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Pathological and apoptotic studies of reproductive tract in commercial layer chicken with aflatoxicosis

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Abstract
The reproductive system of female layer chicken was targeted and damaged by various mycotoxins which results in drop in egg production. Mycotoxins, especially aflatoxin (AF) causes drop in egg production by causing damage in hepatic parenchyma and tubular glands of oviduct. The present study was undertaken to identify the level of toxin in feed, pathomorphological and apoptotic changes in oviduct of commercial layer chicken with aflatoxicosis. Feed samples from various layer flocks with drop in egg production were submitted for toxin analysis and representative tissue samples from dead birds were collected in 10% NBF for histopathology and apoptosis. Out of 71 flocks, the AFB1 and AFB2 was detected in 6 flocks and the level of AFB1was 10-20 ppb and AFB2 was 8-10 ppb were identified. The clinical signs of mild anorexia and drop in egg production (9-17%) were observed. The external and internal quality of egg were also seen in affected flocks. Post mortem lesions observed were ascites, more number of atretic follicles and marked atrophy of oviduct. Histopathologically, ovarian follicles showed degenerative changes and follicular atresia. The oviduct showed hypoplasia of tubular glands with degenerated glandular epithelial cells. The apoptotic cells were more in isthmus and uterus and minimum in infundibulum, magnum and vagina.

Keywords: Aflatoxicosis, apoptosis, layer chicken, oviduct, pathology

Introduction
Aflatoxins are toxic metabolites produced by the fungus *A. flavus* and *A. parasiticus* during their growth on feed grains (Coulombe, 1991) [1]. The major aflatoxins are AFB1, B2, G1 and G2, with AFB1 being the most common and toxic is produced in greater quantities than the others (Rizzi *et al.*, 2001) [4]. AFB1 causes decreased egg production in layers (Leeson *et al.*, 1995) [12] and broiler breeders (Manafi, 2011) [2]. In laying hens the effects of exposure to AF are a dose-dependent decrease in egg production and egg quality with increased susceptibility to salmonellosis, candidiasis and coccidiosis (Oliveira *et al.*, 2002) [3]. The main manifestations of chronic aflatoxicosis in layers are reduced egg production and weight and increase in liver fat levels (Rosmaninho *et al.*, 2001) [5]. Decreased in production performance and egg quality indicates that aflatoxin is toxic to layer chicken at a concentration of 1mg/kg. Despite increasing eggshell strength, aflatoxin reduces egg mass, resulting in a product with lower commercial value (Siloto *et al.*, 2011) [6]. Dietary AFB1 causes reduction in eggshell weight of laying chicken reported that a reduction in the eggshell weight of layers exposed to 2.5 mg/kg of dietary aflatoxin B1. Diets contaminated with mycotoxins are reported to cause liver malfunctioning, thus negatively affecting liver fat synthesis and transport of yolk precursors (Zaghini *et al.*, 2005) [11].
Grossly, dark red colored, congested, and atretic ovaries with a few matured follicles. The developed follicles were distorted and pedunculated. In few birds follicles were found ruptured and the contents were spilled in to abdominal cavity. However, in some cases, there were small, non-functional ovaries with small dark brown colored follicles. The oviducts were normal in size. The serosal vessels were congested in all the cases. The mucosal folds were normal in appearance and in few birds it was dry and congested (Srinivasan, 2007) [7]. Vijayalingam *et al.*, 2017 [9] recorded aflatoxicosis in a desi chicken unit with the history retarded egg production with production of poor quality egg includes small sized, yolkless and watery albumin. The Oviduct was very pale and small. The mucosa of the magnum and shell gland portions was dry and less folded. Ovary showed varying sizes of ruptured and atretic follicles. Toxicological analysis of the feed sample showed the presence of AflatoxinB1 (158ppb). Microscopically, ovary showed the presence of atretic follicles and the uterine portion of oviduct had atrophic changes in the shell glands.

Wang *et al.*, 2013 [10] studied AFB1 induced oxidative stress and apoptosis in broiler spleen and he observed increased percentage of apoptosis cells by flow cytometry and the occurrence of apoptotic cells by TUNEL assay in spleen of broiler chicken fed with 0.3 mg/kg of AFB1 for 21 days. However, a limited information was available on the pathomorphology and apoptosis in reproductive tract of layer chicken affected by aflatoxicosis. Hence the present study was undertaken to investigate the pathomorphological and apoptosis changes in various parts of oviduct in commercial layer chicken affected with Aflatoxin associated drop in egg production.

Materials and Methods

Flock history

The study was conducted in commercial layer poultry farms located in Namakkal region of Tamil Nadu during the period from January 2021 to November 2022. A total of 1400 white leghorn layers, above 20 weeks of age from 71 flocks with the history of drop in egg production were examined for reproductive tract pathology. All the flocks were vaccinated as per standard vaccination schedule. The flocks were inspected during the period of increased percentile of drop in egg production and the information regarding age, strain of chicken, flock strength, method of rearing, vaccination history, source of feed and water, production performance including time of peak production, percentage of production, production drop and mortality were collected.

Necropsy, Histopathology and Apoptosis

The dead birds suspected for aflatoxicosis were collected, surface disinfected and necropsies were performed as per standard procedure. Representative tissue samples were collected and fixed in 10% Neutral buffered formalin for histopathology and apoptosis. Samples were processed as per the standard techniques and 4 µm sections were prepared for histopathology and apoptosis. The sections were stained with Haematoxylin and Eosin for histopathological examination. Apoptotic cells were detected by terminal deoxynucleotidyl transferase mediated dUTP nickend-labelling (TUNEL) stain using a commercial ready-to use kit (In Situ Cell Death Detection Kit, ABCAM, HRP-DAB).

Feed Analysis

Feed samples were collected from suspected flocks were submitted for multitoxin analysis to Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal.

Result and discussion

Out of 71 flocks with the history of drop in egg production, the Aflatoxin was detected in feed samples from 6 flocks. The level of AFB1 ranges from 10-20 ppb in all 6 flocks and AFB2 ranges from 8-10 ppb in 3 flocks. The clinical signs of dull and depressed, mild anorexia and drop in egg production (9-17%) with a greater number of small eggs (5-7%), uneven eggs were noticed. No mortality was recorded other than regular mortality. The egg weight, specific gravity, shape index, albumen index, Haugh unit and shell thickness was significantly reduced in affected flocks (Table-1). The same observations were recorded in white leghorn hens to fed with 1ppm of aflatoxinB1 for 42 d (Verma *et al.*, 2004) [8]. Post mortem lesions recorded were presence of clear watery fluid in the abdominal cavity indicating liver damage, more no. of atretic follicles (Fig.1 & 2), ruptured and flaccid ovarian follicles and presence of yolk material in peritoneal cavity were observed. Oviduct showed marked atrophy and the weight and length of the oviduct was markedly reduced (Fig.3 & Table-2). The infundibulum was thickened and showed moderate congestion, and the magnum, isthmus and uterine mucosa was pale and edematous (Fig.4). Similar findings were recorded earlier by Srinivasan (2007) [7] and the feed contained 0-260 ppb of AFB1. Histopathologically, ovarian follicles showed degeneration changes, congestion and follicular atresia. The infundibulum showed hyperplasia of ridge epithelium, edema, infiltration of inflammatory cells and severe congestion in tunica muscularis (Fig.5). Magnum showed hypoplasia of tubular glands (Fig.6). The isthmus showed mild tubular gland hypoplasia with degenerative changes in tubular gland epithelium. The nucleus of degenerated epithelial cells showed apoptotic changes (Fig.7). Uterus showed severe loss of tubular glands, congestion and severe vacuolar degeneration in the existing tubular gland epithelial cells (Fig.8). The histopathological observations were concurred with the findings of Vijayalingam *et al.*, 2017 [9]. The intensity of apoptotic cells was moderate in tubular glands of isthmus and more in uterus (Fig.9 & 10) and it was minimum in infundibulum, magnum and vagina (Wang *et al.*, 2013) [10].

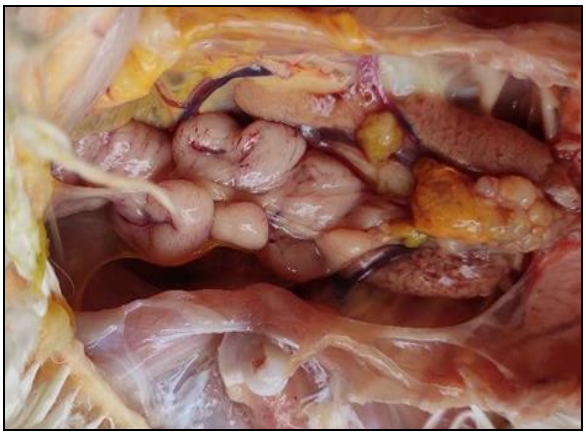

Fig 1: Aflatoxicosis: Ovary and oviduct showing severe atrophy

Fig 2: Aflatoxicosis: Ovary showing more no. of atretic ovarian follicles

Fig 3: Aflatoxicosis: Oviduct showing marked atrophy

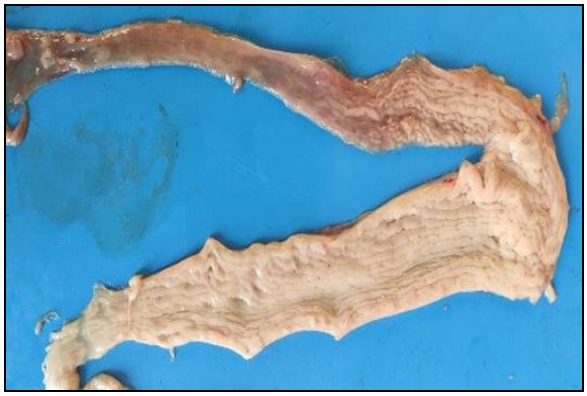

Fig 4: Aflatoxicosis: Infundibulum and magnum: Mucosa was thickened and oedematous

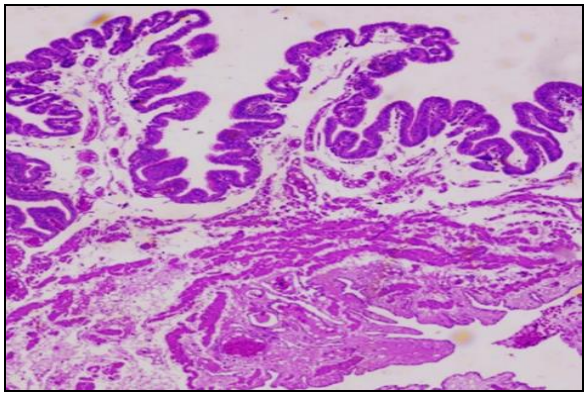

Fig 5: Aflatoxicosis: Infundibulum showing hyperplasia of ridge epithelium and congestion in tunica muscularis. H & E x40

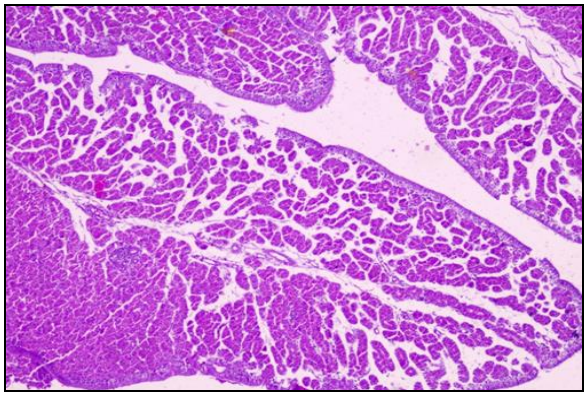

Fig 6: Aflatoxicosis: Magnum showing metaplasia of surface epithelium and moderate hypoplasia of tubular glands. H & E x40

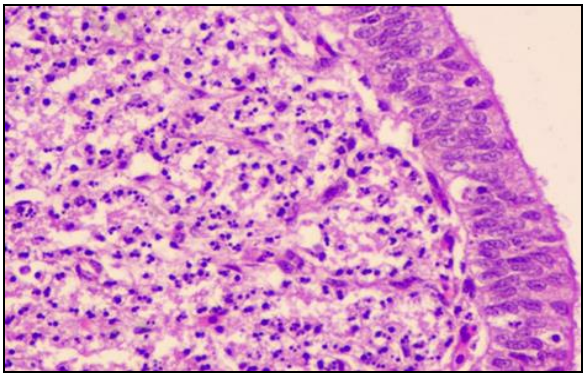

Fig 7: Aflatoxicosis: Isthmus showing loss of tubular glands and more no. apoptotic bodies (nuclear fragmentation) in tubular gland area. H & E x400

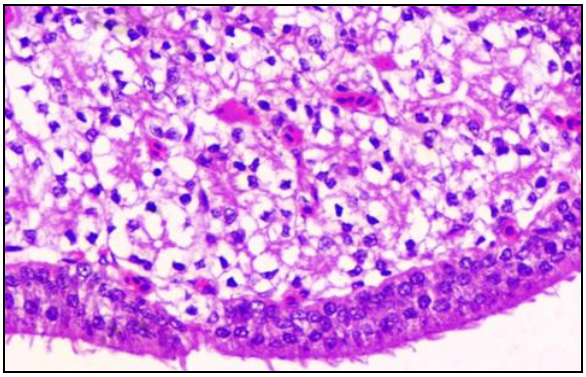

Fig 8: Aflatoxicosis: Uterus showing loss of tubular glands, severe degeneration of tubular gland epithelium and congestion. H & E x400

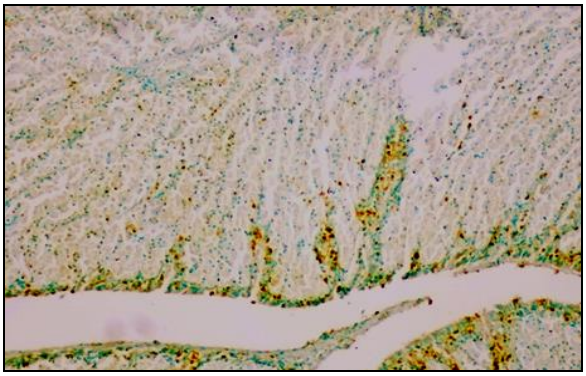

Fig 9: Aflatoxicosis: Apoptosis: Isthmus - Dark brown staining in mucosal lining and tubular gland epithelium indicates TUNEL positive (Apoptotic) cells – x100 (Background stain – Methyl green)

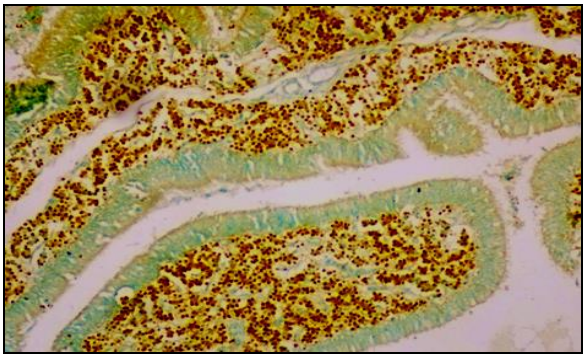

Fig 10: Aflatoxicosis: Apoptosis – Uterus - Dark brown staining in tubular gland epithelium indicates TUNEL positive (Apoptotic) cells – x100 (Background stain – Methyl green)

Table 1: Effect of aflatoxicosis on egg quality performance in layers

Egg quality parameters	Control	Aflatoxicosis
Weight	58.00±0.72	43.72±2.06
Specific gravity	1.05±0.00	0.87±0.01
Shape index	75.47±0.26	67.74±1.17
Albumen index	0.10±0.01	0.10±0.00
Haugh unit	91.07±0.98	68.48±2.44
Shell thickness	0.33±0.04	0.28±0.01

Table 2: Effect of aflatoxicosis on morphometry of oviduct in layers

Morphometry	Control	Aflatoxicosis
Weight	59.16±0.82	40.00±7.38
Length	64.95±0.70	49.16±4.12
Infundibulum	9.00±0.68	7.66±0.72
Magnum	34.50±0.87	26.25±2.53
Isthmus	10.28±0.37	9.16±0.97
Uterus	9.16±0.45	6.75±0.57
Vagina	2.50±0.22	2.33±0.21

Conclusion

In the present study ovarian follicular atresia and oviduct atrophy were recorded in layer chicken with Aflatoxicosis. Out of 71 flocks, 6 were affected with aflatoxicosis and the AFB1 level detected was 10-20 ppb. The clinical signs of mild anorexia and drop in egg production (17%) with a greater number of small eggs and uneven eggs (5-7%) were observed. No mortality was recorded other than regular mortality. The egg weight, shape index, albumen index, Haugh unit and shell thickness was significantly reduced. Post mortem lesions recorded were presence of clear watery fluid in the abdominal cavity, more no. of atretic follicles and the oviduct showed marked atrophy. Histopathologically, oviduct showed severe edema, degeneration and tubular gland hypoplasia. This might be due the effect of the toxin on the hepatic parenchyma and tubular glands of oviduct. Oviduct showed large numbers of TUNEL positive apoptotic cells in the uterus indicates that aflatoxin induce programmed cell death in oviduct and act as powerful stimulant of apoptosis.

References

1. Coulombe RA. Aflatoxins. In Mycotoxins and Phytoalexins. R. P. Sharma and D. K. Salunkhe, ed. CRC Press Inc., Boca Raton, FL; 1991. p. 103-143.
2. Manafi M. Effect of Feeding Different levels of Aflatoxin in the Diets on Production Performance of Broiler Breeders. Adv. Environ. Biol. 2011;5(13):3843-3848.
3. Oliveira CAF, Rosmaninho JF, Butkeraitis P, Correa B, Reis TA, Guerra JL, *et al*. Effect of low levels of dietary aflatoxin B1 on laying Japanese Quail. Poult. Sci. 2002; 81:976-980.
4. Rizzi L, Pezzi P, Simioli M, Formigoni A. Le micotossicosi in buiatria. In 33rd Proceedings of the Societa˜ Italiana di Buiatria. Editografica, Bologna, Italy; 2001. p. 437–451.
5. Rosmaninho JF, Oliveira CAF, Bittencourt ABF. Efeitos das micotoxicoses cronicas na produçao avícola. Arquivos do Instituto Biológico. 2001;68(2):107-114.
6. Siloto EV, Sartori DRS, Oliveira EFA, Sartori JR, Fascina VB, Berto DA. Performance and Egg Quality of Laying Hens Fed Diets Containing Aflatoxin, Fumonisin and Adsorbent. Braz. J Poultry Sci. 2012;13(1):21-28.
7. Srinivasan P. Pathology of Reproductive organs with special reference to oviduct in layers. Ph.D., thesis submitted to TANUVAS, Chennai; 2007.
8. Verma J, Johri TS, Swan BK, Ameena S. Effect of graded levels of aflatoxin and their combination on the performance and immune response of broilers. Br. Poult. Sci. 2004;45:512-518.
9. Vijayalingam TA, Rajesh NV, Ilavarasan S. A Case Report of Aflatoxicosis in a Country Chicken Unit with Retarded Egg Production. Int. J Curr. Microbiol. App. Sci. 2017;6(12):3359-3364.
10. Wang F, Shu G, Peng X, Fang F, Chen K, Cui H, *et al*. Protective Effects of Sodium Selenite against Aflatoxin B1-Induced Oxidative Stress and Apoptosis in Broiler Spleen. Int. J Environ. Res. Public Health. 2013;10:2834-2844.
11. Zaghini A, Martelli G, Roncada P, Simioli M, Rizzi L. Mannanoligosaccharides and aflatoxin B1 in feed for laying hens: effects on egg quality, aflatoxin B1 and M1 residues in eggs, and aflatoxin B1 levels in liver. Poultry Science. 2005;84:825-832.
12. Leeson S, Atteh JO. Utilization of fats and fatty acids by turkey poults. Poultry science. 1995 Dec;74(12):2003-10.