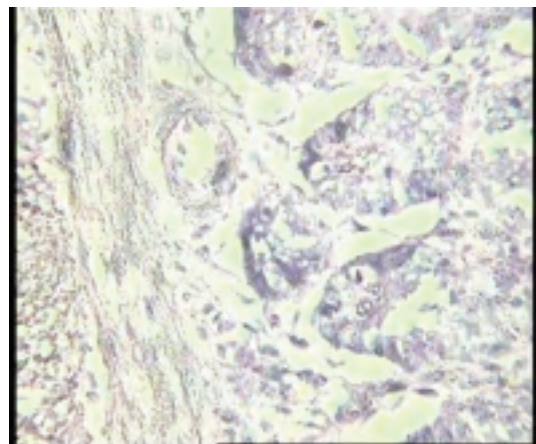
Dense infiltration by L and P (Test)



Infiltration by granular structures suspicious of carcinoma

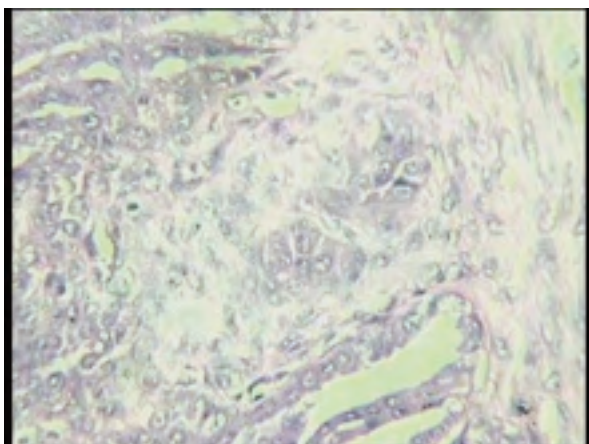


Normal Intestinal mucosa (Control)

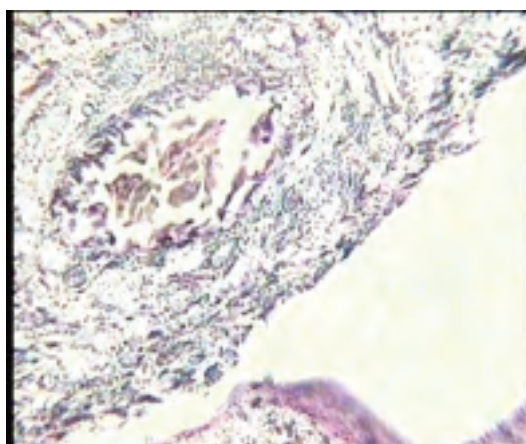


Atypical granular structures some with enlarged nuclei (Test)

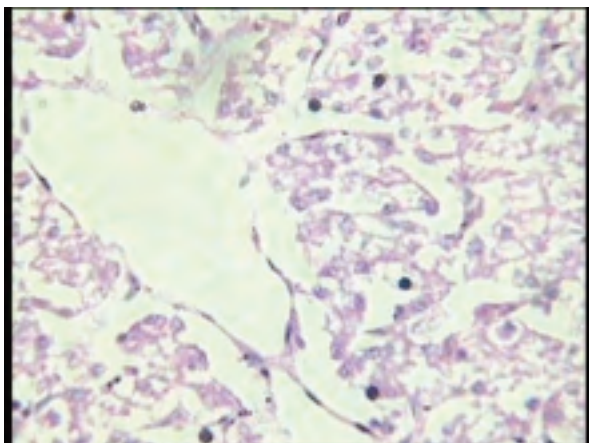
Figure. Histopathological analysis of various organs of hens with aflatoxicosis.



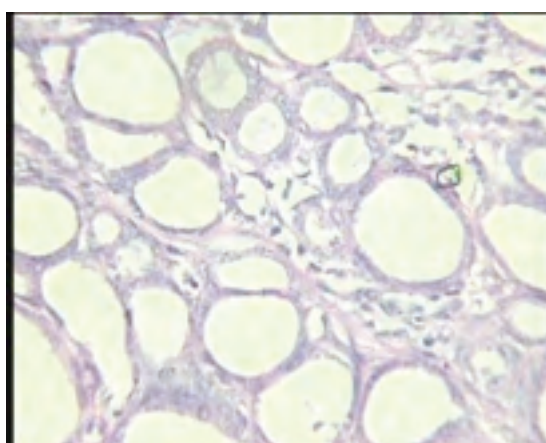
Stomach cells (Control)



Infiltration of glandular structure (Test)



Renal tissue with abnormalities (Test)



Intestine mildly dilated glandular structure (Test)

Figure. (Continued)

The present study concludes that the importance of toxin production by strains isolated from animal feed does not necessarily imply the presence of aflatoxin. However, it indicates a potential risk for a possible contamination with aflatoxins. Furthermore, if these feeds represent a good substratum for aflatoxins production and if the abiotic factors (especially moisture and temperature) are appropriate, the contaminant hazard tends to increase.

Quality of poultry food plays the most important role in the poultry farming as its share is 70%. Good

quality food and resistant strain of chicks can lead to greater production and more profit for the poultry farmer. Poultry industry in Tamilnadu has expanded tremendously during the last few years. However, the acute shortage of chicken meat has pushed its price steeply upwards. It is suggested that use of chicks resistant to aflatoxicosis will help in minimizing problem of poor growth rate and poor feed conversion, which perhaps are the 2 most important factors in poultry management.

## References

1. Clifford, J.I., Rees, K.R.: Aflatoxin: a site of action in the rat liver cell. *Nature*, 1966; 209: 312-313.
2. Javed, T., Bennett, G.A., Richard, J.L., Dombrink- Kurtzman, M.A., Côté, L.M., Buck, W.B.: Mortality in broiler chicks on feed amended with *Fusarium proliferatum* culture material or with purified fumonisin B1 and moniliformin. *Mycopathologia*, 1993; 123: 171-184.
3. Ostrowski – Meeissner, T : Effect of contamination of diets with aflatoxins on growing ducks and chickens. *Trop. Anim. Health Pro.*, 1983; 15: 161-168.
4. Petkov, R., Tacheva, T., Borisova, L.: Paraclinical research on broiler chickens treated with aflatoxin B1 and microbiological studies of the meat and liver obtained from them. *Vet. Med. Nauki*, 1985; 22: 58-64. (article in Bulgarian with an abstract in English)
5. Otim, M.O., Mukiibi-Muka, G., Christensen, H., Bisgaard, M.: Aflatoxicosis, infectious bursal disease and immune response to Newcastle disease vaccination in rural chickens. *Avian Pathol.*, 2005; 34: 319-323.
6. Alexopoulos, C.J., Mims, C.W., Balckwell, M: *Introductory Mycology*. 4<sup>th</sup> edn., John Wiley and Sons, Inc., 1966; 10-25.
7. Allen, T.C.: Hematoxylin and eosin. In: *Laboratory Methods in Histotechnology*. American Registry of Pathology, Washington. 1994; 53-58.
8. Gugnani, H.C.: Ecology and taxonomy of pathogenic aspergilli *Front. Biosci.*, 2003; 8: 346-357.
9. Robens, J.F., Richard, J.L.: Aflatoxins in animal and human health. *Rev. Environ. Contam. Toxicol.*, 1992; 127: 69-94.
10. Parlat, S.S., Yildiz, A.O., Oguz, H.: Effect of clinoptilolite on performance of Japanese quail (*Coturnix coturnix japonica*) during experimental aflatoxicosis. *Br. Poult. Sci.*, 1999; 40: 495-500.
11. Parlat, S.S., Ozcan, M., Oguz, H.: Biological suppression of aflatoxicosis in Japanese quail (*Coturnix coturnix japonica*) by dietary addition of yeast (*Saccharomyces cerevisiae*). *Res. Vet. Sci.*, 2001; 71: 207-211.
12. Theophilus, A., Nandkumar, C., Sudhakar Rao, G.V., Titus George, V., Balachandran, C.: Co-occurrence of aflotoxicosis and infectious bursal disease in chicken. *Indian Vet. J.*, 2005; 82: 935-937.
13. Richard, J.L., Cysewski, S.J., Pier, A.C., Booth, G.D.: Comparison of effects of dietary T-2 toxin on growth, immunoenic organs, antibody formation, and pathologic changes in turkeys and chickens. *Am. J. Vet. Res.*, 1978; 39: 1674-1679.
14. Huff, W.E., Wyatt, R.D., Hamilton, P.B.: Nephrotoxicity of dietary Ochratoxin A in broiler chickens. *Appl. Microbiol.*, 1975; 30: 48-51.
15. Reddy, D.N., Rao, P.V., Reddy, V.R., Yadgiri, B.: Effect of selected levels of dietary aflatoxin on the performance of broiler chickens. *Indian J. Anim. Sci.*, 1984; 54: 68-73.